





## MARGE STARAST

Influence of cultivation techniques  
on productivity and fruit quality  
of some *Vaccinium* and *Rubus* taxa



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## LIST OF SYMBOLS

C	– carbon
Ca	– calcium
ca	– circa
Cl	– chlorine
cm	– centimeter
Cu	– copper
DM	– dry matter
dm <sup>-3</sup>	– per cubic decimetre
DW	– dry weight
g	– gram
g <sup>-1</sup>	– per one gram
h	– hour
H	– hydrogen
ha	– hectare
ha <sup>-1</sup>	– per one hectare
I.E.	– international equivalents
K	– potassium
kcal	– kilocalorie
kg	– kilogram
LSD	– least significant difference (statistic)
m	– meter
M	– mole
Mg	– magnesia
mg	– milligram
min <sup>-1</sup>	– per one minute
ml	– milliliter
mm	– millimeter
N	– nitrogen
n	– term, data
nm	– nanometer
O	– oxygen
°C	– degree Celsius
°E	– eastern longitude
°N	– northern latitude
P	– phosphor
p	– probability (statistic)
plant <sup>-1</sup>	– per one plant
R	– correlation coefficient
rpm	– revolutions per minute

S – sulphur  
SSC – soluble solids content  
t – ton  
TA – titratable acidity  
v/v – volume per volume  
w/v – weight per volume

## I. LIST OF ORIGINAL PUBLICATIONS

- I. **Starast, M.**, Karp, K., Paal, T. 2002. The effect of using different mulches and growth substrates on half-highbush blueberry (*Vaccinium corymbosum* x *V. angustifolium*) cultivars ‘Northblue’ and ‘Northcountry’. *Acta Horticulturae* 574: 281–286.
- II. **Starast, M.**, Karp, K., Vool, E., Moor, U. 2005. The cultivation of half-highbush blueberry under organic farming condition. *Egyptian Journal of Agricultural Research* 83(1): 155–168.
- III. Karp, K., Noormets, M., **Starast, M.**, Paal, T. 2006. The influence of mulching on nutrition and yield of ‘Northblue’ blueberry. *Acta Horticulturae* 715: 301–305.
- IV. **Starast, M.**, Kõljalg, U., Karp, K., Vool, E., Noormets, M., Paal, T. 2006. Mycorrhizal colonization of half-high blueberry cultivars influenced by cultural practices. *Acta Horticulturae* 715: 449–454.
- V. **Starast, M.**, Karp, K., Vool, E., Paal, T., Albert, T. 2007. Effect of NPK fertilization and elemental sulphur on growth and yield of lowbush blueberry. *Agricultural and Food Science* 16 (1): 34–45
- VI. **Starast, M.**, Karp, K., Vool, E., Moor, U., Tõnutare, T., Paal, T. 2007. Chemical composition and quality of cultivated and natural blueberry fruit in Estonia. *Vegetable Crops Research Bulletin* 66: 143–153.
- VII. Vool, E., Karp, K. Moor, U., **Starast, M.** 2007. Yield quality in some taxa of the genus *Rubus* depending on the cultivation technology. *European Journal of Horticultural Science* 72(1): 32–38.

**Table 1.** Author’s contribution to each paper (%)

	I	II	III	IV	V	VI	VII
Idea and design	60	80	30	90	70	100	20
Sampling	100	90	80	100	60	90	10
Chemical analyses	90	–	80	–	80	60	10
Data analysis, statistics	100	100	30	100	60	100	0
Writing	90	90	40	90	80	90	10

## 2. INTRODUCTION

### 2.1. Cultivated *Vaccinium* and *Rubus* taxa

In commercial agriculture the major blueberry-producing species are lowbush, highbush and rabbiteye blueberries. These species are long-lived woody perennials predominantly out-crossing, native to North America. Rabbiteye blueberry (*Vaccinium ashei* Reade) has a lower chilling requirement (generally less than 300 hours of chilling), low cold resistance and thereby cultivars of this species are suitable for the cultivation in southern regions. Highbush blueberry (*Vaccinium corymbosum* L.) is a native to North America, upright, 2 m tall, crown-forming shrub. Fruit ranging 3–20 mm are blue-black berries with many seeds. Many of cultivars have been developed, primarily for commercially valuable fruit characteristics and seasonality. Lowbush blueberry (*Vaccinium angustifolium* Ait.) is native to eastern and central Canada and the northeastern United States. It is a low spreading deciduous shrub growing up to 60 cm tall, though usually 35 cm tall or less. Lowbush blueberry canopy development and expansion occur by rhizomes. The fruit, which is quite variable in color and size, may be dull or glossy black, although with a good bloom turn blue. The berries are ca 2–12 mm in diameter. In the U.S. and Canada where lowbush blueberry cultivation is primary, there is big difference between highbush and lowbush blueberry production systems. Lowbush blueberry growers manage the stands of wild plants for fruit production, not improved plant varieties like highbush growers. (Vander Kloet 1988; Bruederle *et al.* 1991; Trehane 2004)

Interspecific hybridization has been an important aspect in the development of the commercial cultivars and many combinations of species have been crossed (Lyrene & Balhgtton 1986). The lowbush habitat is considered dominant when hybrids between highbush and lowbush genotypes occur (Finn & Luby 1986). This hybrid is named half-highbush blueberry and it is particularly cold hardy, having inherited the ability to survive the harsh winters of northern areas (Luby *et al.* 1989). They are most suitable for small-scale commercial cultivation in cold areas. One of their advantages is that they have inherited medium-sized, more easily picked fruit from a highbush blueberry while retaining the ability to rest undamaged under thick blankets of snow, which comes from their lowbush parentage. Blackberries and raspberries are a diverse group of species and hybrids in the genus *Rubus* L. (Parksepp 1977; Jennings 1988). They are members of the *Rosaceae* Juss. family. *Rubus* is one of the most diverse genera of flowering plants in the world, consisting of 12 subgenera, some with hundreds of species. Blackberry is the most taxonomically complex of any *Rubus* spp. *R. ursinus* Cham. & Schlect. is native to the Pacific Northwest, and has been useful in producing commercial cultivars grown in that region. In Europe, there are some species in the *Moriferi* section that have been used to produce cultivars of local importance; they are referred to as the aggregate species *R. fruticosus* L. agg. Blackberry cultivars are classified as

thorny or thornless. Red raspberry (*R. idaeus* L.) is mainly cultivated *Rubus* spp. The European subspecies of this group is designated *R. idaeus* subsp. *vulgatus* Arrhen., whereas the North American red raspberry is termed *R. idaeus* subsp. *strigosus* Michx., or more simply *R. idaeus* (European) and *R. strigosus* (North American). Raspberry cultivar ‘Tomo’ has been bred in Estonia and has good winter-hardiness and disease resistance. It is the most widely cultivated raspberry in Estonia (Kikas *et al.* 2002). Black raspberry (*R. occidentalis* L.) is fairly straight-forward, being a good species of its own. Its range overlaps that of *R. strigosus*, but extends further to the south (Parksepp 1977; Jennings 1988). Black raspberry cultivar ‘Jewel’ is cultivated in Estonia also (Starast *et al.* 2004). Some of the most important commercially grown brambles are actually blackberry and red raspberry hybrids. Examples include cultivars ‘Boysenberry’, ‘Loganberry’, and ‘Youngberry’ (Jennings 1988).

## **2.2. Cultivation of *Vaccinium* and *Rubus taxa* in the world**

Blueberry cultivation originated in North America when Native Americans burned wild stands of native lowbush blueberry in eastern North America to increase production (Strik 2005). The cultivation of highbush blueberry started in the late 1800s and early 1900s. Cultivated blueberries were introduced to other regions of the world from North America in the 1900s: 1923 to the Netherlands, 1924 to Germany, 1949 to New Zealand, late 1940s to Poland, 1951 to Japan, 1959 to the United Kingdom, early 1980s to Chile, late 1980s to early 1990s to southwestern Europe, and 1990s to China. Nowadays blueberries have become a major crop worldwide. Strong markets for processed and fresh fruit have resulted in good returns for growers and an increase in planted area. New cultivars, better adapted to “nontraditional” growing areas, have expanded production worldwide. In many regions, harvest of native blueberry species from wild has also increased.

Lowbush blueberry is cultivated only in North America where it is native and the plants are managed as a wild crop. In 2003 there were 26,484 ha of lowbush in the United States and 43,465 ha in Canada (Strik 2005). Whereby, only 0.02% of lowbush blueberries are produced organically. Average yields were 3 to 11 t ha<sup>-1</sup>. Total lowbush blueberry production in North America in 2003 was 91,400 t with approximately 99% sold for processing.

North America accounted for about 75% of the planted area and 82% of total highbush blueberry production in the world in 2003 (Strik 2005). Typical yields for well-managed, mature highbush blueberry plantations were 7 to 20 t ha<sup>-1</sup>. Total highbush blueberry production in North America in 2003 was 104,690 t with approximately 60% sold for fresh market. There is tremendous interest in blueberries in Asia especially in China where plantings are expected to increase steadily (Yu *et al.* 2006). South America accounted for about 11% and Europe

about 10% of the world total cultivated area (Strik 2005). There were an estimated 1,100 ha in Poland, 1,350 ha in Germany, 410 ha in France, 300 ha in the Netherlands, 250 ha in Spain and Portugal, 65 ha in Italy and 15 ha in the United Kingdom.

In North European countries such as Norway, Sweden, and Finland, the cultivation of blueberries is also being considered. The climate conditions of North Europe are suitable for the lowbush and half-highbush blueberry cultivation (Bläsing 1989; Haffner & Vestrheim 1994; Hiirsalmi 1989; Paasisalo *et al.* 1994). In Finland there were 109 blueberry growers (total area 30 ha) in 2000 (Manssila 2001).

In Estonia lingonberry (*Vaccinium vitis-idaea* L.), cranberry (*Oxycoccus palustris* Pers.) and bilberry (*Vaccinium myrtillus* L.) are well known like wild berries. The species of blueberry from North America have not been cultivated on production plantation before nineties. Kask and Piir (1980) have recognized that some cultivars of blueberry should be regarded as perspective berry crops in Estonia. At the Department of Horticulture, Estonian Agricultural University (now Estonian University of Life Sciences), a half-highbush blueberry cultivation project was started in 1997. In recent years there has been an increasing interest in setting up blueberry plantations. As a result of changes in agricultural policies, there is a large amount of arable land left out of crop production, which might be suitable for blueberry cultivation. In 2005 23 blueberry farmers (with the total area of 70 ha) got the subvention from The Estonian Agricultural Registers and Information Board (ARIB) (inquire from ARIB), but in reality the total area of cultivated blueberries extend more than 100 ha nowadays.

Cultivation of red raspberry is an important high-value horticultural industry in many European countries because it provides employment in agriculture, and indirectly in food processing and confectionary (FAOSTAT 2005). Most raspberry production is concentrated in the northern and central European countries, although there is an increasing interest in growing cane fruits in southern Europe, e.g. in Greece, Italy, Portugal and Spain. The major raspberry production regions are Russia, Poland, Hungary, Serbia, Germany and also the Pacific Coast of North America. Blackberry, which is mostly cultivated in America, has not gained popularity in Europe because of its poor winter-hardiness and difficulties in fruit handling due to thorns on the stems (Parksepp 1977; FAOSTAT 2005). Raspberry, on the other hand, has longer cultivation history in Nordic countries, including Estonia. However, blackberry is not completely a new cultivated plant in Estonia. During 1954–1959 yield facts were collected and biochemical components of fruits were observed (Parksepp 1977). Blackberry cultivar ‘Agawam’ was investigated as a new species in the Polli Horticultural Institute in Estonia in 1950s. It was named as a very productive cultivar in Estonian conditions and was in a list of perspective cultivars in 1954–1962.

### **2.3. Soil acidity specification for blueberry cultivation**

A blueberry plant needs acidic, well-aerated, sandy-textured soil high in organic matter for growth (Korcak 1986). The plants grow in full sun to partial shade, but those in open sites produce more flowers (Vander Kloet 1988). Lowbush blueberries are calcifuges and typically found on podzolic soils that are considered infertile to other crops (Sanderson *et al.* 1996) with soil pH in the vicinity of 4.0 to 5.5 (Hall *et al.* 1964). As a result, wild blueberry areas usually exhibit characteristics more similar to forest systems than tilled agricultural systems (Eaton 1988).

Experiments have also shown that blueberry cultivars differ in tolerance to alkaline growing conditions (Austin & Bondari 1993; Finn *et al.* 1993). In the case of too acidic soil (pH<3.5) plant growth and yield have been reduced greatly (Spiers 1984). A major factor limiting blueberries to acid soils is considered to be their inability to efficiently utilize iron at high soil pH (Brown & Draper 1980). However, according to Haynes and Swift (1986) the deficiency of magnesium rather than iron appeared to limit the growth in soils with high soil pH.

There are several possibilities to lower soil pH. In experiments soil acidity was significantly increased by tannic acid, ferrous sulfate (Gough 1994), or aluminum sulfate (Peterson *et al.* 1987), acidic irrigation water (Szpunar 1985) and gypsum (Sanderson *et al.* 1996). Soil pH has been reduced by using combined primary and secondary papermill sludge, which is a good potential source of carbon and other nutrients to restore a low level of organic matter in sandy soils supporting native lowbush blueberry (Gagnon *et al.* 2003). In the U.S. and Canada sulphur is widely used to decrease soil pH (Gough 1994). Sulphur applications should be made at least some months prior to planting, because it takes time for sulphur to reduce pH. Elemental sulphur applied once (1120 kg ha<sup>-1</sup>) reduced the pH value from 6.1 to 5.4 after two years (Spiers & Braswell 1992). Haynes and Swift (1986) concluded that the addition of organic matter and an acidifying agent (sulphur) to the planting hole could greatly increase the early growth and yields of blueberries in mineral soil. Cranberry grew the best in the medium treated with 1.5 t of sulfur per ha (Szpunar 1985).

### **2.4. Nutrition requirements of blueberries**

The use of some fertilizers, including urea and ammonium sulphate will cause the pH of soils to become more acid also (Gough 1994). Ammonium is a better source of nitrogen for a blueberry plant than nitrate (Townsend 1967). Nitrate can increase the concentration of aluminum in the roots to toxic level, while ammonium nitrogen will reverse the trend (Peterson *et al.* 1987). There is also some evidence which indicate that the nitrate inhibits growth of mycorrhizae, while the ammonium stimulates it (Korcak 1988). However fertilization

requirements of blueberries are relatively smaller than other berry crops, but results from the investigation indicate that multiple fertilizer applications can improve the nutrient status, growth, development and the yield of blueberries, and may be worth including it in commercial cultural management practices (Percival *et al.* 2003). In experiments carried out in Poland, the most severe frost damage of the bushes were observed on plots fertilized with 150 kg N ha<sup>-1</sup> and the results obtained indicate that the optimal nitrogen dose for highbush blueberry is about 50–100 kg ha<sup>-1</sup> (Smolarz & Mercik 1989). Kozinski (2006) has found that nitrogen fertilization rates higher than 60 kg N ha<sup>-1</sup> decreased the yield. The results from the lowbush blueberry experiment showed that nitrogen formulation can influence the yield potential of lowbush blueberries, and must be considered in fertility management practices (Percival & Privé 2002). To determine fertilizer application rates it is very important to consider specific soil conditions of the arable land (Hanson 2006). Highbush blueberries are grown in many non-native regions, and often on soils that have been modified by acidification or organic matter additions. These varied soil conditions can potentially affect N dynamics and uptake by blueberries, and may partly explain why N application rates and timings vary in different regions. The efficient use of N is an important goal that can reduce production costs and the potential for adverse environmental impacts.

