Factors Affecting the Anthocyanidin Concentration in Fruits of *Vaccinium myrtillus* L.

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Cover: Micropropagated *Vaccinium myrtillus*
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Abstract

Vaccinium myrtillus, a small dwarf-shrub native to northern Europe has received much attention during recent decades because of its economic value and as a result of the health promoting substances found in the fruits. The work outlined in this thesis focused on the effects of exogenous factors such as temperature, light and nutrient availability on the concentration of anthocyanidins in the fruits. The research also examined whether the biosynthesis of anthocyanidins in *V. myrtillus* fruits (bilberries) is mainly under genetic or environmental control. This information is intended to be used in developing the cultivation of the species.

   The concentration of anthocyanidins in bilberries was found to be sensitive to changes in temperature and day length, with higher concentrations in fruits exposed to high mean temperatures (16°C) compared to low mean temperatures (11°C) and under long day (24 h) compared to short day (12 h) conditions. However, anthocyanidin concentrations were higher in the fruits of plants growing under a closed forest canopy compared to those in direct sunlight, certain years. Anthocyanidin concentrations were also found to be positively correlated with thermal sum (after first of May each year) up to 1350 degrees (C); above this value, concentrations decreased.

   Even though anthocyanidin concentrations in bilberries were affected by climatic factors, the results obtained clearly show that anthocyanidin biosynthesis in bilberries is primarily under genetic control. Clones of plants originating from different latitudes differed in anthocyanidin concentration even when they were grown under the same climatic conditions and at the same latitude for a period of more than ten years. Higher anthocyanidin concentrations were found in fruits from clones with a more northerly origin compared to those from further south. The same pattern was found in wild populations.

   The results gathered during the work underlying this thesis indicate that with selection of suitable genotypes and with controlled growth environment, the anthocyandin concentration in bilberries could be greatly enhanced compared to natural levels and concentrations could probably be stabilized thus reducing variation between years. In combination with the strong market for bilberries there are good prospects for future cultivation of *V. myrtillus*.

Keywords: Altitude, Bilberry, Climate, cyanidin, delphinidin, Genome, Geographical origin, Latitude

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Dedication

To my late grandfather and his love of nature.
To the fog coated spruce forests, the golden mires and the sun kissed lakes of northern Hälsingland.

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Andreas Åkerström, Åsa Forsum, Kimmo Rumpunen, Anders Jäderlund & Ulla Bång (2009). Effect of Sampling Time and Nitrogen Fertilization on Anthocyanidin Levels in *Vaccinium myrtillus* Fruits. *Journal of Agricultural Food Chemistry*, 57 (8), 3340-3345.

II Andreas Åkerström, Ulla Bång & Anders Jäderlund. Anthocyanidin Concentration in *Vaccinium myrtillus* Fruits (Bilberries) - Effects of Temperature and Light in Controlled Environment. (Manuscript)

III Andreas Åkerström, Laura Jaakola, Anders Jäderlund & Ulla Bång. Effects of latitude-related factors and geographical origin on anthocyanidin concentration in fruits of *Vaccinium myrtillus* L. (Bilberries). *Journal of Agricultural Food Chemistry* (Accepted)

IV Anders Jäderlund, Andreas Åkerström & Ulla Bång. Influence of altitude and light availability on the concentration of anthocyanidins in fruits of *Vaccinium myrtillus* L. (Bilberries). (Manuscript)

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The contribution of Andreas Åkerström to the papers included in this thesis was as follows:

I   Planning and executing the experiment jointly with the co-authors, chemical analysis of samples and statistical analysis of data and writing the manuscript.

II Planning and executing the experiment jointly with the co-authors, statistical analysis of data and writing the manuscript.

III Planning the experiment jointly with co-authors, statistical analysis of data and writing the manuscript.

IV Planning the experiment jointly with the co-authors, statistical analysis of data and actively taking part in writing the manuscript.

Abbreviations

ROS   Reactive Oxygen Species
PAL   Phenylalanine Ammonia Lyase
ANS   Anthocyanidin Synthase
UFGT   UDP glucose-flavonoid 3-o-glucosyl transferase
PAR   Photosynthetically Active Radiation, $400 - 700$ nm
UDP   Uridine diphosphate
m.a.s.l.   Meters above sea level

1   Introduction

Vaccinium myrtillus and *V. vitis-idaea* are the most important wild berry species in Northern Europe (Sjörs, 1989; Morazzoni & Bombardelli, 1996). Historically, due to its good storage properties, *V. vitis-idaea* has been the most important wild berry in Sweden, but at the end of the last century interest in *V. myrtillus* increased (Jonsson & Uddstål, 2001). *V. myrtillus* has a long history of use in European folk medicine (Morazzoni & Bombardelli, 1996). A diet rich in berries, fruits and vegetables is associated with a reduced risk of developing diseases such as certain types of cancer (Block *et al.,* 1992 Steinmetz & Potter, 1996; Terry, 2001; Fresco *et al.,* 2006; Rajamanickam & Agarwal, 2008) cardiovascular disease (Van Poppel *et al.,* 1994; Kris-Etherton *et al.,* 2002; Hu & Willett, 2002; Bazzano *et al.,* 2003; Vattem *et al.,* 2005; Hu, 2009), age-related neurodegenerative diseases (Youdim *et al.,* 2000; Ramassamy, 2006; Shukitt-Hale *et al.,* 2008; Berglöf*,* 2008) and eye diseases (Vattem *et al.,* 2005). The soluble and insoluble dietary fiber, vitamin C and E, foliate, carotenoids, selenium and phenolic compounds found in fruits and vegetables are shown to be involved in the prevention of degenerative diseases (Feeney, 2004; Abdel-Aal & Akhtar, 2006; Szajdek & Borowska, 2008). The interest in phenolic compounds in "functional foods" has increased dramatically over recent years due to their antioxidant properties. Phenolic compounds are present in all plants and can therefore be considered to be a natural part of our diet (Bravo, 1998) (Table 1). Evaluating phenolic antioxidants in plants requires powerful and accurate analytical instruments since the compounds are often present in complex mixtures. Raw natural phenolic antioxidants are often present in rather low concentrations and are, thus, less potent than pharmaceutical drugs, but are always present in our diet, providing long-term effects (Espín et al., 2007). The fruit and leaves of several commercially important *Vaccinium* species are

rich in phenolic compounds, especially anthocyanins and other flavonoids (Kalt & Dufour, 1997; Jaakola, 2003; Percival, 2006). Many of the anthocyanins present in bilberries (the fruits of *V. myrtillus*) have received attention because of their antioxidant properties, and thus their capacity to reduce free oxygen radicals or reactive oxygen species (ROS). Compared to cultivated North American high bush blueberries (*V. corumbosum)*, *V. myrtillus* contains much higher concentrations of anthocyanins and total phenolics, three times and two times greater, respectively (Giovanelli & Buratti, 2009). Moreover, no allergenic compound has so far been found in the fruits. Therefore, the fruit of *V. myrtillus* can be considered to be an excellent functional food.

Table 1. *Main sources of some natural phenolic compounds in our diet.*

Phenolic class	Food source
Hydroxybenzoic acids	Berries, grapes, orange, kiwi fruit, apple, peach, grapefruit, pears, cherries, potatoes, cereals, olives, vegetables, wine, beer, herbs, spices
Hydroxycinnamic acids	Apples, pears, cherries, plums, peaches, apricots, white grapes, kiwi fruit, tomatoes, coffee, white wine, cider, citrus juice, potatoes, olives, vegetables, cereals
Flavonols	Sweet red pepper, parsley, red wine, citrus fruits
Flavanones	Citrus fruits
Flavan-3-ols	Apples, apricots, peaches, plums, cherries, green and black tea, red wine
Proanthocyanidins	Grapes, cherries, apples, pears, blueberries, raspberries, blackberries, cider, red wine, beer, cocao bean, cereal
Anthocyanidins	Berries, red grapes, red-colored fruits, blood oranges, plums, eggplant, onion, red wine
Ellagic acid and ellagitannins	Raspberries, blackberries, strawberries, nuts

Source: Original table from Vasco, 2009.

