HASKAP (*LONICERA CAERULEA* L.) PLANT RESPONSE TO VARYING FREQUENCY AND RATE OF PYROLIGNEOUS ACID WITH ADDED LIQUID SEAWEED EXTRACT

by

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DEDICATION

This thesis is dedicated to God Almighty for his loving kindness and never-ending faithfulness.

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ABSTRACT

Haskap (*Lonicera caerulea L.*) is a new plant in Canada with many investment potentials. A field experiment was conducted to determine the effects of frequency (weekly, bi-weekly, and tri-weekly) and rates (2, 4, 6, 8, and 10%) of pyroligneous acid application (PA) with added 0.35% liquid seaweed extract (LSE) on haskap plant growth, yield, berry quality, and powdery mildew disease management. Control treatments were water alone, 4% PA alone, and 0.35% LSE alone. The tri-weekly application of 2% and 4% PA + 0.35% LSE enhanced haskap growth and yield. The 2%, 4%, and 6% PA + 0.35% LSE treatments improved haskap berry quality. The weekly application of 10% PA + 0.35% LSE reduced disease incidence and severity by 8.3%. The tri-weekly application of PA increased revenue by 42% compared to other frequencies.

Keywords: Biostimulant, berry yield, leaf tissue nutrients, berry tissue nutrients, organic acids

LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Percentage(s)
wt%	Percentage weight
°C	Degree Celsius
N	Nitrogen
P	Phosphorus
K	Potassium
Ca	Calcium
Mg	Magnesium
Cu	Copper
Fe	Iron
Zn	Zinc
Mn	Manganese
Na	Sodium
B	Boron
min	Minute
hr	Hour
sec	Seconds
cm	Centimeters
g	Grams
µl	Microliter
ml	Milliliters
m	Meters
mm	Millimeters
L	Liters
ha	Hectares
<	Less than

>	More than
rpm	Revolutions per minute
mg/L	Milligram per liter
μS/cm	Micro siemens per centimeter
MT	Metric tonnes
®	Registered trademark
OD	Optical density
CAD$	Canadian dollars
USD$	United States dollars
USA	United States of America
EC	Electrical conductivity
TDS	Total dissolved solids
TA	Titratable acidity
Inc.	Incorporated
LSD	Least significant difference
2-D	2- Dimensional
Ltd.	Limited
NPK	Nitrogen, Phosphorus, and Potassium
PCA	Principal component analysis
UV	Ultraviolet
DPPH	2,2-diphenyl-1-picryl-hydrazl-hydrate
ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonicacid)

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CHAPTER 1
INTRODUCTION

1.0 Thesis Overview

Haskap (*Lonicera caerulea L.*), also known as sweet berry honeysuckle, blue honeysuckle, and honeyberry, is a relatively new plant in Canada with many potential for investment (Bors, 2009). Haskap belongs to the genus *Lonicera* (*Caprifoliaceae* family) of which the genus comprises more than 200 species (Munro, 2020). The plant is considered sacred by the Hokkaido natives of Japan, Kuril Islands, China, and some communities in Russia, who regard it as the "portion of life" or the "golden antidote for infinite youth and long life" (Lefol, 2007; Lauritzen et al., 2015). In recent times, haskap has gained popularity and has been cultivated extensively in Europe, particularly Slovenia, Poland, Slovakia, and the Czech Republic (Senica et al., 2018). Naturally, the plant thrives well in mountainous and low-lying wet areas located in North Asia, woodlands in Europe, and North America (Bors et al., 2012). Some introduced commercial cultivars in North America include Borealis, Tundra, and Indigo Gem. Current cultivars being bred in North America comprise of Boreal Blizzard, Boreal Beast, Boreal Beauty, Aurora, and Honey Bee (Dudonné et al., 2015).

Haskap berries contain vitamins (A and C), carbohydrates and essential minerals (i.e., calcium, phosphorus, potassium, and magnesium) also common to other berries but high in anthocyanin and phenolic compounds in the former (Terahara, et al., 1993; Basu et al., 2010; Dudonné et al., 2015). A study by Bakowska-Barczak et al., (2007) showed increased levels of phenolic compounds (1,111.17 mg/100 g) in haskap which was significantly higher than that found in purple raspberries (*rubus odoratus*) (638.09 mg / 100 g), strawberries (*Fragaria* x *ananassa*) (437.04 mg/100 g) and bilberries (*Vaccinium myrtillus*) (778.47 mg/ 100 g). Also, phenolic compounds have become subjects of extensive research due to its prospective impact on human health (Gołba et al., 2020). As such, the haskap berries have been labelled as a "superfood" in Atlantic Canada due to the presence of high amounts of these nutritional qualities and health-promoting benefits (Richie, 2015). Additionally, health-promoting effects of haskap berries such as anti-inflammatory, anti-diabetic, anti-cancer, cardiovascular protective and antioxidant properties have been discovered through *in vitro* and *in vivo* studies (Tsuda et al., 1994; Chen et al., 2005; Ding et al., 2006; Wang et al., 2008; Petroni et al., 2017). For instance, Celli et al. (2014) reported that haskap berry juice improves the metabolic and vascular health of humans reduces glaucoma and prevents anaemia and hyperactivity in children (Lefol, 2007).

Products of haskap berries offered on the market comprise of wine, gelatine, yoghurt, jams, ice cream and candies (Liu et al., 2018). Notably, as of December 2018, a third country (Japan) in the European Union Economic Partnership Agreement (EPA) had listed haskap fruit as a customary food under its regulations (EFSA, 2018). In 2019, 334 MT of haskap berries were marketed in Canada with a farm gate value of CAD$ 2,309,000 (CHD, 2019). However, growers are faced with limited information on agronomic recommendations like cultural and management practices such as fertilizer and weedicide application to achieve optimum yield (Bors, 2009). Generally, growth and yield of haskap are reliant solely on synthetic chemical fertilizer application; and powdery mildew, which is the primary disease of haskap plants, is controlled with synthetic fungicides (HGANS, 2018).

Currently, sectors involved in agricultural and horticultural activities are up against the task of improving crop production to feed the rising population which is expected to reach 10 billion by 2050 (UN, 2017); as a result, this increases the use of agronomic chemicals resulting in adverse effect on the environment and human wellbeing. (Searchinger et al., 2013; Noroozlo et al., 2019).

Moreover, the use of sustainable and environmentally friendly biological materials like biostimulants for enhancing crop growth is becoming increasingly crucial in fruit production. Biostimulants from sources like plants contain substances which are used as additives for inorganic fertilizers. This in turn helps sustain the ecosystem and the economy (Souri and Bakhtiarizade, 2018; Noroozlo et al., 2019). Biostimulants consist of diverse plant materials and microorganisms that stimulate plant tolerance to abiotic stress, promote growth, increase yield and improve plant quality when applied to seed, growth media, soil or plants (Jardin, 2015). The positive effects of biostimulants and biofertilizers like seaweed extract, beneficial fungi (arbuscular mycorrhizal and bacteria (N-fixing bacteria strains in the genera *Rhizobium* spp. *Azospirillum* and *Azotobacter*); humic and fulvic acids; pyroligneous acid, and inorganic acids has been shown (Jardin, 2015). For instance, liquid seaweed extract (LSE) has been used to boost growth in horticultural crops (Parađiković et al., 2018). Positive results like improved stress tolerance, seedling and transplant vigour, vegetative growth, nutrient acquisition, and distribution in plants have been reported in strawberry (*Fragaria* x *ananassa*) with the use of biostimulants (Parađiković et al., 2018).

Another under-exploited potential natural biostimulant is pyroligneous acid (PA), which is also referred to as wood vinegar. PA is an organic liquid obtained through the process of pyrolysis

(Mathew and Zakaria, 2015; Grewal et al., 2018), and composed of organic acids, alcohol, phenolic, alkane and ester compounds(Wei et al., 2010). This bio-product from various sources of plant biomass as the feedstock has been shown to boost growth, fruit size, fruit weight, and seed germination in some plants like rockmelon (*Cucumis melo* L. *cv.* Golden Langkawi) (Mohan et al., 2006; Zulkarami and Mohammed 2011). However, PA stimulatory effect on haskap growth and yield is understudied. This is the focus of my research in determining the effects of frequency and rate of PA with added LSE on haskap production.

1.1 Research Gap

The application of PA in enhancing growth and yield of haskap could be a novel and promising approach for the thriving haskap industry of Nova Scotia and Canada at large. Nevertheless, scientific information on the optimal rate, frequency, time of application, mechanism of action, bio-pesticidal capabilities of PA on haskap is lacking. Although PA has phenolic compounds and antioxidants, its combinative effect with LSE which contains phytohormones and vitamins on haskap plants has not yet been documented. A successful and positive outcome will provide haskap growers with a lower cost of haskap production with increase farm returns while promoting environmental sustainability.

1.2 Overall Project Objective

The overarching goal of the research was to determine the effect of PA with added LSE on haskap plant growth, berry productivity, berry fruit quality, and powdery mildew disease development.

The specific objectives of the research were to:

1. determine the effects of frequency and rate of PA application with added LSE on plant height, photosynthetic activity, macro- and micro-nutrients in haskap leaves, electrolyte leakage, flower count, and yield and financial analysis of PA application.
2. determine the effect of frequency and rate of application of PA with added LSE on postharvest berry fruit quality, powdery mildew disease management.

1.3 Research Hypothesis

Based on unpublished preliminary studies conducted by Dr Abbey in the Compost and biostimulants laboratory, it is hypothesized that PA with added LSE at 0.35% will have a positive impact on haskap plant growth, berry fruit yield, and prevent powdery mildew development.

1.4 Thesis Organization

The thesis is organized in a manuscript format which consist of six chapters. Chapter 1 is the Introduction, and the next is Chapter 2, which reviews the literature on the origin, distribution, and potential benefits, botany, life cycle, environmental conditions suitable for haskap growth, and the management practices that enhance such growth. It expands on the use of biostimulants with emphasis on PA and LSE based on its composition and stimulatory roles in plants. The research gap was explained in this chapter as well as potential and future prospect. Chapter 3 addresses the effects of PA on plant height, photosynthetic activity, macro- and micro-nutrients in haskap leaves, electrolyte leakage, flower count, berry yield and 100 berry weight. Chapter 4 addresses the effect of frequency and rate of application of PA on postharvest berry fruit quality, and powdery mildew disease management. Powdery mildew affects haskap plant after harvest and may increase spore load and infection severity the next growing season. As such, it was included in Chapter 4. Chapter

5 contains the economic benefit analysis of using PA with added LSE for haskap production. Chapter 6 contains synthesis of the research project supported by references and appendix from all sections. Future research recommendations are also included in this chapter.

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CHAPTER 2
LITERATURE REVIEW

2.0 Origin, Distribution, and Potential Benefits Of Haskap

The term haskap (*Lonicera caerulea*), also known as "Hasakapu," "Hascup," "Haskappu," and "Hasukappu," was first given by the Ainu natives of Hokkaido, Japan. In the language of Ainu natives, haskap means "a collection of berries on a branch" (Thompson, 2006; Lefol, 2007). Haskap is native to northeastern Asia, Russia, and Siberia, and usually located in mountainous and low-lying wet areas (Bors et al., 2012). In Russia, haskap is a prominent berry but gradually gaining ground in parts of Northern Europe due to its ease of cultivation, early growth ability, exceptional flavors, and unique nutritional values (Plekhanova, 2000; Hummer, 2006). Initial attempts to domesticate haskap in eastern and northern Russia started from 1913 to 1915. However, extensive research on breeding for the best varieties occurred in the 1950s-1960s at the Siberian Horticultural institute (Barnaul) and the Vavilov Research Institute of Plant Industry (VIR) (Plekhanova, 2000). At that juncture, Russian plant breeders focused on breeding improved haskap varieties with the focus on increased fruit size and nutritional values to suit consumers' demand. The breeders achieved their objective through the assembling of different germplasm and cross-breeding between *Lonicera caerulea* subspecies *kamtschatica* and *edulis* led by Dr. A. K. Skyortsov (Plekhanova, 1999; Maxine M. Thompson, 2006). Such a vast compilation of haskap germplasm resulted in the production of about 500 different genotypes (Hummer et al., 2012).

Haskap's early introduction to North America began in Oregon, USA in the 1950s, with perception as a decorative plant (Rupasinghe et al., 2012). Research championed by Dr. Thompson Maxine in Oregon, USA focused on the health and financial analysis of haskap (Bors, 2008). Likewise, the late 1990s saw more breeding programs being undertaken at the University of Saskatchewan, of which 8,000 diverse collections of haskap hybrid varieties were produced. This breeding program paved way for commercial production in Quebec, Nova Scotia, Ontario, and British Columbia. At present, 34 species of *Lonicera* species can be found in North America out of which 16 were pioneered by research and 18 are native species (Lieurance & Cipollini, 2013). Additionally, the fruit has been used as conventional medicine for years by the natives of Japan, China, Russia, the Kuril Islands, and some parts of Asia (Plekhanova, 2000). The Ainu natives of Hokkaido, who hunted for survival, adored haskap due to its therapeutic qualities (Lefol, 2007), thereby recognizing it as "the solution of survival" (Maxine M. Thompson, 2006).

The dark-blue berries contain high amounts of anthocyanin and ascorbic acid than most berries such as blueberries, which are known for their health boost benefits (Celli et al., 2014). For instance, *in vivo* and *in vitro* studies revealed that polyphenols extracted from haskap berries are efficient against oxidative stress and inflammation induced by abnormal lipid metabolism i.e. lipopolysaccharide (LPS)-induced inflammation, and ultraviolet-induced skin damage (Raudsepp et al., 2013; Vostalova et al., 2013; Takahashi et al., 2014; Wang et al., 2016). Moreover, haskap extracts have been shown to have antimicrobial properties against *Kocuria rhizophila,* and *Bacillus subtilis* (Raudsepp et al., 2013). Predominant antioxidants in the berries have been shown to reduce the process of aging due to its ability to scavenge reactive oxygen groups (Duthie, 2007). Likewise, daily intake of haskap juice has been shown to enhance neurocognitive activities, reduce bone defects, insulin resistance and cancer cell growth (Devareddy et al., 2008; Gordillo et al., 2009; Krikorian et al., 2010; Stull et al., 2010). Also, consumption of haskap juice can reduce the probability of developing stroke and dementia due to the presence of polyphenols. The polyphenols reduces these risks (thus, stroke and dementia) by initiating survival genes and signaling surge, monitoring of mitochondrial function, and decrease in neuroinflammation (Jones et al., 2012). Haskap consumption also helps prevent melanogenesis (Celli et al., 2014). The juice could be considered as a probiotic as it has been shown to have no effect on human gut microflora (Raudsepp et al., 2013).

2.1 Botany of Haskap

Haskap belongs to the genus, *Lonicera* (*Caprifoliaceae* family) and species *caerulea*. The genus consists of more than 200 species and are known for their ornate vine and bushy plant growth form, which usually produce unappetizing yellow to red bulbous fruits (Figure 2.1). Common species related to the *Caprifoliaceae* family include *Linnaea* L. (e.g., twinflower), *Sambucus* L. (e.g., elderberries), and *Viburnum* L. (e.g., squash berry) (Munro, 2020). Nonetheless, haskap is an exception due to its edible berries compared to species in the *Caprifoliaceae* family (Bors et al., 2012). Haskap plants have a chromosome set of n = 9 with its subspecies *boczkarnikovae* and *edulis* being diploid while subspecies *venulosa, stenantha, pallasi, emphllocalyx,* and *kamtschatica* are tetraploid (4n). Thus, haskap plants contain four sets of chromosomes (Lamoureux et al., 2011). The native populations of haskap in Asia have both diploid (2n) and tetraploid species (Plekhanova, 1992). Notably, tetraploid haskap plants have more substantial genetic diversification than diploids due to the higher number of chromosome levels, in addition high productivity and longer life span (Dawson, 2017). Also, tetraploids haskap populations tend to dominate alpine and northern terrains (e.g. northern Russia, Mongolia, Kazakhstan) with diploids plants limited to southern territories (e.g. southeastern Siberia, Japan, northeastern China) (Plekhanova, 1992; Miyashita et al., 2011).

Haskap bush height ranges from 0.8 to 3.0 m with the development of offshoots, which tend to be sturdy with knots (Figure 2.1). These knots can be with or without pubescence depending on the variety (Plekhanova, 2000). Nonetheless, leaves of haskap are dark green, large, and oval-shaped (with or without pubescence). The plant produces perfect flowers with five anthers, one stigma, and two fused ovaries (Hummer et al., 2012) (Figure 2.1). These flowers are large, tubular to funnel-shaped and light yellow (Hummer, 2006). The flowers of haskap appear with the initial development of the vegetative components, with floral growth occurring in the axils of developing second-year shoots (Hummer et al., 2012; Dawson, 2017)). Flowers of haskap are self-incompatible and need insects for cross-pollination (Hummer et al., 2012). Bumblebees (*Bombus sp.*), honeybees (*Apis mellifera* L.), and solitary bees have been documented as typical pollinators of haskap plants (Bozek, 2012). Fruiting usually begins within two years of cultivation with increased berry fruit yield as plant grows and increase bush size (Plekhanova, 1992). Notably, the plant has low yields per plant (2 to 4 kg) (Plekhanova, 1994) with fruit weight ranging from 0.4 to 2.0 g (Suzuki et al., 2007).

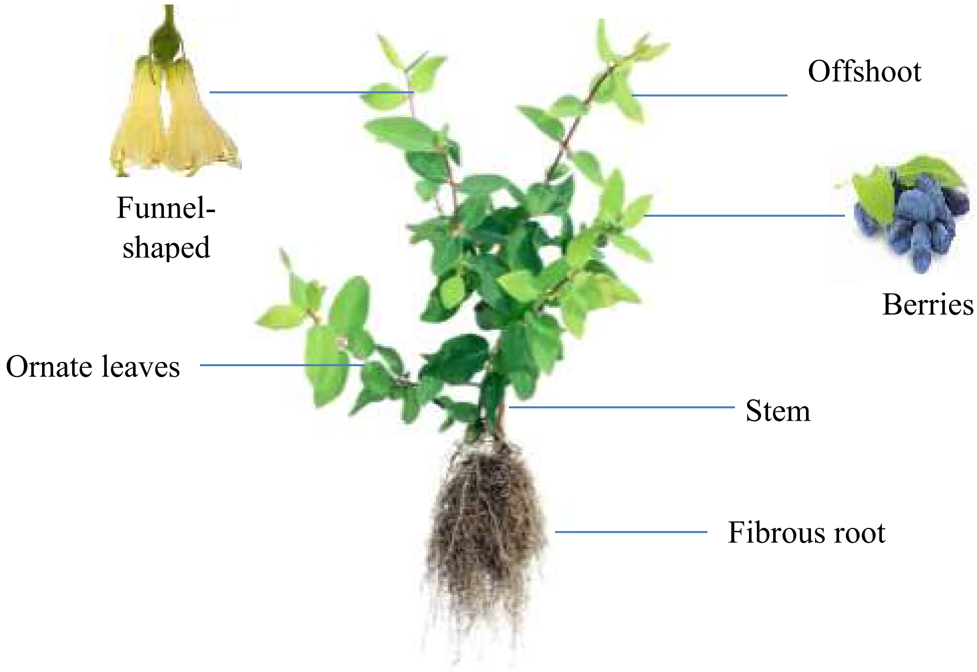

Figure 2. 1 A haskap plant with leaves, funnel-shaped flowers, berries, and fibrous root (Honeyberry, 2019)

The axillary buds of haskap plants develop in an upright series on the stem with the basal two buds containing the flower initials for the next season (Hummer et al., 2012). The apical buds of haskap mostly remain dormant for 3 to 5 years (Renata, 2001). Also, the plant produces 12 to 15 vertical branches that emerge from the pinnacle giving the plant a spherical figure (Plekhanova, 1992). A well implanted haskap plant can have an extensive fibrous root system of 1.5 m in diameter (Renata, 2001).

2.2 Life Cycle

The lifecycle of a plant involves the germination phase, reproduction phase, and the end of the reproduction phase depending on whether the plant is an annual, biennial, or perennial. Haskap is a perennial plant and starts its lifecycle from the seed stage, which may not be the case under commercial production (Fig. 2.2 A). The seeds are nursed in peat moss or perlite mixture for a year or two (Fig. 2.2 B) (Mersey, 2015). Developed haskap plants grow at an average rate of 5 to 6 mm/day, while developing plants grow at the rates of 9 to 15 mm/day depending on the genetic characteristics, management practices and environmental conditions (Fig. 2.2 C) (Plekhanova, 1989). Bud emergence into a new vegetative shoot can develop at 50 cm to 90 cm in a lone season (Hummer et al., 2012). Bloom in haskap begins in the latter parts of April to early May in the southwest region of Nova Scotia, where climatic conditions such as temperature may have a significant impact on the flowering time and duration of haskap plant development (Bożek, 2007; Dawson, 2017). For instance, leaf bud breaking was recorded in Saint Petersburg, Russia when temperatures fell between 2.5°C to 3.8°C and flowering occurred at 9.5°C to 11.7°C (Plekhanova, 1989). Haskap flowers usually remain viable for 48 hrs. after opening, and the flowering period for a variety can range from 7 to 15 days (Fig 2.2 D) (Bors et al., 2012). Usually, bees are needed to aid in pollination (Fig. 2.2 E) (Frier et al., 2016). Oblong fruits with waxy coating develop after pollination has occurred (Fig. 2.2 F) (Plekhanova, 1992). After harvest, the leaves begin to wither (Fig. 2.2 G), and the plant enters a state of dormancy when the production cycle is over (Fig. 2.2 H). Haskap plants can abate after 20 years of production but can be revived by the removal of old branches and stem to boost new shoot growth (Plekhanova, 1989).

