CHARACTERIZATION OF *Ribes nigrum L.* FRUIT QUALITY IN RELATION TO FRUIT MATURITY, GENOTYPE AND GROWING LOCATION

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Abstract

Two separate studies were conducted on Prince Edward Island to determine how fruit cultivar and harvest timing can influence black currant berry quality during organic production. The overall goal of both studies was to characterize these influences and to recommend cultivars and harvest timings for achieving optimum quality across different black currant cultivars including berry size, acidity, total soluble solids and the distribution of biologically active compounds. In 2012, there was an interaction effect (cultivar x harvest timing) on berry size, total soluble solids (^{o}Brix), cyanidin 3-*O*-glucoside and delphinidin 3-*O*-glucoside. Overall, Whistler and Ben Sarek had larger berry size compared to Titania, Ben Tirran and Ben Alder. Titania total soluble solids, total antioxidant capacity and total anthocyanin content increased as currants were left on the bush but sample berry size decreased. Titania currants reached peak ^{o}Brix between 10 to 18 days after currants turned black. Whistler had the highest total soluble solids when compared to Ben Sarek and Ben Alder in 2011. Overall, Whistler had less titratable acidity compared to other cultivars. No cultivar recommendation can be made for optimizing antioxidant capacity, anthocyanin content or phenolic content because the cultivar effect was only present during the 2012 field season.

List of Abbreviations Used

100BW:	the weight of 100 individual berries
C3G:	cyanidin 3-O-glucoside
C3R:	cyanidin-3-O-rutinoside
CAE:	citric acid equivalents
CafAcid:	caffeic acid
ChloroAcid:	chlorogenic Acid
D3G:	delphinidin 3-O-glucoside
D3R:	delphinidin 3-O-rutinoside
D3RE:	delphinidin 3-O-rutinoside equivalents
DPPH:	2, 2-diphenyl-1-picrylhydrazyl
DRE:	delphinidin 3-O-rutinoside equivalents
ECG:	epicatechin gallate,
EGC:	epigallocatechin
EGCG:	epigallocatechin gallate

FC:	folin-ciocalteu
FerAcid:	ferulic acid
FRAP:	ferric reducing antioxidant power
FT:	Farmington, PEI
FW:	fresh weight of fruit at harvest
GAE:	gallic acid equivalents
GDD:	growing degree days
HPLC:	higher performance liquid chromatography
HR:	Hunter River, PEI
IC50:	half maximal inhibitory concentration
IsoferAcid:	isoferulic Acid
M3G:	malvidin 3-O-glucoside
Mo:	heteropolyphosphotugstae-molybdate
MS:	mass spectrometry
MSt:	Mount-Steward, PEI
OACC:	Organic Agriculture Centre of Canada

P3G:	peonidin 3-*0*- glucoside
P3G1:	petunidin 3-*0*-glucoside
Q3G:	quercetin -3-*0*-glucoside
Qarabglu:	quercetin -3-*0*-grabinoglucoside
QRh:	quercetin -3-*0*-rhamnoside
QuR:	quercetin -3-*0*-rutinoside
TA:	titratable acidity
TAC:	total antioxidant capacity
TAN:	total anthocyanins
TE:	trolox equivalents
TPC:	total phenolic content
TSS:	total soluble solids, measured as ^{o}Brix

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Chapter 1: Introduction

With increasing concerns on long distance transportation of foods as well as healthy dietary habits, local fruit production is becoming more popular. However, producers and consumers are looking for crops that are suitable for our local climate and soil conditions. European black currants (*Ribes nigrum* L.) are an insect pollinated self-fertile (though limited) berry species belonging to the Grossulariaceae family. Native to Europe and Asia, there exists many diploid species of *Ribes* with certain genotypes being considered cold hardy (Hummer & Dale, 2010). In 2011, Russia, Poland and Ukraine produced 365, 169 and 26 thousand tonnes of currants, respectively (FAOSTAT, 2012). Many black currants get processed into juice, jams, jellies and liqueurs (Hummer & Dale, 2010). North American production is much lower by comparison (FAOSTAT, 2012), which could be explained by the legislation passed during the 1900's. Black currants were banned from production in many areas in North America following the spread of white pine blister rust (WPBR) (*Cronartium ribicola* J. C. Fischer) from European white pine seedlings. This disease originally spread from Asia across the continent to England, where seedlings were infected with the disease. Today, WPBR and powdery mildew resistance are common breeding objectives for most black currant breeders as these diseases are common in many growing regions.

Canadian small fruit production was estimated to be worth $424 million in 2009 (with $57 million generated in Atlantic Canada); however, most fruit production is

focused on blueberries, cranberries, grapes, strawberries and raspberries. To date, black currant production statistics are too small in Canada to be recorded separately. This is surprising since black currant consumption is becoming increasing popular in other parts of the world (Hansen, 2007). In 2007, farmers on Prince Edward Island (PEI) began growing black currants, targeting both local and export markets. To date, despite Titania being considered the most popular cultivar in PEI, it is now considered inferior to many new cultivars being bred in Poland (Pluta, Urawicz, & Krawiec, 2008). Although Canadian fruit breeders have created Whistler and Blackcomb (two new cultivars bred in British Columbia (BC), it is often difficult to discern if a cultivar bred in a specific growing region will find the same success in different climatic regions (ex. BC vs PEI). As such, it is important to investigate possible factors that could affect black currant fruit in order to improve the Canadian black currant industry. The objective of this research was to investigate the effect of harvest timing, cultivar and farming location on berry quality characteristics. The specific berry characteristics observed in this study include: berry size, titratable acidity (TA), total soluble solids (TSS), total phenolic content (TPC), total antioxidant capacity (TAC), total anthocyanins (TAN), flavonols, catechins and phenolic acids.

Nutrient density

In today's competitive marketplace, growers are often seeking crops with the potential for value-adding (Rupasinghe, 2014). The basic nutrient and bioactive phytochemical profiles vary among fruit crops. In Japan, black currants have been

categorized as “super fruits” (Hansen, 2007). Black currants have a wide profile of biologically active phytochemicals (bioactives) which allow them to provide a variety of beneficial health effects in additional to its many traditional value-adding applications such as jams, juices and alcoholic beverages (Barney & Hummer, 2005). Besides high vitamin C levels, research has shown that black currants contain high levels of polyphenols when compared to other fruits (Borges, Degeneve, Mullen, & Crozier, 2010; Wu, Gu, Prior, & McKay, 2004). Many of the polyphenolic compounds found in black currant have been shown to provide protection against cardio-vascular diseases and help with ocular blood flow (Basu, Rhone, & Lyons, 2010; Ohguro, Ohguro, & Yagi, 2013; Wallace, 2008). Compared to other commercially available fruits such as raspberries or even blueberries, black currants contain many biologically active phytonutrients which make the fruit desirable as a nutraceutical (Borges *et al.,* 2010). Nutraceutical products are a multi-billion dollar industry which continues to grow annually (Statistics Canada, 2007). A nutraceutical is a product or compound isolated from food that can be used for creating beneficial health products (Health Canada, 1998). A fruit can be classified as a “functional food” if consumption provides a health benefit(s) beyond basic nutritional functions. These benefits come in the form of prevention and treatment of disease and serve as a complementary or alternative treatment (Health Canada, 1998).There are many functional foods currently on the market but black currants are recognized for many health benefits (Molan, Liu, & Plimmer, 2014; Nakaishi & Matsumoto, 2000; Ohguro *et al.*, 2013).

Berry quality characteristics

Berry size is a fundamental characteristic that can influence many practical aspects of harvesting and marketing as it is directly linked to fruit growth and development. Black currants are small (roughly 5 mm diameter)and have a green pigment at fruit-set but will increase in size and pigment over the course of development as cells divide (roughly 10 mm diameter) (Hummer & Barney, 2002). Temperature and precipitation can influence water balance within fruits which can indirectly affect berry size (Coombe, 1976). Fruit size will increase throughout fruit development until ripening. Cultivars can vary in fruit size, making cultivar selection important at the initial stages of business development (Pedersen 2007; Pluta, Urawicz, & Krawiec, 2008). The most common measure for berry sizing is 100-berry weights. Black currant berry size can vary by as much as 70 g 100 berries^{-1} (Pluta & Pruski, 2010). Cultivars with larger berries could be potential candidates for "U-PIC" farms. Not all cultivars have been characterized according to size with limited reported values being inconsistent within academic literature. This could be explained by experimental location differences (e.g. Poland compared to Estonia). Berry size and anthocyanin content can be negatively correlated, indicating that smaller berries could have higher anthocyanin content (Krüger, Dietrich, Hey, & Patz, 2011). Additionally, berry size has been negatively correlated with TAC. The relationship between antioxidants and berry size could be a consequence of an increased skin to volume ratio (Krüger *et al.*, 2011).

In many fruits, acid: sugar ratio has a significant influence on flavour. Juice pH and TA are indicators of acid content while TSS is a rough estimate of sugar content (Simson & Straus, 2010b). TA can be calculated using a laboratory pH probe and auto-titrator respectively, whereas TSS is measured using a portable refractometer (Thompson, 2003). TSS is a non-specific measurement of all soluble solids suspended in solution, given as ^{o}Brix (or % Brix as 1 ^{o}Brix often means 1.0 % sugar by weight with fruit). By definition, Brix is a measurement of 1 g of sucrose in 100 g of solution but can include many different kinds of sugars, acids, salts, proteins and pigments (Margalit, 2005). Fruit soluble solids are mainly sugars and acids. TSS is especially important in the winemaking industry as winemakers will not use fruits with ^{o}Brix values of less than 15 as it would require too much additional sugar for ethanol conversion which can make flavour too simple or bland. TA is another important quality characteristic for winemaking as balance between sugar and acidity can significantly influence wine flavour (Margalit, 2005). Black currants contain mostly citric acid so TA is often quantified using % CAE (citric acid equivalents) (Anttonen & Karjalainen, 2006; Zheng, Yang, Tuomasjukka, Ou, & Kallio, 2009). Overripe black currants can have lower TA levels (Rubinskienė, Viškelis, Stanys, Šikšnianas, & Sasnauskas, 2008).

Bioactive compounds in fruits have become a focal point of many researchers (as well as the functional food industry) due to their nutraceutical status and the health benefits associated with consumption (Lindsay, 2000; Wallace, 2008). There are numerous bioactive compounds present in black currant; phenolics are the most

abundant and have been the most studied (Hellström *et al.*, 2010; Wu *et al.*, 2004). Black currants seem to contain higher concentrations of phenolics compared with other commercial fruit including but not limited to blueberries, blackberries, grapes and strawberries (Borges *et al.*, 2010; Wu *et al.*, 2004; Zatylny, Ziehl, & St-Pierre, 2005). Given the presence of high phenolics, black currant growers can be more competitive in the nutraceutical fruit market. Total phenolic concentration can vary between black currant cultivars (Libek & Kikas, 2001; Wu *et al.*, 2004). For example, flavonols in black currants such as myricetin, quercetin and kaempferol will vary among cultivars, ranging from 8.9 to 25.5 mg 100 g^{-1} of fresh weight (Mikkonen *et al.*, 2001). Anthocyanin levels can vary among cultivars with differences as high as 1500 mg 100 g^{-1} (total anthocyanin content) (Hellström *et al.,* 2010; Yang *et al.*, 2010). Furthermore, total antioxidant activity (TAC) can also be affected by cultivar (Sablani *et al.*, 2010). The most abundant phenolic compound in black currants, are anthocyanins (Nielsen, Haren, Magnussen, Dragsted, & Rasmussen, 2003b; Slimestad & Solheim, 2002; Wu *et al.*, 2004). Anthocyanins are water-soluble pigments derived from flavonoids via the shikimic acid pathway (Chalker-Scott, 1999). In addition to other factors, anthocyanins are responsible for a currant's namesake color (cyanidin and delphinidin monoglucosides are associated with red and blue-violet color respectively). Black currant anthocyanins are typically found as 3-*O*-monoglucosides such as cyanidin-*O*-glucoside (Borges *et al.*, 2010; Wu *et al.*, 2004). These compounds are present in both black currant juice and skin (Bishayee *et al.*, 2011; Nielsen *et al.*, 2003b). Overall, anthocyanins have been

linked to a reduction in glaucoma symptoms (Ohguro *et al.*, 2013) and cardiovascular disease (Basu *et al.*, 2010).

Black currant bioactives are often analyzed using spectrophotometric assays or liquid chromatography (Wu *et al.*, 2004). As functional foods become more popular, breeders are considering higher antioxidant capacity or phenolics as new secondary breeding objectives. The primary objective of most black currant breeders is disease resistance. Total antioxidant capacity assays measure the antioxidant capacity of black currant extracts. There are two TAC assays commonly used for fruit analysis: FRAP and DPPH. FRAP (ferric reducing ability of plasma) assay measures the reduction of TPTZ to a blue coloured product in which high blue coloring is an indicator of high antioxidant levels (Benzie & Strain, 1996). Values are presented as μmol of TE (Trolox equivalents) g^{-1} FW. Where FRAP examines the single electron transfer ability of antioxidants, the DPPH (1, 1-Diphenyl-2-picrylhydrazyl) radical scavenging assay examines the hydrogen electron transfer ability. The free radical form of DPPH can be rendered inert by antioxidants (Blois, 1958). The amount of DPPH in its free radical form is inversely proportional to the effectiveness of the antioxidant; less free radical DPPH indicates increased effectiveness of antioxidants. Results are given as IC50 values (concentration required to reach 50 % inhibition). Total phenolic assays (TPC) utilize Folin-Ciocalteu reagent to measure the reduction of Mo (V) to Mo (IV). This gives an estimate of the total phenolics found within the sample (Lowry, Rosebrought, Farr, & Randall, 1951). It is important to note that non-phenolic reducing compounds present in black currants,

such as ascorbate, can affect the phenolic results. The total phenolic assay measures the total phenolics present in black currant extracts as µmol of GAE (Gallic acid equivalents) g^{-1} of FW. Total antioxidant capacity and TPC assays are non-specific analyses, measuring a total approximation of antioxidants and phenolics. High performance liquid chromatography (HPLC) is precise and has become increasingly popular for analysis of polyphenolics present in fruits (Borges *et al.*, 2010; Hellström *et al.*, 2010; Wu *et al.*, 2004). HPLC separates compounds of interest in the sample assisting their detection. Anthocyanins are the main compound of interest in black currants. Black currant extracts can be analyzed with external standards of cyanidin *3-O*-glucoside, cyanidin *3-O*-rutinoside, delphinidin *3-O*-glucoside and delphinidin *3-O*-rutinoside because these anthocyanins are the most abundant (Anttonen & Karjalainen, 2006; Wu *et al.*, 2004). Anthocyanin concentration is expressed as mg 100 g^{-1} FW. The pH-differential technique is another method for determining total monomeric anthocyanins in fruit extracts. It roughly quantifies total anthocyanins by analyzing anthocyanin structural changes that occurs at different pH values (Lee, Durst, & Wrolstad, 2005).