## 2.5. Mycorrhizal status of blueberries

Nutrient uptake of blueberry plants is significantly influenced by the fact that they live in symbiosis with mycorrhizal fungi (Read & Stribley 1973). The roots of *Ericaceae* are fine, threadlike and devoid of hairs (Vander Kloet 1988). Thereby ericoid mycorrhiza is an essential partner to involve in nutrient uptake, especially of phosphorus (Pearson & Read 1973; Maronek *et al.* 1981) and nitrogen (Stribley & Reade 1974; Stribley & Read 1976). Effective uptake of nitrogen by ericoid mycorrhiza fungi from different sources in peat-based soils was described – both free ammonia and nitrate fixing pathways are more efficient for fungi compared to the plants (Pearson & Read, 1975), especially under low pH (Leake & Read 1990).

Some of the ericoid mycorrhizal host plants (*Calluna* and *Vaccinium*) were reported for high tolerance to different environmental stresses like heavy metal toxicity (Bradley *et al.* 1982; Yang & Goulart 1997).

Forming a type of endomycorrhizae, the fungi of ericoid mycorrhiza fill young cortical cells of the plant root with branched and coiled hyphae (Read 1983). The unsubserved epidermal cells in the distal parts of these roots are occupied by a fungal symbiont, which can constitute up to 80% of the root biomass in this region. Largent *et al.* (1980) have found that 61% of *Vaccinium* plants examined had a mycorrhizal system present in the root tips. The fungi produce extracellular enzymes that break down organic matter, enabling the

plant to assimilate nutrients mineralized from organic compounds present in the colloidal material surrounding roots (Perotto *et al.* 1995).

It is verified that fungi from very different taxa can form ericoid mycorrhiza. Couture *et al.* (1983) confirmed the ability of *Oidiodendron* spp. to form ericoid mycorrhizas with aseptically grown *Vaccinium* species. Only one other endophyte of ericoid mycorrhiza, *Hymenoscyphus ericae* (Read) Korf & Kernan (Kernan & Finocchio 1983) = *Pezizella ericae* (Read 1974) has been identified (Pearson & Read 1973). In addition to these records of fungi isolated from typical ericoid mycorrhiza, there are a few reports in the literature of colonization of ericaceous roots by fungal taxa which also form arbuscular- (Koske *et al.* 1990; Dighton & Coleman 1991) and ectomycorrhiza (Largent *et al.* 1980; Dighton & Coleman 1991).

The establishment of blueberry culture has been enhanced by the inoculation of plants with ericoid mycorrhizal fungi apparently rare in native soils (Powell & Bates 1981). Inoculation has been demonstrated to increase growth of micropropagated plants (Jansa1 & Vosátka 2000) and blueberry cuttings in the nursery (Powell & Bagyaraj 1984; Scagel 2005).

Ericoid mycorrhizal fungi are characterized by very slow growth (Duddridge & Read 1982). This study shows a possible role of other microorganisms, which may slow down the development of the fungus to a certain extent. Under field conditions the colonization of blueberry varies significantly with a cultivar (Eynard & Czesnik 1989; Czesnik & Eynard 1990) and the rate of fertilizer application (Powell 1982). Generally mycorrhiza activity is decreased with increasing levels of fertilizers (Powell 1982). Blueberries grown in soils with high organic matter and low pH usually have higher mycorrhizal colonization (Haynes & Swift 1985; Blasing 1989; Eynard & Czesnik 1989; Czesnik & Eynard, 1990; Yang *et al.* 1998; Li *et al.* 2006b).

## **2.6. Mulches and soil amendment techniques in cultivation of *Vaccinium* and *Rubus* taxa**

Weed control is one of the problems in blueberry production (Jensen & Kimball 1985). Hexazinone was approved for weed control in blueberry plantation, but the use of the herbicides introduced some problems, including shifts in weed species, the development of resistance, soil degradation on vegetation-free soils (Jensen & Yarborough 2004), and large damages of young seed-grown lowbush blueberry plants (Starast *et al.* 2005b). Mulch application as weed controller can minimize problems associated with herbicides (Jensen & Yarborough 2004) and it is a practice often used by organic growers to keep down weeds (Jordan 2004). Therefore mulching may offer a particularly effective management alternative to reduce both costs and environmental impacts. The potential of mulches to improve soil structure, to increase organic matter, and to establish patterns of nutrient cycling more similar to natural ecosystems has also been

recognized (Tukey & Schoff 1963; Roe 1998). Mulching increases the availability of soil nutrient resources such as nitrogen (Clarkson 1960), soil moisture conservation, soil temperature, and protects soil from rapid fluctuations of temperature (Makus *et al.* 1995; Robinson 1988; Borland 1988).

Mulches are considered essential for blueberry production on mineral soils and numerous studies have been undertaken to evaluate the effect of mulches. On rabbiteye blueberries grown on a fine sandy loam, mulching increased plant height, and shoot and root weights (Patten *et al.* 1988). Beneficial effect of mulching on the raspberry and blackberry plant root system has been noticed (Percival *et al.* 1998). The raspberry root system is located uppermost soil layer, similarly of blueberry plants. Therefore mulching can be used in cultivation of both cultured plants. The positive effect of mulches is noted on highbush blueberries (Haynes & Swift 1986; Kozinski 2006; Wu *et al.* 2006) and low-bush blueberries (Sanderson & Cutcliffe 1991). Skirvin and Otterbacher (1999) have found that sawdust and chopped cornstalks were particularly effective. Leaf mulch (of oak, locust, beech, maple, sycamore and some pine needles) also performed well, but had to be replenished yearly rather than once every two years. Wood chips and straw produced the lowest yields and shortest plants. Pine bark and well composted manure or stable bedding have been reported to work well (Eck 1988). However, the latter materials may increase soil pH. Korcak (1988) cites mulches maintain a more constant pH of media, and in some cases contribute to the soil acidification. Gustavsson (1999) has pointed that some organic mulches, especially peat moss and pine needle litter, decrease soil pH. Patten *et al.* (1990) have demonstrated living mulch interrow area could be used to produce significant quantities of mulch for rabbiteye blueberries and can be alternative for organic cultivation. At the same time the use of straw mulches provides an environment friendly cultivation option (Ramarkrishna *et al.* 2006).

However, apart from any benefits of organic mulching, there are also some disadvantages. Mulching is expensive (Hasing *et al.* 2004), and higher levels of fertilization are needed (Tiquia *et al.* 2002). Mulching also prevents the soil from emitting heat, which may lead to a lower temperature at the mulch surface and increased frost injury during the blossom time. In a trial the minimum average temperature was 1.8°C lower on the surface of with sawdust mulched soil compared to bare soil during two weeks in May (Gustavsson 1993). Mulching with plastic foil would probably not increase the risk of frost injury. A promising alternative to organic mulching is the use of synthetic mulches (Gustavsson 1999). Black plastic gave higher values for soil temperature compared with organic mulch (Truax & Gagnon 1993), but it depended on color of mulch (Decoteau *et al.* 1988). Experiments with highbush blueberry show that root growth is impaired by temperatures above 18 °C (Abbott & Gough 1987). Polyethylen mulch increased stem volume (Green *et al.* 2003) and it reached early flowering (Ramarkrishna *et al.* 2006). Plastic foil mulch benefited lingonberry growth (Saario & Voipio 1997) and promoted fruit yield (Gus-

tavsson 1999). However, some researchers have showed meager effect on plant yield (Tiwari *et al.* 2003). Mulches have influence on chemical composition of fruits. It is stated that using Black Mypex film reduces content of titratable acids in strawberries (Neuweiler *et al.* 2003). In Estonian conditions it has been found that growing strawberries with plastic mulch and additional fertilization, will increase the content of vitamin C in berries (Moor *et al.* 2004). When polyethylene mulch has amortized, it is important to remove plastic before it deteriorates and decomposes into the soil and pollutes the environment.

In addition organic mulch amendments affect physical, chemical and biological processes of soil. It is generally accepted when high C:N organics are incorporated into soils, the balance between nitrogen mineralization and immobilization by soil microbes is strongly changed (Johnson 1992; Yang *et al.* 1998; Tiquia *et al.* 2002). It changes the plant growth and development. Peat and sawdust influenced the growth and yield of highbush blueberries (Ochmian *et al.* 2006). Odneal and Kaps (1990) have found that pine bark was as good as sphagnum peat for preplant incorporation, with no differences in plant height, spread, a number of canes or plant yield or the size. Rotted sawdust in the planting hole has also proven to be a good material for preplant incorporation (Eck 1988; Peterson *et al.* 1987). Organic material application with mineral soil reduced soil pH (Ochmian *et al.* 2006) and improved nutrient uptake of a blueberry (Goulart *et al.* 1995; Katakura & Hirota 2004).

Estonia is very rich in mires (Orru 1995) and peat production is developed here. Peat is available and relatively inexpensive. Due to large-scale wood-entrepreneurs sawdust is also facile provision. At the same time black plastic mulch is widely used in berry cultivation too (Värnik 2001).

## **2.7. Winter and frost hardiness of blueberries**

Winter hardiness of blueberry species and cultivars is very important for introducing and commercial cultivation in North Europe (Haffner & Vesterheim 1994; Paasisalo *et al.* 1994). The studies of overwintering cereal crops suggest that freezing tolerance is a complex, quantitatively inherited trait with additive and dominance components and frost sensitivity is partially dominant (Sutka 1981). Winter survival in the field is influenced by multiple factors which, in turn, are influenced by uncontrollable environmental parameters (Hummel *et al.* 1982). For example, ultimate winter survival of overwintering plants depends on a number of factors such as fall-timing of growth cessation, freezing tolerance at the cold acclimation state, the tolerance to fluctuating temperature in late winter and early spring, chilling requirement, wind desiccation, snow cover, etc. and thus may exhibit annual variation (Fowler & Gusta 1979; Fear *et al.* 1985). Whereas the evaluation of winter survival in the field may be important for breeding populations, it is often problematic to draw conclusions about the genetics of freezing tolerance at a coldacclimated state (one component of

winter survival) based on the data on winter survival (a trait with several components). One such study dealing with the heritability of winter injury in a woody perennial found large general combining ability by year interaction effects, and confounding effects of two traits measured in field conditions, namely growth cessation and winter injury (Fear *et al.* 1985). These authors concluded that screening for cold hardiness under controlled conditions would be desirable, and no conclusions were attempted regarding additive, dominance and epistatic effects. In a recent survey in the U.S., lack of freezing tolerance and susceptibility to spring frosts were identified as the most important genetic limitations of blueberry cultivars (Moore 1993). One of the goals of United States Department of Agriculture blueberry breeding programs is to develop cultivars with freezing tolerance suitable for northern regions to broaden the climatic adaptation of blueberries.

Lowbush blueberry and cultivars of northern highbush blueberries require the greatest number of winter chilling hour and therefore most suitable for areas with long, cold winters (Trehane 2004). Highbush blueberry plants damage usually occurs when temperatures fall to around  $-29^{\circ}\text{C}$ , well-acclimated canes can withstand an extreme of  $-40^{\circ}\text{C}$ . Half-highbush blueberries ‘Northblue’ survive  $-30^{\circ}\text{C}$ , ‘Northcountry’  $-35^{\circ}\text{C}$  and they need 800 chilling hours (Gough 1994). Dangerous time for blueberry plants is spring with the flowers receiving the frost damage. Four hours exposure to  $-3.5^{\circ}\text{C}$  resulted in 80% and 60% moderate or severe damage to open and closed lowbush blueberry flowers, respectively (Hicklenton *et al.* 2002). While they are still enclosed in their buds, flowers can tolerate temperatures down to  $-6^{\circ}\text{C}$ , (Lin & Pliszka 2003). In Nordic countries worst damages have been a problem in flowering time for lingonberries (Lehmushovi 1977) and bilberries and it often regulated the yield (Paal 1999).

## **2.8. Chemical composition of blueberries, raspberries and blackberries**

In recent years, increasing attention has been paid to consumers’ health, nutritional aspects (vitamin content, mineral elements, antioxidants, etc.) of horticultural products. A diet rich in fruits and vegetables offers protection against some most spread diseases such as cardiovascular, cancerous and many age-related degenerative diseases (Ames *et al.* 1993). Fruits have long been regarded as having considerable health benefits, due to their antioxidant content, which can protect a human body against cellular oxidation reactions. It is important to characterize different types of fruit for the content of single specific antioxidant compounds (Wang *et al.* 1996). The blueberries are recognized as one of the most valuable fruit due to its high antioxidative activity (Prior *et al.* 1998; Mainland *et al.* 2002). For example, lowbush blueberry has a higher antioxidant activity than sweet cherry, potato (purple), wheat germ, and

ginseng root (Velioglu *et al.* 1998). Wu *et al.* (2004) have showed that a one-cup serving of wild blueberries has more antioxidant capacity than the same serving of cranberries, strawberries, plums, raspberries and even cultivated blueberries. Ehala *et al.* (2005) state bilberries exhibit the highest level of total phenolics followed by lingonberry, black currant, strawberry, cranberry, and red currant in Estonia. Phenolic substances such as flavonoids are the most common compounds in fruits and vegetables and have strong antioxidant capacity (Halliwell *et al.* 1992; Wang *et al.* 1997; Gazzani *et al.* 1998). The berries, such as blueberries, are significant sources of flavonoids – anthocyanins. Blueberries contain the following anthocyanins: 3-glucosides, galactosides and arabinosides of delphinidin, cyanidin, petunidin, peonidin and malvidin (Mazza & Miniati 1993; Gao & Mazza 1994). Malvidin content in the fruit of lowbush blueberry (Gao & Mazza 1995; Raudsepp 2007) and cultivar ‘Northblue’ was the highest (Raudsepp 2007). Bilberries had more delphinidin and cyanidin. Moreover, cyanidin-3-galactoside was a common anthocyanin, but it was not found in bog bilberry (*V. uliginosum* L.) (Li *et al.* 2006a). Phenolics have considerable influence on berry color, but Gao and Mazza (1994) found chlorogenic acid was the major colorless phenolic of lowbush and highbush blueberries.

Blueberries contain vitamins A, B, C, E, PP and carotenoids, which significantly contribute to their total antioxidant capacity (Wang *et al.* 1996; Prior *et al.* 1998; Kalt *et al.* 1999; Proteggente *et al.* 2002).

