

**THE EFFECT OF FERTILIZING AND MODIFIED
ATMOSPHERE STORAGE ON BLUEBERRY
(*VACCINIUM* SPP.) FRUIT QUALITY**

VÄETAMISE JA MODIFITSEERITUD ATMOSFÄÄRIS
SÄILITAMISE MÕJU AEDMUSTIKA (*Vaccinium* spp.)
VILJADE KVALITEEDILE

ANGELA KOORT

A Thesis
for applying for the degree of
Doctor of Philosophy in Agriculture

Väitekirj
filosoofiadoktori kraadi taotlemiseks
põllumajanduse erialal

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Estonian University of Life Sciences

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LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following research papers which are referred to by their Roman numerals (I–III in the text). Papers are reproduced by the kind permission of the journals concerned.

- I Koort, A.;** Starast, M.; Tasa, T. 2016. Half-highbush blueberry ‘Northblue’ plant growth in the juvenile stage: dependence on fertilizers in organic conditions. *ISHS Acta Horticulturae* 1137: 67–73.
- II Koort, A.;** Starast, M.; Põldma, P.; Moor, U.; Mainla, L.; Maante–Kuljus, M.; Karp, K. 2020. Sustainable Fertilizer Strategies for *Vaccinium corymbosum* x *V. angustifolium* under Abandoned Peatland Conditions. *Agriculture*, 10 (4), 121.
- III Koort, A.;** Moor, U.; Põldma, P.; Kaiser, C.; Starast, M. 2018. Comparison of Regular Atmospheric Storage versus Modified Atmospheric Packaging on Postharvest Quality of Organically Grown Lowbush and Half-Highbush Blueberries. *Sustainability*, 10(11) (3916), 1–18.

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Paper	Idea and study design	Data collection/ analyses	Data analysis	Manuscript preparation
I	MS	MS, AK , TT	AK , MS	AK , MS, TT
II	MS	MS, AK , KK	AK , MS, PP	AK , MS, PP; UM, LM, MMK, KK
III	MS	MS, UM, PP	AK , MS, PP, UM	AK , UM, PP, CK, MS

MS – Marge Starast; **AK** – **Angela Koort**, TT – Tea Tasa; KK – Kadri Karp; UM – Ulvi Moor; PP – Priit Põldma; LM – Leila Mainla; MMK – Mariana Maante–Kuljus; CK – Clive Kaiser

ABBREVIATIONS

ASC	ascorbic acid content
ACC	total anthocyanin content
ACY	anthocyanin content
DW	dry weight
DM	dry matter
FW	fresh weight
LSD	least significant difference
NS	non-significant
SPAD	Soil Plant Analysis Development
SSC	soluble solids content
SSC/TAC	soluble solids content and titratable acids content ratio
T	temperature(s)
TAC	titratable acids content
TA	titratable acids
TPC	total phenol content
Min	mineral fertilizer 6-14-23
SD	standard deviation
ANOVA	one-way analysis of variance
PCA	principal component analysis
Org.	organic matter %
n.a.	not analyzed
RA	regular atmospheric storage
LDPE	low-density polyethylene
MA	modified atmosphere
Min	Mineral fertilizer 6-14-23
Org 1	Biolan 3-1-7
Org 2	Biolan 4-1-2
Org 3	Compost Kanakaka 5-3-16
Org 4	Monterra 9-1-4
L*	lightness
C*	chroma
h*	hue angle

1. INTRODUCTION

Land and water management practices in the restoration of peatlands are complicated by spatial diversity in geochemistry, regional and irregular climate variations, non-implementation of water quality assessment policies for pollution control and land use (Monteverde *et al.*, 2022). Sustainable management of abandoned peatlands has to be based on thoughtful use of territories and long-term decisions (Konstantinova *et al.*, 2019). Activities related to land-use management can be divided into two categories: the first involves gaining economic benefits through berry production for food or tree plantation for energy, while the second focuses on restoring the natural functions of mires or transforming them into other natural areas. Revegetating abandoned peatlands with blueberries could reduce the negative impacts of these environmentally sensitive areas, but also provide livelihood for local communities.

There are a few cultivated plants that grow well on acidic peat fields. Blueberries (*Vaccinium* spp.) thrive in low pH and have also low Ca requirements relative to other temperate fruit crops (Nestby and Retamales, 2020). The blueberry cultivation studies on abandoned peat fields in Estonia began in the late 1990s (Starast *et al.*, 2002). Since then, different studies have been carried out: the effect of mineral fertilizers on productivity of lowbush blueberry was studied by Starast *et al.* (2002), Shanskiy (2006) and Albert *et al.* (2011); the comparison of the effect of mineral and peat soil on productivity and physiochemical characteristics of half-highbush and lowbush blueberry genotypes was studied by Starast *et al.* (2007, 2017) and by Tasa *et al.* (2012). The economical aspects of growing lowbush blueberries in peatlands was studied by Vahejõe *et al.* (2010). Since the habitat biodiversity plays an important role in revegetating abandoned peatlands, a study about pollinators and nectar production on peat fields and mineral soil was carried out by Starast *et al.* (2014), while the occurrence of mosses, lichens and arthropods in lowbush blueberry plantations on abandoned peat fields was studied by Tasa *et al.* (2015). The regional disease study was carried out by Starast *et al.* (2009). However, the effect of organic fertilizers on half-highbush blueberry (*Vaccinium* × *atlanticum* E. P. Bicknell) has not been studied before.

The peculiarity of Estonia is that most of the blueberry cultivation on abandoned peatlands follows organic farming principles in terms of plant protection, as diseases and pests have not yet caused economically significant yield losses, thus making organic technologies easily adoptable. Since previous studies with blueberry cultivation on abandoned peatlands have been carried out with synthetic mineral fertilizers, it is important to study sustainable fertilizer strategies for organic blueberry cultivation.

In Estonia, blueberries are mostly sold as fresh market fruit. However, extending marketing period with modified atmosphere packages and retaining high fruit quality for longer period could provide Estonian consumers with local blueberries for extended period. Several postharvest studies have been performed with highbush blueberry cultivars (*Vaccinium corymbosum* L.) in controlled atmosphere storage (Beaudry *et al.*, 1992; Hancock *et al.*, 2008; Alsmairat *et al.*, 2011). However, there are very few postharvest studies concerning organically grown half-highbush blueberries from abandoned peatland conditions. It is known that blueberry storage potential is species-specific (Liu *et al.*, 2019). So far, there is limited knowledge regarding the effect of modified atmosphere storage on lowbush and half-highbush blueberries.

2. BLUEBERRY CULTIVATION

2.1. Blueberry cultivation and yield quality on abandoned peatlands

Blueberry consumption is increasing worldwide due to its high content of bioactive compounds, particularly polyphenolic compounds. Anthocyanins have the abilities to enact antioxidation, anti-inflammation and insulin sensitization (Yang *et al.*, 2022). Anthocyanins are bioactive flavonoid compounds that are beneficial against many chronic diseases, and therefore blueberry is one of the fruits that is popular for its taste and richness in anthocyanins (Routray and Orsat, 2011). These compounds are blue, red, or purple pigments that are found in plants, especially flowers, fruits, and tubers. In acidic conditions, anthocyanin appears as a red pigment, while a blue pigment occurs in alkaline conditions. Although the polyphenolic compound content is species-specific, the weather i.e., temperatures, precipitation and sunshine hours during the vegetative period also have an effect on biochemical and growth parameters (Albert *et al.*, 2011; Paal *et al.*, 2011; Strik, Vance and Bryla, 2017). However, the biochemical composition of blueberries depends also on cultivation method and the soil type (Starast *et al.*, 2007; Ramst and Orru, 2009).

The blueberry species suitable for growing under northern climatic conditions include lowbush blueberry (*Vaccinium angustifolium* Ait.) and cultivars of half-highbush blueberry (*Vaccinium* × *atlanticum* E. P. Bicknell). Half-highbush blueberry is an interspecific hybrid of lowbush (*V. angustifolium* Ait.) and highbush (*V. corymbosum* L.) blueberries that has inherited the ability to survive the harsh winters of northern areas (Luby *et al.*, 1986). Half-highbush blueberry cultivar ‘Northblue’ belongs to the recommended cultivar list in Estonia due to the suitability for the region (“Eesti puuvilja- ja marjakultuuride soovitussoortiment”, 2020). A previous study demonstrated that the ‘Northblue’ cultivar is winter-hardy and performs well under cold climatic conditions. While this cultivar produces big berries, it contains lower amounts of anthocyanins and other polyphenolic compounds compared to half-highbush blueberry ‘Northcountry’, which has smaller berries (Starast *et al.*, 2007). Two highbush cultivars, ‘Reka’ and ‘Puru’, are included in the recommended cultivar list (“Eesti puuvilja- ja marjakultuuride soovitussoortiment”,

2020) and are considered in the group of northern blueberry cultivars. However, winter damage, especially caused by fluctuating temperatures in January and February, is among the major causes of blueberry yield loss in Estonia (Tasa *et al.*, 2012).

When recultivating abandoned peatlands with blueberries, it is important that the remaining peat layer is at least 30 cm of thickness and the area cannot be flooded for more than 90 days a year (Konstantinova *et al.*, 2019). In Estonia, peatlands with a depth greater than 30 cm cover an area of about 915,000 ha (Leibak and Paal, 2011), of which about 5000 ha are abandoned peat mining areas, while on another 11,000 ha, mining is still in progress (Ramst and Orru, 2009; Barthelmes *et al.*, 2015).

The content of available mineral elements in peat soil is low, because organic matter mineralization after decomposition is a time-consuming process (Lappalainen, 1996; Schowalter, 2016). Therefore, blueberry growth on peat soil is highly fertilizer dependent (Vahejõe *et al.*, 2010; Albert *et al.*, 2011; Karlsons and Osvalde, 2019). The effect of mineral fertilizers on the cultivation of blueberry in peat fields has been studied before, showing the growth and yield of blueberry plants increased significantly (Paal *et al.*, 2011). Blueberry plants need acidic soil, of pH_{KCl} 4.0-5.5, which is also rich in organic matter (Korcak, 1988). Some researchers have concluded that blueberries grow well in highly acidic peat soil $\text{pH}_{\text{KCl}} > 4.0$ without the need of liming (Paal *et al.*, 2011). Moreover, growing blueberries in peat soil provides better nutrient supply compared to mineral soil, resulting in improved growth and higher yields (Tasa *et al.*, 2012), and blueberry plantations located on peatlands exhibit lower disease indices (Starast *et al.*, 2009).

A limiting factor for blueberry cultivation in peatlands is the availability of suitable fertilizing and harvesting machines. Typically designed for different soil types, these machines are often large, sturdy, and heavy, making them unsuitable for use in peat soil under abandoned peatland conditions. However, different solutions are under development at the Estonian University of Life Sciences (Arak and Olt, 2020). In addition, to increase the cost-effectiveness of blueberry cultivation, all technological operations should be automated. In the framework of product development, the portable spot-fertilizing device, a portable contact-type weed control device, a motoblock-type blueberry harvester and a blueberry sorting device were developed for the peat field blueberry cultivation (Olt *et al.*, 2013).

However, problems in organic blueberry cultivation on abandoned peatlands are related to the organic fertilizer selection that would provide plants with optimal amount of nutrients while being suitable for peatland conditions. In organic farming, animal manure is often utilised as organic fertilizers. Manure may lead to contamination problems including nitrogen emission to the atmosphere, chemical and microbial contamination of water bodies, and odour nuisance (Sims and Wolf, 1994). A promising approach for reducing these problems is the drying and pelletizing of manure (López-Mosquera *et al.*, 2008). As a result, manufactured organic fertilizers have become widely available in the market and are used in organic farming with various cultural plants. For blueberry cultivation, blood-(Clark and Zheng, 2020), feather-(Strik *et al.*, 2019) and fish solubles (Strik *et al.*, 2019), and different composted pelleted manures, for example pelleted chicken and cattle manure (Lillerand *et al.*, 2021; Tan *et al.*, 2023), are widely studied, however most common plant-based fertilizers are municipal yard waste (Strik, Vance and Finn, 2017), paper mill sludge (Lafond, 2008) and chipped ramial wood (Marty *et al.*, 2019) etc.

Blueberry forms symbiosis with ericoid mycorrhizal fungi, which decompose the organic matter in the soil (Matsubara *et al.*, 2004; Montalba *et al.*, 2010), therefore organic fertilizers are important for the soil microorganism and mycorrhizal symbiosis. Mycorrhizal distribution in blueberry plantations depends on the cultivar. Studies in Estonia revealed that the mycorrhizal colonization was higher on the roots of half-highbush ‘Northblue’ compared to cultivar ‘Northcountry’, with a positive correlation between mycorrhizal colonization and growth and yield (Starast *et al.*, 2006). When managed organically, research with a number of crops suggested a consistent reduction in soil-borne diseases (Matsubara *et al.*, 2004), increased defense mechanisms of plants such as antioxidant production (Del Amor *et al.*, 2008), and an increase in both microorganism diversity and biological activity in the soil (Van Bruggen and Semenov, 2000). Organic fertilizer increased the soil biota activity, mycorrhizal colonization, and leaf antioxidant content relative to conventional N source, and improved tolerance to soil pathogens (Montalba *et al.*, 2019). Similar results have shown recent studies, where organic fertilization generally has positive influence on soil fungi, but not all organic fertilizers may be equally beneficial (Vahter *et al.*, 2022). However, there is little information available about organic fertilizers for half-highbush blueberry cultivation that are applicable for Estonia’s peatland conditions.

Berries could be marketed as healthier and more valuable if the synthetic pesticides and fertilizers are avoided; moreover, fertilization in peatlands is essential for blueberry cultivation (Albert *et al.*, 2011), therefore organic fertilizing strategies should be applied. The most immediate opportunity for marketing blueberries may be in promoting blueberries as a healthy and environmentally conscientious food.

