V. ashei, 13 % *V. constablei* and 2 % *V. angustifolium*. Breeders are finding that the use of *V. darrowii* has dramatic impacts on fruit quality, and it only takes two or three generations to restore winter hardiness (Hancock et al, 1995). The complex genetic background of modern breeding populations also makes testing of superior genotypes across broad climatic zones imperative to finding their optimal adaptive zone, particularly for selections of southern and intermediate highbush families. For example, a few years ago we split our breeding families between Oregon and Michigan, and evaluated them independently at each location (Finn *et al.*, 2003). We used a diverse array of families with varying amounts of southern species blood in their heritage. The elite families that emerged in Michigan where also elite in Oregon, but there were a number of elite families in Oregon that proved poorly adapted to the heat and cold in Michigan. Had we relied on Michigan screens, we would have disregarded some important families.

One other important change in highbush breeding is the move towards patenting and licensing blueberry varieties. Today, only the USDA breeding program does not license their varieties, and they are headed in this direction. This move has come primarily as a means to support further breeding work, as State resources dwindle. Licensing may save some public programs from extinction due to diminishing state support, but it will also restrict the availability of new varieties.

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FRUIT DEVELOPMENT IN *VACCINIUM* SPECIES
VACCINIUM SUGU AUGĻU ATTĪSTĪBA

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Abstract

Fruit development and ripening represent one of the most complex developmental processes in plants. Functionally, the role of fruits is to cover the developing seeds and promote the dispersal of mature seeds through the production of attractive colour, flavour and aroma compounds. After fertilisation, the first phases of fruit development include the division and the expansion of the cells. The ripening phase is initiated after the completed seed maturation. Tissue softening and accumulation of flavour compounds, aromatic volatiles and pigments occurs during the ripening phase. The quality of fruits is determined by the different developmental steps via the signalling cascade that is responsible for the metabolic and structural changes during the ripening phenomenon. Genus *Vaccinium* is widespread over the world and it includes many economically important cultivated and wild berry species. Fruits of the *Vaccinium* species are non-climacteric and anatomically they belong to false berries, many-seeded fleshy fruits in which the inferior ovary along with floral tube ripens into an edible pericarp. *Vaccinium* berries are especially rich with phenolic compounds that are known to possess antioxidative properties. This review focuses on characteristics of fruit development in both cultivated and the wild *Vaccinium* species.

Kopsavilkums
Augļa attīstība un nogatavošanās ir viens no sarežģītākajiem attīstības procesiem augos. Funkcionāli augļa uzdevums ir pasargāt jaunattīstītās sēklas un veicināt to nogatavošanos, reizē veicinot arī to iekrāsošanos, atbilstošai garšai un aromātam. Pēc apaugļošanās, pirmajās augļa attīstības fāzēs, tiek veicināta šūnu dalīšanās un augšana. Augļa nogatavošanās fāze sākas pēc tam, kad beidzas sēklu nobriešana. Augļa audi paliek mīkstāki un garšas un aromātu veidojošo savienojumu un pigmentu uzkrāšanās notiek nogatavošanās fāzes laikā. Augļa kvalitāti nosaka dažādi attīstībā izmantotie signāli, kas atbild par vielmaiņas un strukturālām pārmaiņām nogatavošanās laikā. *Vaccinium* ģints ir plaši izplatīta visā pasaulē un tajā iekļautas daudzas ekonomiski svarīgas kultivētas un savvaļas sugas. To augļi anatomiski pieder pie neīstām ogām – daudzsēklu mīkstajiem augļiem. Ogas ir īpaši bagātas ar fenola savienojumiem, kam, kā zināms, piemīt antioksidantu īpašības. Šajā rakstā dots augļa attīstības raksturojums gan kultivētām, gan savvaļas *Vaccinium* sugām.