Blueberries are an excellent source of phytochemicals that are believed to have significant biological activity (Schmidt *et al.* 2005). Anthocyanins have been found significantly to suppress the growth of cultured tumour cells and have been shown to have greater inhibitory effect than other flavonoids (Kamei *et al.* 1995; Mazza 2000). Antioxidative and anticarcinogenic effects of *Vaccinium* berries are partly proposed to be due to phenolic compounds in these berries (Häkkinen & Törrönen 2000; Zheng & Wang 2003; Rimando *et al.* 2004). Blueberry extracts ameliorate age-related declines in neuronal and cognitive function, common in disorders such as Alzheimer disease (Youdim *et al.* 2000). Antioxidants from blueberries have been linked with anti-aging (Joseph *et al.* 2003), anti-cancer (Schmidt *et al.* 2004), heart-health benefits (Norton *et al.* 2005) and vision improvement properties (Camire 2000; Kalt 2006). Apart from biological properties, natural flavonoids are also of interest in the food, cosmetic, and pharmaceutical industries, as they can be used as substitutes for synthetic antioxidants (Moure *et al.* 2001). The anthocyanin content of blueberry fruit has been characterized well, the chemical content of the vegetative parts used in supportive therapy for diabetes has not been ignored. Witzell *et al.* (2003), Percival (2006) and Harris *et al.* (2007) have found the leaf and stem extract of blueberry contained a high concentration of flavonoids and other polyphenol compounds. Health benefits of blueberries have been probably known for thousands of years. By Trehane (2004) blueberries were known from age-old times in North America and Indians used the berries widely for medical purposes. Bilberry is widespread in Estonian

wildlife and by historical traditions the fresh berries, plant leaves and dried berries are used as a medical plant (Pogen, 1977).

In addition to named components the contents of dry matter, sugars, acids and color are important parameters for high quality fresh and processed blueberries (Austin & Bondari 1993; Remberg *et al.* 2006). Moreover chemical composition is strongly affected by the specific plant genotype, but it can also be affected by cultivation conditions (environmental and cultivation techniques) of the plant (Jones & Hartley 1999; Kalt *et al.* 2001; Łata *et al.* 2005; Skupień 2006). The interaction of these different factors in determining the quality and chemical composition of a specific fruit should be established to characterize better agronomic production and information to the consumer.

Blueberries contain a number of beneficial phytochemicals contributing to health. The most immediate, and perhaps the greatest opportunity for marketing blueberries may be in promoting blueberries as a healthy food. For this purpose it is essential to use right methods in blueberry cultivation that will not pollute the environment and decrease fruit quality.

The main constituent of the raspberry fruit is water (ca 87%) (Green 1971). A typical ripe raspberry fruit contains 5–6% sugar. The main sugars are glucose, fructose and a smaller amount of sucrose. These compose the major soluble component of the juice. Citric acid is the second largest component of the soluble fraction; raspberries contain very little malic acid, but at least 10 other acids in trace amounts. A large number of volatile compounds are found in raspberry fruit, but most are present at less than 10 ppm. Raspberry fruits contain small amounts of vitamins; only vitamin C is present at a significant level (Remberg *et al.* 2007). The color of raspberry fruit is imparted by anthocyanins (Green 1971). The anthocyanin molecule in raspberry consists of cyanidin and pelargonidin. Fruits with a preponderance of pelargonidin glycosides have an orange-red color, as opposed to a deep red color with cyanidin glycosides. Total anthocyanins content ranges from 0 to 400 mg 100g<sup>-1</sup> (Cheplick *et al.* 2007).

Genetical characteristics of cultivar and weather conditions of growing seasons also influences raspberry biochemical components (Riaz & Bushway 2007). Fruits grown under warm, dry summers (daytime temperatures near +25°C) are sweeter, less acid, more aromatic, and more deeply colored (Green 1971). Hot weather (temperatures greater than +30°C) will reduce the aroma of the fruit, and wet weather will reduce the sugar content.

Blackberries are notable for their high nutritional contents of dietary fiber, vitamin C, vitamin K, folic acid, and the essential mineral manganese (Wada & Ou 2002; Hager *et al.* 2008). Blackberries rank highly among fruits for antioxidant strength, particularly due to their dense contents of polyphenolic compounds, such as ellagic acid, tannins, quercetin, gallic acid, anthocyanins and cyanidins. Of the ripe fruits tested, on the basis of wet weight of fruit, blackberries may be a richer source for antioxidants than raspberry (Shiow

Wang & Hsin-Shan Lin 2000). Halvorsen *et al.* (2006) reported that blackberry is at the top of more than 1000 antioxidant foods consumed in the United States.

## 2.9. Hypotheses and aims

Taking into consideration the traits and requirements of environmental conditions of lowbush blueberry and half-highbush blueberry we hypothesized that it would be possible to cultivate these taxa in the Estonian conditions. In North Europe, there is a great need for experiments with other hardy blueberry species like lowbush blueberry and its hybrids. Experimental results with lowbush blueberry in Canada and U.S. might not be applicable in North Europe. The lowbush blueberry is native in North America, but not in Europe. Therefore, it is necessary to study the influence of fertilization on the soil and development of plants after establishing a plantation.

We hypothesized that mulching and soil amendments would improve growing conditions, plant productivity, mycorrhizal status of blueberries and chemical composition of *Rubus* taxa.

The quality and chemical composition of fruits are conditioned both by genetic and environmental factors. Bearing this in mind we hypothesized blueberry fruit size, color and chemical content depend on species or cultivars and on environmental conditions.

The main aims of the present investigation were to evaluate:

- the effect of elemental sulphur and acidifying NPK fertilizers on lowbush blueberry plants grown in former arable lands with pH initially too high for blueberry (Paper V);
- the influence of different cultivation methods (mulches, peat application into the soil) on winter hardiness, productivity and nutrition status of half-highbush blueberry cultivars ‘Northblue’ and ‘Northcountry’ and possibility to grow them in organic and intensive farming condition (Papers I, II, III);
- the effects of different production practices on the level of mycorrhizal colonization in field-grown cultivars ‘Northblue’ and ‘Northcountry’ (Paper IV);
- the chemical composition of different *Vaccinium* taxa from both natural and cultivated sites (Paper VI).
- the chemical composition of different *Rubus* taxa depending on cultivation methods (Paper VII).

### 3. MATERIAL AND METHODS

#### 3.1. Experimental sites and plant material

The half-highbush blueberry (*Vaccinium corymbosum* x *V. angustifolium*) experiment was carried out in Tartu County, South Estonia (58°15'N, 26°43'E). The plantation was established in June, 1997 with one-year-old *in vitro* cultivated plants. The cultivars 'Northblue' and 'Northcountry' were used. The gap between individual plants was 0.7 m and the space between two rows was 1.5 m. By WRB classification (2006) the soil in the experimental area was Enti-Umbric Albeluvisol, and the texture was sandy. The experimental design was a randomized complete block with 3 replications and 10 plants per plot. (Papers I, II, III, IV)

Experiments with lowbush blueberries (*V. angustifolium* Ait.) were established in 1999 at Kärļa and Vasula. Kärļa (58°19'N and 22°16'E) is situated on Saaremaa island in West Estonia. The soil type in the experimental site is Umbri-Endogleyic Albeluvisol (Word Reference Base for Soil Resources, 2006), the texture is loamy sand and the plough layer is 18–25 cm thick. Vasula is situated in South Estonia in Tartu County (58°28'N and 26°44'E). The soil type in the experimental area is Endoeutri-Haplic Luvisol (Word Reference Base for Soil Resources, 2006), the texture is sandy loam and the plough layer is 24–30 cm thick. Experimental plantations were established with two-year-old blueberry seedlings. The space between the rows was 1 m and plant-to-plant spacing was 0.8 m. The experimental design was a randomized complete block with 3 replications and 15 plants per plot. In 2002 experimental areas were mulched using pine bark crumbs. (Paper V)

For the analysis of chemical composition the bilberry (*V. myrtillus* L.) fruit were harvested from a natural habitat situated in South Estonia in Võru County (57°37'N and 27°4'E). The lowbush blueberry fruit were collected from two different areas: one plantation was located on mineral soil (Võru County in South Estonia 57°37'N and 27°4'E) and the other one on peat (abandoned peat pits) soil (Harju County in North Estonia 59°9'N and 25°19'E). Half-highbush blueberry fruit were collected from a commercial farm in Tartu County, South Estonia (58°12'N, 26°41'E). (Paper VI)

The biochemical content of two *Rubus* cultivars were investigated: the raspberry (*Rubus idaeus* L.) 'Tomo' and the blackberry (*Rubus fruticosus* coll.) 'Agawam'. The experiment was established in autumn, 1999 in Estonian Agricultural University's experimental garden (58 °23 ' N, 26 °44 ' E). The soil was Stagnic Luvisol (Word Reference Base for Soil Resources, 2006), which texture is sand clay with humus layer 20–30 cm. (Paper VII)

## 3.2. Sampling

Data were collected:

- from the half-highbush blueberry experiment in 1998, 1999 (Paper I), 2001 (Papers III, IV), 2002 (Paper III), 2003 (Paper II),
- from the lowbush blueberry experiment in 2001, 2002, 2003 (Paper V),
- for chemical composition experiment in 2003 (Paper VI).
- for *Rubus* experiment in 2001, 2002, 2003, 2004 (Paper VII).

## 3.3. Peat and mulch applications

In the half-highbush blueberry trial different cultivation combinations were used:

- mineral soil without mulch (control);
- mineral soil with sawdust mulch;
- mineral soil with peat mulch;
- mineral soil with plastic mulch;
- ground mixture (mineral soil + peat) with peat mulch;
- ground mixture (mineral soil + peat) with plastic mulch. (Papers I, II, III, IV)

Each plant received 10 l peat into the growing substrate before plantation (mineral soil + peat). Plastic mulch (black polyethylene) was placed before planting. Organic mulch treatments were applied at a 70-cm-wide band centred on the plant row immediately after planting. The ground was covered with a 5-cm layer of peat or sawdust mulch. In springs of 2000 and 2003, additional mulch was added. We did not use fertilizer and pesticides on the experimental field in any of these years. (Papers I, II, III, IV)

Following cultivation combinations were used in *Rubus* trial:

- mineral soil without mulch (control);
- mineral soil with mulch (black woven ground cover, 100 g m<sup>-2</sup>, UV, 100% polypropylene). (Paper VII)

## 3.4. Fertilizers and fertilization rates (Paper V)

The acidifying fertilizers ammonium sulfate – (NH)<sub>2</sub>SO<sub>4</sub> (N-21%, S-24%), superphosphate – Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> and CaSO<sub>4</sub> 2H<sub>2</sub>O (P-9%, S-12%) and potassium sulphate – K<sub>2</sub>SO<sub>4</sub> (K-34%, S-18%) and elemental sulphur (S; 100%) were used in the experiment. The quantities of the fertilizers were calculated according to the nutrient content of the soil and suggestions worked out by Lehmushovi and

Ylämäki (1999). Fertilizer application rates in the different treatments were the following:

1. In Kärla 1) control, without fertilization; 2) 25 N, 18 P, 51 K kg ha<sup>-1</sup>  
3) 1000 S kg ha<sup>-1</sup> 4) 1000 S + 25 N, 18 P, 51 K kg ha<sup>-1</sup>.
2. In Vasula 1) control; 2) 11 N, 15 P, 34 K kg ha<sup>-1</sup> 3) 1000 S kg ha<sup>-1</sup>  
4) 1000 S + 11 N, 15 P, 34 K kg ha<sup>-1</sup>.

In both experiments elemental sulphur was applied once in the early spring of 2000. In Kärla NPK fertilizer treatments were used in the spring of 2000 and 2001. In Vasula the above-mentioned treatments were used in 2000. In 2001 only ammonium sulfate (at the rate of 25 N kg ha<sup>-1</sup>) was applied to the NPK plots, because potassium and phosphorus contents of the soil were high.

### **3.5. Analyses of soil nutrient content and pH**

Soil samples were taken close to the plants from the soil layer from the depth of 20 cm (Papers I, V) or 0–5, 5–10 and 10–15 cm (Paper III). Soil pH<sub>KCl</sub> was determined using an Evikon pH meter. Using standard methods (Official Methods of Analysis, 1990) soil analyses were fixed in the Laboratory of Plant Biochemistry at Estonian Agricultural University. The content of phosphorus (P) (ammonium lactate extractable), potassium (K) (ammonium lactate extractable), calcium (Ca) and magnesium (Mg) (1 M ammonium acetate extract, pH 7.0) was determined (Paper V). Organic matter concentration (%) in soil was also analysed by loss on ignition (Paper V). Three soil samples were analysed in every variant and one sample consisted of ten subsamples.

### **3.6. Leaf nutrient content and chlorophyll meter measurements**

The nutrient (nitrogen – N%; phosphorus – P%; potassium – K%; calcium – Ca%; magnesium – Mg%) content in lowbush blueberry leaves was determined in the Laboratory of Plant Biochemistry at the Estonian Agricultural University (Paper V). Leaf samples were collected at harvest time, in the beginning of August. N concentration of air-dried samples was determined by the Kjeldahl method. The method involves the digestion of a sample in sulphuric acid using the Kjeldahl Cu catalyst to convert the protein nitrogen to ammonium sulphate. Ammonia is liberated by alkaline distillation using an automatic analyser Kjeltec Auto 1030. P, Ca, and Mg concentrations were measured by Kjeldahl digest using the flow injection analyser “FIAstar 5000”. K concentration was determined flame photometrically by an air-acetylene flame. P was determined at the wavelength 720 nm by the Stannous Chloride method and Ca at the

wavelength 570 nm using o-Cresolphthalein Complexone, 8-Hydroxyquinoline to mark magnesium and 2-amino-2-methyl-propanol-1 as a buffer. Mg was determined by Titan Yellow at the wavelength 540 nm. All nutrient concentrations were expressed on a dry weight basis (% DW). Three samples in every variant were analysed and one sample consisted of leaves from ten plants.

Chlorophyll content of the plants was measured using the portable Hydro N-Tester (SPAD-500) chlorophyll meter, Minolta Camera Co., Ltd. Japan (Papers II, III, V). It permits rapid and non-destructive determination of leaf chlorophyll content by measuring leaf transmittance. Leaves of the same age and position on the plant were used. Leaves from the middle part of one-year-old shoots (not fruit bearing) were chosen for measuring. Each reading consisted measurements from 3 or 10 different plants depending on the experiment specification and one sample consisted of 30 leaf measurements on the average. The young leaves with non-uniform color were left aside. N-Tester readings were measured in the beginning of August, at harvest time.

### **3.7. Measuring of plant status and yield**

In spring (in the end of April), winter hardiness was estimated using a nine-point scale (1 point – very low winter hardiness, all branches damaged up to the soil level; 9 points – very high winter hardiness, branches not damaged). We observed 5 individual plants in every replication. (Paper I)

In the end of vegetation period (in September or October), the blueberry plant height (cm) and width (cm) were measured (Papers I, II, IV, V). The plant width was measured across and along the row and its average was calculated. The number of shoots (longer than 15 cm) per plant was counted (Papers II, IV, V). Three or ten plants were measured in every replication.

The half-highbush blueberry fruit ripen gradually, so the yield was picked four times from the end of July to the end of August (Papers II, III, IV). In the figures total yield per plant is presented. The lowbush blueberry was harvested once in August (Paper V). The yield was weighed from 3 or 10 different plants in every replication. The yield was harvested by hand. At the same time average fruit weight (g) from each plant was calculated.