2.2. Blueberry storage

The postharvest quality of blueberries is affected by diverse physical, physiological, and pathological processes and aspects of blueberry deterioration including decay, shrivelling, and softening (Paniagua *et al.*, 2014). Blueberries are susceptible to the attack of different phytopathogens (Bell *et al.*, 2021). Major causes of postharvest spoilage are fungal decay and physiological changes (Cappellini *et al.*, 1982). The most common postharvest fruit rots of blueberries are *Botrytis cinerea*, *Alternaria*, *Fusarium*, *Penicillium*, *Cladosporium* and yeasts (Tournas and Katsoudas, 2005; Umagiliyage *et al.*, 2017). Storing blueberries in a CO₂-enriched atmosphere proves to be an effective method for extending their postharvest life and inhibiting postharvest decay without fungicidal treatments (Smittle and Miller, 1988). Modified atmosphere packaging (MA) has the potential to provide low O₂/high CO₂ regimes, but the packaging must maintain the appropriate atmospheric composition over a range of temperatures commonly encountered between harvest and consumption (Beaudry *et al.*, 1992) to maintain the sensory and nutritional quality (Chiabrando and Giacalone, 2008; Moggia *et al.*, 2014). Although several studies have found no significant differences in storing blueberries in the range of 0-5 °C (Hancock *et al.*, 2008; Alsmairat *et al.*, 2011; Paniagua *et al.*, 2014), the most popular storage temperature is close to 0 °C (Cappellini *et al.*, 1972; Sanford *et al.*, 1991; Kader, 2002). At this temperature, the blueberry may be stored in a regular atmospheric storage for a maximum of 2 weeks. By raising the storage humidity to 85-89%, the postharvest life can be extended for up to 6 weeks (Giacalone and Chiabrando, 2012; Mattos *et al.*, 2012). Hypobaric storage of 0.025 MPa could prolong the storage life of blueberries up to 50 days and retain fruit quality, bioactive compounds and antioxidant enzymes (James *et al.*, 2022). Ozone treatment is another option to reduce postharvest spoilage and extend storage life (Jaramillo-Sánchez *et al.*, 2019). Ozone treatment at 18 mg O₃ L⁻¹ for 10 min could be an

alternative for reducing fungal decay of blueberries without causing an excessive weight loss during refrigerated storage.

The use of active packaging that is able to interact with fruit through releasing or absorbing substances can be a relevant approach to preserve the quality and extend shelf-life of fruit (Bugatti *et al.*, 2020). An active packaging based on polyethylene filled with a nano-carrier of salicylate showed inhibition of mold development and a reduction of the respiration rate of fruits, as well as extended blueberry fruit shelf-life up to 13 days. Sulfur dioxide (SO₂) application showed good results in mold inhibition during extended storage as SO₂-emitting box liners provided very good decay control (Saito *et al.*, 2020).

The application of biological control methods to decrease pathogen populations by using beneficial microorganisms (bacteria, fungi, and yeast) and other natural origin compounds like chitosan as an alternative biopolymer to control pathogens at postharvest stage has increased (Bell *et al.*, 2021). In a study, where biodegradable films based on corn starch, chitosan and the active film containing grape seed extract were used, the post-harvest weight loss of packed blueberries during the refrigerated storage was reduced compared to the fruits stored in the synthetic containers (Bof *et al.*, 2021).

Edible coatings such as liposome encapsulated limonene could be a good alternative postharvest treatment for extending the storage life of blueberries (Umagiliyage *et al.*, 2017). An *in vivo* study of liposome coatings demonstrated their ability to protect berries against spoilage over a nine-week storage period at 4 °C, where storage loss was decreased by one third by the end of storage.

A large volume of postharvest work has been performed with highbush blueberry cultivars, with particular reference to controlled atmosphere storage (Beaudry *et al.*, 1992; Yahia, 2009). However, blueberry storage is species-specific (Liu *et al.*, 2019). For instance, the cultivar 'Bluecrop' exhibits higher firmness and undergoes a slower softening process than 'Sierra' during cold storage. There is limited knowledge regarding the modified atmosphere storage of lowbush and half-highbush blueberries.

3. HYPOTHESES AND AIMS OF THE STUDY

Half-highbush blueberry cultivar ‘Northblue’ is suitable for cultivation in Estonia due to its winter hardiness. However, there is little information of suitable organic fertilizers for half-highbush blueberry cultivation on abandoned peatlands. In Estonia blueberries are mostly consumed fresh and the period when domestic blueberries are available, is relatively short. Modified atmosphere packages should enable to prolong blueberry postharvest life and therefore prolong the marketing period of local blueberries. However, there is little information about the effect of modified atmosphere packages on half-highbush and lowbush blueberry fruit quality. Based on previous research and knowledge, the following hypotheses were set:

- organic fertilizers are feasible alternatives to mineral fertilizers on peatlands in terms of ensuring half-highbush blueberry cultivar ‘Northblue’ growth and yield;
- compared to mineral fertilizers, the type of organic fertilizers affects the content of health-beneficial compounds for humans in blueberry fruits;
- postharvest life of lowbush blueberry and half-highbush blueberry fruits may be extended by using modified atmosphere packaging.

Based on the hypotheses, the aim of the research was to find out the effect of:

- different organic fertilizers (based on plant extracts and chicken manure) on the plant growth, yield and biochemical parameters of fruits of half-highbush blueberry ‘Northblue’ under peatland conditions;
- modified atmosphere (MA) packages on the external quality and the nutritional value of the fruits of organically grown lowbush blueberry and half-highbush blueberry ‘Northblue’.

4. MATERIAL AND METHODS

4.1. Half-highbush blueberry 'Northblue' plant growth and yield (I, II)

4.1.1. Experimental site and treatments

The experiment was established in 2006 on the territory of Hiie Farm in Ilmatsalu village, South-Estonia (58° 23'N, 26° 31'E, H: 33 m) (Paper I, II). One-year-old half-highbush blueberry (*Vaccinium* × *atlanticum* E.P. Bicknell) cultivar 'Northblue' plants were planted in the spring of 2006 on an abandoned peat-extraction field (Figure 1). Experimental plants were planted at 1.0 x 1.5 m spacing. The peat soil of the experimental area belongs to the soil subgroup Fibri-Dystric Histosol (IUSS Working Group WRB, 2006), with a residual peat layer that was 1.0-1.5 m deep. According to the classification of Estonian vegetation site types (Paal, 1997) this area is classified as an oligotrophic heavily drained bog of raised bog site type. This bog was previously used for industrial peat production until the beginning of 1980s.

Fertilizers were selected based on their composition suitability for blueberry cultivation. Mineral fertilizers were chosen due to their common use among berry producers.

Six different fertilizers were used in the plantation during the growth of young plants in 2006-2009 (Paper I):

- Mineral fertilizer Cropcare 6-6-19 as a control;

The following fertilizers are certified organic fertilizers:

- Algomin 2.5-0.2-0.2 is based on marine algae *Lithothamnium calcareum*. The organic matter content in this fertilizer is 3% and the pH is 8.8;
- Viva 5-1.7-6.2 is specially developed for berry crops, containing by-products derived from corn and grapes processing, along with natural inorganic potassium salt;
- Biolan 2.0-1.2-2.0 contains composted chicken manure and vinasse extract. In production process, the fertilizer is sterilized at over 90 °C;

- Kemira Bio 4-2-3 contains of chicken manure, seaweed, feather, straw and inorganic biotite;
- Chicken manure compost 5-3-16 contains chicken manure, commercial organic fertilizer which is sterilized at high temperature.

During the harvest years, 2011-2015, one mineral and four organic fertilizers were used (Paper II):

- Mineral fertilizer 6-14-23 (plus Mg 3%, S 11%, B 0.05%, Cu 0.1%, Fe 0.1%, Mn 0.7%, Mo 0.01%, Zn 0.01%) (commercially available as Cropcare). Fertilizer 6-14-23 has been commonly used in conventional berry production in Estonia. Mineral fertilizer was considered as a control. Min in the text.
- The following fertilizers are certified organic fertilizers:
- Biolan 3-1-7 contains mainly composted chicken manure and 9% of vinasse extract (potassium rich by-product of the sugar industry) and molasses. Org 1 in the text;
- Biolan 4-1-2 contains chicken manure compost and seaweed meal (plus Cu 0.01%, Fe 0.1%, Mn 0.04%, Zn 0.02%). Org 2 in the text;
- Compost Kanakaka 5-3-16 is a chicken manure compost. Org 3 in the text.
- Monterra 9-1-4 is maltose based organic fertilizer (plus Mg 0.3%, S 3.0%, B 0.015%, Cu 0.1%, Fe 0.1%, Mn 0.7%, Mo 0.01%, Zn 0.01%). Org 4 in the text.

Fertilization was conducted once per year around the plant crown area at the beginning of May (the start of the growing season in Estonia). In 2006-2007 the fertilization rate was 0.24 g N/plant, in 2008–1.0 g N/plant and in 2009–2.4 g N/plant. In the harvest years, the fertilization rate with organic fertilizers was 70 kg/ha N, an additional 50 kg/ha N was given with feather meal 14-1-0. The total N fertilization rate per year was 120 kg/ha in each fertilizer treatment. The fertilizers used in the experiment, both organic and mineral, were granulated.

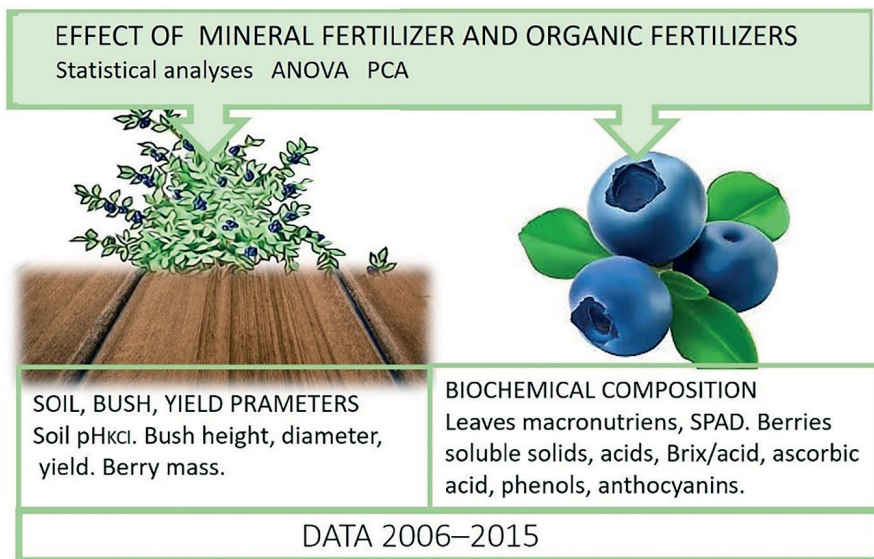


Figure 1. Overview of the parameters and performed statistical analyses from the peat field experiments.

4.1.2. Weather conditions

Based on Estonian Weather Service reports, monthly (May-September) temperatures, precipitation and total sunshine hours of the experimental area from 2011-2015 (except 2014) compared to the mean of 1981-2010 are shown in the Table 3 (II) (Estonian Weather Service, n.d.). In 2011, the temperatures were slightly higher compared to the 30-year mean values, especially in June and July (17.7 and 20.5, respectively), and there was less precipitation. 2013 was also slightly warmer compared to the 30-year mean values and had less precipitation and more sunshine hours from June to September. In 2012, there was more precipitation in May and August-76 and 103 mm, respectively. In 2015, there was less precipitation in June, July and August; however, there were more sunshine hours in August compared to the 30-years mean.

4.1.3. Measurements and analyses

Annually, at the end of the vegetation period (the end of September) the canopy diameter (cm) and height (cm) of the blueberry bushes were measured 2006-2015. The plant diameter was measured across and along the row and its average was calculated. The number of shoots (longer

than 15 cm) per plant was counted. Ten plants were measured in each treatment. The experimental design was a randomized complete block. Each treatment had four replicates with 10 plants per replicate.

Soil samples were taken close to the plants (from three plants from each replicate) from the soil layer at the depth of 20 cm. The pH was measured from the soil suspension with 1M KCl (1:5 w/v) using the Evikon pH meter.

The chlorophyll content was evaluated using the portable chlorophyll meter SPAD-500 (Minolta Camera Co. Ltd. Japan). SPAD (Soil Plant Analysis Development) is an indirect indicator of nutrient, especially nitrogen content in the leaves, and is suitable for blueberry chlorophyll content evaluation (Starast, 2008). Leaves of the same age and position (from the central part of non-fruit-bearing shoots) on the plant were used; young leaves with uneven color were left aside. Each reading consisted of measurements from 10 different plants and one sample consisted of 30 leaf measurements on the average. SPAD readings were performed at the beginning of August (blueberry harvesting time in Estonia), 2009.

The content of macronutrients (nitrogen – N%; phosphorus – P%; potassium – K%; calcium – Ca%; magnesium – Mg%) in the blueberry leaves was determined in the Laboratory of Plant Biochemistry at the Estonian University of Life Sciences. Leaf samples were collected at the beginning of August. N concentration of air-dried samples was determined by the Kjeldahl method (“ISO - 13.080.10 - Chemical characteristics of soils”, n.d.). 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 Kjeltac 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 air-acetylene flame. P was determined at the wavelength 720 nm by the Stannous Chloride method and Ca at the wavelength 570 nm using oCresolphthalein Complexone, 8-Hydroxyquinoline to mask 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). Four sample replicates in all fertilization treatments were analysed and one sample consisted of leaves from ten plants.