Fruit maturity

Many new black currant cultivars have not been adequately studied for growing in Atlantic Canada. Unlike other berries, it is difficult to visually determine black currant fruit maturity because there is no easy visual distinction between early and late ripening. In order to facilitate the assessment of fruit maturity, TSS (^{o}Brix) measurements using a hand refractometer is commonly used. It is difficult to use TSS as

a maturity indicator for new cultivars as each individual cultivar can vary in TSS values. Choosing a standardized ^{o}Brix measurement might result in lower potential Brix values for some cultivars. For example, Gofert and Bona differ significantly in ^{o}Brix values (13.4 vs. 18.5^{o}Brix) (Pluta and Pruski 2010). This makes it difficult to decide when to harvest both of these cultivars unless cultivar specific TSS habits are known; does Gofert only reach a maximum of 13.4^{o}Brix or is it capable of reaching higher TSS values like Bona? Each cultivar has its own set of berry characteristics which should be known prior to large scale planting. It is important to choose harvest timings that allow for each cultivar to perform optimally. Waiting for a ^{o}Brix value to increase is often chosen as the most suitable course of action, especially in the grape industry (Vian, Tomao, Coulomb, Lacombe, & Dangles, 2006). This might be applicable to black currants as they are considered non-climacteric which means that currants do not ripen post-harvest. “Peak” ripeness can only be reached on the bush reaffirming the importance of proper harvest timings. In general, ripe fruits will senesce if left on the bush for prolonged periods of time (Taiz & Zeiger, 2010). It is unclear as to which berry quality characteristics are significantly affected by senescence. One of the goals of this research is to maximize peak quality by picking berries at the optimal time, where TSS, TAC, TPC and berry size are at their highest without compromising berry firmness which affects harvestability. After the initial year of vegetative growth (from seed or cutting), black currants require 120 to 140 frost-free days before fruiting (Hummer & Dale, 2010). In Canada, flowering (raceme inflorescence) occurs in May-June, with bees and various other insects needed as pollinators. After pollination, each individual flower ovary becomes an individual

currant. The ovary wall turns into an edible pericarp layer resulting in a fruit being set on the branch (Gould *et al.*, 2008). Fruiting will only occur on wood that is at least one year old; however, it has been noted that one year old wood produces the most fruit when compared to second and third year wood (Hummer & Dale, 2010). After fruit set, berry pericarp starts to enlarge, chlorophyll starts degrading and anthocyanin biosynthesis increases as fruits mature and ripen. Once a currant has reached 100% visual black pigmentation, it will undergo ripening where cell walls soften (increased pectinase activity) and additional pigments and sugars are produced and stored in vacuolar tissues. After a fruit reaches horticultural maturity (ready to eat), tissues components will slowly get catabolised thereby starting the gradual process of senescence (Coombe, 1976). The rate and magnitude of these changes can vary among cultivars, with some cultivars capable of reaching horticultural maturity much faster than others. Identifying these cultivars will allow black currant farmers to stagger their harvest by planting "early" and "late" cultivars. Total phenolics and antioxidant capacity will accumulate throughout development until horticultural maturity (Tabart, Kevers, Pincemail, Defraigne, & Dommes, 2006). There is evidence that overripe berries (past horticultural maturity) contain more bioactives (Rubinskienė, Viskelis, Jasutiene, Duchovskis, & Bobinas, 2006; Rubinskienė *et al.,* 2008). However, black currants become increasingly difficult to harvest as time progresses as they lose firmness and fall off the bush. As well, if they are too soft, they may be more susceptible to harvest damage since the harvesting machines operate by beating and shaking the currants off the bush. Considering that many farmers use machine harvesting, berry firmness is a high priority;

therefore, harvest timings and cultivars that meet a required firmness (for machine harvesting) are first priority. This “firmness” threshold could be determined using a penetrometer (Rubinskienė *et al.*, 2008). TSS values are important for growers as they are often indicative of maturity and flavour. Many PEI farmers conduct fresh market business so TSS could be considered the second priority. The added health benefits from anthocyanins, TAC and TPC will be considered third, fourth and fifth priority respectively. Additional compounds like flavonols, catechins and phenolic acids can be considered lower priority because they are less abundant in black currants. In PEI, some black currants may undergo drying and sugar infusing, which makes berry size, juice pH and TA less important. When currants are suspended in a sugar solution, they can undergo isotonic exchange. This can make berry size (weight) increase as sugars exchange water with the exterior osmotic sugar solution (Shi, Pan, McHugh, & Hirschberg, 2008). Other than water, soluble solids like acids could be pulled out of fruit cells, which can influence residual berry pH. Total soluble solids is a potential maturity indicator and may also reduce sugar-infusion costs as TSS levels are adjusted during processing (Shi, 2007). In summary, optimal berry quality can be evaluated based on the following criteria in order of importance: firmness, TSS, anthocyanins, TAC, TPC, size, TA, flavonols, catechins and phenolic acids.

Cultivar

Black currant production, breeding and research are significantly more advanced in Europe than in North America. Getting new foreign cultivars into Canada is difficult

and thereby limits Canadian black currant research and production. Additionally, cultivars can lose their disease and insect resistance over time (Libek & Kikas, 2001). This forces breeders and plant nurseries to change stock regularly. Canadian growers who plant older cultivars have access to cultivar growth information but risk evolving insects and pathogens. Contrary to this trend, Titania, a popular black currant cultivar, was originally bred in 1981 by Pal Tamas and has persevered as one of the most common cultivars on the market. Many research institutes still use Titania for comparison. Cultivar selection is one of the most important steps in berry production because genetics can significantly influence berry characteristics (Anttonen, Hoppula, Nestby, Verheul, & Karjalainen, 2006; Tabart *et al.*, 2006; *Wu et al.*, 2004). Identifying cultivars with suitable berry qualities is necessary for black currants to become more marketable both overseas and here in Canada. Breeding programs in other countries have yielded many new cultivars but many of them have not been tested under the same growing pressures present in Canada.

White pine blister rust is the predominant black currant disease on PEI. Ben Hope was planted in PEI before disease resistance was properly verified, resulting in significant crop failure (S. Cousins, personal communication). In order for organic farms to succeed, farmers must grow disease resistant cultivars. Titania has become the standard common cultivar grown on Canadian farms but many newer cultivars have not been adequately tested in PEI (e.g. Whistler, Blackcomb, etc). It is important to note the genetic differences can result in significant physical and chemical differences. For

example, phenolics biosynthesis/accumulation can be influenced by genetics (Chalker-Scott, 1999; Wu *et al.*, 2004; Yang *et al.*, 2010).

Site

Cultivar, climatic conditions and agronomic practices are important factors that can affect fruit quality (Khoo, Clausen, Pedersen, & Larsen, 2012; Yang *et al.*, 2010). While cultivar genetics have been shown to have a significant effect on crop yield and berry quality characteristics (Krüger *et al.*, 2011; Pluta *et al.*, 2008; Pluta & Pruski, 2012), there is some evidence that climate and site effects may also have an influence (Anttonen *et al.,* 2006; Mikkonen *et al.*, 2001; Vian *et al.*, 2006). Even in a relatively small bioregion such as the Maritimes of Canada, there can be considerable variability in soil type and climate among sites. Open field trials are subject to many site-specific effects that could potentially influence berry quality. These site specific effects can include but are not limited to management history of the field, background fertility and soil properties (clay, sand, etc.). These effects can influence soil organic matter content and availability of macro and micro nutrients (like nitrogen or phosphorus). Rain can influence soil nitrogen levels as well, which is an important detail for rain-prone Atlantic Canada. The degree of influence is relatively unknown and difficult to quantify due to many contributing factors. Soil types like clay, sand or humus can improve or impede bush growth which could indirectly affect berry qualities like size or yield. Furthermore, differences in soil fertility have been shown to influence anthocyanin content in grapes (Vian *et al.*, 2006). Plants do not have a developed immune system thereby are

depending heavily on secondary metabolites for defence (e.g. anthocyanins). As such, secondary metabolites are an important part of plant-environment interactions (Lev-yadun & Gould, 2009). Cultivation site can affect phenolic content in black currant fruits resulting in differences of up to 77% (Anttonen *et al.*, 2006). Microclimates could influence berry quality by mediating factors like sunlight or wind. Even site specific management practices can affect berry quality as organic berries have been shown to contain higher ascorbic acid levels and cancer cell inhibition (Khoo *et al.*, 2012). The amount of uncontrollable factors that occur at different farming locations makes it difficult to determine which factor is responsible for berry quality changes seen across sites. Attempting to identify some of these factors is an objective of this research.

In this specific study, sampling occurred over a time period of three weeks in order to allow cultivars to reach optimal performance and to see if the effect of fruit maturity and cultivar interacted. The intention of this research is to assess a wide range of cultivars for commercial production in Canada and to determine the optimal harvest time for cultivars.

The overall goal of this research

To characterize black currant fruit maturity and genotype, with the purpose of optimizing berry quality at harvest.

Objectives

1. To determine the effect of fruit maturity on berry quality.
2. To determine the effect of genotype on berry quality.
3. To determine the effect of farming site (growing location) on berry quality.

By analyzing these effects, recommendations could be made to farmers to determine which cultivar and harvest timing is appropriate for black currants depending on their end use of the fruit.

Chapter 2.The effect of fruit maturity on *Ribes nigrum* L. berry quality

Abstract:

Fruit maturity (harvest timing) can influence black currant berry quality. The overall goal of this study was to determine how fruit maturity affects berry quality and to identify beneficial management practices that influence berry size, acidity, total soluble solids (TSS) and polyphenol concentration. Results have indicated that fruit maturity and growing location significantly affects berry quality. The average size of sampled Titania currants started decreasing (roughly 10%) 10 days after currants turned black. Titania currants reached higher soluble solids (17.1^{o}Brix) 18 days after turning black in 2011 and 10 days after turning black in 2012 (19.1^{o}Brix) compared to other harvest timings. Black currants might reach a ^{o}Brix plateau after receiving a specific amount of heat units but more field seasons are needed to verify this. Black currants picked at later harvest timings had higher antioxidant capacity and anthocyanin content. Growing location was shown to affect black currant soluble solids and antioxidant capacity ($P<0.05$). Farmers interested in maximizing TSS, total antioxidant capacity and anthocyanin content could consider a later harvest timing but risk losing yield to berry drop.

2.1 Introduction

Horticultural maturity or ripeness of black currant (*Ribes nigrum* L.) is difficult to determine visually. Unlike other berries, currants have a time period after full black colouration where organoleptic and nutritional components continue to change while

the berries retain the same appearance (Hummer & Dale, 2010; Rubinskienė *et al.*, 2008). Identification of a reliable maturity indicator is important for farmers to determine optimum harvest timing. Unfortunately, many interactions between berries, variables of interest and their growing locations can become confounded by uncontrollable environmental factors like heat units or precipitation (Koch, 1986; Yang *et al.*, 2010; Zheng *et al.*, 2009). This means that experimental replication across farms or even countries, is necessary to truly understand how physiology of fruits changes independently of its environment. Berry quality components may include: berry size, total soluble solids (TSS, measured as ^{o}Brix), titratable acidity (TA), total antioxidant capacity (TAC), total phenolic content (TPC), total anthocyanins (TAN), flavonols, catechins and phenolic acids. Black currants often achieve 15% TSS (Brix) making them suitable for winemaking. Black currants are also known for high vitamin C but they contain many other biologically active (bioactives) components such as anthocyanins (Wu *et al.*, 2004) which give berries their deep color and provide health benefits by protecting against various chronic conditions (Basu *et al.*, 2010; Wallace, 2008). Black currants contain more bioactives than many commercially available fruits (Borges *et al.*, 2010) making currants suitable for nutraceutical markets. Nutraceutical products (biologically active food components used for health products) are a growing industry (Statistics Canada, 2007) requiring Canadian farmers to grow crops rich with bioactives. Determining potential proxies for non-perceivable benefits such as bioactive antioxidants or phenolics could allow farmers to predict these benefits *in situ* without laboratory equipment.

Development of black currant fruit can include several stages: flowering, pollination (Kaldmäe, Libek, Kikas, & Arus, 2010), fruit set (green), maturation (color change from green, red to black with increasing size) and ripening (changes after currants are 100% black coloured). Inadequate pollination can result in fruit abortion after initial fruit set which is common in black currants as they are poor self-pollinators (or even completely self-infertile in some cases) and thus require insects for pollination (Barney & Hummer, 2005; Hummer & Dale, 2010). Partially pollinated currants can abort and drop during maturation with an off-pink colour. Successful pollination (when enough pollen falls into the stigma) begins a chain of processes that causes fertilized ovules to become small seeds and for the ovary itself to begin cell division resulting in a small green currant which continues to develop physically and chemically throughout June, July and August. Seeds are suspended in the mesocarp layer which grows over time. In ripening fruit, chloroplasts are slowly converted to chromoplasts (Bouzayen, Latché, Nath, & Pech, 2010) and anthocyanin pigments are produced and stored in vascular tissue (Taiz & Zeiger, 2010). Fruit tissue development is driven by pollination and heat (Taiz & Zeiger, 2010). If flowers were successfully pollinated during May, tissues will continue to development of characteristics until respiration remains constant or decreases (Taiz & Zeiger, 2010). Pectin content (responsible for maintaining rigid berry structure) will be gradually broken down by pectinase resulting in soft edible fruits. Berry chemical components can be influenced by many factors including but not limited to fruit maturity, cultivar and growing location (Zhao, 2007). Ultimately, final berry quality of black currants is dependent on harvesting berries at a level of ripeness

when fruits exhibit their optimal berry characteristics. Furthermore, the degree of ripeness could impact post-harvest storage (Zhao, 2007). Black currants are non-climacteric (they ripen without ethylene and peaks of cellular respiration) (Brennan, Gordon, & Lanham, 1998) which means berry quality is not likely to improve post-harvest. Producers can therefore control quality characteristics by managing their harvest timing. Characterizing the stages involved in black currant maturation and ripening can give growers insight into how berry attributes change over time and which response variables are most influenced by harvest timing. Some properties predominantly change pre-colouration including berry size, chlorophyll, anthocyanin pigmentation, and TPC (Zhao, 2007). Some characteristics can continue to increase during post-colouration including TAN or TSS. Black currants are known for high vitamin C but they contain many other biologically active components (Wu *et al.*, 2004). Cyanidin and delphinidin monoglucosides are the most abundant bioactive components in black currants (Brennan *et al.*, 1998; Wu *et al.*, 2004). It is well understood that anthocyanins develop and change over time in most fruits but it is unclear to what magnitude they change during the harvest season after they turn black. There has been some indication that anthocyanins might increase during this time period (Rubinskienė *et al.*, 2008). Some characteristics like TA change continuously throughout fruit development while others do not have a consistent pattern. Evaluating currants during the black post-colouration stage is both practical and essential for allowing characteristics like TSS or TAN to reach suitable levels (Rubinskienė *et al.*, 2008).