For the determination of dry weight of blueberry roots, a soil sample was taken under the canopy of blueberry plant with a cylindrical (6 cm diameter, 15 cm length) stainless steel corer (Paper V). Roots were separated from the sample. After that they were washed under tap water and dried in a drying oven at a temperature of 105°C until constant weight was achieved. The root dry weight was calculated ( $\text{g dm}^{-3}$ ). Ten samples were taken in every replication.

### 3.8. Measuring of mycorrhizal colonization (Paper IV)

Two root samples were taken from five plants. Blueberry plants were sampled by removing small sample with a cylindrical (29 mm diameter, 150 mm length) stainless steel corer. Roots were separated from samples by washing the soil through a coarse screen. The 10% w/v KOH was used to clear roots, and 0.005% trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) was used for staining (Brundrett *et al.* 1996). From each root sample, five root segments of 5 mm long were randomly chosen and examined with a light microscope. The assessment of mycorrhizal colonization under the light microscope was done by randomly selecting microscope field of view and cross-hair positions. On each root segment, three observation fields were studied and the number of hyphae which were across centre horizontal axis was counted. The diameter of root segments were also measured (mm).

### 3.9. Measuring of fruit quality

The blueberry fruits were harvested on 5th, 6th, and 7th of August, 2003. 100 berries from individual taxon were weighed and the diameter measured using calipers. The average berry weight (g) and diameter (mm) were calculated. (Papers VI, VII)

Dry matter (DM) was determined using a  $10 \pm 1$  g sample drying it in a thermostat (Co. Memmert) at  $105^\circ\text{C}$  to a constant weight. Fruit moisture ( $\text{g } 100 \text{ g}^{-1}$ ) was calculated on a dry weight and fresh weight basis. (Papers VI, VII)

The titratable acidity was measured by neutralizing 0.1 M NaOH solution (automatic titrator, Mettler Toledo DL 50 Randolino). Titratable acids were expressed as citric acid ( $\text{g } 100 \text{ g}^{-1}$ ). (Papers VI, VII)

Soluble solids were analyzed using a Pocket Pal-1 refractometer (Co. Atago) (Papers VI, VII).

For the determination of anthocyanins, 10 whole berries were crushed and 10 g of the crushed fruit were soaked in an extracting solution containing HCl (0.1M): $\text{C}_2\text{H}_5\text{OH}$  (96%) = 15: 85 (v/v). Solutions were shaken and held at  $5^\circ\text{C}$  for 24 h. After settling,  $2 \times 2$  ml of the clear supernatant was pipetted into 50 ml volumetric flasks and made up to volume with extracting solution. The anthocyanins were determined using the modified pH differential method of Francis (1982) and Fuleki & Francis (1968). The Thermo Spectronic Helios  $\beta$  spectrophotometer was used for measuring the absorbance. The extinction coefficient of malvidin-3-glycoside (28 000) was used (Wrolstad 1976).

The content ( $\text{mg } 100 \text{ g}^{-1}$ ) of thiamin (vitamin  $\text{B}_1$ ), riboflavin (vitamin  $\text{B}_2$ , vitamin G), nicotinic acid (a form of vitamin  $\text{B}_3$ , vitamin PP), nicotinamide (a form of vitamin  $\text{B}_3$ , vitamin PP), pyridoxine (a form of vitamin  $\text{B}_6$ ), pyridoxal (a form of vitamin  $\text{B}_6$ ) and folic acid (vitamin  $\text{B}_9$ ) was analyzed at the laboratory of Department of Food Processing (Tallinn University of Technology) using

standard methods (Water Soluble Vitamins 2003). The content of fat ( $\text{g } 100 \text{ g}^{-1}$ ), protein ( $\text{g } 100 \text{ g}^{-1}$ ), glucose ( $\text{g } 100 \text{ g}^{-1}$ ), fructose ( $\text{g } 100 \text{ mg}^{-1}$ ) and the total energy value ( $\text{kcal } 100 \text{ g}^{-1}$ ) was determined by standard AOAC (AOAC 1990) methods at the Veterinary and Food Laboratory.

For the determination of ascorbic acid (vitamin C), 10 g of crushed fruit were taken for analysis. As described by Paim & Reis (2000), 60 ml of metaphosphoric and acetic acid (3%  $\text{HPO}_3$  + 8%  $\text{CH}_3\text{COOH}$ ) was added immediately to the fruit to avoid vitamin C breakdown in the air. The slurry was shaken in 125 ml plastic bottles with leakproof screwcaps for one hour using a reciprocating shaker at 190–200  $\text{rpm min}^{-1}$ . Vitamin C was titrated iodometrically using an automatic titrator (Mettler Toledo DL 50). (Paper VI)

Ascorbic acid content of *Rubus* taxa was determined with the modified Tillman's method, where 2 g of fruit material was first pounded and then 1% HCl was added. Next it was filtered and measured in two replications (each 10 ml, 1 ml 1% KJ and 1 ml 1% soluble starch was added). (Paper VII)

Surface color of fresh blueberries was measured with a reflectance colorimeter (Model CR-400, Minolta Co., Ltd.). In each replication ten berries were measured. The mean of three color readings for each berry was calculated. Color of the fruit was expressed as  $L^*$  (lightness; black = 0, white = 100),  $a^*$  (redness, red = +60, green = -60),  $b^*$  (yellowness, yellow = +60, blue = -60),  $C^*$  (chroma, saturation, vividness), and  $h$  (hue angle). (Paper VI)

### 3.10. Statistical data analysis

All measurements and analyses were made in three replications. One- (Papers I, IV, VI, VII) and two-way (Papers II, III, V, VII) analyses of variance (ANOVA) were used for data analysis. The least significant difference (LSD) at the 95% confidence level was calculated for variants. The calculation of standard deviation was used in some objective. Significant ( $p < 0.05$ ) differences were marked by asterisk (\*) (Papers I, IV, V) or by letters (a, b, c...) (Papers III, VI, VII) in tables and figures.

To find out relationships between different parameters correlations and regression analyses were applied (Papers IV, V). Statistically significant differences were marked as follows:

NS – non significant correlation

\* – significant correlation at  $p < 0.05$

\*\* – significant correlation at  $p < 0.01$

\*\*\* – significant correlation at  $p < 0.001$

## **4. RESULTS AND DISCUSSION**

### **4.1. The effect of NPK fertilization and elemental sulphur on the growth and the yield of lowbush blueberry**

#### **Soil pH and its relationships with exchangeable nutrient content**

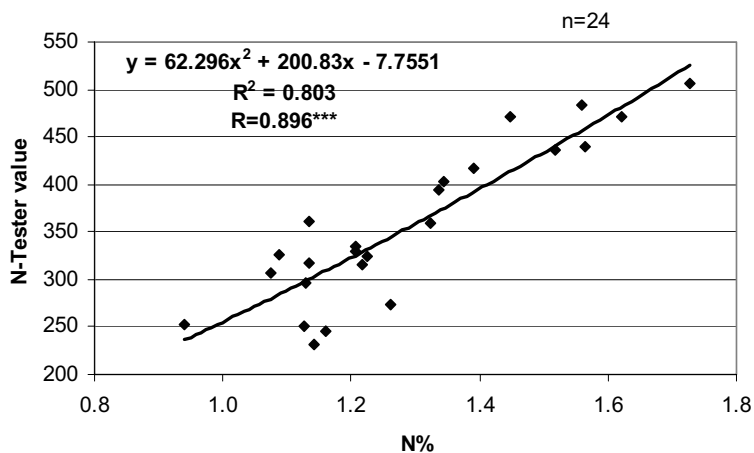
By the results (2001) we can say soil pH was decreased significantly when S was applied in the Kärle plantation where the soil pH was lower and the soil texture was lighter (Paper V). In the Vasula plantation (soil was initially less acidic) the application of NPK fertilizer increased soil acidity. In the following year, the soil was considerably more acidic in all fertilized plots compared to the control one. In 2003 the effect of sulphur and NPK fertilizer decreased, except for NPK fertilizers at Kärle. In the latter plantation there was no difference between the control and sulphur-treated plots. The application of NPK fertilizer had more effect on soil pH. In Vasula, the soil pH was significantly lower when sulphur was used. The activity of soil organisms and the mobility of plant nutrients depend partly on the pH of soil. In most cases the application of mineral fertilizers and S significantly lowered soil pH in experimental areas. Thus, the content of plant-available macronutrients such as P, Ca and Mg of the soil changed as well (Paper V). Similar results when changing soil pH with sulphur has been recorded elsewhere (Haynes & Swift 1986; Owen *et al.* 1999).

To increase the effect of fertilizers higher quantity is often recommended. However, a higher amount of fertilizer for blueberries is not advisable in the Nordic countries, since it could prolong the growing period. One-year-old shoots will not lignify before the first frosts and the tips of shoots will be frozen. Long growing period of blueberry bushes is also one of the reasons why blueberry plants suffer from winter damage, especially young bushes during the first few years of growth (Karp *et al.* 2000). At our experimental sites significant winter damages of plants were not observed.

#### **Leaf nutrient content and N-Tester measurements**

The influence of sulphur on supplementation of leaf nutrients appears in two years after the application of S (Paper V). Spiers and Braswell (1992) have reported that sulphur increased the concentrations of leaf N, P, K and Mn. The results of this study have shown that sulphur increased Mg and Ca content of blueberry leaves in Kärle (Paper V). The application of NPK fertilizer and S significantly increased K, Ca, Mg content in Kärle and Ca, Mg content in the Vasula experiment. According to N-Tester readings NPK fertilization had an

effect on N-content of blueberry leaves. It can be assumed that it was caused by the increase of pH in soil, because the correlation between soil pH and N-Tester readings was found. Several experiments with other horticultural crops have indicated that fertilization increases chlorophyll meter readings (e.g. Sandoval-Villa *et al.* 1999). The application of fertilizers showed a trend to increase N-tester readings (Paper V). In Kärle, NPK fertilizers alone or in combination with S increased N-Tester reading, but a sulphur application alone had no effect in the first experimental year (Paper V). The opposite effect was observed at the Vasula plantation. In 2003 (the third experimental year) chlorophyll meter readings in Kärle were 315–506 and in Vasula 272–391. NPK fertilizers application without S increased N-Tester reading of blueberry leaves in both Kärle and Vasula. It shows that there is a strong relationship between chlorophyll meter readings and N content in leaves (Porro *et al.* 2001). The same influence and relationship were indicated in our experiment (Fig. 1; Paper V).



**Figure 1.** Relationship between the N-Tester value and N content (%) of lowbush blueberry leaves in 2002 (data from Starast *et al.* 2002), 2003 (data from Paper V) and 2004 (data from Starast *et al.* 2004) (\*\*\*) –  $p < 0.001$ ).

### Plant growth and yield

In 2001, the application of fertilizers and sulphur did not affect plant height in Kärle (Paper V). At the same time NPK and NPK + S fertilizers had a significant positive influence on the plant width. In the following two years the above-mentioned treatments increased the plant height and width in the Kärle experiment. In every study-year the application of fertilizers and S significantly increased plant sizes in Vasula. In Kärle, shoots grew better with the application of NPK and NPK + S fertilizers all these years, and the number of shoots was more than doubled compared to a control group. In 2001, S application signi-

ificantly effected the growth of shoots in Vasula. The next year shoot development was poor. In 2003, fertilization and S application increased the number of shoots. On average, the growth of shoots was better in Kärle compared to Vasula. The vegetative growth of blueberry bushes depended significantly on N and P content of leaf (except shoot number) and there was a positive correlation with N-Tester reading (except plant height).

To ensure high yields later on, it is important to create optimal conditions for vegetative growth during the first years. In the first crop year the yield per bush was greatly variable. The yield in Kärle was higher than in Vasula. A significant positive influence of NPK fertilization on the yield was found in both Kärle and Vasula, but fertilization did not increase the berries weight. The yield showed a positive relationship with N-Tester reading and N content in leaves. (Paper V)

As it is known soil reaction plays an important role in blueberry cultivation. Blueberries need acidic soil for optimal growth (Hall *et al.* 1964; Spiers 1984). The quantity of blueberry yield depends on soil acidity (Spiers 1984, Austin & Bondari 1992). Our results have confirmed it as well: the plant size and the yield of blueberry bushes depended significantly on soil reaction (Paper V). Probably low pH of soil makes nutrients more available for blueberries. The predominance of  $\text{NH}_4$  in the soil at low pH may be an important factor contributing to the preference of blueberries to acid soils, since they are known to prefer  $\text{NH}_4$  instead of  $\text{NO}_3$  as their major source of N (Korcak 1988). Presumably uptake of micronutrients was also improved, because soil acidification caused by the addition of S, resulted in increases of extractable Mn and Fe levels (Haynes & Swift 1986). At the same time, Spiers and Braswell (1992) have explained the application of only acidic fertilizers was not enough to achieve the desired pH of the soil. More acid soil can promote the growth of mycorrhizal fungi (Perotto *et al.* 1995) and thereby blueberry productivity increases. However, pH was not the only factor, because in one fertilized plot in Vasula the soil acidity was higher, but the yield was still very low (Paper V). We suppose the soil texture and climatic conditions might have influenced the results, because they were different in the two experimental sites.

## **4.2. The effect of mulches and peat-in-soil applications on winter hardiness, productivity and nutrition status of half-highbush blueberry cultivars ‘Northblue’ and ‘Northcountry’**

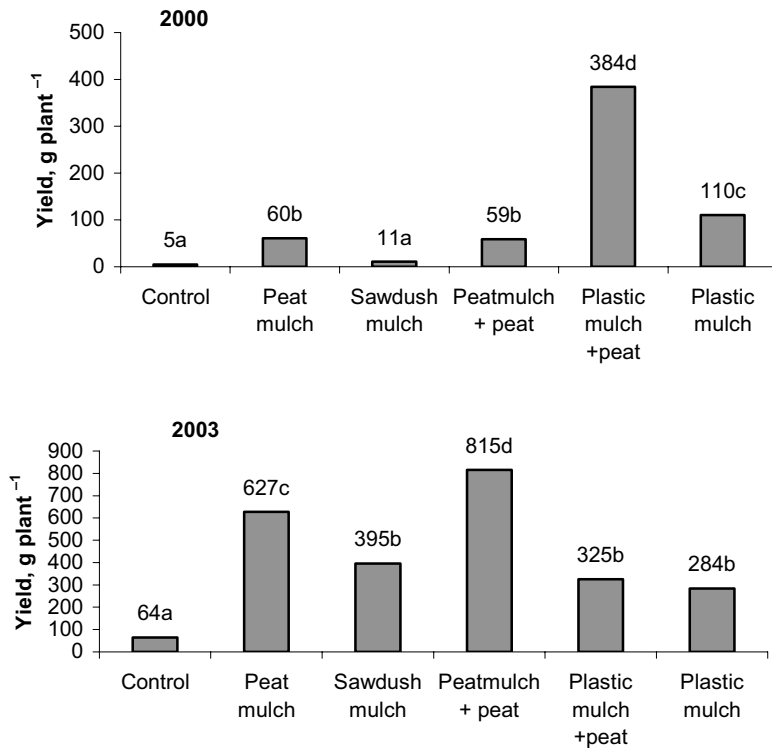
### **Winter hardiness**

The results have shown the winter damage of half-highbush blueberry cultivars ‘Northblue’ and ‘Northcountry’ were not very large, no plants died (Paper I; Karp *et al.* 2000; Tasa 2002). By 3-year experiments it turned out that the use of peat (used as mulch or applied into soil) did not influence the winter hardiness

of half-highbush blueberry. In the case of plastic mulch, the winter hardiness of 'Northcountry' decreased, though the cultivar 'Northcountry' was more winter hardy than 'Northblue'. A good cold hardiness of the vegetative components is a trait of the half-highbush blueberry (Luby *et al.* 1989). However Dhanaraj *et al.* (2007) have found many differences how plants endure freezing in a cold room and in the field environment, field plants are slightly more cold hardy than cold room-treated plants. Winter survival in the field is influenced by multiple factors such as fall-timing of growth cessation, freezing tolerance, tolerance to fluctuating temperature in winter, wind desiccation, snow cover, etc. (Fear *et al.* 1985). In our experiment winter hardiness was problematic in a young plantation where plants grow very intensely (Papers I; Karp *et al.* 2000; Tasa 2002). One-year-old shoots did not finish growing in time, they were herbaceous, not ligneous, and the first frost damaged the shoots in autumn. In the experiment plants had the first yield on the fourth growing year. After that the vegetative growth of plants was not so vigorous, the shoots lignified quicker and blueberry plants had no considerable winter damages. In Estonian climate in addition to low temperatures, fluctuating temperatures can be damaging as well. Actually it can be more problematic as the highbush blueberry experiment has showed (Karp *et al.* 2006; Karp *et al.* 2008). Blueberry plants had less winter damages after extremely cold winter than after fluctuating temperatures winter when late-winter thaws followed by strong colds. Once the chilling requirement has been met, buds are able to grow when the weather warms and followed freezes can cause severe injury to vegetative and flower buds.