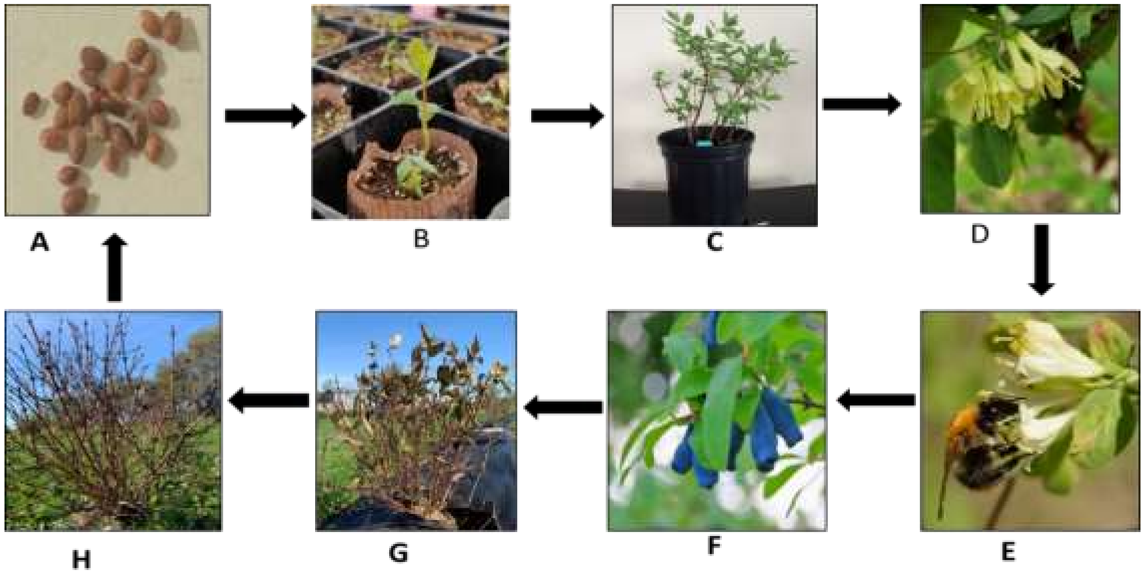

Figure 2. 1. The lifecycle of the haskap plant (Honeyberry, 2019). (A) Haskap seeds (B) Haskap seedling, (C) Matured Haskap plant, (D) Haskap flowers, (E) Pollination of Haskap flowers by bees, (F) Haskap berries, (G) Withering of haskap leaves, (H) Haskap plant in a state of dormancy.

2.3 Factors Affecting Plant Productivity

Nutritional, physical characteristics and yield of haskap berries vary with changes in the environment, climate, genotype, plant maturity, and horticultural practices (Jackson and Lombard, 1993; Prange and DeEll, 1995; Ojeda et al., 2002). However, research on the specific effects of these factors on plant growth, productivity, and berry quality of the haskap plant is limited.

2.3.1 Environmental Factors

Haskap plant growth and berry yield are significantly influenced by variation in environmental conditions (Bors, 2012). The plant can be grown in mountainous areas and thrive well in temperate regions such as the northern parts of Canada (Bors et al., 2012; Hummer et al., 2012). The plants can survive at a minimum temperature of -45ºC (Lefol, 2007) with preference to well aerated and adequately drained soil (Lefol, 2007; Bob et al., 2012). The plant can also adapt to various soil types such as sandy loam, sand, and clay with 5% organic matter and requires a pH range of 5.5 to 8.0 to thrive well (Plekhanova, 1992).

2.3.2. Management Practices

To date, challenges of haskap production have been reported to include susceptibility to sunburn, water stress, spider mites, botrytis, and powdery mildew (Dawson, 2017). Nevertheless, acceptable management practices account for their optimum productivity and berry quality. The growth of haskap plants varies from plump to low growing dome shapes based on their genetic traits. Thus, irrespective of the plant's nature, growing haskap in perennial rows is an effective means for establishment of the roots. This aids in the uptake of nutrients with less competition. The recommended spacing between plants is approximately 1.3 m, and 3.0 m between rows concerning the growth pattern of the variety. Such spacing allows for the easy access and use of farm machinery like tractors and harvesting equipment. The spacing helps in the reduction of disease transfer from infected plant to others. The growing of perennial grass cover between rows is an effective means of curbing weed growth and improving the overall well-being of plants. Such growth also allows for the limitation of dust and mud while creating level grounds for foot traffic and tractor use. It also ensures less incidence of diseases, increase in plant growth and promotion of yield output. (Gerbrandt, 2015). Additionally, coco mats, landscape fabric, plastic covers are among the few materials that help prevent weeds from competing with haskap plants for nutrients, moisture, and space.

Although there is limited research on fertilizer recommendations for haskap plant growth, some researchers recommend the application of fertilizer in spring to ensure nutrient uptake for plant growth and development (Bors, 2008). Irrigation is vital for plant establishment in the first three (3) years but rarely needed after plant establishment. However, continuous irrigation could result in shallow root growth, thereby making the plant vulnerable to drought stress fall due to heavy top (Bors, 2008). Pruning is another essential management practice in haskap production (Elford, 2019). This practice is best performed in the fall season when haskap plants are dormant. The practice involves the removal of mature branches to make room for dormant buds to develop (Gerbrandt, 2015). This promotes upright growth, improves plant vigor, enhance fruit yield, and natural harvest of haskap berries (Rachel Cheverie, Horticulturist, Perennia Innovation Center, Kentville, NS; Personal Communication, September 21, 2019).

2.4 Improvement of Plant Growth Using Biostimulants

Plant biostimulants are organic substances that boost plant tolerance to abiotic stress, promote growth, increase yield, and improve plant quality (Jardin, 2015). Such biostimulants include humic acid, fulvic acid, protein hydrolysates, seaweed extract, beneficial fungi, and plant growth-promoting rhizobia (Halpern et al., 2015). Generally, biostimulants extracted from plants can stimulate molecular, biochemical, and physiological plant reactions in minute concentrations. Such responses include enhanced floral development, increased plant growth, development, and yield, improved functional and dietary value of consumable portions (Bulgari et al., 2015; Calvo et al., 2014; Halpern et al., 2015). Moreover, extracts from sources like wood, seaweed, and vermicompost have been known to trigger essential processes that improve plant tolerance to biotic and abiotic stresses such as drought, heat, cold, and salinity stress and water deficits (De Pascale et al., 2017).

Biostimulants tend to elicit such response through various processes like improved buildup of osmolytes (e.g. betaine, glycine, sorbitol, and proline); enhanced nutrient absorption and utilization attributable to developed root volume, length, and biomass. Similarly, biostimulants stimulate such response through increased photosynthetic capacity and leaf water retention; increased antioxidant enzymatic activities (malondialdehyde, catalase (CAT) and superoxide dismutase (SOD) and decreased transpiration rate (Calvo et al., 2014; Battacharyya et al., 2015; Colla & Rouphael, 2015; Rouphael et al., 2017). Studies have shown that nutrient uptake of biostimulants in plants is stimulated by assembling biological minerals in the soil for plant use (Fawzy et al., 2012). Biostimulants application can be done either by soil drench or foliar application depending on its nature (granules, liquid solution or powder), chemical composition, and desired result on horticultural plants like tomato (*Solanum lycopersicum*), bell pepper (*Capsicum annuum L.*), and strawberry (*Fragaria x ananassa*) (Parađiković et al., 2018). Likewise, biostimulants can boost nutrient accessibility for specific mineral substances via fractional alteration of the physiochemical components of soils (e.g., cation exchange capacity, pH, and mineral nutrients) (De Pascale et al., 2017).

Biostimulants offer the opportunity for many researchers to focus on testing their effects on plants, especially on cereal, vegetables, and fruits due to the high value placed on organically grown, pesticide-free fruits and vegetables (Parađiković et al., 2018). The global market for biostimulants is estimated at $ 1 billion (USD), with a projected yearly increase of 13% in European Union countries like Germany and Sweden (Calvo et al., 2014; Colla and Rouphael, 2015). In North

America, the biostimulants market was estimated to worth $ 378.79 million (USD) in 2018 with a projected growth of $ 646.66 million (USD) by 2023 (NABM, 2019).

Regardless of the significant exposition on the beneficial use and economic impact of biostimulants or biofertilizers, further studies are required regarding their rate of application, method of application (drench or foliar), and frequency of application (plant phenological stages, early or late application). However, crop management activities, plant response, and environmental conditions may affect the efficacy of biostimulants or biofertilizers application (Rouphael & Colla, 2018; Souri & Tohidloo, 2019). Furthermore, pyroligneous acid (PA) and liquid seaweed extract's (LSE) ability to enhance crop growth and yield are currently applied and studied on annual plants like strawberry and not on perennial plants like haskap. Hence, there is the need to investigate haskap response to these biostimulants (i.e., PA and LSE).

2.4.1 Pyroligneous Acid

Pyroligneous acid (PA) also known as wood vinegar, wood distillate, or bio-oil has been used for many years by farmers as biostimulant, biopesticide, biofertilizer, and antimicrobial agent (Souza et al., 2012). PA is a harnessed product of burnt biomass from the process of pyrolysis (Mungkunkamchao et al., 2013) using feedstock such as agricultural biomass, bamboo, algae, woody plants, and herbaceous plant biomass. PA consists of about 200 water-soluble compounds including organic acids, alcohol, phenolic, alkane, and ester compounds, of which 50% is comprised of acetic acid (Wei et al., 2010). The bio-product consists of three main polymers, which are hemicellulose (65%-75%), cellulose, and lignin (18%-35%) (Balat et al., 2009). The physical and chemical makeup of PA is dependent on the feedstock, temperature, size of materials, and the amount of heat applied during the pyrolysis process (Mohan et al., 2006; Mathew & Zakaria, 2015).

The process of pyrolysis involves the thermal breakdown of materials (e.g. organic or plant biomass) under near absence or reduced oxygen supply which results in the making of biochar, pyroligneous acid, smoke, and several gases. The various techniques used for pyrolysis process is dependent on the duration of burning and heating levels (°C/sec) (Grewal et al., 2018). Some essential components which determines the constituents of pyrolysis products include temperature, feedstock, technology utilized in production, and heating rate (Mathew & Zakaria, 2015). Generally, the ideal temperature range for the formation of pyrolysis products lies between 625 to 775 K (Balat et al., 2009). The process of pyrolysis can be categorized as fast or slow depending on the gaseous, liquid, and biochar products obtained (Grewal et al., 2018). Slow pyrolysis results in the decomposition of organic bonds with the conversion of plant waste material into biochar and organic gases (Mansur et al., 2013). The process of slow pyrolysis yields 30 wt. % of liquid, 35 wt. % of biochar, and 35 wt.% of gases. Slow pyrolysis for biomass degradation is conducted at an approximate temperature of 300°C where vapor residence time range from 5 to 30 mins (Steiner et al., 2008). The gaseous by-products released during the process of slow pyrolysis of plant biomass include tar, volatiles, and water vapour which are liquified and stored using cold traps and strainers (Mansur et al., 2013). The condensed liquids are then sedimented for a period of three months of which the aqueous liquid portion obtained is known as pyroligneous acid or wood vinegar (Steiner et al., 2008). On the other hand, fast pyrolysis is suggested for increased liquid yield (Grewal et al., 2018). Fast pyrolysis is considered effective as biomass are speedily converted to biofuel due to increased heat exposure (i.e. 500°C) with reduced vapor time (< 5 sec)

(Tiilikkala et al., 2010). The process of fast pyrolysis usually produces 15 to 25 wt. % of biochar, 60 to 75 wt. % of bio-oil and 10 to 20 wt. % of gasses (Mohanet al., 2006).

Agricultural use of PA has been on the rise since it is environmentally friendly and has been extensively reported to boost plant growth, quality, and yield (Zanetti et al., 2004). For instance, some researchers have proposed that foliar application of PA results in the increase of chlorophyll content and lustrous look of plant leaves which as result, increase photosynthesis including and the synthesis of amino acids and sugars (Grewal et al., 2018). Similarly, foliar application of PA significantly increased the yield of cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*), and cole crops (*Brassica oleracea* var. *capitata* f. *rubra*) (Mu et al., 2006). Likewise, foliar application of PA improved crop yield for most rice farmers in Thailand (Yoshimura et al., 1995; Katoda and Niimi, 2004; Jun et al., 2006). Also, foliar application of PA as a biostimulant improved seedling growth as well as the yield of tomatoes (Kulkarni et al., 2007; Kulkarni et al., 2008). The application of PA caused an increase in fruit size, weight, and sweetness of fruits and vegetables (Mohan et al., 2006; Zulkarami et al., 2011; Oramahi et al., 2013; Tuntika et al., 2013).

It is worth noting that PA was reported to have both antimicrobial and antibacterial properties due to increased amounts of organic acids and phenolic compounds (Lee et al., 2011; Mmojieje & Hornung, 2015). For instance, the application of PA repressed *Erwinia carotovora pv. carotovora* and *Xanthomonas campestris pv. citri* pathogens which are known to cause detrimental yield loss in horticultural crops (Chalermsan & Peerapan, 2009). Also, the application of PA repressed *Ralstonia solanacearum* (a bacterial pathogen), which causes plant wilting due to the presence of guaiacols and phenol compounds in the PA obtained from *Cryptomeria japonica* (Hwang et al., 2005).

Moreover, compounds like methyl formate and methyl acetate in PA stimulate plant development and growth. Studied rates of PA application include 0.01%, 0.02%, 0.1%, 1.5%, 3.0%, 6.0%, 10%, 20%, and 30% with the latter rate considered harmful to plant health (Zulkarami et al., 2011; Petter et al., 2013; Travero & Mihara, 2016; Luo et al., 2019). Also, frequencies considered for PA application include 7, 14 and 28 days (Petter et al., 2013). In relation to yield increase, a study by Travero and Mihara (2016) showed that 30% of PA applied to soybeans increased yield by 3.1 tons/ha with no effect on plant growth. Nonetheless, application of 10% of PA in combination with local formulation on rockmelon (*Cucumis melo* var. *cantalupensis*) improved plant growth and fruit yield but became toxic at a higher rate of 30% (Zulkarami et al., 2011). The application of 10% of PA in combination with local formulation on rockmelon also improved fruit sweetness, fruit diameter and fruit weight (Zulkarami et al., 2011). Growth-stimulating effects such as improved panicle formation in organic rainfed rice were noticed when PA was combined with cattle manure (Anan et al., 2014). PA enhanced hypocotyl and radicle growth in chrysanthemum and watercress when used as a priming agent (Mu. et al., 2003). Also, PA applied after 14 days effectively controlled *P. includes* but had little effect on the yield components of soybeans (*Glycine max*) (i.e. number of seeds per pods and number of pods per plant) (Petter et al., 2013).

Many studies have shown the benefits of PA on several crops including rockmelon, tomato, rice, and cucumber but not on haskap plants. PA contains phenolic compounds and organic acids while LSE contains phytohormones, vitamins and antioxidants, which have been individually tested. Their combinative effect of plants including haskap is understudied. The application of PA might be crucial in enhancing haskap growth, yield, and berry quality. Hence, this project seeks to determine the response of haskap plants (Indigo gem) to varying rates and frequency of PA.

2.4.2 Liquid Seaweed Extract

Seaweed extract is an ecologically friendly biological waste that is becoming increasingly important in crop production (Rayorath et al., 2008; Ramkissoon et al., 2017). Seaweed comprises about 10,000 species of the red (Rhodophyta), brown (Phaeophyta), and green (Chlorophyta) marine macroalgae (Khan et al., 2009). Due to its distribution and abundance, brown seaweed extracts (*Ascophyllum nodosum*) are commercially produced and commonly used in agriculture for plant growth. Studies on commercialized portions of brown seaweed extract revealed a considerable range of inorganic and organic chemical components including mannitol, nutrient minerals, alginic acid, vitamins, antioxidants, and phytohormones (Baardseth, 1970). Many researchers have shown that brown and green seaweed extracts have plant growth-promoting components, which contain 3 to 10% proteins, trace amounts of vitamins C and A, a variety of minerals, 40 to 70% carbohydrates, 2 to 4% phospholipids and glycolipids, 4 to 8% polyphenols and plant hormones, which are used as biostimulants and nutrient supplements to promote plant growth and yield (Khan et al., 2009; Chojnacka et al., 2012; Sharma et al., 2012).

Brown seaweed (*Ascophyllum nodosum*) extract contains chemical compounds that regulate growth and development of vascular plants (Khan et al., 2009). Plant growth hormones from brown seaweed extract like auxins, cytokinin, and gibberellins promote plant growth and development (Crouch & Van Staden, 1993; Rayorath et al., 2008; Craigie, 2011). The presence of betaine-like compounds and betaines have also been shown to promote plant tolerance to abiotic stress such as drought, frost, and salinity stresses (Blunden & Gordon, 1986; Wang et al., 2003). These compounds are osmolytes, and act as osmoprotectant against cellular damage. Also, osmolytes like mannitol are found in brown seaweeds act as protective compounds in response to environmental stressors (Battacharyya et al., 2015). However, the underlying mechanism by which seaweed extract affected signaling of hormones in plants remains unknown (Ghaderiardakani et al., 2019). Furthermore, high amounts of phenolic compounds in brown seaweed have been reported to prevent abiotic stress in plant cellular components and thus, promote plant and crop performance (Khan et al., 2009). Phenolic compounds play a vital role in enhancing the scavenging of radicals such as superoxide, single oxygen, alkoxyl, hydroxyl and peroxyl (Battacharyya et al., 2015). Various research studies have demonstrated that seaweeds are efficient antioxidants due to the presence of phlorotannin other than ascorbic acid, catechin, dieckol, and resveratrol (Shibata et al., 2003).

Moreover, the high phosphorus (P) content of brown seaweed extract stimulates the rapid growth and development of plant roots, which increases nutrient uptake in deep layers of the soil (Biswajit et al., 2013). Additionally, brown seaweed extract enhanced the photosynthetic efficiency in citrus plant leaves and resulted in improved movement of carbohydrates from the leaves into developing fruits, suggesting a biostimulatory effect (Gómez-Cadenas et al., 2012). Also, apple trees (*Malus domestica*) treated with brown seaweed extract exhibited high chlorophyll contents in its leaves, thereby reducing varying times of fruit-bearing (Spinelli et al., 2009). Dissolvable extracts from seaweed were efficient in promoting primary root formation in *Arabidopsis thaliana* at low concentrations but exhibited inhibition of germination and root growth when applied at high concentrations due to the presence of aluminum compounds (Al^{3+}) (Ghaderiardakani et al., 2019).

Seeds of *C. frutescence* primed with brown seaweed extract enhanced seed germination percentage, seedling weight, and seedling vigor index due to a reduction in electrolyte exposure. This led to an improvement in activities of DPPH (2,2-diphenyl-1-picryl-hydrazl-hydrate) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonicacid)) assays and total phenol contents in primed seeds (Dutta et al., 2019).

Several studies suggest the benefits of seaweed extracts through foliar application methods. For instance, foliar application of seaweed extracts increased tomato (*Solanum lycopersicum*) fruit yield by 30% of fresh weight over the control (Crouch & Staden, 1992). Thompson seedless grape (*Vitis vignifera* L.) had a steady increase in yield over a 3-year study period of consistent seaweed extract application. In this study, seaweed treated grape plants recorded 39% increase in weight, 13% increase in fruit size, and 60% increase in yield compared to the untreated plants (Norrie & Keathley, 2006). Furthermore, foliar application of seaweed extract had positive effects on the number of leaves per plant, shoot length, antioxidant activity, total phenolic, flavonoid content, and yield of cowpea (*Vigna unguiculata*) (Vasantharaja et al., 2019). Apart from promoting growth and development in plants, brown seaweed extracts have a positive influence on the biological, chemical, and physical components of soils, which in turn promote plant growth. For instance, seaweed extract improves soil health by promoting development of soil micro-organisms and enhancing water holding capacity in soil (Khan et al., 2009).

Although there are accessible studies of liquid seaweed extract (brown seaweed) on horticultural plants like strawberry, grapes and apples, there is limited research on its agronomic impact on haskap plants when combined with PA. The presence of growth promoting nutrients in liquid seaweed extract makes it an interesting component to consider for this study.

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CHAPTER 3
HASKAP (LONICERA CAERULEA) PLANT GROWTH AND YIELD RESPONSE TO VARYING FREQUENCY AND RATE OF PYROLIGNEOUS ACID APPLICATION WITH ADDED LIQUID SEAWEED EXTRACT

3.0 ABSTRACT

The use of environmentally friendly biological materials to promote plant growth and yield is becoming increasingly crucial in agriculture due to changes in climate and agroecological systems. A field study was conducted to determine the effects of pyroligneous acid (PA) with added liquid seaweed extract (LSE) at different rates (2, 4, 6, 8, and 10%) and frequencies (weekly, bi-weekly, and tri-weekly) on haskap plant growth and yield. 4% PA alone, 0.35% LSE alone, and water alone were used as control treatments. The growth and yield response to weekly, bi-weekly, and tri-weekly applications of PA with added LSE increased flower count, fresh leaf weight, photosynthetic capacity (Fv/Fo), chlorophyll content, and 100-berry weight. The 8% PA + 0.35% LSE increased berry yield by 90.7% compared to all the controls. The tri-weekly application of PA increased percentage yield by 95%. The interaction between frequency and rates of PA application had positive effects on flower count, dry leaf weight, chlorophyll content, and plant height. Principal component analysis (PCA) biplot revealed positive influence of bi-weekly application of PA + 0.35% LSE on chlorophyll, anthocyanin, plant height, fresh and dry leaf weights irrespective of the application rate. The tri-weekly application of PA + 0.35% LSE was positively associated with berry yield. Overall, the tri-weekly application of 2% and 4% PA + 0.35% LSE was positively associated with fresh and dry leaf weights, anthocyanin content, flower count, photosystem II (Fv/Fm), and berry yield. Further studies are required to understand the mechanism of nutrient uptake *via* foliar application of PA + LSE.

Keywords: biostimulant, chlorophyll content, berry yield, 100-berry weight, leaf nutrients

3.1 INTRODUCTION

Haskap (*Lonicera caerulea L.*) is a popular plant native to China and Russia but currently being cultivated in North America due to its distinctive edible qualities (Bob Bors et al., 2012). Haskap belongs to the genus*, Lonicera* (*Caprifoliaceae* family), which comprises more than 200 species (M. M. Thompson, 2008). Haskap plants are winter hardy with self-incompatible flowers (Basu et al., 2010). Haskap berries contain high amounts of vitamins A and C, potassium, fibre, phenolic compounds, and antioxidants (Basu et al., 2010). The haskap varieties currently produced in Canada include 'Berry Blue', 'Borealis', 'Tundra', 'Aurora', 'Jolanta', 'Boreal Blizzard', 'Boreal Beast', 'Honey bee', and 'Indigo gem' (Rupasinghe et al., 2015; Khattab et al., 2017; Rupasinghe et al., 2018). The plant is relatively new in Canada with lots of investment potential, but growers are faced with limited information on the nutrients requirements, and disease control methods of haskap (Bors, 2009). The use of biostimulant to enhance haskap plant growth and yield is also understudied, which was the core of this study.

