AUTECOLOGICAL AND GREENHOUSE STUDY OF WILD AND CULTIVATED HASKAP (*Lonicera caerulea* L.) IN SASKATCHEWAN

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ABSTRACT

Haskap (*Lonicera caerulea*) is a new crop for much of the world including Canada. This study investigated Haskap's autecological conditions in three habitats in Saskatchewan and utilized some of that information to design greenhouse experiments to better understand environmental and soil interactions for this crop. The hypothesis of this study was that environmental factors associated with the growth of wild Haskap and greenhouse studies with the cultivar 'Tundra' could improve the production practices of cultivated Haskap. This thesis may be the first study to investigate *L.c.* autecology, pH levels in hydroponics and greenhouse fruit production. Average *in situ* shoot growth of *L.c.* ssp. *villosa* varied significantly among the study areas. The site with peat/organic soil had greater macronutrient levels and greater shoot growth compared to the two sites with forest Luvisol soil. The site with organic soil had soil temperatures with a daily average of 4.0-12.9°C, air temperatures 8.1-19.4°C, relative humidity 58.6-91.3% and rainfall averages of 80 mm during May to July. Seven *L.c.* subspecies and the cultivar 'Tundra' were grown hydroponically in pH levels ranging from 5 to 9. Dry weights of leaves, stems and roots were measured. The influence of pH on growth was significant for all genotypes with each genotype having the highest dry weights at pH 6 and the lowest at pH 9. Subspecies *stenantha* and 'Tundra' had significantly greater growth than the other genotypes. Two-year-old 'Tundra' seedlings fertilized with a higher rate of N, P, K (1762.5, 334.5 and 403.5 mg/kg respectively) was significantly higher with a total dry weight of 38.27 g per plant compared to the 24.11 g per plant of the control. It was demonstrated that 'Tundra' and Japanese originated seedlings could produce fruit in a winter and spring greenhouse. Compared to field studies, 'Tundra' fruit was a bit smaller in size, but soluble solids and acidity levels were similar. Lower temperatures and use of bumble bees as pollinators were factors contributing to the success in greenhouse production.

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This endeavor would not have been possible without my family's and friends support and I am extremely grateful to them. Thank you.

DEDICATION

This thesis is dedicated to you, the reader of this sentence, who is interested in this wonderful plant and berry and wishes to build a better world tomorrow.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ANOVA	Analysis of variance
C	Carbon
CH_4	Methane
CO_2	Carbon dioxide
d	Day
DM	Dry matter
DMY	Dry matter yield
EC	Electrical conductivity
GDD	Growing Degree Days
hrs	Hours
K	Potassium
L.c.	Lonicera caerulea
LSD	Least significant difference
N	Nitrogen
N_2O	Nitrous oxide
NH_4	Ammonium
NH_4-N	Ammonium nitrogen
NO_3	Nitrate
NO_3-N	Nitrate nitrogen
P	Phosphorus
POM	Particulate organic matter
PRS	Plant roots simulator
PVC	Polyvinyl chloride
S	Sulphur
SMB	Soil microbial biomass
SOC	Soil organic carbon
SOM	Soil organic matter
SPP	Multiple species
SSP	Subspecies
TOC	Total organic carbon

1.0 INTRODUCTION

Lonicera caerulea L. (abbreviated as, *L.c.*) also known as blue honeysuckle or Haskap, is a new emerging fruit (Bors 2009, 2015) with known health benefits (Rupasinghe et al., 2012, Khattab et al., 2020). *L.c.* has been regarded as a superfruit due to its high biological activity of the components containing three times more antioxidants than wild blueberry (Arus and Kask 2007, Bakowska-Barczak et al., 2007, Rupasinghe et al., 2012). Recent studies suggest high levels of flavonoids, anthocyanins, pigments, vitamin C and minerals that encourage its use as a fresh and processed food (Sabitov 1986). From early times, the *L.c.* has been used for the prevention and treatment of arteriosclerosis, hypertension, liver disease and gastritis (Kolasin and Pozdnyakov 1991).

The *L.c.* is a mesophytic shrub adapted to the cold climate of the northern hemisphere (Rüdenberg and Green 1969, Plekhanova 1992). Taxonomists have long known wild blue honeysuckles, with the fruits harvested from both the wild habitats and gardens and used for traditional food and medicine in Russia (Plekhanova 1998), Japan (Nakajima 1996), China (Tang 2012) and Eastern Europe (Jurikova 2012). Inhabitants of Siberia and the Russian Far East have used it fresh and frozen for food, jams, juices, compotes, syrups and as a natural dye (Kolasin and Pozdnyakov 1991). In the 1980s, the Michurin I. V's All-Russian Cultural Center in the Siberian region, which has a cold climate, began research on the introduction of new varieties of *L.c.* and the development of new varieties (Popova 2000). Initially, in the 1990s, Canadians were growing a few Russian cultivars, but more recently, most growers are planting varieties developed at the University of Saskatchewan (USask). The world market of *L.c.* was estimated to reach $500 million CAD a year in the past five years (O'Connor 2015). The worldwide functional food industry is estimated to grow to $50 billion US dollars in the near future (Basu 2007).

Growing *L.c.* has become the latest trend in Canadian gardening, and it is getting the attention of farmers and fruit researchers in recent years due to its health benefits and good flavour. *L.c.* produces the first fruit to ripen in early summer, which can be harvested mechanically. The berries

are suitable for various processing methods. *L.c*. has high adaptability to environmental stresses and bears fruit every year for 20 years or more. It is cold tolerant and does not have many diseases and pests. With its gaining popularity, the *L.c*. orchards have spread worldwide in countries such as the USA, Canada, Russia, Japan, Czech Republic, Poland and China (Auzanneau 2017) beyond its native origins in northern regions of North America, Asia and Europe (Hummer 2006, Lamoureux 2011).

As *L.c*. has attracted interest by orchardists, it is worthwhile to study this species in its native environment (i.e. autecology) as well as under controlled conditions. Autecology is a methodology to learn from nature and from this knowledge farming practices can be improved. The autecological theory is based on three laws: the law of optimal conditions, the independence of ecological species and the law of limiting factors. The law of the optimal conditions studies the phenomenon of the tolerance of any living thing under the influence of any ecological factor. Optimum conditions occur when the numerical values of certain environmental factors are the most suitable for the life activities of living organisms, however in this study the emphasis is on the first law.

The University of Saskatchewan's fruit program has been breeding Haskap since 2001 and has had several projects involved in collecting and breeding a diverse germplasm collection (Bors 2009, Bors 2012, Bors 2015). Other PhD projects have involved studying the morphological, biochemical traits of fruits and phenological adaptation in British Columbia (Gerbrandt 2017). Genotypes with potential for use in breeding programs for the secondary metabolites with nutraceutical importance present within fruit and leaves of Haskap were investigated (Dawson 2017). Although The University of Saskatchewan has been gathering wild Canadian Haskap plants from across the country since 2007 (Bors 2009) and used them in breeding (Bors 2010), sites from which the wild plants were gathered have not been studied (Bors 2009). Concurrently with this thesis, there was a project where farm sites were visited with tissue and soil samples taken and analyzed with a goal of better understanding nutritional disorders. Some small-scale preliminary fertilizer experiments were conducted (Bors 2009).

But this thesis takes a different approach. It begins with an intensive autecological study of three wild Haskap habitats in Saskatchewan. It used the gained knowledge to define the optimal pH level for growing Haskap in hydroponics. It also investigated *L.c*. in winter and spring greenhouses for

fresh berry production. Growth responses of seedlings to a macro mineral fertilizer for nursery production was also assessed.

As one of the first (if not the first) study to investigate autecology and greenhouse production of haskap, this study provided understanding for researchers and farmers that should be useful in developing production protocols that could lead to better and more precise agricultural practices.

1.1 Research goals, hypothesis and objectives

The first goal of this study was to conduct an autecological study of wild haskap *Lonicera villosa*, growing in the wild in Saskatchewan, Canada. This information was the foundation for subsequent cultivation experiments in the greenhouse (Figure 1.1).

The hypothesis is that information regarding environmental parameters, such as soil, light, temperature and moisture of wild haskap sites, can be used to improve production practices of cultivated haskap.

The objectives are:

- To conduct an autecological study of wild Canadian *L.c.* to determine what environmental conditions are associated with optimal plant growth;
- to apply the results of the autecological study and determine the effect of pH on the biomass of wild and cultivated blue honeysuckle;
- to apply the results of the autecological study and determine the response to mineral fertilizers on *L.c*. growth and nutrient uptake;
- to apply the results of the autecological study and grow *L.c.* and harvest haskap berries in winter and spring greenhouses;

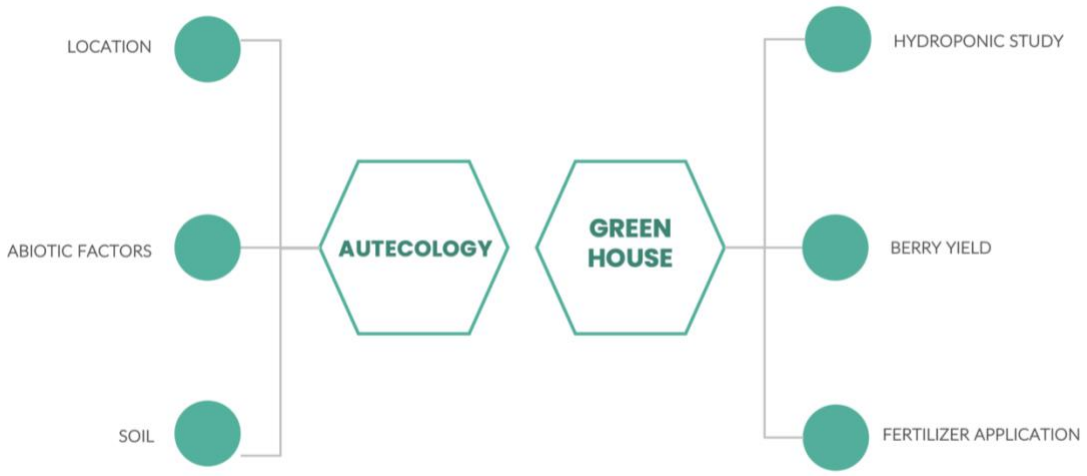


Figure 1.1. Main research components of this thesis. Autecological studies assessed wild Haskap sites in Saskatchewan. Autecological information determined specific parameters of the greenhouse experiment, testing the hypothesis of this thesis

1.2 Practical significance of the research

It is noteworthy to mention that there have not been any published papers on autecology, pH specific nor greenhouse fruit production of *L.c.* The practical significance of this research is to better understand the biological and physiological features of blue honeysuckle, the effect of the external environment and mineral fertilizer application on its growth and production. *L.c.* cultivation is relatively new in some regions of the world such as Canada, the United States and Europe. The recommended levels of nutrients in soil, leaves, pH levels in soils and other environmental conditions are currently not well known for growers. Part of this study involved investigation of growth rates of seven subspecies of *L.c*. that could provide useful information for breeders. It is estimated there are 700 haskap orchards in Canada. Therefore, the need for improvement of knowledge about cultivation is significant. It can cost approximately $10,000-15,000/acre for an orchard establishment, with maturation in four to six years (Iheshiulo 2018). Research that can reduce management costs or speed up production would be beneficial to growers.

2.0 LITERATURE REVIEW

2.1 General description of wild blue honeysuckle (*Lonicera spp*)

The genus was named *Lonicera* in Latin in memory of German physician and botanist Adam Lonizer (1528-1586) by the Swedish botanist Carl Linnaeus. Researchers have noted about 150-250 *Lonicera* species belonging to the family of Caprifoliaceae Juss (Rehder 1903, Gizdyuk 1981, Plekhanova 1990, Kuminov 1994, Skvortsov and Kuklina 2002). Blue honeysuckle is mainly distributed in the relatively cold regions of the northern hemisphere with four seasons of the year. Researchers note that the study of honeysuckle plants dates to early ages. Michurin (1935), one of the pioneers of honeysuckle research, began the study of blue honeysuckles in 1909 in the Tambov region of Russia, and recommended the use of this plant in orchards.

Lonicera plants is diverse and widely distributed in temperate cold climates. For an example 51 species of *Lonicera* have been named in Russia (Plekhanova 1978, 1990). In Mongolia highlands the three most common species are *Lonicera altaica Pall. Ex DC*, *Lonicera tatarica* (*Lonicera tatarica L*) and *Lonicera maackii* (Rupr.) Maxim and are widely distributed (Jamiyandorj and Tsendeekhuu 2020). In North America, some *Lonicera* species such as *Lonicera japonica*, *Lonicera maackii*, *Lonicera morrowii* and *Lonicera tatarica* have been considered as invasive species causing changes to the natural ecosystem (Schierenbeck 2004, Whitehead and Bowers 2013). While some species of *Lonicera* is invasive, *Lonicera caerulea* is cultivated for fruit production.

Blue honeysuckle (*Lonicera caerulea. L.*) is a shrub which grows 1.5-2.0 meters tall reaching in some cases 4 meters (Skvortsov and Kuklina 2002). Young shoots and branches are sparsely covered with bare or short stiff hairs. The leaves are simple, opposite, bare, oblong-oval, with a wedge-shaped round base, blunt apex, hard hairs on both sides. The flowers are bisexual, epigenous, tubular, white yellow. The ovary is inferior, closely attached to the calyx and deeply seats in the pedicel, the petals have a composite division, 13-18 mm long. The filament is attached to the petals base by a pedicel. The stamen is equal to or slightly longer than the petals, and the pistil is protruding beyond the petals. The fruit is a berry and averages about 1 cm long, oval-round, dark

blue, in pairs. Blooms occur in late April and early May resulting in seeds that are oval-round, flattened, small, 2-2.5 mm long, (Bors 2014)

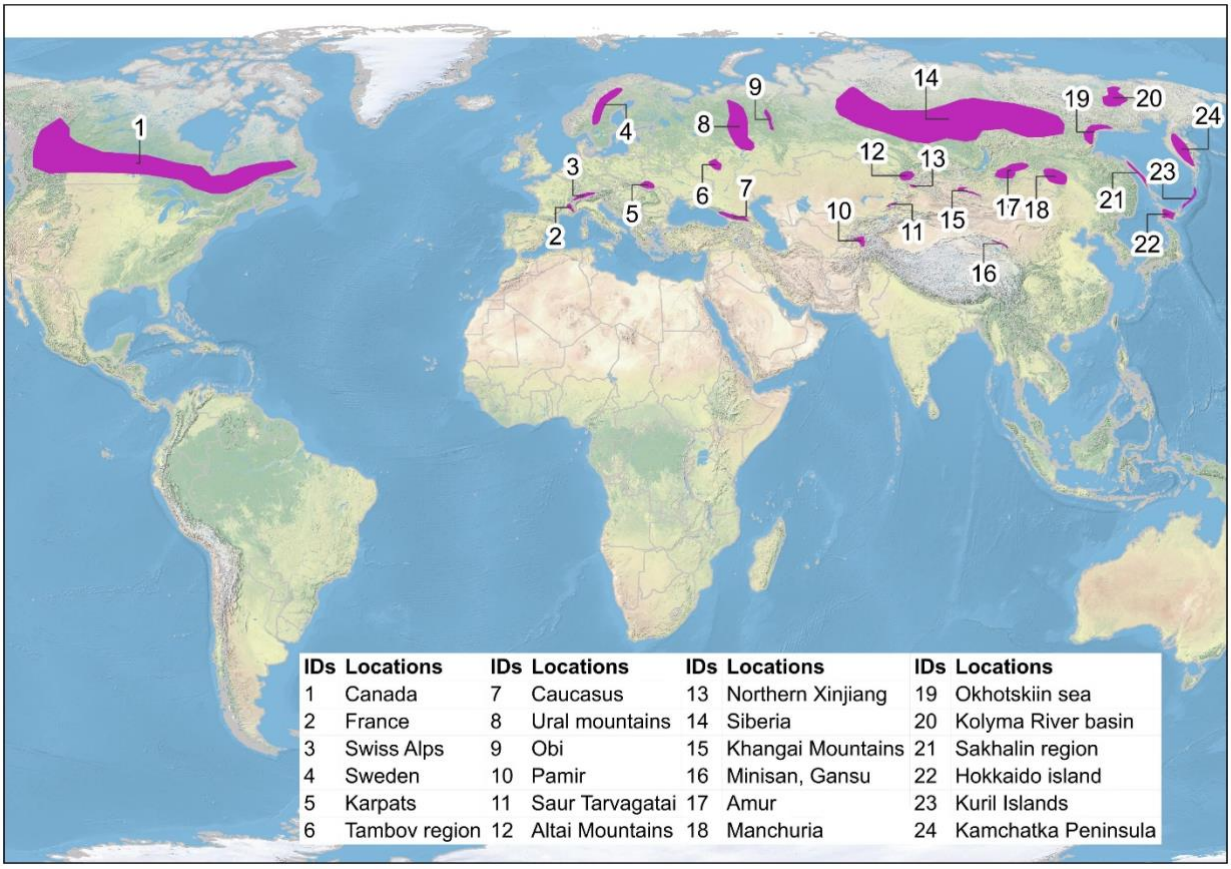
The fruit is high in sugar content with a range of 10 to 20 Brix and an average 16.6 depending on growing years and varieties (Bors, 2015). The fruits also contain vitamins C and B_1, B_2 and B_6. In the past and in the present fruits used in folk medicine for gastrointestinal disorders, cholecystitis, edema and high blood pressure. It is one of the few plants that have a hemostatic effect. When fully ripened, the fruit can be used fresh or processed at home.

Many *Lonicera* species are not edible. However, 10-15 *Lonicera* including *caerulea* and have been found to be edible (Plekhanova 1994). According to the analysis of bioactive components, *L.c*. has more polyphenol and anthocyanin's compared to other fruit (Qian et al., 2007, Svarcova et al., 2007, Bakowska-Barczak 2007, Dawson 2017). Similar studies showed that polyphenol and antioxidants are the highest in three varieties of *L.c*., i.e., 'Borealis', 'Indigo Gem' and 'Tundra', compared to other fruit (commercially farmed blueberry, wild blueberry, strawberry, red grapes, raspberry, blackberry and partridgeberry). The total phenolic content was positively correlated with the total content of flavonoids, suggesting a strong linkage and may also reflect the antioxidant qualities of these fruits (Rupasinghe 2012).

In North America the taxonomy of *Lonicera caerulea* has been debated and has changed over time. Wild Canadian blue honeysuckle, *Lonicera villosa* (Michx) Poem and Schultes, has been classified into four varieties: *Lonicera villosa var. villosa, Lonicera villosa var. tonsa, Lonicera villosa var. solonis and Lonicera villosa var. calvescens* (Scoggan 1979). However, Lahring (2003) considered *villosa* to be a sub species of *Lonicera caerulea*. It blooms as early as April and generally ripens by mid-June, while in western Russia, other L.c. bloom from May to June and ripen in late June and mid-July. Wild blue honeysuckles from the highlands of Mongolia, bloom in early June and ripen in mid-August. This plant is tolerant to heat, cold and drought, does not require high soil fertility, and belongs to the category of moist-dry plants. In some cold and rainy years, the fruits ripen unevenly which may be related to the Growing Degree Days (GDD) of that region and season (Jamiyandorj and Tsendeekhuu 2020). All the subspecies of *L.c.* have the characteristic of blooming earlier than most other plants and tolerating frost on flowers.

2.2 Distribution and genetic characteristics of wild blue honeysuckle

It is hypothesized that *L.c*. may have originated from the mountainous systems of Central Asia and the current geographical distribution could have occurred after the Pleistocene (Skvortsov and Kuklina 2002). The primary center of origin of the plant is considered to be in Southeast Asia, and later it was distributed to the Himalayas of the northern hemisphere, the highlands of Central Asia and the temperate regions of North America, Canada and Europe (Plekhanova 1990).



IDs	Locations	IDs	Locations	IDs	Locations	IDs	Locations
1	Canada	7	Caucasus	13	Northern Xinjiang	19	Okhotskiin sea
2	France	8	Ural mountains	14	Siberia	20	Kolyma River basin
3	Swiss Alps	9	Obi	15	Khangai Mountains	21	Sakhalin region
4	Sweden	10	Pamir	16	Minisan, Gansu	22	Hokkaido island
5	Karpats	11	Saur Tarvagatai	17	Amur	23	Kuril Islands
6	Tambov region	12	Altai Mountains	18	Manchuria	24	Kamchatka Peninsula

Figure 2.1. Geographical distribution map of wild blue honeysuckles complied from Michirin 1935, Bochkarnikova 1979, Hara 1983, Skvortsov 1986, Plekhanova 1994, 2000, Smirnov 2002, Sheiko 2008, Bors 2009, Kang et al., 2018, Jamyandorj and Tsendeekhuu 2020.

Cultivars of *Lonicera caerulea* are mainly bred from germplasm obtained from Primorsky range, Altai Mountains, Kamchatka Peninsula, (Plekhanova 2000) Hokkaido and Kuril Islands (Bors 2009).

L.c. has been found at high elevations including 650 meters in the Kolyma river basin in Russia, 1000 meters in Kamchatka, 1400 meters in the southern Kuril Islands, 1300-1600 m (Stanovoye podnyatiye) in Northern Xinjiang in China, 1850 meters in Karpats in Ukraine, 2300 meters in

Saur-Tarvagatai in Kazakhstan, 2400 meters in Caucasus, 2600 meters above sea level in Swiss Alps, east of Ukraine and 2700 meters in Khangai Mountains of Mongolia, 3200 meters in Minisan, China's Gansu province, near lake Yashikul in the Pamir mountains and 3800 meters in the northeast of Afghanistan respectively (Skvortsov 1986). It was also found on sandy beaches and the bare hills planted with young trees for forestation purposes in Sakhalin region, considered to have excess moisture (Smirnov 2002).

In Japan, Haskap is spread over the island of Hokkaido where it occurs in areas transitioning from the forest, usually on the upper forest border, volcanic ash accumulations, along rivers and wetlands (Hara 1983). *Lonicera caerulea* ssp. *empyllocalyx (Maxim.)* which belongs to the Japanese ecotype Nakai, were also encountered in Monyeron, a small island located southeast of Sakhalin (Bochkarnikova 1979). In the Kuril Islands it was abundant in non-shady or beach areas, meadows, groves, bamboo land, wetlands, mountain peaks and logged areas, however, only one plant was found at the bottom of deciduous forest (Sheiko 2008).

Lonicera caerulea ssp.*caerulea* grows in arctic and boreal regions and is common in the areas where forests transfer to high mountainous areas. Geographically, it was recorded in about 131 places in the Kuril Islands and Kamchatka Peninsula, Yakutia, Stikhon-mining both the Sayan Mountains and the Altai Mountains, the Ob River, the Urals and Pyechora, North Divina Valley in Russia, Hokkaido, Japan, Xinjiang, Northern China, Manchuria, Tajik, Kyrgyz and Kazakh territory, Pyrene, France in Europe (Skvortsov 1986) Scandinavia and the Alps surroundings (Plekhanova 2000), Okhotskiin sea area (Skvortsov 1986).

In Canada, it has been found in every province except British Columbia (Bors 2012). *L.c.* was found in open areas, near forests and seasonal streams, openings in the deciduous boreal forest where fallen trees were decomposing, high calcium soils and disturbance areas near road construction, it mainly grows in areas where trees are doing poorly, in wet areas and usually partly shaded areas (Bors 2009). Relatively little growth near coniferous forests may be related to the lack of seed renewal (Nedolujko 1984). Thus, the distribution of the honeysuckle reflects its wide adaptability and appears to have the potential to be grown in many places when the right growing conditions are created.

A study of polyploidy by Plekhanova (2000) covered 156 accessions of *Lonicera caerulea* sub-types and showed that there are both diploid (2n=18) and tetraploid (4n=36) types. Miyashita et al., (2011) inferred that diploid populations occur in lower elevations and are more rare than tetraploid. When 374 accessions of *Lonicera caerulea* ssp. *villosa* were tested, all except four were found to be diploid (Bors, 2014). Skvortsov (1986) attempted to show the number of chromosomes in the *L.c*. by geographical location in Russia. Their geographical distribution also revealed that tetraploid types more common over diploid, and tetraploids were mostly found in the northern, high mountain regions. Three locations of diploid types were identified of which *Lonicera caerulea* ssp. *illiensis* (2n=18) were found in the valley of Ili river in Kazakhstan, Central Asia and around China's Xinjiang province, *Lonicera caerulea* ssp. *boczkarnikowae*, (2n=18) was distributed at the Amur River source, Zyeyaa and Buryeyaa water reservoirs in Transbaikal region and around the Pryejyevaliskii mountain edge in South Ussuriysky region. *Lonicera caerulea* ssp. *edulis*, (2n=18) occurred also in the Amur and the upper part of Zyeyaa River in Transbaikal region. Based on her study of morphology, anatomy and biochemistry of 583 species, cultivars and forms with origin from the northern European part of Russia, Siberia and the Transbaikal region during 1976-1998, Plekhanova (1994) concluded *Lonicera caerulea* ssp. *pallasii*, *Lonicera caerulea* ssp. *kamtschatica* of Eurasia, *Lonicera caerulea* ssp. *villosa* of North America, *Lonicera caerulea* ssp. *emphyllocalyx* of the Japanese Islands are closely related of phylogenetically. However, Plekhanova likely mistook the 'Bugnet' cultivar bred in Canada for being *villosa* as that subspecies is very different from the others (Bors, personal communication, 2022).

A study of *L.c.* morphology showed *Lonicera caerulea* ssp. *iliennis, Lonicera caerulea* ssp. *stenantha (diploid), Lonicera caerulea* ssp. *edulis* have narrow leaves, *Lonicera caerulea* ssp. *emphyllocalyx, Lonicera caerulea* ssp. *pallasii, Lonicera caerulea* ssp.*villosa* wide leaves*; Lonicera caerulea* ssp. *edulis (diploid)* has relatively small flowers (13-16mm), *Lonicera caerulea* ssp.*kamtschatica, Lonicera caerulea* ssp. *emphyllocalyx* have relatively large flowers; *Lonicera caerulea* ssp. *senantha* has a relatively short pedicel while *Lonicera caerulea* ssp. *kamtschatica* flowers have a relatively long (15mm) pedicel (Plekhanova 1994).

Plekhanova (1994) studied the change in anatomical characteristics of leaves of different ssp's such as the size of the stomata and palisade coefficient, leaves thickness and the number of mesophyll membranes. *Lonicera caerulea* ssp. *emphyllocalyx, Lonicera caerulea* ssp. *pallasii,*

Lonicera caerulea ssp. *kamtschatica* had the same mesophyll anatomical trait with similar leaves structure of thin and medium thick leaves, large upper and lower epidermal cells, and differed by fewer cells with larger stomata. Diploid varieties of *Lonicera caerulea* contain similar leaf structures such as small epidermal cells, large stomata and thin leaves as their tetraploid counterparts. However, compared to diploids, tetraploids have a longer period of growth each season and also have larger numbers of flowers per shoot and greater fruit volumes (Plekhanova 1994). According to Skvortsov (1986) northern plants have a faster seasonal developmental cycle. In the far northern taiga zone, shoots were thick and hairy with a dark greenish colour, but in more southern locations or lower elevations shoots were less hairy with lighter brown and pinkish colour.

2.3 Biological features of the wild blue honeysuckle

2.3.1 Phenophases

Long-term observation of phenophases of *L.c.* subspecies showed they were very similar to each other, with growth, flowering and fruit ripening starting and finishing very early compared to other fruit crops (Plekhanova 2000). Bud break and flowering under temperate climates of B.C. Canada were different according to genotypes of Russian, Kuril and Japanese origin. Russian and Japanese genotypes were earliest in flowering onset, then at budbreak (Gerbrandt, 2017). Dawson (2017) reported that across two years, berries of six cultivated genotypes of *L.c.* matured in 42-49 days after flowering depending on heat requirement, but there were no differences in berry weights.

The time of flowering is influenced by climatic conditions (most importantly temperature) and genetics. Evidence shows large differences in flowering time (more than 2 weeks) of the same varieties in different years. The flowering time also differs from 7-15 days between varieties (Dawson 2017). *L.c.* begins to flower at the beginning of May in Saskatchewan Canada and the end of April in Poland (Gawronski et al., 2014). Since the plant is not self-pollinating, for cross-pollination to occur compatible varieties which flower at the same time need to be close by (Frier et al., 2016).

Blue honeysuckles start bearing fruit from the second year of plantation and maximum yield (3-5 kg) can occur from 8-15 years. Plants can bear fruit up to 30 years (Dawson 2017) or longer with rejuvenation through pruning (Plekhanova 1989).

Factors limiting crop yield can include a disturbance of the plant dormancy period, secondary flowering in late summer or fall, winter damage, disease and pests, plant age, etc. In some cases, flowering and fruit-bearing age varied between the different locations within plants due to shoot underdevelopment (Plekhanova 1994). Blue honeysuckles are self-incompatible so lack of compatible cultivars in the field can also reduce yields. When self-pollination occurs, the pollen tube growth stops at two-thirds of its total length of the style. Under cross-pollination, pollen tubes reach ovules in 12 hours (Plekhanova 1994) and fertilization occurs 16 hours later (Plekhanova 2000).

The University of Saskatchewan Fruit Program has been utilizing genetic differences of subspecies to breed cultivars to ripen at different times. Thus, reducing risks of inclement weather at bloom time and to extend the harvest season.

2.3.2 Water

According to a study in Michurinsk, Russia, the water holding capacity of *L.c*. leaves differed among varieties by allocating water to the most active organ (in this study to the fruit) when there was a shortage of the water. The most vulnerable stage was the fruit development stage but water consumption reduced after the fruit was fully ripened (Kirina 2009).

In a study (Jurikova et al., 2009) of irrigated and non-irrigated *L.c.* in Nitra, Slovakia in Eastern Europe, the respiratory rate of *L.c.* (*L.c.* ssp. *kamtschatica* and *L.c.* ssp. *edulus*) was high during early stages of fruit ripening due to high cell division in the fruit. Later, when the colour of the fruit changed from green to blue, respiration decreased sharply, but when the fruit began to soften, it increased slightly again.

The respiratory rise was most evident under irrigated conditions in which full ripening of the fruit coincided with the peak of respiration and the period of softening of the fruit. Organic acids increased at the early stages of fruit ripening and decreased when fruit softened while increasing again at the time of fully ripening of fruit. However, sugar content decreased at the early fruit ripening stage, reached its peak at the fruit ripening stage and slowly decreased further (Jurikova et al., 2009). Dawson (2017) found a similar trend during fruit ripening but did not investigate after optimum ripeness.

2.3.3 Cold tolerance and dormancy

One of the special features of *L.c.* is its ability to tolerate the cold. This plant tolerates winter temperatures down to -50°C (Bob Bors, personal communication, 2021), while the flowers can tolerate shocks down to -8°C (Shchekotova 2002) for 8-9 hours (Gizdyuk 1981). Haskap flowers usually face downward which would protect them from getting wet from mist or rain. However, when researchers sprayed water into the flowers that were at -2°C temperature, flowers were damaged (Gusta and Bors personal communication). Thus, Haskap flowers are likely avoiding frost down to -8°C.

A Hokkaido study of *Lonicera caerulea* ssp. *emphyllocalyx* Nakai, documented seasonal changes which showed that bud tip water and sugar content were correlated and cold resistance may be related to raffinose and amount of stachyose (Hiroyuki 1998). The increase of freezing tolerance in *Lonicera caerulea L* tissues was dependent on raffinose and/or stachyose composition since the raffinose family of oligosaccharides have a cryoprotectant role in cold-acclimated plant cells (Imanishi 2000). The plants were cold resistant at least down to -40°C and flowers and resisted cold damage at -8°C (Ochmian 2008). However, cold hardiness varies by regions such as Central Europe, Northern Europe, Canada and the US (Becker and Szakiel 2019). *L.c.* is native to Siberia, northeastern Asia and Canada, which includes places that get colder than -50°C. The native habitat includes high mountains and wet areas with rivers and bodies (Celli 2014).