Berries were collected in 2011-2013 and in 2015. In 2014, the blueberry plants had severe frost damage, therefore there was no harvest. For the berry mass (g) measurements, uniform and disease-free berries from the first harvest were weighed using the Scaltex SAC 51 scale. Berry mass was calculated as a mean of 30 fruits from each treatment replicate. For the yield calculation (g plant^{-1}), each year plants were hand-harvested several times due to different ripening time (Figure 2), with ca one-week intervals. The criteria for determining the stage of maturity were the fruit's full blue coloration. The yield of each bush from each harvest was weighed.

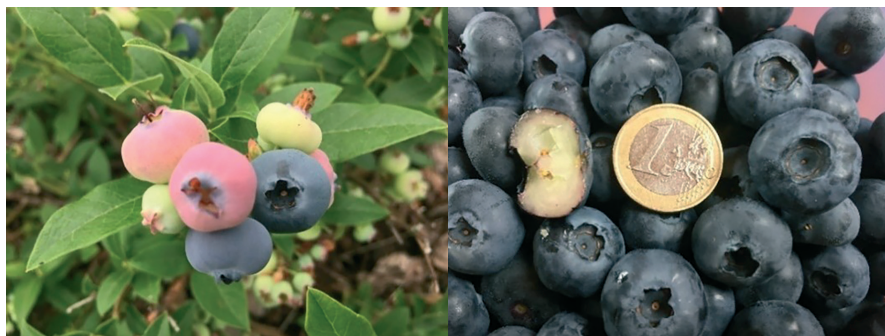


Figure 2. The berries of the half-highbush blueberry ‘Northblue’ ripen gradually. The flesh of fully ripened fruit is white and the anthocyanins are in the fruit skin (K. Karp).

Blueberries were harvested in the first weeks of August. Biochemical analyses were conducted from the first harvest (Figure 2). All the analyses and measurements were performed in three replicates. For preparing the laboratory samples, 250 g of berries were taken from each of the treatment replicate and pureed. Analyses were conducted on fresh berries one day after first harvest. Biochemical analyses were expressed by fresh weight (FW).

The total phenol content (TPC) was determined using the Folin-Ciocalteu method (Wrolstad *et al.*, 2005) with a Shimadzu UV Visible Spectrophotometer UVmini-1240. Ethanol-acetone (7:3) solution was used as the solvent to extract the phenolic compounds. In the experiment, 0.3 mL of the fruit extract was mixed with 7.7 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent (1:1 solution with water). After 1 min, 1.5 mL of a 2% sodium carbonate solution was added. Samples were held for 2 h at 22 °C and the absorbance was read at 765 nm. The TPC was expressed as mg of gallic acid per 100 g of FW.

The total anthocyanin content (ACC) was estimated using the pH differential method (Wrolstad *et al.*, 2005). Absorbance was measured with a Shimadzu UV Visible Spectrophotometer UVmini-1240 at 510 nm and at 700 nm in buffers at pH 1.0 (HCl 0.1 N) and pH 4.5 (citrate buffer). The extraction solution contained hydrochloric acid (0.1 M) and ethanol (96%) in v:v ratio of 15:85. The results were expressed as mg of cyanidin-3-glycoside per 100 g of FW.

A Pocket Pal-1 digital hand-held refractometer (Atago) was used for soluble solids content (SSC) measurements. SSC was estimated as °Brix. Titratable acids content (TAC) was analyzed using a standard acid-base titration method (Wrolstad *et al.*, 2005). An aliquot of sample (40 mL) was titrated with 0.1 M NaOH solution to a phenolphthalein endpoint (pH 8.2). EasyPlus Titration (Mettler Toledo) was used for measuring (with electrode DG 111-SC for endpoint detections). TAC was expressed as mg citric acid per 100 g of fruit FW, as citric acid is the dominant organic acid in blueberries, using the milliequivalent factor of 0.064 for the citric acid. The SSC/TAC was calculated by dividing soluble solids by titratable acids content. For the determination of ASC, hydrochloric and acetic acids were immediately added to the fruit puree to avoid ascorbic acid breakdown in the air. Ascorbic acid content (ASC) was titrated with the solution of 2,6-dichlorophenolindophenol (Ranganna, 1986) using an automatic titrator (EasyPlus, Mettler-Toledo International Inc.), and expressed as mg 100 g⁻¹ FW.

4.1.4. Statistical analysis

All analyses were carried out on three parallel samples for each variable and data were expressed in tables as the mean value \pm standard deviation (SD). The data were evaluated by one-way analysis of variance (ANOVA), and the means were compared using a Fisher's least significant difference (LSD) test at a 95% probability level. Different letters indicate significant differences ($p < 0.05$). A principal component analysis (PCA) was performed on all the measured parameters. Analyses were performed using standardized mean data (10 plant's mean data per replicate). All analyses were performed using Statistica for Windows version 12.0 (StatSoft, Inc., Tulsa, OK, USA).

4.2. Blueberry storage (III)

4.2.1. Plant material and storage conditions

The berries were collected in 2008 from Marjasoo Farm from Tartu County South Estonia (58°12'N, 26°41'E). Two species of blueberry were investigated: the lowbush blueberry (*Vaccinium angustifolium* Ait.) and the half-highbush blueberry (*Vaccinium* × *atlanticum* E.P. Bicknell) cultivar 'Northblue'. Bushes were grown organically in the soil subgroup Fibri-Dystric Histosol (IUSS Working Group, 2006) with a residual peat layer that was 1.0-1.5 m deep. Uniform, disease-free blueberries at commercial maturity (beginning of August) were hand-picked into regular-atmosphere 250-g perforated polyethylene terephthalate "plastic" punnets (Infia TR80/58 mm, Produce Packaging, HL Hutchinson Ltd. England). Punnets were designed for soft and highly perishable fruits such as cherries and tomatoes (Fresh Produce Packaging Solutions, n.d.). The mass of the perforated punnets was 8 g and the dimensions were 143 × 96 × 58 mm. The punnets had four circular perforations (diameter 8 mm) at the bottom and the lid had eight oval perforations (20 × 5 mm). Treatments included:

1. a control, consisting of four regular atmospheric storage (RA) punnets only;
2. four regular atmosphere punnets sealed in a 30 µm thick low-density polyethylene (LDPE) modified atmosphere bag (product of Estiko, Estonia);
3. four regular atmosphere punnets sealed in an Xtend® modified atmosphere blueberry bag (Stepac, Israel).

One treatment consisted of six replicate bags (four punnets in one bag). All treatments, including the control, were stored at 3 ± 1 °C for six weeks at Estonian University of Life Sciences. Relative humidity ranged from 96 to 98%. The fruits were analysed on the day of harvest and then 1 replicate bag consisting four boxes of blueberries was destructively sampled each week. Postharvest shelf-life was considered terminated when either the berries were too soft (firmness below 6.0 points), when shrivelling was $\geq 10\%$, or when the decay was $\geq 5\%$. For each treatment, the shelf-life was considered terminated at different times: in regular atmosphere punnets, it was 22 days for lowbush blueberry and 28 days for half-highbush 'Northblue'; in LDPE modified atmospheric

packaging, it was 22 days for lowbush and 37 days for the half-highbush blueberry 'Northblue'. For Xtend® modified atmospheric packaging, it was 37 days for both species.

4.2.2. Measurements and analyses

Blueberries were stored in the forced-air experimental coolstore of the Estonian University of Life Sciences. During storage, O₂ and CO₂ concentrations were measured using a hand-held gas analyser OXYBABY V (WITT-Gasetchnik GmbH & Co KG, Germany). O₂ and CO₂ concentrations (%) were measured nine times from within each closed system. The integrity of each bagged system was maintained as an impermeable rubber septum was placed on the outside of each modified atmospheric bag and, through the septum, a gas analyser needle was inserted, and an aliquot of air was drawn out for both O₂ and CO₂ concentrations. O₂ and CO₂ concentrations in the natural atmosphere were measured above the berries from the headspace immediately above the perforated holes in the punnets.

Fruit firmness, shrivelling, and decay were determined at the end of experiment. Firmness was evaluated on a sub-sample of 10 berries by hand rolling, using a 1-9 scale (1 = berry ruptures on touch, 4.5 = berry surface depressed on touch, 9 = berry is firm, not yielding to touch). Shrivelling of the fruits was determined visually and was expressed as a percentage of all fruits in a punnet. Fruit decay was visually evaluated and it was expressed as a percentage of all fruits in a punnet. Any berries with visible mould growth were considered decayed. Pathogens were not identified in the experiment.

A trained sensory panel of 10 assessors was used for the sensory descriptive analysis. Prior to the sensory evaluation, assessors attended a discussion and training session, in which they were introduced to the experiment-specific criteria for sensory analyses. Evaluation criteria were conducted with modifications from the study of Schotsmans *et al.* (2007).

Fruit quality characteristics were determined for each of the four punnets per replicate. All berries with visual disease symptoms were counted and removed. For chemical analyses, 100 g of the remaining uninjured and disease-free berries from each replicate were pureed using a hand-held

blender (Turbo MR 5550 M FP, Braun GmbH, Spain). Dry matter (DM) was determined using a 10 ± 1 g sample and drying in a thermostat (Modell 400, Memmert GmbH + Co.KG Co.) at 105 °C to a constant weight. Fruit DM content (%) was calculated on a DW and FW basis.

SSC was analysed as previously described (p. 22).

TA was measured as previously described (p. 22).

Anthocyanin content (ACY) was measured as previously described (p. 25), except solutions were shaken and held at 5 °C for 24 h. Total ACC was determined spectrophotometrically using a Thermo Spectronic Hellios β spectrophotometer (Thermo Scientific Inc., UK) by determining the difference in the absorbance between solutions of pH 1.0 and pH 4.5 at emissions of 510 and 700 nm (Giusti and Wrolstad, 2001). Values are expressed as mg cyanidin-3-glucoside equivalents per 100 g FW using a molar extinction coefficient of 26,900 L mol⁻¹ cm⁻¹.

Both the external (fruit surface/exocarp) and internal (flesh/mesocarp) color were recorded using a reflectance colorimeter (Model CR-400, Minolta Co., Ltd., Japan). Two readings per fruit were taken on opposite sides of each of the 10 fruits from each replicate (from four punnets). In order to measure the color of the fruit surface, the natural wax coating was removed mechanically (Kalt *et al.*, 1995), and the same fruits were measured with a wax and without a wax coating. For fruit flesh color measurements, each fruit was bisected and measurements were taken immediately to avoid discoloration. The 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), and h* (hue angle). The color intensity of the fruit is measured by C*, where higher values represent more intense color. The h* conforms to the values from 0° to 360°, where 0° is red, 90° is yellow, 180° is green and 270° is blue. A white plate was used for calibration (Illuminants C: $Y = 92.6$ $x = 0.3134$ $y = 0.3196$; illuminants D65: $Y = 92.6$ $x = 0.3160$ $y = 0.3324$).

4.2.3. Statistical analysis

The data were evaluated by one-way analysis of variance (ANOVA), and the means were compared using a Fisher's least significant difference (LSD) test at a 95% probability level. Different letters indicate significant differences ($p < 0.05$). Data of O_2 and CO_2 from two blueberry species that were measured from different modified atmosphere packages (LDPE film and Xtend® film) were statistically analysed at the end of the storage period. Depending on the storage conditions (RA, LDPE and Xtend® film) firmness, shrivelling, and decay were compared at the end of the storage period as well. DM, ACC, TA, SSC, SSC:TA and the color parameters ($L^*a^*b^*$, C^* and h^*) were statistically compared in pre-storage and after storage depending on the storage conditions (RA, LDPE, Xtend® film).

5. RESULTS

5.1. The effect of organic fertilizers on soil and on half-highbush blueberry 'Northblue' vegetative and yield (I, II)

5.1.1. Soil and vegetative parameters

All the fertilizers impacted soil pH (Table 1, I). Organic fertilizer Algomin increased pH from 3.5 to 5.2, while mineral fertilizer Cropcare decreased soil pH to 3.1. The use of Biolan, Kemira and chicken manure compost resulted in more stable and similar pH to initial value, ranging from 3.5 to 3.7.

In the young plantations, the height of cultivar 'Northblue' plants ranged from 15.8 to 62 cm (Figure 1, I). The highest bushes were in the Cropcare and lowest in the Algomin treatment. Plants were also wider with the use of Cropcare fertilizer (59.2 cm) compared to the Algomin (20.1 cm), Viva (48.5 cm) and Biolan (51.1 cm) fertilizers. There was also positive effect on the shoot formation when the Cropcare fertilizer was used, forming twice as many shoots (4.6) compared to the Algomin (2.2). In the mature plantation, fertilizers did not have an effect on bush height, ranging from 98 to 105 cm, nor on diameter, ranging from 75 to 118 cm (Table 4, II).

SPAD chlorophyll meter readings in the young plantation were highest when the mineral fertilizer Cropcare was used (34.6), while the organic fertilizers caused lower SPAD readings, ranging from 30.4 to 32.2 (Table 1, I). Nutrient content of the leaves was following: 1.19-1.66 N%; 0.09-0.12 P%; 0.32-0.40 K% (Table 2, I). Mg content ranged from 0.37-0.45%. In the mature plantation, leaf nutrient content was following: 1.18-1.42 N g 100 g⁻¹; 0.08-0.10 P g 100 g⁻¹, 0.28-0.50 K g 100 g⁻¹ (Table 2, II). Mg content ranged from 0.17-0.26 g 100 g⁻¹.

5.1.2. Yield parameters

Berry mass did not differ significantly in 2011 and 2013 between the treatments, ranging from 2.0 to 2.3 g in 2011, and 1.3-1.6 g in 2013 (Table 4, II). In 2012, the use of the Org 1, Org 2 and Org 4 fertilizers

had a positive effect on berry mass compared to the use of Org 3. In 2015, the use of Min and Org 3 had a positive effect on berry mass compared to the results for the use of Org 2 and Org 4.