Pyroligneous acid (PA) is a harnessed product of burnt wood from pyrolysis (Mungkunkamchao et al., 2013). Its solution has murky brown to yellowish colour with an acidic pH depending on the feedstock (Mathew & Zakaria, 2015). Also known as wood vinegar, PA consists of about 200 water-soluble compounds containing organic acids (catechols, guaiacol, and syringols), essential oils, alcohol, phenolic, alkane, ketones, and ester compounds (Wei et al., 2010). It also consists of three main polymers, specifically hemicellulose (polyose), lignin, and cellulose (Balat et al., 2009). Source of materials for pyroligneous acid production include bamboo, agricultural biomass, algae, woody plants, and herbaceous plant biomass (Wei et al., 2010). Some studies have shown the use of PA as a priming agent, soil modifier, deodorizer, sterilizing agent, fertilizer, biostimulant, and antimicrobial agent (Mu. et al., 2003; Souza et al., 2012). For instance, PA has been shown to promote the growth of plants, flower production, and enhance fruit flavour (Zulkarami et al., 2011; Lu et al., 2019). Likewise, foliar application of PA has been documented to enhance seedling growth (Kulkarni et al., 2007). However, there is no reported study that explored the potential use of PA on perennial plants like haskap.

Seaweed extract has been used to boost plant growth in horticultural crops (Parađiković et al., 2018). As a biostimulant, seaweed contains auxins, vitamins, cytokinin, and abscisic acid, which affect cellular metabolism in plants resulting in increased crop yield and growth (Crouch & Staden, 1992). Positive results have been reported in strawberry (*Fragaria x ananassa*) plant growth (Parađiković et al., 2018). With high amounts of phenolic compounds, extracts from seaweed have been reported to protect plant cellular components when under stress while promoting plant immunity and improving crop performance (Khan et al., 2009; Cook et al., 2018). For example, Turan and Kose (2004) reported that foliar application of brown seaweed extract on grapevine (*Vitis* sp.) improved uptake of copper. Similarly, Crouch et al. (1990) confirmed iron uptake in lettuce (*Lactuca sativa*) through foliar application of seaweed extract. New studies have also demonstrated the stimulating effect of seaweed extract on plant immune response (Cook et al., 2018).

Several research have focused on natural biostimulant effects on plants like cereal, vegetables, and fruits (Parađiković et al., 2018). The use of PA in agriculture has been extensively reported but not for perennial plants such as haskap. A preliminary investigation demonstrated an increase in the efficacy of PA when mixed with LSE (unpublished). However, haskap response to PA with added LSE is unknown. Therefore, there is the need to investigate the biostimulant effects of PA with added LSE on the growth and yield of haskap plants under field conditions.

3.2 MATERIALS AND METHODS

3.2.1 Field Location, Soil Type, and Climatic Conditions

The field experiment was conducted at North 45 Orchards in Cloverdale, Stewiacke valley (latitude 45.18, longitude -63.22) located in Colchester county of Nova Scotia, Canada. The study was performed in the 2019 and 2020 growing seasons. The soil types in Cloverdale comprise of alluvium, loam to silty clay, and loam (Webb et al.,1991). The soil surface coverage contains less than 2 % of rocks and 0.01 % of stones. The soil types are imperfectly drained to poorly drained hence the soil remains wet for significant parts of the growing season in Cloverdale (Webb et al.,1991). Cloverdale has an average soil pH of 4.9 and a mixed form of forest vegetation (Gaye & Margret, 1994). The vicinity experiences the northern temperate zone weather conditions due to its closeness to the Atlantic Ocean.

3.2.1.1 Experimental Materials and Design

Haskap 'Indigo gem' was used for the experiment. The plants were transplanted at the age of two in October 2017 and were 4- and 5-year-old bushes at time of the studies in 2019 and 2020. Compound fertilizer containing 15% nitrogen (N), 15% phosphorus (P), and 25% potassium (K), (15-15-25 NPK) was applied in two splits for 2019, with the first application being 86.43 kg/ ha and 101.36 kg/ ha for the second application. In 2020, 54.43 kg of Micro-Essential® fertilizer was applied at the end of May and mid-June. The pyroligneous acid was obtained from Proton Power Inc. (Lenoir City) with its source from white pine biomass. The seaweed extract was obtained via kind donation by Kevin Shiell, Director of formulations, Acadian Plant health.

The experiment was arranged in a 3 x 8 factorial complete randomized block design with 3 replications. The PA with added LSE treatments were 2 % of PA + 0.35% of LSE, 4 % of PA + 0.35% of LSE, 6 % of PA + 0.35% of LSE, 8 % of PA + 0.35% of LSE, and 10 % of PA + 0.35%

of LSE. The controls were water alone, 4% PA alone and 0.35% LSE alone. These rates were applied at frequencies 7 (weekly), 14 (bi-weekly) and 21 (tri-weekly). All treatments applications were done by hand using a sprayer with 3.8 litres capacity. The leaves were sprayed until it dripped. However, days with rainfall were avoided as much as possible to prevent the washing of nutrients from plant leaf.

3.2.1.2 Field Management

Weed control was done using 1 L of Post Ultra mixed with 200L water/ha, which was applied between mid-May and end of July every year. Spacing between plants was 1.3 m between plants and 3.0 m between rows. During the fruiting season, nettings are used to prevent birds from feeding on the ripe berries. The plants are rainfed. The plots were grower managed. The experimental units were made up of 5 plants with 2 plants as buffers. The total number of haskap plants used were 120.

3.2.2 Plant Growth and Berry Yield Analysis

3.2.2.1 Plant height

Plant height was taken from the tip of the shoot to the ground level with a meter rule.

3.2.2.2 Fresh and Dry leaf matter content

Relatively young, fully expanded leaf samples were harvested from the 3rd and 4th nodes from the tip of a branch per treatment in mid-June for leaf dry weight analysis. Leaf samples were taken 2 to 3 hrs after sunrise and kept in labelled paper bags and placed in a cooler with ice for preservation. The fresh weight of the leaf samples was taken using an Ohaus Navigator® XT portable balance (ITM Instruments Inc., Canada) and placed in labelled paper bags. Measured leaf samples were then oven-dried at 65°C for 72 hrs using Millner 100 oven dryer (Cole-Palmer Co., USA) and the leaf dry weight were recorded.

3.2.2.3 Flower count

Flower count was conducted on three plants. The number of flowers per treatment were counted and recorded. The flower count was used to estimate potential berry yield.

3.2.2.4 Berry yield

Ripe haskap berries were harvested by hand from three plants per treatment in each treated block (3 blocks). The berries were weighed in the field before storing in coolers with icepacks and transported to a walk-in refrigerator on campus. One-hundred berries per treatment were selected randomly and weighed using the portable balance to obtain 100-fruit berry weight. Percentage change in berry yield with reference to farmers practice was calculated as [(average yield (Research) – Farmer's average yield) / Farmer's average yield x 100].

3.3.1.5 Leaf Anthocyanin and Chlorophyll content

The leaf anthocyanin content index (ACI) was determined using Anthocyanin content meter (Opti-Sciences Inc., New Hampshire, USA). The chlorophyll content of haskap leaf was determined using a SPAD 502 Chlorophyll meter (Konica Minolta, Inc., Illinois, USA).

3.2.2.6 Leaf chlorophyll fluorescence

Leaf chlorophyll fluorescence are generally used to examine plant photosynthetic activities. The chlorophyll fluorescence was determined using a portable OS30p+ Chlorophyll fluorometer (Opti-Sciences Inc., New Hampshire, USA) before taking samples for further testing. The mid-section of the 3rd and 4th young, fully expanded leaves excluding the midrib were clipped with a dark adaptation clip for 25 mins preceding measurement of chlorophyll fluorescence indices. The data taken involved variable fluorescence (Fv = Fm – Fo), where Fm and Fo represent the maximum and minimum fluorescence, respectively. The maximum quantum efficiency was determined by the ratio of variable fluorescence to maximum fluorescence (Fv/Fm). Also, the ratio of efficiency of electron donation to photosystem II was determined by the ratio of variable fluorescence to minimum fluorescence (Fv/ Fo) (Kalaji et al., 2011).

3.2.2.7 Electrolyte leakage

Electrolyte leakage was measured according to the method described by Huo et al. (2016) with some modifications. Six young leaves were collected from two plants per treatment and washed thoroughly with deionized water to remove surface-adhered electrolytes. The samples were cut into strips, placed in closed vials with deionized water, and incubated for 24 hrs. at 25°C. The initial electrical conductivity of the solution (EC_1) was determined by an EC 500 ExStik conductivity meter (EXTECH Instrument, Taiwan). Samples were then autoclaved at 120 °C for 20 min, and the final electrical conductivity of the solution (EC_2) was measured after cooling. The percentage of electrolyte leakage was determined by the formula $\% EC = EC_1 / EC_2 \times 100$.

3.2.2.8 Mineral nutrient analysis

Selected leaves from each treatment were flash frozen in liquid nitrogen and sent to the Nova Scotia Department of Agriculture Laboratory Services, Truro for leaf tissue nutrients analysis. The leaves total macro-nutrients and micro-nutrients) were determined using the inductively coupled plasma spectroscopic method and the combustion method (AOAC, 2003).

3.2.3 Statistical Analysis

Data were analyzed with Minitab (Minitab 19 Statistical software, USA). Means with significant differences at $P < 0.05$ were separated using Fisher and Tukeys LSD method at alpha = 0.05. Graphs were drawn using Microsoft Excel. A biplot of principle component analysis (PCA) was performed using XLSTAT Version 19.1 to determine the association between treatment and frequency on the growth and yield parameters measured.

3.3 RESULTS AND DISCUSSION

3.3.1 Plant Growth Analysis

Frequency (i.e., weekly, bi-weekly, and tri-weekly) of PA application had significant ($P < 0.05$) effect on haskap plant growth and yield components (Table 3.1). Frequency of PA had significant ($P < 0.05$) effect on flower count, fresh leaf weight, photosynthetic capacity (Fv/Fo), chlorophyll content, and berry yield. However, the rates of PA application had no significance ($P > 0.05$) on the growth and yield components excerpt marginal ($P = 0.05$) effect on chlorophyll content (Table 3.2). Haskap plants treated bi-weekly and tri-weekly with PA recorded the highest number of flowers compared with the weekly treated plants, which recorded the least flower count (Table 3.1). The bi-weekly treatment of haskap plants increased fresh leaf weight and chlorophyll content with the least from the weekly treatment of plants (Table 3.1). The application rate of 10% PA + 0.35% LSE increased chlorophyll content in comparison with the control (Table 3.2). Values of chlorophyll fluorescence was used to evaluate photosynthetic activities of the haskap plants in response to varying frequency and rate of PA application. Haskap plants treated weekly had higher photosynthetic capacity (Fv/Fo) compared with the tri-weekly treated plants with the least effect from the bi-weekly application. Values for maximum quantum efficiency of Photosystem II (Fv/Fm) ranged from 0.79 to 0.80 and was not significantly ($P > 0.05$) different for the different frequency and rate of PA application treatments. A plant is considered healthy if its measured Fv/Fm value ranges from 0.75 to 0.85 (Bolhar-Nordenkampf & Oquist, 1993). Hence, this suggests that the application of PA could have promoted haskap plant health and growth. Some studies have reported the positive influence of PA application on the growth of okra (*Abelmoschus esculentus*), tomatoes (*Solanum lycopersicum*) and rockmelon (*Cucumis velo* var *cantalupensis*) (van Staden et al., 2006; Zulkarami et al., 2011). Additionally, PA is considered rich in organic acids, alcohol, alkane, and ester compounds, which could have enhanced plant development and growth (Zulkarami et al., 2011). Likewise, compounds like methyl formate and methyl acetate in PA stimulate plant growth and development (Travero & Mihara, 2016). These organic compounds modulate plant growth by influencing protein synthesis, ion assimilation, hormonal balance, and respiratory metabolic rate (Gulzar et al., 2016; Latif et al., 2017). Hence, the increase in chlorophyll content, photosynthetic capacity, fresh leaf weight, and flower count with frequency of PA application can be attributed to the possible influence by organic acids. Equally, some studies suggested the likeness of PA to other plant growth regulators due to certain bioactive components such as karrikins (Senaratna et al., 1999; Gardner et al., 2001; Chiwocha et al., 2009). Gardner et al. (2001) suggested that the presence of organic acids, alcohol, many ester compounds, and heavy metals in PA may influence plant growth and yield. Some studies have indicated that PA interacts with plant hormones like cytokinins, ethylene, gibberellins, and abscisic acid in thermo-dormant and photoblastic seeds (van Staden et al., 2000)

Table 3. 1. Effect of the frequency of pyroligneous acid (PA) with added liquid seaweed extract (LSE) on haskap growth and yield components.

Frequency	**Flower count**	**Fresh leaf dry weight (g)**	**Leaf dry weight (g)**	**SPAD value**	**Plant height (cm)**	**ACI**	**Fv/Fm**	**Fv/Fo**	**Berry yield (g)**	**100-berry weight (g)**
F 7	91.13 b	19.38b	18.48 a	35.11 b	64.28 a	7.61 a	0.79 a	3.92 a	207.30 b	69.70 b
F 14	110.11 a	23.85 a	18.73 a	35.64 a	66.66 a	7.88 a	0. 78a	3.78 a	274.80 ab	76.50 ab
F 21	121.47 a	20.15 a	18.73 a	35.57 a	66.13 a	7.53 a	0.79a	3.91 a	351.00 a	79.30 a

Frequency = weekly application (F7), bi-weekly application (F14), and tri-weekly application (F21); Fv/Fm=maximum quantum efficiency of Photosystem II; Fv/Fo = photosynthetic capacity; SPAD value = chlorophyll content; ACI = anthocyanin content index.

Means that do not share a letter are significantly different.

Table 3. 2. Effect of the rates of pyroligneous acid (PA) with added liquid seaweed extract (LSE) on haskap growth and yield components.

Rates (%)	**Flower count**	**Fresh Leaf weight (g)**	**leaf dry weight (g)**	**SPAD value**	**Plant height (cm)**	**ACI**	**Fv/Fm**	**Fv/Fo**	**Berry yield (g)**	**100-berry weight (g)**
T1	101.26 ab	21.83 a	18.61 abc	34.97 b	62.73b	7.82 a	0.79 a	3.87 a	292.00 a	78.60 a
T2	116.26 ab	21.50 a	18.94 abc	35.56 ab	67.55 a	7.90 a	0. 78a	3.84 a	299.30 a	73.40 a
T3	117.48 ab	21.11 a	20.44 a	35.18 b	65.31ab	7.94 a	0.79 a	3.91 a	289.80 a	73.40 a
T4	102.15 ab	20.72 a	17.94 bc	35.22 b	64.08 ab	7.43 a	0.79 a	3.88 a	248.90 a	76.60 a
T5	121.22 a	21.44 a	18.11 abc	35.34 b	67.27 a	7.54 a	0. 79a	3.89 a	343.30 a	77.90 a
T6	98.37 ab	21.39 a	17.16 c	36.18 a	64.51 ab	7.96 a	0.79 a	3.86 a	260.70 a	70.40 a
T7	114.37 ab	20.66 a	18.33 abc	35.44 b	66.76 ab	7.27 a	0.78 a	3.88 a	212.70 a	72.40 a
T8	89.44 b	20.33 a	19.61 a	35.63 ab	67.32 a	7.55 a	0.78 a	3.87 a	275.10 a	78.70 a

T1 = control (water); T2 = 2% PA + 0.35% LSE; T3 = 4% PA + 0.35% LSE; T4 = 6% PA + 0.35% LSE; T5 = 8% PA + 0.35% LSE; T6 = 10% PA + 0.35% LSE; T7= 4% PA alone and T8 = 0.35% LSE alone; PA = pyroligneous acid and LSE = liquid seaweed extract; Fv/Fm= maximum quantum efficiency of Photosystem II; Fv/Fo = photosynthetic capacity; SPAD = chlorophyll content; ACI = anthocyanin content index.

Table 3. 3. Interaction mean effects and p-values of flower count, dry leaf weight, chlorophyll content, plant height, berry yield and 100 berry weight as affected by pyroligneous acid (PA) with added liquid seaweed extract (LSE).

Growth and Yield Parameters						
Treatment interaction	**Flower count**	**Leaf dry weight (g)**	**SPAD value**	**Plant height (cm)**	**Berry yield (g)**	**100-berry weight (g)**
F7 T1	140.67 abcd	19.17 abcde	35.11 defghi	64.00 bcde	35.11 defghi	77.50 abc
F7 T2	117.11 abcde	19.50 abcde	36.67 abc	72.00 a	36.67 ab	71.33 abc
F7 T3	145.67abc	22.00 a	35.21 defgh	60.53 de	35.21 defgh	72.00 abc
F7 T4	113.22 abcdef	16.67 def	33.89 i	60.90 cde	33.89i	67.66 abc
F7 T5	93.22 defg	19.50 abcde	35.71 abcdef	66.10 abcd	35.71 abcdef	75.50 abc
F7 T6	103.56 bcdefg	17.50 cdef	35.15 defgh	57.93 e	35.15 defgh	53.17 c
F7 T7	134.33 abcd	15.33 f	35.07 defghi	64.90 abcde	35.07 defghi	63.50 bc
F7 T8	95.00 cdefg	18.17 bcdef	34.09 hi	67.90 abc	34.09 hi	77.00 abc
F14 T1	76.44 efg	17.67 cdef	34.45 ghi	60.33 de	34.45 ghi	77.83 abc
F14 T2	91.00 defg	20.50 abc	34.91 efghi	64.07 bcde	34.91 efghi	76.33 abc
F14 T3	140.67 abcd	20.33 abc	35.73 abcdef	68.30 ab	35.73 abcdef	75.33 abc
F14 T4	117.11 abcde	19.50 abcde	36.28 abcd	68.00 abc	36.28 abcd	71.17 abc
F14 T5	145.67abc	17.67 cdef	35.17 defgh	70.33 ab	35.17 defgh	79.17 abc
F14 T6	113.22 abcdef	16.00 ef	36.84 a	66.73 abcd	36.84 a	81.50 ab
F14 T7	93.22 defg	18.00 bcdef	35.22 defgh	65.43 abcd	35.22defgh	70.67 abc
F14 T8	103.56 bcdefg	20.17 abcd	36.54 abc	70.10 ab	36.54 abc	80.13 ab
F21 T1	134.33 abcd	19.00 abcdef	35.36 cdefg	63.87 bcde	35.36 cdefg	80.33 ab

Treatment interaction	Flower count	Leaf dry weight (g)	SPAD value	Plant height (cm)	Berry yield (g)	100-berry weight (g)
F21 T2	95.00 cdefg	16.83 cdef	35.11 defghi	66.60 abcd	35.11 defghi	72.50 abc
F21 T3	146.33 abc	19.00 abcdef	34.59 fghi	67.10 abcd	34.59 fghi	73.00 ab
F21 T4	128.78 abcd	17.67 cdef	35.50 bcdefg	63.33 bcde	35.50 bcdefg	91.00 a
F21 T5	107.22 bcdefg	17.17 cdef	35.15 defgh	65.37 abcd	35.15 defgh	79.00 abc
F21 T6	120.44 abcde	18.00 bcdef	36.58abc	68.87 ab	36.58 abc	76.50 abc
F21 T7	76.44 efg	21.67 ab	36.02 abcde	69.97 ab	36.02 abcd	83.16 ab
F21 T8	91.00 defg	20.50 abc	36.25 abcd	63.97 bcde	36.25 abcd	78.83 abc
P-value	0.001	0.053	0.000	0.031	0.956	0.903

ns, indicates no significance at $P < 0.05$.

Frequency = weekly application (F7), bi-weekly application (F14); tri-weekly application (F21); Treatment = T1 = control (water); T2 = 2% PA + 0.35% LSE; T3 = 4% PA + 0.35% LSE; T4 = 6% PA + 0.35% LSE; T5 = 8% PA + 0.35% LSE; T6 = 10% PA + 0.35% LSE; T7= 4% PA alone and T8 = 0.35% LSE alone; SPAD value = chlorophyll content.

Means that do not share a letter are significantly different.

The interaction effect model represents the combinative effects of frequency and rates on haskap growth and yield. The present study revealed significant ($P < 0.05$) interaction effect between frequency and rates of PA on chlorophyll content, flower count, leaf dry weight, and plant height (Table 3.3). The chlorophyll content was increased by treatments 2% and 10% PA + 0.35% LSE applied weekly and bi-weekly, respectively. Nonetheless, the application of 6% PA + 0.35% LSE applied bi-weekly recorded the least chlorophyll content. This suggests that the application of PA at minimum or higher rates like 10% have the potential of increasing chlorophyll content and improving plant growth. However, further increase in PA rate (i.e. > 10%) could be detrimental to plant growth as reported by Mmojieje & Hornung (2015). Plant leaf pigments like chlorophyll are vital for the trapping of light, photoinhibition, and boosting effectiveness in the photosynthetic system of plants (Zhujun et al., 2019). Hence, the increase in chlorophyll content with PA application could possibly be due to a boost in cell development and increased nutrient uptake (Mungkunkamchao et al., 2013). Some studies suggested that the application of PA via foliar method enhances chlorophyll content, which leads to an increase in photosynthesis and synthesis of amino acids and sugars (Grewal et al., 2018). Thus, the frequency of PA application may have caused an increase in photosynthetic activities resulting in the increase in leaf fresh weight (Table 3.1). A typical response of plants to seaweed extract is the increase in chlorophyll levels which was evident in this study. This effect has been documented in plants like strawberry and grapevine (Spinelli et al., 2010; Fan et al., 2013; Jannin et al., 2013). Plant molecular studies have proven that an increase in chlorophyll content can be attributed to the presence of betaine compounds in LSE, which contributes to an increase in chloroplast production and reduction in plant leaf senescence (Rayorath et al., 2008; Nair et al., 2012; Jannin et al., 2013). Hence, the combination of PA + 0.35% LSE could have elicited an increase in the chlorophyll content of haskap leaves.

Weekly application of 4% PA+ 0.35% LSE increased leaf dry weight, which was reduced by the 4% PA alone applied at weekly intervals (Table 3.4). The 4% PA + 0.35% LSE applied weekly yielded higher leaf dry weight with the least by the 4% PA alone applied weekly (Table 3.3). Also, haskap plant height was increased with the weekly application of 2% PA + 0.35% LSE but was reduced by the application of 10% PA + 0.35% LSE applied at the same frequency. Interestingly, the presence of ester and polyphenols in PA stimulates plant development (Zulkaramiet al., 2011), which might have enhanced the haskap plant height following the application of PA.

3.3.2 Berry Yield Analysis

Berry yield in the present study was significantly ($P < 0.05$) influenced by PA application. There was a significant ($P < 0.05$) increase in yield for frequency of PA application but not rate of PA and their interaction. Tri-weekly application of PA increased yield output followed by bi-weekly application with the least recorded by the weekly application (Table 3.1). Thus, frequency of PA application could have positive or negative effect on crop yield which in this study was positive.