The objectives of this experiment was to find the optimal fruit maturity of Titania cultivar of black currant grown in Prince Edward Island to maximize berry size, total soluble solids, total antioxidant capacity (TAC), total phenolic content (TPC), total anthocyanins (TAN), flavonols, catechins and phenolic acids while minimizing acidity.

2.2 Methods

2.2.1 Experimental site

Field trials took place at two farms near Farmington and Hunter River, PEI Canada. Both experimental sites were well-drained sandy loam. The Hunter River site is a gently north-sloping field surrounded by tree windbreaks while Farmington is gently west-sloping with some wind breaks. Background soil fertility levels were assessed at both farm locations by collecting soil samples 15 cm below ground level at each plot and submitting these samples to the PEI soil testing lab (Table 2.1). Climate data was collected from nearby Environment Canada weather stations (Table 2.2) in 2011 and 2012. Temperature sensors were installed on the sites in 2012 to permit calculation of growing degree days and to determine the differences between the closest weather station and on-site measurements.

2.2.2 Fruit sampling

Three plots of five Titania black currant plants were repeatedly harvested at each site during August at four (2011) and five (2012) stages of maturity to determine physical and chemical properties. Harvest timings were chosen based on berry surface colouration reflecting the potential harvest period for Titania currants and to monitor

the change in berry properties. During the 2011 field season, currants were picked at 3, 6, 10 and 18 days after 95% of the berries on the bush turned black (August 2nd, 5th, 9th and 17th respectively). After analyzing the 2011 data, the optimal range for Titania seemed to be closer to the later timings so the harvest timings were adjusted to reflect that during the following season. In 2012, currants were sampled at 6, 10 14, 18 and 22 days after 95% of the berries on the bush turned black (August 6th, 10th, 14th, 18th and 22nd respectively).

At each maturity stage, berries were sampled randomly from these five plants to create a composite sample from each plot. Samples (200-300 g) were collected in a freezer bag and stored in a cooler with cold packs until returning to campus where they were stored in a fridge overnight. Fresh sub-samples were taken the following day to determine size, TSS, and TA. A second sub-sample was taken for TAC, TPC, TAN and HPLC analysis. Prior to extraction, this sub-sample was dipped in liquid-nitrogen and stored at -80^{o}C in order to prevent any changes during storage until analysis.

2.2.3 Berry size, total soluble solids, titratable acidity, total antioxidant capacity, total phenolics and total anthocyanins

Berry size was measured by a modified 100 berry weight technique outlined by Pluta *et al.* (2008), where three replicate measurements of 50 berries were weighed. Total soluble solids were measured using a digital Atago hand refractometer (Atago Co. Ltd., Japan) as outlined by Libek and Kikas (2001). Titratable acidity was measured using a 785 DMP Titrino autotitrator (METTLER TOLEDO Mississauga, Canada). Two mL of

sample were diluted with 28 mL of water and titrated using 0.1M NaOH (results are presented as % CAE (% citric acid equivalents)).

The TAC, TPC and TAN of berries were determined using total phenolic extracts of 80% methanol (modified from Kapasakalidis, Rastall, & Gordon, 2006). During 2011, samples of 100 g were blended with 160 mL of solvent using a laboratory blender (Model HBB908, Hamilton Beach Brands, Inc. Glen Allen, VA) and topped off to a final volume of 200 ml. The sample was poured through four layers of cheesecloth and the resulting extract was centrifuged at 4,000 rpm for 20 minutes. The resulting supernatant was collected for analysis. During 2012, samples of 50 g were blended with 160 mL of solvent (only 50 g were used due to limited berry supply related to the increased number of harvest timings). A total of 35 mL of supernatant was collected for analysis. Berry extracts were stored at -20^{o}C until analysis.

Total antioxidant capacity was measured using the ferric reducing power of plasma (FRAP) (Benzie & Strain, 1996) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH)assays (Benvenuti, Pellati, & Bertelli, 2004; Blois, 1958). A modified method (Lowry *et al.*, 1951) using a Folin-Ciocalteu reagent was used to determine TPC. Method modifications to FRAP, DPPH and FC are outlined in Rupasinghe *et al.* (2012). Both TAC and TPC were quantified using a Fluostar Optima Spectrophotometer (Model FLUOstar OPTIMA, BMG Labtech, Durham, NC).

Total anthocyanin content (TAN) was determined using the pH-differential technique outlined by (Lee, Durst, & Wrolstad, 2005) using a spectrophotometer (Agilent Cary 100 series, Agilent Technologies, Santa Clara, CA).

2.2.4 Quantification of major anthocyanins using HPLC/MS

Total monomeric polyphenols were identified and quantified by liquid chromatography - mass spectrometry (LC-MS/MS) analysis as described by Rupasinghe *et al.* (2008). Analysis was carried out using a Waters Alliance separation module (Waters, Milford, MA, USA) coupled with a Micromass Quattro micro API MS/MS system and controlled with Masslynx V4.0 data analysis system (Micromass, Cary, USA). A Phenomenex Luna C_{18} column (150 mm x 2.1 mm, 5 µm) was used with a Waters X-Terra MS C_{18} guard column.

The analysis of the flavonol, flavan-3-ol, phenolic acid, and dihydrochalcone compounds was done by electrospray ionization in negative ion mode (ESI-), with a capillary voltage of 3000 V, nebulizer gas (N_2) temperature of 375^{o}C, and flow rate of 0.35 mL min^{-1}. Anthocyanin compounds were analysed by electrospray ionization in positive ion mode (ESI+), with capillary voltage 3500 V, nebulizer gas at 375^{o}C, and flow rate of 0.35 mL min^{-1}. The cone voltage (25-50 V) was optimized for individual compounds.

2.2.5 Statistical analysis

Repeated measures analysis of variance (ANOVA) was used to compare: berry size, TSS, TA, TAC, TPC and TAN of black currants harvested at different harvest timings (four timings in 2011 and five timings in 2012). Currants were analyzed using repeated measures in PROC MIXED (SAS 9.3). Differences were considered significantly at $\alpha=0.05$. Both sites were examined together but separated by years as extreme seasonal differences could confound results. Annual data was combined for Pearson-correlations.

2.3 Results

2.3.1 Site effects

Site effects were considered during this experiment to examine the strength of fruit maturity effects at two growing locations. There were no significant site by timing interactions. For certain response variables, individual site effects were significant during both field seasons. This could be the result of different abiotic factors such as soil fertility levels, leaf nutrients or growing season temperature and precipitation.

In other trials at this site, Hobson, Lynch, Pruski and Hammermeister (2012) found that the Farmington site was generally more fertile than the Hunter River site, but based on leaf tissue analysis both sites were deficient in nitrogen, phosphorus, potassium, copper, zinc and boron (Table 2.1). Nutrient deficiencies increased across years (data not included). These deficiencies could explain why some berries attributes were different from other reported results as stress can affect plant secondary

metabolism (Lila, Kellogg, Grace, & Yousef, 2013). Total anthocyanins were higher at Hunter River which had more nutrient deficiency.

Growing degree days were significantly different at both farming locations. Hunter River had an additional 400 growing degree days. This means that currants at Hunter River received more heat units than at Farmington which could explain fruit ripening differences. Both farming locations had different microclimates which could explain this difference (Hunter River was more sheltered by a windbreak). Differences between government weather stations and on-site stations was observed. Local microclimates could have significant influences on temperature and hence GDDs.

2.3.2 Fresh berry analysis (berry weight, TSS, TA)

Berry size generally decreased over time (Table 2.3) however there were few statistically significant differences unless comparing with the last timing date where berries were the smallest (Table 2.6, 2.7). In 2011, site effect and site x time interaction effects on berry size were marginally significant (Table 2.3). In 2012, there was a marginal timing effect on berry size, indicating that smaller currants were being sampled at later harvest timings (Table 2.4). Overall, berries were larger in 2011 (99 g 100 berries^{-1}) than in 2012 (73 g 100 berries^{-1}). In regards to environmental factors, berry size was positively correlated (>60%) with total precipitation and negatively correlated with growing degree days (>80%) (Table 2.8). In regards to response variables, berry size was positively correlated with TPC (Table 2.8). Berry size was negatively correlated with TSS, TAC and TAN.

Total soluble solids generally increased with time in both years but with few significant differences between timings (Table 2.6, 2.7). In 2011, TSS values were highest at the latest harvest timing (Table 2.6). In 2011, both farms followed similar trends but in 2012, TSS values at Hunter River were lowest 6 days after 95% but reached a plateau 10 days after 95% black and maintained similar TSS values throughout the rest of the sampling period indicating a site effect (Table 2.4). The interaction effect was not significant. It is possible this was caused the low number of farms being utilized in this study (n=2). However, TSS values peaked 10 days after 95% black at Farmington during 2012. While all berries were picked black, Titania currants ranged from 13 to 21 ^{o}Brix between the two field seasons depending on the timing of sampling. Total soluble solid levels were higher in 2012 (18.6 ^{o}Brix) than 2011 (15.1 ^{o}Brix). Total soluble solids were positively correlated with GDDs and negatively correlated with precipitation (Table 2.8). Total soluble solids were correlated with some response variables as well. Total soluble solids were positively correlated with TAC and TAN but were negatively correlated with berry size and TPC (Table 2.8).

There were no significant differences in TA among timings or site effects during the 2011 field season (Table 2.3). In 2012, there were significant differences in TA measurements between harvest timings (Table 2.5). Currants picked 6 days after 95% black had significantly more TA than those picked at 18 days after 95% black. Farmington currants were significantly more acidic during the first harvest timing.

Titania currants averaged 3.91 % CAE in 2011 and 3.88 % CAE in 2012. Titratable acidity was not correlated with any environmental or response variables.

During the 2012 field season, decreased berry firmness was observed 10 days after currants turned black (data not shown). Upon inspecting Titania currants post-harvest, about 5-10 % of the currants were damaged by machine harvest. It is assumed that this % value would increase as currants become increasing softer as they ripen.

2.3.3 Post-extraction (TAN, TAC, TPC, anthocyanins, flavonols, catechins, phenolic acids)

In 2011, TAC was affected by both harvest timing and site effects (Table 2.4). Total antioxidant capacity was the highest in berries sampled at the latest date (1387.5 mg TE 100 g^{-1} fresh weight (FW)on August 18th) (Table 2.6).In 2012, there was no significant timing, site or sitextiming interaction effect on TAC. There were no significant differences in DPPH values (data not shown).There were no significant difference in TPC among harvest timings in either field season (average value was 21 GAE mg 100 g^{-1}FW). Phenolic content was slightly higher at Farmington than Hunter River (22.28 and 19.21 GAE mg 100 g^{-1} FW respectively) in 2011, although the site effect was only marginally significant (Table 2.3). In 2012, there was a significant sitextiming interaction effect where Hunter River currants had significantly more phenolic content 22 days after turning black when compared to most other Hunter River and Farmington samples.

Anthocyanins (photometric) were significantly affected by fruit maturity stage. Later timings had higher values (in some cases, nearly double) (Table 2.6, 2.7). In 2012,

there was a site effect which indicated that TAN values were higher in berries picked from Hunter River when compared to Farmington (201 compared to 179 mg D3RE 100 g^{-1}FW). Total anthocyanins were positively correlated with GDDs, TSS, TAC and Total anthocyanins measured using HPLC (Table 2.8). Total anthocyanins were negatively correlated with berry size.

Of the measured standards, currants contained mostly cyanidin and delphinidin glycosides. Cyanidin *3-O*-rutinoside (C3R) was significantly influenced by fruit maturity and site effects (Table 2.5). Currants picked 22 days after turning black contained an additional 4.11 mg 100 g^{-1} FW of C3R compared to those picked six days after turning black (Table 2.7). Currants picked from Hunter River had more C3R than Farmington (24.93 vs 22.73 mg L^{-1} FW).Currants had relatively higher amounts of cyanidin *3-O*-glucoside, delphinidin *3-O*-glucoside and delphinidin *3-O*-rutinoside (2.38, 6.93 and 21.72 mg 100 g^{-1} respectively) when compared to other measured bioactives but no relationship was found between these response variables and factors of interest. Only trace amounts of other measured compounds were found.

2.4 Discussion

2.4.1 Overall effects of fruit maturity state on berry quality

It is well understood that fruits gain mass through maturation (fruit set until full coloration) (Coombe, 1976) but it is unclear as to how weight changes during the final stages of ripening (after black colouration). Berries within a single Titania bush varied from 0.5 to 1.3 g per berry and sampled berries were smaller at later harvest timings

during both field seasons. Larger blueberries have been shown to ripen more quickly (Barker & Collins, 1963). Berry drop was evident on the soil under the black currant bushes. The average size of berries at later sampling date could be smaller because larger berries have dropped, leaving berries of smaller size on the bush. Having said this, the sample taken from the bush would still represent that which would be harvested by the farmer. Water transpiration and evaporation from the berries may be another explanation. During prolonged heat, water can transpire out of fruit tissues or be transported back into leaf tissue in order to prevent damage or drying (Simson & Straus, 2010a). This might be the case during the second field season as it was warmer and drier than 2011 and why "overripe" currants during that year had reduced weight. Reduced berry weight at later harvest timings has been previously recorded (Rubinskienė *et al.*, 2008). Berry splitting and dropping was observed in 2011 which could have been caused by above average precipitation before harvest. Overall, it appears berry size increase primarily occurs before the berries are 95% black. Berry drop and possibly transpiration losses in dry conditions most likely account for the smaller berry size observed at later harvest timings. With regards to correlations, precipitation is most likely responsible for size differences seen across both growing seasons. It is difficult to discern if there is a critical precipitation time as all precipitation periods leading up to 95% black were correlated with larger berries. This might also explain the difference in sizes between reported results for Titania (Khoo *et al.*, 2012; Pedersen, 2007; Pluta *et al.*, 2008). Genetic factors may play a larger role in how currants size up over time (Krüger *et al.*, 2011; Pedersen, 2007; Pluta & Pruski, 2012). At horticultural

maturity, some black currant cultivars have significantly larger berries when compared to Titania (Krüger *et al.*, 2011; Pedersen, 2007; Pluta & Pruski, 2012).