The fertilizers had a significant effect on the yield, which ranged from 285 to 2043 g plant⁻¹ (Table 4, **II**). The use of the Org 2 treatment resulted in the highest yield in 2011 and 2013. In 2012, a higher yield was obtained from the Min, Org 2 and Org 1 treatments compared to that of the Org 3 and Org 4 treatments. In 2015, the yield ranged from 723 to 1046 g plant⁻¹, and no effect of fertilizing was observed.

5.1.3. Fruit biochemical parameters

The TPC ranged from 134 to 220 mg 100 g⁻¹ FW, and the effect of the fertilizer was significant (Table 5, **II**). Cultivar 'Northblue' fruits produced under the Min treatment had a higher TPC in 2012, 2013 and 2015 compared to those produced under the Org 1 treatment. The Org 2 treatment resulted in statistically lower fruit TPC in 2012 and in 2015 compared to that of the fruits produced under the Min treatment. The ACC ranged from 48 to 136 mg 100 g⁻¹ FW. The use of Min resulted in statistically lower ACC in fruits in 2012 and in 2013 compared to the use of the Org 4 treatment. In 2015, the Org 4 treatment resulted in higher ACC compared to Org 1 treatment.

The SSC ranged from 9.6 to 11.9 °Brix, with a significant effect of the fertilizers (Table 5, **II**). In 2011, the SSC was statistically lower with the use of the Org 1 and Org 2 fertilizers, where Org 4 treatment resulted in the highest SSC. The Min treatment resulted in the highest SSC in 2012 compared to the Org 2 and Org 4 treatments. In 2013, fertilizing with Org 1 and Org 2 resulted in the lowest SSC compared to the other treatments. In 2015, a similar trend continued, where Org 1 resulted in the lowest SSC compared to the Org 3 and Org 4 treatments. TAC did not have any statistical differences between the treatments in 2011, 2013 and 2015; however, in 2012, the TAC was statistically different with the use of Min fertilizer compared to the organic fertilizers. SSC/TAC varied between the treatments in 2012, where the highest SSC/TAC was achieved with Min fertilizer.

ASC had high variance in the experiment, ranging from 4.5 to 18.3 mg 100 g⁻¹ FW (Table 5, **II**). In 2011, the Org 4 treatment resulted in statistically

higher ASC compared to the other treatments, where the lowest results were with the use of Org 2. In 2012, there was an opposite effect, where the Org 4 treatment resulted in relatively low ASC compared to the Min treatment. In 2013, a similar trend continued, where Min treatment resulted in higher ASC compared to the Org 4 treatment, and to the other organic fertilizers' ASC. In 2015, Min resulted in a higher ASC compared to the Org 4 treatment.

The PCA showed that the first principal component (PC1) explained 44% of the total variance in the data, and the second principal component (PC2) explained 23% (Figure 1, **II**). The PC1 and PC2 explained 68% of the variance in the data for both Figure 1a (**II**), b. PC3 explained 12% of the variance in the data in Figure 1a and described the negative correlation of ACC (data not presented in figure). PC1 was more related to the SSC, TAC, SSC/TAC, berry mass, dry matter and yield, whereas total PC2 was more related to the TPC, bush height and bush diameter in Figure 1a (**II**). TPC was positively correlated with 2011 in PC2; while yield, berry mass and TAC were positively correlated in PC1 and were highest in 2012.

PCA demonstrated that experimental years distinguished more clearly than different fertilizers, and that their relative importance in determining fruit characteristics was larger (Figure 1, **II**). For instance, high yield and large fruits were characteristic of 2012, and high total polyphenol content was characteristic to 2011. The PCA also showed a close positive relationship between the berry mass and TAC of fruits and a negative relationship between berry mass and SSC of the fruit.

5.2. The effect of modified atmosphere storage on blueberry postharvest quality (III)

5.2.1. Postharvest life, O₂ and CO₂ concentrations

Regular atmospheric (RA) storage punnets at 3 ± 1 °C and a relative humidity over 90% resulted in a postharvest life of 22 days for lowbush blueberries and up to 28 days for 'Northblue', but no longer (Figure 1, **III**). Both the LDPE and Xtend® packages had a positive effect on 'Northblue', prolonging the postharvest life for up to 37 days. The LDPE packaging did not extend the postharvest life of lowbush blueberry compared to RA, whereas Xtend® packaging resulted in a postharvest

life of up to 37 days. The half-highbush blueberry ‘Northblue’ had a longer postharvest life compared to the lowbush blueberry in the LDPE film.

The lowest O₂ concentration was 13% and the maximum CO₂ concentration was ca. 9.3% (Figure 2 and 3, **III**). The O₂ concentration in the MA packages did not have a significant difference: the O₂ concentration dropped to 15.7% in the LDPE film bag and to 13.9% in the Xtend® bag for lowbush blueberries (Figure 2, **III**). For the half-highbush blueberry, the O₂ concentration at the end of the experiment decreased to 14.0% in the LDPE film bag and to 13.0% in the Xtend® film bag. CO₂ increased to 3.4% in the LDPE film and to 8.5% in the Xtend® for lowbush blueberries. For ‘Northblue’, the CO₂ content in the LDPE film was significantly lower (4.8%) compared to Xtend® (9.4%).

5.2.2. Changes in fruit postharvest quality

The fruits stored in the Xtend® film remained firmer compared to the fruits stored in the RA for the lowbush blueberry, scoring 6.0 and 5.0 points, respectively (Table 1, **III**). There was no significant difference in the ‘Northblue’ fruit firmness between storage types, ranging from 7.0-7.3. The ‘Northblue’ fruit remained firmer compared to the lowbush blueberry fruit.

The highest percentage of shrivelling for the lowbush blueberry was in the RA storage (13.0%) compared to Xtend® (5.0%) and to LDPE (3.0%) (Table 1, **III**). The ‘Northblue’ had more shrivelled fruits in the Xtend® film (2.0) compared to the RA (0.1%) and LDPE film (0.1%). The fruits in the Xtend® film had a higher percentage of shrivelling, 5.0% for lowbush blueberry and 2.0% for ‘Northblue’.

There were also less decayed lowbush blueberry fruits compared to the ‘Northblue’ (Table 1, **III**). For lowbush, there was 0.1% of decayed fruits when stored both in the RA and in the Xtend® film, and 0.4% of decayed fruits when stored in the LDPE film. The percentage of decayed fruits for the ‘Northblue’ was significantly higher compared to lowbush blueberry: 15.0 % when stored in the LDPE film, 7.0 % when stored in the Xtend® film, and 3.0% when stored in the RA packages.

5.2.3. Fruit biochemical parameters after storage

Fruit DM content ranged from 13.2 to 15.1 mg 100 g⁻¹. DM content in both blueberry taxa was higher in the fruits stored in the Xtend® packages (15.1 for lowbush and for 14.5 mg 100 g⁻¹ for ‘Northblue’) compared to the fruits stored in the LDPE packages (13.6 and 13.2 mg 100 g⁻¹) (Table 2, **III**). Similar trend was observed with the SSC, where the Xtend® package had positive effect on both lowbush and ‘Northblue’ compared to the LDPE package. However, in the case of ‘Northblue’, the effect was different, where the RA treatment had an increase in the SSC (14.4 mg 100 g⁻¹) compared to both MA packages (12.9 mg 100 g⁻¹ in LDPE and 13.9 mg 100 g⁻¹ in Xtend®).

The TA content ranged from 0.14 to 0.27 mg 100 g⁻¹ for lowbush blueberry, and 0.66 to 0.89 mg 100 g⁻¹ for ‘Northblue’ (Table 2, **III**). The fruits stored in the Xtend® package had significant increase in TA content of both taxa compared to LDPE and RA storage.

The SSC:TA ratio had high variance in the experiment, ranging from 52 to 94 for lowbush blueberry and from 15 to 20 of ‘Northblue’ (Table 2, **III**). The lowbush blueberry fruits stored in the Xtend® package had almost half of the SSC:TA (52) of the RA (94). For ‘Northblue’, similar trend was observed, where both of the MA packages caused decrease in ratio compared to the RA.

ACY content of ‘Northblue’ fruits increased during storage in all of the storage types, ranging from 101 to 103 mg 100 g⁻¹ (Table 2, **III**). For the lowbush blueberry, the ACY was higher in the RA (151 mg 100 g⁻¹) compared to the fruits stored in MA packages (96 mg 100 g⁻¹ in LDPE and 110 mg 100 g⁻¹ in Xtend®). However, for ‘Northblue’ the storage type did not have an effect.

5.2.4. Fruit color

The lowbush blueberry and half-highbush blueberry ‘Northblue’ fruit surface with wax was lighter when stored in the in the LDPE film (L* 29.5 for lowbush and 28.9 for ‘Northblue’) compared to the fruits stored in the Xtend® film (L* 25.6 and 27.0, respectively) (Table 3, **III**). The lowbush blueberry fruit flesh was lighter in color in the RA (L* 41.3) compared to the fruits stored in the LDPE (L*36.8) and in the Xtend®

(L* 36.3) films. For 'Northblue', the fruit flesh was lighter in color in the LDPE film (L* 55.7) compared to the RA (L* 48.0).

During storage, the chroma value of blueberry taxa was affected differently: for lowbush blueberry, flesh color was affected and for 'Northblue', surface colour without wax was affected (Table 3, **III**). The lowbush fruit color with wax had the highest C* value in the RA (C* 6.3) compared to the Xtend® film (C* 5.5). For the 'Northblue' the C* was highest before storage (C* 4.7) compared to the RA after storage (C* 4.1), but there were no differences between the storage types. The lowbush blueberry fruit stored in the Xtend® film had higher chroma values (C* 2.3) compared to the other storage types (C* 1.2 in a RA and C* 1.4 in the LDPE film). Chroma measured from fruit flesh was higher from the fruits stored in the Xtend® package for both lowbush and 'Northblue' when compared to the fruits stored in RA and in the LDPE film.

The h* from the fruit with wax was higher in the Xtend® film (h* 297) compared to the RA (h* 277) and to the LDPE film (h* 280) for lowbush blueberry (Table 3, **III**). For 'Northblue', there was no differences between storage types and fruit hue angle with wax, but the fruit h* values without wax were approximately three times higher in the fruits stored in the Xtend® (h* 330) compared to the fruits before storage (h* 116). The fruit flesh h* decreased in the fruits stored in the RA (h* 94) compared to the fruits stored in the LDPE (h* 103) for 'Northblue'. For lowbush blueberry, hue angle from flesh decreased in the fruits stored in the RA (h* 44) and in the LDPE film (h* 68), and had an increase in the fruits stored in the Xtend® film (h* 200).

6. DISCUSSION

6.1. The effect of organic fertilizers on soil and on half-highbush blueberry 'Northblue' vegetative and yield parameters (I, II)

6.1.1. Soil and vegetative parameters

Present experiment showed that in addition to providing nutrients to the plants, fertilization also changes the pH of the peat soil. Blueberry plants are calcifuges and their cultivation is strongly influenced by the soil pH. The optimal soil pH for blueberries ranges from pH 4.8 to 5.5 (Korcak, 1988). However, previous studies conducted in Estonia have concluded that if the soil pH falls below recommended levels (pH=2.6), there is no need for pH increase through liming, because lower pH is also suitable for the plants (Paal *et al.*, 2011). In contrast, raising the pH could have a negative effect on the blueberry growth instead. This trend was also observed in our study, where the soil pH continued to increase with Algomin fertilizer, which consequently suppressed the overall plant growth. Since the mineral fertilizer decreased pH, it could be suggested that the low soil pH was related to the vital plant growth and shoot formation. Our results affirmed that blueberry plants grown on peat soil prefer higher soil acidity. An earlier study has reported that synthetic fertilizers, which is suitable for blueberry production, decrease the soil pH, while organic fertilizers increase the soil pH (Warman and Shanmugam, 2008). Three organic fertilizers in our study, Biolan, Kemira Bio and chicken manure compost, contain a significant proportion of chicken manure. Chicken manure has a neutral reaction (Dikinya and Mufwanzala, 2010). The drying and pelletizing process also raises the pH of chicken manure (López-Mosquera *et al.*, 2008). These results indicate that the use of the mentioned fertilizers leads to an increase in soil pH. However, in our study the soil pH increased significantly in the first year, but decreased and stabilized in the following years. Previous studies have concluded that chicken manure does not always influence the soil pH similarly and it is more dependent on the soil type where the fertilizer is used (Dikinya and Mufwanzala, 2010).

The experimental plants were affected by the winter damage, and therefore the bush height results were fluctuating in both growth stages.

It has been stated that blueberries are more susceptible to winter damage on peat soil compared to mineral soil, although the 'Northblue' have performed better on peat soil compared to the mineral soil (Tasa *et al.*, 2012). Half-highbush blueberry 'Northblue' reach an average height of 65 cm by their tenth year of growth (Luby *et al.*, 1986). However, experiment conducted on mineral soil, showed that the plant height reached ca. 40 cm by their seventh year (Starast *et al.*, 2005), and 50 cm by the sixth year of growth on the peat soil (Tasa *et al.*, 2012). In present study, blueberry plants reached full height (around 100 cm) when matured. Since the half-highbush blueberry is progeny of highbush blueberry, it matures similarly, as canopy growth increases over time and reaches a plateau at full plant maturity, after about eight years (Hanson, 2006; Messiga *et al.*, 2018). Based on previous, data from present studies indicate that the fertilizers had positive effect on plant growth.