Key words: berries, fruit development, *Vaccinium.*

Introduction

Fruit development and ripening, typically preceded by successful flower pollination, represent one of the most complex and important developmental processes in plants. Functionally, the role of fruits is to cover the developing seeds and promote the dispersal of mature seeds by frugivore animals through the production of attractive colour, flavour and aroma compounds in addition to nutritional value. After fertilisation, the first phases of fruit development include the division and the expansion of the cells. The ripening phase is initiated after seed maturation has been completed. Tissue softening and accumulation of flavour compounds, aromatic volatiles and pigments occurs during the ripening phase (Brady 1987, Giovannoni 2001, 2004).

Fruits can be classified into climacteric and non-climacteric fruits according to the differences in the respiration rate and the production of the plant hormone ethylene during the ripening phase. According to the structure of the pericarp, fruits are classified as non-dehiscent (fleshy) or dehiscent (dry) fruits. Most fruits develop from a gynoecium that contains one or more carpels. In pseudocarpic fruits, organs other than the gynoecium (eg. receptacle bracts, floral tube, or the enlarged axis of the inflorescence) participate in the formation of the fruit (Gillaspy *et al.* 1993, Giovannoni 2004). The genus *Vaccinium* is widespread over the world with about 450 species of evergreen and deciduous woody plants varying from dwarf shrubs to trees. Most *Vaccinium* species originate from the cooler areas of the Northern hemisphere, although tropical species also exists. Well-known members of the genus are cultivated northern or southern highbush blueberries (*V. corymbosum* hybrids), lowbush (*V. angustifolium*) or rabbiteye blueberries (*V. ashei Reade*) and cranberries (*V. macrocarpon*), in addition to commercially utilized wild bilberries (*V. myrtillus*) and lingonberries (*V. vitis-idaea*). Other better known wild species are European cranberries (*V. oxycoccos*, *V. microcarpum*), odon (*V. uliginosum*) and numerous other especially American wild *Vaccinium* species. The *Vaccinium* species require soil with low pH and they grow most abundantly in heaths, bogs and acidic woodlands.

Pollination. Most northern highbush blueberries are self-pollinated, meaning that the pollen of the same individual plant can lead in successful fruit development. However, for many cultivars cross pollination produces higher fruit set and larger fruit. Southern highbush blueberries are only partially self-pollinated (Krebs and Hancock 1990). Hokanson and Hancock (2000) tested self-fertility in controlled hand self- and cross-pollinations with individual *V. corymbosum*, *V. angustifolium* and *V. myrtilloides* plants and detected that all three species showed a significant reduction in self fruit set and in the proportion of fertilized ovules that developed into mature seed in self compared to outcross fruit.

Rabbiteye and lowbush blueberries are also largely self-infertile and therefore cross pollination is required for the good fruit set and berry size. Bumble bees and wild bees pollinate blueberry flowers naturally. Cranberries are self-pollinated but insect or wind disturbance is needed for the pollen release (Rieger). Most wild *Vaccinium* species are cross pollinated by insects. Self-pollination is possible in bilberry and lingonberry but it reduces seed production (Nuortila *et al.* 2002). Fruit development. The fruit of the *Vaccinium* species is fleshy, round or oval by shape, they contain several to many seeds and belong in the category of berries. More strictly, the *Vaccinium* fruits are classified as false or epigynous berries, in which the fruit is formed from an inferior ovary, but the floral tube ripens along with the ovary. In true berries the entire ovary wall ripens into a soft pericarp (Fahn 1990). The fruits can grow clustered like blueberries and lingonberries or singly in branch or leaf axis like bilberries or cranberries.

Most *Vaccinium* species growing in the Northern hemisphere flower from May to June and produce berries from July to October. The fruit development of blueberries takes 45 to 90 days, and varies a lot between different species, cultivars, and growth locations. The growth of most blueberries, especially later ripening cultivars, has been found to exhibit a double sigmoid pattern (Godoy *et al.* 2008). Cranberry fruits mature in 60-120 days after fertilization, depending on the cultivar and weather. Among the cultivated blueberries highbush blueberries reach maturity faster (typically 45-75 days) compared to lowbush or rabbiteye blueberries (Rieger).