V. myrtillus bushes cover about 20% of Sweden's productive forest land (Johansson, 1993). The average fruit yield of *V. myrtillus* in Scandinavia has been estimated to be over 500 million kg • year^{-1}, of which only five to eight percent is exploited by man (Salo, 1995). However, for most berry producing plant species, yields are influenced by several environmental

factors including light (Chalker-Scott, 1999; Martinussen *et al.,* 2009, Uleberg *et al.,* 2009) and temperature (Kardell & Eriksson, 1990; Martinussen *et al.,* 2009; Uleberg *et al.,* 2009) and the spatial and temporal variation in berry yield is very high (Salo, 1999; Wallenius, 1999). Resources may have to accumulate in plants for many years before abundant flowers and berries can be produced. A similar pattern seems to exist for the concentrations of anthocyanins in bilberries (Åkerström *et al.,* 2009) and natural fluctuations in fruit yields have been reported several times for *V. myrtillus* (Laine & Henttonen, 1983; Sedlås, 2000).

1.1 The plant in focus

The genus *Vaccinium* contains over 200 species of evergreen and deciduous woody plants varying from dwarf shrubs to trees. Many are economically important cultivated small fruit species, like North American high bush blueberries (*V. corrumbosum*) and cranberries (*V. macrocarpa*). *V. myrtillus* (Figure 1) grows wild in Europe and Asia and is most abundant in Northern and Eastern Europe.

Figure 1. Vaccinium myrtillus, stems b fruits (Photo: Andreas Åkerström, 2004)

The ecological range of *V. myrtillus* is wide with regard to both soil moisture and light conditions. It can be found on both dry and moist sites, in sunny openings as well as in shade, and at low altitudes as well as in more alpine areas (Atlegrim & Sjöberg, 1996; Timoshok, 2000). *V. myrtillus* is a mesomorphic plant, i.e. it produces the most abundant yields in quite low light conditions and does not benefit from direct sunlight (Salo, 1995). The productivity of *V. myrtillus* reaches its maximum at a relatively low photosynthetic light ratio and a nonlinear relationship has been found between productivity and photosynthesis and this seem to be characteristic of *Vaccinium* species (Väisänen *et al.*, 1977). Plants are also sensitive to changes in other abiotic factors such as nitrogen (Hester *et al.,* 1991; Percival *et al.,* 2003; Percival & Sanderson, 2004) light availability (Atlegrim, 1991; Martinussen *et al.,* 2009) and temperature (Stewart & Bannister, 1973; Laine & Henttonen, 1987; Raatikainen & Vänninen, 1988). These factors may, directly or indirectly affect the morphology, reproduction and chemical composition of fruit and leaves. Yearly growth of *V. myrtillus* starts early to maximize use of the short north European growth season (Mooney & Billings, 1960) and flowering usually starts two or three weeks after snowmelt (Tolvanen, 1997). *V. myrtillus* is insect pollinated (Linné, 1755) and flowering usually starts in three-year-old shots (Tolvanen, 1997).

1.2   Secondary metabolites

There are four major groups of secondary metabolites (substances not directly involved in primary growth) in plants: terpenoids, alkaloids, sulfur-complexes and phenolics. In this thesis the compounds under consideration, the anthocyanins, belong to the phenolic group. More than 8000 different phenolic compounds have been identified (Bravo, 1998) and the main groups of phenolics in fruits are phenolic acids, coumarins, tannins and flavonoids (Macheix et al., 1990; Bravo, 1998; Hounsome et al., 2008). Phenolics are biologically active (often toxic) compounds; the formation of glycoside may be one method of storing phenols in the plant in a non-toxic form (Harborne, 1964). The fruit and leaves of *V. myrtillus* are rich in phenolic compounds, especially flavonoids (Jaakola, 2003), and their bioactive properties have received much attention in recent years. Flavonoids, which are diphenylpropanes (C6-C3-C6) (Bravo, 1998) are divided into seven subclasses: flavonol, flavones, flavan-3-ol,

proanthocyanidin, anthocyanin, isoflavone and flavanone. The structural differences between these subclasses relate to modifications of the C–ring (Taiz & Zeiger, 1998) (Figure 2).

The bioactive part of the compounds, the aglycone, is the non–sugar component remaining after replacement of the glycosyl group from a glycoside by a hydrogen atom. The removed sugar group is then known as the glycone. The aglycone of an anthocyanin is called an anthocyanidin, for example delphinin (anthocyanin) and delphinidin (anthocyanidin) (Figure 2).
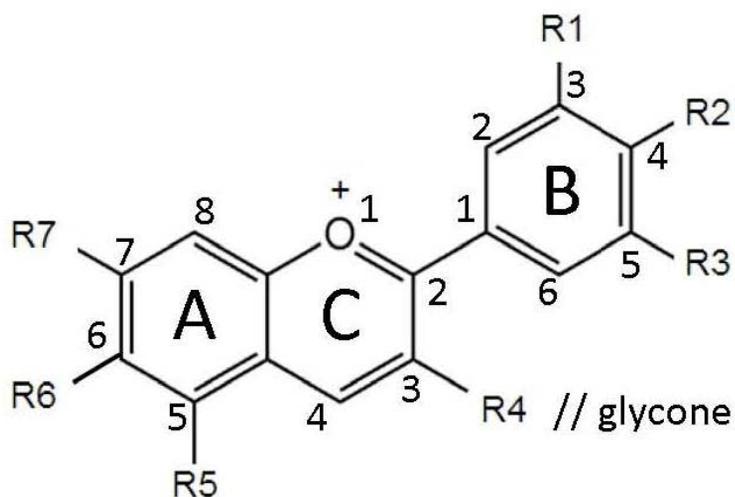
Anthocyanidin	R1	R2	R3	R4	R5	R6	R7
Delphinidin	OH	OH	OH	OH	OH	H	OH
Cyanidin	OH	OH	H	OH	OH	H	OH
Petunidin	OH	OH	OCH_3	OH	OH	H	OH
Peonidin	OCH_3	OH	H	OH	OH	H	OH
Malvidin	OCH_3	OH	OCH_3	OH	OH	H	OH

Figure 2. Schematic structure of anthocyanidins (agycones). Capital letters A, B and C represent the different carbon rings. R(x) represents the different reaction points in the molecule.

1.2.1 Anthocyanin

The bright red colours of autumn and the red, blue and purple colours of flowers, fruits and berries are due to plant pigments called anthocyanins. The supposed biological functions of anthocyanins include UV-protection (Mazza & Minitati, 1993, Jaakola *et al.*, 2004). Of all the anthocyanidins, it has been suggested that cyanidin plays the major role in defence against high solar radiation in *V. myrtillus* (Chalker-Scott, 1999). However, in an ecological context, the role of anthocyanins as visual markers to pollinators is perhaps their best known function (Harborne, 1975). Because of their use as natural colorants and health promoting agents, anthocyanins are of considerable interest to the pharmaceutical and food industries. Several environmental factors such as light, UV-radiation, temperature, and nutrient status contribute to the biosynthesis of anthocyanins (Strack, 1997; Prior *et al.*, 1998; Winkel-Shirley, 2002; Jaakola *et al.*, 2004).

The composition of anthocyanins in *Vaccinium* species is in general highly complex (Macheix et al., 1990) and concentrations of most anthocyanins are much higher in the fruits than the leaves of *V. myrtillus* (Jaakola *et al.*, 2004).