Data of the first experiment showed trend between SPAD reading and Mg content in plants. Shaahan *et al.* (1999) stated that the SPAD chlorophyll meter can be used to assess Mg-status of some fruit trees. Earlier experiment results have also showed correlation between SPAD readings and nitrogen content of plant leaf tissue for various species (Porro *et al.*, 2001) including lowbush blueberry (Starast *et al.*, 2007). However, there was no trend between nitrogen content and SPAD reading. The leaf nutrient analysis showed similar results from both young and mature plantation. When considering optimum ranges on nutrients by Trevett (1972) and Hart *et al.* 2006) the nitrogen content reached the limits only with Algomin treatment in a young plantation and lacked in mature plantation. P was insufficient in both growth stages. The Ca and Mg content stayed within the recommended range. Similar results were obtained in a previous study, where the plants lacked N and P content, but the Ca stayed in recommended range (Tasa *et al.*, 2012). It has been pointed out earlier that evaluating half-highbush blueberry nutrient uptake is complicated when using nutrient levels established for other blueberry species (Warman and Shanmugam, 2008). Applied sufficiency levels are specific for lowbush blueberry and therefore these ranges may not be directly extrapolated to half-highbush blueberry (Trevett, 1972). All blueberries have relatively low nutrient requirements (Korcak, 1988), but there is still a need to work out critical nutrient levels for half-high blueberry to provide effective and environmentally friendly production (Starast *et al.*, 2007; Routray and Orsat, 2011).

6.1.2. Yield parameters

Although fertilizer had a significant effect on yield, the experimental year had a stronger impact, resulting in over sevenfold differences in yield. These yearly variations were caused by weather conditions. The effect of the experimental year was demonstrated by PCA, showing that the experimental year's weather had a strong effect on fruit parameters, while the fertilizer type did not have a strong correlation with the variables. Previous studies have suggested that different environmental conditions affect blueberry mineral nutrition (Ranganna, 1986), but so do the year, site and cultivar interaction (Tasa *et al.*, 2012). Once the plants reach full maturity, the yield fluctuates from year to year as a result of weather conditions and pruning (Strik *et al.*, 2017). As the abandoned peatlands in Estonia have similar organic matter content and soil acidity, the field-to-field variation of these areas is more modest than on mineral soil, and the yearly weather has a more significant impact on the fertilizer's effects on yield (Paal *et al.*, 2011; Tasa *et al.*, 2012). In our study, in 2011 and 2013, the temperatures were slightly higher and there were less precipitation and more sunshine hours from June to September in 2013 compared to the 30-year mean values. In both years, the highest yield was achieved with the Org 2 treatment. However, in 2013, the yield was rather low compared to other experimental years. In an earlier study conducted with lowbush blueberry on peat soil with mineral fertilizer it was also demonstrated that the effect of the fertilizer was weather-dependent (Albert *et al.*, 2011). Despite the weather conditions in our study, the use of Org 2 fertilizer gave a stable yield performance in many experimental years. All of the fertilizers used in our experiment were granulated, but differed in composition; the Org 1, Org 2 and Org 3 contained chicken manure, and the Org 4 contained maltose, but also differed in phosphorus and potassium content. With reference to the different fertilizer compositions, the timescale of fertilizer decomposition may also have been different, as well as weather dependent. In 2011, there was less precipitation from May to August, which may have had a negative effect on nutrient uptake and plant growth.

Organic fertilizers used in the study showed positive results compared to the mineral fertilizer. Although organic fertilizer Org 2 had lower potassium and phosphorus content compared to the mineral fertilizer, it resulted in similar vegetative growth and a high yield. As described earlier, the Org 2 fertilizer contained seaweed. Many studies have stated

a wide range of beneficial effects of seaweed extract application on plants, such as early seed germination and establishment, improved crop performance and yield, increased resistance to biotic and abiotic stress, and the improved postharvest shelf-life of perishable commodities (Hankins and Hockey, 1990; Blunden, 1991; Norrie and Keathley, 2006) Khan *et al.*, 2009. Seaweed contains macro- and micro-elements, amino acids, vitamins and growth hormones like cytokinins, auxins, and abscisic acid, that have an effect on cellular metabolism in plants resulting in improved growth and yield (Durand *et al.*, 2003; Stirk *et al.*, 2003; Ördög *et al.*, 2004). Seaweed also affects the chemical, physical and biological properties of soil, which all influence plant growth. Its extracts improve soil health by enhancing the moisture-holding capacity, increasing the growth of beneficial soil microbes, and encouraging the growth of beneficial fungi to stimulate mycorrhizal development (Kuwada *et al.*, 2006; Khan *et al.*, 2009). It was found that seaweed oligosaccharides, which are produced by the enzymatic degradation of alginic acid, significantly improved the hyphal growth and elongation of arbuscular mycorrhizal fungi, but also activated their infectivity on trifoliolate orange seedlings (Ishii, 2000). Since the organic fertilizer Org 2 had a lower P and K content compared to mineral fertilizer, the positive performance could be related to the seaweed concentration, which may have a beneficial effect on soil biota, mycorrhizal development and plant growth, although this needs further research. Since the abandoned peatlands are environmentally sensitive areas, organic fertilizers that contain seaweed, could reduce the P and K use in blueberry plantations, whilst also minimizing the CO₂ footprint of blueberry production/consumption life cycle.

6.1.3. Fruit biochemical parameters

The biochemical composition of blueberry fruits was affected by the fertilizer type and the year. The use of the Min treatment had higher TPC in 2012, 2013 and 2015 compared to the organic fertilizer Org 1. Valuable antioxidants in blueberries include phenolic compounds, the major role of which is to protect organisms against the oxidative stress induced by free radicals (You *et al.*, 2011). Previous study have showed that organically grown blueberry 'Powderblue' had a higher TPC compared to conventionally grown cultivars. Another study conducted with different high-bush cultivars compared blueberry TPC from conventional and organic farms and found that there were no statistical differences between the cultivation methods, except that

there were significantly higher tannin levels in plants grown under organic cultivation (Gonçalves *et al.*, 2015). In another study, organically grown blueberry yielded significantly higher TPC, malic acid, ACC, antioxidant activity and sugars (fructose and glucose), than fruit from the conventional production (Wang *et al.*, 2008). In the present study, the use of Min fertilizer increased TPC during some of the experimental years compared to some organic fertilizers. A study established in Korea with different half-highbush cultivars, resulted 'Northblue' TPC of 247.8 mg 100 g⁻¹ (Kim *et al.*, 2013), which indicates that our TPC results were similar and are species-specific.

There was high variability in ACC caused by the treatments. The use of the organic fertilizer Org 4 resulted in higher ACC in most of the experimental years compared to Org 1 and Min. Cultivation practices are the main factors that affect the concentration of anthocyanins in fruits depending on the genotype (Routray and Orsat, 2011). Researchers have found that the ACC and total phenol content accumulated in different blueberry cultivars was either higher or comparable in the case of organically grown cultivars compared to conventionally grown rabbiteye blueberries (You *et al.*, 2011). A similar study that focused on the effect of different cultivation practices on highbush blueberries, stated that the ACC was significantly higher in organically cultivated blueberries (Wang *et al.*, 2008).

ASC was more affected by the experimental year than by the fertilization method. A previous study with 'Northblue' has stated an ASC of ca. 15 mg 100 g⁻¹ FW (Starast *et al.*, 2007), which is similar to our results. The use of Min fertilizer tended to result in a higher ASC. In 2012 and 2015, the Min treatment resulted in a higher ASC in fruits compared to the Org 4. In 2013, the use of mineral fertilizers resulted in the highest ASC compared to organic fertilizers. An earlier study concluded that climatic factors like light intensity and temperature are the most important in determining the final ASC (Lee and Kader, 2000). It has been reported that the cooler climate and higher light intensity tend to increase the content of ASC (Albert *et al.*, 2011). In our study, the temperatures were slightly higher with less precipitation in June and July 2011 compared to the 30-year mean, and the ASC was lower compared to other experimental years.

The fertilizers used in our experiment had a different effect on the biochemical contents due to the different composition of the fertilizers. The Min fertilizer had higher potassium (19%) content compared to organic fertilizers. Previous studies suggest that adequate potassium nutrition greatly influences the synthesis of sucrose and starch in different fruits, berries and vegetables, for example in apple (Fediala *et al.*, 2015; Mosa *et al.*, 2015) and in strawberry (Ahmad *et al.*, 2014). Potassium levels have different effects on organic acid metabolism depending on the plant species (Flores *et al.*, 2016) and also strengthen photosynthesis at the source to supply the sink with enough carbohydrates (Beruter and Feusi, 1997). In an experiment with apples, K fertilization promoted higher fruit mass, higher Ca²⁺, SSC, and lower TAC (Zhang *et al.*, 2018). In our study, the K content in the leaves was optimal with the use of mineral fertilizer and the Org 1. Org 1 fertilizer also had a higher K content in the fertilizer (7%) compared to the other fertilizers used in the study. According to Hart *et al.* (2006), the optimum K range in blueberry leaves is 0.41%-0.70%. Despite the higher K content in the Min and Org 1 fertilizer, no clear trends in the effect on biochemical content were observed. Although in some experimental years, the use of Min treatment resulted in higher TPC and ASC, decreased TAC and increased SSC/TAC content, this effect was not observed each experimental year.

6.2. The effect of modified atmosphere storage on blueberry postharvest quality (III)

6.2.1. Postharvest life, O₂ and CO₂ concentrations

The half-highbush blueberry 'Northblue' had a longer postharvest life compared to the lowbush blueberry in the LDPE package, which suggests that the suitability of the film is species-specific. Early studies have shown that postharvest shelf-life of blueberries is strongly correlated with genetics (Song *et al.*, 1992). However, in the case of Xtend® package, the postharvest life was similar for both taxa.

The lowest O₂ concentration recorded was 13% and the maximum CO₂ concentration was ca. 9.3% in present experiment. Even different cultivars of the same species can exhibit different respiration rates (Fidler and North, 1967; Song *et al.*, 1992; Mattos *et al.*, 2012), which was also observed in our study. However, the O₂ and CO₂ concentrations

did not reach the recommended levels suggested by Kader (2002) and Mattos *et al.* (2012), which is O₂ concentrations between 2-5% and CO₂ between 12-20%.

CO₂ concentration increased to 3.4% in the LDPE package and to 8.5% in the Xtend® for lowbush blueberry, indicating greater CO₂ transmission rate of LDPE film. The same phenomenon was noticed with 'Northblue', where the CO₂ concentration in the LDPE film was significantly lower compared to the CO₂ concentration in the Xtend® film. In an earlier study with raspberries, the O₂ concentration was also somewhat higher in the Xtend® film compared to LDPE: 15.7 and 14.9%, respectively (Moor *et al.*, 2014). By the end of the experiment, the O₂ concentration reached 15.1% in the LDPE and 13.1% in the Xtend® bags, where the CO₂ concentration was 5.9% in the LDPE and 7.3% in the Xtend® at the end of the experiment. In another study with strawberries, the O₂ decrease and the CO₂ increase was more rapid in the LDPE film compared to the Xtend® film, which suggests that LDPE film is less permeable to respiration gases (Moor *et al.*, 2012). In the present study results were similar, indicating that the LDPE bag's CO₂ concentration did not reach the desired levels, which may have resulted in a poorer storability for the lowbush blueberry. An early study showed that the O₂ within the low-density polyethylene films containing same mass of blueberries decreased with the increasing temperature and vice versa, which indicates that the activation energy of the film O₂ permeation was less than the activation energy of the fruit (Beaudry, 1992). Previous studies have also reported that the steady-state O₂ and CO₂ levels depend on film permeability and the product respiration rate and that the temperature dependence is determined by the film type and commodity physiology (Beaudry, 1992; Song *et al.*, 1992). Different varieties of the same product exhibit specific respiration rates (Mattos *et al.*, 2012) and the success of the MA packaging greatly depends on the accuracy of the predictive respiration rate (Kader, 2002). The MAP storage with the starch films by Giuggioli *et al.* (2017) helped to control the changes in post-harvest physicochemical properties, such as the pH and TA, but also maintained the antioxidant and nutritional values of fruits after 15 days of storage. In another experiment with three highbush blueberry cultivars 'Coville', 'Blueray', and 'Jersey', the fruit respiration rates in the modified atmosphere decreased with the increasing CO₂, but were little affected by changes in O₂ (Song *et al.*, 1992). On the contrary, Beaudry *et al.* (1992) suggested that the respiration

is minimally affected by levels of CO₂ below the approximate 20 kPa that accumulated in the films under hypoxic conditions. In their work with highbush blueberry 'Bluecrop', the oxygen consumption decreased in response to the decreasing temperature and decreasing steady-state O₂, where the shape of the O₂-dependent respiratory curves changed with temperature, where at the higher temperatures, the O₂ uptake did not appear to approach saturation even at the highest levels of steady-state O₂ generated and, as a result, the fruits were found to be more sensitive to restricted O₂ availability as temperatures increased. Hall and Forsyth (1967), on the other hand, indicated that the longer the fruits were left on the bushes, the lower their rate of respiration would be. In our experiment, berries from both taxa were picked at the same time and there were no differences in the berry ripening stages. During the experiment, the O₂ consumption and CO₂ production were similar with both taxa at a temperature of 3 ± 1 °C but they were influenced by the MA films. At the same time, the concentration of CO₂ did not increase up to the critical level in any of the used MA films. For the half-highbush blueberry, the limits had not been worked out until now, but earlier studies have mentioned that for highbush and lowbush blueberries, the suitable CO₂ concentration in storage ranges from 5% to 15% when kept at 5 °C or below (Beaudry *et al.*, 1992; Giacalone and Chiabrand, 2012). Analogous parameters for O₂ concentration are between 1 and 10%. In our study, the oxygen concentration was higher in both films for both taxa during the experimental period. It may be concluded that the LDPE film is not suitable for lowbush blueberry because it did not extend the postharvest life and the gas concentrations did not reach to desired levels. Although the O₂ did not decrease and CO₂ did not increase as expected, especially in LDPE film, the positive effect was that the anaerobic respiration did not take place during storage in both packages. While the LDPE film did not prolong the postharvest life of lowbush, it did extend the postharvest life of 'Northblue' by 9 days.