The cold tolerance of the *L.c.* was highly dependent on winter temperature fluctuations. For example, in St. Petersburg, Russia, during the winter of 1986-1987, daily average air temperature was +5°C to +10°C for an extended time period, but when temperatures dropped to -42°C to -46°C, this led to stems and shoots freezing with subsequent plant death (Plekhanova 2000). This scenario often resulted in tip kill of branches, reducing flower numbers and yields (Kuklina 2006). In such sudden weather changes (St. Petersburg), the least tolerant subspecies were *Lonicera caerulea* ssp. *edulis* and *Lonicera caerulea* ssp. *boczkarnikowae* from Primorsky Krai.

It is a generally accepted principle that cold resistance and timing of dormancy are directly related (Weiser 1970). Temperature is one of the main factors which can influence growth cessation and dormancy development (Kalcsits et al., 2009) and a specific feature of the *L.c.* is that its dormancy period is short (low chilling requirement). Depending on the geographical origin, the phenomenon of re-flowering in warming periods in late autumn or in winter, after the growing season, is commonly observed. This was observed in St. Petersburg (Plekhanova 2000), Moscow

(Skvortsov 1986) and Ukraine (Mezhensky 2009). Interestingly, re-flowering was occasionally observed in *Lonicera caerulea* ssp. *edulis and Lonicera caerulea* ssp. of the Kuril Islands origin and there were occasions when flowering occurred after harvest season and immature fruit developed (Skvortsov 1986).

2.3.4 Pests and diseases

In cultivated *L.c.* there are little to no insect and disease pest of economic importance. However, a study by the Central Botanical Garden of the Russian Academy of Sciences in Moscow (Upadyshev 2009) 67% of varieties were found to be infected with multiple viruses. Variety 'Yuliya', 'Izbrannitsa', 'Viritskaya Krupnaya', 'Lazurnaya', 'Salut' and 'Nimfa' have been infected with two viruses, mostly with Tomato Black Ring Virus (TBRV) and Raspberry Ring Spot Virus (RpRSV), TBRV and Strawberry Latent Ring Spot Virus (SLRSV). Variety 'Start', 'Parabelskaya', 'Sinniya Ptitsa' were infected with three viruses TBRV, SLRSV and Cucumber Mosaic Virus (CMV). The variety 'Kamchadalka', 'Berel', 'Goluboyue Verteno' were infected with four viruses and variety 'Roksana' and 'Pavlovskaya' with five viruses. Out of 146 cultivars tested for viruses' infections, there was the presence of an average 11.1% - Arabic Mosaic Virus (ArMV), 12.2% - RpRSV, 17.6% - SLRSV and 33.1% - TBRV. In studies in Saskatchewan, the U of SK fruit program identified powdery mildew but did not identify the genus and species. Nevertheless, this provided opportunity to select potentially resistant parents for breeding (Bors 2013). Botrytis has been one of the major diseases of this crop in Japan and Oregon (Thompson, 2006).

According to pest control research conducted in Moscow, the *L.c.* was affected by the following insects: *Parthenolecanium corni Boush, Chionaspis salicis L., Tetranychus urticae Kosh., Archips rozana L., Archips podana Sc., Lithocolletis emberi zaepennella Bouch., Platyptilia calodactyla Den., Athrips mouffetella L., Aleyroodes lonicearae Walk., Semiaphis lonicerae Sieb* (Naumova 2009). In Saskatchewan, birds, voles, mice, rabbits and deer have all been observed to cause damage to *L.c.* plants (Bob Bors, personal communication, 2021).

Sheiko (2008) pointed out the special role of canopy shading on susceptibility of *L.c.* to fungal disease and insect pests in Sakhalin, Russia. Yield was decreased for *L.c.* that was growing in the shady and densely forested areas while honeysuckles growing under a deciduous tree stopped

bearing fruit, growing significantly late and plants were attacked by insects. When growing among other shrubs, the leaves in the middle and lower parts of the plant died and were covered with "grey matter". The same symptoms were observed when the normally fungus-resistant species *Lonicera caerulea* ssp. *xylosteum L*. was growing in the shade (Egorova and Sheiko, 2003). Studies have shown that the "grey matter" was caused by the fungi *Cytospora lonicerae* Grove, which also had the saprotrophs *Conyothyrium* and *Microsphaeropsis.*

2.4 Breeding and genetics of blue honeysuckle and implications for cultivation

In the last two decades, the influx of genetic material into North America from Eurasia and particularly from Japan has greatly contributed to the development of new varieties of this unique orchard and production (Thompson 2006a). Breeding was an important step and played a key role in the cultivation of blue honeysuckle. In Russia, cultivation of *L.c*. began in 1913-1915, intensified during 1950-1960 and expanded in 1972-1990 from a collection of wild forms in north and eastern parts of Eurasia and became a major initiative at the Vavilov Institute of Plant Industry in St. Petersburg and at the Fruit and Vegetable Institute in Barnaul, Siberia (Plekhanova 2000) as well as in Tomsk and Vladivostok (Skvortsov 1986). This work resulted in the first (F1) and second-generation (F2) of the best breeding 'Start', 'Goluboye Vyertyenno' and 'Siniya Ptitsa' varieties with origin from the Kamchatka Peninsula in the 1980s (Plekhanova 2000). The collection of the All-Russian Horticultural Research Institute[1] contains 74 accessions and Bryskin conducted an economic and biological assessment on 67 of them in 2007.

The University of Saskatchewan, Canada, also has one of the largest collections, with 32 varieties of Russian selections, 50 from the nurseries of North American and Russian research institutes, and 50 from expeditions to Japan (Hokkaido), the United States, and Canada. Also, about 3000 seedlings have been raised from various sources. Much material was obtained from the Vavilov Institute. The source material of the wild fruit of the species is being maintained as live plants (Bors and Thomson 2012). Varieties released by the University of Saskatchewan such as 'Tundra', 'Borealis', 'Indigo Gem', 'Aurora', 'Boreal Beast', 'Boreal Beauty', 'Boreal Blizzard', 'Honey Bee' are attaining great interest from producers and gardeners. Improved varieties are anticipated to be released.

[1] named after IV Michurin of the Russian Federation

One interesting observation by Skvortsov (1986) was that fruit taste (bitterness) varied among *L.c*. planted from seeds from a botanical garden in Toulouse, Pyrenees in France. He selected and bred two of the least bitter fruit plants and in the four plants of the next generation the fruit bitterness disappeared. He concluded that the bitter taste of the fruit was induced by part of alleles in a single locus and the disappearance of the bitter taste might be a recessive trait. It has been observed that fruit maturity period, fruit size and bitterness are highly inherited from the seedlings from the parent plant (Plekhanova 1994).

Based on her breeding experience Plekhanova (1987) defined the desirable characteristics of germplasm for creating future Table 2.1.

Table 2.1. Characteristics useful for breeding of Lonicera caerulea. Based on Plekhanova 1987

Germplasm Type	Characteristics useful for breeding
Kamchatka	Cold resistance, large fruit and bittersweet taste, high vitamin C
Kuril Inlands	long shoot dormancy period, cold-resistance, high vitamin C content and late maturity characteristics
Maritime	early maturity, high yield, high polyphenol concentrations
Altai-Sayan Mountains	high yield, low fruit spillage, drought tolerance
Northwest Russia	long shoot dormancy period, cold resistance, low fruit release

Later Bors (2009) made an assessment of basic germplasm groups which included positive and negative attributes, also included mechanical harvesting characteristics such as uniformity of ripening and how strongly plants hold onto their fruit (Table ***2.2***).

Genetic resources were placed in 3 groups based on Random Amplified Polymorphic DNA analysis germplasm Research showed 83.9 % of bands were polymorphic and that the groups had some similar morphological characteristics (Naugzemys et al., 2007):

a) *L.c.* ssp. *xylosteum* will grow more than 3 m high, fruit ripens late in the summer and but flavor is not suitable for food,
b) *Lonicera caerulea, Lonicera caerulea* ssp. *altaica, pallasii, stenantha* have early bud break and produce big blue fruit, dormancy period of generative shoots is short,
c) *L.c.* ssp. *kamtschatika* is short (0.8 meters), Bloom time and fruit maturation is late and bud dormancy period is long.

Table 2.2. Characteristics of different Lonicera caerulea germplasm useful for breeding. Adapted from Bors 2009

Types	Advantages	Disadvantages
Russian cultivars	Uniform ripening	Tubular, smaller fruit
	Most can be harvested by shaking	Plants quit growing by end of June
	Upright plants	
	Productive	Some can be bitter
	Early ripening	
	Tart flavour common	
	Flavor variable but many are good	
Japanese	Larger more rounded fruit	Uneven ripening
	Longer period of active growth	Most plants hold on to fruit too tightly
	Productive	
	Late ripening	
	Tends to be resistant to leaf diseases	
	Flavour variable but many are good	
Kuril	Uniform ripening	Low productivity
	Late ripening	Short plants
	Sweet, pleasant flavour	Most plants hold on to fruit too tightly
	Larger, round fruit	
	Highly resistant to leaves diseases	
	Leaves stay green and healthy through summer	
Canadian	Early ripening	small fruit size
	Brighter blue than other types	Most plants have drooping branches
	Most are sweet, pleasant flavoured	
	Well adapted to Canada	
	Mostly round fruit	

L.c. easily cross-pollinates regardless geographic origin with fruit set rate up to 30-90%, however intercrossing plants with different ploidy levels had only about 0.1-8.0% success rate (Plekhanova 2000). Tikhonova and Sorokin's (2009) study of the *L.c.* pollen stored in liquid nitrogen (-196°C) showed the pollen had a germination rate of 1.9-39.6 % and concluded the growing capability of the pollen could be driven by weather conditions during the pollen development period. Because of early flowering, of *L.c.*, Frier (2016) concluded primarily Bombus *spp* Queens (not workers) are pollinating blue honeysuckle. It was found that individual *Bombus spp* often visit and could tolerate the colder temperatures during *L.c.* flowering and visited more flowers per time interval compared to honeybees.

According to a study of shoot re-growth on the one-year-old shoots conducted in the Russian north-eastern region, inflorescence and fruit occurred in the terminal and lower shoots in *Lonicera caerulea* ssp. *kamtschatica, Lonicera caerulea* ssp. *emphyllocalyx, Lonicera caerulea* ssp. *pallasii, Lonicera caerulea* ssp. *villosa, Lonicera caerulea* ssp. *edulis (diploid)*. The same pattern was observed in *Lonicera caerulea* ssp. *iliennis, Lonicera caerulea* ssp. *altaica* of Central Asian origin. However, 2-3 waves of growth were observed in the following year and new shoots never formed in the upper shoots. Thus, *Lonicera caerulea* ssp. *iliennis, Lonicera caerulea* ssp. *altaica*'s fruit formation usually occurred mainly at the base and middle of annual shoots (Plekhanova 1994).

In terms of yield potential, *L.c.* ssp. *altaica, turczaninowii* and tetraploid *edulis* produced more shoots, while ssp. *emphyllocalyx* produced more flowers and berries per shoot (Plekhanova 1994). There have been numerous studies of *L.c*. germplasm characteristics, but studies were always performed on plants under cultivated conditions. While Bors (2009) suggested wild Canadian ssp. *villosa* may be better adapted to Canadian conditions, prior to this thesis there have not been any studies investigating ssp. *villosa* in its natural environment.

Based on the percentage of drop by the shaking method and fruit damage levels, varieties may differ significantly in mechanized harvesting potential (Kanarsky et al., 2013). There was no difference in ethylene content of leaves different regions, but ethylene levels did vary in fruit. Generally, ethylene concentration in fruit and leaves increased at the start of fruit development, reached its peak at full maturity and subsequently decreased, while a higher rate of ethylene production resulted in greater fruit drop. Preharvest fruit drop rate studied by Leisso et al., (2021) on 15 Haskap cultivars varied by genotype but not influenced by plant growth regulators (1-napthaleneacetic acid and aminoethoxyvinylglycine). Haskap berries are poorly suited for conventional storage which was related to high water concentration in tissues, high metabolism, high respiration with moisture loss, and poor development of epidermal tissues (Goudkovsky et al., 2009). It was emphasized that uniformity of fruit ripeness was most important for storage (Goudkovsky et al., 2009). Depending on the variety and environmental conditions of the growing year, the fruit was able to maintain freshness in a simple refrigerator (4°C) for 15-77 days (Fefelov et al., 2009).

Handpicking and manually sorting fruit to market and processors is not productive (Thompson 2006). This method is labour-intensive, repetitive and should be implemented in a short time of

harvesting. Using hyperspectral imaging analysis, a potential new harvesting system has been developed by Fu et al. (2014) for identifying overripe berries. The system is expected to increase the productivity per worker from 1.5 kg/h to 10.4 kg/h with not much-added cost (Fu et al., 2014). This method is a substitute for RGB colour imaging analysis since overripe berries cannot be distinguished from ripe berries since they have the same exterior colour. The idea of mechanically harvesting *L.c.* was first mentioned by Bors et al. (2003). Breeding for mechanical harvesting became a major goal of the U of SK breeding program which included evaluation of 17 Russian cultivars and other germplasm for characteristics deemed important for mechanical harvesting. In 2009, mechanical harvesting was demonstrated for the first time (Bors 2009). Today, most growers of *L.c.* are mechanically harvesting their fruit.

2.5 Medicinal constituents of blue honeysuckle

L.c. has been gaining much interest in recent years due to its health benefits. At the University of Saskatchewan, a three-year study investigated a wide range of breeding material for phenolics, anthocyanins, and flavonoids (Bors 2015). Nutraceutical levels were also investigated each week during the growing season in both fruit and leaves (Dawson 2017). Both these studies found a great deal of variability among genotypes. For example, some genotypes had 300% higher concentrations of anthocyanins than other genotypes (Bors 2015).

Secondary metabolites have been known to increase under stressful environmental conditions. It was suggested that because *L.c.* evolved in far northern areas under more UV light (longer summer days, less ozone), cold and often wet conditions, it resulted in *L.c.* plants evolving to have more nutraceutical compounds (Dawson 2017).

The medicinal constituents (Table 2.3) of *Lonicera* species have been investigated by many researchers. *L.c.* berries are important to maintain human health, containing bioactive properties such as vitamins, minerals and secondary metabolites. *L.c.* has been used as traditional medicine for centuries in China, Japan and northern Russia (Kaczmarska 2015). The plant was known as the "Elixir of life" by Ainu Aboriginals in Hokkaido (Celli et al., 2014). While raw materials were used to treat fever, headaches, urinary tract diseases (Kaczmarska 2015), detoxicating, detumescence and visual improvement in China (traditional) (Dong 2013, Jin et al., 2006), fruits also were used to treat coronary heart disease, respiratory infections, liver and gallbladder disorders,

gastrointestinal and eye diseases (Ochmian et al., 2012, Caprioli et al., 2016, Becker and Szakiel 2019). Leaves and fruits were infused to treat bacterial and viral infections of the oral cavity and throat.

In a study of mineral content of *L.c.* leaves Boyarskikh (2013) concluded the elemental accumulation varied, throughout various regions. It is may due to the concentrations of available macro and microelements in the soil. The pharmacological and nutritional value of this species was considered valuable due to its ability to accumulate both Cu and Zn (Boyarskish 2013).

The health benefits of *L.c.* has been attributed to its high content of polyphenols such as anthocyanins, antioxidants, triterpenoids (Table2.3). The health benefits of anthocyanins have been investigated extensively in the last 20 years and haskap fruit contains more anthocyanins than many others including blueberries (Rupasinghe 2012).

Table 2.3. Studies investigating potential medicinal usage of Lonicera caeruleas's bioactive components

Potential usage	Reference
Anticarcinogenic	Hou 2004, Gruia 2008, Jin 2006
Cardioprotective and hepatoprotective	Chaovanalikit 2004, Martin 2014, Vostalova 2013
Anti-inflammatory	Jin 2006, Rupasinghe 2015, Wu 2015
Protective effects against UVA and UVB induced skin and DNA damage	Svarcova 2007, Svobodová 2008, 2009, 2010, Vostalova 2013
Abnormal lipid metabolism	Takahashi 2014
Antibacterial	Palikova 2008, Raudsepp 2013, Kula 2013
Improved glucose metabolism and insulin sensitivity and reduced cholesterol accumulation	Liu 2018

2.6 Agronomic and fertilization aspects of blue honeysuckle cultivation

The nutrient requirements for *L.c.* (wild and cultivated) are poorly understood. In nature, plant-available nutrients come from two main sources, one is the break down of soil minerals and the other is mineralization of organic matter. Productivity of this fruit crop depends on adequate

amounts of essential nutrients in the soil to serve as components of organic compounds, energy storage, plant structures, enzyme cofactors, and in electron transfer (Taiz 2006).

Twenty-one elements are functional nutrients for all or some plants to grow and reproduce. These include carbon C, hydrogen H, oxygen O, nitrogen N, phosphorus P, potassium K, sulfur S, calcium Ca, magnesium Mg, iron Fe, chlorine Cl, sodium Na, boron B, manganese Mn, copper Cu, cobalt Co, zinc Zn, molybdenum Mo, silicon Si, nickel Ni and vanadium (V) (Quigg 2008). Of these, seventeen are essential for all plants.

Nitrogen is a major element required in relatively large quantities, influencing the growth yield and quality of fruits (Yadong 2009). It constitutes in cells primarily as amino acids and nucleic acids. A deficiency will limit plant growth. In spring and early summer, N is utilized by aerial organs (bud and bark) in fruit trees, while in late summer and early fall, N is stored in roots (Tagliavini and Millard 2005). Therefore, during their growth and development period in early spring, the highest amount of N is required (Patrick et al., 2004). While N fertilizer usage in spring enhances only the vegetative growth, summer usage enhances the vegetative plus reproductive growth (Christensen et al., 1994).

Nitrate is water-soluble anionic form that is involved in the formation of chlorophyll in plants and the formation of cells in the body. It is considered an available form of nitrogen that can be absorbed into the root by nearly all plants. The main source of nitrate is soil organic matter, which is constantly formed during the mineralization process. The rate of mineralization of organic matter depends on its composition and a complexity of ecological factors, relief, and nature of land use. The formation and accumulation of nitrates in the soil are ecological factors that determine plant nutrition, metabolism, product quality, and crop quality. Nitrogen reserves in the soil and the amount used by plants depends on the activity of microorganisms that convert nitrogen compounds.

The ammonium form of nitrogen in the soil is converted to nitrate by microorganisms that undergo nitrification, creating another source of nitrogen fertilizer. Ammonium is also an available form of nitrogen for plants as it can be absorbed by roots. When the conditions for nitrification are favourable such as in warm, well aerated soils, nitrogen in the soil is converted to nitrate in two to three days (Chernikov et al., 2000). Ammonium ions also occur in soils in exchangeable and non-

exchangeable forms and, as with potassium, there is evidence for use of both exchangeable and non-exchangeable sources in the rhizosphere.

Phosphorus is part of compounds involved in energy transfer processes in the plant such as ATP and is one of the most important nutrients. It is involved in germination, root development, fruit maturity stages, fruit quality and improves N absorption (NSDA 2010a). Phosphorus stimulated the maturation of fruit and increased root growth. It is involved in energy transfer from the light reactions to the carbon fixation reactions of photosynthesis and transforming sugar to starch and starch to sugar processes (Winkler et al., 1974, Sharma et al., 2011). Phosphorus is also an important constituent of the component of plant cells, such as phospholipids of plant membranes and sugar-phosphate intermediates of respiration and photosynthesis. As a component of nucleotides, its structure plays an important role in energy metabolism (ATP etc), DNA and RNA.

Potassium is present in plants in the form of K^+ ions and plays an important role in controlling the osmotic potential of plant cells. It also activates many enzymes which are involved in respiration and photosynthesis (Hasanuzzaman 2018). Potassium increases the monosaccharides of fruits and increases the cold tolerance of plants. It also interacts with calcium and magnesium to improve plant nutrition and nutrient uptake with ammonium nitrogen. It constitutes the highest

Table 2.4. Eight mineral element concentrations in the fruit of Haskap varieties and comparison to the other fruits.

Fruit	**Ca**	**P**	**Na**	**K**	**Mg**	**Mn**	**Cu**	**Zn**
	----------(%)----------					----------(ppm)----------		
Haskap Borealis	0.14	0.21	0.02	1.47	0.08	10.45	6.35	8.65
Haskap Indigo Gem	0.33	0.17	0.02	1.13	0.11	10.59	3.61	8.33
Haskap Tundra	0.55	0.24	0.02	1.39	0.15	12.30	3.40	11.89
Partridgeberry	0.08	0.08	0.02	0.49	0.04	146.51	4.78	10.00
Blueberry	0.08	0.09	0.02	0.39	0.04	114.87	3.10	7.50
Blackberry	0.12	0.16	0.02	0.83	0.12	58.05	8.20	13.92
Strawberry	0.25	0.40	0.02	2.01	0.16	40.23	18.19	14.17
Raspberry	0.13	0.24	0.02	1.27	0.16	24.89	4.21	18.58
Red table grape	0.09	0.12	0.02	0.90	0.04	5.49	7.90	3.75

Adapted from Rupasinghe et al., 2012

concentration of element in many berries, after phosphorus, magnesium and calcium while sodium constitutes the lowest amounts (Table 2.4) (Jurikova et al., 2007, Rupasinghe et al., 2012). Plekhanova et al (1998) reported values ranging between 300 and 500 mg K/kg fresh weight (FW),

Sochor et al, 2014 ranging between 3000 and 5000 mg/kg dry weight while another study reported 10 and 15 mg/kg (Plekhanova 1998).

Potassium is involved in plant growth, roots and cell elongation, longevity and ability to tolerate stressful environments such as water stress (Ebrahimi 2012, Trejo-Tellez and Gomez-Merino 2014). Potassium is required for the transportation, production and storage of carbohydrates in grapevines (Winkler 1974, Spectrum Analytical 2011b). Deficiency of K leads to lack of vine growth, low yield, pre-mature leaves drops and delayed ripening (Conradie and Saayman 1989).

In the Sylamore Experimental Forest in Northern Arkansas, studies were done on the influence of fertilization rate on the ornamental species *Lonicera japonica*. Nitrogen fertilizer increased vegetation yield and crude protein content level in leaves, but fruit yield declined. Fertilization with phosphorus also increased crude protein contents of leaves, while potassium, calcium and phosphorus content of leaves decreased as nitrogen level was increased (Segelquist 1975).

Japanese growers used manure as a main nutrient supply for *L.c.* at farms visited (Bors 2012). Although a few fertilization papers on *L.c.* were found in the literature, the optimum fertilization rate is still not well defined. Even less studied and defined is the relationship between the *L.c.* fertilization rate and the plant morphology and distribution among yield components. The assumptions about blue honeysuckle's morphological responses to fertilization are nearly all based on a study conducted in Michurinsk, Russia by Belosohova and Belosohov (2010). The availability of soil nutrients plants depends on cultivation conditions, pH, fertilization regimes and climate variability.

2.7 Environmental effects on growth

Many researchers have noted that the majority of *L.c.* growth phenophases in natural and artificial environments takes place in early spring and in a very short time compared to other fruit plants (Retina 1973, Gizdyuk 1981, Plekhanova 1990, Belosokhov 1993, Popova 2000 and Yakovleva 2003). *L.c.* plant growth period varies depending on subspecies and genotypes but is generally similar within a few days for cultivars in the same germplasm group but can be weeks apart for different groups of cultivars (Gerbrandt 2017). In eastern Kazakhstan, for example, the vegetation period from *L.c.* budbreak to leaf fall begins in May and lasts 158 to 167 days (Bakaeva 1989). In Northwestern Russia, growth begins in the first ten days of April and ends in mid-May.

Flowering lasts for 7 to 15 days (Plekhanova 1990) and the vegetation period is 160 to 190 days (Kuminov 1994).

2.7.1 Morphology of the plants

L.c. cultivars can reach up to 2 m in height and 1.5-2 m in width. Leaves shapes vary significantly among varieties and range from 1-4 inches long. The weight and length of the fruit vary with variety and climatic conditions, from 0.3-3.8 g and 2-3 cm respectively. *L.c.* is shade tolerant but for maximum yield, full sun exposure has been recommended. It can grow on sandy, clay, peaty and slightly acidic soils and the favourable pH range is 5.5-8 (Dawson 2017).

2.7.2 Adaptation to climate and temperature changes

The global average temperature is expected to rise between 1.4-3.8°C by 2100 (Houghton 2007). Global warming is not the same across locations and the boreal forest (where most *L.c.* can be found in the wild) is expected to experience the greatest global warming among any forest ecosystem over the next 50-100 years (Bronson et al., 2009). The boreal forest is the second largest forest biome on earth, covering $15.8x10^8$ ha (Gower et al., 2001) and contains more carbon than any other forest biome (Gower et al., 1997). If the greatest warming occurs in the region, an important question would be how tree phenology is affected by increased temperature (Hansen 1996). Phenology is one of the plant traits changed by climate (Badeck 2004) and trees are reliable indicators of climate change (Donnelly et al., 2004).

Since low temperatures limit the growing season in the boreal forest, an increase in temperatures may lengthen the growing season (Van Breemen et al., 1998). This means a longer growing season might influence gaining more carbon (Cao and Woodward 1998, Euskirchen et al., 2006) in the forest biome and could balance the increasing amount of atmospheric CO_2 (Bonan et al., 1992, Bridgham et al., 1995, Keyser et al., 2000). Therefore, it is important to investigate the effect of warming on the phenology of boreal forest trees including *L.c.*

Bronson (2009) examined the climate effect on bud burst and annual shoot growth of black spruce trees in northern Manitoba, Canada. Photoperiod activates ontogenetic development (Myking and Heide 1995) but the direct effect of air temperature and photoperiod on phenology is not well studied (Linkosalo 2000). Air temperature is a good predictor of bud burst and shoot

elongation once ontogenetic development begins (Schwalm and Ek 2001). The empirical result by Bronson suggests soil and air warming caused a longer growing season which led to earlier bud burst and greater shoot lengths for black spruce by the third year. Thus, there was an increased amount of carbon uptake by boreal black spruce trees. There was no effect on bud burst when boreal soil was +5°C warmer than previous years (Bergh and Linder 1999). Other studies have shown approximately 17.5 days of earlier bud burst with warmed air temperatures between 2.8 and 5.6°C in Norway spruce stand (Slaney et al., 2007). A delay of 10 days in leaf fall on Quercus species occurred when temperatures were 4°C warmer than average (Nakamura 2010) but no changes occurred in the senescence of seedlings leaves (Morin et al., 2010) in open-field warming experiments. Shoot dynamics and photosynthetic machinery was impacted by longer growing seasons and warmer springs (Jarvis and Linder 2000). It was also reported than warming increased nitrogen mineralization (Lukewill and Wright 1997, Rustad et al., 2001, Stromgen 2001).

2.7.3 Drought tolerance

Due to climate change, changes in precipitation patterns, prevalence and harshness of drought are expected to increase in the northern hemisphere (IPCC 2014). Drought is one of the most important stress factors in determining tree growth and productivity globally (Peng 2011). Drought could inhibit the elongation and division of mesophyll cells, accompanied by smaller leaves area and stomatal size (Wang et al., 2016).

Effects of drought on related *Lonicera* species has been evaluated by Xu et al. (2017). In their study with drought and elevated O_3 effects on *Lonicera maackii*, have concluded both decreased photosynthetic variables and stomata size affected injury symptoms in leaves of *Lonicera maackii*. It was emphasized that moderate drought may influence the plants to adapt to climate change.

2.7.4 Influence of light conditions in canopy gaps on forest regeneration

There's some evidence that shade tolerance vary within L.c. Chlorophyll *a* and *b* in leaves and their role in photosynthesis (photosynthesis I and II) play a key part in capturing and transferring solar light quantum energy and ultimately producing plant carbohydrates. Chlorophyll *a* is a primary electron donor in the photosynthesis and chlorophyll *b* is part of the light-harvesting complex in the chloroplast. An increase in chlorophyll *b* increases the plant's shade tolerance or

light adaptation capability (Hugh et al., 1997). Also, photosynthetic activity is closely linked with some morphological characteristics such as leaf-surface density which can increase dry matter accumulation (Grizodub 2009).

A leaf composition study of *L.c.* varieties at the Krasnokutsk Institute of Horticulture in Ukraine, revealed that leaf-surface density was directly related to the ratio of chlorophyll *a* and *b*. Chlorophyll *a* and *b* ratio was low for varieties with smaller leaves and it was assumed that smaller leaves was an adaptation to high irradiance. Also, some varieties had larger canopy size resulting in high yields while other varieties that were smaller were able to produce high yields due to the intensive activities of the chlorophyll (Grizodub 2009).

L.c. is often found on the edge or areas around wetlands where forest canopy has gaps. In a study of a long-term effect of gamma radiation on 56 naturally growing shrub taxa, Dugle et al (1984) reported *Lonicera villosa* was sensitive to gamma radiation. The canopy gaps in a forest play a key role in the regeneration of forest by promoting light changes in the forest. This may change the air, soil temperature and soil moisture of the forest and the minimum resource requirements for each tree species differ (Nagel and Svoboda 2008, Nagel et al., 2010, Zhu et al., 2014a, b). Gaps may be formed by local disturbances including competition among the species, insect herbivory interactions, soil conditions and flooding (Englemark 1993, Morin and Laprise 1997, Cumming 2000, Comeau 2003, Maclaac et al., 2006). Gaps created differ in dimension and shape depending on disturbance events and time (Kucbel et al., 2010 which leads to a variety of conditions and processes (Fahey and Puettmann 2008, Ye and Comeau 2009).

The gap size and location determine the light availability horizontally and vertically inside the forest gaps. The size of gaps changes as trees grows especially those located at the forest edge. A few models developed to study diffuse light in forests include Nakashizuka (1985), Canham (1988) and Dai (1996). The model developed by Nakashizuka (1985) has a few disadvantages; 1) they used the diffuse light which cannot represent the total light, 2) they assumed the light distance to the center is the same, but horizontally, light decreases from the gap center to the edge (Voicu and Comeau 2006), and 3) they did not consider the geographical location and therefore this has limited application. Canham (1988) developed a gap light index (GLI) and it is considered to be more realistic. Dai (1996) developed a new gap light index (GAPLI) which is advantageous due to its consideration of geographical, geometrical, vegetation-structural, climatic, and temporal factors.

Beam radiation changes are affected by the geographical location of the site (longitude and latitude), the size and shape of gaps, cloudiness of the region, and canopy height and amount of coverage. The results showed light levels differ with height (Domke 2007).

One of the important factors influencing the yield of *L.c.* is the photosynthetic activity of the leaves. *L.c.* belongs to a group of plants that are relatively sun-loving in terms of the light regime. The quality and quantity of *L.c.* yield depends on genetics and characteristics of the variety in certain environments, and it is essential to optimize the relationship between growth process and photosynthetic apparatus by stimulating photosynthesis of leaves (Zhidyokhina 2009). Popova (2000) found that the productivity of blue honeysuckle photosynthesis depends 33.7% on the genetics and characteristics of variety, 19.6% on the weather condition of the growing season, and 46.7% on its interaction effects.