1.2.2 Biosynthesis of anthocyanins

Anthocyanins are the end product of the flavonoid pathway and flavonoids are in turn synthesized through the phenylpropanoid pathway (Figure 3). Through a series of enzymatic steps starting with phenylalanine ammonia lyase (PAL) in the phenylpropanoid pathway leucoanthocyanins are converted to anthocyanidins catalyzed by anthocyanidin synthase (ANS). Then, as a finale step, the anthocyanidins are stabilized into anthocyanin form by 3-O-glucosylation using UDP glucose-flavonoid 3-o-glucosyl transferase (UFGT) as catalyst (Taiz & Zeiger, 1998).
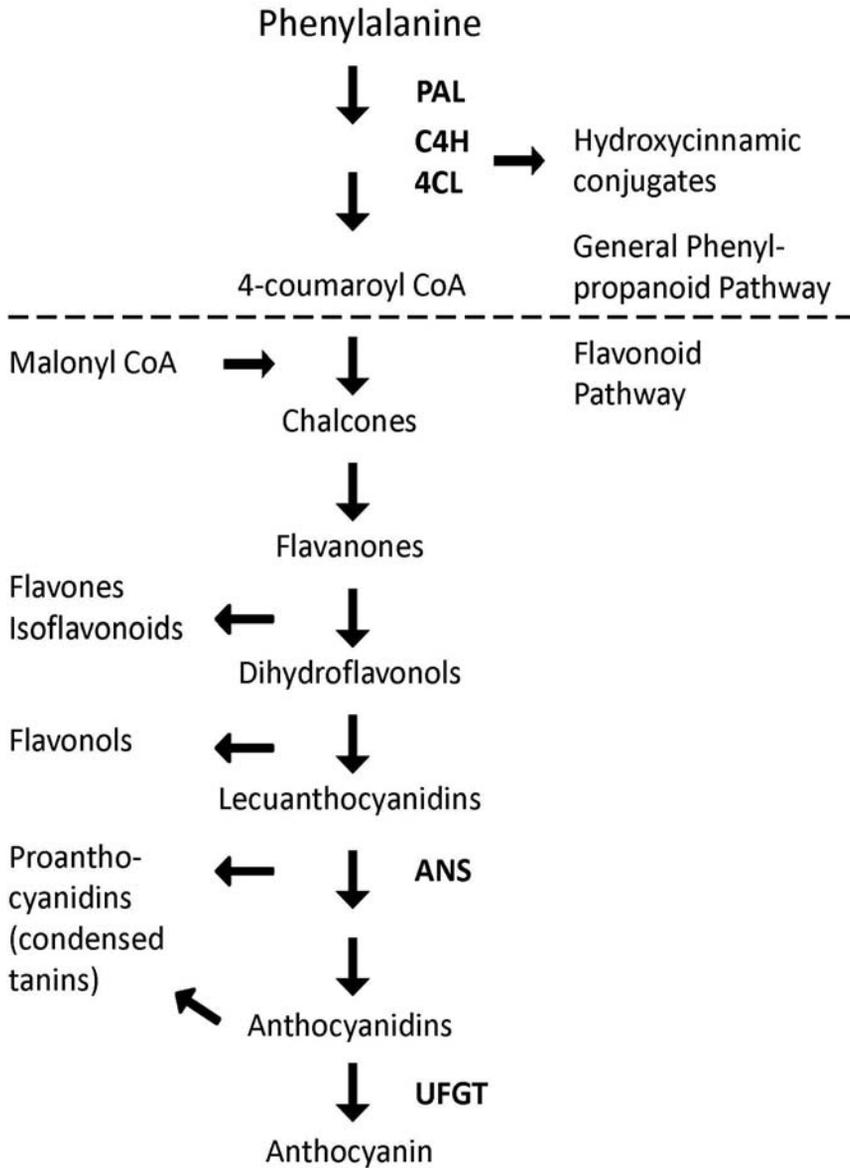
Figure 3. Schematic view of the biosynthetic pathway through which anthocyanins are formed.

Among the anthocyanin derivatives, O-glycsides are mainly found with hexoses or pentoses almost always attached at the C–3 position (represented by R4 in figure 2) (Castaneda-Ovando *et al.,* 2009). In organic chemistry, a hexose is a monosaccharide with six carbon atoms ($C_6H_{12}O_6$) whereas a pentose has five carbon atoms. However, di– and tri–glycosides also exist, as well as acylated anthocyanins (Clifford, 2000; Castaneda-Ovando *et al.,* 2009). During hydrolysis the anthocyanins release their aglycon, the anthocyanidin (figure 2). The number of anthocyanins is 15-20 times greater than the number of anthocyanidins (Mazza & Miniati, 1993). In general, anthocyanidins seem to be less sensitive to variations in sample pre-handling and extraction conditions than the anthocyanins (Nyman & Kumpulainen, 2001). Delphinidin, cyanidin, petunidin, peonidin, pelargonidin and malvidin are considered to be the most significant anthocyanin acglycones (De Pascual-Teresa & Sanchez-Ballesta, 2008). All of these are present in *V. myrtillus* except pelargonidin which is found in species such as *Rubus idaeus* (Raspberry) and *Fragaria* spp*.* (Strawberry) (Wang & Zheng, 2001).

1.3   Environmental factors and their impact on anthocyanidin formation

"Recognition of the relationship between environmental factors and plant growth and function is of prime importance in the manipulation of ecological systems" (Väisänen *et al.*, 1977)

Biosynthesis and accumulation of secondary compounds are regulated internally within the plant and controlled by both external and genetic factors. The most important environmental factors determining photosynthetic rates (and thus synthesis of phenolic compounds) are light and temperature, as long as water and nutrient supplies are adequate (Emmingham & Waring, 1977).

1.3.1 Temperature and light

For most plant species that produce fruits, summer temperatures have a great influence on fruit maturation. It has been reported that reduced temperatures increase the yield of anthocyanins in *V. myrtillus* (Martinussen *et al.,* 2009) and in other species (Chalker-Scott, 1999; Winkel-Shirley,

2002) and the ratio between anthocyanin synthesis and degradation seems to be strongly affected by temperature (Shaked-Sachray *et al.,* 2002).

In general, light stimulates synthesis of flavanoides, especially anthocyanins and flavones (Macheix *et al.,* 1990). Long-day conditions (24 h light) have been shown to increase anthocyanidin concentration in fruits of *V. myrtillus* compared to short-day conditions (12 h light) (Martinussen *et al.,* 2009). Furthermore, the synthesis of tannins, which are also phenolic substances, may be indirectly influenced by increased light availability since PAL, the primary enzyme in the synthesis, is stimulated by red light and ultraviolet radiation (Waterman *et al.,* 1984; Larsson *et al.,* 1986).

Atlegrim & Sjöberg (1996) suggested that the more abundant flowering and higher production of berries combined with lower recourse allocation to vegetative growth on sunny sites can be interpreted as a trade-off between reproductive input and vegetative growth. *V. myrtillus* has a carbon-based defence system (Coley *et al.,* 1985) in which phenols play a major role (Laine & Henttonen, 1987; Jonasson *et al.,* 1986). Vegetative tissues of *V. myrtillus* plants growing in open sites have been found to have significantly higher carbon content than those growing in shaded sites or in closed forest (Atlegrim & Sjöberg, 1996). Changes in the carbon content of plants are expected to cause a shift in the allocation of limiting resources and thus affect growth, reproduction, nutrient concentration and the chemical defences of plants (Vänninen *et al.,* 1988; Bazzaz *et al.,* 1987; Pearcy *et al.,* 1987). In shaded forests, low light availability may result in reduced defence before nutrients become limiting (Atlegrim & Sjöberg, 1996). Furthermore, Atlegrim & Sjöberg (1996) recorded higher levels of total phenolics in *V. myrtillus* growing in the open compared to closed forest. Thus, the increased carbon and phenol content in vegetative parts of *V. myrtillus* plants growing in clear-cuts may be interpreted as a response to protect leaf enzymes and membranes from damage by ultraviolet radiation (Moorthy & Kathiresan, 1997; Rose, 1989).

However, the effects of temperature and light on anthocyanin production may sometimes be hard to separate. It has been suggested that large differences between day time and night time temperatures may promote anthocyanidin formation (Lätti *et al.,* 2008), which was suggested to be the explanation for the results obtained by Atlegrim & Sjöberg (1996). Furthermore, Jaakola *et al*. (2004) found that *V. myrtillus* leaves growing in direct sunlight had elevated anthocyanidn concentrations compared to

shaded leaves on the same branch. This may be an effect of light, temperature or a combination of the two.