6.2.2. Changes in fruit postharvest quality

The lowbush blueberry fruits in the Xtend® film stayed firmer compared to the RA. This result is an expected outcome, as the respiration rate is higher in the RA and the atmosphere has a major role in accelerating the moisture loss, although the loss of firmness may have been affected by the subsequent compression of the berries in the box during the storage. Prange *et al.* (1995) showed that the firmness of the lowbush blueberry

decreased over time, especially after 42 days (1.6-3.4 points in the 0-5-point scale). However, they suggested that an O₂ concentration of 1-5% may also improve the firmness retention with a storage time >28 days. Paniagua *et al.* (2014) reported that the storage atmosphere influenced the firmness of the blueberries, however, its effect varied among the cultivars and storage temperature. For instance, 'Brigitta' had firmer fruits in CO₂ of 10% in both 2.5% or 20% of O₂ compared to air at 4 °C. In our study, the CO₂ concentration was higher in the Xtend® film for both taxa, but only the lowbush blueberry stayed firmer in the Xtend® film compared to the RA. There was no significant difference between the firmness of 'Northblue' fruits stored in both MA films. However, the firmness of the 'Northblue' fruits was slightly higher compared to the lowbush fruits. Half-highbush blueberry 'Northblue' had a longer postharvest life compared to the lowbush blueberry, which could also be correlated to a better firmness performance. As mentioned previously, the lowbush blueberry had softer fruit compared to the half-highbush blueberry, which is a function of the genetic difference of these two taxa and agrees with earlier studies claiming that the firmness is determined by the genetics of the cultivar (Alsmairat *et al.*, 2011; Paniagua *et al.*, 2014). Ballington *et al.* (1984) stated that when the blueberries are grown in a single location and year, the genetic factors are more important than the environmental differences within the field. The firmness/softening depends mainly on hemicellulosic depolymerization (Vicente *et al.*, 2007). However, in another study researchers have stated that the elasticity/turgidity is more related to the internal turgor pressure regulated largely by the cuticular wax properties (Fava *et al.*, 2006), because wax is a very good barrier to the excessive water loss (Connor *et al.*, 2002). Based on this knowledge, the interaction between wax and the gradient was also observed in our study, where the half-highbush 'Northblue' with a high epidermal wax concentration had less shrivelling, but the fruits were also firmer.

In the present study, genetic variation had a significant influence on the firmness. Anatomical differences between the blueberry cultivars could influence CO₂ and O₂ diffusion into the blueberry tissue, affecting the internal gas concentration and contributing to the genetic variability in response to the atmospheric change (Paniagua *et al.*, 2014). The parental phenotype in the blueberry often determines progeny firmness characteristics (Edwards *et al.*, 1974). The lowbush blueberry produces a soft-fruited progeny (Finn and Luby, 1992) and has softer fruits compared

to highbush blueberry (Ballington *et al.*, 1984). The mentioned species are both ancestors of the half-highbush blueberry used in our experiment, thus, concerning the firmness, the half-highbush blueberry 'Northblue' performed similarly to the highbush blueberry. It was found in an earlier study, that the half-highbush cultivar 'Polaris' produced berries as firm as the highbush 'Duke' (Ehlenfeldt and Martin, 2002), where the two half-highbush blueberry cultivars, 'St. Cloud' and 'Friendship', had lower firmness values. The half-highbush blueberry cultivars that possess significant amounts of lowbush blueberry ancestry seem to show a propensity for producing softer fruits. A previous study results showed that after the harvest, berry turgidity becomes more important than firmness (Giongo *et al.*, 2013). In the aforementioned study, the hybrid 'Northblue' was placed in a group which was characterized by a low texture performance with a high elasticity and deformable structure, which may lead to the perception of gumminess by consumers. The storage index for the texture dynamics of 27 cultivars (both highbush and hybrid) was employed, where 'Northblue' ranked slightly below average. Although the half-highbush blueberry 'Northblue' did not have good postharvest properties in this particular study, we may conclude that it has better texture dynamics than the lowbush blueberry, since the half-highbush blueberry 'Northblue' performed better concerning firmness than the lowbush blueberry.

The lowbush blueberry had a higher shrivelling percentage than the half-highbush blueberry 'Northblue' fruits, which can indicate higher water loss of lowbush blueberry fruit compared to the half-highbush 'Northblue' blueberry. There was also a significant interaction between the MA films and fruit shrivelling. The highest percentage of shrivelled lowbush fruits was in the RA compared to the fruits stored the Xtend® and in the LDPE films. High water loss from the fruit is correlated with the high transpiration intensity (Mattos *et al.*, 2012), which can lead to extensive shrivelling and loss in marketable berries. Water loss results in a loss of gloss, fruit shrivelling, and a decrease of firmness (Giacalone and Chiabrande, 2012). Weight losses of 5% lead to wilting and poor texture, and the taste is considered critical for blueberry marketability (Almenar *et al.*, 2008). Based on previous, it may indicate that the Xtend® film had better gas and transpiration exchange compared to LDPE film, which also led to more shrivelled fruits. When comparing the overall postharvest life performance, the genetic factors were more important than the O₂ and CO₂ concentration in the films. Even though

the packaging had significant effect on shrivelling, the half-highbush blueberry 'Northblue' had less shrivelled fruits compared to the lowbush blueberry, also the half-highbush performed better with both MA films. Previous studies support these results, stating that the postharvest berry water loss is genotype specific (Yan and Castellarin, 2022) and have also resulted in different fruit weight and size changes among genotypes (Yan *et al.*, 2023).

The lowbush blueberry had less decayed berries compared to the half-highbush blueberry 'Northblue'. Postharvest diseases of blueberries are usually caused by fungi, with anthracnose (*Colletotrichum acutatum*) being the most common fungal disease, followed by alternaria rot (*Alternaria* spp.) and grey mould (*Botrytis cinerea*) (Cappellini *et al.*, 1982; You *et al.*, 2011). In an experiment with highbush blueberry, there were no differences in the postharvest behaviour between fruits from organic or from conventional fertilization (Echeverría *et al.*, 2009). Previous studies have shown that lowbush blueberries show little decay during the storage, which is defined as the presence of visible mould (Sanford *et al.*, 1991). In the modified atmospheric storage, the berries continue to respire the trapped air until the CO₂ concentration rapidly approaches the critical 10-15% level necessary to inhibit the *Botrytis* growth (Giacalone and Chiabrande, 2012). A study conducted by Paniagua *et al.* (2014) confirmed that after 6 weeks of storage, the low oxygen concentrations (2.5% O₂ + 10% CO₂) significantly reduced the decay for the cultivar 'Maru' in comparison to the air storage and both the controlled atmospheres decreased the decay for 'Brigitta'. High CO₂ concentrations suppress decay, weight loss, and softening (Prange *et al.*, 1995). This was also observed in our study, where the CO₂ concentration was higher in the Xtend® film compared to the LDPE film and, correspondingly, there were less decayed berries. Similar results have obtained in an early study with highbush cultivars, where CO₂ increase decreased the visible decay and at 15% CO₂ the decay was virtually absent, and the increase of unmarketable berries was more related to a loss of firmness than due to the visible decay level (Prange *et al.*, 1995). We observed similar trend with lowbush blueberry, where the increase in the unmarketable berries was more related to a loss of firmness and shrivelling rather than because of visible decay.

A low O₂ concentration during the blueberry storage has very little effect on the decay organism activity or survival at levels above the

fermentation threshold of most commodities (Mattos *et al.*, 2012). Another study has found that the modification of storage atmospheres induces plant defensive responses and increases disease resistance in postharvest commodities (Zheng *et al.*, 2008). A negative plant response to the MA packaging is seen when the respiration is reduced so low that it induces fermentation (Mattos *et al.*, 2012). As mentioned before, for the lowbush blueberry, there were less decayed berries compared to the half-highbush blueberry 'Northblue' (Table 1, **III**). The fungal growth of the half-highbush blueberry 'Northblue' could have been suppressed by lowering the storing temperature since the previous studies have demonstrated that storing them at 0 °C has the great benefit of maintaining the quality. In the study by Paniagua *et al.* (2014), a higher temperature (4 °C in comparison to 0 °C) resulted in more rot incidence from 5 weeks onwards for 'Brigitta' and after 4 weeks onwards for 'Maru'. Earlier works have also suggested that minimal mechanical damage and storage at 0 °C gives the advantage of maintaining the quality of the highbush (Cappellini *et al.*, 1972) and lowbush blueberries (Sanford *et al.*, 1991; Jackson *et al.*, 1999). The reported enhanced temperature conditions during blueberry storage could also be beneficial for the half-highbush blueberry 'Northblue' storage since the decay percentage was high when the fruit was stored at 3 ± 1 °C.

6.2.3. Fruit biochemical parameters after storage

At the end of the experiment, fruit DM content in both blueberry taxa was significantly higher in fruits stored in Xtend® films compared to the RA storage, which indicated that the water loss may have been higher in Xtend® films. Kalt and McDonald (1996) have described the chemical composition of several lowbush blueberries ('Blomidon', 'Cumberland', 'Fundy'), which had higher average dry matter content compared to the blueberries in our study. A lower fruit dry matter content may have been caused by the climate conditions, cultivation techniques, but may also be due to the genetic differences between the lowbush species.

The SSC increased in the fruits stored in the Xtend® for both lowbush and half-highbush blueberry, and decreased in the fruits stored in the LDPE. An earlier study compared four different MA packages with a highbush blueberry 'Lateblue' and found that the microperforated (1 mm Ø) film and non-perforated film affected the total SSC positively (Chiabrando and Giacalone, 2008).

The Xtend® package caused an increase in the TA content of both blueberry taxa, which may have been influenced by the dry matter content decrease during storage. Zheng *et al.* (2008) studied the impact of high oxygen (40%, 60%, 80%, and 100%) in MA on highbush blueberry ‘Duke’ and reported significantly lower TA within all the O₂ types, but also with storage at 5 °C for 9, 14 or 35 days compared to the initial value. Duan *et al.* (2011) stated that during postharvest storage, acid metabolism is converted to starch and acid to sugar, resulting in a decrease of the TA and an increase of soluble solids, however this trend was not observed in the present study. A study, which evaluated the production mode on biochemical composition, showed that blueberries produced in organic farming were less acid and less sweet, but had a more intense blue color and were less elastic when compared with blueberries produced in conventional cultivation, but also resulted in genetical variation when considering biochemical composition during the storage (Gonçalves, 2015). In another study, highbush blueberry ‘Lateblue’ had high TA content compared to other cultivars, when fruits were stored in the microperforated film (Chiabrando and Giacalone, 2008). The half-highbush blueberry ‘Northblue’ in the present study also has a higher TA content compared to the lowbush blueberry and is more similar to the highbush ‘Lateblue’ TA content from the mentioned study, which indicates to the genetic variation. The increase of the TA content in fruits stored in the RA and MA films could also have an impact on the taste properties. Beaudry (1992) reported that blueberries should contain >10% soluble solids, 0.3-1.3% titratable acidity, and have an SSC:TA ratio between 10 and 33, where 0.1% decrease in the acid concentration is known to be equivalent to a 1% increase in the perceived sweetness in the blueberry fruit. In our study, the lowbush blueberry had a decrease of soluble solids and an increase in the titratable acidity in the fruits stored in the LDPE film, which indicates that the taste of fruits probably became more acidic.

Lowbush blueberry anthocyanin content was significantly higher in the fruits stored in the RA compared to the fruits stored in the MA films, which indicates that the MA inhibited the ACY biosynthesis. The ACY content in half-highbush blueberry ‘Northblue’ fruits did not have statistical difference between storage types. Anthocyanin and flavonol concentrations change during postharvest storage and these changes are genotype specific (Yan *et al.*, 2023). Same MA films as in our study were used with raspberry ‘Polka’ (Moor *et al.*, 2014) and with three different

strawberry cultivars (Moor *et al.*, 2012), where raspberries stored in LDPE packages had significantly lower anthocyanin content compared to the fruits stored in RA.

6.2.4. Fruit color

Fruit surface with wax was darker in color of both taxa when stored in the Xtend® film compared to the fruits stored in the RA. The decrease of L* value (lightness) indicated that fruits stored in Xtend® were more ripe at the end of experiment. The ‘Northblue’ fruit surface with wax was lighter when stored in the LDPE film compared to the fruits stored in the RA and to the Xtend® film. It is likely that the surface wax affects the L* value (i.e., higher amounts of surface wax might lighten the fruit), as well as the visual perception of the fruit color (e.g., blue chroma) (Saftner *et al.*, 2008). Color is a complex quality characteristic affected by the quantity and structure of the surface waxes (Albrigo *et al.*, 1980) and the anthocyanin content of blueberries (Kushman and Ballinger, 1975; Yan *et al.*, 2023). However, in the present study, the surface color without wax had no difference with respect to the L* values for both taxa. In a study with two highbush blueberry cultivars, ‘Duke’ had an increase in L* value, while ‘Bluetta’ had a slight decrease in lightness during storage (Eum *et al.*, 2013). When comparing these results to our study, it can be suggested that the lowbush blueberry and half-highbush blueberry ‘Northblue’ have a higher L* value compared to the highbush cultivars ‘Bluetta’ and ‘Duke’, which again reflect the genetic differences.