Although there was no significant ($P > 0.05$) difference of treatment effect on yield, 8% PA+ 0.35% LSE increased yield with the least effect from 4% PA alone (Table 3.2). This probably explain the synergistic effect of PA and LSE on berry yield. This further suggests that the 8% PA+ 0.35% LSE may have influenced the uptake of nutrients for plant use causing an increase in yield. A study by Zulkarami et al., (2011) showed that yield of rock melon (*Cucumis melo* var Cantalupensis) increased when 10% PA was used in combination with local fertilizer formulation, suggesting that PA alone may not influence yield. Oddly, the interaction effect for water alone applied tri-weekly increased yield output (Table 3.3). The least yield output was recorded for 6% PA+ 0.35% LSE treatment applied weekly

The percentage change in yield with reference to the farmer's average was 90.7% for plants treated with the 8% PA+ 0.35% LSE with the least recorded by plants treated with the 4% PA alone at 18.1% (Figure 3.1). However, plants treated with water alone (negative control), 2% and 4% PA+ 0.35% LSE were not significantly ($P > 0.05$) different with respect to percentage change in yield (Figure 3.1). The tri-weekly application of PA recorded 95% increase in yield with the least from the weekly application of PA i.e. 15.2% (Figure 3.2). The increase in yield coincided with similar studies in which yield increased with foliar application of PA on tomatoes and rock melon (Kulkarni et al., 2008; Zulkarami et al., 2011). For instance, foliar application of PA significantly increased the yield of cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*), and cole crops (*Brassica oleracea* var. *capitata* f. *rubra*) (Mu et al., 2006). Likewise, foliar application of PA improved crop yield for most farmers in Thailand (Yoshimura et al., 1995; Katoda & Niimi, 2004; Jun et al., 2006). Additionally, a study by Travero & Mihara (2016) showed that PA applied to soybeans (*Glycine max*) increased yield by 3.1 tons/ha with no effect on plant growth. Likewise, the application of PA improved yield in organic rainfed rice combined with cattle manure (Anan et al., 2015). Similarly, increase in yield has been shown with foliar application of LSE on pear (*Pyrus spp*.) (Colavita et al., 2011), mango (*Magnifera indica*) (Mohamed & El-Sehrawy, 2013), banana (Karthikeyan & Shanmugam, 2014), and apple (Basak, 2008).

Nonetheless, rate and frequency of application had no significant ($P > 0.05$) effect on 100-berry weight. This suggests that poor weed management and varying weather conditions could have played a significant role in the berry development process, affecting berry weight. Comparatively, yield output per plant from existing studies was comparatively higher than that obtained from this study. For instance, the yield output per plant from these studies ranged from 500 g to 1,500 g (Plekhanova, 2000; Malodobry et al., 2010; Miyashita & Hoshino, 2010; Ochmian et al., 2012).

The plants used for this study were relatively young (4-5 years), and yield increase per plant could be expected in the coming seasons.

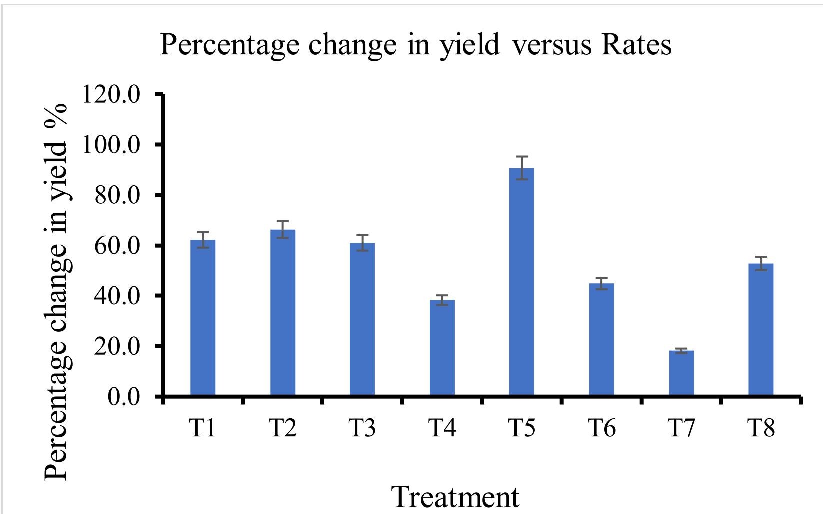


Figure 3. 1. Percentage change of haskap yield in response to rates of pyroligneous acid (PA) with added liquid seaweed extract (LSE) application T1 = control (water); T2 = 2% PA + 0.35% LSE; T3 = 4% PA + 0.35% LSE; T4 = 6% PA + 0.35% LSE; T5 = 8% PA + 0.35% LSE; T6 = 10% PA + 0.35% LSE; T7= 4% PA alone; T8 = 0.35% LSE alone; LSE = liquid seaweed extract.

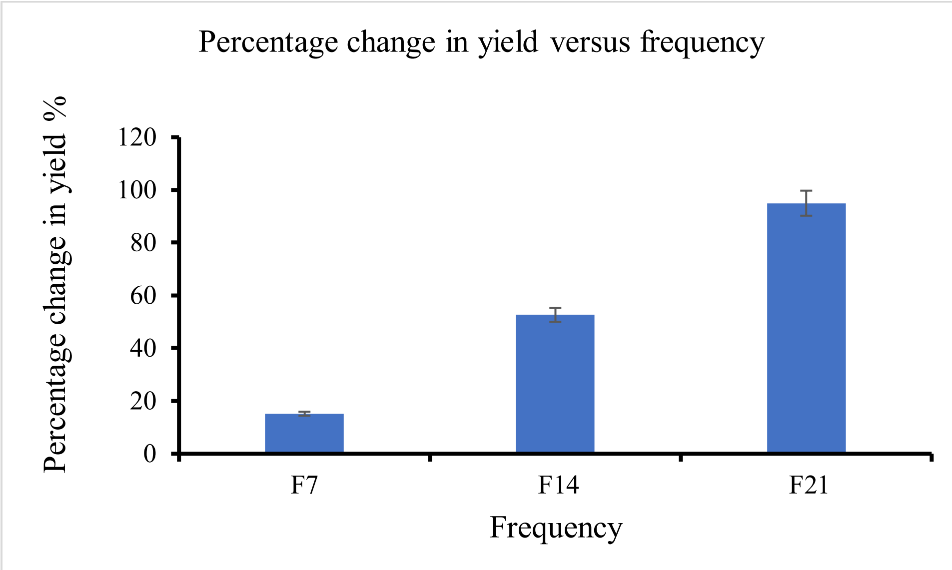


Figure 3.2. Percentage change of haskap yield in response to frequency of pyroligneous acid (PA) with added liquid seaweed extract (LSE) application F7 = weekly application; F14= bi-weekly application; F21= tri-weekly application.

3.3.3 Leaf Nutrient Analysis

The 8% and 10% PA + 0.35% LSE treatment increased nitrogen (N) content of the haskap leaves compared to the other treatments (Table 3.4). Nitrogen plays important roles in plant respiration, cell division and growth (Zekri, 1995). Hence, the increase in N content with the 10% PA + 0.35% LSE application may have contributed to increased chlorophyll content. This may have equally enhanced berry yield with the application of the 8% PA + 0.35% LSE due to increased photosynthetic activities with increased N content. However, the leaf tissue calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P) contents, and sodium (Na) were not significantly ($P > 0.05$) affected by variation in the PA rate (Table 3.4). The decreased plant tissue content of Ca, K, Mg, and P with increase in PA rates may have resulted in decreased berry yield. For instance, calcium partakes in auxin, gibberellic acid and abscisic acid signalling to control cell division, cell growth and fruit set (Yu et al., 2006). Therefore, Ca reduction may have caused less fruit set which might have reduced berry yield. A study by Zulkarami et al. (2011) indicated that increased leaf Mg content corresponded with increased fruit development and fruit weight of rock melon following PA application. Hence, leaf tissue Mg content reduction probably may have affected haskap berry weight.

The application of 2% and 8% PA + 0.35% LSE increased leaf tissue boron (B) content whilst the control (water alone) recorded the least leaf tissue B content (Table 3.4.1). The presence of increased leaf tissue B content results in leaf, flower, and fruit development (Dell & Huang, 1997; Kot, 2009). Therefore, the increase in plant tissue B content may have increased chlorophyll content, flower count and berry yield with decreased PA rates (thus < 8%). However, the rate effect on copper (Cu) content in haskap leaves was not different. Interestingly, haskap leaves treated with water alone increased levels of iron (Fe) in comparison with 4% PA + 0.35% LSE treatment. Reduced Fe content was observed in plants treated with LSE alone. Leaf tissue manganese (Mn) was increased with the application of 2%, 4%, and 6% PA + 0.35% LSE. The least Mn content was observed for haskap plants treated with water alone (Table 3.4.1). Leaf tissue Mn are generally needed in minute amounts for plant use (Broadley et al., 2012). Hence, an increase may be detrimental to plant growth and development. Increased Mn contents have been reported to decrease photosynthetic rate and inhibit the absorption and translocation of mineral nutrients like Mg, Ca, Fe, and P (Amao & Ohashi, 2008; Blamey et al., 2015; Lešková et al., 2017). Thus, an increase in Mn content with 2%, 4%, and 6% PA + 0.35% LSE may have reduced uptake of Mg, Ca, Fe, and P thereby influencing plant growth and yield.

Notably, the 6% PA + 0.35% LSE increased zinc (Zn) content compared with plants treated with 2% PA + 0.35% LSE and 0.35% LSE alone. Haskap plant treated with 8% PA + 0.35% LSE observed the least Zn content. Overall, the increase in certain leaf tissue nutrients suggests that PA may have improved the uptake of nutrients for plant use. A study by Hiyasmin et al. (2016) suggested that the acidic property of PA due to presence of acetic acid may have aided in the assimilation of mineral nutrients by plants. Tuntika et al. (2013) reported that PA has minute amounts of N, which may not elicit the same response as N fertilizers in leaf chlorophyll measurement. This could possibly be related to a decrease in chlorophyll content with decreased PA rates. Nevertheless, the synergistic effect of PA and LSE may have enhanced the presence of the macro- and micro-nutrients resulting in improved plant growth based on increased chlorophyll content and fresh leaf weight.

Table 3. 4. Haskap leaf macro-nutrients composition on a leaf dry weight basis as affected by pyroligneous acid (PA) with added liquid seaweed extract (LSE).

Rate (%)	**N (mg/L)**	**Ca (mg/L)**	**K (mg/L)**	**Mg (mg/L)**	**P (mg/L)**	**Na (mg/L)**
T1	2.02	1.70	0.60	0.57	0.16	0.00
T2	1.99	1.76	0.59	0.58	0.16	0.01
T3	2.03	1.68	0.54	0.55	0.15	0.01
T4	1.91	1.57	0.63	0.53	0.15	0.01
T5	2.07	1.62	0.58	0.53	0.15	0.01
T6	2.05	1.58	0.62	0.55	0.16	0.01
T7	2.02	1.64	0.67	0.52	0.17	0.00
T8	2.07	1.69	0.63	0.55	0.16	0.01

T1 = control (water); T2 = 2% PA + 0.35% LSE; T3 = 4% PA + 0.35% LSE; T4 = 6% PA + 0.35% LSE; T5 = 8% PA + 0.35% LSE; T6 = 10% PA + 0.35% LSE; T7 = 4% PA alone; T8 = 0.35% LSE alone; PA =

pyroligneous acid LSE = liquid seaweed extract; N = nitrogen; Ca = calcium; K = potassium; Mg = magnesium and P = phosphorus.

Table 3.4. 1. Haskap leaf micro-nutrients composition on leaf dry weight basis as affected by pyroligneous acid (PA) with added liquid seaweed extract (LSE)

Rate (%)	B (mg/L)	Cu (mg/L)	Fe (mg/L)	Mn (mg/L)	Zn (mg/L)
T1	36.13	7.69	104.01	92.21	22.90
T2	39.94	7.50	84.62	143.25	25.55
T3	38.79	7.77	95.34	163.97	22.80
T4	37.51	7.61	86.33	142.88	26.04
T5	39.00	7.47	87.40	118.65	21.14
T6	37.51	7.65	85.30	132.75	22.58
T7	36.24	7.47	88.12	106.24	24.91
T8	38.50	7.54	83.24	129.64	25.12

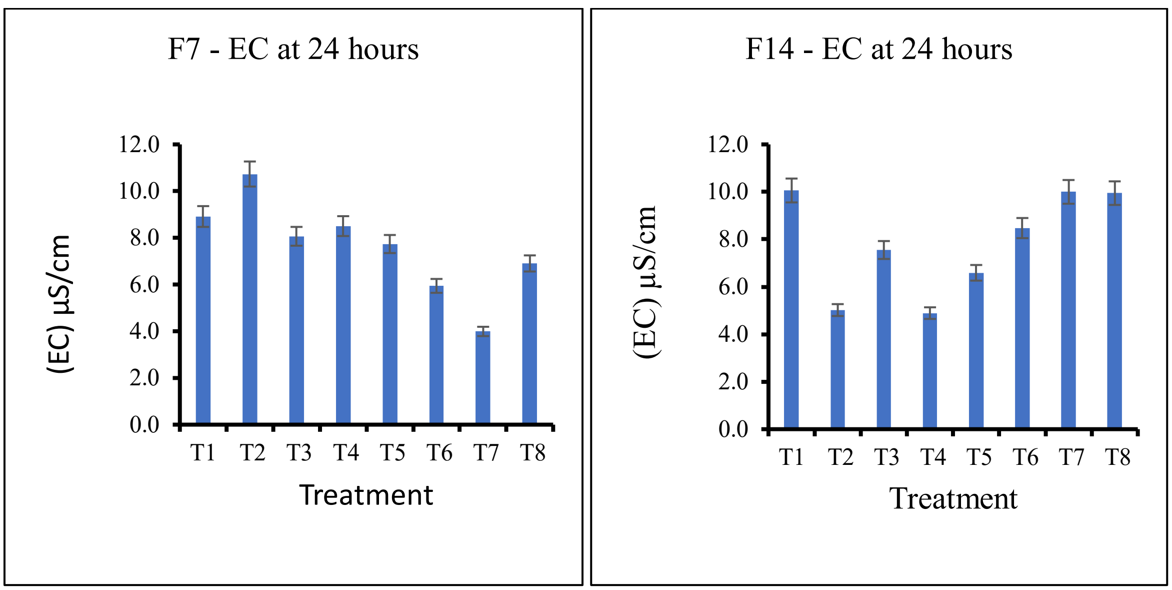
T1 = control (water); T2 = 2% PA + 0.35% LSE; T3 = 4% PA + 0.35% LSE; T4 = 6% PA + 0.35% LSE; T5 = 8% PA + 0.35% LSE; T6 = 10% PA + 0.35% LSE; T7 = 4% PA alone; T8 = 0.35% LSE only; PA = pyroligneous acid; LSE = liquid seaweed extract; B = boron; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc.

3.3.4 Electrical Conductivity, Total Dissolved Solids, and Salt Content

Generally, electrical conductivity (EC) measures the overall ionic concentration in solution while total dissolved solids (TDS) represent the aggregate concentration of these mineral components in a solution (Phonphan et al., 2014; Rahman et al., 2015). The weekly application of the 2% PA + 0.35% LSE increased the EC of leaves in haskap plants by 10.7% while the 4% PA alone reduced it by 4.0% after 24 hrs (Figure 3.2). Plants treated with water alone, and 4% and 6% PA + 0.35% LSE moderately increased EC by 8.9 μS/cm, 8.5 μS/cm, and 8.1 μS/cm, respectively with weekly application of PA (Figure 3.3). The EC of haskap plants treated with water alone, 4% PA alone, and 0.35% LSE alone increased by 10.1 µS/cm, 10.0 µS/cm, and 9.9 µS/cm with bi-weekly application, respectively (Figure 3.3). However, the 2% and 6% PA + 0.35% LSE applied bi-weekly reduced EC by 5.0 µS/cm and 4.9 µS/cm, respectively (Figure 3.3). Tri-weekly foliar application of water alone on haskap plant increased EC by 8.9 µS/cm after 24 hrs. (Figure 3.2). However, the 6% PA + 0.35% LSE reduced EC by 6.3 µS/cm. The increase in EC levels with frequency of PA application may have negatively affected leaf dry weight and yield of haskap (Table 3.1). Increased EC levels decrease the fixation of carbon dioxide in plants due to reduced stomatal conductance (Xu et al., 1994). This resulted in reduced leaf dry weight because of reduced leaf area and photosynthetic capacity stemming from the closure of stomata (Tadesse et al., 1999). Hence, the reduction in leaf dry weight which was evident in this study with frequency and rate of PA. Tadesse et al. (1999) suggested that decreased leaf area and photosynthesis may have resulted in decreased tomato (*Solanum lycopersicum*) yield with increased EC levels.

Application of water alone to the haskap plants recorded higher TDS content by 9.0 mg/L with weekly application of PA. However, the 10% PA + 0.35% LSE reduced TDS by 4.1 mg/L (Figure S1). The application of water alone, 4% PA alone, and 0.35% LSE alone increased TDS content of haskap plants by 6.7, 6.7, and 6.4 mg/L, respectively with bi-weekly application. The treatment of 2% and 6% PA + 0.35% LSE reduced TDS content by 3.3 and 3.2 mg/L with bi-weekly application. The tri-weekly application of water alone increased TDS content by 8.9 mg/L while the 6% PA + 0.35% LSE reduced TDS content by 6.3 mg/L. The increase in TDS with PA application may be associated with the increase in N, B, Mn, Zn, and Fe. This may have improved overall growth of haskap.

The weekly application of 2% PA + 0.35% LSE increased salinity by 15.9 mg/L while 4% PA alone reduced salinity by 6.2 mg/L (Figure S2). The treatment of water (control), 4% PA alone, and 0.35% LSE alone increased salinity of the haskap plants by 10.2, 9.6, and 9.5 mg/L, respectively with bi-weekly application (Figure S2). The treatment of haskap plants with 10% PA+ 0.35% LSE increased salinity by 9.0 mg/L while 6% PA + 0.35% LSE reduced salinity by 6.3 mg/L. The increase in salinity (i.e. > 10 mg/L) may have caused an increase in the distribution of nutrients for haskap plant growth over berry development (Ieperen, 1996). This may have resulted in decreased berry yield with subsequent effect on berry weight.



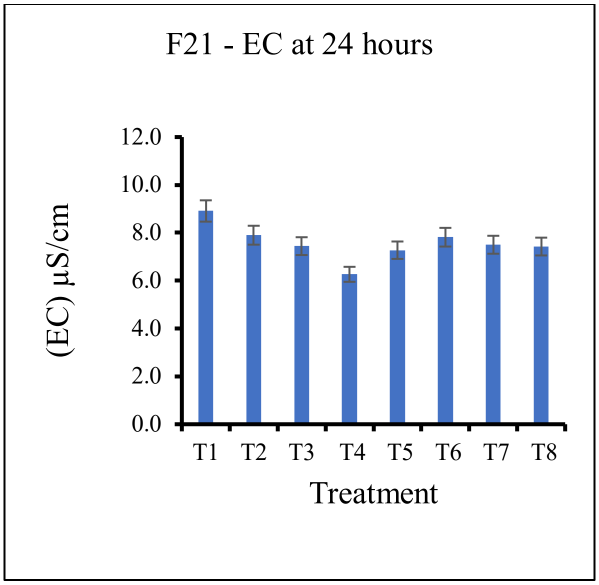


Figure 3. 3. Effects of pyroligneous acid (PA) with added liquid seaweed extract (LSE) rates and frequency on electrical conductivity in haskap plant leaves T1 = control (water); T2 = 2% PA + 0.35% LSE; T3 = 4% PA + 0.35% LSE; T4 = 6% PA + 0.35% LSE; T5 = 8% PA + 0.35% LSE; T6 = 10% PA + 0.35% LSE; T7 = 4% PA alone; T8 = 0.35% LSE alone, F7= weekly application; F14 = bi-weekly application; F21 = tri-weekly application; LSE= liquid seaweed extract. Vertical in lines on the bars represent standard error of the means.

3.3.5 Principal Component Analysis Biplot

Typically, the two-dimension (2-D) principal component analysis (PCA) biplot shows the relationship between the different variables (Figure 3.4). The PCA biplot for rates showed projections of the plant growth and yield parameters in the factor space (i.e., F1 and F2) and explained approximately 48.4% of the total variations in the dataset. Plant growth and yield parameters that were clustered together suggested positive relationships, while parameters that separated suggested a negative relationship. Parameters located at right-angles suggested no relationship. Parameters assembled with a treatment indicated that that rate influenced them, while parameters that were distant from a rate indicated that that rate did not influence them. The PCA biplot revealed that the control application positively affected TDS, EC, and salt due to increases in Ca, Fe, and Mg concentration. This suggests that the presence of high TDS and salt levels can be attributed to the presence of high concentrations of cations, namely, Ca, Fe, Mg, and N.

The 2% PA + 0.35% LSE application had a positive influence on Mg, N, Cu, fresh and dry leaf weight, flower count, anthocyanin content, and berry yield but effects on Fv/Fm, Fe, Ca, salt, EC, and TDS were moderate (Figure 3.3) (Ahmad et al., 2009; Janan et al., 2016). Nevertheless, the 2% PA + 0.35% LSE application negatively influenced plant height, Mn, Na, B, and Fv/Fo. The increased concentration of Cu with treatment 2% PA + 0.35% LSE might have negatively influenced plant height as an increase in Cu could inhibit plant growth (Miotto et al., 2014).

Furthermore, the 4% PA + 0.35% LSE had a positive influence on Fv/Fm, B, flower count, and berry yield. Thus, an increase in flower count and leaf tissue nutrient B may have resulted in a positive influence on berry yield. However, 4% PA + 0.35% LSE negative influence on chlorophyll content, Fv/Fo, Mn, Na, B, and plant height may have resulted from increased leaf tissue Cu.

Also, the 6% PA + 0.35% LSE positively influenced chlorophyll content and plant height but had negative influence on other plant growth and yield parameters. This may account for the reduction in berry yield with the 6% PA + 0.35% LSE treatment. Likewise, the 6% PA + 0.35% LSE may have promoted haskap plant growth at the expense of berry development.

The 8% PA + 0.35% LSE positively influenced Fv/Fo, flower count, B, Mn, and Na, with a moderate effect on plant height. Such influence with Fv/Fo and leaf tissue B may account for the increase in plant height and flower count. The presence of increased B content results in leaf, flower, and fruit development (Dell & Huang, 1997; Kot, 2009). However, increase in Mn may have contributed to decreased chlorophyll content (Amao & Ohashi, 2008).

The 10% PA + 0.35% LSE positively influenced leaf tissue Zn, K, and P, plant height and chlorophyll content. The influence of 10% PA + 0.35% LSE on leaf tissue Zn, K, and P may have increased photosynthetic rate with increased chlorophyll content. This may have resulted in an increase plant height.