2.7.5 Effect of genotype and environmental interaction

The biochemical composition of fruits vary with location. Oranges planted in northern regions had 31 mg/100 g higher concentrations of ascorbic acid than those from southern areas (Kim et al., 2015). The biochemical content in the blue honeysuckle berry varied between cultivars in a study performed in Slovenia (Senica et al., 2018). Significant differences between the regions were found with the highest vitamin C content being 8.86 mg/g and the lowest was 6.56 mg/g (Marta et al., 2020). A range of fruit characteristics such as dry matter, weight of 50 berries, length and diameter, organic acid, phenolics, ascorbic acid and sugar contents were also reported in variety ‘Aurora’ grown in six locations in Slovenia and Croatia. These compounds increased and decreased with changes in environmental factors, noteworthy was that total sugar content was positively related to increased light intensity (Senica et al., 2018).

The biochemical composition of the *L.c.* is influenced by genetic characteristics of the different varieties, climatic conditions, geographical location of the crop, agrotechnical treatments (irrigation and fertilization), and harvest period (Kaczmarska et al., 2015).

The biochemical profile of fruits determines their edible quality and health benefits and is strongly affected by environmental conditions, harvest date, and genotype (Hoppula and Karhu 2006). Climatic conditions can have a significant effect on total acidity. Auzanneau (2018) reported a higher content of phenolic compounds in 2014 under longer sunshine hours and lower

precipitation. Ochmian (2010) investigated the yield and chemical composition of *L.c.* fruit depending on ripening time. The berries from same varieties and same bush harvested late were bigger with higher quantities of soluble solids and polyphenols. The higher temperatures during the development of late berries influenced the enlargement, however, as the season progressed firmness, the amount of titratable acidity and L-ascorbic acid decreased. Dawson (2017) observed that the total content of secondary metabolites in each blue honeysuckle berries increased with increasing GDD's however, concentrations of quercetin-3-glucoside decreased as berries size swelled taking in water during the final ripening stage. Rupasinghe et al. (2012) found that the three University of Saskatchewan cultivars tested had higher levels of flavonoid and phenolics than blueberry, lingonberry and blackberries. In another study, a *L.c.* sample had the highest total phenolics of 13 fruit crops (Bakowska-Barczak 2007).

2.8 Effect of soil on growth

Soil characteristics such as aeration and water absorption, water retention, and nutrient supply have a major impact on plant health and survival (Brown et al., 2021, Kumaragamage et al., 2021, Whalen 2021). Soil is composed of unconsolidated minerals and organic matter generated under the influence of parent materials, climate and macro- and microorganisms over a period of time (Walley et al., 2021). The texture/composition of soil is determined by the ratio of its three components, sand, silt, and clay and composed of iron oxides, carbonates, clays, silica, and humus. Soil temperature and colour are unstable due to external and internal factors. Specific resistance is defined as the ability to resist electrical current. Soil properties vary depending on the depth at which they are located.

2.8.1 Soil organic matter

Organic matter plays a role in nutrient storage of nutrients, improving tilth, air and water movement, water retention and availability, erodibility, pesticide efficacy and decomposition processes in soil (Gregorich et al., 1994). Maintenance of adequate soil organic matter levels is therefore considered imperative to sustain soil quality and agricultural productivity. According to Smith et al. (2000), Canadian soils are considered to have lost about 25-35% of their C due to cultivation and the replacement of native perennial vegetation with annual crops. On the other hand, land-use change from annual crops to perennial grasses increases soil organic C level by

sequestering C into the soil (Gebhart et al., 1994, Mensah et al., 2003). The particulate organic matter fraction and soil organic matter is considered an active organic matter pool that participates in the release of nutrients and is an early indicator of the influence of management change on soil organic matter content (Cambardella and Elliott 1992). Blue honeysuckle orchards, like most orchards can benefit by growing a shelterbelt, which can reduce wind erosion of soil, can potentially increase organic matter, and have other benefits. For examples, caragana shelterbelts have potential on carbon sequestration in Saskatchewan 1.3-2.7 Mg C ha^{-1} $year^{-1}$ (Amichev et al., 2016).

2.8.2 Soil pH and electrical conductivity (EC)

Soil environmental pH has a significant impact on plant growth. Soil pH is dictated by hydrogen ion concentration (soil acidity) and is a key variable that impacts many different chemical and biological properties in soil including soil microorganism and plant roots growth (Brady and Weil 2002, Havlin et al., 2005). Some sources of soil acidity are precipitation, CO_2 evolved from microbial respiration, nutrient uptake, leaching, clay minerals, soluble salts, and fertilizers (Havlin et al., 2005). Soil pH differentially influence plant species. In a forage system in central Alberta, the annual application of 100 kg N ha^{-1} of ammonium nitrate for five years lowered the pH of the soil at 0-7.5 cm depth, decreasing as rate of N increased (Agriculture Agri-Food Canada 1993).

In Saskatchewan Canada, a soil pH map was created by soil sampling and testing of 8000 samples in 1982 using a modified grid system and 15,000 samples between 1983 to 1986 through routine soil sampling surveys. According to the map, 710,000 hectares were classified as having a pH of less than 5.5; 2,040,000 hectares with a pH of 5.5-6.0; 5,222,000 hectares with a pH of 6.1-6.7; 9,878,000 hectares with pH of 6.8-7.5 and 10,507,000 hectares with pH of greater than 7.5 (Rostad et al., 1987). Thus, almost half of the soils may be considered alkaline. In a recent study of 14 sites of haskap grower sites in Saskatchewan, *L.c.* was being grown in soils ranging from 5.2 to 8.5 although plants were always found to be unhealthy if pH was 8.0 or higher (Bors 2019). On soil surveys done in Alberta moss peat soil, pH was 4.5 to 5.5 (Bowser et al., 1962) and 6.2 (Doughty 1941).

Electrical conductivity (EC) is a measure of soil salinity. Salinity is known to be generally related to the downslope movement and discharge of soil water containing dissolved salts. Salinity

will restrict the growth of many crops due to its osmotic effect on holding back water from the plants (Larney et al., 1994). Salinity may also restrict the activity of soil microorganisms, which in turn will affect the turnover of elements such as C and N (Campbell 1978). Bors (2009) noted that wild *L.c.* could be in areas in low lying areas close to the ocean.

2.8.3 Effect of soil moisture on growth

Tree phenology is controlled by many genetic and environmental factors (Kilpelainen et al., 2019). The amount of moisture in the soil depends on many factors, such as the mechanical structure and physical properties of the soil, the quantity of organic matter and the air relative humidity. The growth of terrestrial organs and roots systems depends on the amount of physiologically available water. In terms of moisture requirements, the *L.c.* belongs to a group of vegetation that grows largely in moist soils and coniferous forests on near rivers, lakes and other wetlands, but it also grows in arid and foothill areas. According to Bannikov (1996), the evaporation coefficient of the *L.c.* was on average 500 units or in other words, 500 grams of water to produce 1 gram of dry matter.

2.8.4 Effect of soil temperature on shoot growth

The active development of any type of crop depends on its biological characteristics, geographical origin and it is limited by specific temperature regimes and ranges, growing most rapidly within their optimal temperature range. The temperature requirements of plants change depending on its age, and each organ, such as the leaves, roots, and fruits, requires different optimum temperatures. For example, temperature requirements of roots system are generally lower than leaves. Scots pine (Pinus sylvestris) aboveground biomass was lower at soil temperatures 17°C than at 13°C (Domisch et al., 2001). However, other studies observed Scots pine greatest growth observed at 12°C (Vapaavuori et al., 1992) and 15 °C (Lyr and Garbe 1995). Moreover, Norway spruce shoot elongation slowed the growth of roots and when shoot growth decreased root growth increased (Lahti et al. 2005).

2.8.5 Summary

In summary, there are numerous studies on blue honeysuckle as a circumpolar species with a large diversity mainly distributed in relatively cold regions. Botanical descriptions abound for

various subspecies of *L.c.* which further indicate the species has diversity. There are a number of scientific articles about *L.c.* as an exciting fruit with great potential for health benefits. They conclude *L.c.* is a good candidate crop in the northern areas because of good flavour, cold tolerance, early ripening in the summer, low susceptibility disease and pests, high adaptability to environmental stresses, the possibility for harvesting mechanically, and availability of field production practices.

However, *L.c.* has been not studied specifically in the wild habitat. There are many references in the Russian literature that *L.c.* grows well in soils with weakly alkaline and neutral pH, yet there are no concrete scientific experiments or convincing data in this area. Moreover, studies are rare on *L.c.* for greenhouse production including hydroponics and fertilization under controlled conditions. Collectively, this information might be useful for improving production practices.

3.0 AUTECOLOGY STUDY- WILD BLUE HONEYSUCKLE (*LONICERA CAERULEA* SSP. *VILLOSA*) GROWTH AT THREE SASKATCHEWAN SITES, AND THEIR ASSOCIATION WITH SOIL AND LEAF PROPERTIES

3.1 Introduction

Although *Lonicera caerulea* is being widely planted as a new berry crop across Canada, one of its wild relatives *Lonicera caerulea* ssp. *villosa* has not been extensively studied in its native habitat and no autecological studies have been done on European and Asian subspecies either. This subspecies is not only present in Saskatchewan but is also present in every province except BC and is considered a rare plant. Therefore, defining the environmental factors that govern the success of this *L.c.* subspecies can provide opportunities for protection of this plant, conservation of genetic resources in its native environment, use of beneficial agricultural properties in the development of new varieties, learning of growth characteristics and to improve low-cost fruit production.

In recent studies, the impact of climate change on boreal forests includes outbreak of pests and frequent fires, thawing permafrost, drought and elevated temperature causing stress on biomes (Leona 2019). Boreal forest and climate change are interlinked and as the wild blue honeysuckle (*Lonicera caerulea* spp. *villosa*). *L.c.* spp. *villosa* is indigenous to Saskatchewan's boreal forest, it is important to study the dynamics of its growth in relation with the surrounding environment.

Among the ecological factors, temperature has one of the decisive influences on plant growth and development. Temperature is considered the kinetic basis of biochemical reactions within living organisms, and the rate of metabolism in living organisms is directly related to temperature. Temperature varies depending on the season of the year and latitude of the geographical location and also varies on a daily basis.

The environmental properties also influence growth of leaves, roots, and fruits and with optimal temperatures varying between species. The amount of water and moisture in the soil for example depends on many factors, such as the mechanical structure, physical properties, amount of organic matter, the roots suction capacity, and relative humidity of ambient air. In terms of moisture requirements, *L.c.* fits into the moist plant category (Bannikov 1996) and it grows mainly in moist soils, coniferous forests and open areas on the banks of rivers and lakes, and in some cases in mountainous areas (Bors, personal communication, 2019).

The only Saskatchewan studies involving wild *Lonicera caerulea* ssp. *villosa* have been surveys to characterize ecological zones for common vegetation and soil characteristics (McLaughlan et al., 2010) and the sites of this research belongs to the Boreal Plain ecozones. *Lonicera caerulea* ssp. *villosa* appears in lists of plants in various ecosites but was not part of any detailed research to better understand this species. This chapter investigates many environmental components of *villosa in situ* with a greater focus on soil nutrients and physical properties and how they may impact shoot growth. In particular, this chapter focusses on the macronutrients N, P, K and organic carbon levels in the soil and found in leaves.

Macronutrients play an important role in plant metabolism and growth. Nitrogen is involved in the composition of simple and complex proteins, which are the main components of plant protoplasm. Nitrogen requirements are low in the early stages of plant growth. However, consumption will increase as perennial plants age. Plant nitrogen is the most abundant green mass in the stems and leaves, during which amino acids and proteins are synthesized and nitrogen is absorbed from the soil (Choijamts 2006). Phosphorus is another important nutrient for plants. Phosphorus is distributed and absorbed in varying amounts in plant organs. For example, the amount of phosphorus in the root mass of *L.c.* was 2.5 to 3.0 times higher than in leaves and stems (Kondratyev 2008). Potassium is present in plants in the form of ions and does not participate in the formation of organic compounds in cells. Approximately 20 % of the potassium absorbed by plants is metabolized within the cell by cytoplasmic colloids, up to 1.0 % by non-metabolic absorption of potassium in mitochondria, and about 80 % is excreted in cellular juices and easily excreted in water. Potassium improves plant water retention, resists transient drought, and increases cold tolerance by increasing the osmotic pressure of cell sap.

There are many indicators of soil chemical properties. Soil organic matter plays an important role in soil productivity and has numerous physical, chemical, and biological benefits (Hatten and Liles 2019). It is involved in the storage of nutrients, improving tilth, improving availability of air and water, erodibility and decomposition processes in soil (Gregorich et al., 1994). Maintaining proper levels of soil organic matter is considered essential to maintaining soil quality and plant productivity.

Soil pH is determined by measuring the reaction of the soil, specifically the concentration of hydrogen ions in the soil solution and it is a variable that affects a variety of chemical and biological properties in the soil (Brady and Weil 2002, Havlin et al., 2005). Soil acidity is influenced by the degree of precipitation, creation of CO_2 by respiration of soil microbes, the clay minerals concentrations, salts, and fertilizers. Soil pH greatly influences the uptake of nutrients by plants (Havlin 2005). Soil hydrogen ion concentration (pH) is an important factor for growth of plants and soil microbes, with the optimums commonly varying between 5.5 to 6.5 (Islam et al., 1980, Köpp et al., 2011).

Electrical conductivity (EC) is a measure of soil salt content. Excess salinity limits the growth of many plants as it has an osmotic effect on plant water retention (Larney et al., 1994, Nemali and van Iersel 2004). Also, salinity can limit the activity of soil microorganisms, which in turn affects the cycling of elements such as carbon and nitrogen (Campbell 1978).

This chapter's hypothesis is that *L.c.* has a large variation in growth in the forests of northern Saskatchewan. The growth of wild *L.c.* may be associated by the amounts of soil properties.

3.2 Materials and methods

An autecological study of wild *L.c.* spp. *villosa* was conducted during the growing seasons of 2014 and 2015. Due to financial and labor constrain, three study sites were initially chosen from seven sites by difference in geographical location, angle of sunlight, amount of water, and physical properties of soil in the spring of 2014. Plots of 20 x 30 meters were selected and divided into three blocks each consisting of four *L.c.* spp. *villosa* plants and marked with red type bands.

3.2.1 Temperature and precipitation in the general area of the study regions

Weather data (Table 3.1) at Waskesiu Lake region (located closest to the three sites) showed the mean monthly temperatures were in April 2.51°C and June 2.02°C warmer in 2015 than 2014. However, May, July, and August temperatures were nearly 8.13°C and 8.67°C, 18.04°C and 18.23°C, 17.27°C and 16.93°C respectively in 2014 and 2015. But monthly precipitation was 123 mm in June and 67.4 mm in July 2014, whereas 71.8 mm in June and 120.8 in July 2015.

Table 3.1. Climate data of Waskesiu Lake, Saskatchewan, Year 2014 and 2015

		Average Max Temp (°C)		Average Min Temp (°C)		Average Mean Temp (°C)		Precipitation (mm)	
		Year							
		2014	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Months	Jan	-10.63	-8.61	-23.23	-17.91	-16.95	-13.27	22.5	19.20
	Feb	-14.26	-12.66	-26.15	-24.19	-20.23	-18.45	10.70	17.70
	Mar	-4.18	1.79	-17.93	-9.35	-11.07	-3.79	8.30	8.90
	Apr	4.33	9.49	-5.33	-3.50	-0.49	3.00	74.20	24.00
	May	14.04	16.09	2.35	1.47	8.13	8.67	50.30	10.60
	Jun	18.83	22.28	8.81	9.38	13.84	15.86	123.40	67.40
	Jul	23.73	23.83	12.29	12.59	18.04	18.23	71.80	120.80
	Aug	22.88	22.31	11.62	11.50	17.27	16.93	48.90	83.00
	Sep	16.37	15.82	5.35	5.79	11.02	10.80	13.10	77.80
	Oct	9.46	10.79	0.54	1.76	5.02	6.30	6.30	40.20
	Nov	-7.93	0.63	-13.88	-6.10	-10.93	-2.73	50.10	10.00
	Dec	-7.69	-6.62	-14.42	-13.10	-11.08	-9.85	8.50	6.80

Source: climate.weather.gc.ca

3.2.2 Study site descriptions and its distinguishing feature

Three sites were in the forest near Prince Albert National Park, approximately 300 km north from Saskatoon, Saskatchewan, Canada. The first site will be designated as SPF (short for Shady, Peat & Flat) located at 54°10'1.423"N & 105°57'33.487"W; the BLF site (Bright, Luvisol & Flat) is at 54°8'16.692"N & 105°57'1.692"W and the BLS site (Bright, Luvisol & Sloped) site: 53°54'31.817"N & 105°54'6.678"W) (Figure 3.1). All three sites were located within the Boreal Plains ecozone. That ecozone is described as containing trembling aspen and white spruce, wetlands or peatlands, spruce bogs and tamarack fens scattered across the landscape (McLaughlan 2010).

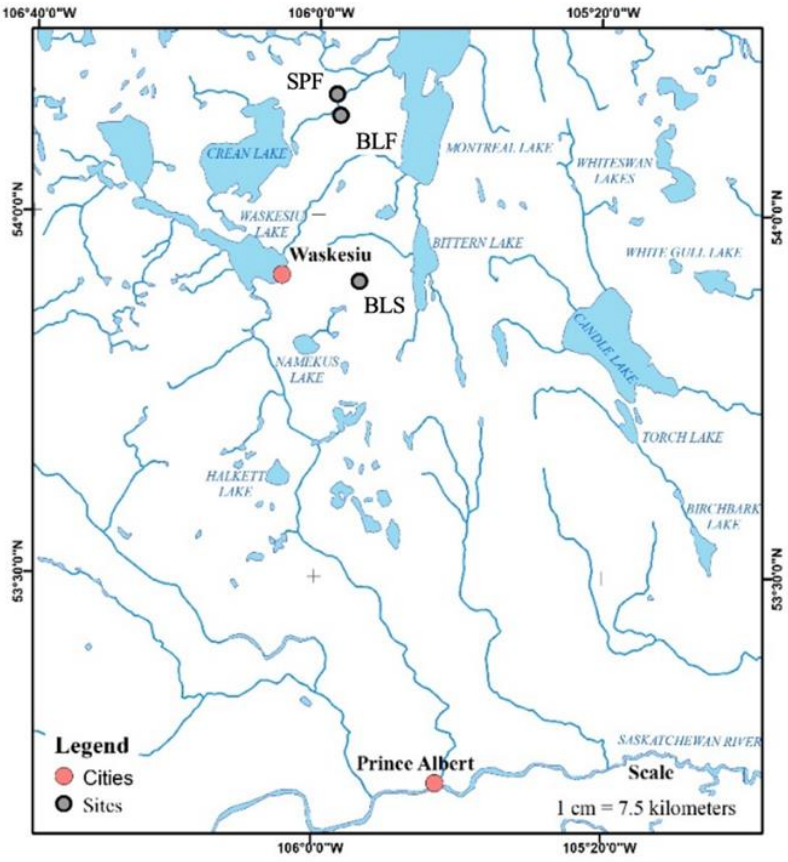


Figure 3.1. Three study locations of wild blue honeysuckle (*Lonicera caerulea* spp. *villosa*) in the vicinity of lake Waskesiu, Saskatchewan

SPF site - Description and its distinguishing features

The SPF site is located on the south bank of the Crean River, along the northern edge of the forest in the north of Prince Albert, Saskatchewan, approximately 300 km north of Saskatoon at 54°10'1.423"N and 105°57'33.487"W. The surface is level.

The trees in the south-east part of the site block most of the morning sunlight except for some light that comes in at sharp angles under the canopy. However, in the evening, the sun strikes at more direct angles (Figure 3.2). It is well lit during the daytime but also shaded by trees up to 15 meters high for most of the day. This area experiences relatively constant daylight. The soil at the site consists primarily of peat and the water table is very close to the surface, at around 45 cm below the soil surface.

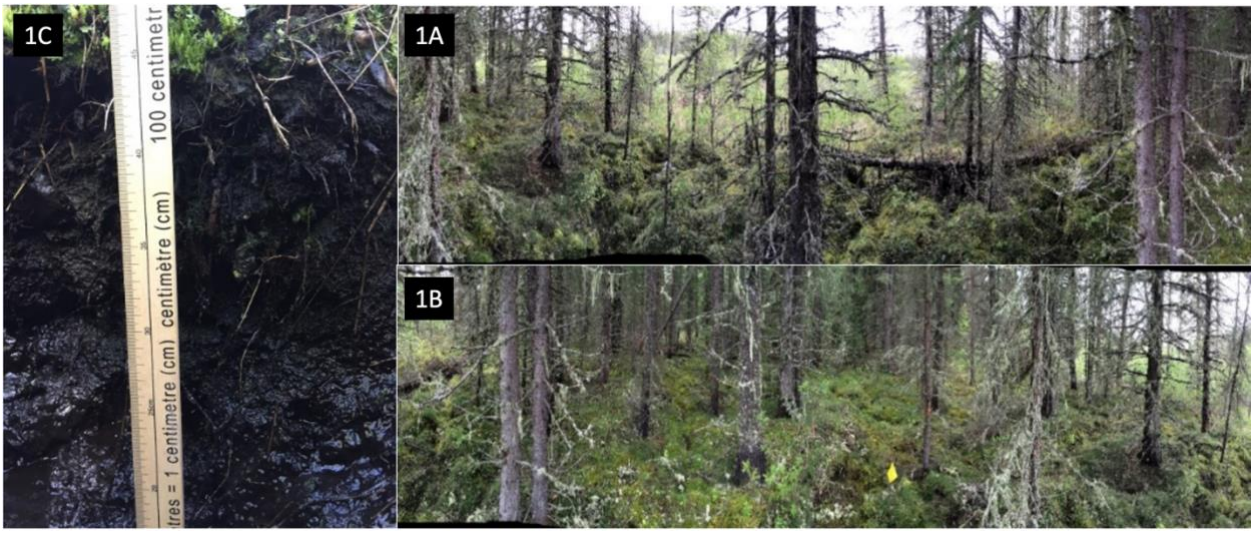


Figure 3.2. Photos of the environmental scenery taken from the center of Site SPF Panoramic view facing east. Photo 1A was facing East-Northeast while Photo 1B was facing West-Southwest June 17, 2014 at 10:16 AM. Photo 1C shows an 80 cm trench that revealed a peat soil. The site was located along the Crean river.

BLF site - Description and its distinguishing features

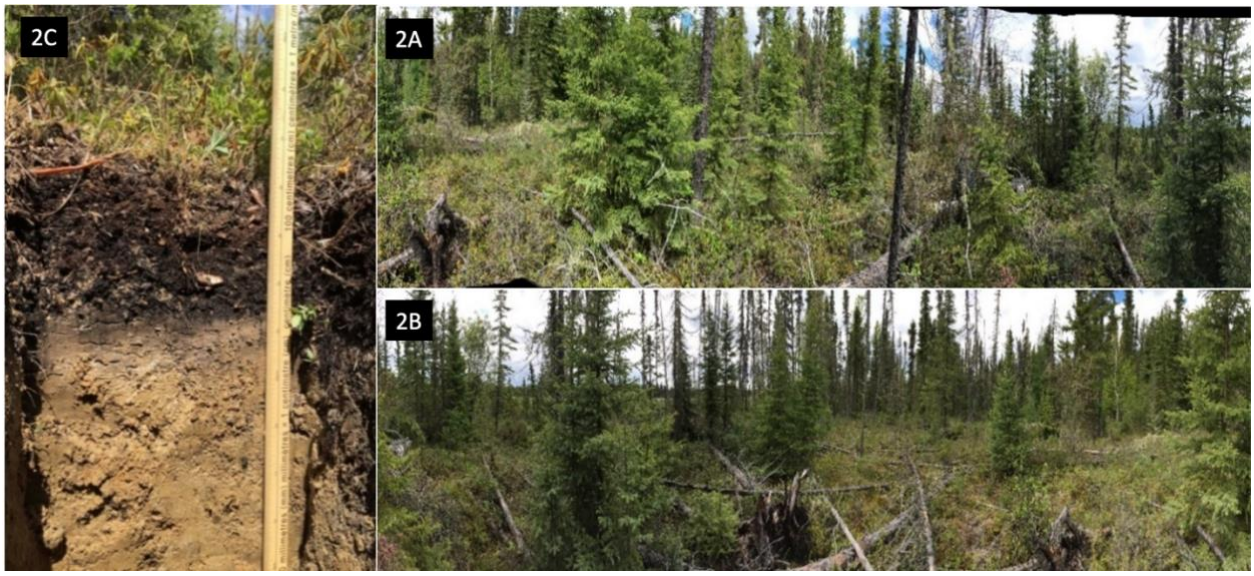


Figure 3.3. Photos of the environmental scenery taken from the center of Site BLF Panoramic view facing east. Photo 2A was facing East-Northeast while Photo 2B was facing West-Southwest June 17, 2014 at 11:30 AM and soil section (Photo 2C: June 6, 2015 at 12:21 PM) of the BLF site. An 80 cm deep trench revealed soil Bt horizon was clay. The site was open, level, swampy, with a high light intensity.

The BLF site of the study was located approximately 5 km south of the SPF site on the coordinate of 54°8'16.692"N and 105°57'1.692"W.

The site is in a relatively flat forested wetland. Due to its open location, it is exposed to direct sunlight and plants are heated during the day. The A horizon of the area was rich in organic matter, well-drained and has a 25-28 cm thick forest Gray Luvisol soil. The thickness of the B horizon is about 5-6 cm. The subsoil is did not have organic matter and has a heavy clay structure with water blocking effects (Figure 3.3).

BLS site - Description and its distinguishing features

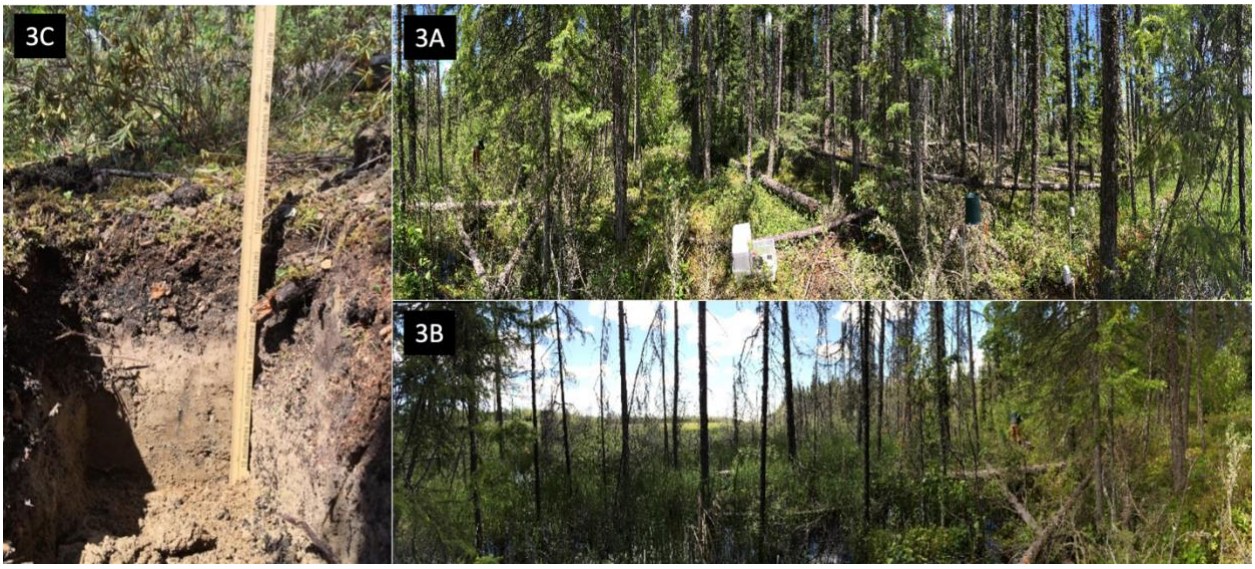


Figure 3.4. Photos of the environmental scenery taken from the center of Site BLS Panoramic view facing east. Photo 3A was facing East-Northeast while Photo 3B was facing West-Southwest June 17, 2014 at 12:04 AM and soil section (Photo 3C: June 6, 2015 at 1:57 PM) of the BLS area. An 80 cm deep trench revealed soil Bt horizon was clay. The site had an east slope that was partly waterlogged.

The BLS site was located approximately 25 km south of the BLF site at 53°54'31.817"N and 105°54'6.678"W. The north part of the site is adjacent to a forest while the south part borders a small pond. Due to the south-facing slope, the area received more sunlight than the other two locations. The A horizon of this area was well-drained forest Gray Luvisol soil dark with organic matter and distributed in thin layers (Figure 3.4), the Bt horizon was a thick clay soil and the boundaries of the transition were visible.

3.2.3 Plant material and shoot growth

Shoot growth of *Lonicera caerulea*. spp. *villosa* was measured at three) different locations (Figure 3.1). Each site contained three blocks with four plants per block for a total of 36 plants that

were evaluated for two years and unit of observation was each block. Initial data were collected at weekly intervals during the eight-week observation period. Measurements were performed on three shoots of each plant. The lengths (mm) measured were of new growth formed each growing that occurred in the mid portion of each plant. Lengths were measured by a digital caliper (axGear, WA, USA).

Microclimate conditions of each site were monitored and recorded by sensors and data loggers 2014 and 2015 (Figure 3.5).

Air temperature: Hourly measurements of air temperature were obtained using WatchDog Data Logger Model 450 Temperature and Relative Humidity (Spectrum Technologies, Inc., IL, USA) installed in the field with a radiation shield cap prior to the start of vegetation period in late-May until end of study period for eight weeks.

Relative humidity: Hourly measurements of soil temperature were obtained using WatchDog Data Logger Model 450 Temperature and Relative Humidity (Spectrum Technologies, Inc., IL, USA) installed in the field with a radiation shield cap prior to the start of vegetation period in late-May until end of study period for eight weeks.

Dew point: Hourly dew point was calculated using air temperature and relative humidity by MS Excel Office 365 software using the Lawrence method, (2005).

Solar radiation: Hourly measurements of solar radiation were obtained using a Silicon pyranometer model #3670 and digital data recorder (WatchDog Data Logger Model 200; Spectrum Technologies, Inc., IL, USA) installed in the field with a radiation shield cap prior to the start of the vegetation period in late-May until end of study period for eight weeks. The obtained measurement unit of Watt/m^2 was converted to μmole.m^2/s using coefficient of 4.57 (Thimijan and Heins 1983).

Soil temperature: Hourly measurements of soil moisture were obtained using Watermark soil moisture sensor model #6669 and a digital data recorder WatchDog Data Logger Model 450 Temperature and Relative Humidity (Spectrum Technologies, Inc., IL, USA) installed in the field with a radiation shield cap prior to the start of vegetation period in late-May until end of study

period for eight weeks. Sensors were only obtained in 2015, so data were collected only for the 2015 season.

Figure 3.5. Measuring instruments installed in the selected study area. A-Automatic rain gauge WatchDog 1120, B-WatchDog data logger built with air temperature and air humidity sensors and connected to the soil temperature and moisture sensors, C-Solar radiation sensor, LightScout Silicon Pyranometer, Spectrum Technologies, D-Second backup WatchDog data logger built with air temperature and air humidity sensors and connected to the and soil temperature and moisture sensors. All equipment was from Spectrum Technologies Inc., Aurora, IL, USA.

Soil moisture: Hourly measurements of soil moisture were obtained using Watermark soil moisture sensor model #6669 and a digital data recorder WatchDog Data Logger Model 450 Temperature and Relative Humidity (Spectrum Technologies, Inc., IL, USA) installed in the field with a radiation shield cap prior to the start of vegetation period in late-May until end of study period for eight weeks. Due to the sensors obtained in 2015, data were collected only for the season 2015.