The hue angle measured from the lowbush fruit with wax was higher in the Xtend® film compared to the fruits stored in the RA and in the LDPE film. Hue angle is a good measure of blueberry color and the blue color of the fruits has been suggested as the best criteria of fruit maturity and decision-making regarding harvesting time (Routray and Orsat, 2011; Eum *et al.*, 2013). The higher hue angle values indicate bluer colors. For half-highbush, the h* values without wax were approximately three times higher in the Xtend® compared to the initial value, which indicates that the fruits became bluer during storage due to the anthocyanin increase. The dark blue-purplish color of the blueberry fruit surface is mainly correlated to the high levels of delphinidin and malvidin glycosides (Grace *et al.*, 2019; Chai *et al.*, 2021). Changes in fruit surface color have been observed during postharvest study in several genotypes, where some showed an increased hue angle, indicating

changes toward more blue-purplish color hues, while others showed a decreased hue angle, indicating a purer blue color hue (McLellan *et al.*, 1995; Yan *et al.*, 2023). Differences in color changes during postharvest have been previously reported toward darker blue hues (Chu *et al.*, 2018). In the Eum *et al.* study (2013), the hue angle of highbush cultivars 'Bluetta' and 'Duke' were similar during the storage, but also similar to our experiment's h^* values.

For both blueberry taxa, the Xtend® film had a pronounced effect on the fruit surface color. Chroma values from fruit flesh were higher in Xtend® compared to LDPE. The decrease of C^* and h reflect the changes in fruit color, from green to dark-blue/purple (Lin *et al.*, 2020). The color of the fruit is also an important quality factor influencing fresh-market value and acceptability by the consumers (Faria *et al.*, 2005; Sinelli *et al.*, 2008; Duarte *et al.*, 2009). Half-highbush 'Northblue' fruit's C^* values with wax in the present study indicated that fruits were fully ripe, since the C^* values with wax decreased compared to the pre-storage. Similar results in terms of C^* measurements, was reported with different ripening stages of cultivar 'Northblue', where fully ripe fruit C^* values were accordance to our measurements (Lin *et al.*, 2020). Highbush blueberry fruit C^* values decreasing from immature to fully ripe fruit (Smrke *et al.*, 2023), which also support our results that fruits were darkening during storage.

Earlier studies have reported that the blueberry surface color correlates well with the measurements of SSC, ACC, and TAC (Kalt *et al.*, 1995; Kushman and Ballinger, 1975; Yan *et al.*, 2023). In the present study, both lowbush and half-highbush blueberry 'Northblue' TA content and SSC were higher in the fruits stored in the Xtend® film compared to the initial value, which can also be associated to color values, as the fruit surface color with wax had a higher degree of redness and hue angle of the fruits stored in the Xtend® film compared to the pre-storage.

7. CONCLUSIONS

Blueberry growth and yield depends on the cultivation technology and yearly weather conditions. The effect of mineral fertilizers on cultivated blueberry growth and biochemical content on abandoned peatland has been studied before. Since the sustainable cultivation technology has become more relevant, it is important to find out suitable organic fertilizers for blueberry cultivation on abandoned peatland conditions.

In the present thesis, the effect of organic fertilizers on half-highbush blueberry cultivar 'Nortblue' plant growth and fruit quality on abandoned peatland were studied. In addition, postharvest life of lowbush and half-highbush blueberries using modified atmosphere packaging were investigated.

Based on the results obtained from fertilization experiments with half-highbush blueberry 'Northblue', it can be concluded:

- Algomin fertilizer based on marine algae *Lithothamnium calcareum* content increased the soil pH up to 5.2 and suppressed the plant growth in juvenile growth stage (I).
- Organic fertilizers containing chicken manure increased the soil pH in the first year, but it stabilized in the following years within the range of pH 3.5 and 4.0, resulting in comparable vegetative growth and yield when compared to plants receiving mineral fertilizer (I).
- Seaweed-containing organic fertilizer ensured yearly stable, similar or higher yield compared to mineral fertilizer despite its significantly lower potassium and phosphorus content (II). Thus, this type of fertilizers could be used as environmentally friendly alternatives to mineral fertilizers for fertilizing blueberries in abandoned peat fields.
- The experimental year was more important in determining fruit parameters than the fertilizer type (II). The use of seaweed-based organic fertilizer resulted in similar total polyphenol content in fruits as mineral fertilizer (commercially available as Cropcare 6-14-23) treatment.

Based on the results from modified atmosphere (MA) experiment with lowbush and half-highbush blueberry 'Northblue', it can be concluded:

- Compared to regular atmosphere conditions, the Xtend® package prolonged the postharvest life of lowbush blueberries for 15 days and half-highbush blueberries for 9 days (III). Low-density polyethylene (LDPE) package did not prolong the postharvest life of lowbush blueberries, but extended the postharvest life of 'Northblue' for 9 days.
- The CO₂ content was significantly higher in Xtend® film compared to the LDPE at the end of the storage. It can be concluded that lowbush blueberries need a higher CO₂ concentration (III) for retaining postharvest quality compared to half-highbush blueberry 'Northblue'.
- Both MA packages had a negative effect on the taste-related properties of blueberries irrespectively of the taxa (III). The SSC:TA ratio of the blueberries in the MA decreased both compared to the initial value and compared to the RA-stored blueberries by the end of storage.
- The content of anthocyanins increased significantly with all storage conditions irrespectively of the taxa (III). However, for lowbush blueberries compared to RA, both the MA packages suppressed anthocyanin biosynthesis, whereas the 'Northblue' anthocyanins in MA were no different from RA.
- The genetic differences were more important concerning fruit firmness, shrivelling, and decay. Both MA packages had an impact on the firmness and the shrivelling, but the half-highbush blueberry 'Northblue' fruits were firmer and less shrivelled compared to the lowbush (III).

The hypotheses of the fertilizing study were partially confirmed, because not all the fertilizers were suitable for blueberry cultivation on abandoned peatland conditions. Modified atmosphere package experiment showed that package effect is species specific, as LDPE package did not prolong the postharvest life of lowbush blueberries, but extended the postharvest life of 'Northblue'

Recommendations based on results and further research objectives:

Since blueberry producers are primarily interested in high yield in mature plantation, the seaweed-based low nutrient fertilizer Org 2 (Biolan 4-1-2) could be recommended for organic production in peatland areas due to its more stable yield performance and due to the lower impact to peat pH. Seaweed-based fertilizers that contain auxins can increase yield even with a low nutrient content.

Further studies should be conducted to find suitable fertilizers that contain cytokinins and auxins and are suitable for blueberry cultivation. As well to find the effect of blueberry cultivation to the the CO₂ emission on abandoned peatland areas. In young plantation, macro element (N, P, K) content in the leaves indicated insufficiency of nutrients, therefore, higher fertilizer amounts per plant require further studies.

Since blueberries are marketed as health promoting food, yield quality during postharvest storage is equally important to prolonging postharvest storage period. Modified atmosphere package Xtend® prolonged postharvest life of both genotypes and therefor it could be recommended for blueberry storage. Further studies should be conducted to study the metabolic differences among blueberry taxa and to match the respiration of the product with the permeation rates of the packages. The use of biodegradable films should be considered in order to ensure sustainability.

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SUMMARY IN ESTONIAN

VÄETAMISE JA MODIFITSEERITUD ATMOSFÄÄRIS SÄILITAMISE MÕJU AEDMUSTIKA (*Vaccinium* spp.) SAAGI KVALITEEDILE

Sissejuhatus

Mahajäetud turbatootmisalade jätkusuutlik kasutamine peab olema läbimõeldud ja lähtuma pikaajalistest otsustest (Konstantinova *et al.*, 2019). Turbamaad saab kasutada tootmisistandike rajamiseks või loodulike koosluste taastamiseks. Happelistel turbaaladel hästi kasvavaid kultuurtaimi on vähe. Aedmustika (*Vaccinium* spp.) sordid kasvavad madala pH juures ja on teiste parasvöötme taimedega võrreldes ka vähese kaltsiumivajadusega (Nestby and Retamales, 2020). Seega on mahajäetud freesturbaalade negatiivse keskkonnamõju vähendamise üks võimalusi alade taimestamine aedmustikaga. Eestis algasid aedmustika katsed mahajäetud freesturbaaladel 90ndate lõpus (Starast *et al.*, 2002). Aja jooksul on tehtud erinevaid katseid: uuritud on mineraalväetiste mõju ahtalehise mustika saagile (Starast *et al.*, 2002; Shanskiy, 2006; Albert *et al.*, 2011) ning mineraalmulla ja turbapinnase mõju poolkõrge ja ahtalehise mustika genotüüpide saagile ja viljade biokeemilisele koostisele (Starast *et al.*, 2007, 2017; Tasa *et al.*, 2012). Vahejõe *et al.* (2010) koostasid majandusanalüüsi ahtalehise mustika kasvatamise kohta turbaväljal. Kuna bioloogiline mitmekesisus mängib turbaalade taimestamise ja jätkusuutliku keskkonna majandamise juures suurt rolli, uurisid Starast *et al.* (2014) tolmeldajaid ning nektari tootmist turbavälja tingimustes. Samuti on uuritud sammalde, samblike ja lüljalgsete esinemist ahtalehise mustika istandikus (Tasa *et al.* 2015). Regionaalse taimahaiguste seireuuringu viisid läbi Starast *et al.* (2009). Seni pole uuritud orgaaniliste väetiste mõju aedmustikale mahajäetud turbaalade tingimustes.

Eesti mustikakasvatases pole haigused ega kahjurid probleeme tekitanud, mistõttu on ka mahetehnoloogiaid kasutusele võtta lihtsam kui teiste marjakultuuride puhul. Senistes teaduskatsetes mahajäetud freesturbaaladel on kasutatud mineraalväetiseid, seetõttu on oluline leida jätkusuutlikud väetamisstrateegiad, mis vastaksid mahetootmise nõuetele ja sobiksid aedmustika kasvatamiseks neil aladel.

Eestis müüakse aedmustikaid peamiselt värskest ja turustusperiood on sõltuvalt liigist kaks kuni neli nädalat. Modifitseeritud atmosfääris säilitamine peaks võimaldama kohaliku aedmustika müügiperioodi oluliselt pikendada. Aedmustikate säilivus sõltub liigist ja sordist. Maailmas on peamiselt uuritud kännasmustika (*Vaccinium corymbosum* L.) säilivust kontrollitud atmosfääris (Alsmairat *et al.*, 2011; Beaudry *et al.*, 1992; Hancock *et al.*, 2008). Mahajäetud freesturbaväljal maheviljeluse tingimustes kasvanud poolkõrge aedmustika säilivust aga ei ole varem uuritud.

Hüpoteesid ja eesmärgid

Lähtuvalt varasematest uuringutest püstitati järgmised hüpoteesid:

- Aedmustikakasvatuses on mõned orgaanilised väetised sobilikud alternatiivid sünteetilistele mineraalväetistele turba tootmise järel mahajäetud freesturbavälja tingimustes.
- Võrreldes mineraalväetistega tagavad orgaanilised väetised viljades tervisele kasulike biokeemiliste ühendite kõrge sisalduse.
- Poolkõrge ja ahtalehise mustika saagi säilivusaega on võimalik pikendada säilitamisega modifitseeritud atmosfääris.

Doktoritöö eesmärkideks oli välja selgitada järgmist:

- Erinevate orgaaniliste väetiste mõju poolkõrge mustika 'Northblue' kasvule, saagile ja saagi biokeemilisele koostisele mahajäetud freesturbavälja tingimustes.
- Modifitseeritud atmosfääri pakendite mõju mahedalt kasvatatud ahtalehisele ja poolkõrge mustika 'Northblue' saagi välisele kvaliteedile ja biokeemilisele koostisele.

Katsematerjal ja meetodika

Doktoritöös on kasutatud poolkõrge mustika sordi 'Northblue' kohta ajavahemikus 2006–2015 kogutud katseandmeid. Kasvatuskoht oli mahajäetud freesturbaväljale rajatud Hiie Talu tootmisistandikus Ilmatsalu külas Lõuna-Eestis (58° 23'N, 26° 31'E, H: 33 m).

Juveniilses kasvufaasis (2006–2009) kasutati kuut väetist: kontrolliks oli mineraalväetis Cropcare (6-6-19), ja mahevätised olid järgnevad:

- Algomin (2.5-0.2-0.2);
- Viva (5-1.7-6.2);
- Biolan (2.0-1.2-2.0);
- Kemira Bio (4-2-3);
- Compost Kanakaka (5-3-16).

Matuurses kasvufaasis (2011–2015) kasutati viit väetist: kontrolliks oli mineraalväetis Min (6-14-23), kaubandusliku nimega Cropcare, ja mahevätised olid järgnevad:

- Org 1 (3-1-7), kaubanduses Biolan 3-17;
- Org 2 (4-1-2), kaubanduses Biolan 4-1-2;
- Org 3 (5-3-16), kaubanduses Compost Kanakaka;
- Org 4 (9-1-4), kaubanduses Monterra.

Modifitseeritud atmosfääris säilitamise katse jaoks korjati ahtalehise ja poolkõrge aedmustika sordi 'Northblue' marjad freesturbaväljale rajatud Marjasoo Talu tootmisistandikust (58°12'N, 26°41'E). Katses kasutati kahte modifitseeritud atmosfääri pakendit: 1) 30 µm läbimõõduga polüetüleen pakendit (LDPE), (Estiko, Eesti) ja 2) spetsiaalselt mustikate säilitamise pakendit Xtend®, (Stepac, Iisrael). Modifitseeritud atmosfääriga pakendites säilitatud marjade kvaliteeti võrreldi tavaõhus säilitatu omaga.