Interestingly, the 4% PA alone and the 0.35% LSE alone had a positive influence on plant tissue K and Zn but moderate influence on chlorophyll content and P concentration. This suggest that the influence on plant tissue Zn could be linked with moderate effects on chlorophyll content. However, the use of 4% PA alone did not influence the growth and yield of haskap.

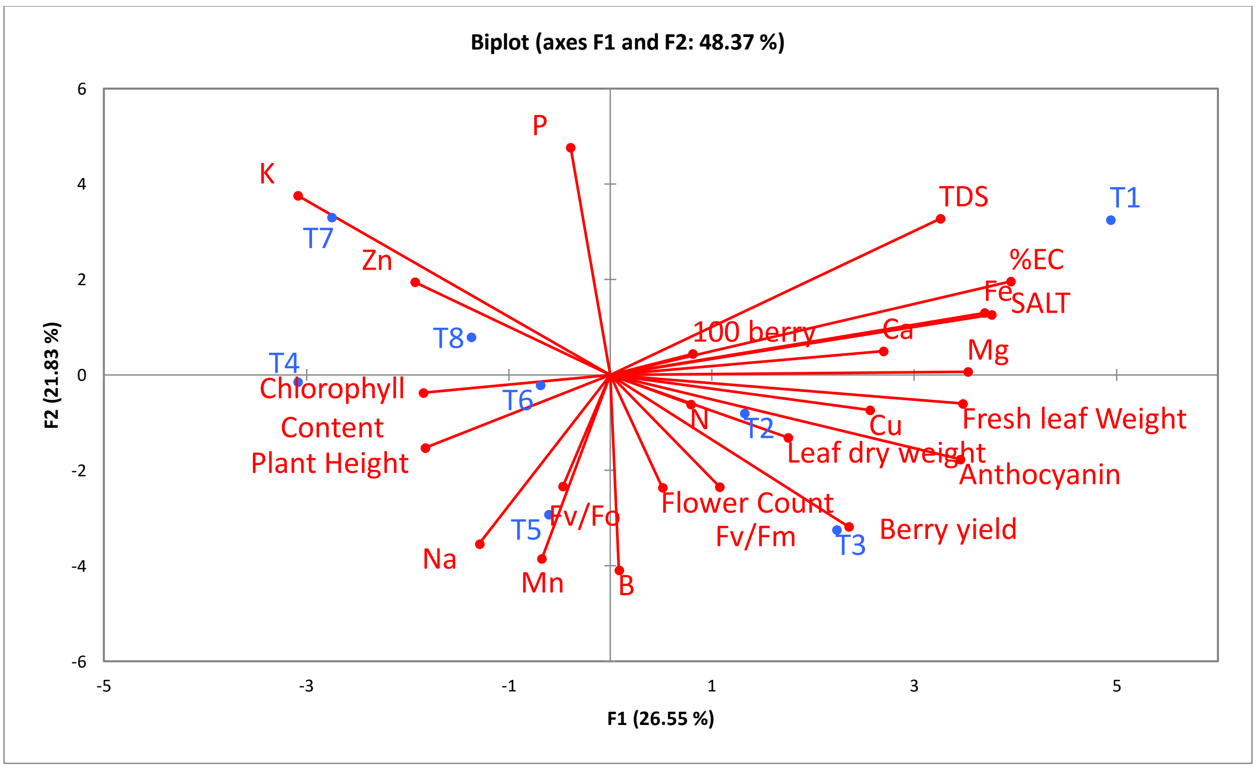


Figure 3. 4. A two-dimensional (2-D) principal component analysis biplot (PCA) showing relationships amongst pyroligneous acid (PA) with added liquid seaweed extract (LSE) treatments and growth and yield parameters of haskap 'Indigo Gem'. TDS = total dissolved solids; EC = electrical conductivity; Fv/Fm = maximum quantum efficiency of Photosystem II; Fv/Fo = photosynthetic capacity; K = potassium, P =

phosphorus; N = nitrogen; Ca = calcium; Mg = magnesium; L B = boron; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc;. T1 = control (water alone); T2 = 2% PA + 0.35% LSE; T3 = 4% PA + 0.35% LSE; T4 = 6% PA + 0.35% LSE; T5 = 8% PA + 0.35% LSE; T6 = 10% PA + 0.35% LSE; T7 = 4% PA alone; T8 = 0.35% LSE alone; PA = pyroligneous acid; LSE = liquid seaweed extract.

3.4 CONCLUSION

In conclusion, the application of varying frequency and rate of PA is a novel approach. The preliminary findings suggests that plant growth and yield responses to weekly, bi-weekly and tri-weekly of PA with added 0.35% LSE were significant ($P < 0.05$) for photosynthetic capacity (Fv/Fm), chlorophyll content, flower count, fresh leaf weight, and 100-berry weight. However, rates of PA had marginal ($P = 0.05$) effect on chlorophyll content. The percentage change in yield with farmer's average was increased by about 91% with the 8% PA + 0.35% LSE application but was reduced by about 18% with 4% PA alone. The 2-D principal component analysis (PCA) biplot revealed the positive influence of bi-weekly application of PA on haskap plant growth with increases in anthocyanin, fresh and dry leaf weight, plant height and chlorophyll content (Figure S3). The PCA also revealed positive influence of tri-weekly application of PA + 0.35% LSE on berry yield, 100-berry weight, and Mn content (Figure S3). The PCA showed positive influence of the 2% and 4% PA + 0.35% LSE on haskap plant growth and berry yield.

The current study suggested that frequency of PA application is important in the promotion of haskap plant growth due to the presence of some organic compounds. The increase in berry yield indicates possible synergistic effect of PA with LSE on berry development. Thus, the addition of PA with added LSE could have increased haskap berry yield. The bi-weekly application of 2% and 4% PA + 0.35% LSE may enhance haskap growth. However, tri-weekly application of 2%

and 4% PA + 0.35% LSE may increase yield. Nonetheless, further studies are needed to understand the mechanism of nutrient uptake via the foliar mode of application of PA + 0.35% LSE.

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3.6 SUPPLEMENTARY

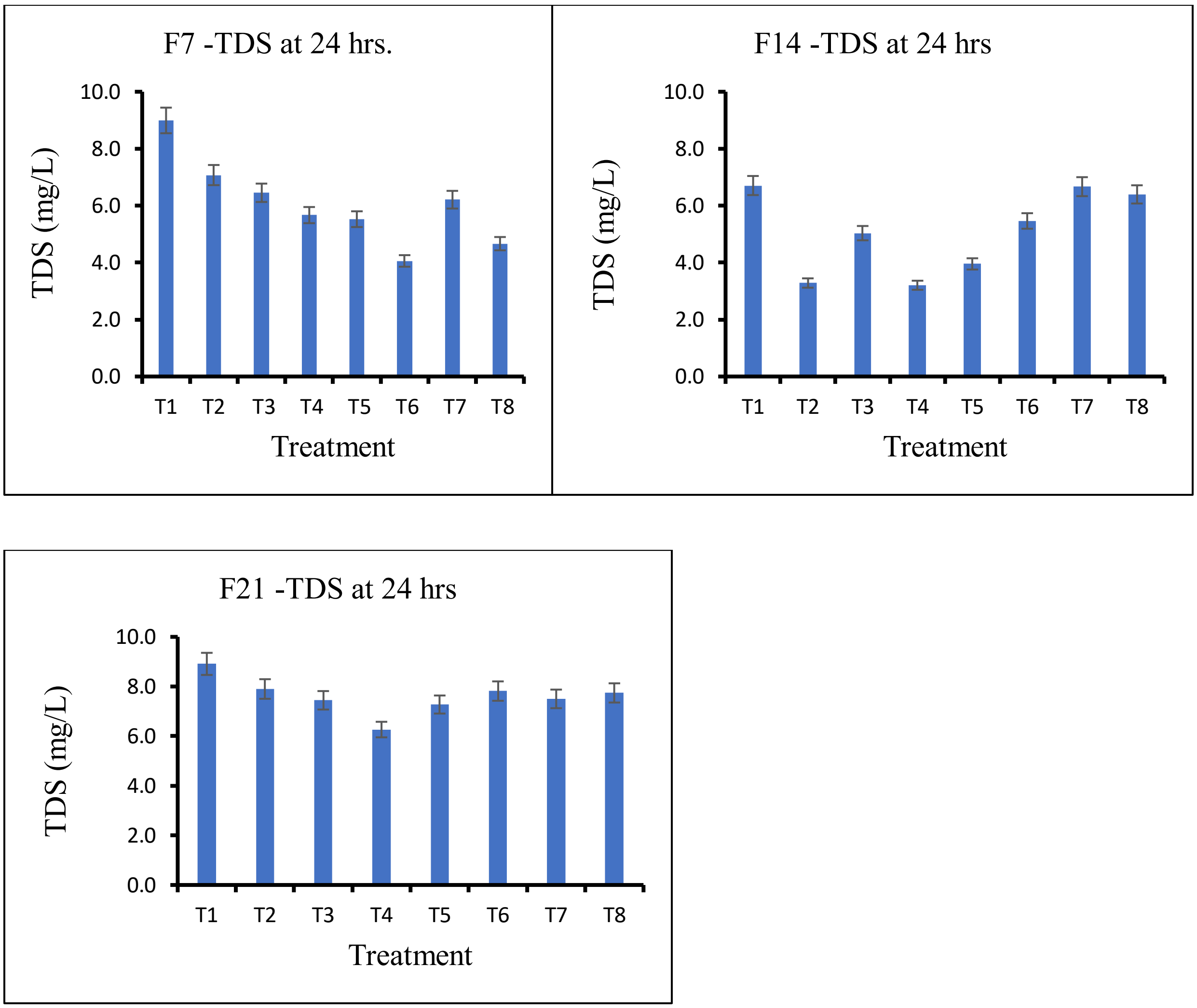

Figure S1 Influence of frequency and rates of pyroligneous acid (PA) with added liquid seaweed extract (LSE) on TDS in haskap plant leaves T1 = Control (water); T2 = 2% PA + 0.35% LSE; T3 = 4% PA + 0.35% LSE; T4 = 6% PA + 0.35% LSE; T5 = 8% PA + 0.35% LSE; T6 = 10% PA + 0.35% LSE; T7 = 4% PA alone; T8 = 0.35% LSE alone; F7- weekly application; F14 – bi-weekly application; F21 – tri-weekly application; LSE= liquid seaweed extract.

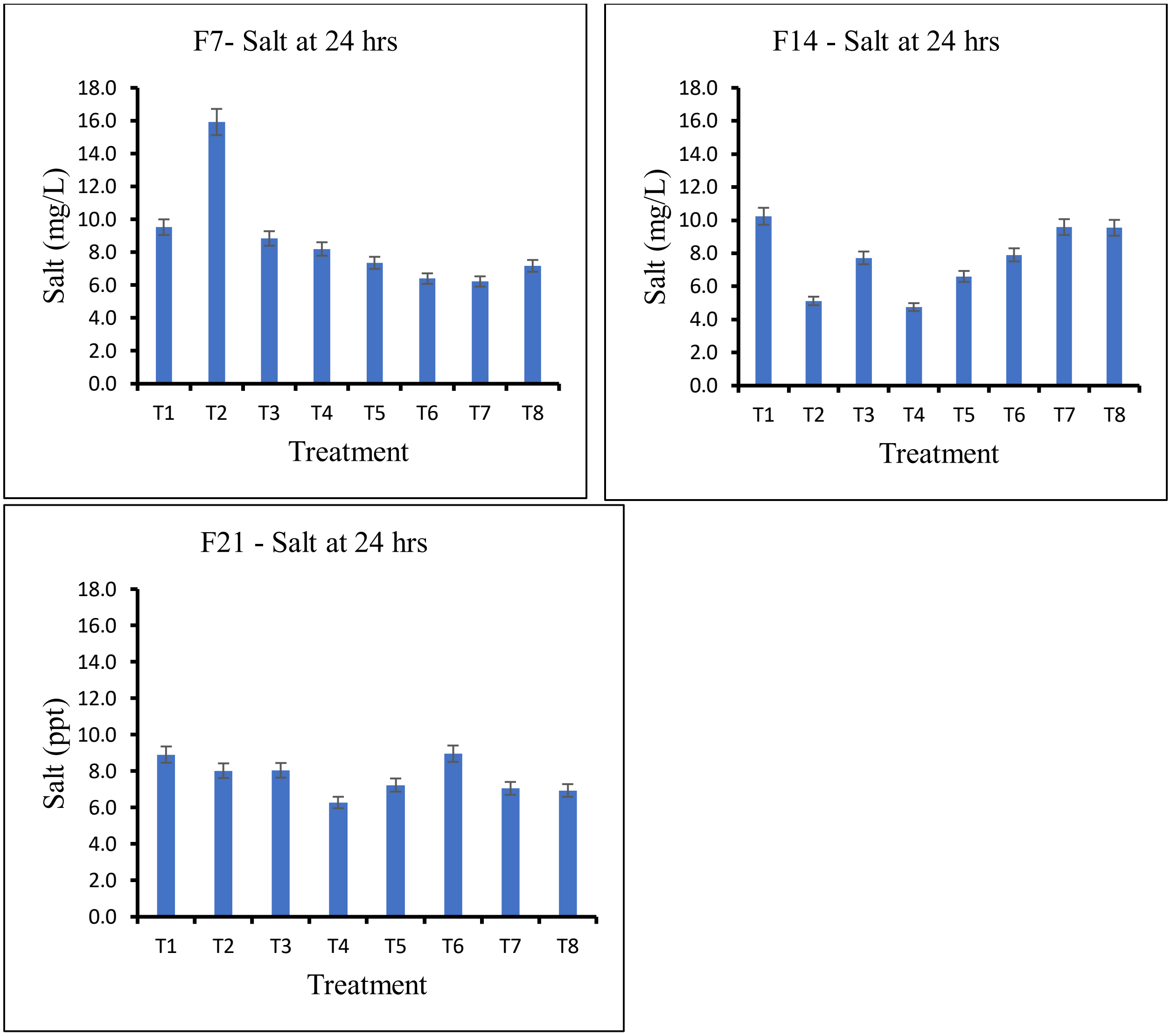

Figure S2 Influence of frequency and rates of pyroligneous acid (PA) with added liquid seaweed extract (LSE) on salinity in haskap plant leaves T1 = Control (water); T2 = 2% PA + 0.35% LSE; T3 = 4% PA + 0.35% LSE; T4 = 6% PA + 0.35% LSE; T5 = 8% PA + 0.35% LSE; T6 = 10% PA + 0.35% LSE; T7 = 4% PA alone; T8 = 0.35% LSE alone; F7- weekly application; F14 – bi-weekly application; F21 – tri-weekly application; LSE= liquid seaweed extract.

Vertical in lines on the bars represent standard error of the means

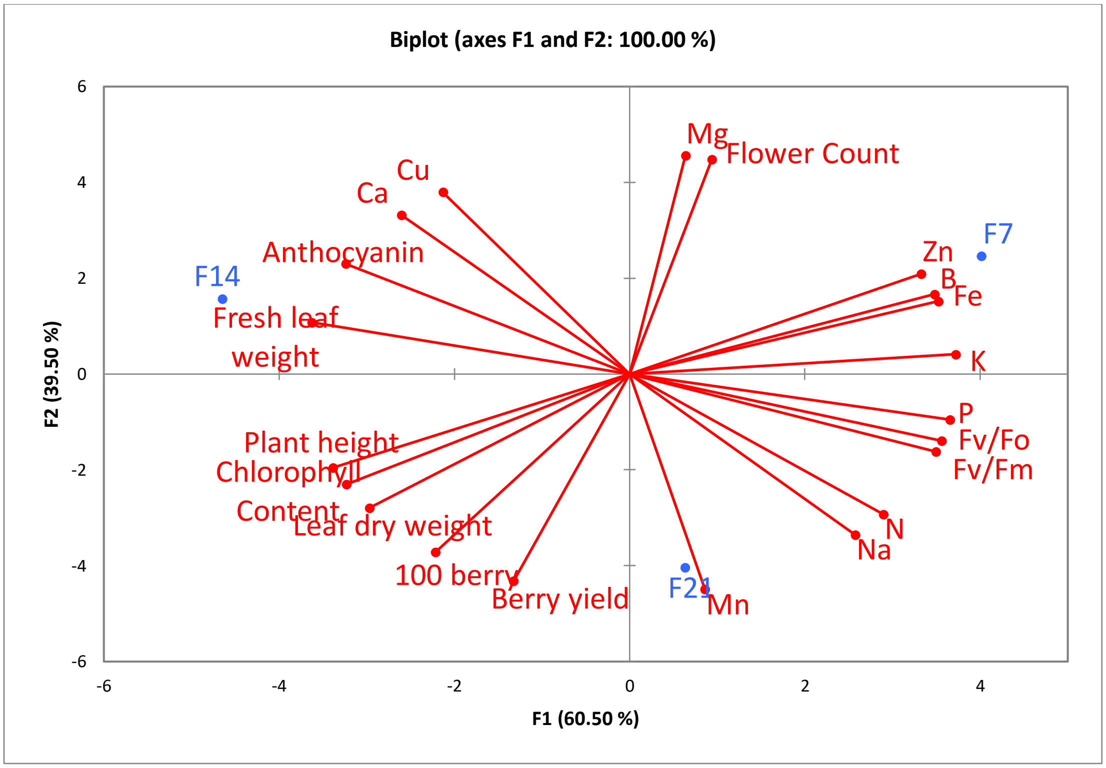

Figure S3 A two-dimensional (2-D) principal component analysis biplot (PCA) showing relationships amongst pyroligneous acid (PA) with added liquid seaweed extract (LSE), frequency, growth, and yield parameters of haskap 'Indigo Gem'. TDS = total dissolved solids; EC = electrical conductivity; Fv/Fm = maximum quantum efficiency of Photosystem II; Fv/Fo = photosynthetic capacity; K = potassium, P = phosphorus; N = nitrogen; Ca = calcium; Mg = magnesium; B = boron; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc. F7- weekly application; F14 – bi-weekly application; F21 – tri-weekly application; PA = pyroligneous acid; LSE = liquid seaweed extract.

CHAPTER 4
HASKAP (*LONICERA CAERULEA*) BERRY QUALITY AND POWDERY MILDEW DISEASE RESPONSE TO VARYING FREQUENCY AND RATE OF PYROLIGNEOUS ACID APPLICATION WITH ADDED LIQUID SEAWEED EXTRACT

4.0 ABSTRACT

The use of pyroligneous acid (PA) has gained widespread attention owing to its prospects in enhancing fruit quality and curbing disease spread. However, the effect of PA with added liquid seaweed extract on haskap fruit quality and powdery mildew disease is not reported. A field study was conducted to determine the effects of pyroligneous acid (PA) with added liquid seaweed extract (LSE) at different rates (2, 4, 6, 8, and 10%) and frequencies (weekly, bi-weekly, and tri-weekly) on haskap berry quality and powdery mildew disease management. Control treatments were 4% PA alone, 0.35% LSE alone, and water alone. Variations in the frequency and rates of PA with added 0.35% LSE significantly ($P < 0.05$) increased berry quality and reduced powdery mildew disease on the plant. The weekly application of 10% PA + 0.35% LSE reduced disease incidence and severity by 8.3% on the plants after harvest. A principal component analysis (PCA) biplot revealed a positive influence of weekly PA application on the haskap berry quality with increases in berry juice nutrients, pH, and Brix°. The PCA showed positive influence of the 2%, 4%, and 6% PA + 0.35% LSE treatments on haskap berry quality. Overall, weekly application of 2%, 4% and 6% PA + 0.35% LSE positively influenced berry tissue nutrients, titratable acidity, maturity index, pH, Brix°, total dissolved solids, and salinity. Further studies are required to elucidate the underlying mechanisms by which PA + 0.35% LSE enhances haskap berry quality *via* foliar application.

Keywords: Phenolic compounds, Brix°, Maturity index, Organic acids, and Berry tissue nutrient

4.1 INTRODUCTION

Haskap (*Lonicera caerulea L.*) belongs to the *Caprifoliaceae* family which has only a few domesticated species known for fruit production (Bell & Williams, 2019). *Lonicera caerulea L.* is much preferred for production due to its edible berries (Hummer et al., 2012). The fruits of haskap develop from large, tubular to funnel-shaped and light-yellow flowers which appear with the vegetative components (Hummer et al., 2012; Dawson, 2017). Haskap fruits are purple to dark blue in colour with oblong to slender shapes covered in a waxy coating, which develop after pollination has occurred (Plekhanova, 1992). Nutritionally, haskap berries contains high amounts of vitamins (A and C), potassium, fibre, phenolic compounds, anthocyanins, and antioxidants (Wojdyło et al., 2013; Zhao et al., 2015). The anthocyanin and ascorbic acid contents of the berries are higher than most berries, such as blueberries, strawberries, and raspberries which are crucial for their health boosting benefits (Celli et al., 2014). Some *in-vivo* and *in-vitro* studies have suggested haskap berry extracts as remedy for health issues including neurodegenerative diseases, cardiovascular diseases, cancer, osteoporosis, and inflammatory diseases (Rupasinghe et al., 2012; Bruce, 2014; Caprioli et al., 2016). Other health benefits include preventing anaemia, reducing glaucoma effects, and averting hyperactivity in children (Lefol, 2007).

Plant biostimulants are organic substances that stimulate plant tolerance to abiotic stress, promote growth, increase yield, and improve plant yield quality (Jardin, 2015). Biostimulants include humic acid, fulvic acid, protein hydrolysates, seaweed extract, beneficial fungi, and plant growth-promoting rhizobia (Halpern et al., 2015). Many attempts are made to utilize plant biostimulants in crop production due to the rapid population growth, poor crop growth, low yield and the recurrent issues of environmental stresses affecting crop productivity (Parađiković et al., 2018). Although such studies have focused on enhancing the growth and yield of crops, studies involving biostimulant use to improve crop and berry quality are limited. Moreover, biostimulants are applied either as soil drench or foliar, based on the desired result and chemical composition (Parađiković et al., 2018). The foliar application method has been utilized as an essential means to supplement plant nutrient requirements and considered ecologically friendly (Fernández & Eichert, 2009).

Among these biostimulants pyroligneous acid (PA) and seaweed extracts are well known and utilized to enhance crop growth and productivity. PA, also known as wood distillate, wood vinegar, or bio-oil, is a by-product of charred wood obtained from pyrolysis (Mungkunkamchao et al., 2013). This bio-product plays an essential component in agriculture as it use in conventional agriculture increase plant growth and development, and promote yield (Hussain & Abbasi, 2018). PA comprises approximately 200 water-soluble compounds including organic acids (catechols, guaiacol, syringols), alcohol, essential oils, ketones, phenolic, alkane, and ester compounds (Wei et al., 2010). PA is produced from numerous organic materials including agricultural biomass, algae, herbaceous plant biomass, and bamboo (Mu et al., 2003). Various studies have demonstrated the stimulatory effects of PA as a soil modifier, priming agent, deodorizer, antimicrobial agent, sterilizing agent, growth promoter, and yield promoter (Souza et al., 2012; Mungkunkamchao et al., 2013; Mmojieje & Hornung, 2015; Souza et al., 2018) . Similarly, PA promotes the growth of flowers and improve fruit flavour (Zulkarami et al., 2011; Lu et al., 2019).