### **Plant growth and yield**

Mulch use had a positive effect on the growth and yield of half-highbush blueberry plants. In the young plantation blueberry plants had the best growth if peat or plastic mulch was applied (Paper I). The positive effect of sawdust mulch did not appear on the first experimental years (Paper I), but since the fifth year the plant productivity increased significantly (Fig. 2; Paper II; Tasa 2002). However, if plastic mulch was used, the increment of growth was short, the yield did not increase more and berry weight decreased from the sixth year (Fig. 2; Paper II; Starast *et al.* 2003; Starast *et al.* 2004). After a eight-year experimental period we could see the blueberry plants had the best growth and yield when peat mulch was used (Papers I; II; III; Starast *et al.* 2001; Tasa 2002; Starast *et al.* 2003; Starast *et al.* 2005c). Our results demonstrate clearly that peat amendments into the soil before planting will increase plant growth. The average yield of five years was 68% higher when adding mulches. The half-highbush blueberry cultivar 'Northblue' had a higher yield compared with 'Northcountry'. Cross pollination is preferred with majority blueberry cultivars including 'Northcountry' and 'Northblue' (Luby *et al.* 1989). However, the cultivar 'Northblue' fertilized well in self-pollination condition and the yield of the plant was high (Järvela 2003).



**Figure 2.** Average influence of cultivation technology on plant yield (g) of half-highbush blueberry in 2000 (Starast *et al.* 2002) and 2003 (Paper II).

We can draw the conclusion organic matter in a planting hole can greatly increase early growth and yields of highbush blueberries in mineral soil (Haynes & Swift 1986). Pine bark is often used as mulch material in a commercial blueberry plantation. Kozinski (2006) has pointed the sawdust had more positive influence on highbush blueberry yield than bark mulch. The positive effect of sawdust mulch has been found also in a lowbush blueberry trial (Sanderson & Cutcliffe 1991). Wu *et al.* (2006) have concluded bark and sawdust mulch increased highbush blueberry productivity, but moss mulching was found to be the best. The positive influence of peat and plastic mulches on plant productivity is found also with other *Vaccinium* species (Butkus *et al.* 1989; Scibisz & Pliszka 1989; Saario & Voipio 1997; Gustavsson 1999). Spiers (1986) has found that for rabbiteye blueberries, mulch was the most important component, followed incorporated peatmoss and irrigation.

## Soil pH and plant nutrition status of plants

Mulch treatments influenced significantly soil pH values. In a five-year-old plantation of these experimental plots where peat mulch was used the soil acidity was the highest in uppermost soil layer (0–5 cm) mainly (Paper III). In the control plot (without mulch), the soil pH remained the same as at the start – with pH 5.9. The plots with plastic mulch had a similar pH with control plots. The soil was more acidic in the plots mulched with sawdust, but the influence of sawdust was significantly lower when compared to peat mulch. Earlier experimental years showed that the soil pH decrease was the greatest in the variant where the soil was amended with peat in addition to peat mulch (Paper I).

Most of blueberry roots are located in the uppermost soil layer (Vander Kloet 1988). The analysis of roots location showed that in the depth of 10–15 cm the root rate was higher when sawdust (5%) or plastic (7%) mulch was applied (Starast *et al.* 2003). At the same time soil moisture was lower in sawdust mulch variant and soil temperature was higher in plastic mulch (19°C) variant (Tasa 2002). The roots located mostly (98%) in the 0–5 cm depth and only 0.7% of roots were deeper than 10 cm when peat mulch was used (Starast *et al.* 2003). If peat was added into the growing substrate before planting, the total root weight increased 8%, although in the surface level (0–5 cm) root weight decreased significantly. Wu *et al.* (2006) have indicated peat mulch did increase root dry weight and the root depth. In our experiment the use of peat mulch inveigled roots into uppermost soil layer (0–5 cm) specifically (Starast *et al.* 2003) where soil pH was lower (Paper III). If plant roots situated mostly in uppermost soil layer (0–5 cm), they became especially susceptible to drought stress. To avoid such stress, the irrigation system should be used in the blueberry plantation where peat mulch is applied. Adequate soil moisture is required in fruit development period, when, at the same time, new flower buds are formed (Alan Erb 1993).

The use of organic mulches (Gustavsson 1999) or the application of organic material into the soil will reduce soil pH (Ochmian *et al.* 2006). Katakura and Hirota (2004) have indicated peat-moss plays an important role in blueberry growth, as soil pH decreased and the nutrient uptake of blueberry increased. Based on the chlorophyll meter readings the nutritional condition of plants was significantly higher in all mulched variants (Papers II; III). Compared to peat mulch this value was lower in plastic mulch variant (Paper II). However, if peat was mixed with soil, the chlorophyll meter readings increased 17% in plastic mulch variant. Leaf analysis affirmed a better nutrient (N and P) uptake in mulched variants (Starast *et al.* 2005c). On average the application of organic mulches increased soil organic matter content 35% and if peat was applied into the soil, the value increased 20% (Starast *et al.* 2004). The quality of organic amendments can greatly influence soil N mineralization processes (Yang *et al.* 1998). The amount of nitrogen, released from organic matter available for plants, is determined by the net balance between nitrogen mineralization and

immobilization by soil microbes (Johnson 1992; Tiquia *et al.* 2002). The balance between nitrogen mineralization and immobilization is strongly influenced by the C:N ratio of the decaying organic material (Facelli & Pickett 1991; Tate 1995). The population of soil microbes is generally carbon-limited, and the addition of organic carbon to the soil stimulates the population growth of microbes until they eventually become limited by available nitrogen (Aber 1992). Since soil microbes are stronger competitors for nitrogen than are plants, when the C:N ratio of organic material is high (above 30:1), much of the available nitrogen pool will be immobilized by soil microbes, and therefore made unavailable to plants. Conversely, decomposition of organic matter high in nitrogen (C:N ratio lower than 30:1) increases the availability of nitrogen for plants by releasing nitrogen in excess of microbial demands. Sawdust is characterized by very high C content and high C:N ratio (Huang *et al.* 2004), C:N ratio of peat is lower (Comont *et al.* 2006). In a young blueberry plantation nitrogen deficiency explained poor growth of blueberry plants in the sawdust mulch variant, compared with peat mulch, because sawdust mulch increased the content of organic matter, but nitrogen content of soil was lower than in peat mulch variant (Paper I; Starast *et al.* 2002). Corbin and Antonio (2004) revealed the same problem with sawdust addition. Fertilization can relax the competition between plants and microbes, thus stimulating plant growth (Aber 1992), but in our experiment fertilization was not used. In the older plantation the increase of plant productivity was not so intensive in plastic mulch variant compared to peat mulch (Papers II; III). We can say nutrient deficiency appears in the plants growing in less fertile, light texture soil and when mineral nutrient and organic fertilizer were not applied. Nutrition problems did not appear in leaf analyses (Starast *et al.* 2004), but low N-tester readings affirmed it (Paper II).

### **Mycorrhizal colonization**

Statistical analysis assured that more hyphal fragments were numbered in the roots of 'Northblue' which was cultivated with plastic mulch (Paper IV). The average number of hyphae of cultivar 'Northblue' was significantly higher than in 'Northcountry'. The number of hyphae was higher in both cultivars if mulch was used. The mulch application increased 42% of mycorrhizal colonization as an average. The vegetative growth of a blueberry plant was strongly dependent on the number of hyphae and the positive correlation was seen between the yield and hyphal number. The diameter of observed roots was between 0.1 to 0.9 mm. A significant increase in the root diameter of a half-highbush blueberry was related to an increase in the number of hyphae in the root.

The observations of half-highbush blueberry roots showed all samples of roots were infected by mycorrhizal hyphae, but the degree of infection was different (Paper IV). It is claimed that some ericoid mycorrhizal species (*Oidiodendron*) can form intracellular hyphal coils (Read & Kearly 1999; Dalpe 1991). In our trial hyphal coils have not been observed. The cultivars of half-

highbush blueberry differed by mycorrhizal colonization (Paper IV). Eynard and Czesnik (1989) observed a higher level of mycorrhizal colonization on the roots of highbush blueberry cultivar 'Berkeley' and 'Herbert' in comparison with 'Bluecrop' and 'Darrow'. Also Scagel and Yang (2005), Czesnik and Eynard (1990) have affirmed the variation in root colonization with the cultivar. Mulch using increased significantly a number of hyphae and more colonized plants had a higher productivity in the experiment (Paper IV). Some other scientists have indicated a positive influence of mulching on mycorrhiza as well (Goulart *et al.* 1995; Scagel & Yang 2005). The soil amendments with peat showed a similar influence on mycorrhizal infection of blueberry cultivar 'Northland' (Tang XueDong *et al.* 2005). Mulches help to stabilize soil moisture (Blatt 1992). Jeliaskova and Percival (2003) have suggested that drought stress had no effect on mycorrhizal colonization levels of lowbush blueberry. Inoculation with an ericoid mycorrhizal isolates increased production of blueberries but there is variation of different isolate influence (Koron & Gogala 2000; Eccher & Noe 2002; Noe *et al.* 2002; Yang *et al.* 2002; Starrett 2003). However Yang *et al.* (1998) detected that field inoculation with *Oidiodendron maius* had no positive effect on highbush blueberry plant growth. More root growth, as determined by dry weight, was observed in mycorrhizal ones of highbush blueberry than non-mycorrhizal (Yang *et al.* 1996). The result of this study indicates a significant positive correlation between root diameter of half-highbush blueberry and a number of hyphae (Paper IV). Scagel and Yang (2005) have supplemented that root colonization by ericoid mycorrhizal fungi ranged from 0.5 to 44% of total root length. Colonization generally increased with increasing plant age with the highest levels of colonization in roots from the upper 15 cm of soil in younger plants and the highest levels of colonization in roots at the 15–30 cm depth in older plants. In our experiment it was a five-year-old plantation and the root samples were taken from upper 15 cm soil, because most blueberry roots were close to the soil surface (Paper IV). Stevens *et al.* (1997) have noted mycorrhizal infection intensity increased during the growing season. We took samples for observation only at the harvesting time, in August (Paper IV).

The influence of fertilization on mycorrhizal colonization in blueberry roots is not clear. When no mulch was employed, increasing N rate decreased mycorrhizal infection in the roots when plants were mulched, effects were inconsistent (Goulart *et al.* 1995). Using sawdust mulch and pre-planting amendment with sawdust had no relationship between mycorrhizal infection and N application. However, when either one or the other of the sawdust treatments was applied or neither, then there was a strong correlation between mycorrhizal infection and N level (Goulart *et al.* 1997). The combination effects of N, P, and K, in the form of ammonium sulfate, superphosphate, and murate of potash, respectively, on the mycorrhizal infection of wild blueberry in cropping stage of production were investigated. N, P, and K were applied at 0, 30, and 60 kg/ha in several different combinations. Mycorrhizal infection levels

did not differ among the studied treatment combinations (Jeliaskova & Percival 2003). Applying N + Mg + K had no effect on the level of mycorrhizal infection of roots, but P application had a slight effect (Scibisz *et al.*, 1990). Eccher *et al.* (2002) observed a strong interaction between mineral fertilization and mycorrhizal fungus: some of the tested strains doubled the effect of fertilizers as compared to others.

It is known that native blueberries populations have higher mycorrhizal infection than the commercial (Stevens *et al.* 1997). Thereby selected cultivation techniques must help to hold and increase mutually beneficial metabolic relationship existing between fungus and the plant root.

### **4.3. Chemical composition and quality of fruits**

#### **4.3.1. *Vaccinium taxa***

In the experiment the half-highbush blueberry cultivar ‘Northblue’ produced the largest berries (Paper VI). There was no significant difference in berry weight between bilberry and lowbush blueberry grown in different soils. An average berry weight of ‘Northcountry’ was smaller than ‘Northblue’ and bigger than lowbush blueberry and bilberry. Both ‘Northblue’ and ‘Northcountry’ are hybrids of a lowbush and a highbush blueberry and it is typical that ‘Northcountry’ have bigger berries (Lubi *et al.* 1989). ‘Northblue’ resembles highbush blueberries while ‘Northcountry’ is more like the lowbush blueberry.

Dry matter content was higher in the berries of ‘Northcountry’ (16.5%) and lowbush blueberry (15.8%) grown on mineral soil (Paper VI). The berries of bilberry (89.0%) and lowbush blueberry (90.2%), grown in peat soil contained more water. Skupień (2006) found in highbush blueberries average dry matter content ranged from 14.5% to 15.8%, as Mladin *et al.* (2006) showed ranging between 11% and 16%. Rejman and Pliszka (1991) have noted that 100 g of a fresh highbush blueberry fruit contains 83.4 g of water.

The amount of fat and protein was lower in ‘Northblue’ berries. Protein content was the highest in bilberry (0.67 g 100g<sup>-1</sup>) fruits (Paper VI). The titratable acidity was significantly different among taxa. It was the highest in bilberries and the lowest in ‘Northcountry’ berries. The berries of lowbush blueberry, grown in peat soil, had significantly higher protein and titratable acidity, compared with berries harvested from mineral soil. Mladin *et al.* (2006) found the acidity of blueberries could have extreme values from 0.4% to 2.3%. The berries of rabbiteye blueberry are more acidic than cultivars of highbush and lowbush blueberries (Prior *et al.* 1998). It was found in this study that the highest titratable acidity was in bilberries. Highbush blueberry contained 0.6 g 100g<sup>-1</sup> of protein and 0.6 g 100g<sup>-1</sup> of fat (Rejman & Pliszka 1991).