As previously observed (Rubinskienė *et al.*, 2008), TSS values were significantly influenced by harvest timing. Soluble solids from later harvest timings for Titania were significantly higher than some previously recorded values (16-20 compared to 14^{o}Brix) (Giongo, Grisenti, Eccher, Palchetti, & Vrhovsek, 2008; Libek & Kikas, 2001). Differences in Titania seen between previous research and this experiment could be the result of environmental factors such as GDD differences or precipitation. During fruit ripening, TSS might increase in most fruits predominantly due to an increase in sugars (a large contributor to the ^{o}Brix).This coincides with a decrease in acid, which is synthesized pre-colouration as a means to combat disease or pests (Iversen, Pedersen, & Brandt, 2000). The trends seen in this research follow similar patterns. Total soluble solids increased overtime with a notable spike at 10 days after 95% of the fruit turned black during 2012. This could have been caused by environmental factors such as daily temperature, precipitation or picking time. Total soluble solids reached a plateau afterwards giving ^{o}Brix-oriented farmers a wide window for picking. Farmers could invest in a portable ^{o}Brix meter in order to keep track of ^{o}Brix measurements. The general TSS trend indicates that TSS will hit a plateau and change very little after that point. If farmers notice that ^{o}Brix values are no longer increasing substantially, they should harvest as berry drop increases over time which can decrease yield. There is a distinction between long term (bloom to 95% black) and short term (after 95% black) factors that can affect

TSS values. Changes in TSS can be affected by short term events like precipitation (Rubinskienė *et al.*, 2006). It has been noted that ^{o}Brix values can decrease following a rain event as a result of fruit sugar and acid dilution. Sugar movement from the plant leaf tissues into fruits can be influenced by temperature. Temperature plays a key role in diurnal changes which can affects berry characteristics like TSS (Koch, 1986; Simson & Straus, 2010a). Dry conditions like those seen in 2012 can cause water to move from fruits to leaves or to be lost through the fruit itself via transpiration. Water loss can increase TSS content which could be the case during the 2012 summer growing season. In contrast, 2011 was very wet during harvest at 10 days after turning black which could explain why ^{o}Brix values dropped. Heat units in April and May might affect ^{o}Brix values in August. This could be the case when comparing 2011 and 2012 field seasons. More GDD's in spring 2012 might have resulted in larger TSS values of 18-20^{o}Brix compared to the 2011 values of 15-17^{o}Brix (Table 2.2, 2.3, 2.5). The GDDs pre-harvest could be used to predict peak ^{o}Brix values for the coming season. For PEI, seasons with GDDs above the LTAs could result in Titania currants reaching 16-20^{o}Brix.The contrary is also a possibility as seasons with pre-harvest GDDs below LTAs could result in Titania currants reaching 13-16^{o}Brix. The use of GDDs was investigated as an alternative to surface color for approximating ripeness. There was a correlation between the number of GDD's between each harvest and soluble solids (Table 2.7). This means that GDD's might be a good maturity indicator as surface color is unreliable after the initial color change. Both years had a moderate negative (significant but less than 60% correlation) correlation between TSS and TA. This was expected as currants become sweeter and less acidic

throughout ripening. Fresh currant vendors might consider later harvest timings to allow currants to develop a sweeter flavour but, as mentioned previously, later harvest timings run the risk of berry drop and, consequently, lower yields. Environmental factors might influence fruit maturity which, in turn, affect berry quality like TSS. This might also explain the TSS differences between PEI results and other countries. For example, Titania currants grown in Lithuania showed different TSS results for Titania but reported similar correlations (Rubinskienė *et al.*, 2006). Berry quality changes during maturation and ripening in Lithuania progressed similarly to PEI's 2011 season. Overripe currants in Lithuania reached 16.9 ^{o}Brix which was similar to the value of 17.1^{o}Brix (the last harvest timing was used for comparison) obtained from PEI during the 2011 season. However, 2012 overripe Titania currants reached a TSS value of 19^{o}Brix. It is important to include seasonal data with each field season as it could explain the difference in reported values. Furthermore, defining 'horticultural maturity' is important as it often used as the end point before sampling. Considering the non-climacteric nature of currants, choosing the best end-point is very important as it can significantly affect berry quality like TSS. Rubinskienė *et al.* (2006), also reported similar relationships between TSS, precipitation and temperature. Precipitation seems to decrease average TSS which could be the direct result of physical removal due to weakening peduncle or an indirect result of dilution from increased berry size when exposed to higher precipitation. It might be worthwhile to do an experiment involving irrigation and berry size as controlled factors and investigating whether or not there is an interaction between the two (and does this interaction influence berry qualities like TSS or TA). Temperature and GDDs could have a

positive relationship with TSS. The increase in TSS could be explained by the fact that heat is the primary catalyst for many biological pathways during fruit growth and development. Total soluble solids is a measurement of many soluble solids (sugars, acids, salts and proteins) so it is possible that heat units modify these solids during maturation and ripening. Growing degree days and TSS were positively correlated meaning that more heat leads to more soluble solids within each fruit. Growing degree days and precipitation opposite effects on soluble solids (Table 2.8). Accumulated GDDs after 95% black had the highest correlation with TSS. This could be caused directly by heat units or indirectly from decreased berry size. Berry size decreased over time and had a strong negative relationship with TSS (Table 2.8). Total soluble solids were strongly correlated with total antioxidant capacity (TAC) during both field seasons. Farmers looking to optimize TAC could consider high ^{o}Brix as an indicator of high potential TAC but more research would be needed to verify this (more than a correlation). It is possible that the correlation is a result of another factor that affects both TSS and TAC positively.

Titratable acidity was only affected by harvest timing during the 2012 field season. As currants ripened, there was less acid present 18 days after 95% black when compared to 6 days after 95%. Previous results have shown that acids decrease as fruits ripen from green to black (Rubinskienė *et al.*, 2008) but the magnitude of August acidity changes were uncertain. There were no significant correlations between environmental or response variables. The difference between years could be explained by the change

in sampling procedure. The addition of more harvest timings in 2012 might have allowed better acidity characterization.

During 2011, currants had significantly higher antioxidant capacity at 18 days after 95% black (Table 2.6). Titania currants had high TAC (20-60 µmol TE g^{-1} FW) compared to and raspberries (7.57 µmol TE g^{-1} FW), blueberries (16.24 µmol TE g^{-1} FW), blackberries (15.03 µmol TE g^{-1} FW), strawberries (8.00 µmol TE g^{-1}) (Rupasinghe *et al*., 2012). Black currant results were comparable to haskap (28-50 µmol TE g^{-1} FW) (Rupasinghe *et al*., 2012). Previous research indicates that size and TAC have a negative correlation which could explain why the highest TAC is highest at the smallest sampled berry size (Krüger *et al.*, 2011). Results from this research have indicated a strong negative correlation between berry size and TAC (Table 2.8). As many antioxidants are found in black currant skin, it is possible that smaller currants have a higher skin to volume ratio which resulted in higher TAC (Krüger *et al.*, 2011).

In previous studies, TPC has been shown to be the highest in green unripe fruits (Beekwilder *et al.*, 2005; Rubinskienė *et al.*, 2006; Zhao, 2007). Phenolic content decreases during the pre-colouration “green” stage as colour changes from green to black in most berries (Zhao, 2007). Green fruit was not analyzed in this study, but the results from both field seasons indicated that TPC did not significantly change after currant bushes reached 95% black (Table 2.3 and 2.4). There was a marginally significant difference in TPC between farming locations (Table 2.3). Considering GDDs, berries ripening at Hunter River may have been more advanced which could explain why berries

grow at this location had lower TPC than those grow in Farmington. It is difficult to characterize how TPC is changing as it is a total measurement of all phenolic components. It is possible that differences in soil fertility status affected phenolic content in some way. In general, synthesis of most plant phenolics are stimulated in response to stress conditions (Chalker-Scott, 1999; Pascu, Morariu, Caulet, Efrose, & Sfichi-Duke, 2011); therefore, the marginally significant difference between sites could be the result of soil fertility differences. Cyanidin glycosides (one of the most abundant phenolic compounds and also an anthocyanin) was also affected by site conditions. It is possible that cyanidin glycosides, as a large contributor to total phenolic content, caused total phenolic content as a whole to be affected by site conditions.

Total anthocyanin content increased significantly after currants turned black resulting in significant differences particularly between the early and late harvest timings (during both extremely different seasons). The highest quantities of anthocyanins were discovered at the latest harvest timing which was expected (Krüger *et al.*, 2011). Farmers interested in maximizing TAN could consider later harvest timings. Black currant anthocyanins (ranged from 150-230 mg 100 g^{-1} FW) were much higher than reported values for cranberries (78 mg 100 g^{-1} FW), red raspberries (40-50 mg 100 g^{-1} FW) and strawberries (15-75 mg 100 g^{-1} FW) and results were comparable to previous result for black currants (130-400 mg 100 g^{-1} FW),blackberries (100-400 mg 100 g^{-1}FW) and blueberries (25-500 mg 100 g^{-1} FW) (Manach, Scalbert, Morand, Rémésy, & Jiménez,

2004). Black currants are known for high anthocyanin content and it was expected that they outclass other fruits in this category (Borges *et al.*, 2010; Manach *et al.*, 2004).

In agreement with previous reports, delphinidin glycosides are some of the most abundant anthocyanins in black currants but remain relatively unchanged after currants turn black. Cyanidin glycosides, however, were influenced by growing location. Cyanidin glycosides seem to be more influenced by the site specific environmental conditions than fruit maturity after color change. Nielsen *et al.* (2003a) found that black currant anthocyanin rutinosides were more readily absorbed in humans than glucosides which is beneficial for consumers as most anthocyanins discovered in this research were C3R and D3R.

Considering both field seasons, berry quality seems to improve significantly overtime. During 2011, many of the flavour elements (TSS and TA) improved after 10 days. During 2012, currants picked 18 days after 95% black and 22 days after 95% black did not differ significantly which could imply that quality begins to plateau at that point. A key characteristic that was not considered empirically was berry firmness but it was observed that berry firmness started to decrease 10 days after 95% black. Many characteristics seem to constantly improve but berries eventually become too soft and non-harvestable. There were significant differences between this research and previous research results (2.3 compared to 20 mg of C3G 100 g FW^{-1} (Wu *et al.*, 2004). The extraction procedures, harvest timing and cultivar differences could serve as possible explanations for the discrepancy in values. The rate of increase in TAN over time was

significant as well. The nutraceutical application of these results could be of interest considering anthocyanins doubled over two weeks during ripening in 2011.

2.4.2 Maturity indicators

TSS values are commonly used as a maturity indicator but results have indicated that Brix values can significantly change overtime. It is possible to harvest when farmers measure a ^{o}Brix value that is suitable for their own purposes. For example, winemakers should not harvest too early as Brix values are generally lower right after black colouration. Growing degree days are another maturity indicator for fruit crops (Carlson & Hancock, 1991) and were calculated from on-site temperature stations in 2012. The results indicate that accumulated GDDs might be a good indicator of fruit maturity because they correlated with many response variables including TSS and TAN (Table 2.5).

2.5 Conclusion

Fruit harvest timing and farm location had a significant influence on black currant berry quality. Leaving black currants on the bush after they turn black improves some berry quality attributes (TSS, TAC, TAN, etc) but could potentially compromise others (yield, firmness). Delaying harvest can substantially increase TSS which may benefit certain markets, however, TSS can vary significantly between years. Results from this research have indicated that GDDs could be a good maturity predictor. Farmers might consider investing in temperature monitors in order to predict potential seasonal TSS ranges and/or anthocyanin content. Prolonging berry ripening can be used as a

strategy to increase content of anthocyanins. However, it is important to consider that berry drop will increase as black currants ripen so delaying harvest can result in lower yields. Considering site conditions like GDD's is important as it affects berry quality. When examining black currant research results, it is important to consider how different berry quality can be across different growing locations and further research could be conducted into which specific factors (e.g. heat or water stress) are responsible for berry quality differences between sites (e.g. irrigation trial). Excluding bioactives, overall berry quality does not seem to change substantially between 10 and 18 days after turning black. Based on the results of two field seasons of Titania, harvesting should be done a minimum of one week after 95% black colouration. Establishing a machine-harvest threshold based on firmness should be considered during future field seasons in order to maximize TSS or TAN.

Table 2.1 Soil and leaf tissue results from Farmington (FT) and Hunter River (HR) Prince Edward Island soil testing facility (2012). Results are presented in mg kg^{-1}.

Soil	*Site	Organic matter	pH	P_2O_5	K_2O	Ca	Mg	B	Cu	Zn	S	Mn	Fe
	FT	3.1	5.6	290	95	473	25	0.4	1.3	1.0	13	40	203
	HR	3.2	6.4	124	47	795	71	0.3	0.6	0.2	9	32	133
Leaf		N^x	P^x	K^x	Ca^x	Mg^x	B	Cu	Zn				
	FT	2.4	0.2	1	1.5	0.2	8.8	3.8	13				
	HR	2.1	0.2	0.6	1.4	0.4	10	3.2	13				

*FT=Farmington and HR=Hunter River sites. ^{x}Results are presented as a %.

Table 2.2 Weather data at Farmington and Hunter River sites.