The development of bilberries from flower to ripe fruit lasts usually 55 to 70 days, varying between different years. The different phases of bilberry fruit development and ripening are presented in figure 1. Typically, the beginning of fruit development involves divisions and expansions of the cells (stages 2 – 3). During the ripening (stages 4 – 6) the accumulation of anthocyanins, and other secondary metabolites and sugars as well as the softening of the cell walls occur. Compared to the bilberry, lingonberry flowers and fruits ripen later during the season, approximately 78 – 84 days after full blossom (Gustavsson 2001).

Phenolic compounds. The berries of the *Vaccinium* species are known especially for their high content of phenolic compounds that are strong antioxidants and possess health beneficial properties. *Vaccinium* berries are among the best sources of anthocyanins and proanthocyanidins (Ovaskainen *et al.* 2008). Therefore, the accumulation of phenolic compounds has been one of the focus areas in the study on the fruit development of the *Vaccinium* species. The composition of flavonoids and other phenolic compounds in fruit development and ripening has been analysed in bilberries, cranberries, and blueberries (Jaakola *et al.* 2002, Vvedenskaya and Vorsa 2004, Castrejon *et al.* 2008, Celik *et al.* 2008). The overall profile of phenolic compounds at their different stages of development shares similarities between the examined *Vaccinium* species.

Figure 1. Fruit development and the ripening of bilberry from pollination to ripe fruit.

At the beginning of fruit development proanthocyanidins and flavonols are the main flavonols in *Vaccinium* fruits. At the onset of the ripening, the content of proanthocyanidins decreases at the same time anthocyanins begin to accumulate. A high content of proanthocyanidins at the early phases of fruit development has also been detected from fruits other than the *Vaccinium* species. Proanthocyanidins have been suggested to provide protection against fungal pathogens and the predation of unripe fruits (Harborne 1997).

The levels of flavonols are more constant over the period of fruit development, although especially qurcetin glycosides are found to be in slightly higher level at the beginning of the fruit development. In addition to quercetin glycosides, many *Vaccinium* berries contain myricetin glycosides (Määttä-Riihinen *et al.* 2004). In the bilberry, myricetin glycosides were found to accumulate during the ripening phase, along with anthocyanins (Jaakola *et al.* 2002). Of the phenolic compounds, in addition to flavonoids *Vaccinium* berries contain hydroxycinnamic acids. Blueberries contain high levels of caffeic acids (Määttä-Riihinen *et al.* 2004). The antioxidant activity during fruit development has been shown to be associated with the total phenolic content at the different developmental stages (Castrejon *et al.* 2008, Celic *et al.* 2008).

Other nutraceuticals. There is a lot of information on the nutritional value of *Vaccinium* berries, but it is mainly focused on ripe fruits. Only a limited number of studies report the contents of the nutritional compounds during fruit development. Cano-Merdano and Darnell (1997) analysed sugar accumulation during the development of rabbiteye blueberries. Glucose and fructose are the main sugars in *Vaccinium* fruits, whereas the contents of sucrose are low (Cano-Merdano and Darnell 1997, Viljakainen *et al.* 2002). According to studies of rabbiteye blueberries, the levels of sugars were at their lowest about 20 days after bloom, after which the contents increased up to ten fold. The maximum levels were reached between 60 and 90 days after bloom, reaching the maximum in the ripe fruits.

Celic *et al.* (2008) analysed the content of organic acids at four different maturity stages of cranberries. The most abundant organic acid in cranberries was citric acid (73 %) followed by malic and ascorbic acid. The overall concentration of citric and malic acid increased over the ripening, whereas the ascorbic acid concentration decreased. Regulation of fruit development. Recent discoveries have begun to reveal the developmental cues that are responsible for priming and initiating the ripening in fruit bearing species (Giovannoni 2004, 2007). The tomato (*Solanum lycopersicum*) has been the model system of choice due to its genetic resources and the knowledge that researchers have of its ripening physiology and biochemistry of ripening (Seymour *et al.*, 2008). However, key information has also been obtained from the model plant *Arabidopsis*, and to a lesser extent from strawberries (*Fragaria* spp).