1.3.2 Nitrogen availability

The biological effects of the addition of nitrogen fertilizers to both agricultural (Newbould, 1989) and forest ecosystems (Hartley *et al.,* 1999; Strengbom *et al.,* 2002; Strengbom *et al.,* 2004) as well as on stem and leaf chemistry of *Vaccinium* species are well documented (Percival & Privé, 2002; Percival, 2003; Forsum, 2008). There is some information available regarding the effects of added nitrogen (N) on berry chemistry but there is no consensus in the published results on the effects of N-fertilizer on antioxidant concentrations and antioxidant activity. For example, a fertilizer concentration-dependent increase in antioxidant activity has been recorded in *Cichorium pumilum, Eryngium creticum*, *Pistacia palaestina* and *Teucrium polium* (Azaizeh *et al.,* 2005), while other studies have identified no relationship between fertilizer and antioxidant activity in different tomato (*Solanum* spp) cultivars (Wold *et al.,* 2004) or flavonol content in *Allium cepa* L. (Mogren *et al.,* 2006). In the skin of grapes (*Vitis vinifera* × *V. labrusca*), Okamoto *et al,* (2003) found a relationship described by a convex curve, with the highest amounts of anthocyanins at moderate nitrogen fertilizer concentrations.

   In Sweden, the abundance of natural populations of *V. myrtillus* has been shown to be negatively correlated with nitrogen deposition (Strengbom *et al.,* 2003). It has also been reported that nitrogen fertilization of forest soils leads to accumulation of nitrogen-based metabolites in the vegetative tissues of *V. myrtillus* (Näsholm *et al.,* 1994; Strengbom *et al.,* 2004).

1.3.3 Latitude and altitude

When studying the influence of latitudinal, altitudinal or temporal variations on anthocyanin production environmental factors such as light and temperature are of prime importance. Connor *et al.* (2002) found a significant genotype × location interaction when examining total anthocyanin content in American blueberry cultivars. Strong *et al.* (2009) interpreted the changes in environmental conditions from southern to northern Canada for several dwarf shrubs as a response to cooler and slightly

drier climatic conditions and reduced PAR (photosynthetically active radiation, 400–700 nm) availability by 20%.

For changes in altitude similar changes would be expected as the altitude increase. Friend *et al*. (1989) found no significant effect on photosynthesis in *V. myrtillus* along westerly (200–1100 m.a.s.l.) and southerly (400–1000 m.a.s.l.) altitudinal gradients in Scotland. In a two year study of *V. myrtillus* fruits by Rieger *et al*. (2008) decreasing anthocyanin contents in were found with increasing altitude in Austria (800, 1200 and 1500 m.a.s.l.). In Sweden where altitudinal differences are less pronounced this effect of altitude on anthocyanidins may not be easily detected.

1.3.4 Temporal variations

The content of phenolic compounds in a fruit varies greatly depending on state of maturity (Raven *et al.,* 1999). Published studies have revealed decreasing concentrations of different phenolic acids in fruits of *Rubus fruticosus* (Blackberry) and *Fragaria* spp**.** (Strawberry) as the fruits mature (Sthör & Herrmann, 1975). However, it has been found that anthocyanins accumulate in red fruits at the end of maturation (Macheix *et al.,* 1990) and Prior *et al*. (1998) found that in fruits of North American blueberry and *V. myrtillus*, anthocyanins and total phenolics increased the more mature the fruits were at harvest.

1.4 Genome

Not much has been published about the variations in anthocyanin content in relation to the genome, but it is clear that there is a genetic control of the biosynthesis of secondary metabolites. Significant differences in anthocyanidin concentrations have been found between genotypes and between years for *V. corymbosum* (Cho *et al.,* 2005). Howard *et al*. (2003) found that genotype had a stronger influence than year when studying American blueberry cultivars and selected varieties under cultivation. In red grapevine (*Vitis vinifera* × *V. labrusca*), differences in skin anthocyanin have been found between genotypes (Ortega-Regules *et al.,* 2006). In *V. myrtillus* higher anthocyanidin concentrations have been found in fruits grown under controlled conditions and originating from northern latitudes than those from more southern latitudes (Martinussen *et al.,* 2009). The same pattern

has also been found in wild populations of *V. myrtillus* in Finland (Kähkönen *et al.,* 2003; Lätti *et al.,* 2008).

1.5 Objectives

The project upon which this thesis is based originated from a "think–tank" involving politicians and stakeholders from the four most north easterly municipalities in Sweden. Several areas of interest relating to the development of the region were identified, amongst which exploitation and on farm production of *V. myrtillus* emerged as an opportunity that needed further investigation.

V. myrtillus is one of the most economically important wild berries in Scandinavia and has been shown to be one of the richest natural sources of anthocyanin on the planet. Despite the species importance and the high concentration of health promoting compounds in its fruits most research has focused on the vegetative tissues of *V. myrtillus* or on fruits of cultivated *Vaccinium* spices.

This thesis focuses on two principal areas of interest. The first area deals with how specific climatic conditions affect anthocyanidin concentration in *V. myrtillus* fruits (Papers I & II). The second area is connected to variations in anthocyanidin concentrations over latitudinal and altitudinal gradients and different genotypes (Papers III & IV).

More specifically the aims are covered as follows in Papers I–IV:

I   To investigate how climatic parameters (temperature, precipitation, irradiation and humidity), sampling time and N–fertilization affect anthocyanidin concentration in fruits of *V. myrtillus*.

II To evaluate how different combinations of light regimes and temperature levels affect anthocyanidin concentrations in fruits and certain phenological events of *V. myrtillus*.

III To investigate whether the anthocyanidin concentration in fruits of *V. myrtillus* is predominately under genetic or environmental control.

IV To investigate the effect of altitudinal gradients in combination with different light availabilities on anthocyanidin concentration in fruits of *V. myrtillus*.

2 Material and Methods

2.1 Methodological approaches

In the work underlying this thesis two main approaches for collecting information about the variability in anthocyanin concentration in fruits of *V. myrtillus* were used. 1) Sampling within wild populations (Papers I, III & IV) from locations ranging in latitude from 54° N to 69° N. 2) Experiments in controlled environments, either as a small scale field trial (Paper III) or in climate chambers (Paper II) (Figure 4). In the climate chamber studies wild plant material was used and in the field trial plants had been micro-propagated from seeds (cover photo). It was decided that when the fruits had developed a uniform deep blue skin colour they would be considered to be fully mature.

Immediately after harvesting all sampled bilberries were put in sealable plastic bags or tubes. The samples were placed in an ice-filled cooler for transportation to the laboratory, whereupon they were transferred to a freezer and stored at –20°C until analysed.

Figure 4. Map of Northern Europe showing the locations of the wild population sampled in Papers I and II (□), locations of sampling for Paper III (●1-5), geographical origin of parent plants for Paper III (◆1-6), and of the altitudinal gradients studied in Paper IV (1●). The test field used in the work for Paper III at the University of Oulu, Finland (★) and the climate chambers used in the Paper II study at the Swedish University of Agricultural Sciences, Umeå (O), are also marked.

2.2 Analyses

To circumvent the problem of glycosides interfering with the analysis of anthocyanins, analysis of aglycones after acid hydrolysis has been recommended for plant- based materials (Nyman & Kumpulainen, 2001); this approach has been implemented throughout the research described herein. However, hydrolysis times were modified to improve the extraction of bilberry anthocyanidins (Åkerström *et al.,* 2009). Briefly, material was freeze-dried and finely milled. Fifty mg of the milled powder was then mixed with 2 ml of 2M HCl for extraction of delphinidin, cyanidin, petunidin, peonidin and malvidin.

Hydrolyzed samples were filtered and 0.8 ml of each filtered sample was placed in an auto-sampler. The amounts of delphinidin, cyanidin, petunidin, peonidin and malvidin in each sample were then determined by HPLC. The wavelengths used for quantification were 530 nm for delphinidin and 510

nm for cyanidin, petunidin, peonidin and malvidin. The identity of the compounds corresponding to putative anthocyanidin peaks in the resulting chromatograms was confirmed by comparing their spectra and retention times with those of pure compounds. Total anthocyanidin concentration in the samples was calculated as the sum of all five anthocyanidins analyzed. Anthocyanidin concentrations are presented as mean of two analytical replicates (mg • g^{-1} dry weight).