Precipitation: Hourly measurements of precipitation were obtained using a Tipping bucket rain gauge model #36651 and digital data recorder "WatchDog Data Logger" model #200 (Spectrum Technologies, Inc., IL, USA) installed in the field with a radiation shield cap prior to the start of the vegetation period in late-May until end of study period for eight weeks.

The air temperature (°C), air relative humidity (%), dew point (°C), soil temperature (°C), soil moisture (°C), rainfall (mm) and solar radiation (μmole.m^2/s) data were downloaded every week to a portable computer (SONY Vaio) using a computer program (Specware 9; Spectrum Technologies, Inc).

3.2.4 Soil sampling and analysis

In this study, unit of observation was block and soil was evaluated for nitrate, ammonium, phosphorus, potassium, organic carbon at depths of 0-20 cm and 20-40 cm. Soil pH and electrical conductivity were only measured for depths of 0-20 cm. Sampling was done June of 2014. Soil samples were taken 20 cm away from each plant and consolidated to characterise each block. Using a Dutch auger, cores were taken up to 40 cm depth with the cores segmented into two depth increments (0 to 20; 20 to 40 cm). The cores were placed in plastic bags and put in an insulated container. Later, samples were dried, grinded and stored until evaluation of organic carbon, EC, pH, and extractable nutrients.

Soil extractable nutrients: The KCl extraction to measure NO_3-N and NH_4- N was carried out according to Keeney and Nelson (1982). Five grams of soil was weighed out into extraction bottles, and 50 mL of 2 M KCl solution added. The bottles were then shaken on a rotary shaker for 1 hour at 142 RPM. The solution was then filtered through a Whatman® 454 filter paper into vials. The vials were capped and stored at 4ºC until they were analyzed using a Technicon™ Auto-analyzer sampler II; AAII single-channel colourimeter with 30 mm tubular flow cell with 15 mm internal diameter, 420 nm interference filter, voltage stabilizer, and recorder (Technicon Industrial Systems, Tarrytown, NY 10591).

A Modified Kelowna (MK) extraction was used to determine extractable P and K according to the procedure outlined by Qian et al. (1994a). The extractant solution was prepared by mixing 0.25 M HOAc, 0.25 M NH_4OAc, and 0.015 M NH_4F with a measured pH of 4.9. A known weight of soil (3 g) was placed into 100 mL plastic extraction bottles with 30 mL of the MK extractant solution, then shaken on a rotary shaker at 200 RPM for 5 min. The mixture was run through a Whatman® #454 filter paper into plastic vials and the extractant stored at 4ºC until the samples were able to be colourimetrically analyzed using the Technicon™ Auto-analyzer sampler II.

Organic carbon: Soil organic carbon was determined using a LECO CR-12 Carbon Analyzer (Wang and Anderson 1998). The soil preparation for use in the Carbon Analyzer first involved

grinding the soil to pass through a 40-mesh sieve. A 0.15 g sub-sample was then placed into the furnace at a set temperature of 840ºC. The soil organic carbon was oxidized to CO_2 which was then measured by an infrared (IR) cell (LECO 1987). To prevent drift, the IR cell was calibrated with a known carbon sample (sucrose). To ensure that only organic carbon is measured care was also taken to remove the sample from the furnace after 120 seconds as inorganic carbon begins to decompose after 150 seconds.

Electrical Conductivity (EC) and pH: The procedure for determining the electrical conductivity (EC) and pH followed the techniques of Hendershot and Lalande (1993) and Janzen (1993) respectively. Twenty grams of soil was placed into a plastic bottle and 40 mL of distilled water was added. The bottles were placed on a rotary shaker at 142 revolutions per minute (RPM) for 20 minutes, then left to stand for two hours. The resulting 2:1 distilled water to soil suspension was filtered through a Whatman No.1 filter. The filtrate was analyzed for pH and EC with a Beckman 50 meter for pH and a Horiba ES-12 conductivity ($mS/cm.s^{-1}$) meter for EC.

3.2.5 Leaf nutrient and chlorophyll analysis

In this experiment, leaves were evaluated for total nitrogen, phosphorus and potassium, and chlorophyll *a* and *b*. Individual plants were sampled every year for two years, 8 weeks after bud break. Leaves for the nutrient analysis were labeled and placed in paper bags in a drying cabinet (Precision Scientific) at 50°C for 72 hours. When constant dry weight was achieved, samples were weighed and ground using a micro hammer mill (Culatti AG, Zurich, Switzerland). All samples were stored in a dark room, at room temperature until further analysis.

Total nitrogen, phosphorus and potassium concentration: Total N and P concentrations in the plant dry matter were determined on the dried and ground sample using a standard H_2SO_4-H_2O_2 digestion method (Thomas et al., 1967). This procedure involves taking a plant sample of 0.25 g and placing it in a 75 mL digestion tube and then adding 5 mL of concentrated sulfuric acid. The mixture was then mixed vigorously with a vortex shaker and placed in a block digester at 360°C for 20 min. Then 0.5 mL of 30% ($vol\ vol^{-1}$) H_2O_2 was added to the tubes, which were vortexed a second time and heated at 360°C for another 30 min. The tubes were then removed, cooled and 0.5 mL more H_2O_2 added to the tubes. This heating and cooling procedure was repeated five more times. The last heating procedure involved a one-hour heat treatment to completely remove the last remaining H_2O_2 from the sample. When the 30 min cooling had taken place, the

remaining sample was brought up to volume (75 mL) with deionized water. The samples were shaken and transferred into 50 mL plastic vials for storage and further analysis. Total P and N concentrations were analyzed colourimetrically as phosphate and ammonium in the digest solution using the Technicon^{TM} automated analyzer (Tarrytown, NY). Total Plant K concentration was measured by digesting plant tissue in sulfuric acid-peroxide using T°C controlled (360°C) digestion block (Thomas 1967) followed by flame emission spectrometry.

Chlorophyll extraction followed the Arnon (1949) method. A 0.5 gram fresh tissue was pulverised in a chilled mortar and pestle containing 5 ml of 100% acetone. Then it was filtered through a Mira cloth and centrifuged for 5 min at 3024 rpm to clarify the liquid sample. Samples of 0.05 mL of were added to 0.95 mL of 82% acetone and stored in the refrigerator until further use. The spectrophotometer (Dynamica, Newport Pagnell, UK) was set to measure adsorption at 663 nm for chlorophyll *a*, 645 nm for chlorophyll *b*.

Calculations were done as follows:

Chlorophyll *a* (mg/g fresh weight) = (12.7 A_{663}) - (2.69 A_{645}) g fresh weight* V

Chlorophyll *b* (mg/g fresh weight) = (22.9 A_{645}) - (4.68 A_{663}) g fresh weight* V

Chlorophyll *a*+*b* (mg/g fresh weight) = (20.08 A_{645}) + (8.02 A_{663}) g fresh weight* V

where, V = volume of chlorophyll extract (0.05 mL)

3.2.6 Statistical analysis

Data analysis of shoot growth three sites of Saskatchewan was analyzed by Repeated Measures Analysis of Variance (ANOVA) via GLM and soil properties, leaf and chlorophyll were analyzed by ANOVA using IBM SPSS 28.0 Grad Pack Pro software. For all analysis, post hoc mean separations were established per Tukey HSD test at $p<0.05$ and Fisher's least significant difference. Log 10 transformation was used on all soil properties data and leaf total P data.

3.3 Results and discussion

3.3.1 Association of location on shoot growth

The average growth (Table 3.2) of wild blue honeysuckle (*Lonicera caerulea* spp. *villosa*) shoots varied significantly among the study locations SPF site: 79.74±1.56 mm, BLF site:

48.83±0.77 mm and BLS site: 65.84±1.15 mm (p<0.001). However, Tukey HSD post hoc test showed that there was no significant difference in between site SPF and site BLS in overall two years of observation. In the first 6 weeks of growth the average shoot growth was the highest at 88 mm at the SPF site in 2014, while the average shoot growth at the BLF site was the shortest at 46 mm or 1.9 times lower.

Table 3.2 Average shoot growth (mm) at study locations in the forest near Prince Albert National Park, within the Boreal Plains ecozone, Saskatchewan, May to July of 2014 and 2015

Location	Average of two years (2014, 2015)
SPF site	79.74 ± 1.57* a**
BLF site	48.83 ± 0.77 b
BLS site	65.85 ± 1.15 a
Site	F 17.031, p<0.0001
Year	F 1.137, p=0.307
Site x Year	F 0.658, p=0.536

*Mean standard error, **Means followed by the same letter within a column are not significantly different at α=0.05 (Tukey's HSD). SPF: Shady, Peat & Flat, BLF: Bright, Luvisol & Flat, BLS: Bright, Luvisol & Sloped.

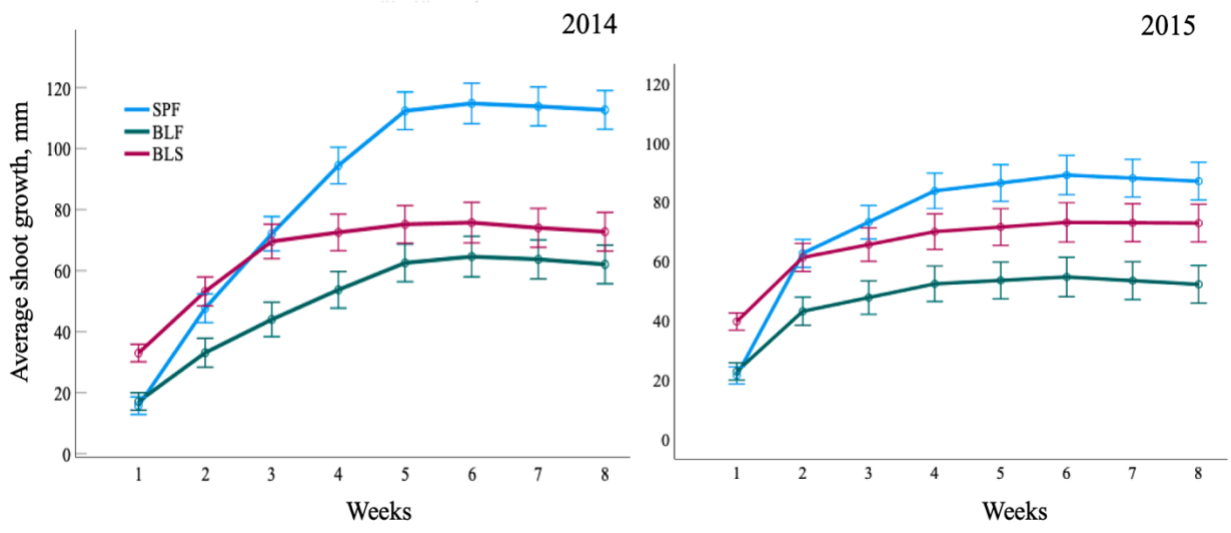


Figure 3.6. Average shoot growth over eight weeks at study locations in the forest near Prince Albert National Park, within the Boreal Plains ecozone, Saskatchewan, May 29 to July 24 of 2014 and 2015

Across all study sites, an intensive increase in shoot growth was observed in the first five weeks (Figure 3.6) but it slowed down after the sixth week. This may be due to the early initiation of terminal bud set, and growth slows down (K. Tanino, personal communication, 2019). Indeed, shoot stems started hardening and changing color to darkening. The result of this study is similar to those reports from the Kirov region in Russia. Firsova (2002) stated that the growth of *L.c.* cultivar's shoots commenced in mid-May and terminated in early July, lasting an average of 53 days; however, average growth was between 15.3 to 31.3 cm.

The shoot growth in 2014 was higher than in 2015 when all sites were averaged. There was no accessible literature data on shoot growth of wild blue honeysuckle (*Lonicera caerulea* spp. *villosa*) but at the U of SK fruit program it is easily observed that *Lonicera caerulea* spp. *villosa* plants are less than one third the size of cultivars. Research on growth rates has been done on *L.c.* cultivars. Shvirst (2016) in Magadan region of Russia found that that over a period of five years 'Amorfa' grew 62.5±29.3 mm, 'Lebedushka' 45.0±5.0 mm and 'Nimfa' 102.5±18.9 mm.

Table 3.3. Microclimate differences between three locations where shoot growth of wild blue honeysuckle (*Lonicera caerulea ssp. villosa*) was studied. The three sites were located near Prince Albert National Park, within the Boreal Plains ecozone, Saskatchewan, Canada. Data was collected from May to July in 2014 and 2015.

Description	Location		
	SPF	BLF	BLS
Air Temperature (°C)	13.35 ± 0.14*	13.46 ± 0.11	14.07 ± 0.11
Air Relative Humidity (%)	63.49 ± 0.37	63.62 ± 0.51	65.85 ± 0.41
Dew Point (°C)	6.05 ± 0.16	6.19 ± 0.17	7.24 ± 0.16
Soil Temperature (°C)	6.18 ± 0.20	7.66 ± 0.16	7.92 ± 0.21
Rainfall (mm)	14.42 ± 0.63	19.48 ± 0.72	7.48 ± 0.34
Soil Moisture (kPa)	19.05 ± 0.36	13.92 ± 0.10	10.29 ± 0.12
Solar Radiation (μmole.m^2/s)	273.24 ± 2.92	569.06 ± 4.75	326.60 ± 3.79

SPF: Shady, Peat & Flat, BLF: Bright, Luvisol & Flat, BLS: Bright, Luvisol & Sloped, *Mean standard error

3.3.2 Variation of microclimatic parameters and shoot growth

The microclimatic parameters varied (Table 3.3) on the sites with wild blue honeysuckle shoot growth (Table 3.2, Figure 3.6). Other studies found that the blue honeysuckle grown in different

locations also impact the primary and secondary metabolites, and it may vary significantly due to environmental factors such as precipitation, temperature, light intensity, and length of vegetation period (Shi et al., 2004, Jaakola and Hohtola 2010, Ni et al., 2013, Senica et al., 2018).

Growth of the shoots were greater at the site SPF with cooler (13.35°C) average air temperature condition compared to the other two sites. Air temperature may affect blue honeysuckle growth differently by genotype. For example, in a study by Shvirst (2016) in Magadan region of Russia, 'Amorfa' grew an average of five years 62.5±29.3 mm, 'Lebedushka' 45.0±5.0 mm, and cultivar 'Nimfa' 102.5±18.9 mm during 2011 to 2015. Growth of shoots differed by years and by cultivars whereas, days with temperature above +15°C 2011 was 32, 2012 was 31, 2013 was 59, 2014 was 46 and 2015 was 17 only. However, in a study of *Lonicera caerulea* cultivars with high temperatures and wet weather, a positive effect on ascorbic acid (vitamin C) content of (Pokorna 2009) was observed. In Polish cultivars (*Lonicera caerulea),* higher temperatures at the end of the season promoted the growth of larger, softer fruits, higher soluble solids, higher phenolic content, lower titratable acidity, and lower ascorbic acid concentrations (Skupien 2009, Ochmian 2013). In some studies of *L.c.*, lower temperatures and wet conditions increased ascorbic acid content (Pokorna 2009).

The average relative humidity in 2015 at BLS site was 60.12% or the lowest compared to the other two sites. Generally, the relative humidity in 2015 was lower from the previous year 2014 (Table 10.2 Appendix). There were no studies involving blue honeysuckle growth and relative humidity. However, in a study of rose (Mortensen and Gislerød 1999), shoot lengths were similar between humidity levels of 75% and 83%, however, when relative humidity was increased to 91%, shoot length decreased to 53.1 cm along with total fresh weight of the biomass.

The average dew point varied from site to site (Table 10.3 Appendix). Moreover, minimum dew points were lower at SPF site by -11.4°C in May, -2.0°C in June and -3.5°C in July in the year 2015 than year 2014. Dew utilization by plants is considered one of the survival strategies to short term moisture shortage in arid, semi-arid environments. In a study using sprinklers in a dew simulation experiment, the dew treatments significantly increased the growth of *Populus euphratica* seedlings (Zhang et al., 2019)

Growth of shoots was greatest at the site with cooler soil temperature 6.18°C compared to the other two sites. The soil temperature varied from site to site significantly (Table 3.3). For three locations, the soil average temperature in 2015 (SPF: 8.4°C, BLF: 8.9°C, BLS: 9.8°C) was slightly warmer than in 2014 (SPF: 10.1°C, BLF: 9.1°C, BLS: 10.6°C) (Table 11.4). In 2014, the minimum soil temperature was in the SPF site (8.4°C), and the maximum was in the BLS site (9.8°C). But in 2015, the BLF site was the coldest (9.1°C) and the BLF site was also the warmest (10.6°C).

The BLF site received the largest amount of precipitation compared to the other two sites. Although, there was less rainfall at other two sites than BLF site, there was a pond next to the BLS site and peat soil with high water holding capacity at the SPF site, which may have supplied additional water to the wild blue honeysuckle plants. Thus, the soil moisture positively effected the shoot growth of wild blue honeysuckle (*Lonicera caerulea* ssp. *villosa*).

Soil moisture sensors were obtained and installed in 2015 and were not available in 2014. There was lack of literature on *L.c.*'s moisture requirements. In this study, average soil moisture varied from 19.05 ± 0.36, 13.92 ± 0.10 and 10.29 ± 0.12 kPa respectively at SPF, BLF and BLS sites. Optimum soil moisture tension is considered for the plants such as Celery 20-30 kPa, Leek 25 kPa, Strawberry 20-30 (Sanders 1997, Gratton and Oster 1992). At the locations of this study, soil moisture tension may have been close to optimum at the SPF site but less than optimum and more water saturated, especially at the BLF and BLS sites.

The solar radiation showed great differences between study sites. At the SPF site, which had the greatest shoot growth, levels of solar radiation varied between190.1 and 452.9 μmole.m^2/s but averaged 272.8 μmole.m^2/s. A similar observation to this study was made by Kontsevoi and Ezhov (1997) who noted that wild *L.c.* shoots did not grow well in direct sunlight and in low fertile, heavy clay soil with high moisture content, instead it grew well under indirect sunlight in a loamy soil.

For SPF and BLS locations, the condition/pattern of solar radiation was similar (Table 11.7). In May 2014, it was 442.8 μmole.m^2/s and 397.1 μmole.m^2/s, but gradually increased during May to July to 536.1 μmole.m^2/s and 635.6 μmole.m^2/s, respectively for SPF and BLS. In May 2015, from 732.6 μmole.m^2/s and 928.6 μmole.m^2/s, it continuously decreased to 394.4 μmole.m^2/s and 640.7 μmole.m^2/s in July, respectively for SPF and BLS. This may be related to the number of cloudy days during both years.

3.3.3 Soil properties and shoot growth

Nitrate (NO_3): The concentration of nitrate (Table 3.4) in the soil of the SPF area was the

Table 3.4. Soil and leaf properties at three sites in the forest near Prince Albert National Park, within the Boreal Plains ecozone, Saskatchewan, Canada, May to July of 2014 and 2015

Soil properties	Location			*F* value	*p* value
	SPF site	BLF site	BLS site		
	----------mg/kg----------				
NO_3 (0-20 cm)	9.55 ± 0.18* a**	0.33 ± 0.01 c	1.74 ± 0.03 b	44.4	<0.0001
NO3 (20-40 cm)	3.96 ± 0.10 a	0.24 ± 0.00 b	0.35 ± 0.01 b	44.8	<0.0001
NH_4 (0-20 cm)	42.23 ± 0.33 a	10.29 ± 0.29 b	9.93 ± 0.29 b	20.6	0.002
NH4 (20-40 cm)	26.34 ± 0.35 a	3.59 ± 0.33 b	2.99 ± 0.04 c	965.9	<0.0001
P (0-20 cm)	17.06 ± 0.42 a	5.09 ± 0.13 b	5.02 ± 0.25 b	12.0	0.008
P (20-40 cm)	3.57 ± 0.10 a	1.51 ± 0.01 b	1.66 ± 0.01 b	12.5	0.007
K (0-20 cm)	189.93 ± 2.72 a	58.11 ± 1.31 b	49.62 ± 2.51 b	16.2	0.004
K (20-40 cm)	46.29 ± 29 a	30.79 ± 0.77 a	53.95 ± 1.49 a	0.8	0.479
SOC % (0-20 cm)	34.48 ± 0.17 a	3.12 ± 0.12 b	7.00 ± 0.38 b	22.9	0.002
SOC % (20-40 cm)	28.56 ± 0.25 a	0.64 ± 0.02 b	0.84 ± 0.01 b	299.2	<0.0001
pH (0-20 cm)	6.53 ± 0.01 a	5.16 ± 0.02 c	5.99 ± 0.03 b	50.6	<0.0001
EC (0-20 cm)	0.39 ± 0.01 a	0.15 ± 0.00 b	0.18 ± 0.00 b	13.6	0.006
Leaf properties	----------mg/g----------				
Total N	17.48 ± 0.12 a	15.92 ± 0.07 b	12.95 ± 0.12 c	42.2	<0.0001
Total P	3.29 ± 0.03 a	2.13 ± 0.03 b	1.73 ± 0.02 b	20.7	<0.0001
Total K	12.82 ± 0.14 b	15.52 ± 0.09 a	11.29 ± 0.18 c	61.8	<0.0001
Average chl *a*	0.45 ± 0.00 a	0.35 ± 0.00 b	0.31 ± 0.00 b	6.0	0.038
Average chl *b*	0.17 ± 0.00 a	0.13 ± 0.00 b	0.11 ± 0.00 b	4.3	0.071
Average chl *a*+*b*	0.62 ± 0.01 a	0.48 ± 0.00 b	0.42 ± 0.00 b	5.5	0.044

*Mean standard error, ** Means followed by the same letter within a line are not significantly different at α=0.05 (Fisher's least significant difference). SPF: Shady, Peat & Flat, BLF: Bright, Luvisol & Flat, BLS: Bright, Luvisol & Sloped. NO_3: Nitrate, NH_4: Ammonium, P: Available Phosphorus, K: Available Potassium, SOC: Soil Organic Matter, EC: Electrical Conductivity, N: Nitrogen, P: Phosphorus, K: Potassium, chl: Chlorophyll.

highest in the 0-20 cm layer (9.5 mg/g) and the lowest in the soil of the BLF area (0.3 mg/kg). The amount at the SPF site was also greater in the 20 to 40 cm layer (3.9 mg/kg). Nitrate uptake level in the plants commonly reflects presence of the nitrate in the soil (Liu et al., 2014) and the reason is nitrate cannot be produced in the plants, except symbiotic bacteria in root nodules of few legumes

(Hipkin et al., 2004). This indicates that the mineralization and supply capacity of organic matter was greater in the SPF site.

Ammonium (NH_4): The content of ammonium in the 0-20 cm soil depth at the SPF site was 42.2 mg/kg, which was 4 times higher than the content of ammonium in the soils of BLF and BLS site in the depth of 0-20 cm (Table 3.4). However, the amount of ammonium in the 20-40 cm layers of soil in all areas was lower, 1.6, 2.8 and 3.3 times respectively at the sites SPF, BLF and BLS than the depth of 0-20 cm. Ammonium being the more prevalent ionic species and more available compared to other nitrogen forms common in some acidic or anaerobic environments (Miller and Cramer 2004). Also, plants require less energy to synthesize organic nitrogen from ammonium than from nitrate (Williams et al., 1987).

Available Phosphorus: The amount of the phosphorus was greatest (Table 3.4) at the SPF, 17.1 mg/g compared to the BLF (5.09 mg/kg) and BLS (5.02 mg/kg) sites at the depth of 0-20 cm. This trend was similar in the depth of 20-40 cm.

Available Potassium: The soil analysis showed that the content of extractable, available potassium in the soil was greater compared to the available nitrogen and phosphorus content at study locations (Table 3.4). The maximum potassium content in the 0-20 cm layer was 189.9 mg/kg in the SPF site and was approximately 3 times lower in the other two sites. The available potassium content in the 20-40 cm depth was lower at SPF and BLF sites compared to 0-20 cm layer, but higher at BLS site.

Soil organic carbon: The concentration of organic carbon in the SPF site soil in the depth of 0 to 20 cm was 34.5 % of organic carbon, consistent with this soil being an organic soil, dominantly comprised of peat. It was 5 times more than in the BLS site (7.0 %) and 11 times more than in the site BLF (3.12 %). Moreover, the amount of organic carbon in the 20 to 40 cm depth was lower in all areas (Table 4.2). Since soil organic carbon mineralization releases chemical energy for the growth and utilization of plants and soil organisms, the mean organic carbon reduction is approximately equivalent to the reduction of microbial biomass (Bleam 2017). Microbial biomass generally increases as soil organic matter content increases. Also, soil organic carbon contributes to soil fertility by cementation of soil aggregates, retention of cations and conservation of nutrients

in organic forms (Lavelle and Spain 2001) which may explain their positive correlations between soil organic matter content and shoot growth.

Soil pH: The soil pH at 0-20 cm depth was location dependant and significantly different (Table 3.4). Soil pH is a one of the major variables and is involved in many chemical processes in soils. In particular, pH affects plant nutrient availability by determining the chemical form of nutrients. Although the optimal pH of the soil is between 5.5 and 7.0 for most plants (Perry 2003), many plants can tolerate environments above and below this limit. The best shoot growth was at the SPF site with pH 6.53. Phosphorus is to some extent more affected by soil pH compared to nitrogen and potassium. At slightly alkaline pH conditions phosphate ions tend to react with calcium and magnesium, whereas at acidic pH condition, phosphate ions react with aluminum and iron to form less soluble compounds. Most of the other micronutrients tend to be less available when soil pH is above 7.5 except molybdenum (Jensen 2010).

Soil electrical conductivity: The electrical conductivity of the soil in the SPF area was 0.39 $mS/cm.s^{-1}$, which is twice as high as in the BLF and BLS areas. However, the results of the electrical conductivity measurements are all below 2 $mS/cm.s^{-1}$ indicating that all threes soils were non-saline (FAO 2022).

3.3.4 The leaf nutrients and chlorophyll

Total nitrogen, and potassium concentration in the leaves (Table 3.4) was significantly different between locations. However, total P concentration in leaves was similar at BLF and BLS sites but different from SPF. Both BLF and BLS had similar levels of P in the soil and had thick clay C horizons and more saturated soils. A lack of aeration may have inhibited P uptake. Phosphorus has been shown to affect the water retention capacity of protoplasm, thereby stabilizing plant water metabolism (Minaev 1990, Agrochemistry 1989).

Chlorophyll *a*, *b* and *a* + *b* showed a similar patten, whereby SPF was different from the other two sites but BLF and BLS were similar. Although chlorophyll *a*, *b* and *a* + *b* was different for SPF, within each site ratios between chlorophyll *a* and *b* were similar: SPF was 2.6:1, BLF was 2.7:1 and BLS was 2.8:1. In a study by Ying et al. (2018) showed that chlorophyll a and b ratio ranged from 0.87 to 15.92, with an average of 2.47 on 823 plant species at natural forest

communities. The chlorophyll *a*:*b* ratio can be a one of the indicators in judging shade tolerant species (Givnish 1988) and shade tolerant species show a lower *a*:*b* ratio under reduced light compared to the high light environments. However, it is not observed in this study.

Thus, the hypothesis is accepted that the L.c. (*Lonicera villosa*) has a large variation in growth at the three sites in the forests of northern Saskatchewan and may be associated by the considerable amounts of environmental, soil and leaf properties. It is worthwhile to note that the observed differences in growth could be due to many other confounding factors.

3.3.5 Conclusion

The results of this study indicate that wild blue honeysuckle (*Lonicera caerulea* spp. *villosa*) can grow in boreal forest environments having a range of soil properties, with soil properties appearing to be important factors controlling their growth. In this observation, the wild blue honeysuckle (*Lonicera caerulea* ssp. *villosa*) shoots grew longest with an average of 80 mm in the area with PAR of 190.1 - 452.9 μmole.m^2/s an average of 272.8 μmole.m^2/s, without direct sunlight, with good soil moisture (21.5-28.9 kPa) supply. The soil temperature at the best site was 4.0-12.9°C, air temperature 8.1-19.4°C, relative humidity 58.6-91.3% and dew point 6.2 -11.3°C. In terms of soil properties, wild blue honeysuckle (*Lonicera caerulea* spp. *villosa*) shoots grew best at a site with organic, peaty soil with about 10 mg/kg of nitrate nitrogen, 42 mg/kg of ammonium nitrogen and 17 mg/kg and 190 mg/kg of extractable available phosphorus and potassium respectively, with electrical conductivity of 0.39 mS/cm.s^{-1} and in the pH of 6.5 at the depth 0-20 cm.

In conclusion, shoot growth varied with location factors and surrounding abiotic environment in a complex matrix. Location and microclimatic parameters such as soil temperature, dew point, soil moisture, air temperature, air relative humidity, precipitation and solar radiation may have contributed to the shoot growth in the study sites. There are many factors that are not measured and are confounded with "location" and environment. For example, growth may be affected by *L.c.* genotype, other soil nutrition than N, P, K, competing vegetation (intra and interspecific) and all of which could vary by location.

Link to the next chapter

In Chapter 3, *L.c*. shoot growth may have association with many environmental, soil and leaf properties. While this chapter helps to understand wild *L.c*. better, many of the environmental components observed are beyond the ability of growers to manipulate for better production. All study sites had significant but closer pH levels, so this study does not provide insight into what range of pH might be optimal to *villosa*. The next chapter investigates pH effects under controlled conditions on growth of different *L.c*. subspecies including *villosa*.

4.0 EFFECT OF pH IN NUTRIENT SOLUTIONS ON WILD AND CULTIVED BLUE HONEYSUCKLE BIOMASS

4.1 Introduction

The previous chapter covered soil pH ranges in the field between pH of 5.16 and 6.53. To further explore the effects of pH on blue honeysuckle growth, an experiment was conducted using a wide range of pH levels in nutrient solutions applied to blue honeysuckle under controlled environment conditions. This chapter also involves a wide range of germplasm.

Soil properties are closely related to factors such as climate, parent material and topography (Jenny 1941). One important property of the soil controlling many processes affecting plant growth is concentration of hydrogen ions in the soil solution (pH). The pH influences availability of nutrients needed for plant growth and has a major impact on soil biogeochemical processes in nature (Brady and Weil 2002, Minasny et al., 2016). The negative logarithm of the soil hydrogen ion concentration gives the pH value that is an indicator of acidity or alkalinity, and ranges from 0 to 14 depending on the concentration content of hydrogen ions. Low pH values indicate high hydrogen ion concentration in the soil solution. The ideal environment for most plants to grow is soil pH between 6 to 7.5, but there are some plant species that grow in acidic soils of pH 4.5 to 5.5.

Soils formed in dry environments are usually alkaline due to limited leaching. Conversely, in humid climates, soils usually have low pH or acidic properties due to weathering and loss of base cations like calcium and magnesium (Brady 2002). Precipitation and evaporation control changes in global soil pH (Slessarev et al., 2016). In addition, the impact of climatic factors on soil pH fluctuations is observed regionally (Brandy 2002, Cheng-Jim et al,., 2014, Chytry 2007). For example, Cheng-Jim et al., (2014) reported that soil pH is negatively related to average temperature and average precipitation. Chytry (2007) also found that soil pH tends to decrease as precipitation increases. At the same time, the relationship between soil pH and soil parameters depends on the site. For example, a study by Moore (1993) found that surface slope and surface moisture index (TWI) had a significant effect on soil pH in Colorado's agricultural landscape. Chen et al. (1997)

found that the main factor influencing the pH of the soil in the mountainous areas of southern Taiwan is the aspect and slope of the surface. Li et al. (2017) found that hydrological activities related to site characteristics and water accumulation may affect regional soil pH.