Seaweed extract is a biostimulant that is obtained from the brown (*Phaeophyceace*), red (*Rhydopyta*) and green (*Chlorophyta*) algae which enhances plant growth in horticultural crops (Parađiković et al., 2018). Seaweeds contain amino acids, lipids, phytohormones, phenols, macro- and micronutrients and vitamins which promotes plant yield and development (Khan et al., 2009).

Seaweed extract has been reported to contain high amounts of phenolic compounds that protect plant cellular components under stress conditions while boosting plant immunity, crop performance, and plant yield (Khan et al., 2009; Cook et al., 2018). Likewise, application of seaweed extract have been reported to enhance yield in several crops including strawberry (Parađiković et al., 2018), apple (Basak, 2008), citrus (*Citrus x latifolia*) (Mohamed & El-Sehrawy, 2013), and olive (*Olea europaea*) (Chouliaras et al., 2009) with the. Additionally, foliar application of seaweed extract on banana (*Musa* spp.) caused an increase in nutrient content, and reduced fruit's moisture content (Karthikeyan & Shanmugam, 2014). Nonetheless, haskap can benefit from the stimulator effect of PA and LSE but understudied.

Crop productivity is significantly constrained by several plant disease. Powdery mildew is one of the destructive disease and its pervasive nature of spread poses a huge risk to the horticultural industry (Glawe, 2008). Powdery mildew disease is caused by a fungal pathogen that thrives best in warm, humid conditions. Globally, approximately 10,000 plant varieties succumb to powdery mildew fungal infection from 650 species (Takamatsu, 2004; 2008). Major symptoms of powdery mildew infection include powdery spotting on the upper and lower leaf surface, coiling of leaves, and leaf necrosis (Cal et al., 2008). Studies have reported its damage to fruit crops like strawberry (*Fragaria* x *ananassa*), melon (*Cucumis melo*), peach (*Prunus persica*), apple (*Malus domestica*), tomato (*Solanum lycopersicum*), and grapevine (*Vitis* spp.) (Dean et al., 2012). Powdery mildew can cause about 30% to 100% reduction in yield of crops (Peetz et al., 2009), as result, for example, increased leaf defoliation, decreased yield, and quality of fruits (Karajeh et al., 2012). Its control is also heavily dependent on synthetic fungicides, which can have toxic effects on the ecosystem, environment, and human health. Hence there is the need to investigate environmentally friendly options for controlling the disease on fruit crops like haskap.

Several research have focused on natural biostimulant effects on plants like cereal, vegetables, and fruits (Parađiković et al., 2018). The use of PA in agriculture has been extensively reported but not for perennial plants such as haskap. Some studies have reported inhibition of fungal growth with PA application (Mourant et al., 2007; Oramahi &Yoshimura, 2013). These studies suggest that PA can aid in the prevention of powdery mildew disease on haskap. However, haskap berry quality and powdery mildew disease management response to PA with added LSE is unknown. Therefore, this study aims to investigate the biostimulant effects of PA with added LSE on the berry quality and powdery mildew disease management of haskap plants under field conditions.

4.2 MATERIALS AND METHODS

4.2.1 Field Location, Soil Type, and Climatic Conditions

The field experiment was conducted in the North 45 Orchards farm in Cloverdale, Stewiacke valley (latitude 45.18, longitude -63.22) located in Colchester County of Nova Scotia, Canada. The study was performed in the 2019 and 2020 growing seasons. The soil types in Cloverdale comprise of alluvium, loam to silty clay, and loam (Webb et al.,1991). The soil surface coverage contains less than 2 % rocks and 0.01% stones. The soil types are imperfectly drained to poorly drained; hence the soil remains wet for significant parts of the growing season in Cloverdale (Webb et al.,1991). Cloverdale has an average soil pH of 4.9 and a mixed form of forest vegetation (Gaye & Margret, 1994). The vicinity experiences the northern temperate zone weather conditions due to its closeness to the Atlantic ocean.

4.2.1.1 Experimental Materials and Design

Haskap 'Indigo gem' was used for the experiment. The plants were transplanted at the age of two in October 2017 and were 4- and 5-year old bushes at time of the studies in 2019 and 2020. Fertilizer containing 15% nitrogen, 15% phosphorus, 15% potassium (15-15-25 NPK) was applied in two splits for 2019, with the first application being 86.43 kg/ ha and 101.36 kg/ ha for the second application. In 2020, 54.43 kg of Micro-Essential® fertilizer was applied at the end of May and mid-June. The pyroligneous acid was obtained from Proton Power Inc. (Lenoir City) with its source from white pine biomass. The seaweed extract was donated by Kevin Shiell, Director of formulations, Acadian Plant health.

The experiment was a 3 x 8 factorial experiment with 8 treatments applied in a complete randomized block design with 3 replications. The PA with added LSE treatments were 2 % of PA + 0.35% of LSE, 4 % of PA + 0.35% of LSE, 6 % of PA + 0.35% of LSE, 8 % of PA + 0.35% of LSE, and 10 % of PA + 0.35% of LSE. The controls were water alone, 4% PA and 0.35% LSE. These rates were applied at frequencies 7 (weekly), 14 (bi-weekly) and 21 (tri-weekly). The haskap plants were foliar treated with these eight rates on weekly, bi-weekly, and tri-weekly basis. Days with rainfall were avoided as much as possible to prevent the washing of nutrients from plant leaf. All treatments applications were done by hand using a sprayer with 3.8 litres capacity. The leaves were sprayed until it dripped.

4.2.1.2 Field Management

Weed control was done using 1 L of Post Ultra mixed with 200L water/ha, which was applied between mid-May and the end of July every year. Spacing between plants was 1.3 m between plants and 3.0 m between rows. During the fruiting season, nettings are used to prevent birds from feeding on the ripe berries. The plants are rainfed. The plots were grower managed. The experimental units were made up of five plants with two plants as buffers. The total number of haskap plants used were 120.

4.2.2 Haskap Berry Quality determination

4.2.2.1 Sample preparation

Total soluble solids (TSS), pH, titratable acidity (TA), electrical conductivity (EC), total dissolved solids (TDS), salinity content, and maturity index of haskap berries were determined immediately after harvest. 100 berries were randomly sampled for carotenoid content, macro-and micro-nutrient analysis. The selected berries were flash-frozen with liquid nitrogen and freeze-dried. The frozen berries were crushed into fine powder using mortar and pestle and stored in a -70°C freezer (Frilabo, Lyon, France) for further analysis.

4.2.2.1.1 Total soluble solids, pH, titratable acidity, and maturity index

A sample of 100 berries from each replicate was crushed using a handheld plant sap press (Spectrum Technologies Inc., Illinois, USA) to extract juice for total soluble solids (TSS), pH, and titratable acidity determination. Total soluble solid content was measured using a calibrated digital handheld pocket refractometer (ITM Instruments Inc., Canada). A drop of the berry juice was placed on the refractometer and the value obtained was expressed as Brix°. pH was determined using EC 500 ExStik conductivity meter (EXTECH Instrument, Taiwan). Titratable acidity was determined by the titration of water extract of haskap homogenate (10 ml) with few drops of phenolphthalein indicator against a 0.1 mol/L of sodium hydroxide solution (NaOH) to an endpoint of 8.1. The titratable acidity of the berries was expressed as the percent (w/v) equivalent to citric acid based on calculations from Sadler & Murphy, (2010).The maturity index (MI) was calculated using the formular,

$$MI = \frac{\text{Total soluble solids (Brix°)}}{\text{Titratable acidity (TA)}}$$

4.2.2.1.2 Electrical conductivity, total dissolved solids, and salt content

Electrical conductivity (EC), total dissolved solids (TDS), and salinity content of the haskap berry extract were determined using EC 500 ExStik conductivity meter (EXTECH Instrument, Taiwan).

4.2.2.1.3 Berry nutrient analysis

100 frozen berry samples of each treatment were sent to the Nova Scotia Department of Agriculture Laboratory Services for further analysis. Complete macro- and micro- nutrients were determined using the inductively coupled plasma spectroscopic method and the combustion method (AOAC, 2003).

4.2.2.1.3.1 Total carotenoid content

Total carotenoid content was determined as described by Harborne, (1973). In brief total carotenoid content was determined using 100 g ground frozen haskap berries. The ground tissue was then topped up with 10 ml of 80% acetone. The solution was vortexed for 5 mins and centrifuged at 3000 rpm for 10 mins. The supernatant was carefully transferred into a new tube and made up to a volume of 10 ml. The optical density (OD) values were measured at 480 nm in Jenway 6405 UV/ Vis Spectrophotometer (Bibby Scientific Ltd, Dunmow, UK) and expressed as mg/gm. The amount of carotenoids in 100 mg berry tissue was calculated as =

$$\frac{4 \text{ x OD values x Total volume of sample}}{\text{Weight of frozen berry tissues}}$$

4.2.3 Powdery Mildew Disease Assessment

Powdery mildew disease severity and incidence were assessed after 3 months of pyroligneous acid application. Disease incidence was recorded based on the presence or absence of the disease spores on the leaves. Disease severity was evaluated based on a 5-point scoring scale (0, 1, 2, 3, and 4), with 4 being the highest infection (Table 4.1). Disease severity was then calculated using the following formula described by (Prithiviraj et al. (1997).

$$\text{Disease Severity} = \frac{\text{Sum of ratings (0– 4 scale)} \times 100}{\text{(Maximum possible score} \times \text{Total number of leaves examined)}}$$

Table 4. 1. Disease severity rating based on infected haskap leaf surface

Infection rating	Leaf area infection (%)
0	No infection
1	25
2	50
3	75
4	100

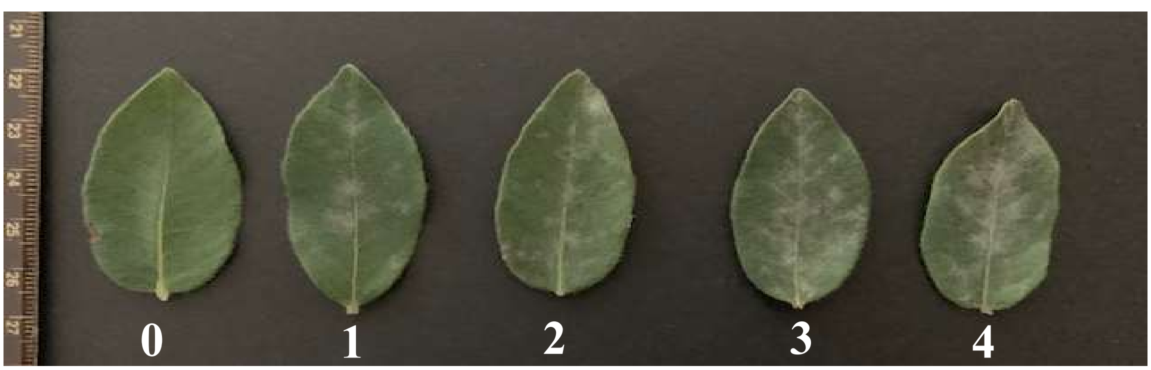

Figure 4. 1. Disease infection rating based on the presence of powdery mildew spores on haskap leaf surface.

4.2.4 Statistical Analysis

All data collected were subjected to analysis of variance (ANOVA) using the General Linear Model function in Minitab (Minitab 19 Statistical software, USA). Means with significant differences at $P < 0.05$ were separated using Tukey's pairwise mean comparison method. Graphs were drawn using Microsoft Excel. A principle component analysis (PCA) biplot was performed using XLSTAT Version 19.1 to determine the association between rates and frequency on the haskap fruit quality parameters measured.

4.3 RESULTS AND DISCUSSIONS

Frequency (weekly, bi-weekly, and tri-weekly) and rate of PA application had significant ($P < 0.05$) effect on the berry quality parameters measured (Table 4.2 and 4.3). Likewise, there was a significant ($P < 0.05$) interactive effect on the berry quality parameters measured (Table 4.4). The interaction effect model represents the combinative effects of frequency and rates on haskap berry quality.

4.3.1 Total Soluble Solids, pH, Titratable Acidity, and Maturity Index

The Brix° content of haskap berry significantly ($P < 0.05$) increased with the bi-weekly application of PA but reduced the tri-weekly application (Table 4.2). The Brix° content was significantly ($P < 0.05$) increased with 8% PA + 0.35% LSE treatment but reduced with 4% PA + 0.35% LSE (Table 4.3). The interaction between bi-weekly application with 2% PA + 0.35% LSE and 4% PA alone significantly ($P < 0.05$) increased Brix° content. However, Brix° content reduced as observed with the weekly application of 4% PA alone (Table 4.4). These can be attributed to the rich complex mixture of organic compounds in PA which may have enhanced the Brix° content of the treated berries as suggested by earlier studies (Zulkarami et al. 2011; Mungkunkamchao et al., 2013).

These also suggests a possible synergistic effect of PA with LSE in enhancing berry Brix° content. The results obtained conformed with a study by Mungkunkamchao et al. (2013) who recorded an increase in Brix° content with PA application on tomatoes (*Solanum lycopersicum*). Also, Zulkarami et al. (2011) recorded significant increase in Brix° content with PA application on rock melon fruits (*Cucumis melo* var. cantalupensis). Some studies have shown PA's role in enhancing sweetness of fruits and vegetables (Mohan et al., 2006; Oramahi et al., 2013; Tuntika et al., 2013). The concentration of soluble solids is an essential component assessed in several commercial fruit species (Mackenzie et al., 2018). Hence, an increase in Brix° content with PA application could improve haskap berry quality, market value, and consumer's preference for the fruit.

The pH content of haskap berry was significantly ($P < 0.05$) increased with tri-weekly application of PA + 0.35% LSE but decreased with weekly application (Table 4.2). The 6% PA + 0.35% LSE treatment increased pH content while 4% PA alone observed a reduction in pH content (Table 4.3). The interaction between tri-weekly application and 6% PA + 0.35% LSE increased pH content while weekly application of 4% alone reduced pH content (Table 4.4). The increase in pH with tri-weekly application of PA suggests that reduced exposure of haskap berries to PA could reduce acidity of berries resulting in increased sugar content or sweetness. However, the weekly application of PA may have increased sour taste in haskap berries due to decreased acidity levels.

Maturity index (MI) is a measure of savour in fruits and is crucial concerning the user's approval and liking for a fruit (Wojdyło et al., 2013). The weekly treatment of PA + 0.35% LSE significantly ($P < 0.05$) increased the maturity index compared with tri-weekly treatment which recorded the least maturity index (Table 4.2). The application rate of 6% and 8% PA + 0.35% LSE significantly

(P < 0.05) increased maturity index by 17%, respectively while 10% PA + 0.35% LSE reduced the maturity index of the haskap berries by 9% (Table 4.3). However, the interaction between frequency and rate showed a significant (P < 0.05) increase in maturity index with bi-weekly application of 8% PA + 0.35% LSE (Table 4.4). Maturity index decreased with the bi-weekly application of the 10% PA + 0.35% LSE. The values obtained for MI in this study ranged from 3.64 to 7.50, which conformed with a study by Wojdyło et al. (2013). However, this can be attributed to increased acidity levels in haskap juice (Wojdyło et al., 2013). This also suggests that the acidic nature of PA may have influenced the decrease in acidity levels of haskap berries which could have affected the MI. Hence, the application of 10% PA + 0.35% LSE may have played a role in reducing the MI of the haskap berries. Also, a reduced PA rate could possibly increase MI and increase consumers preference for haskap berries.

Table 4. 2. Effect of the frequency of pyroligneous acid (PA) with added liquid seaweed extract (LSE) on haskap berry quality components

Frequency	Maturity Index	TA (%)	Brix° (%)	pH	Carotenoid content (mg/gm)	TDS (mg/L)	Salinity (mg/L)	EC (µs/cm)
F 7	4.39 a	2.50 a	12.39 b	3.01 b	0.06 a	8.25 a	6.80 a	11.81 a
F 14	1.98 b	2.47 a	12.72 a	3.03 b	0.06 a	3.66 c	2.77 c	4.45 c
F 21	1.92 b	2.14 b	12.24 b	3.06 a	0.05 a	3.77 b	2.91 b	7.11 b

Frequency = weekly application (F7), bi-weekly application (F14), and tri-weekly application (F21); PA = pyroligneous acid; TA = titratable acidity; TDS = total dissolved solids; EC = electrical conductivity.

Table 4. 3. Effect of the rates of pyroligneous acid (PA) with added liquid seaweed extract (LSE) on haskap berry quality components

Rate (%)	Maturity Index	TA (%)	Brix° (%)	pH	Carotenoid content (mg/gm)	TDS (mg/L)	Salinity (mg/L)	EC (µs/cm)
T1	2.12 c	2.26 ab	12.37 bcde	3.00c	0.05 abc	4.08 f	3.23 d	7.34 c
T2	2.22 bc	2.48 a	12.51 bcd	3.06 abc	0.05 c	4.68 e	3.77 c	7.08 d
T3	2.68 b	2.28 ab	12.14 e	3.07 ab	0.07 a	5.10 d	4.01 c	7.40 c
T4	3.44 a	2.58 a	12.56 bc	3.08 a	0.05 bc	7.05 a	5.56 a	10.03 a
T5	3.45 a	2.01 b	12.81 a	3.03 abc	0.06 abc	6.07 b	3.87 c	6.24 e
T6	1.94 c	2.43 ab	12.64 ab	3.04 abc	0.07 ab	4.09 f	3.28 d	5.09 f
T7	2.58 b	2.54 a	12.35 cde	3.00 c	0.06 abc	5.35 c	5.05 b	9.47 b
T8	2.04 c	2.40 ab	12.22 de	3.01 bc	0.06 abc	4.19 f	3.31 d	5.08 f

T1 = Control (water); T2 = 2% PA + 0.35% LSE; T3 = 4% PA + 0.35% LSE; T4 = 6% PA + 0.35% LSE; T5 = 8% PA + 0.35% LSE; T6 = 10% PA + 0.35% LSE; T7= 4% PA alone and T8 = 0.35% LSE alone ; PA = pyroligneous acid and LSE = liquid seaweed extract; TA = titratable acidity; TDS = total dissolved solids; EC = electrical conductivity.

Table 4. 4. Interaction mean effects and p-values of maturity index, titratable acidity, Brix°, pH, carotenoid content, total dissolved solids, salinity, and electrical conductivity as affected by pyroligneous acid (PA) with added liquid seaweed extract (LSE).

Berry Quality Parameters										
Treatment interaction	**Maturity Index**	**TA (%)**	**Brix° (%)**	**pH**	**Carotenoid content (mg/gm)**	**TDS (mg/L)**	**Salinity (mg/L)**	**EC (µs/cm)**	**DI (%)**	**DS (%)**
F7 T1	4.32 abc	2.49 abcd	11.74 g	2.98 cde	0.06 a	8.08 b	6.64 a	11.52 abc	25.00 a	25.00 abc
F7 T2	4.73 ab	2.29 abcd	12.14 defg	3.08 abcd	0.06 a	8.13 ab	6.62 a	11.61 ab	13.89 cd	13.89 cd
F7 T3	4.27 abc	2.49 abcd	12.27 defg	3.01 cde	0.08 a	8.01 b	6.59 a	11.53 abc	25.00 a	25.00 abc
F7 T4	4.72 ab	2.33 abcd	13.07ab	3.02 cde	0.05 ab	8.26 ab	6.86 a	11.91 a	25.00 a	25.00 abc
F7 T5	5.11 ab	2.16 abcd	12.67 bcd	3.01 cde	0.05 ab	8.29 ab	6.81 a	11.89 a	16.67 bc	16.67 bcd
F7 T6	4.48 abc	2.43 abcd	12.74 abcd	3.01 cde	0.08 a	8.17 ab	6.75 a	11.65 ab	8.33 d	8.33 d
F7 T7	3.66 abc	3.13 a	11.40 g	2.93 e	0.06 a	8.60 a	7.13 a	12.40 a	19.44 abc	16.67 bcd
F7 T8	4.10 abc	2.76 abc	12.23 defg	3.06 bcd	0.06 a	8.49 ab	7.05 a	12.07 a	13.89 cd	13.89 cd
F14 T1	1.73 efg	2.03 bcd	12.60 cde	3.07 abcd	0.05 ab	2.63 g	2.04 e	3.74 g	22.22 ab	30.56 a
F14 T2	1.44 fg	2.29 abcd	13.10 a	3.03 cde	0.07 a	2.48 g	2.00 e	3.56 h	22.22 ab	25.00 abc
F14 T3	1.50 efg	2.33 abcd	12.33 defg	3.04 bcd	0.07 a	2.63 g	2.00 e	3.74 g	22.22 ab	22.22 abc
F14 T4	4.56 abc	2.40 abcd	12.37 defg	3.03 cde	0.06 a	8.21 ab	6.78 a	8.10 d	25.00 a	33.33 a
F14 T5	6.15 a	1.74 d	13.04 abc	3.02 cde	0.07 a	8.17 ab	3.34 d	6.08 e	22.22 ab	22.22 abc
F14 T6	1.38 g	2.43 abcd	12.57 def	3.02 cde	0.07 a	2.52 g	1.91 e	3.53 h	22.22 ab	22.22 abc
F14 T7	1.75 efg	1.93 cd	13.10 a	3.07 abcd	0.05 ab	2.53 g	3.63 cd	6.00 e	19.44 abc	22.22 abc
F14 T8	1.60 efg	2.10abcd	12.23 defg	3.00 cde	0.06 a	2.51 g	1.91 e	3.53 h	22.22 ab	22.22 abc
F21 T1	1.49 fg	2.27 abcd	12.57 def	2.96 de	0.05 ab	2.53 g	1.92 e	11.47 abc	22.22 ab	27.78 ab
F21 T2	1.94 ef	2.92 abc	11.93 efgh	3.07 abcd	0.03 b	4.26 f	3.50 d	10.80 bc	22.22 ab	27.78 ab
F21 T3	3.52 bc	2.02 bcd	11.71 g	3.18 ab	0.07 a	5.40 d	4.10 c	11.77 a	25.00 a	33.33 a
F21 T4	2.10 de	3.08 ab	11.93 efgh	3.21 a	0.05 ab	4.97 e	3.42 d	10.66 c	25.00 a	30.56 a
F21 T5	1.69 efg	2.17 abcd	12.67 abcd	3.08 abcd	0.06 a	2.74g	2.14 e	3.91 fg	25.00 a	33.33 a
F21 T6	1.44 fg	2.43 abcd	12.62 cde	3.09 abc	0.06 ab	2.63 g	2.12 e	3.94 f	22.22 ab	22.22 abc
F21 T7	2.88 cd	2.69 abc	11.81 fg	3.01 cde	0.07 a	5.83 c	4.69 b	12.50 a	22.22 ab	25.00 abc
F21 T8	1.50 efg	2.27 abcd	12.20 defg	2.98 cde	0.06 a	2.69 g	2.02 e	3.83 fg	19.44 abc	25.00 abc
P-value	0.001	0.010	0.000	0.000	0.004	0.000	0.000	0.000	0.112	0.6668

ns, indicates no significance at P < 0.05

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Frequency = weekly application (F7), bi-weekly application (F14) and tri-weekly application (F21); Treatment = T1 = Control (water); T2 = 2% PA + 0.35% LSE; T3 = 4% PA + 0.35% LSE; T4 = 6% PA + 0.35% LSE; T5 = 8% PA + 0.35% LSE; T6 = 10% PA + 0.35% LSE; T7= 4% PA alone and T8 = 0.35% LSE alone ; PA = pyroligneous acid; LSE = liquid seaweed extract; TA = titratable acidity; TDS = total dissolved solids; EC = electrical conductivity; DI = disease incidence; DS = disease severity.