2.3 Experiments

2.3.1 Paper I

Initially in the study described in Paper I, the main objective was to evaluate the effects of different levels of added nitrogen in the form of NH_4NO_3 (0 = C (control), 12.5 kg • ha^{-1} • year^{-1} = treatment N1 and 50.0 kg • ha^{-1} • year^{-1} = treatment N2) on anthocyanidin concentration in bilberries. However, focus shifted during the course of the study to examining the effects of climate parameters on anthocyanidin concentration in fruits of *V. myrtillus*. Sampling was carried out during the years 2005–2007 and on three dates every year (in total nine sampling occasions), early, mid and late in the growing season. The study was conducted in a late successional Norway spruce (*Picea abies* L.) forest and the experimental design consisted of two blocks, with differing levels of water availability (as indicated by the ground vegetation). Each block contained three large randomly placed plots per treatment (C, N1 and N2) with two small fixed sampling spots (50 cm radii) within each plot rather than scattered random sampling. In total 228 samples were collected each containing a minimum of ten fully ripe bilberries.

A weather station within the experimental site automatically collected climate data – air temperature (°C), relative air humidity (%), precipitation (mm), and light radiation (MJ) – every 10 minutes at 1.7 meters above the ground.

2.3.2 Paper II

The purpose of the studies described in Paper II was to evaluate the effect of light and temperature separately on anthocyanidin concentration in bilberries. Plots (40×60 cm) including high densities of *V. myrtillus* shoots were excavated in 2006, from a late succession Norwegian spruce (*Picea*

abies) forest. In total, 20 plots including bilberry stems were collected from the Svartberget Research forest, within an area of 20×20 m. All *V. myrtillus* stems included in the study were regarded as originating from the same population. The twenty plots containing the excavated shoots were put in plastic trays and numbered randomly from one to ten in two series.

Two studies were performed, each conducted over a period of three months. The samples were also exposed to a total of four months cold storage before and between the two studies. In the first study, different light regimes were simulated (Northern and Southern) in two separate climate chambers each containing ten trays. The light regimes represented daily mean light distribution patterns for five years (2001-2005) during the period May to September for Northern (latitude 65°55´ N), NL, and Southern (latitude 55°72´ N), SL, Sweden (SMHI). Temperature settings were identical for both treatments (16°C). In the second study, two different temperature settings were used (Low = 11°C and High = 16°C), representing daily mean temperatures for Northern (latitude 65°55´ N) and Southern (latitude 55°72´ N) Sweden during the same period as in the first study. The light regime used in both temperature treatments was that of the Southern treatment used in the first study.

To avoid possible placement effects within the chambers, the trays in each chamber were moved once a week so that tray number one took the place of tray number two and so on. In all studies water was applied in abundance twice a week. Light (Par quantum sensor, Skye instruments) and temperature (Tiny talk, Intab AB) were recorded automatically every ten minutes. Honeybees were used as pollinators but complementary hand pollination using a brush was also conducted. Bilberries were sampled continuously as they matured.

2.3.3 Paper III

Paper III describes two studies that were performed to evaluate the effect of latitude-related parameters and genome on anthocyanidin concentrations in bilberries. In the first study bilberries were collected from four different locations during the summers of 2007 and 2008 (Gammel Rye: 56°50´ N in Denmark and Kungsör: 59°25´ N; Tönnebro: 61°40´ N and Tjourre: 65°45´ N in Sweden) and from Kvikkjokk, 66°57´ N in 2008 only (Figure 4). All sampled populations were healthy *V. myrtillus* stands, on visible inspection, growing either in the open or in semi-shaded conditions under

either mature spruce or pine stands. In each of the two years ten samples containing a minimum of ten mature bilberries, were collected from the selected sites, making a total of 90 samples.

In the second study bilberries were collected from the Oulu University test field in 2007 and 2008. The *V. myrtillus* plants in the test field were micropropagated from seeds gathered from plant material originating from a transect ranging from northern Germany to northern Finland (Figure 4). Six clones with parents from different geographical origins were sampled. Between five and ten replicates each containing a minimum of ten bilberries was obtained from each clone depending on the number of fully mature bilberries available (in total there were more than 100 samples).

2.3.4 Paper IV

In the study described in Paper IV, mature fruits of *V. myrtillus* were collected along three altitude gradients in northern Sweden, Akkanålke (65°32´ N; 18°59´ E), Galtispuoda (67°07´ N; 17°56´ E) and Kvikkjokk (66°57´ N; 17°48´ E) (Figure 4) with the objective of quantifying the anthocyanidin concentration in the fruits. During the years 2006–2008 Akkanålke was sampled at five elevations between 453 and 738 m.a.s.l. Two sampling altitudes were located above and three below the forest line. At each level below the forest line one open and one tree covered site was chosen for the collection of bilberries. At Galtispuoda and Kvikkjokk three different altitudes were sampled on each mountain in 2008, one open sampling site located above and two open sampling sites located below the forest line.

At each sampling site, five plots of one square meter were randomly distributed 5–15 m apart. At Akkanålke new sampling points were selected each year. Ten to twenty fully mature bilberries were collected from each plot forming one sample. In total 150 samples were collected.

3 Results

3.1 Paper I

In Paper I we reported that total anthocyanidin concentrations were significantly higher in 2007 than in the two previous years and significantly higher in 2005 than in 2006 on all sampling occasions. There were also significant differences between all sampling dates the different years. Total anthocyanidin concentration increased linearly with thermal sum in 2005 and 2007, but decreased between the second and third dates in 2006 which was very warm and sunny (Figure 5). For all studied years a combined regression analysis of total anthocyanidin concentration and accumulated thermal sum gave a good quadratic fit (R^2 = 0.889; P = 0.001) with a maximum concentration at a thermal sum around 1350°C (Figure 5).

There was no significant effect of N–fertilization or estimated water availability on the total anthocyanidin concentration in any of the years. The relative abundance of individual anthocyanidins remained stable over the studied years with delphinidin being the most abundant, accounting for 40–44% of the total content, followed by cyanidin (28–34%), while petunidin, peonidin and malvidin contents were 12% or lower.
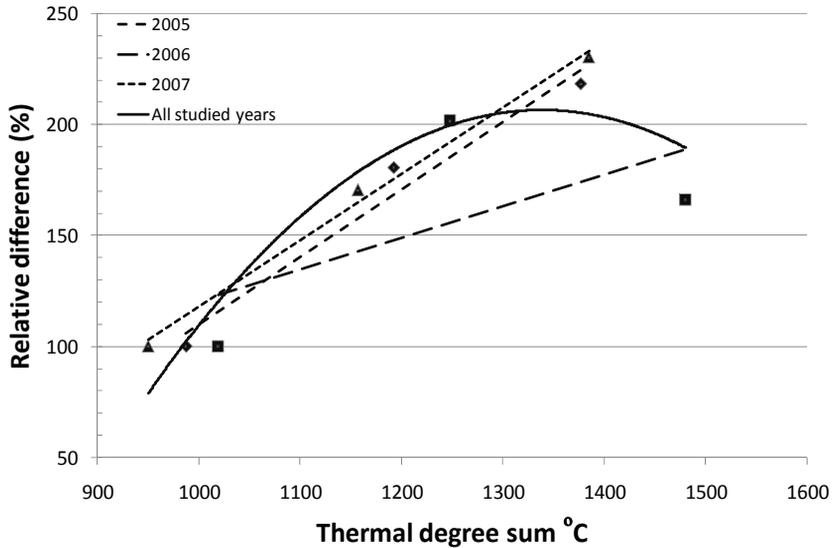
Figure 5. Linear regressions of the relative mean anthocyanidin concentrations on specific sampling dates (% difference from the first sampling date) against the thermal degree sum (calculated as the accumulated daily mean temperature above 5°C from 1st May) for 2005 (◆), 2006 (■), and 2007 (▲). The quadratic regression line is based on data from all studied years (—). Åkerström *et al.,* 2009.