The concentration of the H+ ions in solution play a key role by influencing energy requirements for nutrient uptake and transport (Marschner 1995). The pH of the solution influences the energy requirement by the plant to import ions (H+ ions mostly) across cell membranes and tonoplasts against electrochemical gradients. The reaction environment of the nutrient solution determines the absorption of minerals by the plant, which varies from plant to plant. Reuter et al., (2008) reported that the spatial distribution of soil pH depends on the nature of the minerals in the bedrock and whether the soil developed from rocks such as granite, quartzite, sandstones or calcareous sediments. Fabian et al., (2014) discovered that there was low pH in soils above crystalline bedrock and a high pH in limestone areas.

Low pH soils are a major obstacle to agricultural production as they directly affect plant growth and pH around roots (Fageria and Baligar 2001, Kochian et al., 2004, Fageria and Baligar 2008, Chen 2009). When soil pH is too low, roots growth decreases, plasma membrane permeability increases, and H^+ ion concentrations increase directly (Chen et al., 2009). In addition, aluminum (Al) and manganese (Mn) may reach toxic levels, and calcium (Ca), magnesium (Mg), and phosphorus (P) become deficient (Kochian et al., 2004).

Important biological processes in the soil like organic nitrogen mineralization, nitrification, and biological nitrogen fixation may be hampered by acidity (Brady and Weil, 2002). Roots release H^+ and HCO_3- into the rhizosphere to absorb nutrients and maintain electroneutrality, so when plants are grown in hydroponics the pH of solutions change and buffer solutions are required to maintain pH levels (Brady and Weil 2002).

Soil-free hydroponics-based evaluation methods are widely used to determine the comparative response of genotypes to different stresses (Köpp et al., 2006, 2007a). In this study, the response of *L.c.* to different pH solutions in a hydroponic system was evaluated by investigating uptake of N, P and K into stems, leaves and roots systems in addition to biomass measurements. This is the first time that a *L.c.* study of pH effects in a hydroponic environment has been conducted.

The purpose of the research described in this chapter is to use a hydroponic system in a greenhouse setting to assess the growth of plants and macronutrient uptake as influenced by

different pH levels, when other environmental conditions are held constant. The hypothesis was that different pH levels will affect growth and nutrient uptake of the *L.c.* differently, with reduced growth and uptake under highly acidic and alkaline pH conditions in the hydroponic solution.
This study also investigated seven subspecies of *L.c.* and the cultivar 'Tundra' to determine if they might have different optimum pH levels. If a subspecies of *L.c.* could be found better adapted to high pH, that subspecies might be useful in breeding future varieties for Saskatchewan.

4.2 Materials and methods

This experiment used five pH levels (5, 6, 7, 8, 9) and was arranged in a split block design with three blocks and eight genotypes. The experiments were repeated twice. These included *Lonicera caerulea subspecies: pallasii, stenantha, venulosa, emphyllocalyx, kamtschatica, altacia, villosa* and the commercial cultivar 'Tundra'. Most of the genotypes for this experiment were obtained from Dr. Artem Sorokin of the Vavilov Institute in St Petersburg originally in the form of seeds. Dr. Maxine Thompson provided ssp. *emphyllocalyx*. The University of Saskatchewan had bred 'Tundra' and had gathered ssp. *villosa*. The experiment was conducted in the University of Saskatchewan Agriculture Greenhouse (45 Innovation Blvd, Saskatoon, SK S7N 2T8). Dry matter yield accumulation was determined by growing the honeysuckle plants in a mesh pot with expanded clay pebbles in a plastic container (17 L) with a hydroponic solution (nitrogen 175 ppm, phosphorus 50 ppm, potassium 212 ppm, magnesium 41 ppm, calcium 184 ppm, sulphur 71 ppm, iron 2.6 ppm, zinc 0.3 ppm, boron 0.7 ppm, manganese 0.8 ppm, copper 0.2 ppm and molybdenum 0.07 ppm) based on Resh's (HydroBuddy, 2013) program for tomato plants. Different pH levels (5, 6, 7, 8, 9) were established and maintained using standard pH up and down solutions (General Hydroponics®). Prior to the experiment, in 2014 clonally propagated cuttings of the *L.c.* subspecies and the cv. 'Tundra' were propagated in sufficient amounts and grown in potting mix "SunShine №4" in 5.5 cm x 5.5 cm x 6 cm black plastic seedling pots. Plant roots were washed from the soil mix and repotted in a mesh pot with the pebbles at the start of the experiment. One-year old dormant plants were used and the second-year vegetative growth was assessed. The hydroponic solution was continually aerated with an air pump (dual diaphragm air pump with four outlet channels), tubing (transparent, aquarium tube 4.76 mm in diameter) and an air stone (GC Air Stone with nozzles). The whole experiment had two reps, the first rep started on February 16th of 2015 and the second rep started on April 21st of 2015 (Figure 4.1). The temperature in the greenhouse was set to

20ºC day and 15ºC night. High pressure sodium 400-watt light were used and set for 16 hours day radiation.

Plants were harvested 45 days after the start of the experiment. Leaves, stems and roots (washed) were separated, labeled and placed in a paper bag for further air drying in a drying cabinet (Precision Scientific). After drying at 50°C for 72 hours to constant dry weight, the samples were weighed for the total dry matter in grams on digital scale and later were ground using a micro hammer mill (Culatti AG, Zurich, Switzerland). All samples were stored in a dark room, at room temperature until further analysis.

Figure 4.1. Photo of the hydroponic pH experiment after five weeks with 7 subspecies and one cultivar of blue honeysuckles (*Lonicera caerulea*) grown in a hydroponic system with five different pH environments.

Total nitrogen, phosphorus, and potassium analysis: Total N, P and K concentrations in the plant dry matter were determined using the same methodology described in chapter 4.

Chlorophyll fluorescence (OS30p+, Opti-Sciences, New Hampshire, USA) was measured on three leaves of each plant after 2 hours from clipping the leaves. Chlorophyll fluorescence ratio between variable fluorescence (Fv) and maximum fluorescence (Fm), Fv/Fm was recorded to assess the environmental stress.

Statistical analysis: The SAS (Statistical Analysis System, Version 9.4 for Windows; SAS Institute, Cary, NC) software was used for data processing. All data were subjected to analysis of variance (ANOVA) using PROC MIXED in SAS while pdmix800 SAS macro 29 was used to

assign grouping, blocking and experiments were included as a random effect in the model. Log 10 transformation was used on leaf, stem, root and total dry matter, leaf total P concentration, stem N and P concentration, root P concentration and N, P, K uptake data. A probability level of $p<0.05$ was chosen to establish statistical significance. Differences between treatment means were determined using Tukey's multiple range test and considered significant at $p\leq0.05$.

4.3 Results and discussion

In this study, the relationship between the dry weight of the leaves, stems and roots of *Lonicera caerulea* subspecies, and macro nutrients such as nitrogen, phosphorus and potassium in the plants were explored under different pH environments in hydroponic conditions.

4.3.1 pH and genotypes effects on dry matter weight accumulation of blue honeysuckle

The pH (Figure 4.2, F 17.3, $p<0.0001$) and genotypes (Figure 4.3, F 28.4, $p<0.0001$) had statistically significant effects on the total dry weight of the plants. While pH 6 resulted in highest average dry matter yield, it was not significantly different from pH 5 and 7 (Figure 4.2). For effects of genotype (Fig. 4.3), *Lonicera caerulea* ssp. *stenantha* had the greatest growth, consistent with this subspecies being largest in the field. Cultivar 'Tundra' had more growth compared to most subspecies, reflecting that this cultivar was the result of breeding and selection while the other subspecies were wild selections (Figure 4.3). There were no significant interactions between pH and genotypes (pH x Genotype F 0.7, $p=0.868$). It had been hoped that the experiment might reveal some genotypes better adapted to high pH soils, but it seems that all subspecies react similarly to pH levels.

This study suggests that total dry mass accumulation was active in the honeysuckles grown from weakly acidic to neutral environments, while alkaline pH values of resulted in reduced growth with increasing alkalinity (pH 8 and pH 9). On average, when the alkalinity of the hydroponic solution increased by one unit, the dry mass accumulation of the *L.c.* decreased by more than 20 percent. For example, the dry mass accumulation decreased by 23.2% when pH increased from 7 to 8, and by 20.8% when pH increased from 8 to 9 (Figure 4.2).

Growing *Lonicera caerulea* subspecies *pallasii, stenantha, venulosa, emphyllocalyx, kamtschatica, altaica, villosa* and the cultivar 'Tundra' at five different pH levels showed

significant difference between genotypes in dry matter biomass accumulation (Figure 4.2, Figure 4.3 and Table 4.1).

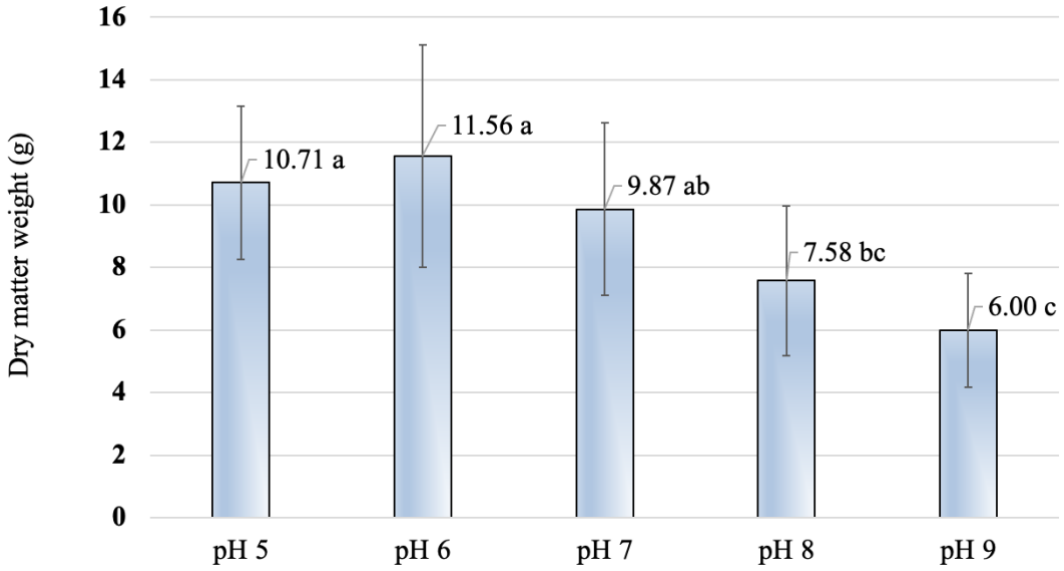


Figure 4.2. Average dry matter (*F* 17.3, *p*<0.0001) of blue honeysuckle (*Lonicera caerulea*) grown in a hydroponic system with five different pH environments. These bars are averages of seven subspecies and one cultivar. *Means followed by the same letter within a column are not significantly different at α=0.05.

The total dry weight of *Lonicera caerulea* ssp. *stenantha* was the highest, followed by *Lonicera caerulea* cv. Tundra, while the total dry weight of other 6 subspecies was significantly lower (Figure 4.3). Anugoolprasert et al. (2012) concluded in their study of sago palm seedlings that pH had no significant effect on dry matter weight although it tended to be 9% lower at pH 3.6 than pH 5.7. Zieslin and Snir's (1989) study with roses found that within a short period of time (9-20 days), no difference was observed in plant and leaf biomass accumulation between pH 4 and 6. In the current study, the preference of blue honeysuckle for soils of neutral to weakly acid pH is clearly shown.

4.3.2 The effect of pH and genotypes on leaf, roots and stems dry weight of blue honeysuckle

The leaf dry weight showed a similar pattern to the total dry matter weight and the experimental plants grew well in pH 5 and pH 6 hydroponic solutions and showed poor growth at pH 8 and pH 9 and lower leaf dry weight compared to pH 7 (Table 4.1). This also suggests that the *L.c.* may

grow better in a slightly acidic environment. Similar studies but with pH adjusted mineral soil (Mistassini loamy sand) found that the *Lonicera caerulea* cv. *Indigo Treat* grew best in slightly acidic soil environments of pH 5.9-6.5 (Tremblay et al., 2019).

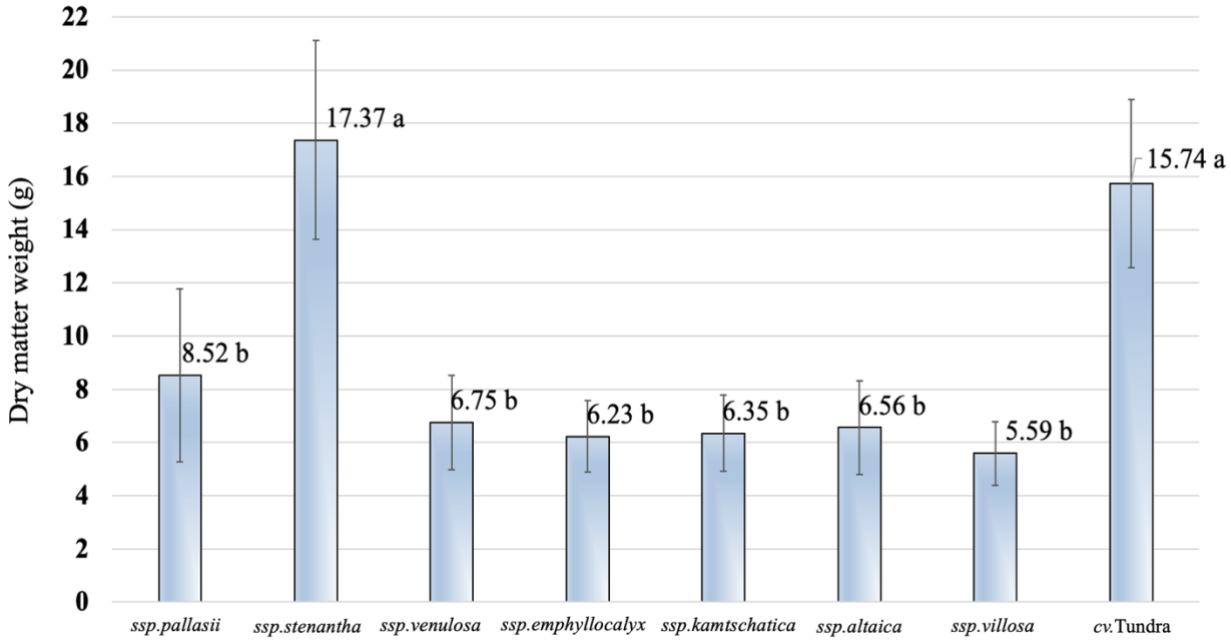


Figure 4.3. Average dry matter weight (*F* 28.4, *p*<0.0001) of eight different blue honeysuckles (*Lonicera caerulea*) genotypes grown in a hydroponic system at University of Saskatchewan Agriculture Greenhouse. *Means followed by the same letter within a column are not significantly different at α=0.05.

Dry weights of honeysuckle plant stems were the highest/greatest in pH 5 and pH 6 environments, but when the pH changed to neutral and alkaline, it decreased. This indicates the blue honeysuckle grows better in a slightly acidic and neutral environment. For 8 different blue honeysuckle genotypes, the stem dry weight of *Lonicera caerulea* spp. *stenantha* species was significantly higher (7.20 mg/g) than others, followed by *Lonicera caerulea* cv. Tundra (4.08 mg/g), and the other 6 species were similar (1.49-2.45 mg/g). Overall, the stems dry weight yield of *Lonicera caerulea* ssp. *stenantha* was 1.7 times higher than cultivar Tundra and on average 3.9 (maximum 4.83 and minimum 2.94) times higher than the other 6 subspecies (Table 4.1). This reflects genetic differences among subspecies in resource allocation and production of different plant parts.

Table 4.1. Leaf, stem and root dry matter of eight different blue honeysuckles (*Lonicera caerulea*) genotypes grown in a hydroponic system with five different pH levels.

Experimental Factors	Leaves		Stems		Roots	
	Mean	SD	Mean	SD	Mean	SD
	g/per plant					
pH*						
5	4.22a	2.26	3.25a	2.44	3.24a	1.99
6	4.33a	3.02	3.67a	2.97	3.56a	2.31
7	3.33b	2.75	2.93ab	2.74	3.61a	2.50
8	2.37c	2.04	2.31bc	2.40	2.90ab	1.62
9	1.77c	0.98	1.81c	1.48	2.42b	1.82
SEM**	0.59		0.51		0.48	
Genotype***						
Lonicera caerulea spp. *pallasii*	2.83b	1.52	2.45c	1.26	3.24bc	1.30
Lonicera caerulea spp. *stenantha*	6.07a	3.73	7.20a	3.67	4.10b	1.98
Lonicera caerulea spp. *venulosa*	2.57b	1.76	1.78cd	1.15	2.40cd	1.03
Lonicera caerulea spp. *emphyllocalyx*	2.24b	1.34	1.82cd	0.74	2.17d	0.90
Lonicera caerulea spp. *kamtschatica*	2.33b	1.43	1.63cd	0.78	2.39cd	0.98
Lonicera caerulea spp. *altaica*	2.50b	1.75	1.88cd	0.99	2.18cd	1.18
Lonicera caerulea spp. *villosa*	2.02b	1.20	1.49d	0.81	2.08d	1.11
Lonicera caerulea cv. Tundra	5.06a	2.95	4.08b	2.23	6.60a	3.16
SEM	0.64		0.57		0.54	
F value, *p*-value						
pH	*F* 29.4, *p*<0.0001		*F* 16.6, *p*<0.0001		*F* 5.42, *p*=0.0004	
Genotype	*F* 19.4, *p*<0.0001		*F* 36.3, *p*<0.0001		*F* 22.0, *p*<0.0001	
pH x Genotype	*F* 1.0, *p*=0.5307		*F* 0.6, *p*=0.9652		*F* 0.6, *p*=0.9292	

* These are averages of all 8 genotypes listed below.
** Means followed by the same letter within a column are not significantly different at α=0.05. (Means were compared using Tukey-Kramer test)
*** These are averages of all 5 pH treatments.
SD: Standard deviation

Root weights followed a similar trend to leaves and stems. The pH 5, pH 6 and pH 7 showed positive effects on root growth and biomass accumulation compared to the pH 8 and pH 9. The highest root dry weight was *Lonicera caerulea* cv. Tundra, followed by *Lonicera caerulea* ssp. *stenantha*. The other six natural subspecies blue honeysuckles were similar to each other and significantly lower (Table 4.1). Similar results were obtained for lettuce growth by Anderson et al., (2017) in a recirculating aquaculture system (RAS) with pH 5.8 having the least biomass in roots fresh weight and dry weight (18% reduction) compared to pH 7.

The effect of pH on the growth of eight different blue honeysuckles (*Lonicera caerulea*) genotypes grown in the hydroponic system with five different pH environments can be seen in

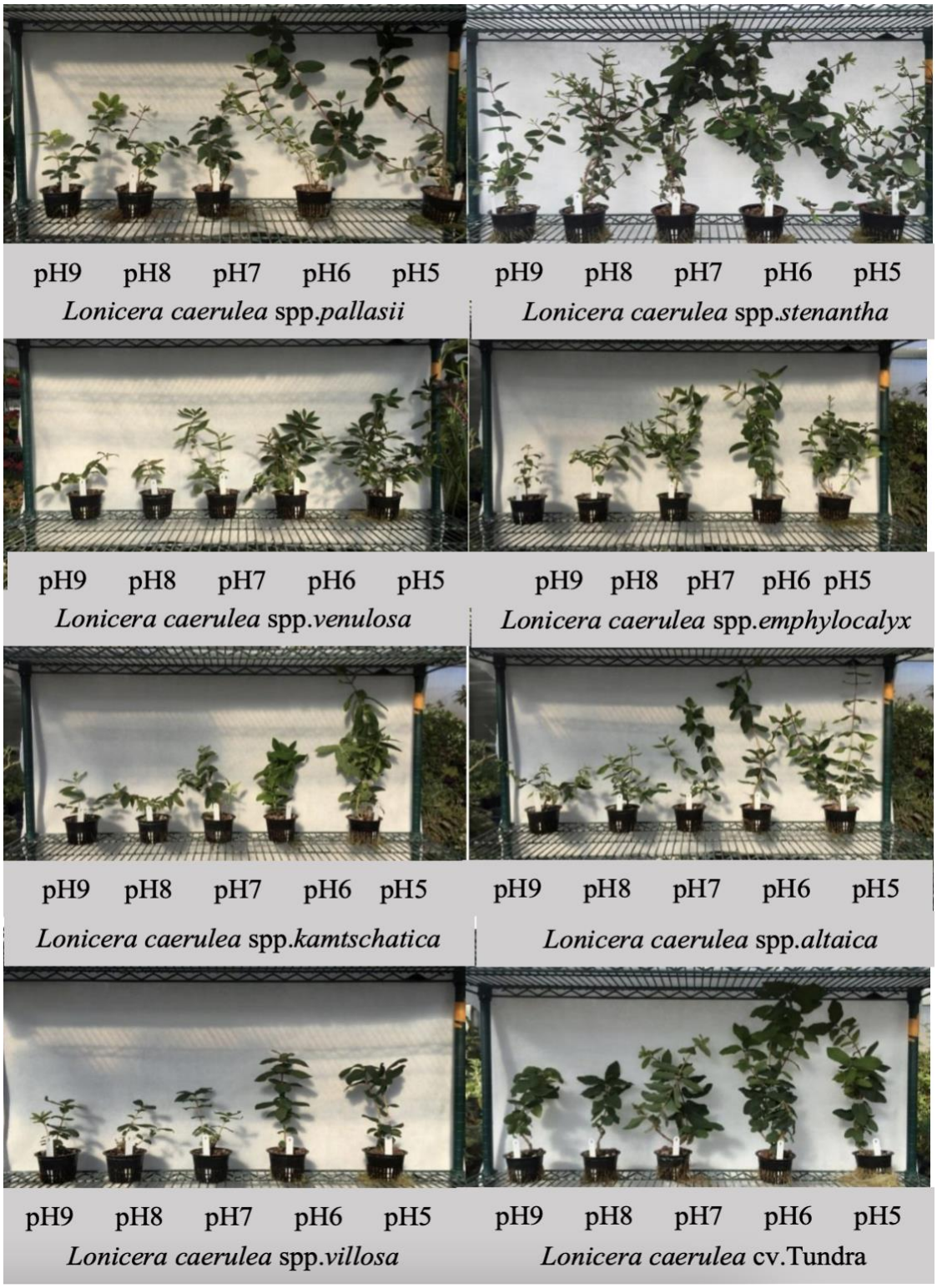


Figure 4.4. Growth of eight different blue honeysuckles (*Lonicera caerulea*) genotypes after 45 days grown in a hydroponic system with five different pH environments at University of Saskatchewan Agriculture Greenhouses.

Figure 4.4. Notably, *Lonicera caerulea* ssp. *stenantha* and *Lonicera caerulea* cv. Tundra growth in pH 5 and pH 6 showed greater dry mass growth of leaves, stems and roots compared to other subspecies. It is remarkable that such differences of growth occurred in only 45 days. Nursery growers of Haskap plants would do well to provide acidic conditions to their plants.

4.3.3 Nitrogen, phosphorus and potassium uptake by blue honeysuckle plants

Nitrogen, phosphorus and potassium total uptake per plant (Table 4.2) was calculated by taking the concentration in the plant multiplied by the plant yield, generally followed the same pattern as

Table 4.2. Total nitrogen, phosphorus and potassium uptake per plant (mg) of eight different blue honeysuckle (*Lonicera caerulea*) genotype grown in a hydroponic system with five different pH environments at University of Saskatchewan Agriculture Greenhouses.

Experiment factors	Nitrogen		Phosphorus		Potassium	
	Mean	SD	Mean	SD	Mean	SD
	mg/plant					
pH*						
5	182.10 a	103.64	47.17 a	29.37	166.81 a	116.60
6	194.10 a	116.70	47.81 a	30.43	171.05 a	135.52
7	145.12 b	113.68	34.39 b	23.41	128.74 b	120.16
8	104.39 c	79.05	20.12 c	13.93	97.25 bc	82.15
9	90.11 c	61.36	16.75 d	11.23	86.29 c	62.39
SEM**	3.42		7.83		4.09	
Genotype***						
Lonicera caerulea spp. *pallasii*	139.14 b	67.21	32.04 b	19.21	119.45 b	57.73
Lonicera caerulea spp. *stenantha*	261.34 a	139.12	55.53 a	37.00	290.42 a	177.35
Lonicera caerulea spp. *venulosa*	110.56 bc	67.34	25.66 bc	18.55	91.02 bc	54.99
Lonicera caerulea spp. *emphyllocalyx*	91.22 c	47.27	22.76 c	13.51	81.35 c	47.24
Lonicera caerulea spp. *kamtschatica*	98.89 c	46.47	25.14 bc	13.42	89.11 bc	46.96
Lonicera caerulea spp. *altaica*	116.91 bc	79.62	29.10 bc	19.30	97.02 bc	62.58
Lonicera caerulea spp. *villosa*	90.43 c	47.36	21.24 c	13.41	76.92 c	39.76
Lonicera caerulea cv. Tundra	248.67 a	122.72	57.29 a	36.40	197.83 a	109.21
SEM	15.77		11.08		7.57	
F value, *p*-value						
pH	*F* 27.5, *p*<0.0001		*F* 55.1, *p*<0.0001		*F* 24.1, *p*<0.0001	
Genotype	*F* 26.5, *p*<0.0001		*F* 19.7, *p*<0.0001		*F* 30.5, *p*<0.0001	
pH x Genotype	*F* 0.83, *p*=0.712		*F* 0.59, p=0.948		*F* 0.80, p=0.754	

* These are averages of all 8 genotypes listed below.
** Means followed by the same letter within a column are not significantly different at α=0.05. (Means were compared using Tukey-Kramer test)
*** These are averages of all 5 pH treatments.
SD: Standard deviation

total dry matter (Table 4.2) and blue honeysuckle leaf, stem and roots concentration of nutrients (Table 4.3, Table 4.4 and Table 4.5). At pH 6 the blue honeysuckle plant showed greatest uptake in all nutrients examined and it tended to slightly decrease in the pH 5 and pH 7. Moreover, when hydroponic solution alkalinity increased to pH 8 and pH 9 nutrient uptake further decreased to the lowest level in this experiment (Table 4.2).

Depending on the genotype and pH level, plants used 2.0-8.8 percent of the macro minerals (NPK) from the hydroponic solution, which may reflect the excessive usage of the nutrients in the hydroponic practice.

4.3.4 Nitrogen, phosphorus and potassium concentration in the leaves of blue honeysuckle

The nitrogen concentration in the leaves was greater at pH 5 and pH 6 and tended to decrease with the increasing alkalinity of the hydroponic solution and the difference was significant between pH 5, pH 6 and pH 7, pH 8, pH 9 (Table 5.3). This trend was also noted with beans (Anugroho 2010) and rose cultivation (Kim 2005). The average nitrogen concentrations were in the leaves of *Lonicera caerulea* ssp. *stenantha* and cultivar 'Tundra' which were significantly different compared to other six subspecies whereas *Lonicera caerulea* ssp. *villosa* had the lowest leaf N levels at 14.84 mg/g of concentration. Although *Lonicera caerulea* ssp. *stenantha* and cultivar 'Tundra' had the most growth, *Lonicera caerulea* ssp. *stenantha* had significantly more N than cultivar 'Tundra', but P and K were similar.

Concentration of the phosphorus was significantly higher under pH 6 whereas at pH 9, it decreased to 2.93 mg/g. Phosphorus concentration decreased when alkalinity of the hydroponic solution increased (Table 5.3). In a study by Kim et al. (2005) the phosphorus concentration at pH 4 was greatest compared to pH 8 and pH 6 in rose cultivation. The current study found that the phosphorus concentration in the leaves of *Lonicera caerulea* ssp. *altaica* was the highest 4.85 mg/g, whereas *Lonicera caerulea* spp. *villosa* had the lowest value (2.75 mg/g).

Concentration of potassium in the leaves in this study showed a different pattern compared to the nitrogen and phosphorus concentration. In the hydroponic solution with pH 5 potassium concentration was greatest compared to the pH 7 solution and was significantly different. Nitrogen

and phosphorous form anions while potassium forms cations and slightly acidic conditions are known to favor uptake.

Table 4.3. Nitrogen, phosphorus and potassium concentration in leaves of eight different blue honeysuckles (*Lonicera caerulea*) genotypes grown in a hydroponic system with five different pH environments at University of Saskatchewan Agriculture Greenhouses.

Experiment factors	Nitrogen		Phosphorus		Potassium	
	Mean	SD	Mean	SD	Mean	SD
			mg/g			
pH *						
5	18.87 a	6.01	3.86 a	1.24	15.33 a	5.16
6	19.33 a	4.10	4.17 a	1.33	14.38 ab	3.68
7	15.55 b	4.57	3.47 ab	1.40	11.94 c	3.76
8	15.82 b	4.85	3.42 bc	2.85	12.90 bc	4.71
9	15.09 b	5.23	2.93 c	2.15	14.68 ab	4.55
SEM**	0.019		0.016		0.025	
Genotype***						
Lonicera caerulea spp. *pallasii*	18.64 ab	4.99	4.04 ab	1.52	14.36 ab	4.48
Lonicera caerulea spp. *stenantha*	20.60 a	5.13	3.60 abc	1.61	16.29 a	6.01
Lonicera caerulea spp. *venulosa*	16.08 bc	4.83	3.61 abc	1.90	13.98 ab	3.97
Lonicera caerulea spp. *emphyllocalyx*	15.30 c	4.45	2.92 bc	0.90	13.02 b	3.84
Lonicera caerulea spp. *kamtschatica*	16.48 bc	3.77	3.50 ab	1.01	14.00 ab	3.88
Lonicera caerulea spp. *altaica*	17.13 bc	6.26	4.85 a	3.47	13.52 ab	3.99
Lonicera caerulea spp. *villosa*	14.84 c	5.81	2.75 c	1.55	12.16 b	5.21
Lonicera caerulea cv. Tundra	16.40 bc	4.64	3.30 abc	1.44	13 45 ab	3.89
SEM	0.030		0.060		0.028	
F value, *p*-value						
pH	*F* 13.3, *p*=<0.0001		*F* 12.3, *p* =<0.0001		*F* 6.0, *p*=0.0002	
Genotype	*F* 7.1, *p*=<0.0001		*F* 4.6, *p*=0.0001		*F* 2.8, *p*=0.009	
pH x Genotype	*F* 0.8, *p*=0.743		*F* 0.9, *p*=0.559		*F* 1.3, *p*=0.148	

* These are averages of all 8 genotypes listed below.
** Means followed by the same letter within a column are not significantly different at α=0.05. (Means were compared using Tukey-Kramer test)
*** These are averages of all 5 pH treatments.
SD: Standard deviation

4.3.5 Nitrogen, phosphorus and potassium concentration in the stems of blue honeysuckle

Total nitrogen and potassium concentrations in stems were not significantly different for pH levels. The amount of phosphorus accumulated in the stems of *Lonicera caerulea* subspecies in different hydroponic solutions ranged from 1.71 mg/g to 2.91 mg/g and tended to decrease as the pH of the hydroponic solution became more alkaline. The amount of phosphorus accumulated in the stems among the genotypes ranged from 1.72 to 2.58 mg/g (Table 4.4). Generally, as expected,

the total nitrogen concentrations of the stems was higher than phosphorus and lower than average potassium, which also reflects similar ratio of NPK in the hydroponic solution which was 3.5:1:4.2 respectively. The total phosphorus, nitrogen and potassium accumulation in stems in *Lonicera caerulea* ssp. *altaica* and *Lonicera caerulea* ssp. *villosa* were higher than others. But the total phosphorus and nitrogen concentration of stems, the lowest was in *Lonicera caerulea* spp. *stenantha* 1.72 mg/g and 6.15 mg/g respectively and potassium concentration was lowest was in *Lonicera caerulea* cv. Tundra 7.26 mg/g (Table 4.4).