Soluble solids content is relatively high in blueberries (Bushway *et al.* 1983). In our experiment it ranged from 10.0 to 14.9 g 100g<sup>-1</sup>, with bilberries having

the lowest value (Paper VI). Prior *et al.* (1998) indicated the soluble solids content in lowbush blueberries was 14.3% which is comparable to the results of our study. They found much wider scope of soluble solids content in particular cultivars of highbush blueberry: 10.0–19.0%. Seasonal variation of soluble solids content in particular cultivars observed in this study reveals that the characteristic is more strongly conditioned by environmental than genetic factors. The sugar value largely ranging (7.4–2.8%) is showed by Mladin *et al.* (2006).

Glucose and fructose account essentially for all the sugar in lowbush blueberries (Barker *et al.* 1963). The berries of bilberry had the lowest level of glucose and fructose (Paper VI). Glucose content was the highest in the berries of ‘Northcountry’ while the fructose content was the highest in the lowbush blueberry. Free sugars, organic acids and amino acids are natural components of many fruits and vegetables and they play an important role in maintaining fruit quality and determining nutritive value. Acids and sugars are important components in a number of food products because of their important influence on organoleptic properties (Ashoor & Knox 1982). Right-time harvesting guarantees optimal ripening and the best quality of berries (Sousa *et al.* 2006). Fresh material with high quality requires little addition of sugars in processing and it is possible to have more natural food product (Wrolstad 1981). Such products are wanted in market recently when ecological mentality expands (Costanza *et al.* 1997)

The total energy values of the five *Vaccinium* taxa ranged from 26 kcal 100g<sup>-1</sup> to 43kcal 100g<sup>-1</sup> (Paper VI). The berries of bilberry had the lowest total energy values. The value was higher in lowbush blueberry and ‘Northcountry’ berries. Usui *et al.* (1994) have suggested the total energy value of lowbush blueberry is 52.5 kcal 100g<sup>-1</sup> and carbohydrate is the major contributor to this value whereas fat and protein content do not influence the total energy value.

In our study the content of vitamins showed a significant variation between cultivars and species. Ascorbic acid concentration was between 15 and 25 mg 100g<sup>-1</sup> (Paper VI). The ascorbic acid content was significantly lower in the berries of the half-highbush blueberry ‘Northblue’ compared with the other berries. However, Noormets *et al.* (2006) detected higher ascorbic acid concentration of the cultivar ‘Northblue’ compared with the lowbush blueberry and ‘Northcountry’. Prior *et al.* (1998) have shown that highbush blueberry has higher levels of ascorbate than lowbush blueberry and bilberry. In our experiment the content of ascorbic acid was higher than in the Prior *et al.* (1998) study. The ascorbic acid content is not a very stable parameter. The content is influenced by many factors of cultivation techniques including pruning, thinning (Lee & Kader 2000). Fertilization and climate conditions have an important influence (Noormets 2006) as well.

The average folic acid concentration of berries was higher in ‘Northcountry’ (Paper VI). Thiamin and pyridoxine contents were lower in bilberry and ‘Northcountry’. The average riboflavine concentration was 2.8 mg 100g<sup>-1</sup> and a

higher level had bilberry, lowbush blueberry from peat soil and 'Northcountry'. The highest content of nicotinic acid was found in lowbush blueberry from peat soil and the lowest level was found in bilberry berries and 'Northblue'. However, bilberry had a higher content ( $0.5 \text{ mg } 100\text{g}^{-1}$ ) of nicotinamide. Bushway *et al.* (1983) have suggested lowbush blueberry fruit are an excellent source of nicotinic acid and vitamin C. According to Rejman and Pliszka (1991) 100 g of fresh fruit contains 0.02 mg of vitamin B<sub>1</sub>, 0.02 mg of vitamin B<sub>2</sub>, 0.3 mg of vitamin PP, 16 mg of vitamin C, 289 I.E.

In our experiment an average content of anthocyanins was  $229 \text{ mg } 100\text{g}^{-1}$  (Paper VI). The lowest content was found in 'Northblue' berries and the highest in bilberry, respectively. Prior *et al.* (1998) reported blueberries to be one of the richest sources of antioxidant phytonutrients, and found a linear relationship between oxygen radical absorbing capacity of four *Vaccinium* species and anthocyanin or total phenolic content. However, it was observed that the antioxidant capacity in *Vaccinium* species and diverse small fruits is more highly correlated to total phenolics than to anthocyanins (Moyer *et al.* 2002), with lowbush blueberries having higher antioxidant capacity than highbush blueberries (Kalt *et al.* 2001; Beccaro *et al.* 2006). Anthocyanin contents are bigger in more colored and well ripened fruits (Jaakola *et al.* 2002). Anthocyanins are located mostly in blueberry fruit peel (Prior *et al.* 1998). However, the flesh of lowbush blueberry is more colored, flavonoid content is high and therefore an average anthocyanin content is bigger than in other cultivated blueberries. Smaller berries have greater fruit peel/flesh ratio that influences total anthocyanin content (Beccaro *et al.* 2006). By our results we can affirm: smaller berries have higher anthocyanin content (Paper VI). Highbush blueberries are reported to have an anthocyanin content 25–495  $\text{mg } 100\text{g}^{-1}$  while bilberry has 300–698  $\text{mg } 100\text{g}^{-1}$  (Mazza & Miniatti 1993). Li *et al.* (2006a) showed that the total content of anthocyanins was 343  $\text{mg } 100\text{g}^{-1}$  in bog bilberry fruit and 147  $\text{mg } 100\text{g}^{-1}$  in lingonberry fruit.

In the experiment the cultivation conditions of lowbush blueberry did not influence anthocyanin content (Paper VI). Plant nutrition (Delago *et al.* 2004) and water availability, temperature and the level of solar radiation influence the synthesis of phenolic compounds essentially (Bilyk & Sapers 1986; Jaakola *et al.* 2004). Besides, unfavorable environmental conditions for plant growth promote phenolic formation. Cultivation techniques influence antioxidant formation. Berries harvested from a peat plantation had the highest antioxidant capacity, the lowest coming from the bushes planted on Cocoa husk (Grajkowski *et al.* 2007).

The actual color of blueberries is deep purple-black, masked by a glaucous covering that gives the fruit a characteristic bluish color (Darrow & Camp 1945), which is responsible for the lightness or darkness of the berry blue color. Light blue color is the desired marketing characteristic (Galleta 1975). Surface color of bilberry was the darkest and less red (Paper VI). Remberg *et al.* (2006) have marked smaller fruits had darker color. The berries of lowbush blueberry

from peat soil were darker than that from mineral soil (Paper VI). The cultivar 'Northblue' had the bluest fruit, compared with the other taxa. The color was more saturated on bilberry than on the other species and cultivars. The value of hue angle (h) was higher in bilberry berries and lower in 'Northblue'. Austin and Bondari (1993) have indicated the color of berries depended on cultivar and it varied in different years. Sousa *et al.* (2006) have got the same results, moreover the fruit of rabbiteye blueberry presented lower values of lightness (L\*) and chroma (C\*) compared with highbush blueberry cultivars.

#### **4.3.2. *Rubus taxa***

Fruit dry matter and titratable acidity content was influenced neither by cultivation technology nor by an experimental year. It has been found before that cultivar characteristics and weather conditions have an effect on titratable acidity content (Haffner *et al.* 2002; Krüger *et al.* 2003). Raspberry fruit soluble solids content was significantly different in variants with mulch and without it in 2001 and 2003: fruits from control variant had significantly higher content (Paper VII). Blackberry fruit was significantly influenced by cultivation technology in 2001, when fruits from control variant had significantly higher soluble solids content. We may say that with cultivation technology we can also influence the content of total sugars in raspberries and blackberries, because earlier experiments (Kallio *et al.* 2000; Gonzales *et al.* 2002) have shown that soluble solids content in fruits is linearly related to the content of total sugars. In 2002 and 2003 raspberry fruit ascorbic acid content was significantly different in variants, whereby fruits from the variant without mulch had significantly higher content (Paper VII). However, blackberry fruit ascorbic acid content was not influenced by cultivation technology. In Estonian conditions average ascorbic acid content in raspberry fruits is recorded to be 31.6 mg 100g<sup>-1</sup> and in blackberry fruits 38 mg g<sup>-1</sup> (Parksepp 1977). Compared to cited findings, ascorbic acid content of fruits from both species was lower in our experiment. Ascorbic acid is the essential bioactive compounds in raspberry fruits beneficial for human health. (Parksepp 1977). Blueberries have no high ascorbic acid content (Paper VI; Prior *et al.* 1998). Thereby these berries are not a primary source of vitamin C for a human.

## 5. CONCLUSIONS AND PRACTICAL RECOMMENDATIONS

- It can be concluded that the amount of fertilizers by this study (Chapter 3.4.) recommended for cultivating half-highbush blueberry was sufficient to lower soil pH to a level suitable for good growth and yield of a lowbush blueberry. Soil acidity increased when elemental sulphur was used, but on loamy sand soil with light texture this treatment did not increase plant productivity. The influence of sulphur on soil pH began to decrease after four years of the application. However, it affected earlier on the soil with light texture.
- Compared with sawdust and peat mulch plastic mulch application decreased the winter hardiness of the half-highbush blueberry cultivar ‘Northcountry’ in a young plantation, although ‘Northcountry’ was more winter hardy than ‘Northblue’. The use of organic mulches and the application of organic material in the soil reduced soil pH and influenced nutrient status in a plant. Using mulches increased the blueberry productivity, the plants had the best growth and yield in variants where peat mulch was used. In a young plantation blueberries had a better growth if plastic mulch was applied. The positive effect of plastic mulch decreased in an older plantation caused by the nutrition deficiency on less fertile soil. The positive effect of sawdust was noticed from the fifth experimental year: the plant productivity increased significantly. The stress of nutrition deficiency determined poor plant growth in a young plantation. Peat amendment in the soil before planting increased plant growth and the average yield was 68% higher with mulch addition.
- Mulch application significantly increased mycorrhizal colonization, besides the number of hyphae in ‘Northblue’ was significantly higher than that of ‘Northcountry’. The higher level of mycorrhizal colonization had a positive influence on the plant growth and the yield.
- Considering the results of the study we can characterize different *Vaccinium* taxa as follows. The berries of bilberry have dark-blue color with acid taste. The fruit contain more anthocyanin. Total energy value is very low. ‘Northblue’ half-highbush blueberry have big, light-blue berries with a low level of fat, protein, anthocyanin and vitamins. The berries of ‘Northcountry’ are of medium size, sweet in taste and contain more folic acid and riboflavin. Lowbush blueberry has small and sweet berries. The cultivation environment influences the chemical composition of the fruit. The cultivation on peat soil increases the content of water, protein, titratable acidity, riboflavin, nicotinic acid and pyridoxine. However, pyridoxal content decreases on the peat soil.

- Cultivation methods influenced the chemical content of *Rubus* fruit. We suggest that fruit from the plants grown without mulch contain more soluble solids.

On the basis of the obtained experimental results it can be concluded that lowbush blueberry and half-highbush blueberry tolerate Estonian environmental conditions and the cultivation of these plants is possible. Thereby using different agronomic techniques can increase plant productivity and quality of berries. The influence of different soil conditions is remarkable. Sulphur can be recommended in nutrient rich soil to increase soil acidity, but not for nutrient poor soil with light texture, where the application of only NPK fertilizers is more effective. In South Estonia on the widely distributed light texture soils, where the soil reaction is less suitable for blueberry. It is possible to obtain a higher yield from half-highbush blueberry plants by using mulches. It is important to point out the influence of different mulches changed during a five-year period. Plastic mulch had a strong positive effect in a young plantation, but the influence disappeared in an older plantation. If sawdust mulch was used in a young plantation, blueberry plant had poor growth. In an older plantation this effect was reverse. For intensive cultivation sawdust and plastic mulches are recommended, but additional nutrition using of fertilizers is needed. In the organic farming condition peat mulch is the best way to get a high yield of blueberries. Irrigation is certainly needed to avoid drought stress of plants. Peat application in the soil can improve the soil condition for blueberries. Thereby plant productivity increases in case of both cultivation types.

It is not known if a portable chlorophyll meter, like N-Tester, has been used to measure blueberry leaves before. In our experiment a strong relationship between chlorophyll meter readings and nutrients content in leaves, plant growth and yield was indicated. Therefore we can recommend N-Tester for the evaluation of blueberry plants.

Considering the results of the study cultivation technologies have been worked out for half-highbush and lowbush blueberries in the Estonian condition. A handbook “Kultuurmustikas ja selle kasvatamine Eestis” (“Blueberry cultivation in Estonia”) (Starast *et al.* 2005a) was published in the Estonian language. Practical recommendations for blueberry cultivators are presented there. The half-highbush blueberry cultivars ‘Northblue’ and ‘Northcountry’ were nominated in the list of recommended fruit and berry cultivars in Estonia (Eesti puuvilja- ja marjakultuuride soovitussortiment 2007).

The blueberry trial plants of seven-eight-year blueberries become old, the vegetative growth decreases and cutting or pruning of bushes is needed. Our work group started with half-highbush blueberry study where different methods of pruning were used. Blueberries have a good resistance to plant diseases. Therefore organical cultivation experiment was established in peat soil in 2006. Good results of studies have inspired us to establish new trials with other half-highbush, northern highbush blueberry and blackberry cultivars. We began to

investigate the carbon balance in a lowbush blueberry plantation in peat soil and how restoration with blueberries helps to restore the biodiversity of flora and fauna in the area of an abandoned peat pit.

In future it is necessary to pay more attention to the chemical composition of processed blueberries and to the possibilities of using berries as raw material for functional food.

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## 7. SUMMARY IN ESTONIAN

### **Kasvatustehnoloogiate mõju mustika (*Vaccinium*), vaarika ja pampli (*Rubus*) produktiivsusele ning viljade kvaliteedile**

Kultuurmustikate kasvatamine on maailmas üha suuremat populaarsust võitnud kuna inimesed on õppinud seda väärtuslikku marjakultuuri hindama. Kõige pikaajalisemad kogemused ahtalehise mustika (*Vaccinium angustifolium* L.) ja kännasmustika (*V. corymbosum* L.) kultiveerimisel on USA-s ja Kanadas. Eestis on kultuurmustikate kasvatamine uus haru aianduslikus ettevõtluses, kusjuures esimesed tootmisistandikud rajati kümme aastat tagasi. Vaarika- ja pampli kasvatusega tegeletakse meil juba pikka aega ning pampli kasvatusega on alustanud esimesed marjatootjad. Käesoleva uurimistöö eesmärgiks oli välja selgitada: 1) väävlit ja happeliste väetiste mõju ahtalehise mustika produktiivsusele (artikkel V); 2) multside ja kasvupinnasele lisatud turba mõju poolkõrge kasvuga mustikasortide (*V. corymbosum* x *V. angustifolium*) 'Northblue' ja 'Northcountry' taimede talvekindlusele, kasvule, saagikusele, hinnates nende sortide kasvatusvõimalusi intensiiv- ja maheviljeluse tingimustes (artiklid I, II, III); 3) erinevate agronoomiliste võtete mõju sortide 'Northblue' ja 'Northcountry' juurte nakatumisele mükoriisaseentega (artikkel IV); 4) erinevate mustika taksonite viljade keemiline koostis sõltuvalt taimede kasvukeskkonnast (artikkel VI); 5) vaarika (*Rubus idaeus* L.) ja pampli (*R. fruticosus* coll.) viljade keemiline koostis sõltuvalt kasvatusviisist (VII). Poolkõrge kasvuga mustikate katseistandik paiknes Tartu maakonnas, Kambjas ning katseandmeid koguti seal 1998, 1999 (artikkel I), 2001 (artiklid III, IV), 2002 (artikkel III) ja 2003 (artikkel II) aastal. Ahtalehise mustika istandikud asusid Tartu maakonnas Vasulas ja Saare maakonnas Kärlal (artikkel V). Andmeid koguti nimetatud istandikest 2001, 2002, ja 2003 aastal. Viljade keemiliseks analüüsiks koguti 2003. aastal hariliku mustika (*V. myrtillus* L.) marjad looduslikust kasvukohast, Võru maakonnast Ruusmäelt (artikkel VI). Ahtalehise mustika vilju koguti tootmisistandikest Võru maakonnast Ruusmäelt (mineraalmullal) ning Harju maakonnast, Ardust (turvasmullal). Poolkõrge kasvuga mustikate viljad korjati Tartu maakonnas Kambja vallas paiknevast tootmisistandikust. Perekonna *Rubus* taksonite katseistandik paiknes Eesti Maaülikooli katseaias, Tarus. Katsetöid teostati 2001, 2002, 2003 ja 2004 aastal.