3.2 Paper II

This work comprised of two studies. In the first total anthocyanidin concentrations were significantly higher under the simulated northern (NL=24 h) than under the southern (SL=20 h) light regime (P = 0.034). Daily mean temperatures were 16.3°C (stdev 0.3) in both treatments. Mean anthocyanidin concentrations were 24.10 and 21.73 mg $\bullet$ g^{-1} DW under the Northern and Southern light regimes, respectively. The relative contents of individual anthocyanidins were similar between light treatments and cyanidin was the only compound that had higher values in the southern compared to the northern light regime (Figure 6).

In the second study there was a significant difference between the two temperature treatments (Low = 11.3°C, stdev 0.5 and High = 16.0°C, stdev 0.3) with higher anthocyanidin values in the plants under the high temperature treatment (P = 0.015). Light settings used were the simulated southern Swedish light regime from the first study (SL, 20 h). Mean

anthocyanidin concentrations were 19.52 and 22.64 mg • g^{-1} DW in the plants exposed to the Low and High temperature treatments, respectively.

The relative contents of individual anthocyanidins in the fruits were similar between temperature treatments (Figure 6). The mean relative content of individual anthocyanidins, irrespective of treatment, was highest for delphinidin (49%), followed by cyanidin (26.5%). Petunidin, peonidin and malvidin had mean relative contents of 12.5%, 2.8% and 9.4%, respectively.
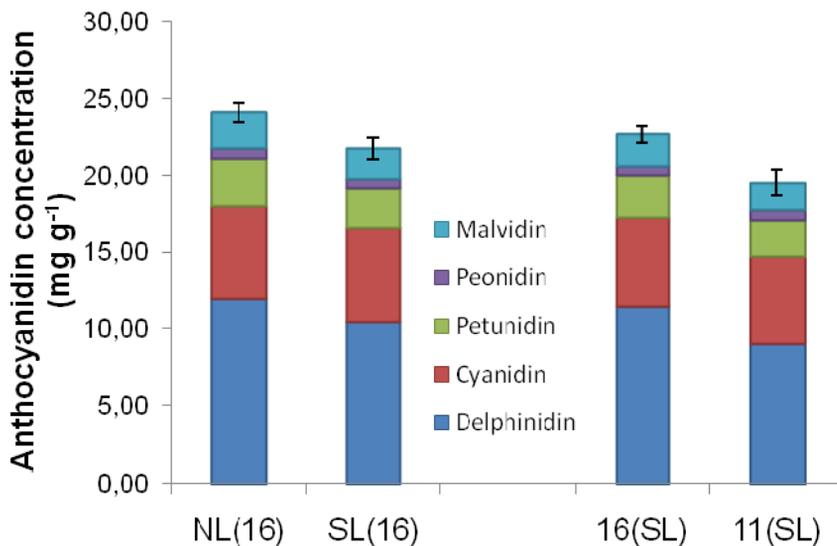

Figure 6. Mean concentrations of individual anthocyanidins and standard error of mean of total anthocyanidin concentration in study 1 (NL(16) and SL(16)) and study 2 (16(SL) and 11(SL)).

3.3 Paper III

In the study of the effect of latitude on anthocyanidins in fruits from wild populations, total anthocyanidin concentration was significantly affected (P < 0.001) and the anthocyanidin concentration was higher in 2008 than in 2007. Total anthocyanidin concentrations at all locations differed from the northern Swedish location (Tjourre, 66°10´ N), with higher values in the

north. Linear regression analysis of total anthocyanidin concentration versus latitude was significant ($P < 0.05$) for the years considered separately and for the two years combined. The R^2 values for 2008, 2007 and both years combined were 0.385, 0.443 and 0.278, respectively.

The mean relative content of individual anthocyanidins, irrespective of latitudinal location was 39%, 31%, 15%, 4% and 10% for delphinidin, cyanidin, petunidin, peonidin and malvidin, respectively.

In the study of how different genotypes affect anthocyanidins, the geographic origin of parent plants affected total anthocyanidin concentrations significantly ($P < 0.001$); concentrations increased with increasing latitude. In addition, values were higher in 2007 than in 2008. Linear regression analysis of total anthocyanidin concentration versus geographical origin for each year separately and both years combined indicated significant P values ($P < 0.05$). The R^2 values for 2008, 2007 and both years combined were 0.295, 0.490 and 0.387, respectively.

The mean relative content of individual anthocyanidins, irrespective of parental origin, was 38%, 35%, 14%, 4% and 9% for delphinidin, cyanidin, petunidin, peonidin and malvidin, respectively.

In both the Latitude and the Geographical origin studies differences could be seen at the level of the anthocyanidins delphinidin and cyanidin, where samples from more northern populations (Tönnebro & Tjuorre) and from clones with the most northern parental origin (Utsjoki) expressed very high relative amounts of delphinidin compared to cyanidin: 42% compared to 27–30%, respectively. However, in samples collected from either more southern populations (Gammel Rye) or from the clone originating from Parkano, cyanidin was expressed in higher amounts than delphinidin: 38–39% compared to 34%, respectively (figure 7).
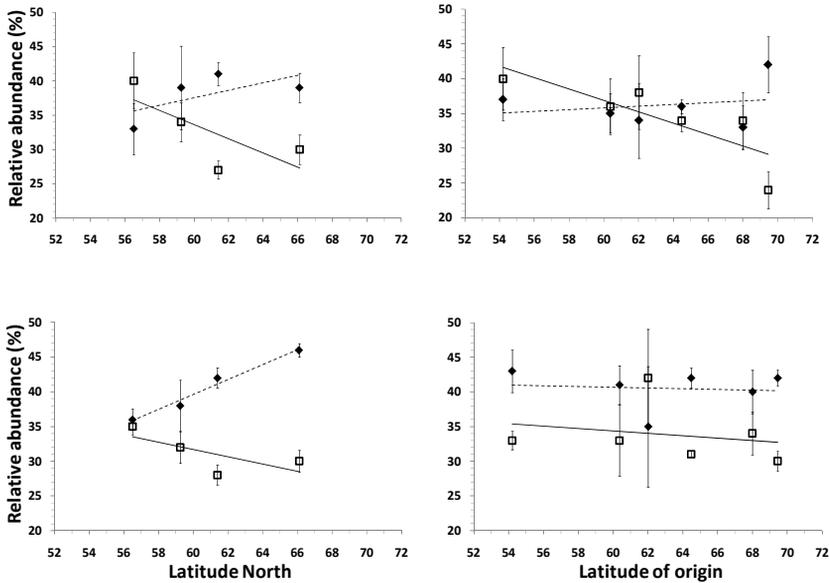
Figure 7. Relative abundance of delphinidin (--◆--) and cyanidin (-□-) as a percentage of total anthocyanidin. Data from the latitude study in 2007 (upper left) and 2008 (bottom left) and from the geographical origin study in 2007 (upper right) and 2008 (bottom right).

3.4 Paper IV

In the altitude study anthocyanidin concentration was highly variable, thus no general trends were found with respect to this variable. However, along the Kvikkjokk altitude gradient the concentrations of total anthocyanidin and all individual anthocyanidins were significantly ($P < 0.05$) affected by elevation: anthocyanidin concentration decreased with increasing height above sea level. In contrast, the concentration of total anthocyanidin along the Galtispuoda gradient demonstrated a positive relationship with altitude. The anthocyanin concentration in bilberries from the five open sites along the Akkanålke gradient fluctuated greatly between years, with the highest total anthocyanidn concentration in 2007; no clear relationship with elevation was found even though some individual sites differed significantly from each other.