Table 4.4. Nitrogen, phosphorus and potassium concentration in stems of eight different blue honeysuckles (*Lonicera caerulea*) genotypes grown in the hydroponic system with five different pH environments at University of Saskatchewan Agriculture Greenhouses

Condition of experiment	Nitrogen		Phosphorus		Potassium	
	Mean	SD	Mean	SD	Mean	SD
			mg/g			
pH*						
5	8.96 a	6.07	2.91 a	1.63	10.38 a	4.01
6	8.04 a	3.19	2.54 a	1.03	10.00 a	3.57
7	7.95 a	5.12	2.11 b	1.07	8.56 a	3.68
8	6.73 a	1.43	1.57 c	0.51	8.99 a	3.69
9	8.15 a	3.88	1.71 bc	0.76	9.01 a	3.89
SEM**	0.027		0.013		0.024	
Genotype***						
Lonicera caerulea spp. *pallasii*	7.22 ab	1.65	2.03 ab	0.86	8.78 bcd	3.52
Lonicera caerulea spp. *stenantha*	6.15 b	1.32	1.72 b	0.69	12.28 a	4.88
Lonicera caerulea spp. *venulosa*	7.03 ab	1.71	1.94 ab	0.94	8.06 cd	2.83
Lonicera caerulea spp. *emphyllocalyx*	6.56 b	1.23	1.85 ab	0.62	8.34 bcd	2.50
Lonicera caerulea spp. *kamtschatica*	8.01 ab	4.30	2.47 ab	1.65	9.08 bcd	3.14
Lonicera caerulea spp. *altaica*	9.84 a	5.87	2.57 a	1.29	10.52 abc	3.64
Lonicera caerulea spp. *villosa*	10.26 a	7.29	2.58 a	1.62	10.77 ab	4.39
Lonicera caerulea cv. Tundra	8.64 ab	4.84	2.17 ab	1.09	7.26 d	2.54
SEM	0.030		0.014		0.026	
*F v*alue, *p*-value						
pH	*F* 2.1, *p*=0.0850		*F* 18.7, *p*<0.0001		*F* 2.3, *p*=0.0585	
Genotype	*F* 5.0, *p*<0.0001		*F* 3.6, *p*=0.0012		*F* 7.1, *p*<0.0001	
pH x Genotype	*F* 0.8, *p*=0.785		*F* 0.9, *p*=0.692		*F* 0.7, *p*=0.8852	

* These are averages of all 8 genotypes listed below.
** Means followed by the same letter within a column are not significantly different at α=0.05. (Means were compared using Tukey-Kramer test)
*** These are averages of all 5 pH treatments
SD: Standard deviation

4.3.6 Nitrogen, phosphorus, and potassium concentrations in the roots of blue honeysuckle

All N, P and K concentrations in the roots were affected by high and low pH. When the hydroponic solution was pH 5, the total phosphorus content of the roots was the highest on average and lowest in an alkaline (pH 9) environment. Similar studies by Anugoolprasert et al. (2012) showed the P concentration in the roots was higher at pH 3.6 and pH 4.5 than at pH 5.7. Higher concentration of P also was observed in the roots in low pH in studies by Kim et al. (2005) with rose plants. At lower pH the $H_2P0_4^{-1}$ form of orthophosphate dominates over the HPO_4^{-2} form in solution. Perhaps the honeysuckle prefers the primary orthophosphate ion over the secondary for uptake by its root membrane carriers or perhaps since this is only observed in the root, that the lower pH interferes with translocation of P from root to shoot (J.J. Schoenau, personal communication, 2022).

The maximum nitrogen concentration in the roots system of the *L.c.* was 23.05 and 23.72 mg/g when the medium of the nutrient solution was weakly acidic pH 5 and pH 6, respectively. When the medium of the nutrient solution was neutral and alkaline, the amount of nitrogen accumulation was uniform and ranging from 19.90 mg/g to 19.31 mg/g. The accumulation of total nitrogen in the roots was not statistically significantly different between genotypes.

The concentration of potassium in the *L.c.* roots significantly differed in pH 5 compared to pH 7, pH 8 and pH 9 and decreased from 20.15 to 17.27 mg/g when the nutrient medium changed from acidic to alkaline, similar to that observed for the leaves. The highest total potassium concentration was 23.72 mg/g in *Lonicera caerulea* ssp. *stenantha* and the lowest was 14.79 mg/g in Tundra. This may be related to the adaptability, biological and physiological characteristics of the subspecies. Similarly, in other studies the potassium concentration in the roots and stems were higher than the leaves in various low pH (pH 5.7, 4.5 and 3.6) solutions in sago palm (Anugoolprasert et al., 2012). However, in this experiment potassium concentration in the roots was lowest (Table 4.5).

Table 4.5. Nitrogen, phosphorus and potassium concentration in the roots of eight different blue honeysuckle (*Lonicera caerulea*) genotypes grown in a hydroponic system with five different pH environments at University of Saskatchewan Agriculture Greenhouses

Condition of experiment	Nitrogen		Phosphorus		Potassium	
	Mean	SD	Mean	SD	Mean	SD
			mg/g			
pH*						
5	23.05 a	6.78	6.71 a	2.47	20.15 a	7.31
6	23.72 a	5.30	6.16 a	1.61	19.04 ab	5.12
7	19.90 b	4.96	5.52 a	3.12	17.51 b	6.85
8	19.31 b	3.89	3.86 b	1.49	16.86 b	6.10
9	19.55 b	4.49	3.60 b	1.20	17.27 b	5.63
SEM**	0.028		0.021		0.030	
Genotype***						
Lonicera caerulea spp. *pallasii*	21.20 a	5.89	5.06 a	1.58	18.69 b	4.89
Lonicera caerulea spp. *Stenantha*	22.26 a	5.00	4.82 a	1.93	23.72 a	7.12
Lonicera caerulea spp. *venulosa*	22.66 a	4.63	5.27 a	2.06	17.12 bc	5.11
Lonicera caerulea spp. *emphyllocalyx*	19.55 a	4.97	5.55 a	2.48	16.81 bc	6.34
Lonicera caerulea spp. *kamtschatica*	18.83 a	4.66	5.32 a	3.45	16.92 bc	5.20
Lonicera caerulea spp. *altaica*	22.50 a	6.39	5.43 a	3.11	18.98 b	6.28
Lonicera caerulea spp. *villosa*	21.72 a	6.51	5.31 a	2.35	18.28 b	6.13
Lonicera caerulea cv. Tundra	20.13 a	4.72	4.59 a	1.91	14.79 c	5.79
SEM	0.031		0.022		0.033	
F value, *p*-value						
pH	*F* 7.5, *p*<0.0001		*F* 27.2, *p*<0.0001		*F* 5.1, *p*=0.0006	
Genotype	*F* 2.2, *p*=0.0336		*F* 0.7, *p*=0.6590		*F* 11.7, *p*<0.0001	
pH x Genotype	*F* 0.6, *p*=0.950		*F* 0.8, *p*=0.773		*F* 1.1, *p*=0.354	

* These are averages of all 8 genotypes listed below.
** Means followed by the same letter within a column are not significantly different at α=0.05. (Means were compared using Tukey-Kramer test)
*** These are averages of all 5 pH treatments
SD: Standard deviation

4.3.7 Chlorophyll fluorescence ratio of the blue honeysuckle

Chlorophyll Fv/Fm ratio showed no significant difference between pH treatments, ranging from 0.711 to 0.752 (Table 4.6). There was a significant difference between subspecies *stenantha and kamtschatica.* All other subspecies and *cv.* Tundra showed intermediate Chlorophyll Fv/Fm ratios.

Table 4.6. Chlorophyll fluorescence ratio (Fv/Fm) of eight blue honeysuckle (*Lonicera caerulea*) genotypes grown in the hydroponic system under five different pH environments at University of Saskatchewan Agriculture Greenhouses

	Mean	SD
	mol/s	
pH*		
5	0.752 a	0.056
6	0.740 a	0.080
7	0.752 a	0.056
8	0.711 a	0.130
9	0.733 a	0.079
SEM**	0.11	
Genotype***		
Lonicera caerulea spp. *pallasii*	0.713 ab	0.072
Lonicera caerulea spp. *stenantha*	0.777 a	0.022
Lonicera caerulea spp. *venulosa*	0.734 ab	0.056
Lonicera caerulea spp. *emphyllocalyx*	0.745 ab	0.067
Lonicera caerulea spp. *kamtschatica*	0.702 b	0.077
Lonicera caerulea spp. *altaica*	0.758 ab	0.046
Lonicera caerulea spp. *villosa*	0.747 ab	0.084
Lonicera caerulea cv. Tundra	0.724 ab	0.171
SEM	0.12	
F value, *p*-value		
pH	F 1.9, p=0.1050	
Genotype	F 2.6, p=0.0143	
pH x Genotype	F 1.1, p=0.3921	

* These are averages of all 8 genotypes listed below.
** Means followed by the same letter within a column are not significantly different at α=0.05. (Means were compared using Tukey-Kramer test)
*** These are averages of all 5 pH treatments.
SD: Standard deviation

An Fv/Fm ratio in the range of 0.790-0.840 is normal for plants without much stress (Maxwell and Johnson 2000). In a study by Johnson et al. (1993), they observed that Fv/Fm ratio on *Spinacia oleracea* was 0.850, *Pisum sativum* was 0.831, *Chemopodium album* was 0.831 and similar studies done by Demmig and Björkman (1987) showed Fv/Fm ratio on *Glycine max* was 0.800, *Gossypium hisutum* was 0.835, *Monstera deliciosa* was 0.842, *Nerium oleander* was 0.816, *Rhizophora stylosa* was 0.777, *Schefflera actinophylla I* was 0.829, *and Typha latifolia* was 0.830. However, there were no previous studies conducted on the blue honeysuckle using Chlorophyll fluorescence (OS30p+, Opti-Sciences, New Hampshire, USA). While this study had some stress caused by

different pH levels, more investigation could be done with *Lonicera caerulea* in order to clarify the range of the normality in this species by imposing other types of stress.

4.3.8 Conclusion

In summary, hydroponic experiments showed that pH of the nutrient solution bathing the roots had a significant effect on total dry weight and nutrient uptake of the blue honeysuckle. However, it is important to note that hydroponic conditions are different than field conditions. Adsorption-desorption and precipitation-dissolution reactions with soil minerals that control solution concentrations of P and K are important in the field and are affected by pH. For available nitrogen, biological processes like N turnover are affected by pH in field soils that are not a factor in hydroponic systems. There was a pattern of increased alkalinity above neutrality causing reduced growth and nutrient uptake, while pH 5 and pH 6 induced the highest dry mass accumulation and nutrient uptake. It indicates that the growth may be enhanced in weakly acidic soil. This enhanced growth would also increase demand for nutrient, hence a similar pattern between growth and nutrient uptake as influenced by pH. The pH variations generally influenced leaf concentrations of N and P in a manner similar to biomass yield but K concentrations were greatest at low and high pH, suggesting that uptake of potassium cations is increased at low and high pH by the honey suckle. Tremblay et al. (2019) observed that *Lonicera caerulea* cv. *Indigo Treat* growth was optimal in slightly acidic soils between pH 5.9-6.5 without the use of nitrogen fertilizers. Among the 7 subspecies, *Lonicera caerulea* ssp. *stenantha* and variety *Tundra* had the highest dry weight, while *Lonicera caerulea* ssp. *villosa* had the lowest. However, responses were similar among subspecies suggesting that optimal pH is similar across all of *L.c.*

The root weight was highest at pH 6 and lowest at pH 9. The ratio between stems and roots biomass averaged 1.00: 1.14, indicating that the root system is usually greater than the weight of above-ground stems for most genotypes. Two notable exceptions were *Lonicera caerulea* ssp. *stenantha* which had more biomass in stems and ‘Tundra’ which had more biomass in leaves and roots Both *Lonicera caerulea* ssp. *stenantha* and cv. ‘Tundra’ had almost twice the total biomass compared to other subspecies in the study. Moreover, *Lonicera caerulea* subspecies showed similar characteristics on dry matter weight, weight ratio of the leaves, stems and roots, for their nitrogen, phosphorus and potassium concentration.

This study confirms the hypothesis that pH will affect growth of the *L.c*., with reduced growth and nutrient uptake at high (alkaline) pH. Some genotypes of the *L.c.* will show different growth than others. Blue honeysuckles, regardless of the subspecies, grow better in hydroponics in pH 5-pH 6 than neutral or alkaline pH. However, when grown in the field, soils vary in their composition and pH will impact availability of the vital macro and micro elements in plants.

Link to the next Chapter

For growers of *L.c.* in gardens and commercial operations, one of the common questions is fertilization. In Chapter 6, *L.c.* was grown with different NPK rates in a closed environment (greenhouse) with a goal of providing practical advice for growers.

5.0 EFFECTS OF MINERAL FERTILIZERS ON THE GROWTH OF BLUE HONEYSUCKLE NURSERY PLANTS

5.1 Introduction

It is important for propagators to provide healthy and vigorous plants for new orchards. Typically, nurseries propagate blue honeysuckle either by tissue culture or by cuttings, but never by seed. The resulting young clones sold the following season are called plugs or liners. Some nurseries will take plugs and grow them for an extra year or two in larger containers resulting in nursery plants. Nursery plants are typically sold at garden centres, but some growers use them in orchards. In this chapter, plug plants were transplanted into larger containers and given various combinations of fertilizers to evaluate responses. The results of this research could directly benefit nursery growers and farmers who wish to fertilize newly planted plugs in the field. In the previous chapter, different pH levels in hydroponic solution were evaluated for their effect on biomass production and nutrient uptake and revealed that generally the yield and nutrient uptake of different genotypes was reduced at alkaline pH. The ability of fertilization to overcome nutritional limitations in blue honeysuckle was investigated and is covered in this thesis chapter. Nurseries do not typically use hydroponic. Consequently, this chapter uses a potting mix commonly used by nurseries.

One of the vital factors to grow young plant is to have well balanced soil nutrients. Nitrogen is involved in the composition of simple and complex proteins, which are the main components of plant protoplasm. Nitrogen requirements are low in the early stages of plant growth. Nitrogen consumption will increase as the root and shoot systems develop (Olson and Kurtz 1982). Plant nitrogen is the most abundant in the stems and leaves, in which amino acids and proteins are synthesized from nitrogen that was absorbed from the soil. During plant growth, proteins are synthesized in young organs, while in older organs, protein is broken down and the resulting products continue to be transferred to young organs (Novoa and Loomis 1981). Due to the different nitrogen metabolism occurring in different parts of the plant, nitrogen is not evenly distributed throughout the plant. Nitrogen compounds are more abundant in areas with high protein synthesis

(Choijamts 2006). A few studies were done on the influence of fertilization and *Lonicera japonica.* N fertilization increased vegetation yield and crude protein content level in leaves (Segelquist 1975). In similar study by Belosohova and Belosohov (2010) with *L.c.* cultivar 'Goluboye Verteno', uptake of P was better when N fertilizer was applied applied in the form of ammonium-nitrate during the wet year of 2006. During the drier year of 2005 P uptake was better when N was applied in the form of urea. The K uptake in 2005 was better when N was applied in the form of ammonium and in 2006 when N was in the form of ammonium-nitrate.

Another major element in plant nutrition is phosphorus. Phosphorus is part of the high-molecular-weight nucleic acids involved in protein synthesis, plant growth, development, and heredity. Phosphorus is contained in organic compounds mainly as ester bonded phosphate. Phosphorus-containing organic compounds are rapidly synthesized. Temperature, aeration, soil moisture, and microbial activity are important factors in plant phosphorus nutrition. Lack of phosphorus slows down the growth of crops, the accumulation of sugars and protein synthesis, and the leaves turn blue-green, sometimes brown or bronze radiant coloured, and the yield decreases. With a normal supply of phosphorus, the plant's ability to withstand extreme cold and winter hardiness improves as the number of carbohydrates in the fruit increases and the strength of the stems increases (Havlin et al., 2014). Phosphorus is distributed and absorbed in varying amounts in plant organs. In the experiment covered in Chapter 5, the roots of *L.c* plants at pH level 5 contained greater phosphorus concentrations than leaves, and lowest was in the stems. For example, the amount of phosphorus in the roots mass of *L.c*. was 2.5 to 3.0 times higher than in leaves and stems (Kondratyev 2008).

Potassium is absorbed and transported within plants in the form of ions and drive cellular expansion and organ movements, such as stomata aperture (Ragel et al., 2019). Potassium also improves plant water retention, resists drought, and increases cold tolerance by increasing the osmotic pressure of cell sap (Wang et al., 2013, Xu et al., 2021).

Determining the optimal fertilization regime for *L.c.* propagation is important. Large, vigorous plants will perform better in the field and excessive fertilizer will be uneconomical and waste of resources. When a nutrient is deficient in the growing medium, the response to added nutrient as fertilizer affects plant growth rates, however, this varies by nutritional demand, the type and amount of fertilizers applied to the soil, environmental conditions and many other factors (Stepura

2012). To this end, it is important to determine the specific dose rates of fertilizers required under specific conditions to create the most favourable conditions for plant growth and development by optimizing the supply of nutrients and achieving sustainable high yields (Vildflush et al., 2005).

In the nursery industry, soil-less potting mixes made chiefly with peat moss with small amounts of vermiculite and perlite are typically used to grow blue honeysuckle and woody plants. Peat moss itself contains nutrients that may be useful for growing blue honeysuckle. Peat moss is naturally a loose substance whereas peat moss in the potting mix is decomposed mainly due to microbial activities which results to about 20% of air space loss, with higher water holding capacity and nearly free of disease, weeds, wood and stone contaminants. Depending on the usage, a macro and micro mineral nutrients may added in the potting mix (Prasad 2022). While nursery growers may use various supplemental water-soluble fertilizers, there are no previous studies investigating fertilizers for blue honeysuckle nursery growers. In this research, the effects of 16 different fertilizer treatments with varying amounts of N, P and K were evaluated for their effects plant nitrogen, phosphorus, and potassium uptake in Haskap. This study has the potential to help nursery growers. The hypothesis for this study is that N, P and K added as fertilizers will have a positive effect on the weight and nutrient uptake of *L.c*. in commercial peat based potting media typically used by nursery growers. The response to fertilization will depend on the relative amounts of N, P and K added and fertilization will increase the plant biomass compared to using the commercial peat potting mixture alone.

5.2 Materials and methods

This randomized complete block design (three blocks) experiment was conducted in the University of Saskatchewan Agriculture Greenhouse (45 Innovation Blvd, Saskatoon, SK S7N 2T8). Prior to the experiment, in 2014 clones of the variety ‘Tundra’ were propagated as cuttings in sufficient amounts and grown in “SunShine №4” potting mix in plastic pots with a volume of 1412 cm^3. Fertilizer was added to the clones already grown in the “SunShine №4” potting mix. Average nutrients and pH in 2015, before the fertilization experiment was pH 6.1, ammonium: 8.9 mg/kg, nitrate: 68.7 mg/kg, available P: 71.8 mg/kg, available K: 149.3 mg/kg in the growing media. A whole trial was repeated one more time and total of 96 one-year old plugs (18-23 cm) were used in each trial. The second-year vegetative growth was assessed.

Sources of NPK for this experiment were urea (46%) for nitrogen, superphosphate (46%) for phosphorus and potassium chloride (62%) for potassium. Experimental plants were fertilized with a water-based solution prepared at a dose rate of 587.5 mg/kg of potting soil mix of urea, 111.5 mg/kg of potting soil mix of superphosphate and 134.5 mg/kg potting soil mix of potassium chloride as a low single dose per plant and applied at once along with the start of the experiment. The single dose rate was doubled for the medium dose and tripled in for the high dose of treatments.

A total of 16 treatments (Table 5.1) were used, consisting of a control (water) and fifteen treatments of low, medium and high fertilizer doses (low N, P, K, low, medium and high NP, low, medium and high NK, low, medium and high PK, low, medium and high NPK). Dry weights and concentrations of NPK in leaves, stems and roots were measured.

Table 5.1. Dosage rates for 16 treatments in mg per kg of potting soil mix.NP, NK, PK, and NPK all had low, medium, and high levels of fertilizers

Fertilization level	Low			Medium			High		
Fertilizer	N (U)	P_2O_5 (SP)	K (PC)	N (U)	P_2O_5 (SP)	K (PC)	N (U)	P_2O_5 (SP)	K (PC)
Dosage	mg/kg								
Control	0.0	0.0	0.0	n/a	n/a	n/a	n/a	n/a	n/a
N	587.5	0.0	0.0	n/a	n/a	n/a	n/a	n/a	n/a
P	0.0	111.5	0.0	n/a	n/a	n/a	n/a	n/a	n/a
K	0.0	0.0	134.5	n/a	n/a	n/a	n/a	n/a	n/a
NP	587.5	111.5	0.0	1175.0	223.0	0.0	1762.5	334.5	0.0
NK	587.5	0.0	134.5	1175.0	0.0	269.0	1762.5	0.0	403.5
PK	0.0	111.5	134.5	0.0	223.0	269.0	0.0	334.5	403.5
NPK	587.5	111.5	134.5	1175.0	223.0	269.0	1762.5	334.5	403.5

N: Nitrogen, P: Phosphorus, K: Potassium, U: Urea, SP: Superphosphate, PC: Potassium Chloride, N/A: No treatment exists

Plants were harvested 45 days after the start of the experiment. Leaves, stems and roots (washed) were separated, labeled, and placed in paper bags for further air drying in a drying cabinet (Precision Scientific). Drying at 50°C for 72 hours achieved constant dry weight samples that were weighed and later ground using a micro hammer mill (Culatti AG, Zurich, Switzerland). All samples were stored in a dark room, at room temperature until further analysis.

Total N, P and K Analysis: Total N and P concentrations were determined using the methods in chapter 4 and based on Thomas (1967) digestion with peroxide and sulfuric acid at 360 degrees C followed by colorimetric analysis.

5.2.1 Statistical analysis

The SAS (Statistical Analysis System, Version 9.4 for Windows; SAS Institute, Cary, NC) software was used for data processing. All data were subjected to fixed effect analysis of variance (ANOVA) using PROC MIXED whereas, blocks and experiments are included as a random effect. Log 10 transformation was used on leaf, stem, root and total dry weight. A probability level of $p< 0.05$ was chosen to establish statistical significance. Differences between treatment means were determined using Tukey's multiple range test and considered significant at $p\leq 0.05$.

5.3 Results and discussion

5.3.1 Fertilization effect on blue honeysuckle leaves, stems and roots dry matter weight

The NPK treatment with the highest rate of added N, P and K resulted in significantly higher (p=0.014) dry matter total weight accumulation compared to the unfertilized control (Table 5.2). Other fertilizer treatments did not significantly increase the dry matter weight of leaves, stems, roots and total weights of the experimental plants, but did result in higher mean dry matter yields (Table 5.2). Some studies have shown that fertilization with nitrogen fertilizers had a negative effect on the yield, quality and biochemical parameters of young plants of *L.c*. (Kondratyev 2008). In the current study, treatments that contained N had a tendency to yield higher than treatments containing P and/or K, suggesting that N availability was a limitation in the potting media. In this experiment the highest rate of nitrogen, phosphorus, and potassium combined resulted leaf dry matter (11.15 g) that was 80% more than the control treatment (6.18 g) while the effect on root dry matter weight was 116% more than the control treatment. In a study by Segelquist and Rogers (1975, fertilization of *Lonicera japonica* with N fertilizer resulted in increased growth and increased leaves protein content but decreased fruit yield. Fruit yield was not measured in the current study, but many studies have shown fruit yield to be more positively responsive to P fertilization than N fertilization depending on soil available P (Skinner et al., 1988).

Table 5.2. Blue honeysuckle (*Lonicera caerulea*) leaves, stems, roots and total dry weight (g) of the cultivar Tundra grown under 16 different fertilization treatment at University of Saskatchewan Agriculture Greenhouses

	Leaves		Stems		Roots		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
				grams				
Treatment								
Control	6.18a*	0.75	10.08a	2.88	7.85a	2.06	**24.11** b**	8.15
L-N	8.65a	1.59	8.35a	2.55	13.91a	6.31	30.91 ab	11.2
L-P	7.64a	1.28	8.03a	1.35	12.05a	3.61	27.72 ab	6.33
L-K	8.63a	2.56	7.83a	1.70	11.98a	2.63	28.44 ab	5.7
L-NP	8.80a	2.58	8.38a	2.38	15.14a	8.56	32.32 ab	12.6
M-NP	8.98a	0.66	8.43a	3.03	13.69a	9.42	31.10 ab	15.2
H-NP	8.67a	3.67	7.78a	2.25	13.92a	8.07	30.37 ab	12.8
L-NK	8.09a	1.80	7.80a	2.16	10.81a	4.34	26.70 ab	8.77
M-NK	8.61a	1.80	7.92a	3.90	10.21a	7.09	26.74 ab	15.2
H-NK	9.79a	3.03	8.60a	2.18	14.73a	3.12	33.12 ab	10.7
L-PK	8.39a	2.82	7.31a	0.86	11.90a	2.40	27.60 ab	3.62
M-PK	10.04a	1.71	9.58a	4.20	12.93a	3.56	32.55 ab	11
H-PK	8.24a	3.04	7.81a	1.71	12.26a	3.65	28.31 ab	6.38
L-NPK	10.44a	2.44	9.05a	3.01	14.17a	2.75	33.66 ab	7.96
M-NPK	9.84a	3.49	9.29a	1.54	15.39a	4.45	34.52 ab	7.33
H-NPK	11.15a	2.69	10.15a	2.62	16.97a	5.40	**38.27 a**	10.27
SEM	1.00		1.03		1.49		2.03	
F, *p*-value	*F* 1.4, *p*=0.160		*F* 0.750, *p*=0.731		*F* 1.5, *p*=0.147		*F* 2.1, *p*=0.014	

L: Low, M: Medium, H: High, N: Nitrogen, P: Phosphorus, K: Potassium, SD: standard deviation. *Means followed by the same letter within a column are not significantly different at α=0.05 and the transformed values "mean log10 (total weight)" are used for mean comparison using Tukey's multiple range test. SD-Standard deviation

Overall, the average dry matter weight distribution among the blue honeysuckle organs leaves, stems and roots were 28, 29, and 43% respectively (Figure 5.1). In a study of rooted cuttings of peach cultivar 'Nemaguard' in different volume containers, the leaves, stems and roots were 46, 33 and 21% respectively when grown in 2.4-liter container. However, when container volumes decreased, leaf percentages increased while stem and root percentages were reduced (Rieger and Marra 1994).

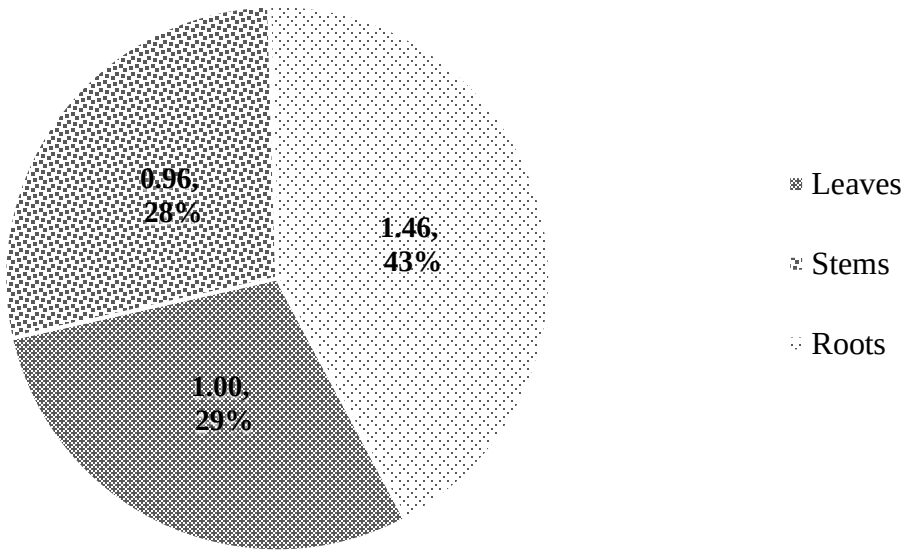


Figure 5.1. Percentage of the leaves, stems and roots of the blue honeysuckle cultivar 'Tundra' when16 different fertilization treatments were averaged.

5.3.2 Effects of fertilization on nitrogen, phosphorus and potassium concentrations of blue honeysuckle leaves

This experiment did not find statistically significant differences (Table 5.3) in the nitrogen concentrations in 'Tundra' blue honeysuckles leaves dry matter (p=0.079). Mean nitrogen concentration range was between 6.55 mg/g and 10.57 mg/g. Treatment with a single dose of K (8.69±0.70 mg/g) and the treatment of triple dose of NPx3 (7.87±2.50 mg/g) showed significant difference when compared between nitrogen concentration in leaves dry matter that may reflect some growth dilution. In a study with strawberries, Daugaard (2001) reported that the tissue N content is less in the strawberry plant during the fruit formation and development stage than other vegetative stages. However, in this case there were no statistically significant differences in the leaf nitrogen concentrations for the other treatments.

The phosphorus concentration in the leaves dry matter (Table 5.3) was not affected by fertilizer treatment (p=0.791). This is consistent with high available P content of the potting medium and lack of any evidence of yield response to phosphorus fertilization. Blue honeysuckle leaves

contained a minimum of 0.93 mg/g and maximum of 1.42 mg/g of phosphorus. Similar to nitrogen and phosphorus concentration in leaves, only non-significant statistical differences were observed in the concentration of potassium accumulated in the blue honeysuckle leaves grown in 16 different fertilization treatments (p=0.302).

Table 5.3. Macronutrient concentrations in the blue honeysuckle (*Lonicera caerulea*) leaves of cultivar 'Tundra' grown under 16 different fertilization treatments.

	Nitrogen		Phosphorus		Potassium	
	Mean	SD	Mean	SD	Mean	SD
			mg/g			
Treatment						
Control	7.60 ab*	4.65	1.26 a	0.63	11.22 a	4.95
L-N	6.22 a	1.29	0.94 a	0.17	10.94 a	2.69
L-P	7.25 ab	0.81	1.16 a	0.31	12.68 a	1.82
L-K	8.69 ab	0.70	0.93 a	0.04	12.32 a	1.94
L-NP	8.77 ab	1.70	1.28 a	0.75	11.35 a	1.77
M-NP	7.03 ab	2.88	1.31 a	0.70	11.35 a	4.19
H-NP	7.87 ab	2.50	1.38 a	0.31	13.17 a	2.89
L-NK	7.83 ab	1.86	1.42 a	0.42	13.48 a	2.80
M-NK	7.80 ab	1.91	1.12 a	0.26	13.84 a	3.56
H-NK	8.06 ab	3.52	1.05 a	0.55	9.41 a	4.71
L-PK	8.74 ab	1.55	1.21 a	0.50	12.77 a	1.47
M-PK	10.57 b	0.85	1.05 a	0.22	11.20 a	1.82
H-PK	6.99 ab	1.70	1.38 a	0.66	14.40 a	2.52
L-NPK	6.93 ab	1.29	1.13 a	0.27	11.88 a	2.10
M-NPK	6.55 ab	0.89	1.16 a	0.23	11.92 a	1.22
H-NPK	7.13 ab	1.65	1.12 a	0.24	11.69 a	1.90
SEM	0.29		0.13		0.34	
F, *p*-value	*F* 1.7, *p*=0.076		*F* 0.8, *p*=0.715		*F* 1.2, *p*=0.302	

L: Low, M: Medium, H: High, N: Nitrogen, P: Phosphorus, K: Potassium, * Means followed by the same letter within a column are not significantly different at α=0.05. Mean comparison used Tukey's multiple range test. SD-Standard deviation

5.3.3 Effects of fertilization on the nitrogen, phosphorus and potassium concentration of the blue honeysuckle stems

In this research, stems were non-responsive to fertilization treatment, with the 16 treatments of fertilizers having no statistically significant effect on the nitrogen (p=0.752), phosphorus (p=0.702) and potassium (p=0.637) concentration in the blue honeysuckle stems (Table 5.4). Concentrations of N, P and K were lower in the stems (Table 5.4) than the leaves, consistent with the stems serving

as transport rather than storage and metabolic process organs. Other studies (Hagen-Thorn 2004) have also shown stems to contain lower concentrations of macronutrients than leaves.