Katsetulemustest selgus, et happeliste väetistega on võimalik alandada mulla pH-d ja seeläbi parandada ahtalehise mustika taimede kasvu ja saagikust (artikkel V). Mulla happelisus suurenes, kui kasutati väävlit, kuid kerge lõimise-ga liivsavi mulla puhul ei suurendanud see mustikataimede produktiivsus. Poolkõrge kasvuga mustikasortide katses selgus, et noores istandikus kilemultš vähendas sordi 'Northcountry' taimede talvekindlust, võrreldes saepuru- ja turbamultšiga (artikkel I). Sort 'Northcountry' oli parema talvkindlusega, kui

'Northblue'. Orgaanilisest materjalist multšid ja turba lisamine kasvupinnasele suurendas mulla happesust ning mõjutas taimede toitainetega varustatust (artiklid II ja III). Multšimine suurendas mustikataimede produktiivsust, kusjuures parima kasvu ja saagikusega olid taimed turbamultši puhul (artiklid I, II, III ja IV). Noores istandikus soodustas kilemultš taimede kasvu, kuid positiivne efekt vähenes vanemas istandikus, mida võis põhjustada toitainete defitsiidist tingitud madal mullaviljakus. Saepurumultši positiivne mõju ilmes aga vanemas istandikus, kusjuures noortel taimedel täheldati toitainete puudusest tingitud tagasihoidlikku kasvu. Turba lisamine mullale, enne istandiku rajamist, parandas mustikataimede produktiivsust keskmiselt 68%. Multšide kasutamine suurendas oluliselt ka mükoriisaseente olemasolu, kusjuures rohkem hüüfe loendati sordi 'Northblue' juurtes (artikkel IV). Suurem mükoriisaseentega nakatumine soodustas oluliselt taimede kasvu ja saagikust. Kasvatuse viis mõjutas vaarika ja pampli keemilist koostist: suurema rakumahla kuivaine sisaldusega viljad saadi multšita kasvanud taimedelt (artikkel VII).

Lähtuvalt kogutud andmetest iseloomustab hariliku mustika vilju tumesinine värvus ja hapukas maitse. Antotsüaanide sisaldus viljades oli kõrge, kuid energiatiline väärtus väike (artikkel VI). Mustikasordi 'Northblue' viljad olid küll suured, kuid rasva, proteiinide, antotsüaanide ja vitamiinide sisaldus oli väike. Sordil 'Northcountry' olid keskmise suurusega viljad, maitselt olid marjad magusad ning sisaldasid rohkem vitamiine B<sub>2</sub> ja B<sub>9</sub>. Ahtalehise mustika marjad olid väikesed, kuid magusad. Kasvukeskkond mõjutas oluliselt viljade keemilist koostist: turvasmullal, ammendatud freesturbaväljal, kasvanud taimede marjad sisaldasid rohkem vett, valke, orgaanilisi happeid, riboflaviini, nikotiinhapet ja püridoksiini.

Kokkuvõtlikult võib väita, et ahtalehine mustikas ning poolkõrge kasvuga mustikad taluvad hästi Eesti keskkonnatingimusi ja neid on võimalik siin edukalt kultuuris viljeleda. Erinevate kasvatustehnoloogiliste võtetega saab suurendada taimede produktiivsust ja viljade kvaliteeti. Mullastikutingimustel on märkimisväärne mõju taimede kasvule ja arengule. Väävlit võib soovitada mullahappesuse suurendajana eelkõige toitainete rikkal mullal. Kerge lõimise, toitainete vaesel mullal on aga efektiivsem füsioloogiliselt happeliste väetiste kasutamine. Kerge lõimisega ja vähehappelistel muldadel saab mustikataimede kasvutingimusi parandada multšimisega. Oluline on märkida, et multšide kasutamine pikema perioodi jooksul mõjutab taimede kasvu aasta-aastalt erinevalt. Kilemultšil on tugev positiivne mõju taimedele noores istandikus, kuid vanemas istandikus see efekt väheneb oluliselt. Saepurumultši puhul on taimede kasv pärsitud nooremate taimede puhul, kuid pikemaegne saepuru kasutamine mõjub positiivselt. Kile- ja saepurumultš sobivad mustikate kasvatamiseks intensiivtehnoloogiast lähtuvalt, sealjuures on vajalik taimede väetamine. Maheviljeluse puhul on sobiv kasutada turbamultši tagamaks mustikataimede kõrge saagikus. Viimasel juhul on vajalik kasutada kunstlikku niisutamist, et vältida taimedel põuastressi. Istutuseelne turba lisamine mullale

parandab mullatingimusi ning seeläbi suureneb oluliselt ka mustikataimede produktiivsus.

Taimede seisukorra hindamiseks saab edukalt kasutada kaasaskantavat klorofüllmeetrit N-tester (SPAD 500). Seda seadet on tarvitatud mitmete kultuurtaimede puhul, kuid pole varem rakendust leidnud mustikatel. Meie katsetes täheldati tugevat seost klorofüllmeetri lugemi ja lehe toitainete sisalduse, taimede kasvuparameetrite ning saagikuse vahel (artiklid II, III ja V).

Käesoleva uurimistöö tulemusi kasutades on koostatud esimene eestikeelne mustikakasvatust käsitlev käsiraamat “Kultuurmustikas ja selle kasvatamine Eestis” (Starast *et al.* 2005a), kust leiavad praktilisi soovitusi nii mustikakasvatajates ettevõtjad kui ka hobiaednikud. Poolkõrge kasvuga mustikasordid ‘Northblue’ ja ‘Northcountry’ on kinnitatud Eestis soovitatud sortide nimekirja (Eesti puuvilja- ja marjakultuuride soovitusisortiment 2007).

Positiivsed katsetulemused on innustanud meie töörühma jätkama ja laiendada kultuurimustikatega seotud uurimistööd. Alustatud on katseid erinevate lõikusviiside rakendamisega vananevas mustikaistandikus. Mustikate hea resistentsus taimehaigustele võimaldas rajada uusi maheviljeluse katseid ka mahajäetud freesturbaaladele. Rajatud on kännasmustikaistandik 15-e põhjapoolsematesse tingimustesse sobiva sordiga ning vaatluse all on ka teised pamplisordid. Mahajäetud turbaväljadele rajatud ahtalehise mustika istandikes oleme alustanud süsinikuringe ja loodusliku mitmekesisuse taastamise alaseid uurinuid. Tulevikus pöörame suuremat tähelepanu töödeldud mustikate keemilisele koostisele, leidmaks sobivamaid sorte funktsionaalse toidu tooraineks.

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## **PUBLICATIONS**

# CURRICULUM VITAE

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**Date and place of birth:** 02.01.1967, Paide, Estonia  
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### Education

1997–1998 Estonian Agricultural University, Faculty of Agronomy (MSc study)  
1991–1997 Estonian Agricultural University (undergraduate study)  
1985–1988 R apina Horticultural College  
1974–1985 Secondary School of Koeru

### Positions held

1997– Estonian Agricultural University, Faculty of Agronomy, Department of Horticulture (since 2005 – Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences), researcher  
1995–1996 Estonian Agricultural University, Faculty of Agronomy, Department of Horticulture, laboratory assistant  
1988–1995 Estonian Agricultural University, Faculty of Agronomy, Department of Horticulture, agronomist

### Main field of research:

Cultivation and quality of berries

### Participation in research projects

- Project of Environmental Investment Centre “Suitability of exhausted peat pits for berry production in West Estonia”. 2006–2007. (Project leader)
- Project of Enterprise Estonia “Blueberries as raw material for functional food”. 2006. (Project leader)
- Project of Enterprise Estonia “Cultivation technologies and selection of low-bush blueberry”. 2001–2005. (Project leader)
- 12 projects in total, 1997–2008 (Participator)

### **Acknowledgments**

- 2002 The prize of a young scientist of Estonian Academic Agricultural Society  
1998 The general prize of the competition of student research work of Estonian Academy of Sciences (MSc thesis – “Arctic Bramble (*Rubus arcticus* L.) – a New Berry Culture in Estonia”)  
1997 The second prize (for graduation paper) of the competition of student research work of Estonian Science Foundation and Ministry of Education

### **Scientific – organizational and administrative activities**

- Member of International Society of Horticultural Science (since 2003)

### **Supervised master’s and bachelor’s theses**

- |             |     |      |  |
|-------------|-----|------|--|
| M. Miländer | MSc | 2008 | The influence of cultivar on the biochemical content of grape  |
| K. Zimmer   | MSc | 2007 | The influence of fertilization on growth and yield of lowbush ( <i>Vaccinium angustifolium</i> Ait.) blueberry in young plantation |
| T. Albert   | MSc | 2005 | The fertilization of the lowbush blueberry ( <i>Vaccinium angustifolium</i> Ait.) on mineral soil                                  |
| E.Vool      | MSc | 2003 | The yield and berry quality of different <i>Rubus</i> taxa   |
| P. Järvan   | MSc | 2003 | The influence of pollination on the productivity of half-highbush blueberry cultivar Northblue                                     |

18 bachelor’s theses in total.

### **Participation in international conferences**

- “9<sup>th</sup> International *Vaccinium* Symposium”. 2008, Corvallis, U.S.
- “The Northern Region Grape Growers and Wine-Makers’ conference”. 2006, Jurmala, Latvia.
- “Effect of Pre- and Post-Harvest Factors on Health Promoting Components and Quality of Fruits and Vegetables”. 2006, Skierniewice, Poland.
- “The Third International Conference of Plant Protection Research Institute”. 2005, Giza, Egypt.
- “Culture of berrybush of *Vacciniaceae*: results and prospects”. 2005, Minsk, Belarus.
- 8th International Symposium on *Vaccinium* Culture. 2004, Oeiras/Portugal; Seville/Spain
- “Fertilizers in context with resource management in agriculture”. Debrecen, Hungary.
- “Cultivation of wild berries”. 2003, Skierniewice, Poland.
- “Small Fruit in the Wild and Culture. August 2002, Kaunas, Lithuania.
- “Foliar Nutrition of Perennial Fruit Plants”. 2001, Merano, Italy.
- “Wild berry culture; an exchange of western and eastern experiences”. 1998, Tartu, Estonia.

## Trainings

- The training of sensory tasting, Tallinn University of Technology, 2008.
- The advanced training course of wild berry cultivation, August 2007, Novosibirsk, Russia.
- The advanced training of self-expression, Estonian University of Life Sciences, 2007.
- The advanced training of lecturers, University of Tartu, 2006.
- The teaching and study in university. Pedagogical University of Tallinn, 2004.
- The advanced training of fertilization. University of Debrecen, Hungary, 2003.
- The advanced training course of blueberries cultivation. Nova Scotia Agric. Coll., Canada, 2000.
- Nordic Postgraduate Course “How to write a scientific paper”. Tartu, Estonia, 2000.
- Nordic Postgraduate Course “Advisory systems for reduced pesticide use in agriculture and horticulture”. Tartu, Estonia, 1999
- Nordic Postgraduate Course “Practical statistical data analysis”. Jelgava, Latvia, 1999.
- The advanced training course of berries cultivation. The Danish Royal University of Veterinary and Agriculture, Kopenhagen, 1999.
- The advanced training course of horticulture. Gjennestad Hort. Coll. Norway, 1996.

## Publications

### Pre-reviewed articles in international journals (ISI Web of Science);

1. **Starast, M.**, Karp, K., Vool, E., Paal, T., Albert, T. 2007. Effect of NPK fertilization and elemental sulphur on growth and yield of lowbush blueberry. *Agricultural and Food Science*, 16 (1): 34–45
2. Vool, E., Karp, K., Moor, U., **Starast, M.** 2007. Yield quality in some taxa of the genus *Rubus* depending on the cultivation technology. *European Journal of Horticultural Science*, 72(1): 32–38.
3. Moor, U., Karp, K., Põldma, P., Asafova, L., **Starast, M.** 2006. Post-harvest disorders and mineral composition of apple fruits as affected by pre-harvest calcium treatments. *Acta Agriculturae Scandinavica, B*, Vol. 56, pp. 179–185.

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1. Moor, U., Karp, K., Põldma, P., **Starast, M.** 2007. Effect of 1-MCP treatment on apple biochemical content and physiological disorders. *Acta Agronomica Hungarica*, 55(1), 61–70.
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3. **Starast, M.**, Karp, K., Vool, E., Moor, U. 2005. The cultivation of half-highbush blueberry under organic farming condition. *Egypt. J. Agric. Res.*, 83(1): 155–168.

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8. Kukin, M., Karp, K., **Starast, M.** 2001. Juurevälise väetamise mõju aedmaasika sordi 'Senga Sengana' saagile. *EPMÜ teadustööde kogumik* 212: 141–144. (in Estonian with English abstract)
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9. Karp, K., **Starast, M.** 2002. Effects of springtime foliar fertilization on strawberry yield in Estonia. *Acta Hort.* 594: 501–505.