		Growing Degree Days[z]				Precipitation (mm)		
Site (Period[y])		2011[x]	2012[x]	2012 site[w]	LTA[y]	2011[x]	2012[x]	LTA[y]
Farmington								
May		95	105	130	132.1[v]	37	19	93.3[v]
June		212	254	307	282	54	53	87
July		363	401	481	409	101	51	85
	3	38	42	43	.	17	6	.
	6	73	88	83	.	19	6	.
	10	120	154	162	.	46	6	.
	14	166	221	243	.	52	8	.
	18	219	286	324	.	68	44	.
	22	277	346	387	.	69	46	.
Bloom to 22		964	1105	1305	.	335	169	.
Hunter River								
May		108	122	138	138	44	23	92
June		237	276	315	295	101	62	90
July		400	447	500	427	73	34	86
	3	40	42/9	42	.	14	7	.
	6	76	93	84	.	25	7	.
	10	126	161	162	.	66	7	.
	14	176	230	237	.	70	17	.
	18	233	300	313	.	91	59	.
	22	296	365	383	.	91	64	.
Bloom to 22		1042	1210	1335	.	309	182	.

[z] Growing Degree Days base 5°C, [y]Period measurement data starts with Bloom on May 16; July data ends at 95% black berries (August 1 in 2011, July 27 in 2012), 3, 6, 10, 14, 18, 22 are the cumulative GDD and precipitation after in days after 95% black (time 0), [x]growing season weather and long term average (LTA) data from Environment Canada weather stations, St. Peters for Farmington and New Glasgow for Hunter River, [w]Temperature data collected on site in 2012, [v]LTA data was collected for the whole month of May.

Table 2.3 ANOVA p-values and overall means for fresh berry quality variables in 2011 in response to site and timing of harvest.

*Responses	Mean	SE	Timing	Site	Site x Timing
Size (g 100 berries^{-1})	98.85	3.4	0.01	0.06	0.08
Total soluble solids (^{o}Brix)	15.1	0.32	0.05	0.78	0.09
Titratable acidity (%CAE)	3.82	0.19	0.45	0.48	0.03

*CAE, citric acid equivalents.

Table 2.4 ANOVA p-values and overall means for berry quality variables measured from extracts in 2011 in response to site and timing of harvest.

*Responses	Mean	SE	Timing	Site	Timing x Site
Spectrophotometric measurements					
Total antioxidant capacity (mg TE 100 g^{-1} FW)	1115	66.5	<0.0001	0.02	0.22
Total phenolic content (mg GAE 100 g^{-1} FW)	20.74	2.18	0.8	0.06	0.28
Total anthocyanins (mg DRE 100 g^{-1} FW)	156.9	8.6	<0.0001	0.33	0.13

*TE, Trolox equivalents; GAE, gallic acid equivalents;

Table 2.5 ANOVA p-values and overall means for berry quality variables in 2012 in response to site and timing of harvest.

Responses	Mean	SE	Timing	Site	Timing x Site
Size (g 100 berries^{-1})	72.8	2.9	0.002	0.05	0.78
Juice measurements					
Total soluble solids (^{o}Brix)	18.6	0.36	0.002	0.001	0.07
Titratable acidity (%CAE)	3.95	0.13	0.058	0.008	0.666
Spectrophotometric measurements					
Total antioxidant capacity (mg TE 100 g^{-1} FW)	1756	102	0.3	0.3	0.3
Total phenolic content (mg GAE 100 g^{-1} FW)	12.11	0.5	0.67	0.11	0.05
Total Anthocyanins (mg D3RE 100 g^{-1} FW)	190	11.1	0.2	0.69	0.36
*Liquid Chromatography/ Mass spec measurements**					
Total phenolic content(mg 100 g^{-1} FW)	57.3	2.69	0.15	0.05	0.52
Total Anthocyanins (mg 100 g^{-1} FW)	55	2.58	0.12	0.05	0.56
C3G (mg 100 g^{-1} FW)	2.33	0.17	0.034	0.006	0.2
D3G (mg 100 g^{-1} FW)	6.9	0.6	0.19	0.15	0.59
D3R (mg 100 g^{-1} FW)	21.4	1.75	0.3	0.19	0.67
C3R (mg 100 g^{-1} FW)	23.2	1.4	0.14	0.01	0.25

TE-Trolox equivalents, D3RE-Delphinidin 3-*O*-equivalents, C3G-Cyanidin 3-*O*-glucoside, D3G-Delphinidin 3-*O*-glucoside, Delphinidin 3-*O*-rutinoside, C3R-Cyanidin-3-*O*-rutinoside. Only trace quantities (<1mg 100 g^{-1} fresh weight) of the following were found: Petunidin 3*O*-glucoside, Peonidin 3-*O* glucoside, Malvidin 3-*O*-glucoside, Quercetin-3-*O*-Glucoside, Quercetin-3-*O*-ArabinoGlucoside, Quercetin-3-*O*-Rhamnoside, *Quercetin-3-*O*-Rutinoside, *Epigallocatechin, Epigallocatechin gallate, Epicatechin gallate, Chlorogenic Acid, Caffeic acid, Ferulic acid, Isoferulic Acid

Table 2.6 Total soluble solids (TSS), total antioxidant capacity (TAC) and total anthocyanins (TAN) in response to timing of sampling in 2011.

Sampling date (**days after 95%)	*Berry weight (g 100 berries^{-1})	*TSS (^{o}Brix)	*TA (%CAE)	*TAC (mg TE 100 g^{-1} FW)	*TPC (mg GAE 100 g^{-1} FW)	*TAN (mg D3RE100 g^{-1} FW)
3	103.5 a	14.2 c	3.9	1025 b	21	119 c
6	103.3 ab	14.9 b	N/A	950 b	20	151 b
10	94.9 ab	14.2 c	3.9	1100 b	21	158 b
18	93.6 b	17.1 a	3.7	1388 a	22	200 a

*results followed by different letters are considered significantly different (α=0.05).
**Days after 95% of the berries on the bush have turned fully black (August 29th)
FW, Fresh weight; TE, Trolox equivalents; D3RE, Delphinidin 3-*O*-rutinoside equivalents.

Table 2.7 The effect of harvest timing on selected berry quality characteristics (2012). Results are considered significant at α=0.05.

*Sampling date	Berry size (g 100 berries^{-1})	TSS (^{o}Brix)	TA (%CAE)	^{x}TAC (mg TE 100 g^{-1} FW)	^{x}TPC (mg GAE 100 g^{-1} FW)	^{y}TPC	^{x}TAN (mg D3RE 100 g^{-1} FW)	^{y}TAN	^{y}C3G	^{y}C3R	^{y}D3G	^{y}D3R
6	73 ab	17.5 b	4.2 a	1309 b	12	53.2 b	174 b	50.8 b	2.2 b	22 b	6.2 b	19.1
10	78 a	19.1 a	3.9 ab	1449 ab	12.1	58.1 ab	195 ab	55.7 ab	2.3 b	25 ab	7.0 ab	22.1
14	78 a	18.7 a	3.8 ab	1368 ab	12	56.7 ab	190 ab	54.5 ab	2.3 b	23 ab	6.7 ab	21.9
18	70 bc	18.8 a	3.7 b	1575 a	12.2	58.1 ab	183 ab	55.7 ab	2.5 b	25 ab	6.5 ab	20.5
22	66 c	19 a	3.8 ab	1518 ab	12.3	60.3 a	209 a	58.1 a	2.7 a	26 a	7.9 a	23.3

*Days after 95% of the berries on the bush have turned fully black (August 30th). TSS, total soluble solids; TAN, Total Anthocyanins, C3G – Cyanidin *3-O*-glucoside, C3R – Cyanidin 3 -rutinoside, FW – Fresh weight, TE-Trolox equivalents, CAE – citric acid equivalents, D3RE – delphinidin 3-*O*-rutinoside

^{y}HPLC/MS analysis measured in mg 100 g^{-1} FW

^{x}Photometric analysis

Table 2.8 Pearson correlation and statistical p-value between environmental factors (accumulated GDD-growing degree days and precipitation since bloom), harvest timing and selected black currant response variables for PEI. Response variables include: size (g 100 berries^{-1}), titratable acidity (TA as %CAE), total soluble solids (TSS as ^{o}Brix), total antioxidant capacity (TAC as mg TE 100 g^{-1} FW), total phenolic content (TPC as mg GAE 100 g-1 FW) and total anthocyanin content (TAN as mg DRE 100 g^{-1} FW). Data was combined from both field seasons and only significant values are shown at p<0.1 (with a correlation above 50%).

Variables	GDD	Precipitation	size	TA	TSS	TAC(FRAP)	TPC(FC)	TAN (pH-dif)	TAN(HPLC)	TPC(HPLC)	C3G	D3G	D3R
Precipitation													
Size	-0.8	0.6											
Juice													
TA													
TSS	0.8	-0.6	-0.8										
Photometric													
TAC (FRAP)	0.8	-0.7	-0.8		0.9								
TPC (FC)	-0.6	0.7	0.8		-0.7	-0.7							
TAN (pH-dif)	0.7		-0.6		0.7	0.7							
HPLC/MS													
TAN	0.5							0.6					
TPC	0.5							0.6	0.1				
C3G	0.7	0.6			0.6		0.5	0.6	0.8	0.8			
D3G								0.2	0.9	0.9	0.8		
D3R								0.5	0.9	0.9	0.6	0.9	
C3R	0.6				0.567			0.6	0.9	0.9	0.9	0.8	0.8

Chapter 3. The effect of genotype and fruit maturity on *Ribes nigrum* L. berry quality

Abstract: Fruit cultivar can influence black currant berry quality during organic production. The overall goal of this study was to assess black currant cultivars grown in PEI and to determine beneficial management practices for achieving optimum quality across different black currant cultivars. Berry qualities of interest included berry size, acidity, total soluble solids and the distribution of biologically active compounds. Whistler, Ben Connan, Ben Sarek and Blackcomb had larger berries (in some cases nearly double the size) when compared to Titania, Ben Tirran and Ben Alder but the average weight of berry samples started to decrease two weeks after currants turned black. Titania, Whistler and Blackcomb had the highest TSS (^{o}Brix) starting 10 days after turning black accounting for differences of up to 4 ^{o}Brix. Whistler was significantly less acidic. Cultivar selection did not affect antioxidant capacity, phenolics or anthocyanins during the wetter and colder 2011 season. During the 2012 season, Blackcomb and Ben Connan had more total phenolic content then Whistler. Blackcomb had higher antioxidant capacity than Ben Sarek.

3.1 Introduction

Cultivar selection is an important first step for fruit orchard design because genetic differences can influence physical and chemical characteristics such as disease resistance, yield and berry quality. Black currant (*Ribes nigrum* L.) cultivars are being constantly improved resulting in new cultivars that have yet to be tested. Constantly

improving cultivars is necessary in order to stay ahead of changing disease pathogens and insects (Libek & Kikas, 2001). Most new black currant cultivars are bred in foreign countries like Poland or New Zealand. Regionally adapted cultivars are needed to account for variability in berry growth and development in different growing locations (Granelli, Mariani, Parisi, & Eccher, 2012; Kähkönen, Hopia, & Heinonen, 2001; Yang *et al.*, 2010). For example, the cultivars Whistler and Blackcomb were bred in British Columbia, Canada for west coast conditions. As such, it is difficult to predict how British Columbian or even Polish black currants will perform on Canada's east coast. Characterizing cultivars could allow Prince Edward Island (PEI) growers to determine which cultivars are most suitable for their growing location. Furthermore, identifying favourable attributes (^{o}Brix, antioxidants, etc) for their chosen end use (winemaking, frozen, fresh, etc) would allow farmers to optimize cultivar selection. The economic value of increased yield, berry size and Brix are important and these traits can be easily observed but biologically active compounds cannot be measured without laboratory assays.

With oxidative stress mediated chronic diseases such as cancer becoming increasingly prevalent, understanding the antioxidant capacity and bioactive polyphenol content of newly introduced fruit crops including black currants is important. Total antioxidant capacity measures how effective black currant extracts are at scavenging carcinogenic free radicals. For black currants, most of the compounds responsible for radical scavenging are phenolic compounds making a measurement of total phenolic

content very important. The most prevalent phenolic compounds in black currants are anthocyanins (Wu *et al.*, 2004). Anthocyanins (water soluble pigments) are effective free radical scavengers and provide numerous health benefits (Kähkönen & Heinonen, 2003) (the most unique being the reduction of age-related macular degeneration and improved retinal blood flow) (Ohguro *et al.*, 2013). Catechins, flavonols and phenolic acids are important biologically active compounds which, like anthocyanins, provide various health benefits. Genetic and developmental differences among currant cultivars might result in unique biochemical profiles. Cultivars have different genotypes and previous studies have indicated that cultivars can vary in fruit maturation rates allowing cultivars to be classified as early, mid-season or late (Pluta, Urawicz, and Krawiec 2008; Rubinskienė *et al.* 2006). Understanding how each cultivar ripens after turning black is essential for developing optimal management practices for each cultivar.

The objectives of this experiment were to characterize berry quality at different fruit maturity stages across seven cultivars (Titania, Ben Connan, Ben Sarek, Ben Connan, Ben Tirran, Ben Alder, Blackcomb and Whistler) and to determine the optimal time of harvest for these cultivars on Prince Edward Island (PEI). Berry quality was considered optimized when berry size, total soluble solids (TSS), total antioxidant capacity (TAC), total phenolic content (TPC), total anthocyanins (TAN) were maximized and titratable acidity (TA) was minimized.

3.2 Methods

3.2.1 Site and Climate

Field plots were located in Mount-Stewart (MSt), PEI on a north facing slope slightly protected with some windbreaks. The experimental site was well-drained sandy loam. Temperature and precipitation climate data were collected from nearby government weather station (Charlottetown) (Table 3.1). Fertility levels were measured by the PEI soil testing lab situated in Charlottetown (Table 3.2). Once 95% of the currants on the bush displayed a black surface colouration, fruits were considered in the "post-colouration" phase of development. Sampling started during post-colouration. Harvest timings were chosen based on berry surface colouration. Timing '0' was chosen when 95% of the berries on a bush were completely black.