Table 5.4. Macronutrient concentration in the blue honeysuckle (*Lonicera caerulea*) stems of cultivar Tundra grown under 16 different fertilization treatments.

	Nitrogen		Phosphorus		Potassium	
	Mean	SD	Mean	SD	Mean	SD
			mg/g			
Treatment						
Control	4.01 a	1.46	0.86 a	0.18	7.47 a	2.34
L-N	3.46 a	1.53	0.81 a	0.37	5.87 a	1.27
L-P	3.13 a	0.55	0.70 a	0.12	5.51 a	0.78
L-K	3.42 a	0.72	0.82 a	0.18	6.39 a	1.57
L-NP	3.89 a	1.16	0.87 a	0.16	6.59 a	1.09
M-NP	4.74 a	2.02	0.87 a	0.13	6.11 a	1.10
H-NP	4.09 a	0.77	0.91 a	0.19	6.52 a	1.15
L-NK	3.59 a	0.55	0.79 a	0.13	6.04 a	0.70
M-NK	4.08 a	1.49	0.81 a	0.22	5.92 a	1.14
H-NK	3.99 a	0.91	0.84 a	0.17	5.99 a	0.99
L-PK	3.90 a	1.81	0.81 a	0.27	6.23 a	0.27
M-PK	4.07 a	1.85	0.81 a	0.20	6.46 a	0.85
H-PK	3.10 a	0.58	0.68 a	0.17	6.05 a	1.18
L-NPK	4.05 a	1.06	0.92 a	0.19	6.26 a	0.70
M-NPK	3.77 a	0.57	0.85 a	0.21	6.59 a	0.30
H-NPK	4.28 a	0.93	0.95 a	0.17	6.63 a	1.08
SEM	0.22		0.09		0.21	
F, p-value	*F* 0.8, *p*=0.655		*F* 0.9. *p*=0.571		*F* 0.8, *p*=0.638	

L: Low, M: Medium, H: High, N: Nitrogen, P: Phosphorus, K: Potassium, * Means followed by the same letter within a column are not significantly different at α=0.05. Mean comparison used Tukey's multiple range test. SD-Standard deviation

Mean concentration of nitrogen, phosphorus and potassium in the blue honeysuckle stems were minimum of 3.10 mg/g, 0.70 mg/g and 5.51 mg/g, maximum of 4.74 mg/g, 0.95 mg/g and 7.47 mg/g respectively (Table 5.4).

5.3.4 Effects of fertilization on the nitrogen, phosphorus and potassium concentration of the blue honeysuckle roots

In this study, nitrogen, phosphorus and potassium concentrations (Table 5.5) had minimums of 5.61 mg/g, 0.93 mg/g and 8.72 mg/g, and maximums of 7.32 mg/g, 1.17 mg/g and 11.15 mg/g respectively in the roots.

Table 5.5. Macronutrient (N,P,K) concentration (mg/g) in blue honeysuckle (*Lonicera caerulea*) root dry weight of cultivar Tundra grown under 16 different fertilization treatments.

	Nitrogen		Phosphorus		Potassium	
	Mean	SD	Mean	SD	Mean	SD
			mg/g			
Treatment						
Control	6.06 a	1.73	1.00 a	0.13	9.81 a	3.51
L-N	7.28 a	2.74	1.05 a	0.20	8.72 a	1.49
L-P	7.10 a	2.19	1.14 a	0.26	11.15 a	2.26
L-K	6.09 a	0.65	0.93 a	0.09	9.55 a	1.39
L-NP	6.76 a	0.98	1.13 a	0.36	9.73 a	0.86
M-NP	7.14 a	1.40	1.17 a	0.18	9.94 a	3.72
H-NP	7.18 a	1.05	1.17 a	0.20	8.72 a	1.78
L-NK	6.94 a	1.26	1.09 a	0.14	10.43 a	1.72
M-NK	7.32 a	1.54	1.06 a	0.26	10.72 a	3.27
H-NK	6.54a	0.63	1.12 a	0.13	9.46 a	2.27
L-PK	5.90 a	1.26	1.02 a	0.19	10.38 a	2.46
M-PK	5.61 a	0.58	0.96 a	0.12	9.18 a	0.41
H-PK	6.39 a	0.80	1.09 a	0.17	10.92 a	2.41
L-NPK	6 50 a	0.70	1.08 a	0.16	9.45 a	1.61
M-NPK	6.73 a	0.60	1.09 a	0.08	10.04 a	1.54
H-NPK	7.10 a	0.99	1.15 a	0.16	10.08 a	1.59
SEM	0.23		0.08		0.20	
F, *p*-value	*F* 1.0, *p*=0.508		*F* 0.9, *p*=0.553		*F* 0.6, *p*=0.835	

L: Low, M: Medium, H: High, N: Nitrogen, P: Phosphorus, K: Potassium, * Means followed by the same letter within a column are not significantly different at α=0.05. Mean comparison used Tukey's multiple range test. SD-Standard deviation

However, there was no significant difference between treatments in concentration of nitrogen (p=0.513), phosphorus (p=0.627) and potassium (p=0.839).

Overall, in this study, leaves had the highest concentrations of macronutrients followed by roots and then stems (Figure 5.2). In this study nitrogen levels were twice as high in leaves as in stems which was similar to studies with blue honeysuckle conducted in Russia where nitrogen accumulated in the leaves was 2-3 times higher than in the stems. (Kondratyev 2008). In the study by Kondratiev there was some variation due to biological characteristics of cultivars and age of plants.

When comparing average uptake of N, P and K in leaves, stems and roots by blue honeysuckle organs (Figure 5.3) N and K had very similar distribution rates. (Marschner et al., 1997), while N and K are more dominant in physiological functions like photosynthesis, protein synthesis and osmotic regulation in the leaves.

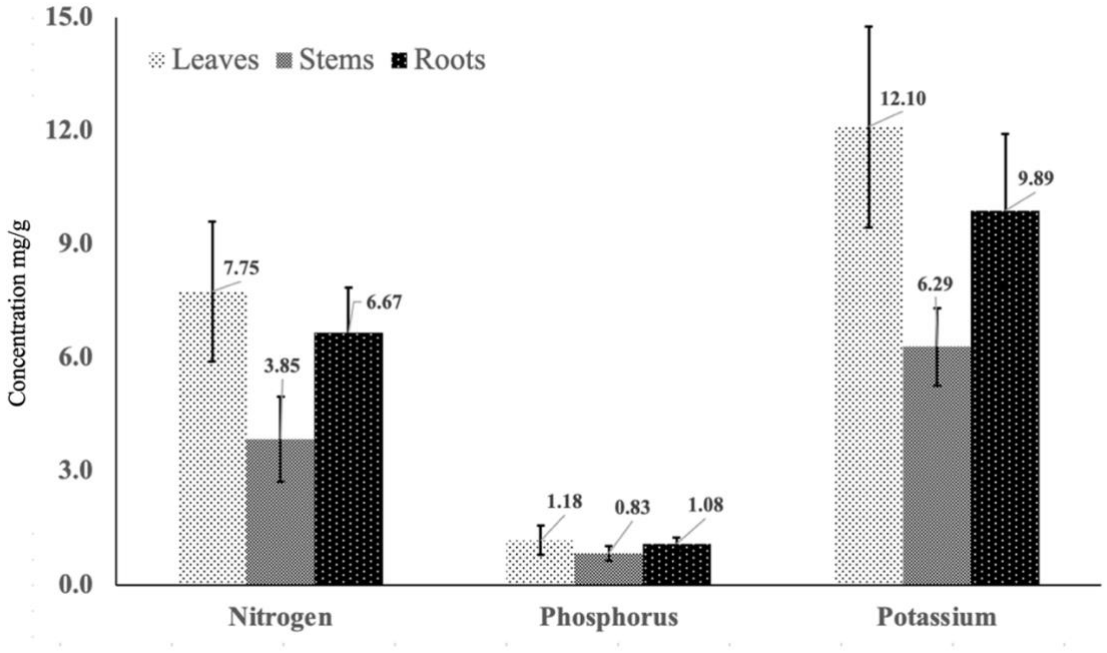


Figure 5.2. Macronutrient concentration (mg/g) in the blue honeysuckle (*Lonicera caerulea*) cultivar 'Tundra' leaves, stems and roots in dry matter, grown under 16 different fertilization treatments

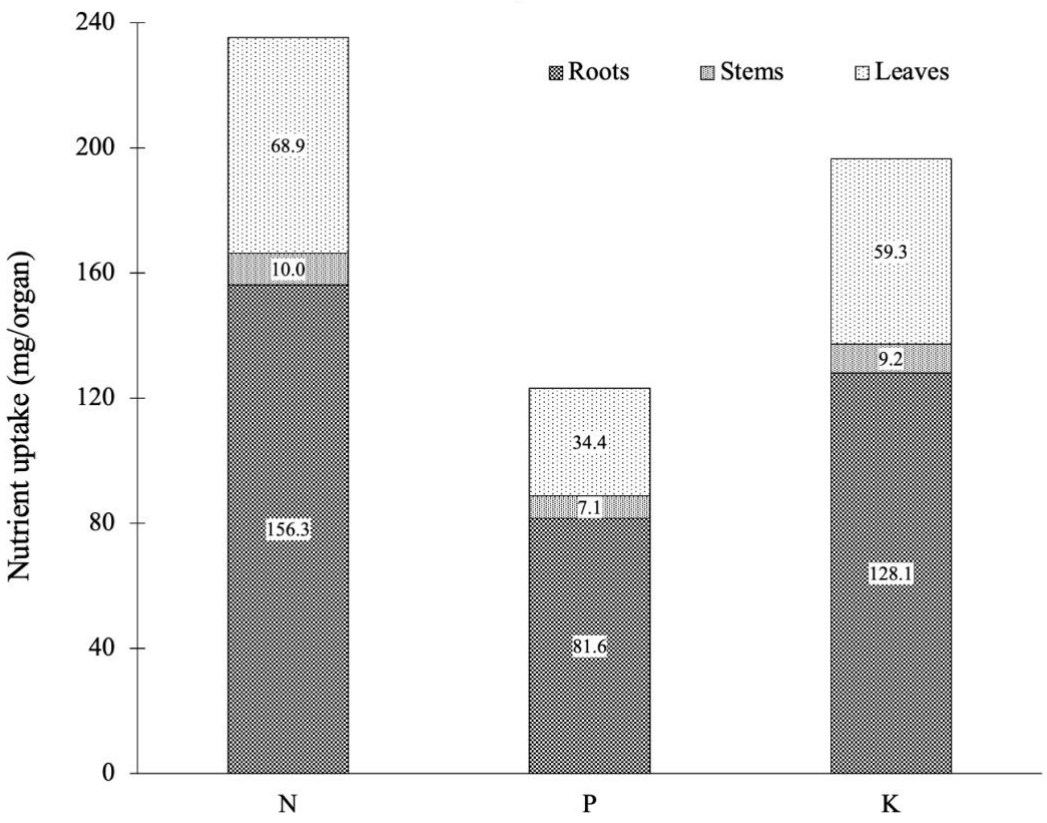


Figure 5.3. Average uptake of nitrogen, phosphorus and potassium in the leaves, stems and roots in the blue honeysuckle cultivar 'Tundra' grown under 16 different fertilization treatments, expressed as percentages of total amounts in plants.

5.4 Conclusion

From this study I accept my hypothesis that fertilization with N, P and K will increase the weight of blue honeysuckle nursery plant grown in Sunshine potting mix #4 with the reported available nutrient levels present in the potting mix. Further, higher rates may increase the growth as well. Among the nutrients, the trend was for the greatest response in yield and plant part nutrient concentration to result from N fertilization. Proportions of N and K were relatively higher in leaves. This study did not investigate carry-over effect of fertilization on growth in subsequent years, and future impacts especially on fruit production would be worth while investigating. This research investigated growth and uptake at the time when shoots had stopped growing, but with leaves having the largest proportion of NPK. Another interesting area for the future study might be to compare NPK distribution at the time of dormancy in plant organs. Chapter 3 showed that growth

of shoots slows after 5 weeks of active growth. However, for the rest of the growing season haskap may allocate and translocate nutrients from leaves to store in roots and stems before going into dormancy. This leads to the future research question that perhaps one of the strategies of the relatively large root system is to sequester carbohydrates and nutrients in the root system in fall to enable rapid growth in the spring and fruit development in early summer. An average root weight in this study accounted for quite a high proportion 43% of total plant weight (Figure 5.1) and as such, storage of nutrient in the roots is significant, but may vary by age in the future. In a meta-analysis Cairns et al. (1997) mean percentage of tree root system of the boreal forest accounted about 27% but differed by soil texture and tree type.

Link to the next chapter

This chapter covers research work involving growth of very young *L.c.* plant in a greenhouse setting. The final research chapter investigates production of berries of the *L.c.* in a greenhouse. In particular, greenhouse production was studied in winter and spring as these could be possible times that growers might want to extend the growing season. Fresh berries in winter or early spring would an excellent time to supply nutrient rich berries to the market when demand is high, especially in winter months of Saskatchewan.

6.0 PRODUCING BLUE HONEYSUCKLE BERRIES IN GREENHOUSES IN WINTER AND SPRING, and THEIR BIOCHEMICAL PARAMETERS

6.1 Introduction

It is important to provide access to food for all four seasons of the year. When growing crops such as vegetables and fruits for commercial production, high yields can be achieved in environments with controlled microclimate factors such as air and soil temperature, light conditions, carbon dioxide and evaporation (Rouphael et al., 2018). A greenhouse can provide optimum conditions for temperature, lighting, ventilation, and irrigation. In cold climates such as Canada, greenhouse growers often choose to either close down during winter or grow plants that can be successful at colder temperatures. Raspberries have been grown successfully in winter greenhouse (Pritts et al., 1999, Dale et al., 2003). It seems reasonable to expect that blue honeysuckle (*L.c.*) could do well in winter greenhouses since it is fast growing during cool temperatures in spring and is quick to ripen its fruit. The cultivation of Haskap under greenhouse conditions can be fully controlled and plants can be protected from external influences. *L.c.* cultivars are obligate outcrossers and only a few cultivars are compatible with their own pollen, but even so they rely on insects to carry pollen for pollination. No more than 3.1 percent of the total flowers of the *L.c.* (Popova 2000) were self-compatible. *L.c.* plants are characterized by cross-pollination, and pollination by bees dramatically increases fruit yield (Kolbasina et al., 1984, Kuminov 1994). The ripening of the blue honeysuckle fruit differs by genotype and study in Saskatchewan fruit matured in 42-49 days after flowering depending on heat requirement (Dawson 2017). The best time to harvest is 5 to 7 days after full ripening. During this time, the weight of the fruit increases, the aroma and taste are intensified, the flesh becomes softer, and the sugar and vitamin content of the fruit increases. It is also noted that if the harvesting period is prolonged, the flesh becomes too soft and sticky (Plekhanova 1990).

In a small-scale preliminary experiment where haskap plants were brought into the greenhouse and hand pollinated. Greenhouse temperatures were about 20-22°C. In that experiment

there was practically no fruit set. In this experiment, lower temperatures were applied to better simulate daily highs during pollination season. Pollen used in the preliminary test was not tested for viability, but the storage method of the pollen has been successful for the fruit program.

The main purpose of this study was to determine the possibility of obtaining berries from *L.c.* in winter. My hypothesis is that fresh berries of the *L.c.* of a marketable yield can be grown in winter greenhouses by mimicking field conditions

6.2 Materials and methods

An experiment to determine whether the *L.c.* berries growth can be achieved in the winter heated greenhouse conditions conducted at the University of Saskatchewan Agriculture Greenhouse (45 Innovation Blvd, Saskatoon, SK S7N 2T8). The experiment consisted of two genotypes cv. Tundra (clones) and Japanese seedlings (grown from seed), two greenhouses (A3 and G), and two seasons (winter and spring). Total of 36 (18 Tundra + 18 Japanese) plants were used per season.

Fully grown, 7 to 8-year-old plants of the cv. Tundra clones and Japanese seedlings were dug from the field at the USASK Horticulture Research Field (2909 14th Street, Saskatoon). 'Tundra' was the bred at U of SK and was recommended for commercial production at the time. The Japanese seedlings originated from seeds Dr. Bors had collected when visiting Hokkaido, Japan. Digging was done on 26 October 2014 when plants had gone dormant. The main part of the root system was sprayed with water to remove soil and transplanted into a Econo Grip 49.93L container (dimension of 43.82×43.82×38.42 cm). Sunshine potting mix #4 was used. Plants were watered and stored in cold storage at an average of -18°C.

The plants were transferred to a winter greenhouse hallway to thaw. To achieve simultaneous flowering, plants of Japanese genotype were brought in the greenhouse 10 days earlier than the variety Tundra because it was known that Tundra blooms earlier. The experiment was carried out in two seasons: winter (mid-January to mid-March 2015) and Spring (mid-March to mid-May 2015) with each cycle lasting about sixty days. At the beginning of the bud breaking plants were transferred from the greenhouse hallway to two greenhouses for the duration of the experiment. Distribution was completely randomized block design. The zone A3 greenhouse was made of glass while the zone G greenhouse is made of plastic. Climatic data outside of the

greenhouse were obtained from the automatic weather station. Greenhouse internal environmental parameters were collected internal data loggers (Table 6.1). High pressure sodium 400-watt light were used and set for 16 hours day radiation.

Pollination was performed using a standard sized "Biobest" hive and colony of the bumblebee species *Bombus terrestris* (Biobest Canada., Ltd). The hives were supplied with BIOGLUC® a ready-to-use sugar solution. Plants were watered once in a day in the mornings.

Fruit harvesting for the total yield per plant was taken when 90 percent of the blue honeysuckle fruit reached harvest maturity which was about 45 days after peak flowering, assuming that maturation timing is similar in the GH compared to the field. Total weights were measured for each plant. Average fruit weights were based on 50 randomly selected berries per plant that were individually weighed.

Table 6.1. Air temperature, integrated photosynthetically active radiation (PAR) and relative humidity in the University of Saskatchewan Agriculture Greenhouse zone A3 and zone G during experimental period, January to May, 2015.

Location in the greenhouse	Air temperature (ºC)		Integrated PAR (μmole.m^2/s)		Relative humidity (%)	
	Zone A3	Zone G	Zone A3	Zone G	Zone A3	Zone G
			January			
Minimum	9.2	13.7	138.5	8.7	2.8	27.8
Maximum	24.4	26.4	2895.1	369.3	62.1	89.6
Mean	17.0	21.8	455.1	78.6	25.2	45.2
			February			
Minimum	9.0	16.1	138.5	10.5	5.2	29.7
Maximum	27.6	30.5	3061.0	557.5	59.1	72.9
Mean	14.3	22.1	531.5	112.0	26.8	46.3
			March			
Minimum	9.0	16.4	146.2	11.9	7.4	29.1
Maximum	31.3	34.8	3494.6	934.1	75.1	90.7
Mean	15.6	23.0	644.4	198.8	41.5	47.5
			April			
Minimum	9.3	15.9	164.1	13.7	1.2	13.4
Maximum	38.4	38.9	4331.0	1099.5	65.8	98.1
Mean	18.1	23.2	754.5	289.3	33.7	39.4
			May			
Minimum	9.7	15.1	165.0	18.7	1.1	14.7
Maximum	36.5	39.7	4412.8	1413.0	78.7	96.7
Mean	19.7	24.7	822.1	312.1	39.0	42.0

Titratable acidity measurements were done on each five berries from each experimental plant using a HI 84432 Fruit Juice Titratable Acidity Meter by Hanna Instruments (Laval Quebec Canada) which measured acidity as % citric acid equivalents. Determination of total sugar in the fruit was conducted using a digital brix meter by Atago Co. Ltd (Tokyo Japan) for each of five berries from each plant. Averages of the five berries were used as an independent sample in statistical analysis.

Total leaf weight of Japanese genotype plants was not determined as plants were needed for breeding. However, all the leaves of Tundra plants were removed weighed for the fresh weight and dried in paper bags using dryer oven (Culatti Ag, Zurich, Switzerland) at +50°C until constant weight.

The SAS (Statistical Analysis System, Version 9.4 for Windows; SAS Institute, Cary, NC) software was used for data processing. All data were subjected to analysis of variance (ANOVA) using PROC MIXED in SAS software by taking greenhouse locations as a random effect. A probability level of $p < 0.05$ was chosen to establish statistical significance. Differences between treatment means were determined using Tukey's multiple range test and considered significant at $p \leq 0.05$.

6.3 Results and discussion

6.3.1 Total fruit yield per plant and average weight of the blue honeysuckle fruit

The results of the experiment showed (Table 6.2) that winter harvest averaged 164.67 g/plant more than spring harvest, however not significantly different (p=0.182) than spring harvest. However, there were significant differences (p=0.0003) in yields of the two genotypes. Total yields of 'Tundra' plants were about 2.25 times greater than Japanese plants. Ochmian (2010) reported that in a field trial the total yield per bush in 'Wojtek' cultivar was 940g on average, 'Brazowa' cultivar was 895g. Bors (2008b) reported 0.5-0.75kg per bush for 3-4-year-old L.c. seedlings. The yield of older plants was 2-5 kg per bush (Lefol 2007). But the above cited yields were from plants in the field and not like this experiment where recently uprooted plants were used. There were no greenhouse studies on Haskap production to compare with this research.

There were no statistically significant differences in average fruit weights in terms of time, locations, and genotypes of blue honeysuckle, ranging about 0.90 to 0.94 g (Table 6.2).

According to a study Bors et al. (2009) conducted on Haskap breeding the cultivar 'Tundra' weighted an average 1.49 g per berries, while single berry weight of other Russian genotypes was between 0.7 to 0.9 g. However, in this study average weight of the single berries was 0.59-0.55 g lower compared to the study by Bors et al. on cultivar 'Tundra' (2009). Fruit sizes in this experiment were somewhat similar to sizes in studies where cultivars were grown outside that ranged from 0.71 to 1.32g/berry (Firsova 2002, Ochmian 2010). The fruit weight and size depend mainly on fruit load, agronomic conditions, and fruit maturity stage (Ochmian 2012) which affects fruits' quality and visual attractiveness.

Table 6.2. Two different blue honeysuckle (*Lonicera caerulea*) genotypes total fresh fruit yield per plant and average fruit fresh weight (g) grown during Winter and Spring, 2015 at University of Saskatchewan Agriculture Greenhouses.

	Total fresh fruit yield (g/per plant)		Average of single fresh fruit weight (g)	
	Mean	SD	Mean	SD
Season				
Winter	450.50 a	262.75	0.94 a	0.21
Spring	285.83 a	166.19	0.90 a	0.07
SEM	6.99		0.19	
Genotype				
Lonicera caerulea cv. Tundra	472.38 a	201.00	0.90 a	0.09
Lonicera caerulea (Japanese origin)	263.95 b	231.20	0.94 a	0.21
SEM	6.99		0.19	
F value, *p*-value				
Season	*F* 4.14, *p*=0.182		*F* 0.34, *p*=0.565	
Genotype	*F* 16.75, *p*=0.0003		*F* 0.14, *p*=0.709	
Season x Genotype	*F* 0.00, *p*=0.974		*F* 1.27, *p*=0.267	

* Means followed by the same letter within a column are not significantly different at α=0.05. SD-Standard deviation. Difference between treatment means were determined using Tukey's multiple range test and considered significant at p≤0.05

6.3.2 Soluble solids content and titratable acidity of the blue honeysuckle fruit

There were significant differences (Table 6.3) in soluble solids content between different genotypes (p=0.029). However, the results of the mean soluble solids content grown in two different seasons did not show significant difference (p=0.537). The soluble solids content of

Lonicera caerulea from Japan was slightly higher at 13.41 ±1.15 ºBrix, which was 7.28% higher than *Lonicera caerulea* cv. 'Tundra' (Table 6.3). Accumulation of sugars is the product of photosynthesis which was mainly dependent on light and to a lesser extent, temperature (Yamaki 2010). Fruits exposed to the sun had a higher sugar content compared with those grown in shade. Moreover, the fruits with the highest quantity of sugars were harvested in the late season in the same genotype (Zorenc et al., 2016). The results of studies in Russia showed sugar content of L.c. varies depended on the variety and the soil and climate of the area. According to Firsova (2002), the sugar content of L.c. ranged from 4.18 to 10.9%, while according to Zimina (2002), the sugar content ranged from 8.1 to 8.8%.

Table 6.3. Two different blue honeysuckle (*Lonicera caerulea*) genotype soluble solids content (ºBrix) and titratable acidity (%) of the fruit grown during Winter and Spring, 2015 at University of Saskatchewan Agriculture Greenhouses

	Soluble solids content (ºBrix)		Titratable acidity (%)	
	Mean	SD	Mean	SD
Season				
Winter	13.18 a*	1.52	2.24 a	0.76
Spring	12.74 a	0.98	2.29 a	0.60
SEM	0.47		0.31	
Genotype				
Lonicera caerulea cv. Tundra	12.5 b	1.29	1.76b	0.18
Lonicera caerulea (Japanese origin)	13.41 a	1.15	2.77a	0.61
SEM	0.47		0.31	
F value, *p*-value				
Season	*F* 0.54, *p*=0.537		*F* 0.25, *p*=0.667	
Genotype	*F* 5.25, *p*=0.029		*F* 52.12, *p*<0.0001	
Season x Genotype	*F* 1.29, *p*=0.265		*F* 0.01, *p*=0.995	

* Means followed by the same letter within a column are not significantly different at α=0.05. SD-Standard deviation. Difference between treatment means were determined using Tukey's multiple range test and considered significant at p≤0.05

The results did not show significant differences (Table 6.3) for titratable acidity grown in different seasons (p=0.667). However, there were highly significant differences in titratable acidity between Tundra and Japanese seedlings (p<0.0001). The titratable acidity of *Lonicera caerulea* (Japan) was 57.39% higher compared to the *Lonicera caerulea* cv. 'Tundra' (1.76). A similar study in the field (Bors et al 2015) reported, that cv. 'Tundra' fruit weight was 1.2, 1.4 g and 1.5 g/berry; soluble solids content was 13,13 and 17 ºBrix) and titratable acidity was 1.6, 1.6 and 2.25%)

respectively for 3 years. Senica et al. (2018) reported the total organic acid was 6.55- 8.85 mg g-1 and had a significant effect on location dependencies. According to Tanaka (1998), the average weight was 0.9g, which was very similar results to this study.

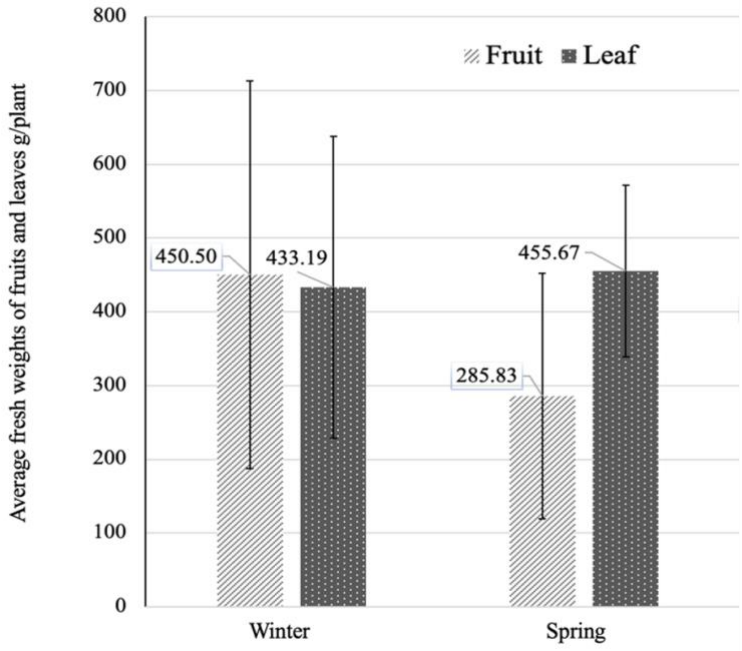


Figure 6.1. Average fresh weights of fruits and leaves of 'Tundra' blue honeysuckle (*Lonicera caerulea*) grown for two seasons at University of Saskatchewan Agriculture Greenhouses

There are several factors that can influence acidity content in the fruit such as genotype, location and environmental condition. One of the strategies of the University of Saskatchewan fruit program is breeding for low acidity. Environmental conditions and different ripening stages may affect the organic acid content (Jurikova et al., 2012, Zorenc 2016). A positive correlation of organic acids with precipitation was observed by Senica et al. (2018). During the fruit ripening period, the number of sugars and anthocyanins increased while the number of organic acids decreased (Jurikova et al., 2009). Mikulic-Petkovsek et al. (2015) suggested fruit sugar was positively correlated and acid levels were negatively correlated affected by light intensity of the location. As a result, photosynthetic activity increased and primary metabolites accumulated.

In this study, ‘Tundra’ had a sugar/acid ratio of 7.10 while Japanese plants were 4.84. Compared to the trials conducted in Slovenia under the same environmental conditions with 17 cultivated and 8 wild berry species, cultivar ‘Tundra’s sugar/acid ratio was higher than 19 of the total 25 fruits species listed (Mikulic-Petkovsel et al., 2015). This seems to indicate that the fruit flavour of greenhouse produced ‘Tundra’ was similar to flavours of field grown haskap.

There was no statistically significant difference (Figure 6.1) between fresh weight of cultivar ‘Tundra’ leaves grown Winter and Spring season ($p=0.514$). This experiment showed winter fruit yields were non-significant than spring fruit yield. Fruit-leaf ratio was almost 1:1 in winter but 1:1.06 in spring.

7.4 Conclusion

The presented work in this chapter showed blue honeysuckle *L.c.* could be produced in winter and spring greenhouse. With this study I accept my hypothesis that the fresh berries of the *L.c.* can be grown in the winter greenhouses and harvested during the high demand season. However, economic feasibility in terms of the yield per plant and fruit price per unit of weight needs to be considered. By controlling environmental factors such as air temperature, PAR and possibly relative humidity it may be possible to influence total soluble solids content and acidity which may directly relate to taste of the marketable fruit.

Compared to other studies involving ‘Tundra’ fruit size was a bit smaller than field grown berries but sugars and acidity levels were very similar. This indicates that it is likely that greenhouse grown fruit would be acceptable to consumers. Lower temperatures and bumbles bees as pollinators were factors contributing to the success. However, the economic viability needs to be determined. Heating of the winter greenhouse is costly and hard to keep greenhouse warm enough but in spring there may be days that need cooling. In the future experiment even lower temperatures might be worthwhile to investigate.