The effects of light availability on fruit anthocyanidin concentration were most pronounced in 2006. In this very sunny year the shaded sites at Akkanålke supported plants with significantly higher concentrations of total

anthocyanidin (P = 0.023) and the individual anthocyanidins delphinidin, petunidin and malvidin (P < 0.003) compared with the shaded sites (Figure 8). In 2007 only peonidin was significantly (P = 0.018) affected by light availability with higher anthocyanidin concentrations in plants growing in open areas. In 2008, total and all anthocyanins were negatively affected by light availability but the differences were not statistically significant (Figure 8). Interestingly delphinidin was expressed at a higher level in plants in shaded sites than open sites during all years, but cyanidin was expressed at lower mean concentrations in shaded sites in two of the three studied years (2006 & 2007) (Figure 8).

Considering all the individual anthocyanidins irrespective of altitudinal gradient or year the relative abundance of delphinidin and cyanidin were greatest, amounting to 39% and 32%, respectively. Petunidin, peonidin and malvidin contents were 14%, 5% and 10%, respectively.
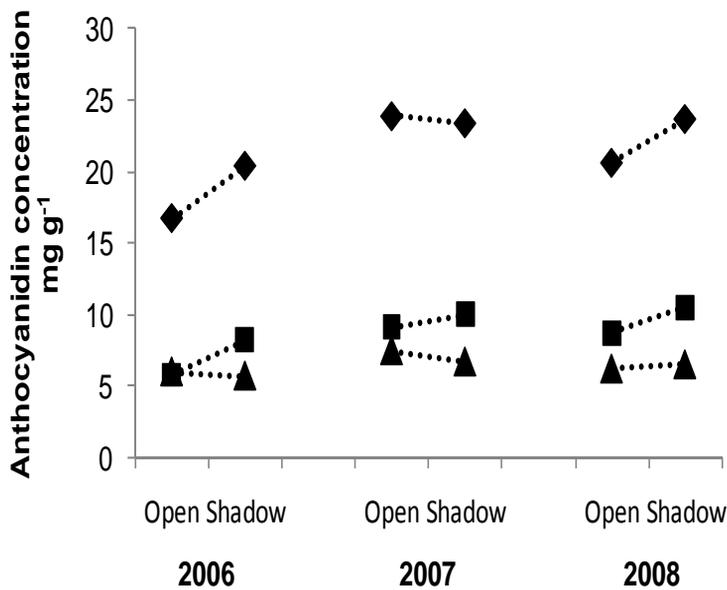

Figure 8. Total anthocyanidin (◆), delphinidin (■) and cyanidin (▲) concentrations (mg • g^{-1} dry weight) in *V. myrtillus* fruits growing in either direct sun light (Open) or under closed forest cover (Shadow) for all studied years (2006–2008).

4 Discussion

4.1 Biotic control of anthocyanidin formation

The specific anthocyanidin concentration within a bilberry is dependent on several factors. These factors can be either biotic or abiotic. During the work with this thesis it was discovered that anthocyanidin synthesis in bilberries is mainly under genetic control (Paper III), but that climatic factors, such as light and temperature may increase or diminish the amplitude of the variation between populations and between years (Papers I, II & IV).

The discovered strong genetic control of anthocyanidin formation in fruits of *V. myrtillus* (Paper III) is in accordance with results obtained for *V. myrtillus* by other researchers. For example, higher concentrations in northern compared to more southern populations (Paper III) have been found in studies of wild populations of *V. myrtillus* by Kähkönen *et al.* (2003) and Lätti *et al.* (2008). The genetic control is also supported by results obtained in a climate chamber study of *V. myrtillus* in which higher total phenol concentrations were obtained from clones with a more northern origin compared to those originating further south but grown under identical conditions (Martinussen et al., 2009).

The ratio of delphinidin to cyanidin differed between clones and populations (Paper III) and could be linked to changes in latitude or geographical origin. There were higher levels of delphinidin in northern populations or clones originating from the north. It has been suggested that UV-light promotes the production of anthocyanidins with a higher degree of hydroxylation (Chalker–Scott, 1999). However, we found no support for this in our data when comparing anthocyanidin levels with metrological data from the Swedish metrological institute (SMHI). Rather, we interpret this

shift as a long term adaptation to local climate that is now imbedded in the genes controlling biosynthesis of anthocyanidins in *V. myrtillus* fruits.

4.2   Abiotic control of anthocyanidin formation

The large variation between years (Papers I & III) and between replicated samples (Papers I, III & IV) indicates that the formation of anthocyanidins is also affected by external factors. This is further supported by the small between replicate variations obtained in controlled environments (Paper II), where variations in micro climate were limited.

4.2.1 Temperature

In Paper II higher concentrations of total anthocyanidins were found in fruits produced under high (16°C) compared to low (11°C) temperature conditions (20 h light conditions). In contrast, Martinussen *et al*. (2009) found higher concentrations of total phenolics in *V. myrtillus* fruits produced under low (12°C) compared to high (18°C) temperature conditions, irrespective of light regime (12 h or 24 h). Riger *et al*. (2008) on the other hand found decreasing anthocyanidin concentrations in bilberries with increasing altitude, suggesting a negative response to lower temperatures. However, other factors such as light input and CO_2 levels also change with increasing altitude thus the results obtained by Riger *et al*. (2008) may be an effect of the combination of changes occurring over an altitudinal gradient.

  Sampling time or timing of harvest is of prime importance if one wants to maximize the anthocyanidin content of bilberries. In Paper I we described a convex relationship between thermal sum (°C) and anthocyanidin concentration. The fact that anthocyanidin accumulates in *V. myrtillus* fruits as they mature has been previously reported by Prior *et al*. (1998) and Jaakola *et al*. (2002), but we also found that the concentrations start to decrease once the thermal degree sum from first of May reaches 1350°C (Paper I).

4.2.2 Light

It has been suggested that the specific light conditions of the more northern latitudes, with midnight sun and a large proportion of red light in the

spectrum promote high biosynthesis of anthocyanidins (Lätti *et al.,* 2008). The results described both in Paper II and by Martinussen *et al.* (2009) support this statement since there were higher concentrations of anthocyanidins (Paper II) and total phenolics (Martinussen *et al.* 2009) in fruits produced under long-day (24 h) compared to short-day (20 h; Paper II and 12 h; Martinussen *et al.,* 2009) conditions.

Furthermore, *Vaccinium* spp. reach their photosynthetic maxima at rather low light intensities (Väisinän, 1977). However, whether light intensity affects anthocyanidin concentrations is uncertain. In Paper II, anthocyanidin concentrations were not significantly affected by a decrease in total light input of 19% (1072 and 762 µmol m^{-2} • s^{-1} PAR). In Paper IV, a significant difference in anthocyanidin concentration between closed forest and open areas was found with higher concentrations in fruits produced under closed canopies in 2006. On the other hand, Jaakola *et al.* (2004) reported higher expression of anthocyanidin related genes in *V. myrtillus* leaves growing in upper, sun-exposed, compared to lower, shadowed, branches of the same bush. These seemingly contradictory results may be explained by the influence of temperature. In our study, Paper IV, the estimated degree sum at harvest in 2006 was very high and according the results obtained in Paper I this might lead to a degradation of anthocyanidins in the fruits. Most likely, temperatures were higher in the plants exposed to direct sun light thereby starting the degradation process earlier compared with shades ones. Other years with lower degree sums which had not passed the estimated threshold level there were no significant differences in anthocyanidin concentrations of samples from open or shaded sites.

4.2.3 Nitrogen

Fertilizer application was found to have no effect on the anthocyanidin concentrations in *V. myrtillus* fruits obtained from wild populations (Paper I). This is in contrast to other studies where concentration dependent effects on antioxidant activity have been found in for example felty germander, *Teucrium polium* (Azaizeh *et al.,* 2005). In addition, convex relationships with the highest anthocyanidin levels after moderate nitrogen additions have been recorded in fruits of grape, *Vitis vinifera* (Okamoto *et al.,* 2003). The different results may be due to the different species, and tissues studied, but may also indicate a chain of priority were the content of secondary metabolites is not always affected by increased nitrogen availability. In

bilberries anthocyanins may already be produced at sufficient levels in the plant, and given a constant climate, increased available nitrogen levels would not result in additional biosynthesis of anthocyanins, but would rather be incorporated into biomass or stored for next the year's growth. This suggestion is supported by Strengbom *et al.* (2004) who found that addition of N based fertilizers led to accumulation of nitrogen in plant tissues of *V. myrtillus*. Furthermore, for American lowbush blueberry (*V. angustifolia*) nitrogen additions over the growing season have been shown to increase the nutrient status of stem and leaves, fruit set and harvestable yield in fields where the initial nutrient status of the plant was sub–optimal (Percival *et al.,* 2003; Percival & Sanderson, 2004).