3.2.2 Experimental Design

The Experimental site at MSt consisted of 210 black currant bushes (planted in 2010) organized into six randomized blocks. Five plants per cultivar (Titania, Ben Alder, Ben Connan, Ben Sarek, Ben Tirran, Blackcomb and Whistle) were planted together in a random order within each of these blocks. With the exception of Titania (which 4 years old), all plants were 2 years old at the beginning of this experiment in 2011. The start date of sampling was determined based on average surface colouration berries on all plants. Individual strigs (black currant fruit set structure) of berries were assessed based on the amount of berries turned black. After fruit set, currants progress from green to red and eventually to black. Timing "0" was set once 95% of the currants across all plots

had turned black. Berry samples and data were gathered at two harvest maturities in 2011 (6 and 10 days)and it was observed that 95% of the currants across all blocks turned black on July 29$^{th.}$Three blocks were harvested six days after 95% turned black and another three blocks 10 after 95% of the currants turned black. A split plot design was used with harvest time as the main plot factor with two levels (6 and 10 days after 95% black) and cultivar as the subplot factor with seven levels (Titania, Ben Alder, Ben Connan, Ben Sarek, Ben Tirran, Blackcomb and Whistle). The results from 2011 indicated that Ben Alder and Ben Tirran had lower results compared to other cultivars and lower than previously reported results (Rubinskienė *et al.*, 2006) so It was decided that more fruit maturity stages should be assessed to allow cultivars to reach their optimal fruit maturity. Berry samples were harvested at four levels of fruit maturity (6, 10, 14 and 18 days after 95% black) in 2012 and it was observed that 95% of currants across all blocks turned black on July 27th.Three blocks were repeatedly harvested six, 14 and 18 days after currants turned black. Due to time restrains, the three other blocks were sampled 10 days after currants turned black. A complete randomized block design was used with three replicates of seven cultivars being harvested at four maturity stages (6, 10, 14, 18 days after 95% black which was on the 27th of July). Three hundred grams of berries were collected from each plant in each block.

3.2.3 Berry size, soluble solids, juice pH and titratable acidity, total antioxidant capacity, total phenolics, total anthocyanins

Analysis procedures were separated into two distinct stages: fresh and post-extraction. Fresh analysis examined berry size, TSS and TA. Post-extraction analysis was conducted for TAC, TPC, TAN and HPLC analysis (which measured anthocyanins, catechins, flavonols and phenolic acids). A modified 100 berry weight technique (the average fresh weight (FW) of 100 berries in grams) was used to evaluate berry size as outlined by Pluta *et al.* (2008), where three replicate measurements of 50 berries were weighed. Total soluble solids were measured using a digital Atago hand refractometer (Atago Co. Ltd., Japan) as outlined by Libek and Kikas (2001). Titratable acidity was measured using a 785 DMP Titrino autotitrator (Mettler Toledo Mississauga, Canada). A 2 ml sample was diluted with 28 ml of water and titrated using 0.1M NaOH (results are presented in % citric acid equivalents (% CAE).

A simple disease assessment was done during 2012 across the cultivar trial. Disease resistance was assessed based on rust % (*Cronartium ribicola* aka ' white pine blister rust') found on leaves post-harvest

The TAC, TPC, TAN and individual metabolites of berries were determined using total phenolic extracts of 80% methanol (modified from Kapasakalidis *et al.*, 2006). During 2011, samples of 100 g were blended with 160 ml (final volume of 200 ml) of solvent using a Hamilton laboratory blender (Model HBB908, Hamilton Beach Brands, Inc. Glen Allen, VA). The sample was poured through four layers of cheesecloth and the

resulting extract was centrifuged at 4,000 rpm for 20 minutes. The resulting supernatant was collected for analysis. During 2012, samples of 50 g were blended with 160 ml of solvent (only 50 g were used due to limited berry supply related to the increased number of harvest timings). A total of 35 ml of supernatant was collected for analysis. Berry extracts were stored at -20^{o}C until analysis.

Total antioxidant capacity was measured using the ferric reducing power of plasma (FRAP)(Benzie & Strain, 1996)and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical; scavenging assays (Benvenuti *et al.*, 2004; Blois, 1958). A modified method (Lowry *et al.*, 1951) using a Folin-Ciocalteu reagent was used to determine TPC. Method modifications are outlined in Rupasinghe *et al.* (2012). Both TAC and TPC were quantified using a Fluostar Optima Spectrophotometer (BMG Labtech, Durham, NC).

Total anthocyanin content was assessed using the pH-differential technique outlined by (Lee, Durst, & Wrolstad, 2005) using a spectrophotometer (Agilent Cary 100 series, Agilent Technologies, Santa Clara, CA).

3.2.4 Quantification of major anthocyanins using HPLC/MS

Total monomeric polyphenols were identified and quantified by liquid chromatography - mass spectrometry (LC-MS/MS) analysis as described by Rupasinghe *et al.* (2008). Analysis was carried out using a Waters Alliance separation module (Waters, Milford, MA, USA) coupled with a Micromass Quattro micro API MS/MS system and controlled with Mass lynx V4.0 data analysis system (Micromass, Cary, USA). A

Phenomenex Luna C_{18} column (150 mm x 2.1 mm, 5 µm) was used with a Waters X-Terra MS C_{18} guard column.

The analysis of the flavonol, flavan-3-ol, phenolic acid, and dihydrochalcone compounds was done by electrospray ionization in negative ion mode (ESI-), with a capillary voltage of 3000 V, nebulizer gas (N_2) temperature of 375°C, and flow rate of 0.35 mL min^{-1}. Anthocyanin compounds were analysed by electrospray ionization in positive ion mode (ESI+), with capillary voltage 3500 V, nebulizer gas at 375°C, and flow rate of 0.35 mL min^{-1}. The cone voltage (25-50 V) was optimized for individual compounds.

In order to use high performance liquid chromatography, unbound sugars were removed from extracts using a solid phase extraction. Extracts were filtered through C-18 Bond Elut 500 mg SPE cartridge (Varian Canada, Mississauga, ON). After passing through the SPE column, the eluate was collected and filtered through a 0.2 µm syringe into two ml amber vials for analysis. Compounds of interest were determined using HPLC coupled to electrospray ionization and mass spectrometry. Extracts were run against known anthocyanins, flavonols, catechins and phenolic acids as standards which include peonidin 3-*O*-glucoside (P3G), malvidin-3-*O*-glucoside (M3G), delphinidin 3-*O*-rutinoside (D3R), cyanidin 3-*O*-rutinoside (C3R), quercetin 3-*O*-glucoside (Q3G), quercetin 3-*O*-arabinoglucoside (Qarabglu), quercetin 3-*O*-rhamnoside (QRh), quercetin, quercetin 3-*O*-rutinoside (QuR), phloridzin, phloritin, epigallocatechin (EGC), catechin, epicatechin,

epigallocatechin gallate(EGCG), epicatechin gallate(ECG), chlorogenic acid (ChloroAcid), caffeic acid (CafAcid), ferulic acid (FerAcid) and isoferulic acid (IsoferAcid)

3.2.5 Statistical analysis

Minitab 16 was used to check normality of residuals. Analysis of variance (ANOVA) was conducted using SAS 9.3 software using PROC MIXED at α=0.05. Treatments were compared using Tukey's multiple means comparison. Both years were combined when calculating Pearson coefficients.

3.3 Results

3.3.1 Weather

Weather varied between both field seasons (weather station Charlottetown A). Precipitation from May through July in 2011 was close to the long term average, and conditions were wet during the berry ripening stages in August (Table 3.1). Precipitation in 2012 was well below average and there was much lower precipitation during the berry ripening period compared with 2011.

3.3.2 Berry size

Berry size was significantly influenced by genotype in 2011 where Whistler, Ben Sarek, Ben Connan and Blackcomb had significantly larger berries than Titania, Ben Tirran and Ben Alder (Table 3.3 and 3.4).

A significant cultivar by timing interaction in berry size was observed in 2012 (Table 3.5). Ben Alder berry size varied significantly across timings compared with other

cultivars, ranging from largest at the 10 days to smallest at 18 days after 95% black. Ben Connan reached a maximum size at 14 days after bushes reached 95% black. Ben Sarek berry size did not seem to be affected by harvest timing. With a notable exception at 10 days after 95%, Ben Tirran was one of the smallest cultivars through the ripening stage. Blackcomb and Titania berry samples had similar sizes throughout the first 14 days of sampling after 95% black but sample size was slightly smaller at 18 days. The largest sample of Whistler currants were picked 14 days after turning 95% black. Compared to most cultivars, Whistler and Ben Sarek had larger berry size throughout sampling. Titania and Ben Connan currants were larger in size during the 2011 field season (102.38 compared to 83.67 g per 100 berries FW) (Table 3.3 and 3.5). June precipitation had a moderate positive correlation with size (Table 3.6). There was no strong correlation (>70%) between size and any other response variables of interest.

3.3.3 Total soluble solids

During the 2011 field season, TSS values were significantly influenced by genotype (Table 3.3). Whistler berries were significantly higher in TSS than both Ben Sarek and Ben Alder, and Ben Alder was lower in TSS than all other berries (Table 3.4). Remaining cultivars displayed values of roughly 13 to 14 ^{o}brix.

In 2012, TSS values were influenced by cultivar x fruit maturity effects (Table 3.5 and 3.7). Ben Alder maintained a TSS value of roughly 15.25^{o}Brix during the first two weeks after turning 95% black after which values dropped to 12.72 ^{o}Brix. Ben Connan reached peak TSS values two weeks after turning 95% black. Harvest timing did not

produce a significant difference in TSS of Ben Sarek at different timings. Ben Tirran had high TSS values 1.5 weeks after turning 95% black. Blackcomb had slightly higher TSS values after the initial week after turning black. Highest recorded TSS values were seen within the Titania cultivar two weeks after turning black.

3.3.4 Titratable acidity

Titratable acidity was significantly influenced by genotype in 2011 (Table 3.3 and 3.4). Whistler had significantly lower TA than all other cultivars. Titratable acidity was significantly higher in Ben Connan than all other cultivars except Ben Tirran. Results from 2012 were similar (Table 3.5). Whistler had significantly lower TA than all other cultivars (Table 3.6). Ben Sarek was significantly more acidic than most cultivars. Fruit maturity stage did not significantly affect TA. In 2012, there was a marginally significant interaction affect. Except for Ben Sarek, all cultivars were less acidic during the 2012 field season.

3.3.5 Bioactive compounds

During 2011, TAC was significantly influenced by harvest timing. Currants picked 10 days after 95% black had significantly more TAC than those picked at only 6 days after 95% black (1377.84 compared to 986.49 mg TE 100 g^{-1} FW respectively). During 2012 (Table 3.5), the cultivar effect was considered significant when $p<0.1$. The only notable difference was between Blackcomb and Ben Sarek (1762.93 compared to 1325.33mg TE 100 g^{-1} FW respectively). There were no significant differences in TAC values between cultivars (Table 3.3 and 3.5). Values were observed to be higher during

the 2012 season (1575.39 mg TE 100 g^{-1} FW Win 2012 compared to 1181.54 mg TE 100 g^{-1} FW in 2011). There was no significant difference in total phenolic content among cultivars during 2011 (mean of 75 mg GAE 100 g^{-1} FW) (Table 3.3). However, total phenolic content was significantly influenced by genotype in 2012 (Table 3.5). Blackcomb and Ben Connan had significantly higher phenolic content than Whistler and Ben Alder (Table 3.7). There were no significant differences in TAN values among cultivars (Table 3.3). Currants averaged 28.32 mg D3RE 100 g^{-1}. All four primary anthocyanins were significantly influenced by genotype (Table 3.5). Other individual phenolic compounds such as Epigallocatechins (EGC) and quercetin 3-*O*-rutinoside were significantly influenced by genotype x fruit maturity and genotype but it is unclear if such quantities less than 1 mg 100 g^{-1} FW could provide health benefits (Table 3.9).

3.3.6 Fruit yield

Yield was difficult to assess in 2011 as plants were only two years old. Although small, Ben Connan and Whistler had significantly higher yields during the 2011 field season (94.43 and 80.23 g $bush^{-1}$ compared to an average of 40 g $bush^{-1}$ across all treatments). Yields jumped significantly once the plants reached their third year of growth; Blackcomb, which had 36.30 g $bush^{-1}$ in 2011, produced 487.25 g $bush^{-1}$. Four year old Titania bushes yielded significantly higher than all other cultivars across both years (roughly 1500 g $bush^{-1}$).

3.4 Discussion

Genetic factors play an important role in fruit growth and development including fruit size so it is not surprising that cultivars with different parentage and genetics had different berry characteristics (Krüger *et al.*, 2011; Pedersen, 2007; Pluta *et al.*, 2008).

Berry size is important because size affects marketability and harvest ability. For fresh market products, larger currants fill baskets quicker and are easier to pick by hand. Whistler, Ben Connan, Ben Sarek and Blackcomb had larger sized berries. According to McGinnis berry crops (original breeders), Whistler is supposed to be smaller than Ben Alder (berrycrops.net). These results were not seen in this research data as Whistler was larger than most cultivars. Many factors influence berry size including but not limited to genetics, pollination and environment (Kaldmäe *et al.*, 2010; Pluta & Pruski, 2012; Zatylny *et al.*, 2005). Titania and Ben Connan currants grown in Germany were smaller when compared to both PEI research years (Krüger *et al.*, 2011). Size differences between genetically identical clones could be the result of growing environments, management practices or a combination of both. Berry weights were used as a proxy for berry size so it is important to consider factors that could influence weight rather than size. Daily changes in water balance could account for some of the variability between sampled times as fruit have high water content (Taiz & Zeiger, 2010). With destructive sampling, the ability to assess the change in size and quality of an individual berry over different harvest times is not possible. New berries were collected randomly at each timing meaning that the sampled population changed between sampling. The sampled

black currant population might have changed between harvest timings due to physical or chemical factors. Wind could have blown larger and riper berries off the bush between harvest timings resulting in the smaller sampled berry size at later timings. The differences seen between reported results for each individual cultivar indicates that factors other than genetics might play a key role in berry weight and size. Differences in precipitation and heat unit accumulation may affect berry size differences between years. It is well documented that fruit size can vary year to year because of many factors such as pollination, fruit load, heat units and precipitation (Kaldmäe *et al.*, 2010; Krüger *et al.*, 2011). Fruit load (yield) did not have a strong negative correlation with berry size. It is possible that the effect of yield on berry size is less significant than genotype. Kruger *et al.*(2011) stated that berry size and total anthocyanins might be negatively correlated however these results have indicated no strong correlation (Krüger *et al.*, 2011).