7.0 GENERAL DISCUSSION AND FUTURE RESEARCH

The genus *Lonicera* primary center of origin is considered to be Southeast Asia where the climate is strongly influenced by the monsoons with a substantial amount of rainfall. Later it may have spread to the Himalayas of the northern hemisphere, to the mountainous systems of Central Asia with a continental arid and semi-arid climate and a great range of hot and cool temperature fluctuations later moving to the temperate regions of Europe and North America (Plekhanova 1990, Skvortsov and Kuklina 2002). In the past, explorers and researchers found *L.c.* mostly in boreal regions and it was common in the areas where forests transfer to high mountainous areas, high elevations, mountain peaks, near lakes, small islands, on sandy beaches, river basins, along rivers and wetlands, waterlogged wet areas and usually partly shaded areas (Michurin 1935, Bochkarnikova 1979, Hara 1983, Skvortsov 1986, Plekhanova 1994, 2000, Smirnov 2002, Sheiko 2008, Bors 2009, Kang et al., 2018, Jamyandorj and Tsendeekhuu 2020).

Numerous factors may have contributed to the succession and dispersal of the *L.c.* throughout the Boreal Forest. Producing the first fruit to mature compared to the other fruits contributes to the seed dispersal possibly by birds and other animals given the high demand and competition for food early in the growing season.

The soil surface landscape of the Boreal Forest is characterized by various slopes and depressions which is one of the main influences on micro-territorial soil formation, diversification, and local hydro-thermal regimes. In the sites of this study, one site has organic peat soil up to 80 cm in depth and the other two sites had forest Luvisol soil with thick clay in a lower horizon. Although soils at the study sites differed by soil properties and nutrient supply and microclimatic factors, the similarity was that the soil at the sites was frozen until late summer, with slow thawing in the edge of the forest, in the forest gap, around small ponds, wetlands and fens with higher moisture regimes. In spring the upper soil horizon warmed up due to being exposed or partially exposed to solar radiation. It is worthwhile to note that the roots of *L.c.* at the natural habitat were shallowly positioned in the upper soil horizon and laterally oriented, helping start the supply of nutrients and water to the above-ground vegetation as early as possible. Sites similar to ones in this

study may play an important role in a successful habitat of *L.c*. by creating an advantage for the *L.c* by slowing or eliminating some early growth species that have deeper roots or tap roots and could not tolerate higher moisture and shade.

In general, adaptation of *L.c* may have developed to avoiding major competition by growing in the least competitive places smartly positioning itself in a time and space of the environmental matrix. Thus, we may generalize that the location is vital for the successful growth and dispersal of the *L.c.* plant in the Saskatchewan Forest. In other words, an adaptable plant simply grows with a strategy of growing at the right time in the right place. Since Boreal Forest biota changes by time, fire, insects, land use and other factors, some locations in the future will most likely disappear while others will be created, and this also may accelerate due to climate change.

A better understanding of plant-environmental interactions is not only important for better management and preservation of natural resources, but it is also important for helping to establish a functioning, sustainable Haskap industry and enhancing the productivity of current production requirements.

In this autecological study of wild blue honeysuckle (*Lonicera caerulea* ssp. *villosa*), important environmental factors at the study locations were the soil temperature and soil moisture assuming that there were sufficient levels of soil nutrients, water, temperature, etc. to limit shoot growth. The growth of shoots varied significantly in different environments and appeared to be influenced by the microenvironment, climate and soil parameters and their interactions and locations.

The effect of an important soil property, soil pH, on honeysuckle growth and nutrition was clarified in this research. The pH effects on blue honeysuckle (*Lonicera caerulea*) growth were one of the research questions as different subspecies of the blue honeysuckle grow globally in a wide range of soils with different pH. This research found that pH has a significant effect on total dry weight and nutrient uptake of the blue honeysuckle. A considerable area of Saskatchewan soils has a pH greater than pH 7.5, around 10.5 million hectares (Rostad et al., 1987) including the University of Saskatchewan Fruit program field which has a pH of 7.8. It is not easy to change the soil pH on a large scale because of soil buffering capacity. However, certain fertilizers, especially sulphuric fertilizers can temporarily influence the lowering of pH. Organic fertilizers depending on composition of the minerals and peat are good sources for lowering pH and act as a nutrient in the longer term, which could be a good strategy in Haskap organic farming and sustainability-wise.

The investigation into different genotypes did not reveal any subspecies that are better adapted to high pH. Without any clear genetic answers to the problem of less growth, it might be better to focus on improving cultural practices. To establish a successful commercial Haskap orchard, planning and assessment of potential orchard sites need to consider soil pH. The pH of Saskatchewan Soils map shows that lower pH soil is mostly located in West-central and North-west Saskatchewan (Rostad et al., 1987). Therefore, those areas may be most suitable for commercial organic Haskap orchards.

This research has shown that the growth of shoots slows after 5 weeks of active growth. This may lead to a recommendation that NPK fertilization is critical in the field in late fall or early spring. In terms of soil nutritional aspects of blue honeysuckle, it may be beneficial to use the application of compound fertilizers at least containing nitrogen, phosphorus and potassium (NPK) together rather than applying individual elemental fertilizers. In this study, the greatest response in honeysuckle yield was observed for nitrogen, although this may differ depending on the available nutrient levels in different media used for potting mixes. Leaves contain about 40% of the N, P, K of the plant, followed by roots and stems. There is a need to determine how much nutrient is in the fruit as they were removed in harvest and need to be replaced to maintain long-term soil fertility.

BEST PRACTICES

In the process of this study, a few "Best Practices" were noted that might be useful for Haskap growers and may help save time and effort if carefully applied in the Haskap industry.

- For the development of a new orchard, choosing a suitable location with a slightly acidic pH is important for successful production in the future. Carefully selected plots will save the growers some fertilizer costs at a minimum and if production is planned as organic farming, the benefits will be far greater.
- Controlling the pH of growing media in a range of '6' may help to produce a vigorously healthy plant with a strong root system. It may be easier to apply this, especially in nursery production than in commercial field orchards. However, applying organic fertilizers and manure may also help to reduce the pH of the more alkaline soils, at least in the root zone. But still, soils need to be tested and rationally applied to prevent exceeding the needs of the nutrients, especially phosphorus.

- The greenhouse production for the winter market needs good planning ahead to prepare plants in the previous season considering choosing a more upright genotype in terms of space usage but needs careful cost and benefit analysis.
- It may be better to start growing Haskap in a container from the beginning for the greenhouse fruit production and air prune the roots, to reduce transplant stress, which will also prevent water logging depending on the bench or floor condition.
- Poly-greenhouse will do as well as glass greenhouse for the Haskap fruit production with appropriate management. It is important to note that one will need at least two different genotypes of Haskap to successfully pollinate flowers. It is equally important to synchronize the flowering of different genotypes by testing in advance in similar conditions for future growth. Pollination by bees is essential.
- By controlling environmental factors such as air temperature, photosynthetically active radiation, and possibly relative humidity, it may be possible to influence total soluble solids content and acidity which may directly relate to the taste of the marketable fruit.
- Balancing light, air circulation and air temperature and the relative humidity of the greenhouse are important to prevent diseases such as powdery mildew.
- Since wild plants survived in partly shaded forests, temporary shading might be an option for regulating temperatures or reducing sunburn of young plants.

FUTURE RESEARCH

Blue honeysuckle leaves have thick cuticles with trichomes on the upper surface and stomata only on the lower surface (preliminary study with SUMP disk, courtesy of Dr. K. Tanino) which may be classified as a plant with a hypostomatous leaf. These characters may need to be explored and further elaboration will be needed on their functions in terms of abiotic stress tolerance and avoidance.

The blue honeysuckle's root system and function is a lesser-known area of study. Since the wild habitat of the *Lonicera caerulea* ssp. *villosa* is a boreal forest and soil-root interaction mostly functions in a fungi-dominated soil environment, this may be an important area for further research investigation. Especially, phosphorus uptake by the blue honeysuckle and possible symbioses with

arbuscular or ectomycorrhizal fungi. If there is a symbiosis, this may lead to an important economic development for the reduction of phosphorus fertilizer usage and impact on organic farming of blue honeysuckle in the future. Another unknown area is the relationship between dissolved organic matter and wild blue honeysuckle in the natural habitat.

Future fertilization studies with macro and micronutrients are vital. It is important to compare different potting mixes and document their properties including available nutrient levels. Regarding the plant materials for experiments, using plants started from seeds rather than softwood cuttings may be worthwhile to investigate. While seedlings would be genetically different (a disadvantage), they don't have to undergo the rooting process and could show a faster response to different fertilizers during their 1st season of growth. Also, there would be no or extremely minimal carry over of nutrients from previous management of mother plants. To better inform Saskatchewan nursery growers on fertilization, extensive research is needed for periods longer than one growing season and with more samples.

Since the pH dependency experiment on the growth of *L.c.* was conducted hydroponically while keeping other factors constant, the actual field conditions would be different, and this experiment should be validated in the natural environmental condition. Studies have not been done on the different pH effects on blue honeysuckle fruit biochemical composition and other qualitative characteristics.

Research in this thesis indicated that fresh berries of blue honeysuckle could be grown in a winter greenhouse with pollinators. However, the economic viability needs to be determined. Genotype selection can be an important factor for high yield. It is important to note that in the future, breeding for upright-oriented varieties may be more suitable for greenhouse production as broad-architecture plants, like 'Tundra' take greater space and reduce light in the lower part of the plant. In addition, to maximize the use of greenhouse space, plants in square or rectangular containers may be preferable. In this experiment, the fruit yield per plant, weight of the fruit, ratio of leaves and fruits of the blue honeysuckle did not show dependence on the location of the greenhouse. It is quite interesting that winter production was much higher than spring production in the greenhouse. This might indicate that optimum temperature levels are worthwhile investigating and that perhaps lower temperature may cause longer flowering time and more fruit set.

In terms of the capital cost of the greenhouse, producers may equally obtain similar results in the poly greenhouses compared to the glass greenhouse. It is worthwhile to mention that out of necessity, plants that were used in the experiment were dug out of the field in the previous season. If those plants had been grown for a few years in containers, they would have less stress and could produce a higher yield than this study. The greenhouse growing method can be a good research method for the future investigation of this interesting plant and the breeding time of new cultivars could be potentially shortened.

8.0 CONCLUSION

The wild habitat of the blue honeysuckle is a complex interrelated domain at different locations in Saskatchewan. Growth of the blue honeysuckle (*Lonicera caerulea* ssp. *villosa*) in the wild may mainly be associated by environmental, soil and leaf properties and other unmeasured factors during the growing season. Different levels of pH showed a considerable effect on the growth of the blue honeysuckle and some genotypes responded differently than others. The commercial cultivar 'Tundra' showed higher growth than wild genotypes except for *Lonicera caerulea* ssp. *stenantha* which showed equally higher growth in an optimal pH in hydroponic conditions. A study of NPK on blue honeysuckle seedling growth showed the application may more effective together (NPK) rather than applying individual elemental fertilizers. Under the conditions of the potting media used, positive response was mainly associated with the nitrogen component of the fertilization treatment. For the fertilization of seedlings in nursery production, it is important to test growing media to better clarify the amount of nutrients needed. As one of the first studies of greenhouse production for fresh haskap, this study provided understanding that should be useful in developing production protocols that could lead to better and more harvest in the future, especially on-demand season for fresh and nutritious fruits.

The blue honeysuckle (*Lonicera caerulea*) is an important fruit-producing perennial plant that has high adaptability and with promising future in Canada. Focusing and supporting more honeysuckle production in areas for example, with optimal soil pH on a provincial and national level may reduce mineral fertilizer usage and contribute to Environment Canada's climate plan which set a target to reduce emissions from fertilizers by 30% below current levels. Moreover, climate change may become one of the major challenges for maintaining the sustainable production of Haskap. To adapt to the changing environment, understanding the plant-environmental interaction, and application of findings and solutions at the production level is a key to resilience and increasing productivity.

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10.0 APPENDIX

Table 10.1. Air temperature (°C) of research plots at three sites with wild blue honeysuckle (*Lonicera caerulea* ssp. *villosa*) in the forest near Prince Albert National Park, within the Boreal Plains ecozone. The sites were located approximately 300 km north from Saskatoon, Saskatchewan, Canada information was collected during the growing season which was from May to July of 2014 and 2015.

Year		2014					
Month		May		June		July	
Time		Day	Night	Day	Night	Day	Night
	Min	1.4	2.3	-2.7	-1.7	2.3	2.7
SPF site	Max	24.0	17.5	29.1	17.9	32.8	20.2
	Mean	15.5	9.3	15.3	8.1	19.4	11.3
	Min	5.3	2.3	-1.7	-3.1	2.7	0.6
BLF site	Max	26.0	13.3	33.6	15.6	34.9	20.2
	Mean	16.9	7.9	16.4	7.7	20.8	10.0
	Min	4.5	4.0	-0.8	-1.7	5.7	4.5
BLS site	Max	26.0	16.0	30.4	20.2	30.8	26.0
	Mean	16.8	9.3	15.2	9.9	20.4	12.7
Year		2015					
Month		May		June		July	
Day/Night		Day	Night	Day	Night	Day	Night
	Min	-6.1	-6.1	-3.1	-3.6	-1.3	-1.3
SPF site	Max	28.7	16.4	31.2	25.2	34.1	26.0
	Mean	15.6	4.1	17.8	9.2	19.3	12.4
	Min	-8.2	-7.6	-4.1	-4.6	-2.2	-2.2
BLF site	Max	30.8	16.8	34.1	23.7	34.1	21.0
	Mean	17.0	2.7	18.6	7.9	19.9	11.7
	Min	-4.6	1.4	-1.7	6.5	1.0	1.0
BLS site	Max	29.1	30.4	35.3	36.2	30.4	30.4
	Mean	10.3	17.2	13.5	18.5	18.8	14.7

SPF: Shady, Peat & Flat, BLF: Bright, Luvisol & Flat, BLS: Bright, Luvisol & Sloped

Table 10.2. Relative humidity (%) of research plots at three sites with wild *Lonicera caerulea* spp. *villosa* in the forest near Prince Albert National Park, within the Boreal Plains ecozone, located approximately 300 km north from Saskatoon, Saskatchewan, Canada, May to July of 2014 and 2015

Year		2014					
Month		May		June		July	
Time		Day	Night	Day	Night	Day	Night
	Min	20.7	32.0	20.7	43.2	23.5	63.3
SPF site	Max	98.7	100.0	100.0	100.0	100.0	99.5
	Mean	58.6	75.2	68.8	90.6	63.5	91.3
	Min	20.7	46.0	20.7	51.7	20.7	59.4
BLF site	Max	100.0	100.0	100.0	100.0	100.0	100.0
	Mean	55.5	79.3	65.8	92.9	57.2	94.2
	Min	20.7	41.4	20.7	47.5	25.0	39.6
BLS site	Max	100.0	100.0	100.0	100.0	100.0	100.0
	Mean	57.8	76.2	71.0	89.9	60.6	89.6
Year		2015					
Month		May		June		July	
Day/Night		Day	Night	Day	Night	Day	Night
	Min	20.7	29.2	20.7	25.0	20.7	35.0
SPF site	Max	99.5	99.5	100.0	100.0	100.0	100.0
	Mean	40.7	69.6	52.4	83.3	65.9	92.7
	Min	20.7	32.5	20.7	33.0	20.7	41.8
BLF site	Max	100.0	98.7	100.0	100.0	100.0	100.0
	Mean	38.7	70.4	50.1	83.5	63.4	92.1
	Min	20.7	20.7	20.7	20.7	20.7	26.4
BLS site	Max	100.0	72.7	100.0	98.7	100.0	100.0
	Mean	52.4	31.7	71.2	49.4	69.8	86.2

Table 10.3. Average dew point (°C) of research plots at three sites with wild blue honeysuckle (*Lonicera caerulea ssp. villosa*) in the forest near Prince Albert National Park, within the Boreal Plains ecozone, located approximately 300 km north from Saskatoon, Saskatchewan, Canada, May to July of 2014 and 2015.

Year		2014			2015		
Month		May	June	July	May	June	July
	Minimum	0.48	-3.3	2.2	-11.9	-5.3	-1.3
SPF site	Maximum	15.6	17.0	16.5	12.8	15.7	21.1
	Mean	6.2	8.2	11.3	1.8	7.4	11.4
	Minimum	0.5	-3.2	0.5	-13.5	-6.9	-2.8
BLF site	Maximum	14.8	18.5	20.2	14.9	18.2	21.3
	Mean	6.4	8.4	11.0	2.0	7.3	11.7
	Minimum	2.2	-2.3	3.3	-11.1	-4.4	-0.2
BLS site	Maximum	14.4	19.0	19.4	14.5	20.3	20.7
	Mean	6.8	8.9	11.8	1.8	8.0	12.4

Table 10.4. Soil temperature (°C) at three sites with wild blue honeysuckle (*Lonicera caerulea* ssp. *villosa*) in the forest near Prince Albert National Park, within the Boreal Plains ecozone, located approximately 300 km north from Saskatoon, Saskatchewan, Canada, May to July of 2014 and 2015.

Year		2014					
Month		May		June		July	
Time		Day	Night	Day	Night	Day	Night
	Minimum	2.7	3.2	0.6	-1.3	1.4	9.4
SPF site	Maximum	6.9	6.9	14.4	13.3	17.5	16.8
	Mean	4.0	4.8	8.0	7.8	12.7	12.9
	Minimum	4.0	4.9	3.2	3.2	-2.2	7.7
BLF site	Maximum	12.5	9.0	19.4	14.1	20.2	14.1
	Mean	7.8	6.0	10.2	8.2	11.0	10.4
	Minimum	4.0	4.0	3.2	3.2	12.1	12.9
BLS site	Maximum	9.0	8.6	23.3	17.1	17.9	17.9
	Mean	5.6	5.5	8.7	8.9	14.9	15.4
Year		2015					
Month		May		June		July	
Time		Day	Night	Day	Night	Day	Night
	Minimum	-0.3	-0.3	0.1	0.1	0.1	0.1
SPF site	Maximum	23.3	10.9	28.7	17.9	34.1	21.3
	Mean	7.8	3.1	13.0	8.0	17.0	11.7
	Minimum	2.3	2.7	4.0	4.9	6.5	7.3
BLF site	Maximum	10.1	9.0	12.9	11.7	15.2	14.8
	Mean	6.7	6.2	9.2	8.8	12.0	11.8
	Minimum	1.4	5.3	3.2	6.5	7.7	9.4
BLS site	Maximum	11.7	14.4	16.8	19.0	20.2	18.3
	Mean	5.4	10.1	8.6	11.6	13.7	14.4

SPF: Shady, Peat & Flat, BLF: Bright, Luvisol & Flat, BLS: Bright, Luvisol & Sloped

Table 10.5. Total rainfall (mm) at three sites with wild blue honeysuckle (*Lonicera caerulea* ssp. *villosa*) in the forest near Prince Albert National Park, within the Boreal Plains ecozone, located approximately 300 km north from Saskatoon, Saskatchewan, Canada, May to July of 2014 and 2015.

Year	2014			2015		
Month	May	June	July	May	June	July
SPF	2.5	79.8	26.7	5.9	42.1	106.0
BLF	2.7	132.6	62.9	9.3	41.6	143.2
BLS	0.9	37.8	57.7	5.6	41.2	17.9

SPF: Shady, Peat & Flat, BLF: Bright, Luvisol & Flat, BLS: Bright, Luvisol & Sloped

Table 10.6. Soil moisture (kPa) of research plots at three sites with wild blue honeysuckle (*Lonicera caerulea* ssp. *villosa*) in the forest near Prince Albert National Park, within the Boreal Plains ecozone, located approximately 300 km north from Saskatoon, Saskatchewan, Canada, May to July of 2015.

Month		May		June		July	
Time		Day	Night	Day	Night	Day	Night
	Min	111.0	113.0	69.0	75.0	78.0	74.0
SPF	Max	0.0	3.0	0.0	13.0	12.0	12.0
	Mean	28.2	28.9	21.5	22.7	22.5	23.4
	Min	15.0	15.0	17.0	17.0	20.0	20.0
BLF	Max	12.0	13.0	12.0	12.0	11.0	12.0
	Mean	13.1	13.9	12.9	13.6	13.7	14.0
	Min	16.0	16.0	14.0	29.0	12.0	11.0
BLS	Max	13.0	13.0	10.0	10.0	9.0	9.0
	Mean	14.4	14.0	11.9	12.0	9.2	9.2

SPF: Shady, Peat & Flat, BLF: Bright, Luvisol & Flat, BLS: Bright, Luvisol & Sloped

Table 10.7. Photosynthetically active solar radiation (μmole.m^2/s) of research plots at three sites with wild blue honeysuckle (*Lonicera caerulea* ssp. *villosa*) in the forest near Prince Albert National Park, within the Boreal Plains ecozone, located approximately 300 km north from Saskatoon, Saskatchewan, Canada, May to July of 2014 and 2015.

Year		2014			2015		
Month		May	June	July	May	June	July
	Min	22.4	22.4	22.4	22.4	22.4	22.4
SPF site	Max	1174.0	3341.6	2935.3	5713.9	3273.9	2980.6
	Mean	442.8	501.8	536.1	732.6	525.6	394.4
	Min	22.4	22.4	22.4	22.4	22.4	22.4
BLF site	Max	3567.3	4245.1	3703.1	3815.9	4335.1	4854.7
	Mean	654.9	887.5	1034.6	1527.7	1095.0	1040.6
	Min	22.4	22.4	22.4	22.4	22.4	22.4
BLS site	Max	3499.7	3793.1	3748.3	3928.8	3816.0	3680.2
	Mean	397.1	507.3	635.7	928.6	776.4	640.7

SPF: Shady, Peat & Flat, BLF: Bright, Luvisol & Flat, BLS: Bright, Luvisol & Sloped

Table 10.8. Percentage (%) of nitrogen, phosphorus and potassium used per plant from the hydroponic solution* by eight different blue honeysuckle (*Lonicera caerulea*) genotype and five different pH environments at Agriculture greenhouse

Experiment factors	Nitrogen	Phosphorus	Potassium
pH			
5	6.1	5.5	4.6
6	6.5	5.6	4.7
7	4.9	4.0	3.6
8	3.5	2.4	2.7
9	3.0	2.0	2.4
Genotype			
Lonicera caerulea spp. *pallasii*	4.7	3.8	3.3
Lonicera caerulea spp. *stenantha*	8.8	6.5	8.1
Lonicera caerulea spp. *venulosa*	3.7	3.0	2.5
Lonicera caerulea spp. *emphylocalyx*	3.1	2.7	2.3
Lonicera caerulea spp. *kamtschatica*	3.3	3.0	2.5
Lonicera caerulea spp. *altaica*	3.9	3.4	2.7
Lonicera caerulea spp. *villosa*	3.0	2.5	2.1
Lonicera caerulea cv. *Tundra*	8.4	6.7	5.5

*Hydroponic solution contained 2.975 g of nitrogen, 0.85 g of phosphorus and 3.604 g of potassium in 17 liter of solution per container.

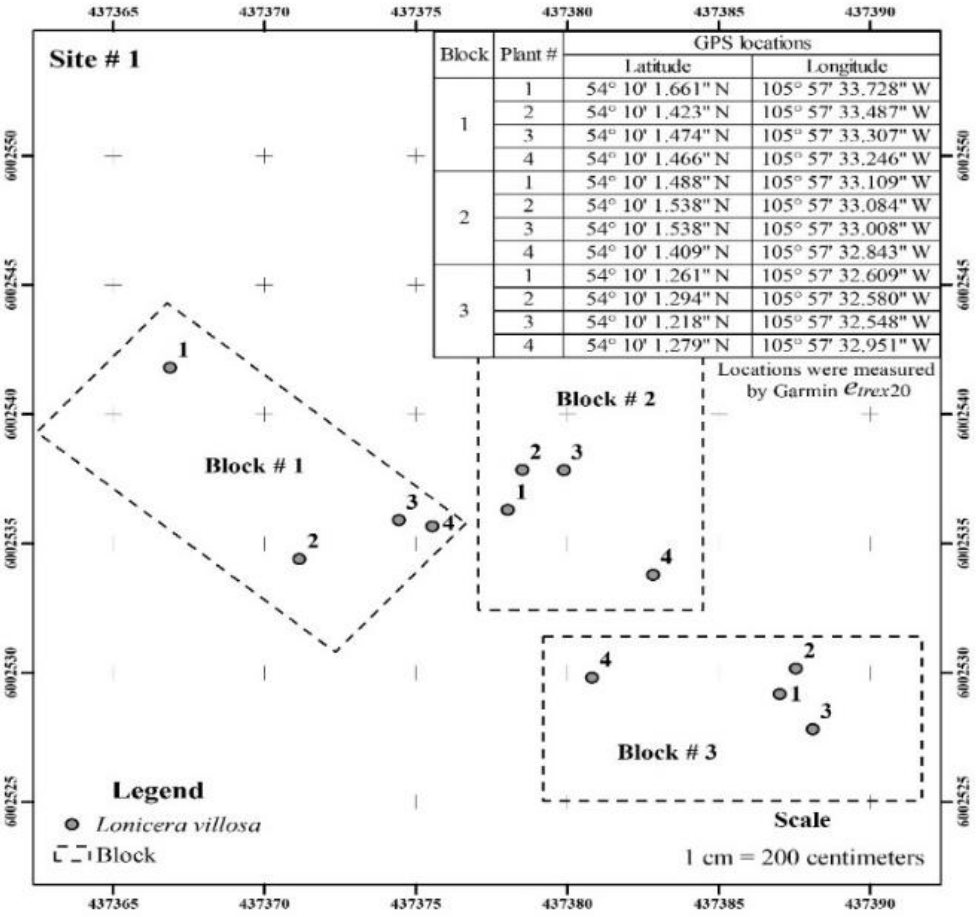
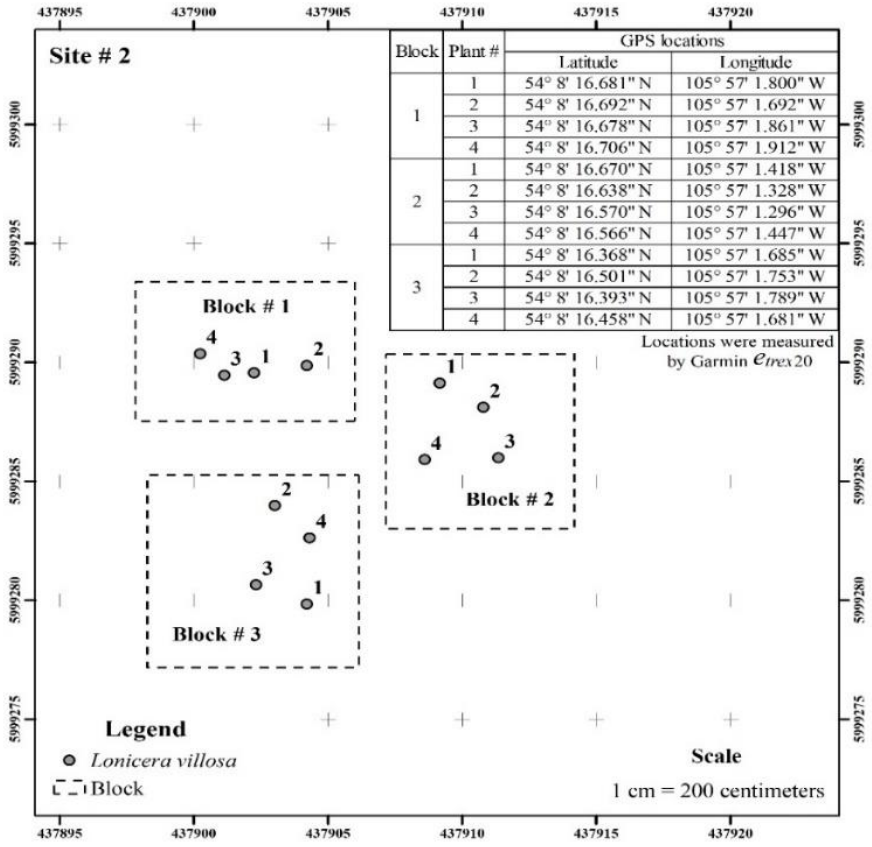


Figure 10.1. Locations and positions *Lonicera caerulea.* spp.*villosa* plants selected in the first experimental area (SPF)



Block	Plant #	GPS locations	
		Latitude	Longitude
1	1	54° 8' 16.681" N	105° 57' 1.800" W
	2	54° 8' 16.692" N	105° 57' 1.692" W
	3	54° 8' 16.678" N	105° 57' 1.861" W
	4	54° 8' 16.706" N	105° 57' 1.912" W
2	1	54° 8' 16.670" N	105° 57' 1.418" W
	2	54° 8' 16.638" N	105° 57' 1.328" W
	3	54° 8' 16.570" N	105° 57' 1.296" W
	4	54° 8' 16.566" N	105° 57' 1.447" W
3	1	54° 8' 16.368" N	105° 57' 1.685" W
	2	54° 8' 16.501" N	105° 57' 1.753" W
	3	54° 8' 16.393" N	105° 57' 1.789" W
	4	54° 8' 16.458" N	105° 57' 1.681" W

Figure 10.2. Locations and positions of *Lonicera caerulea* ssp.*villosa* plants in the second experimental area (BLF)

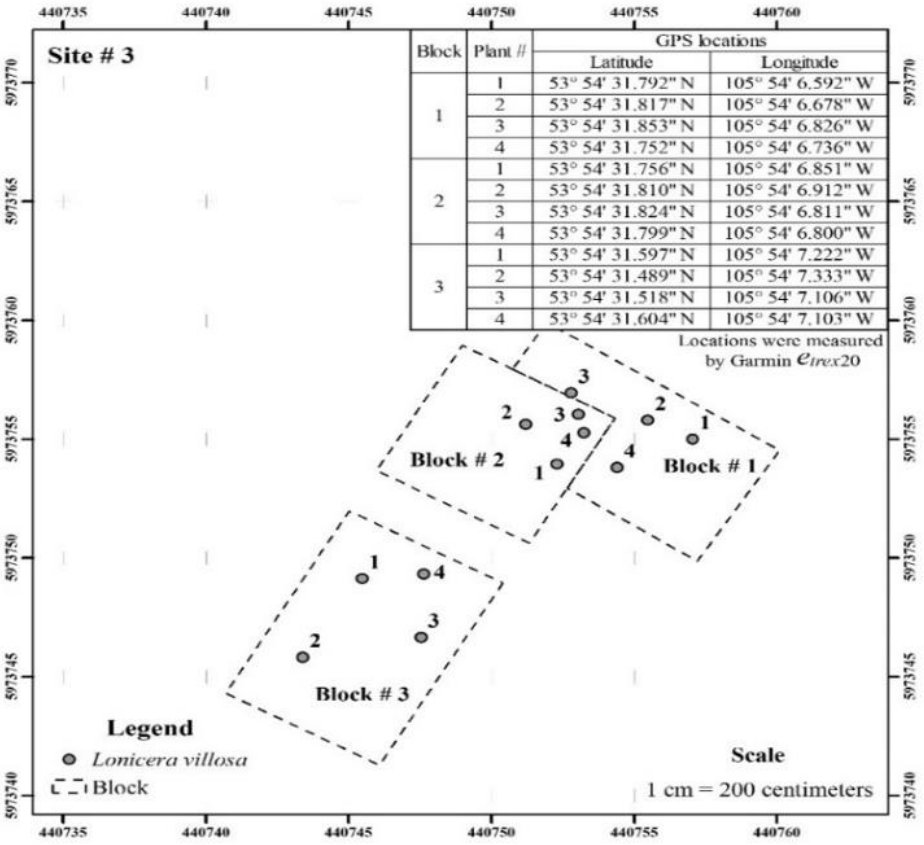


Figure 10.3. Locations and positions of *Lonicera caerulea* ssp.*villosa* plants in the third experimental area (BLS)