#### Articles in proceeding of international conferences

1. **Starast, M.**; Paal, T.; Vool, E.; Karp, K.; Noormets, M. 2005. Effect of hexazinone in young low-bush blueberry plantation. Scientific international conference *Kultura brusnitshnik jagodnikov: itogi I perspektivy*. August 15–19, 2005. Minsk: Smeltok, 2005, 85 – 89
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6. **Starast, M.**, Karp, K., Moor, U., Vool, E., Paal, T. 2003. Effect of fertilization on soil pH and growth of lowbush blueberry (*Vaccinium angustifolium* Ait.). *Fertilizers in context with resource in agriculture. 14th International Symposium of Fertilizers June 22–25, 2003, Hungary*, 628–635.
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2. Moor, U., **Starast, M.** 2007. Õunad ja kemikaalid. *Tervis Pluss*. August, lk. 70–73.
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17. **Starast, M.** Maasikad potis ja rõdukastis. Ajalehe "Maaaleht" lisa "Targu talita". 13.mai 1999. lk 332–333.
18. **Karp, K., Starast, M., Värnik, R.** 1999. Maasikakasvatus kui tasuv alternatiivtootmine väiketaludes. Maakodu 6: 17–18.
19. **Starast, M.** 1999. Kääbusõunapuud – mugav saaki korjata. Aed ja Kodu, 5: 24.
20. **Starast, M., Karp, K., Tasa, T.** 1999. Mitmesugused kasvatustehnoloogiad metsamarjade viljelemisel. – Teaduselt põllule ja aeda. Jäned. 226–230.
21. **Starast, M.** 1999. Sammasõunapuu – baleriina. Aed ja Kodu, 5: 25.
22. **Starast, M.** 1998. Erilisi ripp- ja ronimaasikaid pole olemas. Aiakiri 8: 24–25.
23. **Starast, M.** 1998. Maasikas, kaunis dekoratiivtaim. Ajalehe "Postimees" eriväljaanne "Aed" 30.aprill 1998. lk. 14–15.
24. **Starast, M.** 1997. Mesimurakas istandusse. Aiakiri 3: 42–45.
25. Karp, K., **Starast, M.** 1996. Mesimurakas võib olla perspektiivne. Maakodu 6: 3.

**Book (in Estonian)**

**Starast, M., Karp, K., Paal, T., Värnik, R., Vool, E.** 2005. Kultuurmustikas ja selle kasvatamine Eestis. Tartu: Eesti Põllumajandusülikool.

# ELULOOKIRJELDUS

## MARGE STARAST

**Sünniaeg ja koht:** 02.01.1967., Paide, Eesti  
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### Haridus

- Eest Põllumajandusülikool, põllumajandusteaduse magister, 1998
- Eest Põllumajandusülikool 1997
- Rápina Sovhoostehnikum 1988
- Koeru Keskkool 1985

### Töökogemus

- Eest Põllumajandusülikool, aianduse instituut, alates 1997.a. teadur
- Eest Põllumajandusülikool, aianduse instituut, 1995–1996, laborant
- Eest Põllumajandusülikool, 1988–1995, aiandusagronoom

### Peamised uurimisvaldkonnad:

marjakultuuride agrotehnika ja viljade kvaliteet

### Osalemine uurimisprojektide täitmisel:

- Keskkonnainvesteeringute Keskuse projekt: “Mõnede Lääne –Eesti maha- jäetud turbaalade sobivus marjakasvatuseks”. 2006–2007, EMÜ projektijuht.
- Ettevõtluse Arendamise Sihtasutuse projekt: “Kultuurmustikad spetsiaal- toodete toorainena”. 2006, projektijuht.
- Ettevõtluse Arendamise Sihtasutuse projekt “Ahtalehise mustika kasvatus- tehnoloogiad ja sordiaretus” (2001–2005), projektijuht;  
12 projekti, milles osalenud täitjana, 1997–2008.

### Tunnustused

2002. a. Akadeemilise Põllumajanduse Seltsi noorteadlase preemia  
1998. a. Eesti Teaduste Akadeemia korraldatud teadustööde konkursil pea- preemia magistritööle “Mesimurakas (*Rubus arcticus* L.) – uus marja- kultuur Eestis”  
1997. a. Eesti Teadusfondi ja Eesti Haridusministeeriumi korraldatud üliõpi- laste teadustööde konkursi II preemia diplomitööle

### **Osalemise erialastes organisatsioonides**

- Rahvusvahelise organisatsiooni “International Society Horticultural Science” (ISHS) liige alates 2003. a.

### **Juhendamisel kaitstud väitekirjad:**

- M. Miländer “Viinamarjade keemiline koostis sõltuvalt sordist”, 2008.
- K. Zimmer (Mc.S.) “Väetamise mõju ahtalehise mustika (*Vaccinium angustifolium* Ait.) kasvule ja saagikusele noores istandikus”, 2007.
- T. Albert (Mc.S.) “Ahtalehise mustika (*Vaccinium angustifolium* Ait.) väetamine mineraalmullal”, 2005.
- E. Vool (Mc.S.) “Perekond *Rubus* erinevate taksonite saagikus ja viljade kvaliteet”, 2003.
- P. Järvela (Mc.S.) “Tolmlemise mõju hübriidmustiks sordi ‘Northblue’ produktiivsusele”, 2003.

Juhendamisel kaitstud 18 bakalaureuse tööd.

### **Osalemise rahvusvahelistel konverentsidel**

- “9<sup>th</sup> International *Vaccinium* Symposium”, 2008, Corvallis, USA.
- “The Northern Region Grape Growers and Wine-Makers’ conference”, 2006, Jurmala, Läti
- “Effect of Pre- and Post-Harvest Factors on Health Promoting Components and Quality of Fruits and Vegetables”, 2006, Skiernewice, Poola.
- “The Third International Conference of Plant Protection Research Institute”, 2005, Giza, Egiptus.
- “Culture of Berrybush of *Vacciniaceae*: Results and Prospects”, 2005, Minsk, Valgevene
- “8th International Symposium on *Vaccinium* Culture”, 2004, Portugal/ Hispaania.
- “Fertilizers in Context with Resource Management in Agriculture”, 2003, Debrecen, Ungari
- “Cultivation of Wild Berries”, 2003, Skiernewice, Poola
- “Small Fruit in the Wild and Culture”, 2002, Kaunas, Leedu
- “Foliar Nutrition of Perennial Fruit Plants”, 2001, Merano, Itaalia
- “Wild berry culture; an exchange of western and eastern experiences”, 1998, Tartu, Estonia

### **Erialane enesetäiendus**

- Sensoorse analüüsi algkursus, TTÜ, 2008
- Metsanmarjade kultuuristamine, Novosibirsk, Venemaa, 2007.
- “Eneseväljenduskunsti alused”, EMÜ, 2007
- “Lektori kutseoskuste arendamine”, TÜ ja EMÜ, 2006
- “Õppimine ja õpetamine kõrgkoolis.” 2004.a., TPÜ

- Aiakultuuride väetamisalane enesetäiendus Ungaris. Debreceni Ülikool, Põllumajandusteaduse Keskus, 2003
- Mustikakasvatuse alane täiendus, Novo Scotia Agricultural College, Kanada, 2000
- NOVABA kursus “How to write a scientific paper”, 2000, Tartu
- NOVABA kursus “Advisory systems for reduced pesticide use in agriculture and horticulture”, 1999, Tartu
- NOVABA kursus “Practical statistical data analysis”, 1999, Jelgava, Läti
- Marjakasvatuse alane täiendus Taani Kuninglikus Veterinaaria ja Põllumajanduse Ülikoolis, Kopenhaagen, 1999
- Aiandusala enesetäiendus., Norra, Gjennestad Aianduskolledž, 1996

### **Publikatsioonide loetelu**

#### **Artiklid ajakirjades, mida katab ISI Web of Science**

1. **Starast, M.**, Karp, K., Vool, E., Paal, T., Albert, T. 2007. Effect of NPK fertilization and elemental sulphur on growth and yield of lowbush blueberry. *Agricultural and Food Science*, 16 (1): 34–45
2. Vool, E., Karp, K., Moor, U., **Starast, M.** 2007. Yield quality in some taxa of the genus *Rubus* depending on the cultivation technology. *European Journal of Horticultural Science*, 72(1): 32–38.
3. Moor, U., Karp, K. Põldma, P., Asafova, L., **Starast, M.** 2006. Post-harvest disorders and mineral composition of apple fruits as affected by pre-harvest calcium treatments. *Acta Agriculturae Scandinavica, B*, Vol. 56, pp. 179–185.

#### **Artiklid muudes rahvusvahelistes, eelretsenseeritavates ajakirjades**

1. Moor, U., Karp, K., Põldma, P., **Starast, M.** 2007. Effect of 1-MCP treatment on apple biochemical content and physiological disorders. *Acta Agronomica Hungarica*, 55(1), 61–70.
2. **Starast, M.**, Karp, K., Vool, E., Moor, Tonutare, T., Paal, T. 2007. Chemical Composition and Quality of Cultivated and Natural Blueberry Fruit in Estonia. *Vegetable Crops Research Bulletin*, 66: 143–153.
3. **Starast, M.**, Karp, K., Vool, E., Moor, U. 2005. The cultivation of half-highbush blueberry under organic farming condition. *Egypt. J. Agric. Res.*, 83(1): 155–168.
4. Paal, T., **Starast, M.**, Karp, K. 2004. Influence of different fertilisers and fertilising frequency on the development of *Vaccinium angustifolium* seedlings. *Botanica Lithuanica* 10(2): 135–140.
5. Karp, K., Mänd, M., **Starast, M.**, Paal, T. 2004. Nectar production of *Rubus arcticus*. *Agronomy Research* 2(1): 57–62.
6. Karp, K., **Starast, M.**, Värnik, R. 1997. The Arctic bramble (*Rubus arcticus* L.) – the most profitable wild berry in Estonia. *Baltic Forestry*. 2:47–52.

#### **Artiklid muudes rahvusvahelistes ajakirjades**

1. **Starast, M.**, Karp, K., Vool, E., Paal, T., Moor, U. 2005. Kärpimise mõju ahtalehise mustika seemikute kasvule. *EPMÜ teadustööde kogumik Agronoomia 2005*, 220: 111–113.

2. Albert, T, Karp, K., **Starast, M.** 2004. Väetamise mõju ahtalehise mustika (*Vaccinium angustifolium*) saagile. *Agronoomia* 2004. EPMÜ tead. tööde kogumik 219: 109–111.
3. **Starast, M.**, Vool, E., Karp, K. 2004. Pamplisordi 'Agawam' ja läänevaarikasordi 'Jewel' produktiivsus. *Agronoomia* 2004. EPMÜ tead. tööde kogumik 219: 103–105.
4. Karp, K., Uueni, K. Lipping, P., **Starast, M.** 2004. Mesimuraka taimede saagikus. EPMÜ tead.tööde kog. 219:115–117.
5. Paal, T., Karp, K., **Starast, M.** 2003. Hariliku pohla ja ahtalehise mustika seemne idanemisest ja ahtalehise mustika seemikute väetamisest. *Metsanduslikud uurimised*, 38: 125–133.
6. Noormets, M., Karp, K., **Starast, M.**, Paal, T., 2002. Väetamise mõjust ahtalehise mustika (*Vaccinium angustifolium* Ait.) seemikute saagikujunemisele viljakandvas istanduses ammendatud freesturbaväljal. *Agraarteadus XIII (5)*, 293–303.
7. **Starast, M.**, Karp, K., Tasa, T., Järvela, P. 2001. Multšide ja kasvusubstraadi mõju poolkõrge mustika (*Vaccinium corymbosum* x *V. angustifolium*) sortide 'Northblue' ja 'Northcountry' saagile. – EPMÜ teadustööde kogumik 213: 152–155.
8. Kukin, M., Karp, K., **Starast, M.** 2001. Juurevälise väetamise mõju aedmaasika sordi 'Senga Sengana' saagile. EPMÜ teadustööde kogumik 212: 141–144.
9. Mänd, M., Karp, K., **Starast, M.** 2001. Meemesilase (*Apis mellifera* L.) korjekäitumine mesimuraka (*Rubus arcticus* L) istanduses. – EPMÜ teadustööde kogumik 212: 184–187.
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13. Karp, K., **Starast, M.**, Värnik, R. 2000. Erinevate maasikasortide saagikus ja tulusus. – EPMÜ tead. tööde kogumik 208. 56–59.
14. Karp, K., **Starast, M.**, Tiido, T. 2000. Frost damages of arctic bramble (*Rubus arcticus*) and half-highbush blueberry (*Vaccinium corymbosum* x *Vaccinium angustifolium*) depend on cultivation methods. – *Proceedings of the International Conference: Fruit Production and Fruit Breeding. Tartu, 207*: 244–247.
15. **Starast, M.**, Karp, K., Pae, A. 2000. Orgaaniliste hapete, askorbiinhappe ja suhkrute sisaldus mesimuraka viljades olenevalt sordist ja kasvukohast. – *Agraarteadus XI*. 81–87
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1. Moor, U., Karp, K., Põldma P., **Starast, M.** 2008. Influence of preharvest calcium treatments on apple soluble solids, titratable acids and vitamin C content at harvest and after storage. Acta Hort. 768: 49–55.
2. Kaldmäe, H., **Starast, M.**, Karp, K. and Paal, T. 2006. Effect of donor plant physiological condition on in vitro establishment of *Vaccinium angustifolium* shoot explants. Acta Hort. 715:433–438.
3. **Starast, M.**, Kõljalg, U., Karp, K., Vool, E., Noormets, M., Paal, T. 2006. Mycorrhizal colonization of half-high blueberry cultivars influenced by cultural practices. Acta Hort. 715:449–454.
4. Karp, K., Noormets, M., **Starast, M.**, Paal, T. 2006. The influence of mulching on nutrition and yield of 'Northblue' blueberry. Acta Hort. 715:301–306.
5. Noormets, M., Karp, K., **Starast, M.**, Leis, L., Muru, K. 2006. The influence of freezing on the content of ascorbic acid in vaccinium species berries. Acta Hort. 715:539–544.
6. Karp, K., **Starast, M.**, Kaldmäe, H. 2002. Influence of the age of plants and foliar fertilisation on the yield of strawberry cultivar Jonsok under plastic mulch. Acta Hort., 567: 459–462.
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1. **Starast, M.**; Paal, T; Vool, E; Karp, K. Noormets, M. 2005. Effect of hexazinone in young low-bush blueberry plantation. Scientific international conference Kultura brusnitshnik jagodnikov: itogi I perspektivy. August 15–19, 2005. Minsk: Smeltok, 2005, 85–89
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the 1 Congress of the Int. Seabuckthorn Association. September 14–18, 2003, Berlin. 181–185.

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#### **Populaarteaduslikud artiklid.**

1. **Starast, M.** 2007. Tervistavad mustikad koduaias. Kodukiri. Aed, sept/okt. 2007, lk.36–41.
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7. **Starast, M.** 2003. Mustikas Saaremaal. Maakodu 10: 62.
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9. **Starast, M.** 2003. Viljapuude kasvatamine spaleeril. Ajalehe “Postimees” eriväljaanne “Remont, kodu, aed”. 2003, 26.sept, lk 41–44.
10. **Starast, M.**, Karp, K. 2003. Väetamise ja väävlil mõju mustikataimede kasvule ja mullareaktsioonile. Maamajandus. Juuli 2003, lk. 33–34.
11. **Starast, M.** 2003. Värvikirevad vaarikad. Ajalehe “Postimees” eriväljaanne “Remont, kodu, aed”. 2003, apr. lk. 46.
12. **Starast, M.** 2002. Kääbusviljapuud. Ajalehe “Postimees” eriväljaanne “Remont ja sisustus”, 2002, 27.sept., 46–47.
13. **Starast, M.**, Karp, K. 2001. Poolkõrge mustika (*Vaccinium corymbosum* x *V. angustifolium*) saagikus esimesel saagiaastal. Teaduselt põllule ja aeda. Jäned. 100–103.
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