4.3.2 Electrical Conductivity, Total Dissolved Solids, and Salt Content

Total dissolved solids (TDS), salinity and electrical conductivity (EC) in haskap berries were significantly ($P < 0.05$) enhanced by weekly application of PA but decreased with bi-weekly application of PA (Table 4.2). The 6% PA + 0.35% LSE treatment significantly ($P < 0.05$) increased TDS, salinity and EC content but reduced with 10% PA + 0.35% LSE, and 0.35% LSE alone, respectively (Table 4.3). The interaction between weekly application of the 4% PA alone increased TDS content of the berry juice but was decreased by the bi-weekly application of 2% PA + 0.35% LSE (Table 4.4). Similarly, weekly, and tri-weekly application of 4% PA alone increased salinity and EC content but decreased following biweekly application of 10% PA + 0.35% LSE and 0.35% LSE alone respectively. This suggests that the presence of organic acids and the low pH of the PA may have caused the high EC of the berry juice. The increase in salinity and EC levels with PA application may have had negative effects on berry quality of haskap. For instance, a study by Hernández-Pérez et al. (2020) indicated that increasing EC levels decreased the Brix° content in tomatoes (*Solanum lycopersicum*). Similarly, a study by Tadesse et al. (1999) showed that high EC levels decreased Brix° content in sweet pepper fruit (*Capsicum annuum*). Tadesse et al. (1999) suggested such decrease in Brix° content to reduction in leaf surface area to fruit ratio which may have reduced the photosynthetic capacity resulting in decreased transportation of sugar for plant use. Also, Awang et al. (1993) reported decreased Brix° content with increased salinity levels in strawberry. Therefore, increased TDS could suggest increased nutrients in haskap berry which represent the aggregate concentration of mineral components in a solution (Phonphan et al., 2014).

4.3.3 Berry Nutrient Analysis

The 4% PA + 0.35% LSE treatment increased berry tissue nitrogen (N) content in haskap berries but decreased with 4% PA alone treatment (Table 4.5). This suggests that organic acids present in PA may have influenced N reduction and that the synergistic effect of PA with LSE in increasing N content. The application of 2% and 10% PA + 0.35% LSE treatment increased berry tissue nutrient calcium (Ca) but decreased with water alone and 4% PA alone treatments. Berry tissue calcium in human diet is vital in promoting healthy growth and development of bones (Ross, 2011). Deficiencies associated with lack of calcium includes obesity, osteoporosis, hypertension, and increased risks of developing cancer (Nicklas et al., 2011). Hence, an increase in berry tissue nutrient calcium content with PA application could increase consumer's preference for haskap berries due to possible improvement in health. The berry tissue potassium (K) increased with 10% PA + 0.35% LSE but decreased with 4% PA + 0.35% LSE. This suggests that the application of PA at higher rate has the potential of increasing berry tissue potassium. However, the application of PA at decreased rates may reduce berry tissue potassium. Berry tissue K is essential in promoting healthy functioning of the kidneys, heart, liver, brain cells, and muscles (D'Elia et al., 2011). Hence, its increase with PA application is vital in increasing consumer's preference for haskap berries. The frequency and rate of application on berry tissue magnesium (Mg), phosphorus (P), and sodium (Na) contents were not different from each other which suggest that PA application did not enhance these contents in berry tissue.

The 2% PA + 0.35% LSE treatment increased berry tissue boron (B) content but decreased with 8% PA + 0.35% LSE treatment (Table 4.5.1). Similarly, berry tissue copper (Cu) content increased with 4% PA + 0.35% LSE treatment but decreased with the application of 6% PA + 0.35% LSE.

This suggests that the increasing PA rate may negatively affect berry tissue B and Cu content in haskap berries and that PA supply at low rate may enhance berry tissue B and Cu content. It also suggests that the synergistic effect of PA and LSE may have enhanced effect on berry tissue B and Cu. Turan & Köse (2004) reported that foliar application of brown seaweed extract on the grapevine (*Vitis*) improved its copper absorption. The increase in berry tissue B is important in the field of health as B mineral enhances brain activity, aids in the healing of wounds, and enhances the uptake of magnesium for the body's use (Pizzorno, 2015). Cu aids in the formation of red blood cells, normal functioning of connective tissues and prevention of cardiovascular diseases (Prohaska, 2012). Thus, the increase in berry tissue B with PA application could increase consumers preference for the fruit as significant sources of B nutrients.

Table 4. 5. Haskap berry macro-nutrient composition on a berry dry weight basis as affected by pyroligneous acid (PA) with added liquid seaweed extract (LSE).

Rate (%)	**N (mg/L)**	**Ca (mg/L)**	**K (mg/L)**	**Mg (mg/L)**	**P (mg/L)**	**Na (mg/L)**
T1	0.76	0.13	1.00	0.08	0.16	0.00
T2	0.74	0.15	1.04	0.08	0.17	0.01
T3	0.80	0.14	0.94	0.08	0.17	0.00
T4	0.75	0.14	1.03	0.08	0.16	0.00
T5	0.67	0.12	1.01	0.07	0.16	0.00
T6	0.75	0.15	1.07	0.08	0.17	0.00
T7	0.66	0.13	1.00	0.07	0.16	0.00
T8	0.75	0.14	1.06	0.08	0.18	0.00

T1 = Control (water); T2 = 2% PA + 0.35% LSE; T3 = 4% PA + 0.35% LSE; T4 = 6% PA + 0.35% LSE; T5 = 8% PA + 0.35% LSE; T6 = 10% PA + 0.35% LSE; T7 = 4% PA alone; T8 = 0.35% LSE alone; PA =

pyroligneous acid LSE = liquid seaweed extract; N = nitrogen; Ca = calcium; K = potassium; Mg = magnesium and P = phosphorus.

Interestingly, water alone recorded higher berry tissue iron (Fe) in comparison with 8% PA + 0.35% LSE treatment. The application of 4% PA + 0.35% LSE treatment increased berry tissue manganese (Mn) but decreased following the application of 4% PA alone. Also, berry tissue zinc (Zn) increased with 0.35% LSE only but decreased with the application of 4% PA alone The decrease in berry nutrient Mn and Zn when the 4% PA alone was applied suggests that the presence of certain organic compounds may have caused a decrease in Mn and Zn. Nevertheless, the synergistic effect of PA and LSE in promoting berry development may have enhanced these nutrients resulting in increased levels of macro- and micro- nutrients in the berry juice. For example, the foliar application of LSE on cherry tomatoes enhanced micronutrients like Fe, and Zn (Dobromilska et al., 2008).

Table 4.5 1. Haskap berry micro-nutrient composition on a berry dry weight basis as affected by pyroligneous acid (PA) with added liquid seaweed extract (LSE)

Rate (%)	**B (mg/L)**	**Cu (mg/L)**	**Fe (mg/L)**	**Mn (mg/L)**	**Zn (mg/L)**
T1	3.86	6.53	24.17	14.99	10.69
T2	10.94	7.07	22.69	16.45	11.64
T3	7.20	7.16	21.17	17.34	11.46
T4	10.79	6.32	20.03	15.72	11.19
T5	3.36	6.63	17.28	14.90	10.60
T6	10.85	6.93	19.74	16.73	11.62
T7	7.22	6.44	18.65	12.57	10.54
T8	10.87	6.89	22.27	13.90	12.14

T1 = Control (water); T2 = 2% PA + 0.35% LSE; T3 = 4% PA + 0.35% LSE; T4 = 6% PA + 0.35% LSE; T5 = 8% PA + 0.35% LSE; T6 = 10% PA + 0.35% LSE; T7 = 4% PA alone; T8 0.35% LSE alone; PA = pyroligneous acid; LSE = liquid seaweed extract; B = boron; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc.

4.3.3.1 Total Carotenoid Content

The carotenoid content in haskap berries was significantly ($P < 0.05$) enhanced with the weekly application of PA followed by the bi-weekly PA application with the least from the tri-weekly application (Table 4.2). The 4% PA + 0.35% LSE treatment increased carotenoid content while 2% PA + 0.35% LSE reduced carotenoid content (Table 4.3). The interaction between weekly application of 10% PA + 0.35% LSE significantly ($P < 0.05$) increased carotenoid content but was reduced with the tri-weekly application of 2% PA + 0.35% LSE (Table 4.4). This suggests that an increase in PA rates may enhance carotenoid content. Carotenoids are pigments which inherently exhibit the orange, red and yellow hues in different types of fruits and vegetables (Hiyasmin et al., 2016). Carotenoids are biologically produced by plants and crucial for improving human health (Hiyasmin et al., 2016). Carotenoids have been widely studied due to its roles as an antioxidant and provitamin A activity (Zakynthinos & Varzakas, 2016). These phytochemicals inhibit the occurrence of cardiovascular, neurodegenerative, and cancerous diseases (Rao et al., 1999; Rissanen et al., 2000; Giovannucci et al., 2002). Hence, an increase in carotenoid content with PA application could imply better fruit quality and improved health conditions following its consumption.

4.3.4 Powdery Mildew Disease Assessment

The frequency and rate application of PA had significant ($P < 0.05$) effect on powdery mildew disease incidence management (Figure 4.1). The weekly application of PA decreased the incidence of powdery mildew spores by 18% whereas, the bi-weekly and tri-weekly application increased by 22% and 23% respectively (Figure 4.1). This suggests that weekly application of PA can reduce the spread of powdery mildew spores thereby improving haskap plant health. However, the application of PA at longer intervals like tri-weekly may cause an increase in powdery mildew incidence. Treatment of 6% PA + 0.35% LSE increased disease incidence by 25% while the 10% PA + 0.35% LSE reduced disease incidence by 17.5%. The interaction between weekly application and 10% PA + 0.35% LSE decreased powdery midew disease incidence by 8.3% (Table 4.4). This suggests that increasing PA rate with weekly application may curb the spread of powdery mildew disease due to the possible presence of increased phenolic compounds in PA. For intance, the application of PA at 3% to 4% inhibited the growth of *Sclerotium oryzae* and *Rhizoctonia solani* (Chalermsan & Peerapan., 2009). However, the application of PA at reduced rates (i.e. < 6%) may not be effective in managing powdery mildew incidence on haskap plants.

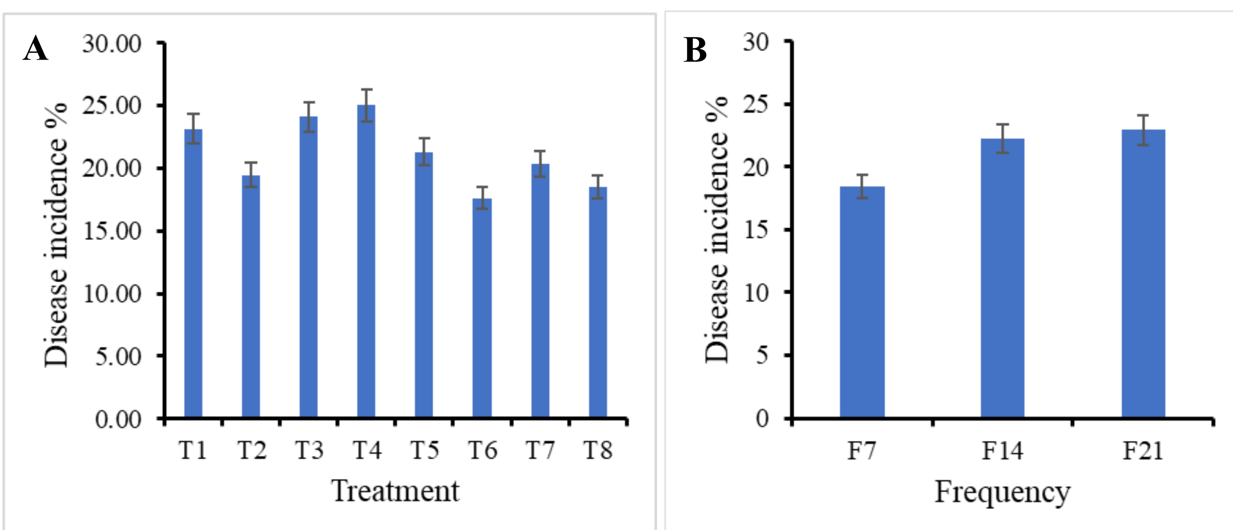

Figure 4. 2. Effects of pyroligneous acid (PA) with added liquid seaweed extract (LSE) rates (A) and frequency (B) on powdery mildew disease incidence on haskap leaves. F7= weekly application, F14 = bi-weekly application, F21 = tri-weekly application; T1 = Control (water); T2 = 2% PA + 0.35% LSE; T3 = 4% PA + 0.35% LSE; T4 = 6% PA + 0.35% LSE; T5 = 8% PA + 0.35% LSE; T6 = 10% PA + 0.35% LSE;

T7 = 4% PA alone; T8 = 0.35% LSE alone; LSE = liquid seaweed extract; Vertical lines represent error bars.

Similarly, the weekly application of PA significatly ($P < 0.05$) decreased disease severity by 18% whereas, bi-weekly and tri-weekly treatrments increased disease severity by 25% and 28% respectively (Figure 4.3). This suggests that weekly application of PA may curb the intensity of powdery mildew disease and thereby promote plant immunity. The 10% PA + 0.35% LSE treatment significantly ($P < 0.05$) reduced powdery mildew disease severity by 17.6% whereas 6% PA + 0.35% LSE treatment increased it by 29.6%. The interaction between weekly application of 10% PA + 0.35% LSE reduced disease severity by 8.3% (Table 4.4). This suggests that the frequent application of PA at increased rates may curb the severity of powdery mildew disease and that synergistic of PA with LSE may have influenced the reduction in disease incidence and severity. Some studies have shown antimicrobial and antifungal effects of PA application due to the presence of organic acids and phenol compounds (Lee et al., 2011; Mmojieje & Hornung, 2015). Likewise, seaweeds contain bioactive components like carrageenans, laminarians, fucans, and fulvans, which confers antimicrobial effect against pathogenic attacks (Klarzynski et al., 2003; Sangha et al., 2010; Vera et al., 2011). These bioactive components have been reported to elicit plant reaction against pathogens by acting as elicitor molecules (Sharma et al., 2014; Shukla et al., 2016). Additionally, phenolic compounds play a vital role in enhancing scavenging radicals such as superoxide, single oxygen, alkoxyl, hydroxyl, peroxyl radicals, and antioxidant activities (Battacharyya et al., 2015). Therefore, the reduction in powdery mildew disease incidence and severity can be attributed to the synergistic effects of these compounds in PA with added LSE.

4.3.5 Principal Component Analysis Biplot

The two-dimension (2-D) principal component analysis (PCA) biplot shows the relationship between the different variables (Figure 4.4). The PCA biplot for rates showed projections of the fruit quality parameters in the factor space (i.e., F1 and F2) and explained approximately 69.5% of the total variations in the dataset. Fruit quality parameters that were clustered together suggest positive relationships, while dataset clusters that are separated suggest a negative relationship. Parameters located at right-angles suggest no relationship. Parameters assembled with a treatment indicated that rate influenced them, while parameters that were distant from a rate indicated that rate have no influence them.

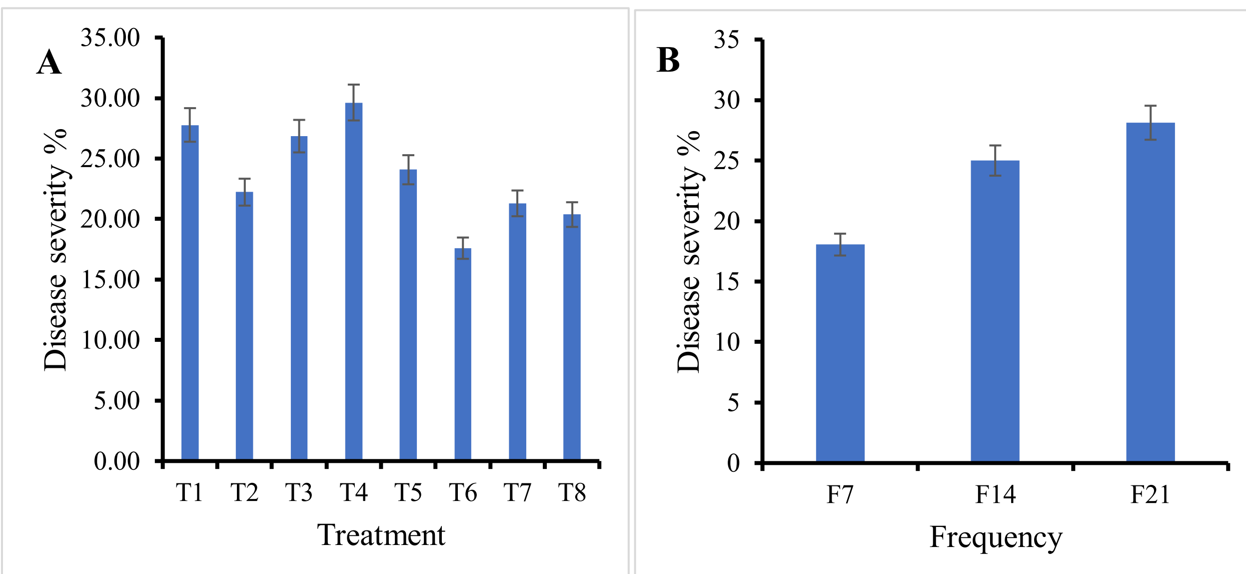

Figure 4. 2. Effects of pyroligneous acid (PA) with added liquid seaweed extract (LSE) rates (A) and frequency (B) on powdery mildew disease severity on haskap leaves. F7= weekly application, F14 = bi-weekly application, F21 = tri-weekly application; T1 = Control (water); T2 = 2% PA + 0.35% LSE; T3 = 4% PA + 0.35% LSE; T4 = 6% PA + 0.35% LSE; T5 = 8% PA + 0.35% LSE; T6 = 10% PA + 0.35% LSE;

T7 = 4% PA alone ; T8 = 0.35% LSE alone; LSE = liquid seaweed extract. Vertical lines represent error bars.

The PCA biplot revealed that the control application positively influenced pH, Brix°, EC, TDS, and salinity in haskap berries. This suggests that the increase or decrease in EC, TDS and salinity may have influenced the pH and Brix levels in haskap berries. The 2%, 4%, and 6% PA + 0.35% LSE treatments positively influenced the berry tissue macro- and micro- nutrients in haskap berries. The 4% PA + 0.35% LSE had moderate influence on EC, TDS, salinity, pH, and Brix°. However, 2%, 4%, and 6% PA + 0.35% LSE treatments had no influenced carotenoid content. The increase in berry tissue macro- and micro- nutrients with 2%, 4%, and 6% PA + 0.35% LSE treatments suggests that PA application at minimal rates (i.e. < 6%) enhances berry nutritional content and quality. Also, synergistic effect between PA and LSE may have possibly contributed to increased berry tissue nutrients in haskap berries. Nonetheless, the application of PA at higher rates (i.e. > 6%) may decrease berry tissue nutrients which may negatively affect fruit quality. The 4% PA + 0.35% LSE had moderate influence on pH and Brix° suggests that the application of PA may have minimal effect on the pH and Brix° content of haskap berries. The organic acids components in PA solution may probably account for such minimal effect on pH and Brix° level.

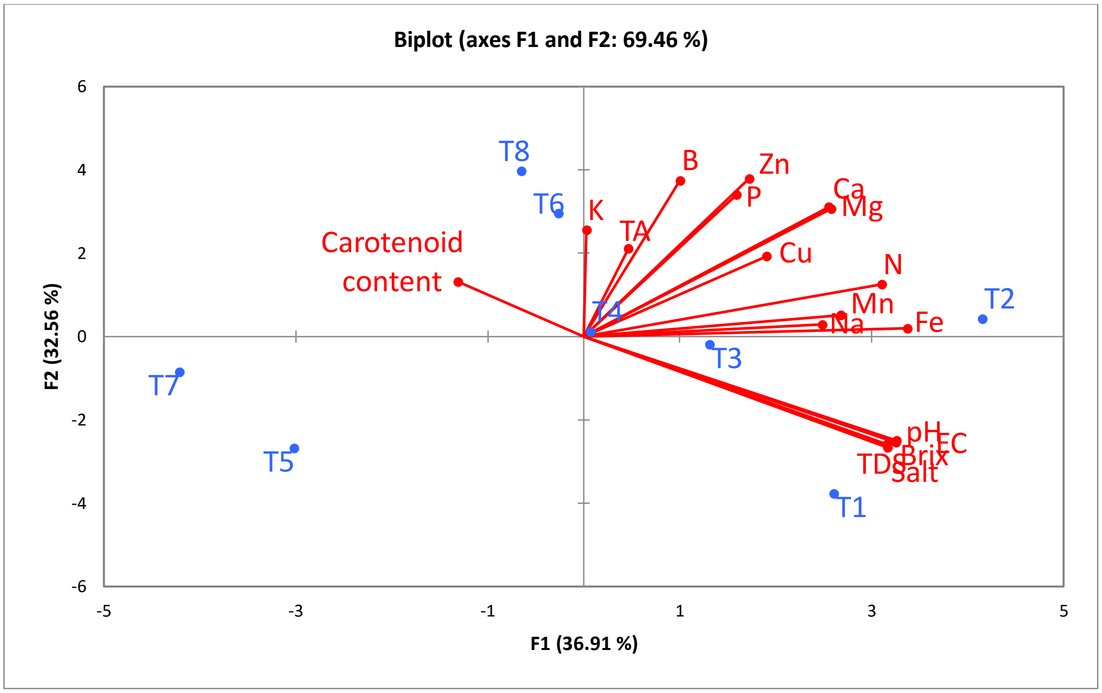

Figure 4. 3. A two-dimensional (2-D) principal component analysis biplot (PCA) showing relationships amongst pyroligneous acid (PA) with added liquid seaweed extract (LSE) treatments and berry quality parameters of haskap 'Indigo Gem'. TDS = total dissolved solids; EC = electrical conductivity; TA = titratable acidity; K = potassium, P = phosphorus; N = nitrogen; Ca = calcium; Mg = magnesium; B = boron; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc;. T1 = Control (water alone); T2 = 2% PA + 0.35% LSE; T3 = 4% PA + 0.35% LSE; T4 = 6% PA + 0.35% LSE; T5 = 8% PA + 0.35% LSE; T6 = 10% PA + 0.35% LSE; T7 = 4% PA alone; T8 = 0.35% LSE alone; PA= pyroligneous acid; LSE = liquid seaweed extract.