Total soluble solid content differences between cultivars has been reported many times so it was expected that genotypes could vary in TSS (Giongo *et al.*, 2008; Pluta & Pruski, 2012; Yang *et al.*, 2010). Acidity can be influenced by genetics which explains why cultivars had different levels of TA (Zatylny *et al.*, 2005). Previous experiments have indicated that TA decreases as fruits ripen but the timing of this process can differ across cultivars (Rubinskienė *et al.*, 2006). It was unclear how the ripening end point is decided for each cultivar. Total soluble solids is commonly used as a maturity indicator but factors other than genetics might contribute TSS making it difficult to pick a cultivar at its highest TSS. The addition of more harvest timings

definitely allowed a longer period in measure individual changes in TSS and TA trends (Table 3.6). Titratable acidity data from both years showed a cultivar x timing interaction indicating that each cultivar ripened differently. The 2011 sampling had lower TSS across all cultivars akin to results for similar cultivars grown in Italy (Giongo *et al.*, 2008). The ratio of TSS to TA can be used as a rough measurement of flavour (Kilburn, 1958). Whistler had high TSS and low TA making it suitable for fresh consumption. Overall, most currants were more acidic than previously established range (2.8-4 % CAE) (Nes, Hageberg, & Opstad, 2008; Zatylny *et al.*, 2005). Based on correlations in this experiment (Table 3.6) and previous harvest timings experiments, precipitation and TSS have a strong negative correlation so low TSS (12-13 ^{o}Brix) was probably caused by above-average precipitation (Table 3.1) (Koch, 1986). This correlation was further exemplified in 2012, where lower precipitation and high GDDs resulted in significantly higher TSS (15^{o}Brix and higher). There were significant differences between field seasons (ANOVA, results not shown); TSS values were generally lower during 2011 compared to 2012 (13.49 vs 16.0 ^{o}Brix respectively). Results were within the reported range of TSS values for black currants (Giongo *et al.*, 2008; Nes *et al.*, 2008; Zatylny & Ziehl, 2005).

Wines often require ^{o}Brix values greater than 15 (Eisenman, 1998; Margalit, 2005). If TSS is too low, additional sugar will be added which can affect wine complexity depending on the type of wine been made. It is difficult to mitigate seasonal extremes but it is possible to choose cultivars and harvest timings. Whistler and Titania might be

good for winemaking as they consistently reached higher TSS levels compared to other cultivars (Table 3.4 and 3.7). Currants destined for a fresh market should have the highest TSS and the lowest TA because it could indicate currants will taste sweeter. Whistler had high TSS and low TA making it suitable for fresh market.

Results from the harvest timing trial and 2011 cultivar trial indicated that harvest timing significantly influenced TAC but results from 2012 indicated no significant difference in TAC across harvest timings (Taylor, Hammermeister, Rupasinghe, & Pruski, 2011). It is difficult to determine what could have caused this difference. It is possible that seasonal differences caused TAC to plateau early making anything changes throughout August non-significant. May precipitation seemed to correlate with TAC which could explain the difference in trends as 2011 had 44mm while 2012 only had 22mm (50% difference). Berries that experience environmental extremes and stress can have higher amounts phytochemical compounds (Lila *et al.*, 2013) which could contribute to TAC and 2011 was above average when compared to the long term precipitation average but two years of data is inconclusive and so it could be a coincidence. Many antioxidants found within currants exist to protect fruit from environmental stress (Gould, Davies, & Winefield, 2008; Lev-yadun & Gould, 2009). Many individual antioxidant compounds and phenolics were positively correlated with TAC. This was expected as most of the TAC in black currants come from phenolics (more specifically, anthocyanins). Total phenolic content was correlated with TAC and did exhibit the same trends. Like TAC, cultivar only influenced TPC during the 2012 field

season (Table 3.3 and 3.5). Overall, currants had high TAC (20-60 µmol TE g^{-1} FW) compared to the reported result for blueberries, blackberries, strawberries and raspberries (16.24, 15.03, 8.00 and 7.57 µmol TE g^{-1} FW respectively). After analyzing the data across black currant cultivars, results were comparable to haskap (28-50 µmol TE g^{-1} FW) (Rupasinghe, 2012). Previous research (Krüger *et al.*, 2011) indicates that size and TAC have a negative correlation but this was not seen in this study.

Overall, black currant anthocyanins (ranged from 150-387 mg 100 g^{-1} FW) were much higher than reported values for red raspberries (40-50 mg 100 g^{-1} FW), cranberries (78 mg 100 g^{-1} FW) and strawberries (15-75 mg 100 g^{-1} FW) and results were comparable to previous result for black currants (130-400 mg 100 g^{-1} FW), blackberries (100-400 mg 100 g^{-1} FW) and blueberries (25-500 mg 100 g^{-1} FW) (Manach *et al.*, 2004). Black currants are known for high anthocyanin content and it was expected that they outclass other fruits. Amongst bioactives, cyanidin glycosides and delphinidin glycosides were the most abundant as predicted (Borges *et al.*, 2010; Slimestad & Solheim, 2002; Wu *et al.*, 2004) but both compounds acted differently based on its sugar moiety. Cyanidin 3-*0*-rutinoside (C3R) was affected by genotype but only Titania had significantly more C3R when compared to Ben Tirran (a difference of C3R by 9.6 mg 100g^{-1} FW). Delphinidin 3-*0*-rutinoside (D3R) was affected by cultivar like C3R but no cultivar had significantly more D3R other than Titania when compared to Ben Tirran (a difference of 8.6 mg 100 g^{-1} FW). There were differences in the relative abundance of C3R, D3R and C3G, D3G

(cyanidin and delphinidin *3-O*-glucoside) indicating that most black currant anthocyanins are rutinosides. This is positive from a nutraceutical perspective as there is some evidence that black currant anthocyanin rutinosides might be more readily absorbed in humans compared to glucosides (Nielsen, Dragsted, Ravn-Haren, Freese, & Rasmussen, 2003a). Although less abundant, C3G and D3G results showed a cultivar x timing interaction but is impractical to propose a management strategy. It is possible that glucosides are more sensitive to changes during ripening compared to rutinosides.

Preliminary results indicated that Blackcomb, Ben Sarek and Whistler might reach higher yields before establishment compared to Ben Tirran and Ben Alder but it is difficult to draw any conclusions as bushes were only two to three years old during sampling (except for Titania) (data not shown). Many currants will drop before fully maturing (off-red color during July) because of some sort of deficient prerequisite which could be environmental but literature did not provide any definitive explanation (possibly spring temperature and pollination or a combination both). Currants require multiple pollinations for fruit set so it is possible that currants with insufficient pollination get aborted by the plant itself. Alternatively, as berries mature, berry drop occurs more readily. Yield was gradually decreasing over time as berries were lost to berry drop as well as physical elements like wind.

Disease resistance was assessed based on rust % (*Cronartium ribicola* aka ' white pine blister rust') found on leaves post-harvest. Ben Alder and Ben Tirran had the highest % rust amongst cultivars (data not shown). Titania, though typically resistant,

showed some signs of rust. Whistler, Blackcomb, Ben Connan and Ben Sarek showed no signs of rust. Rust can cause premature leaf drop which could affect the following year's harvest.

3.5 Conclusion

Berry qualities such as berry size and TSS were affected by genetic differences between currant cultivars. There were also some instances where cultivar x timing effects affected quality but it difficult to recommend a general management strategy as in would be impractical. There were some significant differences in bioactive compounds between different cultivars but it is unclear how biologically relevant quantities less than 1 mg 100 g^{-1} FW are. With the exception of D3R, C3R, D3G and C3G, many bioactive compounds measured were found in trace quantities below 1 mg 100 g^{-1} FW. Overall, Whistler is recommended for growing on PEI as it had superior size, TSS and TA. Seasonal differences caused a significant shift in berry quality indicating that temperature and precipitation stations on-site could allow farmers to forecast seasonal berry quality by comparing their local conditions to LTAs.

Table 3.1 Weather data[y] for Mount Stewart in 2011 and 2012.

	Growing Degree Days[z]			Precipitation (mm)		
Period[y]	2011	2012	LTA[y]	2011	2012	LTA[y]
May	103.45	117.5	136.4[w]	44	22.1	97.7[w]
June	243	278.55	287.8	100.8	35.7	93.2
July	392.65	431.45	417	72.5	38.8	85.8
3	37.7	44.1	.	12.6	9.8	.
6	75.5	92.05	.	23	9.8	.
10	125.45	160.3	.	63	10.2	.
14	174.95	230.15	.	68.2	18	.
18	231.45	301.1	.	93.4	57.8	.
22	293.95	363.9	.	94.2	58.8	.
Bloom to day 22	1033.05	1191.4	.	308.6	155.4	.

[z] Growing Degree Days base 5°C

[y] Period measurement data starts with Bloom on May 16; July data ends at 95% black berries (August 29th in 2011, July 27 in 2012), 3, 6, 10, 14, 18, 22 are days after 95% black (time 0). The growing season weather and long term average (LTA) data from Environment Canada 'Charlottetown A' weather station

[w] full month of May

Table 3.2 Soil results for the cultivar trial at Mount Steward, PEI. Analysed at the PEI soil testing facility located in Charlottetown (2012). Results are presented in mg kg^{-1}.

Site	Organic matter (%)	pH	P_2O_5	K_2O	Ca	Mg	B	Cu	Zn	S	Mn	Fe
			----------(mg kg^{-1}) ----------									
MS_t	3.3	6.0	144	54.3	737	81.7	0.4	1.5	3.0	9.7	31	164

Table 3.3 ANOVA p-values and overall means for black currant berry quality variables in 2011 in response to cultivar and timing of harvest. Results are considered significant at α=0.05. CAE-citric acid equivalents, TE-Trolox equivalents, D3RE-Delphinidin 3-O-rutinoside equivalents. FW-Fresh weight.

responses	mean	SE	cultivar	timing	cultivar x timing
Size (g 100 berries^{-1})	102.3	7.9	<0.001	0.20	0.99
Total soluble solids (^{o}Brix)	13.5	0.3	<0.001	0.13	0.15
Titratable acidity (% CAE)	4.1	0.2	<0.001	0.35	0.01
Total antioxidant capacity (mg TE 100 g^{-1} FW)	1215	215	0.15	0.09	0.27
Total phenolic content (mg GAE 100 g^{-1} FW)	6.9	1.6	0.25	0.64	0.25
Total Anthocyanins (mg D3RE 100 g^{-1}FW)	292.5	63.5	0.05	0.60	0.84

Table 3.4 The effect of cultivar on black currant berry quality averaged across all timings (2011). Means followed by a different letter are considered significantly different at α=0.05. TA-titratable acidity, CAE-citric acid equivalents, TSS-total soluble solids

cultivar	size (g 100^{-1} berries)	TSS (^{o}Brix)	TA (%CAE)
B.Alder	67 d	12.5 c	4.0 bc
B.Connan	115 a	13.5 ab	4.9 a
B.Sarek	122 a	13.2 bc	3.9 bc
B.Tirran	70 cd	13.4 abc	4.4 ab
Blackcomb	113 ab	13.8 ab	4.3 ab
Titania	91 bc	13.9 ab	4.1 b
Whistler	139 a	14.1 a	3.4 c

Table 3.5 ANOVA p-values and overall means for berry quality variables in 2012 in response to cultivar and timing of harvest.

responses*	Mean	SE	Cultivar	Timing	Cultivar x Timing
Size (g 100 berries^{-1})	84.1	9.7	<.0001	0.01	0.00
Juice measurements					
Total soluble solids (^{o}Brix)	16.0	0.5	<.0001	0.01	0.00
Titratable acidity (%CAE)	4.2	0.3	<.0001	0.88	0.11
Spectrophotometric measurements					
Total antioxidant capacity (mg TE 100 g^{-1} FW)	1986.0	277.0	0.03	0.77	0.67
Total phenolic content (mg GAE 100 g^{-1} FW)	9.1	1.9	0.08	0.27	0.49
Liquid Chromatography/ Mass spec measurements					
Total phenolic content(mg 100 g^{-1} FW)	80.6	7.4	0.01	0.06	0.08
Total Anthocyanins (mg 100 g^{-1} FW)	76.6	7.2	<0.01	0.05	0.08
C3G (mg 100 g^{-1} FW)	5.0	0.7	<0.001	0.11	0.01
D3G (mg 100 g^{-1} FW)	14.0	1.7	<0.001	0.03	0.02
D3R (mg 100 g^{-1} FW)	30.7	2.9	0.01	0.15	0.12
C3R (mg 100 g^{-1} FW)	26.6	2.9	0.01	0.08	0.12

TE-Trolox equivalents,D3RE-Delphinidin 3-*O*-equivalents, C3G-Cyanidin 3-*O*-glucoside, D3G-Delphinidin 3-*O*-glucoside D3R-Delphinidin 3-*O*-rutinoside , C3R-Cyanidin-3-*O*-rutinoside . Only trace quantities of the following compounds were found (>1 mg 100 g^{-1} FW): Petunidin 3*O*-glucoside, Peonidin 3-*O* glucoside, Malvidin 3-*O*-glucoside, Quercetin-3-*O*-Glucoside, Quercetin-3-*O*-ArabinoGlucoside, Quercetin-3-*O*-Rhamnoside, Quercetin-3-*O*-Rutinoside, Epigallocatechin, Epigallocatechin gallate, Epicatechin gallate, Chlorogenic Acid, Caffeic acid, Ferulic acid, Isoferulic Acid

Table 3.6 Pearson correlation and statistical P value between environmental factors (accumulated GDD-growing degree days and precipitation since bloom), harvest timing and selected black currant response variables for PEI. Response variables include: size (g 100 berries^{-1}), titratable acidity (TA as %CAE), total soluble solids (^{t}TSS as ^{o}Brix), total antioxidant capacity (TAC as mg TE 100 g^{-1} FW), total phenolic content (TPC as mg GAE 100 g^{-1} FW) and total anthocyanin content (TAN as mg DRE 100 g^{-1} FW). Only Pearson correlations above 50% with a significant value at p<0.1 are shown (data was combined from both field seasons). *Variables contain one year of data only.

variables	GDD	precipitation	size	TA	TSS	TAC (FRAP)	TPC (FC)	TAN (pH-dif)	TAN (HPLC)	TPC (HPLC)	C3G	D3G	D3R
precipitation	-0.6												
size													
juice													
TA													
TSS	0.7	-0.7											
Photometric													
TAC (FRAP)	0.5	-0.6			0.5								
TPC (FC)						0.6							
*TAN (pH-dif)													
**HPLC/MS*													
TAN								N/A					
TPC								N/A	0.9				
C3G								N/A	0.6	0.6			
D3G								N/A	0.8	0.8	0.9		
D3R								N/A	0.9	0.9			
C3R								N/A	0.9	0.9	0.5	0.7	0.9

Table 3.7 The effect of cultivar and harvest timing on fresh berry and juice characteristics (2012). Means followed by a different letter are considered significantly different at α=0.05 throughout the entire column.