4.3 Future agricultural development

Currently the market demand for bilberries is higher than the amount that can be extracted from the forests, and the demand for pure anthocyanidin extracts is also increasing. A possible solution to the shortage of berries on the market is agricultural cultivation of *V. myrtillus*.

Today commercial berry-picking in Sweden falls under the Swedish legislation "Allemansrätten" (All man's right). The term "Allemansrätt" was coined during the 1940s, but the legislation dates back to medieval times and states that each individual has the right to herd animals and to pick berries, mushrooms and herbs in the forests for household consumption.

This legislation is probably one of the main factors explaining why, to date, commercial cultivation of *V. myrtillus* has not been developed. However, as the demand for more berries increases and as commercial berry-picking intensifies conflicts with domestic collectors will arise. There will most likely also be conflicts with other activities in the forest, for example game hunting. It is possible that changes to this fundamental right of the individual will become needed. Furthermore, the high cost of transports over long distances and the long distribution chain, with intermediates and contractors, also leads to the conclusion that farming of *V. myrtilus* is worthy of consideration.

In order to maximize the concentration of anthocyanidins in bilberries, careful selection of high potential plant material is of prime importance, as indicated by the results presented in this thesis and in earlier published work. Populations with a northern origin, such as those from Tjuorre and

Kivkkjokk in Sweden and Utsjoki in Finland, produce very high anthocyanidin concentrations in their fruits (Paper III). Higher anthocyanidin concentrations were also found in northern populations compared to more southern populations by Lätti *et al.* (2008) and Kähkönen *et al.* (2003). At the level of individual anthocyanidins careful selection is just as important. Differences in the relative concentrations of the two most abundant anthocyanidins in bilberries, delphinidin and cyanidin were found (Paper III), with higher relative amounts of delphinidin in more northern, compared to more southern populations; the opposite was the case for cyanidin, with higher relative amounts in the south. This shift has a genetic component but was found to be more pronounced in wild populations indicating a high degree of environmental influence.

Furthermore, during cultivation, light and temperature conditions need to be managed. The results from Paper II, as well as those presented by Jaakola *et al.* (2004) and Martinussen *et al.* (2009), all indicate that certain light conditions are beneficial for anthocyanidin formation. In contrast, in Paper IV we reported that anthocyanidin concentrations were lower in fruits produced by plants in direct sunlight compared to those under a forest canopy one of the years. This result was however; obtained in 2006 when the growing season was very sunny and warm and was probably reflecting effects of temperature (see above and below). Also, taken into account that *V. myrtillus* has a rather low light maximum for photosynthesis (Väisänen *et al.*, 1977) care should be taken so that extreme light is avoided.

The data available about the effects of different temperature levels on anthocyanidin concentrations in bilberry indicate that this relationship is not as straightforward as the effect of day length. In vegetative tissues, anthocyanidin biosynthesis is induced by low temperatures (Chalker-Scott, 1999). However, in reproductive tissues of *V. myrtillus* the results obtained during this research indicate a positive effect of higher daily mean temperatures (Paper II) on anthocyanidin concentrations. Fabric screening and windbreaks could be used to increase mean temperatures and prevent extreme temperatures. Both covers and windbreaks would probably also benefit plant primary production, flower survival and thus harvestable yield.

For bilberry cultivation water availability needs to be abundant and reliable relating to irrigation and a suitable growth substrate. Nitrogen fertilization has no confirmed effect on anthocyanidin concentration in bilberries (Paper I), but has resulted in positive effects on vegetative growth

and harvestable yields in American low bush blueberries (*V. angustifolia*) (Percival *et al.*, 2003).

An agroforestry production system with a combination of wood or canopy production and understory berry production seems to have potential for future *V. myrtillus* cultivation.

4.4   Conclusion

Year had an effect on the anthocyanidin concentration in all the studies were it was considered. In addition, differences in the effect of "year" were found between clones subjected to the same treatments thus indicating complex relationships.

The anthocyanidin concentrations in wild fruits of *V. myrtillus* are highly variable and are affected by both environmental factors, such as light and temperature, and by genotype.

The effect of latitude, with high levels of anthocyanidin in plants from the north compared to those from the south, is primarily an effect of differences between genotypes, although the specific light climate in northern latitudes promotes higher anthocyanidin concentrations compared to a more southern light climate.

The effect of temperature on anthocyanidin concentration in bilberries is less clear and may be dependent on co-variables such as light conditions. However bilberries, like many other small fruits, accumulate anthocyanin as they mature and this can be positively correlated with thermal sum up to a threshold after which anthocyanidin levels start to decrease. Thus, making harvest date very important for the anthocyanidin concentration of the end product.

4.5   Future prospects

Cultivation of *Vaccinuium myrtillus* is still not realised and more knowledge is needed before more generalized management plans can be compiled. However, it is important even at this stage to develop co-operations between scientists, farmers and industry to start co-funded small scale farming of bilberry with the aim of gathering knowledge from both a scientific and practical perspective.

In addition to small scale test farms, more large scale controlled studies are needed in order to evaluate the effect of year and environment on selected clones, and also to examine whether high anthocyanidin concentrations can be achieved at the same time as good harvestable yields. The prospect of cultivating extremely anthocyanidin rich bilberries is intriguing but it is not yet possible to determine the quantity of anthocyanidin that could be present in such a "super–berry". However, the fact that the highest overall mean value of anthocyanidin concentration obtained during this work, (Paper III: Tjuorre, 2007), was more than 10 fold that of the lowest, (Paper I: control treatment, first sampling 2006), might provide a hint about the potential.

5 Svensk sammanfattning

Vaccinium myrtillus (blåbär) är en liten dvärgbuske som växer vilt i norra Europa. Den har uppmärksammats mycket det senaste decenniet på grund av sitt ekonomiska värde som naturresurs och för de hälsosamma bären. Som ett första steg mot att odla blåbär vi har inom detta projekt samlat information om hur de hälsosamma substanserna i bären (antocyanidier) påverkas av temperatur–, ljus– och näringsförhållanden. Vi har också försökt att besvara frågan om i vilken grad bildandet av dessa ämnen i bäret styrs av växtens gener eller av klimatet där de växer.

Mängden antocyanidiner visade sig vara beroende av både temperatur och dagslängd, med högre halter vid högre temperatur (16°C jämfört med 11°C) och under långdags förhållande (24 timmar ljus) jämfört med kort dag (20 timmar ljus). Halterna ökade mot slutet av säsongen up till en gradsumma av 1350°C, varefter halterna minskade. Ett mycket soligt år var koncentrationen högre hos bär från plantor i skuggiga lägen jämfört med sådana från öppna solexponerade växtplatser.

Förutom känsligheten för variationer i klimat är produktionen av dessa ämnen under genetisk kontroll. Halterna av antocyanidiner visade sig vara högre i kloner som härstammade från nordliga populationer än i kloner med mer sydlig härstamning, trots att de fått växa på samma plats och under liknande förhållanden under mer än tio år. Denna egenskap kunde vi också se hos bär från vilda populationer på sin naturliga växtplats.

Resultaten i denna avhandling indikerar att man med rätt plantmaterial och genom att kontrollera odlingsmiljön kan öka halterna av dessa hälsosubstanser i blåbär dramatiskt i jämförelse med naturliga förhållanden samt minska variationen mellan år. Denna möjlighet i kombination med det starka intresset och den uppåtgående marknaden för blåbärsprodukter gör att framtiden för odling av blåbär ser ljus ut.

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