The 8% PA + 0.35% LSE and 4% PA alone had no influence on berry quality components. This finding suggests that the application of 4% PA alone has little effects in enhancing berry quality of haskap. This findings conforms with a study by Zhang et al. (2020) who reported that the individual application of PA had no effect on blueberry (*Vaccinium cyanococcus*) fruit quality. Also, the finding suggests that the application of PA at high rates (≥ 8%) may negatively affect berry quality. However, the application of PA at lower rates (thus ≤ 8%) may enhance the berry quality. It is also possible that high concentration of certain compounds in PA may have negatively influenced berry quality.

The 10% PA + 0.35% LSE and 0.35% LSE alone moderately influenced carotenoid content and berry tissue nutrients but not Brix°, pH, EC, TDS, and salinity in haskap berries. These observations suggest that the increase in PA at 10% with added 0.35% of LSE rate may influence carotenoid content but negatively affect berry juice Brix° and pH content.

4.4 CONCLUSION

In conclusion, the application of PA can be a novel approach to enhance the berry quality of haskap. These findings suggest that varying frequency and rates of PA application with added 0.35% LSE had significant ($P < 0.05$) effect on berry quality and postharvest powdery mildew disease infection of the plants. The weekly application of 10% PA + 0.35% LSE reduced disease incidence and severity by 8.3%, respectively. The 2-D principal component analysis (PCA) biplot revealed the positive influence of weekly application of PA + 0.35% LSE on haskap berry quality with increases in berry tissue nutrients, pH, and Brix°. (Figure S1). The PCA showed positive influence of the 2%, 4%, and 6% PA + 0.35% LSE on haskap berry quality.

The current study suggests that varying frequency and rate of PA application is important in the promotion of haskap berry quality and powdery mildew disease management due to the presence of some organic and phenolic compounds. The increase in berry quality indicates possible synergistic effect of PA with LSE on berry tissue nutrients. The increase in PA rates (thus, > 8%) with added 0.35% may have negative effects on berry quality. However, decrease PA rates (thus < 8%) with added 0.35% LSE may enhance berry quality. The weekly application of 10% PA + 0.35% LSE at the end of haskap berry harvest may curb the incidence and severity of powdery mildew disease and promote plant immunity. Nonetheless, repetition of this experiment on different varieties and locations is needed prior to recommendations.

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4.6 SUPPLEMENTARY

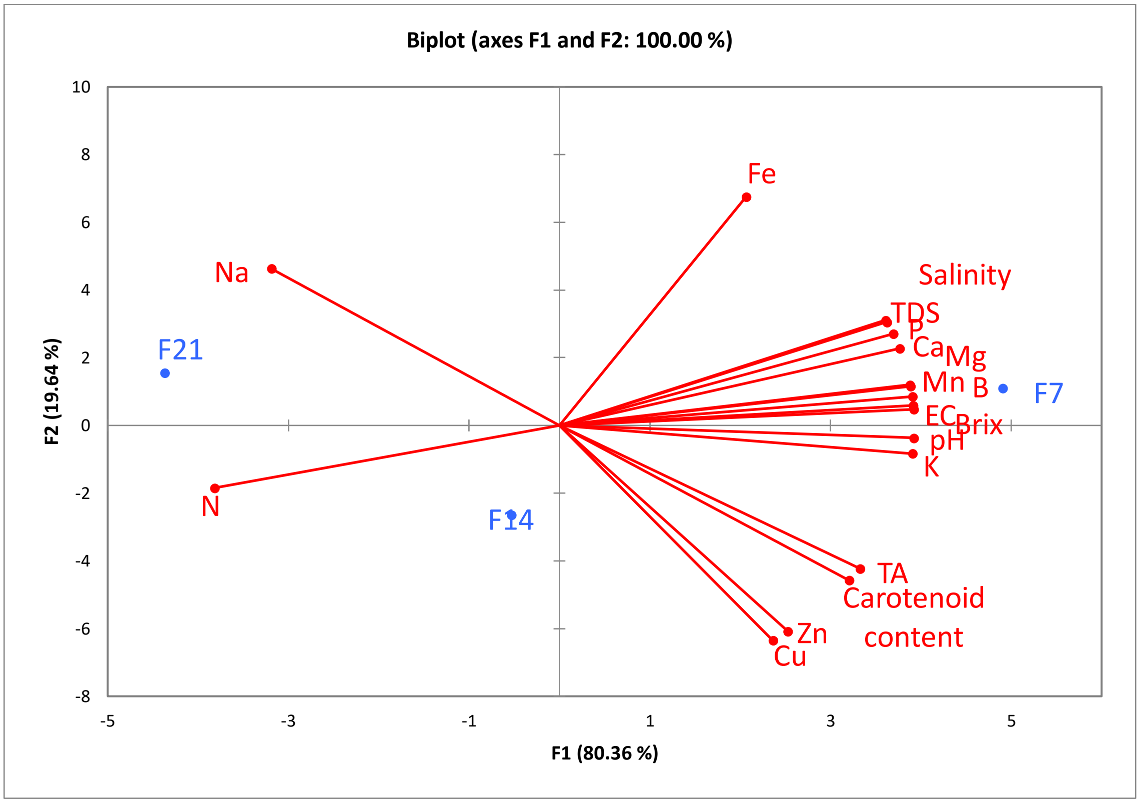

Figure S1. A two-dimensional (2-D) principal component analysis biplot (PCA) showing relationships amongst Frequency of pyroligneous acid (PA) with added liquid seaweed extract (LSE) application and berry quality parameters of haskap 'Indigo Gem'. TDS = total dissolved solids; EC = electrical conductivity; TA = titratable acidity; K = potassium, P = phosphorus; N = nitrogen; Ca = calcium; Mg = magnesium; B = boron; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc; F7 = weekly application; F14 = bi-weekly application; F21 = tri-weekly application.

CHAPTER 5
FINANCIAL ANALYSIS OF HASKAP (*LONICERA CAERULEA*) PRODUCTION IN RESPONSE TO PYROLIGNEOUS ACID APPLICATION

5.0 ABSTRACT

Many studies have demonstrated improvement in crop productivity following the application of pyroligneous acid (PA) as biostimulant. However, there are limited studies on the financial analysis of PA in agriculture. A field study was conducted to assess the economic cost-benefits of the application frequency (weekly, bi-weekly, and tri-weekly) of PA on haskap (*Lonicera caerulea*) plant. In the present study, the rate of PA application was not used in the economic analysis because differences in the haskap berry yield with respect to rate of PA application was not statistically significant ($P > 0.05$). Overall, the tri-weekly application of PA increased revenue by 42% compared to the weekly (25%) and bi-weekly (33%) applications. The benefit-cost ratios (BCRs) suggested that all the three application frequencies were all not cost effective. Nonetheless, the tri-weekly application of PA gave a BCR of 0.78 suggesting possible increase in viability with time as the 4-year old perennial haskap plants continue to grow and mature. However, further analysis is required since the results obtained was based on assumptions that limited the number of factors and conditions that must be considered in the economic analysis.

Keywords: Benefit cost ratio, Revenue, Labour, Yield, and Liquid seaweed extract

5.1 INTRODUCTION

Haskap (*Lonicera caerulea*) is an indigenous plant known to the Hokkaido natives of Japan and some parts of Russia (Bors, 2008; Thompson, 2006). Haskap berries tend to be rich in vitamin (A and C), potassium, fibre and high contents of antioxidants with studies showing its important remedy effects on health conditions like neurodegenerative and cardiovascular diseases, cancer, osteoporosis and inflammatory diseases (Basu et al., 2010; Jones et al., 2012). Products of haskap berries offered on the market comprise of wine, gelatine, yoghurt, jams, ice cream and candies (Liu et al., 2018). Notably, as of December 2018, a third country (Japan) in the European Union Economic Partnership Agreement (EPA) had listed haskap fruit as a customary food under its regulations (EFSA, 2018). In 2019, 334 MT of haskap berries were marketed in Canada with a farm gate value of CAD$ 2,309,000 (CHD, 2019). Despite the economic potential of haskap on the world market, the assessment of the financial analysis of various agronomic inputs include biostimulant application on haskap production is limited.

Plant biostimulants are organic substances that boost plant tolerance to abiotic stress, promote growth, increase yield, and improve plant quality (Jardin, 2015). Generally, biostimulants extracted from plants can stimulate molecular, biochemical, and physiological plant reactions in minute concentrations. Such responses include enhanced floral development, increased plant growth, development, and yield, improved functional and dietary value of consumable portions (Calvo et al., 2014; Bulgari et al., 2015; Halpern et al., 2015). The global market for biostimulants is estimated at $1 billion (USD), with a projected yearly increase of 13% in European Union countries like Germany and Sweden (Calvo et al., 2014; Colla and Rouphael, 2015). In North America, the biostimulants market was estimated to worth $378.79 million (USD) in 2018 with a projected growth of $646.66 million (USD) by 2023 (NABM, 2019). Regardless of the significant exposition on the beneficial use of biostimulants, its economic impact on haskap must be assessed.

Pyroligneous acid (PA) is an organic liquid obtained through the process of pyrolysis (Mathew and Zakaria, 2015; Grewal et al., 2018), and composed of a mixture of chemical compounds such as organic acids, alcohol, phenolic, alkane and ester (Wei et al., 2010). This bio-product is obtained from various sources of plant biomass as the feedstock. Many studies have shown PA can boost seed germination, plant growth, fruit size, and fruit weight. in some plants like rockmelon (*Cucumis melo* L. *cv.* Golden Langkawi) (Mohan et al., 2006; Zulkarami and Mohammed 2011). The utilization of PA has been on the rise since it is environmentally friendly and has been researched to boost plant quality and yield. However, for extensive adoption of PA by farmers and acceptance of its market value, the economic implications of its application must be evaluated and validated over a period of time and under regional conditions through benefit cost analysis (Chan et al., 2011).

Benefit cost analysis is the method for assessing a scheme or venture by likening the financial analysis derived from that project with the financial costs of the project. The rationale of benefit-cost analysis is to assess the fiscal value of a project and use the results obtained to evaluate other projects (Shively & Galopin, 2013). Therefore, this study aims to assess the viability of different frequencies of PA application to haskap production using the benefit-cost analysis.

5.2 MATERIALS AND METHODS

For experimental design, refer to Chapter 3 and Chapter 4.

Enterprise budgets were developed for haskap for each of the PA treatments and frequencies of application. All the costs were based on the expenses at the time they were incurred in 2020 growing season. This financial analysis was performed in consultation with an economic expert. The wholesale price for haskap berries, labour cost for packaging, harvesting, cost of freezing and transportation, herbicide, and beehives were obtained from North 45 orchards based of grower practices in Cloverdale, NS. The fertilizer cost was obtained from purchases made at Truro Agromart. Vehicle rental cost was obtained from Enterprise car rental limited. The fuel cost was based on mileage from Stewiacke to Cloverdale. Labour was based on the number of hours that will be accrued from a particular farm activity using manual labour (spraying and harvesting of haskap berries) from May to August on a hectare of land. The wage rate used is equivalent to the minimum wage rate of Nova Scotia (i.e. the Province in which the research was carried out). The liquid seaweed extract was obtained from Scotian Gold, NS with an estimate of $10/L. The price for pyroligneous acid was estimated $10/L. The miscellaneous components include cost incurred from purchasing stationaries, personal protective equipment, labels, water, flags, hand sanitizers, containers for harvesting, and refreshment during the trips to the field and other transaction costs.

Benefit-cost ratio (BCR) was used to compare the three frequencies of application of PA. The BCR is calculated as the ratio of the benefit (total revenue) to the total production cost. A BCR greater than one (1) means that the project is viable (Shively & Galopin, 2013). On the contrary, a BCR less than one implies that the project is not viable. If the BCR is equal to one, it means there is a break-even. To do this, the expected future cost and benefits of the project must be discounted at a certain rate to account for the time value of money. However, no discounting was done in this work because the experiment was run at a single time period, and there were constraints beyond the scope of the research.

5.3 RESULTS AND DISCUSSION

The benefit cost analysis was performed without fixed costs such as capital (land), seedlings, and land preparation. This is because the full analysis of the viability of this project is beyond the scope of this thesis. The values were not discounted because they were present values and collected in one time period (2020). The weekly application of PA had the highest total cost of production followed by the bi-weekly application while the tri-weekly application had the least cost (Table 5.1). The high total cost of production for the weekly application was largely impacted by the frequent activities carried out on the farm, which included the weekly visit to the farm and PA applications compared to bi-weekly and tri-weekly farm activities.

The fertilizer and herbicide cost were the same for all the frequencies of PA application due to one-time point application. Thus, the purchase and application of fertilizer and herbicide were carried out in a day and not in timely intervals (Table 5.1). Vehicle rental increased for weekly application due to the number of trips (16) required for the field visits during the growing season. The bi-weekly application required eight trips whereas the tri-weekly application required four trips to the field for PA application. Hence, the increase in number of field trips for the weekly application contributed to its high total cost. Likewise, the insurance coverage with every trip influenced the total vehicle rental cost for the weekly application of PA compared to the bi-weekly and tri-weekly applications.

The increase in fuel cost with weekly application of PA occurred due to increased visits to the field and mileage covered between May and August in comparison to the bi-weekly and tri-weekly applications of PA. The cost of beehives was the same for all the application frequencies because it was considered a one-time purchase for use. Hence, the uniformity in price across the frequencies. The cost of labour also increased with the weekly application due to the number of hours accrued from activities carried out in the field during the 4 months growing season. These activities included spraying, harvesting and data collection. Thus, the number of trips coupled with the hours accrued from spraying and hourly rate of pay significantly increased labour cost for the weekly application of PA followed by the bi-weekly and then the least for the tri-weekly application.

Table 5. 1. Total cost analysis of weekly, bi-weekly, and tri-weekly pyroligneous acid (PA) application

Items	**Weekly**	**Bi-weekly**	**Tri-weekly**
Fertilizer	309.50	309.50	309.50
Herbicide	462.90	462.90	462.90
Vehicle rental	2496.00	1248.00	624.00
Fuel	500.00	400.00	300.00
Beehives	1000.00	1000.00	1000.00

Items	Weekly	Bi-weekly	Tri-weekly
labour	8366.67	4183.33	2788.89
Harvesting	697.22	697.22	697.22
Freezing and transportation	855.25	1133.55	1447.88
packaging	1539.45	2040.39	2606.18
Pyroligneous acid	4745.83	2372.92	1581.94
Liquid Seaweed extract	512.50	300.00	155.00
Miscellaneous	2000.00	1500.00	1000.00
Total Cost	**23485.32**	**15647.81**	**12276.29**

All monetary values are in Canadian dollars. BCR = benefit cost ratio; PA = pyroligneous acid

The cost for harvesting was the same across all frequencies because it was considered a one-time event. The mechanized form of harvest was considered for this study. Thus, the estimated hours for harvesting a hectare of land was less in comparison to manual harvesting of berries (i.e. handpicking). The freezing and transportation of haskap berries increased with the tri-weekly application due to increased berry yield output. The tri-weekly application increased percentage yield by 95% compared to control treatments. Hence, more funds were needed to freeze and transport the berries.

Similarly, the cost accrued from packaging haskap berries was influenced by berry yield. The labour required for packaging increased with higher berry yield from tri-weekly application of PA in comparison to weekly application of PA which recorded decreased yield. The weekly application of PA and LSE required the use of more litres of PA and LSE hence the increase in cost. Tri-weekly application required less quantities of PA and LSE. The number of trips to the farm for the application of PA influenced the miscellaneous cost for weekly application of PA. Thus, more funds were required for the purchase of items like water, disposable safety wear, and refreshment due to the nature of the work. However, reduced trips implied less purchases made for these items. Similarly, the tri-weekly application had the highest revenue followed by the bi-weekly application while the weekly application recorded the least revenue (Table 5.2). The revenue values per PA application frequency correlated with their respective yield. That is, tri-weekly had the highest yield, followed by bi-weekly and then the weekly PA application.

The Benefit-Cost Ratios (BCRs) suggested that the three different frequency of PA application were not viable. The weekly application of PA gave the least BCR followed by bi-weekly and then the tri-weekly (Table 5.2).

Table 5. 2. Revenue and BCR for weekly, bi-weekly, and tri-weekly application of PA

	Weekly	**Bi-weekly**	**Tri-weekly**
Revenue	5708.51	7566.08	9664.10
BCR	**0.24**	**0.48**	**0.78**

All monetary values are in Canadian dollars. BCR= benefit cost ratio; PA = pyroligneous acid

However, these results must be interpreted with caution since the analysis was based on assumptions that did take several items and conditions into consideration. For instance, the fixed cost values were not incorporated into the analysis. Also, the haskap plant is still at its early stages (4 to 5 years) of development and have not reached their critical growth stage for optimum berry production. This suggested that there is a potential of higher yield in the future until the peak year when it starts to decline in yield. The values obtained in this study might change if all these factors were taken into consideration. Unfortunately, the direction of change cannot be predicted with the limited information available.

Nevertheless, this result gives a fair idea as to which frequency gave the best benefit at the present stage of development of the haskap plants and will potentially yield the best revenue as the plant develops to its full potential. It is imperative to recognize that the overall benefits of using PA could be significant; firstly, with savings in fertilizer application, the recovery in soil conditions, and increase in yield for future haskap berry production (Chan et al., 2011). Reduced cost in fertilizer purchase and its application could lead to decrease in carbon release linked with the production of synthetic fertilizers specifically nitrogen fertilizers (Davis & Haglund, 1999).

5.4 CONCLUSION

The benefit cost analysis suggested that the application of PA on tri-weekly basis could increase revenue by 42% in comparison to bi-weekly (33%) and weekly (25%) basis. This suggests that the tri-weekly application of PA could provide haskap farmers with beneficial economic returns with increased production.

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CHAPTER 6 CONCLUSION

Many waste materials have been commonly used as fertilizer sources in crop production, but very few waste materials have been exploited for plant biostimulant abilities. Such plant products like wood, seaweed, left-over plant produce have been known to promote crop growth, yield, and fruit quality (Monterumici et al., 2015). Hence this study aimed to investigate the stimulatory effect of biostimulants like pyroligneous acid (PA) combined with liquid seaweed extract (LSE) on haskap (*Lonicera caerulea* L.) growth, yield, fruit quality, and powdery disease management from two seasons (2019 and 2020). The financial analysis of PA application was studied.

The preliminary findings suggested that plant growth and yield responses to weekly, bi-weekly and tri-weekly of PA with added 0.35% LSE were significant ($P < 0.05$) for photosynthetic capacity (Fv/Fm), chlorophyll content, flower count, fresh leaf weight, and 100-berry weight. However, rates of PA had marginal ($P = 0.05$) effect on chlorophyll content. The percentage change in yield with farmer's average was increased by about 91% with the 8% PA + 0.35% LSE application but was reduced by about 18% with 4% PA alone. The 2-D principal component analysis (PCA) biplot revealed the positive influence of bi-weekly application of PA on haskap plant growth with increases in anthocyanin, fresh and dry leaf weight, plant height and chlorophyll content. The PCA also revealed positive influence of tri-weekly application of PA + 0.35% LSE on berry yield, 100-berry weight, and Mn content. The PCA showed positive influence of the 2% and 4% PA + 0.35% LSE on haskap plant growth and berry yield.

Additionally, varying frequency and rates of PA with added 0.35% LSE had significant ($P < 0.05$) effect on berry quality. The weekly application of 10% PA + 0.35% LSE reduced disease incidence and severity by 8.3%, respectively. The 2-D principal component analysis (PCA) biplot revealed the positive influence of weekly application of PA + 0.35% LSE on haskap berry quality with increases in berry tissue nutrients, pH, and Brix°. (Figure S1). The PCA showed positive influence of the 2%, 4%, and 6% PA + 0.35% LSE on haskap berry quality. The benefit cost analysis suggested that the application of PA on tri-weekly basis could increase revenue by 42% in comparison to bi-weekly (33%) and weekly (25%) basis. This suggests that the tri-weekly application of could provide haskap farmers with beneficial economic returns with increased production.

The limitations to this study include varying environmental and soil conditions, which could account for such low berry yield despite PA rate application. Additionally, fruit drop due to removal of bird nettings for harvest could also account for the low yield recorded. This further suggested that the haskap plant may have undergone some level of stress, thereby affecting yield output per plant (MacKenzie et al., 2018). Weeds were also a significant factor that contributed to the yield reduction in this study. Weeds are ubiquitous plants that contend with fruit crops for nutrients, water, light, and space (Khaliq et al., 2014).

The current study suggested that frequency of PA application is important in the promotion of haskap plant growth due to the presence of some organic compounds. The increase in berry yield indicates possible synergistic effect of PA with LSE on berry development. Thus, the addition of PA with added LSE could have increased haskap berry yield. Bi-weekly application of 2% and 4% PA + 0.35% LSE may enhance haskap growth. However, tri-weekly application of 2% and 4% PA + 0.35% LSE may increase yield. Nonetheless, further studies are needed to understand the mechanism of nutrient uptake via the foliar mode of application of PA + 0.35% LSE.

The increase in berry quality indicates possible synergistic effect of PA with LSE on berry tissue nutrients. The increase in PA rates (thus, > 8%) with added 0.35% may have negative effects on berry quality. However, decrease PA rates (thus < 8%) with added 0.35% LSE may enhance berry quality. The weekly application of 10% PA + 0.35% LSE at the end of haskap berry harvest may curb the incidence and severity of powdery mildew disease and promote plant immunity. Nonetheless, further studies are needed to elucidate the underlying mechanisms by which PA + 0.35% LSE enhances haskap berry quality via foliar method.

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