Cultivar	Timing	size	TSS (°Brix)	TA (%CAE)
B.Alder	6	66cde	15.2 abcd	3.95 bc
B.Alder	10	133a	15.1 abcd	
B.Alder	14	75 abcde	15.5 abcd	
B.Alder	18	40 e	12.7 d	
B.Connan	6	78 abcde	15.7 abcd	4.68 ab
B.Connan	10	68 cde	14.5 cd	
B.Connan	14	91 abcde	17.5 ab	
B.Connan	18	73 cde	17.4 abc	
B.Sarek	6	104 abcd	15.5 abcd	4.92 a
B.Sarek	10	112 abc	16.1 abc	
B.Sarek	14	111 abc	16.4 abc	
B.Sarek	18	103 abcd	16.3 abcd	
B.Tirran	6	65 cde	14.5 cd	4.28 ab
B.Tirran	10	92 abcde	17 abc	
B.Tirran	14	66 cde	15.2 abcd	
B.Tirran	18	56 de	15.6 abcd	
Blackhom	6	80 abcde	15 bcd	4.06 b
Blackhom	10	79 abcde	17 abc	
Blackhom	14	77 abcde	16.4 abc	
Blackhom	18	69 cde	15.9 abcd	
Titania	6	72 cde	16.7 abc	4.05 b
Titania	10	88 abcde	15.9 abcd	
Titania	14	83 abcde	18.3 a	
Titania	18	64 cde	18.3 a	
Whistler	6	103 abcd	15.4 abcd	3.31 c
Whistler	10	76 bcde	16.6 abc	
Whistler	14	130 ab	16.2 abc	
Whistler	18	105 abcd	16.4 abc	

Table 3.8 The effect of cultivar and harvest timing on spectrophotometric berry measurements after extraction (2012). Means followed by a different letter are considered significantly different at α=0.05. TAC-total antioxidant capacity (mg TE 100 g^{-1} FW), TPC-total phenolic content (mg GAE 100 g^{-1} FW).

cultivar	timing	TAC	TPC
B.Alder	6	2144	6.4
B.Alder	10	1738	8.9
B.Alder	14	2573	11.8
B.Alder	18	N/A	N/A
B.Connan	6	2244	9.8
B.Connan	10	2327	10.9
B.Connan	14	2169	10.9
B.Connan	18	1928	11.5
B.Sarek	6	1627	10.3
B.Sarek	10	1918	10.0
B.Sarek	14	1522	6.7
B.Sarek	18	1574	8.0
B.Tirran	6	1775	8.7
B.Tirran	10	1968	9.4
B.Tirran	14	1839	8.1
B.Tirran	18	1804	8.6
Blackcomb	6	2149	11.5
Blackcomb	10	2176	9.1
Blackcomb	14	2528	12.6
Blackcomb	18	2049	10.8
Titania	6	2080	5.6
Titania	10	2259	10.9
Titania	14	1936	8.6
Titania	18	2113	7.6
Whistler	6	1476	4.0
Whistler	10	2166	10.3
Whistler	14	1662	6.7
Whistler	18	1878	8.1

Table 3.9 The effect of cultivar and harvest timing on liquid chromatography/mass spec berry measurements (2012). Means followed by a different letter are considered significantly different at α=0.05. TPC-total phenolic content, TAC-total antioxidant capacity, C3G-cyanidin-3-*0*-glucoside, D3G-delphinidin-3-0-glucoside. All responses were measured in mg 100 g^{-1} FW.

cultivar	timing	TPC/MS	TAN/MS	C3G	D3G	C3R	D3R
B.Alder	6	77 ab	74 ab	5.0 abc	13.4 abc	29.9 ab	26.0 ab
B.Alder	10			4.3 abc	11.6 abc		
B.Alder	14			6.1 abc	16.6 abc		
B.Alder	18			3.1 abc	9.0 bc		
B.Connan	6	90 a	86 a	5.8 abc	16.0 abc	33.6 a	28.8 ab
B.Connan	10			4.9 abc	14.7 abc		
B.Connan	14			6.9 a	18.9 a		
B.Connan	18			6.6 abc	19.2 a		
B.Sarek	6	75 ab	72 ab	4.6 abc	11.8 abc	28.2 ab	23.6 ab
B.Sarek	10			5.1 abc	14.5 abc		
B.Sarek	14			5.0 abc	13.1 abc		
B.Sarek	18			6.5 abc	17.3 abc		
B.Tirran	6	71 b	66 b	3.6 abc	10.1 bc	26.4 b	23.0 b
B.Tirran	10			4.1 abc	12.2 abc		
B.Tirran	14			4.1 abc	12.2 abc		
B.Tirran	18			6.0 abc	14.2 abc		
Blackcomb	6	86 b	81 a	5.3 abc	14.5 abc	32.5 ab	27.1 ab
Blackcomb	10			4.6 abc	14.0abc		
Blackcomb	14			6.6 abc	17.7 ab		
Blackcomb	18			6.1 abc	16.9 abc		
Titania	6	81 ab	77 ab	2.7 c	8.2 c	31.5 ab	29.4 a
Titania	10			6.0 abc	17.3 ab		
Titania	14			3.1 bc	10.0 bc		
Titania	18			3.8 abc	11.7 abc		
Whistler	6	86 ab	83 a	5.1 abc	13.1 abc	33.1 ab	29.0 ab
Whistler	10			5.9 abc	17.0 abc		
Whistler	14			5.3 abc	13.8 abc		
Whistler	18			N/A	N/A		

Chapter 4. Conclusion

Black currants have a lot of potential for Canadian growing because they are very cold hardy and have numerous bioactives (mostly anthocyanins). Both studies provided insight into how harvest timing, and by extension fruit maturity, affected black currant quality. The fruit maturity experiment (chapter two) used Titania as a model to examine how various berry quality attributes changed over time. The cultivar experiment (chapter three) expanded the fruit maturity experiment in scope by examining the effects of cultivar, harvest timing and the interaction between both factors. The fruit maturity analysis of Titania make it possible to characterize how berry changes over time while the cultivar analysis characterized each cultivars capacity for change. Results from this experiment definitely showed how soluble solids, antioxidant capacity and anthocyanin content can steadily improve by delaying harvest. However, delaying harvest resulted in smaller currants and potentially lower yields. Deciding on a suitable harvest timing often requires compromise so it depends on the market destination and end use. Heat units (growing degree days) and precipitation were important environmental factors which affected fruit maturity and berry quality at each harvest. This was exemplified by the large variability in berry quality across both years. Titania was a good model because it is one of the most commonly grown cultivars on PEI but it is well understood that genetics play an important role in berry quality so more cultivars were needed for analysis. Of all the cultivars grown in PEI, Whistler was the most

interesting cultivar because it was bred in Canada, had large berries and had favorable taste elements (high soluble solids and low acid).

Black currants must contend with the flavour of other Canadian small fruits. Flavour is often dependent on sugar and acid balance. Black currant might have higher ^{o}Brix values than blueberries and black berries but most currants have significantly more acidity. Whistler had high TSS and low acid making a great fresh eating currant. Furthermore, Whistler was bred in Canada which simplifies distribution (easier for Canadian farmers to get plants). A formal sensory evaluation could determine scientifically which harvest timing and cultivar had the best "flavour" However higher TSS values are typically indicative of sweeter taste which means currants will get progressively sweeter as time passes (such was the case with Titania 14 to 18 days after turning black).Total soluble solids definitely increases in currants over time during August in PEI. It is possible that TSS is primarily driven by heat units but capacity is determined by genetics. Fruit maturity differences can improve TSS values by roughly 1-4 ^{o}Brix. Cultivar can also account for roughly 1-4 ^{o}Brix. Similarly, seasonal differences can account for 1-4^{o}Brix which brings forth the question: How do I grow a black currant with the highest possible Brix? The most controllable factor is genetics (cultivar) followed by harvest timing (fruit maturity). Titania, Whistler and Blackcomb (also Canadian bred) had consistently higher ^{o}Brix values after being black for 10 days. Soluble solids measurements and GDDs are good indicators of fruit maturity as an alternative to individual tasting and surface colour. Total soluble solids can be used to indicate

maturity by determining if currants fall within an acceptable range. Titania for example, can range from 14 to 20 % Brix meaning that farmers should not pick currants before they reach at least 14 ^{o}Brix. However, most harvest timing decisions require compromise. It was observed that delaying harvest made plants more susceptible to berry drop resulting in lower yields. Environments are difficult to control but correlations did show that hotter years might promote higher ^{o}Brix values with wetter years causing the opposite.

Precipitation also was highly correlated with berry size. Berry size was primarily influenced by genetics and environment. Some cultivars simply grow bigger like Whistler, Ben Connan, Ben Sarek and Blackcomb but average sample berry size decreased after two weeks. Cultivar selection is very important for optimizing berry size as variation can be as high as 50% (140 vs 70 g 1000 berries^{-1}). Aside from fruit maturity and genetic effects, site and environmental effects influenced berry quality significantly over the course of two years. This could explain why results in this study deviated from previous studies. In 2012, Titania and Ben Connan berry size was similar to a previous German study (Krüger *et al.*, 2011). However, both years had smaller Titania currants than those grown in Estonia (which had currants of 130 g 100 berries^{-1} FW) (Libek & Kikas, 2001).Seasonal differences in this study resulted in roughly 10-30 g 100 berries^{-1} variation within cultivars (greater precipitation resulted in larger berries). There were significant differences in berry quality between the sites involved in this research so it is just as likely that sites in different countries have different berry quality attributes as

well. For both PEI sites involved in this research, individual temperature microclimates might indirectly affect fruit maturation and ripening at each location, effectively skewing fruit development forward or backwards. This can make currants at one location slightly more or less developed than another creating a difference in overall berry quality. This was seen during both years where the HR site had more heat units possibly creating a small gap in development between it and the FT site. The magnitude of these differences might change across locations but some trends remains fairly consistent (e.g. ^{o}Brix increasing overtime). This is due to phenotypic plasticity which allows currants to exhibit different phenotypic traits (like bigger currants or higher ^{o}Brix) in various environments.

Aside from haskap, most small Canadian fruits don't contain the same bioactive profile as black currants. Overall, black currants had high antioxidant capacity compared to more commercially available fruits like blueberries, blackberries, strawberries, cranberries and red raspberries. This potentially allows black currants to fill a health benefit value-added niche. Many biologically active antioxidants were assessed in black currants in this research. Total antioxidant capacity measured free radicle scavenging ability of extractable compounds in black currants and seemed more affected by fruit maturity and environment differences than genetics. Depending on the season, antioxidant capacity might reach a maximum between 10 days to 18 days after turn black. Soil differences between sites might account for this. Total phenolic content was relatively unaffected by cultivar except for 2012 where Blackcomb and Ben Connan

outperformed Whistler and Ben Alder. Total anthocyanin content was strongly affected by fruit maturity with later harvest timings containing significantly more anthocyanins. In 2011, this resulted in currants picked 18 days after turning black having more than double the amount of anthocyanins when compared to currants picked 3 days after turning black. The fact that fruit maturity differences can account for this much variation makes harvest timing extremely relevant to anthocyanin optimization. As mentioned previously, it is important to consider the risk of lower yields and berry size when waiting leaving currants on the bush for extended periods of time. High performance liquid chromatography confirmed that C3R, D3R, C3G and D3G were the main anthocyanins. As mentioned in both chapters two and three, C3R and D3R were the most abundant which is good for consumers as a previous study had found black currant rutinosides to be more bioavailable to humans than glucosides. For food in general however, it is commonly understood that glucosides are more bioavailable (Manach, Williamson, Morand, Scalbert, & Rémésy, 2005). More research is needed to investigate how black currant anthocyanins are absorbed. It was the glucosides however that seemed to change over time following a pattern similar to TAC. It is possible that rutinosides are responsible for changes in TAC and TAN over time. Some bioactive compounds seemed affected by farming locating. It is possible that microclimate-induced developmental differences are responsible as mentioned above but most bioactives in black currants are secondary metabolites which can respond to stress. It is possible variability in plant stress caused by the fertility differences between both sites elicited a greater secondary metabolic response increasing bioactive content but further

researched is needed to verify this as stress responses are complex. It is important to note that many response variables were based on extractable compounds only. Non-extractable compounds left in currant pomace or seeds provide many benefits which were not explored in this study.

Fruit maturity is difficult to determine and quantify in black currants as there is no visual indicator of maturity. TSS measurements seem to be good surface colouration was chosen as a simplified visual tool to determine when to start counting days. Predicting when currants will mature prior to black colouration is difficult as numerous factors can influence the speed at which currants reach colouration. When comparing 2011 to 2012, the second year currants ripened much faster than the first year. Long term effects prior to colouration seem to determine how fast currants reach black colouration like heat units or precipitation. Short term effects seem to influence currants post-colouration meaning that warm weather during the harvest season makes currants ripen much faster (as seen during 2012). During 2011, currants were still considered not completely ripe on August 9th.In 2012, currants were completely ripe on August 9th. Black currant monocropping simplifies management but "companion" planting could help farmers determine maturity by using visual indicators from different fruiting plants growing with the currants. Gooseberries (*Ribes uvacrispa*) could be used to indicate black currant maturity as both berries require similar heat units. Red gooseberries turn completely red when currants are ready to eat (akin to raspberries).

The benefits of polycropping are well-known and could be applied with black currants to help with market diversity and management.

Future research should focus on breeding as genetics was one of the most important factors in this study. Controlling heat units and water might be another research avenue as seasonal growing degree days and precipitation were correlated with many berry qualities. Aside from outdoor irrigation, greenhouses can allow growers to control heat units and water. Fruit breeding should take place in every province in Canada to account for regional differences. Whistler and Blackcomb seedlings might be a good start as they expressed many desirable traits. It possible that new cultivars could emerge with traits better tailored to the Canadian growing season.

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Appendix A Global black currant production (modified from FAOSTAT 2012)

Countries	2011 Production(tonnes)
World (Total)	*652490*
Russian Federation	364500
Poland	169634
Ukraine	25700
Austria	19960
Denmark	13372
United Kingdom	12060
Germany	9587
France	9367
New Zealand	7486
Asia (Total)	3829
Netherlands	3693
Hungary	2987
Uzbekistan	2200
Finland	2181
Belgium	1687
Czech Republic	1672
Azerbaijan	1500
Estonia	780
Italy	700
Switzerland	550
Latvia	427
Australia	415
Sweden	400
Africa	367
Norway	333
Spain	239
Slovakia	214
Ireland	158
Romania	30
Japan	19
Northern America	0