The current state of the art is that a number of ripening related genes are known from these species including the *ripening inhibitor* (*rin*) and the *Colourless non-ripening* (*Cnr*) (Giovannoni, 2007). The gene at the tomato *ripening inhibitor* (*rin*) locus is a member of the MADS-box *SEPALLATA* (*SEP*) sub-family; *LeMADS-RIN* (Vrebalov *et al.*, 2002). MADS-box genes were previously associated with floral development, but *LeMADS-RIN* is necessary for ripening. The gene at the *Cnr* locus encodes an SBP-box transcription factor (Manning *et al.*, 2006), that is likely to interact with the promoters of the *SQUAMOSA* (*SQUA*) sub-family of MADS-box genes (Lännenpää *et al.*, 2004). These include *TDR4,* which shows enhanced expression during tomato fruit ripening (Eriksson *et al.*, 2004), but has yet to be assigned a function. This regulatory network appears to be conserved across fruit bearing species.

Nonetheless, links between the regulatory factors and the down stream effectors are poorly understood and thus far the studies are limited almost exclusively to the tomato, despite the diverse ripening behaviour of other important fruiting species.

Studies clarifying the molecular basis of fruit development in the *Vaccinium* species have, until recently, been limited and nothing is known about the genes controlling the fruit ripening in the *Vaccinium* species. Some structural genes encoding the key enzymes of the flavonoid biosynthetic pathway have been characterised from cranberry (Polaschock *et al.*, 2002) and bilberry fruits (Jaakola *et al.*, 2002). Just recently, two *SQUA* sub-family of MADS-box genes have been cloned from the bilberry (Jaakola *et al*., unpublished). The other of the two cloned transcription factor genes was related by sequence homology with tomato *TDR4* and Arabidopsis *FRUITFULL* (*FUL*) genes. The functional analyses revealed a hitherto unsuspected link between the SQUA MADS-box gene and the production of secondary metabolites.

Conclusions

The genus *Vaccinium* includes several economically important berry species. Therefore, the study on the quality characteristics over the development and ripening of the fruits can open new commercially exploitable applications in the future. Understanding better the gene x environment interaction during the ripening process is an important part of fruit quality research. Fruit development in various *Vaccinium* species shares several similarities, despite the different growth habits of the plants (e.g. highbush blueberry vs. cranberry). Even though there are differences in the phenolic profiles between the *Vaccinium* species, the trend in the accumulation of the compound groups is the same.

The development of new analytical methods has allowed for the accumulation of the new information in the entire area of plant science. However, most information to date has been gathered from the model species and the most economically important crop species. Along with new efficient sequencing technologies, we will soon have vastly more gene level information available for studying the gene x environment interaction in the various *Vaccinium* berries.

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NORDIC BILBERRY PROJECT
ZIEMEĻVALSTU MELLEŅU PROJEKTS

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Abstract

Wild berries are a characteristic part of Northern nature and a particular speciality of Nordic countries. Wild berries are also a rich and valuable resource that has not yet been exploited in a satisfactory level. Approximately 90 – 95 % of the whole wild berry crop yield is left unpicked in the Nordic forests every year. The challenges of wild berry utilization are similar in Nordic countries - the logistics of berry picking including traceability, fragmented sector structure as well as the high share of unprocessed raw material in export. The Nordic project focusing on bilberry (*Vaccinium myrtillus*) "Bilberry: Towards functional food markets" (2007 – 2009) is a part of the New Nordic Food programme funded by the Nordic Innovation Centre. The programme aims to enhance cooperation and innovation among companies that utilize the natural resources of the Nordic countries. The aim of the project is to improve wild berry production and utilization in the Nordic and global market. To achieve this goal a network between the Nordic experts presenting the different fields of the wild berry sector has been established. The project has focused on marketing research, quality issues, biodiversity and the traceability of wild berries; especially the bilberry. The results of the marketing survey were published in November 2008. The aim of the survey was to generate an overall picture of the companies working with wild berries in Nordic countries and to gather information on the existence and willingness of the berry companies to cooperate in wild berry supply, logistics, marketing and research and development. According to the results, a general agreement for the need of increased cooperation at the Nordic level was highlighted.