Mahevätiste mõju tuvastamiseks poolkõrge mustika sordi 'Northblue' kasvule ja saagile, mõõdeti (**I**, **II**):

- juveniilses kasvufaasis (2006-2009) vegetatiivsed parameetrid: **põõsa kõrgus, laius ja diameeter (I)**. Lisaks tehti mulla- ja leheanalüüsid ning fikseeriti SPAD-i näit;
- matuurses kasvufaasis (2011–2015) vegetatiivsed parameetrid: **põõsa kõrgus, diameeter, marja mass ja saak (II)**. Biokeemilise koostise parameetritest määrati viljade rakumahla kuivaine, antotsüaanide, polüfenoolide ja tiitritavate hapete sisaldus. Maitse kirjeldamiseks arvutati rakumahla kuivaine ja tiitritavate hapete suhtarv.

Poolkõrge ja ahtalehise mustika viljade säilivust võrreldi modifitseeritud atmosfääriga pakendis tavaõhuga pakendiga ning selleks tehti järgmised analüüsid (III):

- mõõdeti O₂ ja CO₂ sisaldust modifitseeritud atmosfääriga pakendites.
- hinnati saagi kvaliteeti (vilja tugevus, närtsimine ja hallitamine).
- määrati antotsüaanide, rakumahla kuivaine, tiitritavate hapete sisaldus, arvutati rakumahla kuivaine ja tiitritavate hapete suhtarv.

Tulemuste kokkuvõte

Aedmustika saak ja kvaliteet sõltuvad kasvatustehnoloogiast ja ilmastikust. Mineraalväetiste mõju mahajäetud freesturbaväljal kasvava aedmustika vegetatiivsetele parameetritele ja biokeemilisele koostisele on juba eelnevalt uuritud. Kuna jätkusuutlik ja keskkonda hoidev taimekasvatuse on ühe olulisem, tuleb välja selgitada, milliseid mahevätiseid saab aedmustika kasvatamiseks kasutada.

Käesolevas doktoritöös uuriti väetamise mõju poolkõrge mustika sordi 'Northblue' kasvule ja viljade biokeemilisele koostisele mahajäetud freesturbavälja tingimustes. Lisaks on vaja leida lahendus mustikate säilivuse pikendamiseks.

Väetuskatse hüpoteesid leidsid osaliselt kinnitust, kuna kõik väetised ei sobi mustika kasvatamiseks. Katse tulemused mahajäetud freesturbaväljal poolkõrge mustika sordiga 'Northblue' olid järgmised:

- Mahevätis Algomin, mis on kõrge anorgaanilise aine sisaldusega ja põhineb merevetikal *Lithothamnium calcareum*, tõstis mulla pH-d kuni 5.2 ja pidurdas taimede kasvu juveniilses kasvufaasis, mistõttu ei sobi kaltsifuugse aedmustika väetamiseks (I).
- Kanasõnnikut sisaldavad väetised tõstsid mulla pH-d esimesel kasvuaastal, kuid pH stabiliseerus järgnevatel aastatel, jäädes vahemikku 3,5–4,0, tagades sarnase vegetatiivse kasvu ja saagikuse nagu mineraalväetise kasutamise korral (I).
- Mereadru sisaldav väetis tagas aastate lõikes stabiilse, sarnase või kõrgema saagi nagu mineraalväetise kasutamise korral, sõltumata märkimisväärselt madalamast P ja K sisaldusest (II). Seetõttu saab seda väetist kasutada keskkonnasõbraliku alternatiivina mineraalväetiste asemel mahajäetud turbavälja tingimustes.

- Kasvuaastal oli olulisem mõju mustikate bioaktiivsete ühendite sisaldusele kui väetamisel (II). Mereadrul põhinev väetis tagas sarnase polüfenoolide sisalduse viljades nagu mineraalväetise Cropcare 6-14-23 kasutusel.

Säilituskatse tulemused ahtalehise ja poolkõrge mustikaga modifitseeritud atmosfääriga pakendites olid järgmised:

- Spetsiaalselt kannasmustikate säilitamiseks välja töötatud modifitseeritud atmosfääriga pakend Xtend® pikendas ahtalehise mustika viljade säilivust 15 päeva ja poolkõrge mustika puhul 9 päeva võrra võrreldes tavaõhus säilitamisega (III). Polüetüleen pakend LDPE ei pikendanud ahtalehise mustika säilivust, kuid poolkõrge mustika marjad säilisid 9 päeva võrra kauem. CO₂ sisaldus oli Xtend® pakendis kõrgem kui LDPE pakendis.
- Sõltumata genotüübist mõjutasid mõlemad modifitseeritud atmosfääriga pakendid maitseomadusi, muutes mustikad hapumaks (III). Võrreldes tavaõhus säilitamisega, oli mahla kuivaine ja tiitritavate hapete suhtarv katse lõpuks väiksem mõlemas modifitseeritud atmosfääriga pakendis.
- Antotsüaanide sisaldus suurenes säilitamise jooksul kõikides katsevariantides, kuid võrreldes tavaõhus säilitamisega oli pakendite mõju ahtalehise mustika antotsüaanide sisaldusele väiksem (III).
- Genotüüpide erinevus ilmnis marjade väliste kvaliteedi näitajate puhul, kus poolkõrge mustika viljad olid võrreldes ahtalehise mustika viljadega vähem närtsinud ja tugevamad (III).

Kokkuvõtteks võib väita, et hüpotees leidis osaliselt kinnitust, mõlemad modifitseeritud atmosfääriga pakendid pikendasid poolkõrge mustika säilivust oluliselt, kuid modifitseeritud atmosfääriga pakendil on liigiti erinev mõju. Tulemustest saab järeldada, et ahtalehise mustika saak vajab kvaliteedi säilitamiseks võrreldes poolkõrge mustikaga kõrgemat CO₂ sisaldust.

Doktoritöö tulemuste põhjal saab välja tuua järgnevad soovitud ja edasist uurimist vajavad teemad:

Täiskandeeas tootmisistandikes on oluliseks näitajaks saagikus, seega võib mereadrul sisaldav madalama toiteelementide sisaldusega maheväetis

Biolan 4-1-2 olla turbaalade taimestamisel mineraalväetisele sobilik keskkonnasäästlikum alternatiiv.

Mustikalehtede makroelementide (N, P, K) sisaldus näitas juveniilses kasvufaasis toitelementide puudust, mistõttu on lisauuringud suuremate väetiskogustega selles kasvufaasis vajalikud. Täiendavalt tuleb uurida ka mustika kasvatamiseks sobivaid auksiine ja tsütokiniine sisaldavaid väetiseid. Lisaks vajab uurimist turbaaladel CO₂ emissiooni vähendamine aedmustikatega taimestamise abil.

Modifitseeritud atmosfääriga pakendite kasutamisega saab pikendada aedmustikate värskest turustamise aega. Modifitseeritud atmosfääriga pakend Xtend® pikendas mõlema genotüübi säilivust, mistõttu saab just seda pakendit aedmustika säilitamiseks soovitada. Keskkonna jätkusuutlikku majandamist arvestades tuleb plastikpakendite asendamiseks uurida biolagunevaid alternatiive.

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Half-highbush blueberry 'Northblue' plant growth in the juvenile stage: dependence on fertilizers in organic conditions

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Abstract

The aim of this research was to find out how different naturally-manufactured fertilizers influence the vegetative growth of young blueberry plants in abandoned peat field conditions. The study was carried out during the years 2006-2009 in southern Estonia. The plantation was established with one-year-old half-highbush blueberry (*Vaccinium corymbosum* × *Vaccinium angustifolium*) 'Northblue' plants on an abandoned peat-extraction field in the spring of 2006. Six fertilizers were used in the experiment, including the synthetic mineral fertilizer, Cropcare (6-6-19), and five natural (containing organic and mineral components) fertilizers: Algomin (2.5-0.2-0.2), Viva (5-1.7-6.2), Biolan (2.0-1.2-2.0), Kemira Bio (4-2-3) and chicken manure compost. Fertilization was carried out once a year (at the beginning of May). In 2006-2007, the fertilization rate was 0.24 g N plant⁻¹; in 2008, it was 1.0 g N plant⁻¹; and in 2009, it was 2.4 g N plant⁻¹. The most vigorous growth was obtained with the mineral fertilizer, Cropcare, where the plant height was 62 cm in 2009. Kemira Bio and Biolan led to the highest growth compared to the other natural fertilizers, 55.3 and 49.2 cm, respectively. The P and K contents in the leaves were the highest with Biolan. The SPAD chlorophyll meter value was also the highest in this treatment compared to other natural fertilizers. There was a pronounced effect of the fertilizers on soil acidity. The soil pH_{KCl} before plantation was 3.5, decreasing to 3.1 with Cropcare and increasing to 4.0 with Viva and to 5.2 with Algomin in a four-year-old plantation. The fertilizer, Algomin, considerably increased soil pH, leading to suppressed plant growth, and the plant height was only 15.8 cm in 2009. It was concluded that Algomin is not suitable for cultivating calcifuge plants such as blueberry. Biolan (2.0-1.2-2.0) and Kemira Bio (4-2-3) fulfill plant growth requirements and can be recommended for organic blueberry production.

Keywords: peat soil, soil pH, plant height, nutrient, abandoned peat field

INTRODUCTION

Estonia is very rich in mires and bogs; therefore, peat production is a well-developed industry sector here (Ramst and Orru, 2009). Currently, thousands of hectares once used for the milling of peat are now out of use because extraction has stopped. Earlier studies have shown that half-highbush blueberry and lowbush blueberry grow successfully and with high productivity in these peat fields (Paal et al., 2011; Tasa et al., 2012). The reason is that blueberries are calcifuges, and these plants need acidic soil with high organic matter content (Korsac, 1988). In harvested peat fields, the soils are very acidic, with pH=2.2-2.6, and the organic matter content is very high – more than 70% (Paal et al., 2011; Tasa et al., 2012). Pure peat has low concentrations of essential plant nutrients, so proper fertilization is needed for effective blueberry production.

In organic farming, manures are often used as natural fertilizers. Using fresh manure may lead to contamination problems including nitrogen emission to the atmosphere, chemical and microbial contamination of water bodies, and odour nuisance (Sims and Wolf,

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1994). Likewise, storage is problematic, as the manure is dumped as waste (López-Mosquera et al., 2008). A promising approach for reducing these problems is drying and pelletizing manure. Therefore, manufactured natural fertilizers are widely present in the market, and they are used in organic farming in the cultivation of many plants.

Blueberry fruit contain several beneficial phytochemicals contributing to human health: polyphenols including anthocyanins, vitamins, carotenoids, dietary fibres, etc. (Kalt and Dufour, 1997; Prior et al., 1998; Starast et al., 2007a). The bioactive component contents of blueberries depend on the cultivation method and environmental conditions in addition to the soil type (Starast et al., 2007a; Routray and Orsat, 2011). It is preferable to use cultivation techniques that do not decrease the quality of blueberry fruit. Berries may be more healthy and valuable if organic farming without synthetic pesticides and fertilizers is used. The most immediate, and perhaps greatest, opportunity for marketing blueberries may be in promoting blueberries as a healthy food. The berries are becoming more and more popular due to consumers' increasing awareness.

The hypothesis of this scientific study was that natural fertilizers are suitable for organic blueberry cultivation in soil with low fertility, high soil acidity and organic matter content.

The aim of this research was to ascertain how different natural, manufactured fertilizers influence the vegetative growth of young half-highbush blueberry plants grown in abandoned peat field conditions.

MATERIALS AND METHODS

The study was carried out in 2006-2009 on Hiie Farm territory of Ilmatsalu village, in southern Estonia (58°23'N; 26°31'E, H: 33 m). One-year-old half-highbush blueberry (*Vaccinium corymbosum* × *Vaccinium angustifolium*) 'Northblue' plants were planted in the spring of 2006 on an abandoned peat-extraction field. Blueberry plants were planted at 1.0×1.5 m spacing. The peat soil of the experimental area belongs to the soil subgroup of Fibri-Dystric Histosols (IUSS Working Group WRB, 2006). The peat layer left in the trial area was 1.0-1.5 m deep. According to the classification of Estonian vegetation site types (Paal, 1997), this area is classified as an oligotrophic (ombrotrophic) heavily drained bog of raised bog site type. This bog was used industrially for peat production (milling was used), but production ceased in the beginning of the 1980s. The experimental area is flat.

Six different fertilizers were used in the experiment:

- the synthetic, mineral fertilizer Cropcare (6-6-19) as a control,
- natural (containing organic and inorganic components) fertilisers: Algomin (2.5-0.2-0.2), Viva (5-1.7-6.2), Biolan (2.0-1.2-2.0), Kemira Bio (4-2-3); chicken manure compost (5-3-16).

In addition to the macronutrients, all fertilisers also contain micronutrients. All natural fertilisers are manufactured and commercially available. Viva is a powder fertiliser, all others pelleted.

Algomin is a fertiliser that is mainly based on the marine alga *Lithothamnium calcareum*. The organic matter content is 3% and the pH is 8.8.

The fertiliser Viva is specially developed for berry cultures, and it contains by-products from the processing of maize and grapes in addition to natural inorganic potassium salt.

Biolan fertiliser contains mainly composted chicken manure and vinasse extract, which is a potassium rich by-product of the sugar industry. During the production process, the fertilizer is sterilised at over 90°C.

Kemira Bio fertiliser consists of chicken manure, seaweed, feather, straw and inorganic biotite.

Chicken manure compost is a commercial organic fertilizer, which is sterilised at high temperature.

Fertilisation was carried out once a year in the beginning of May (the beginning of the growing season in Estonia). In 2006-2007, the fertilization rate was 0.24 g N plant⁻¹; in 2008, 1.0 g N plant⁻¹; and in 2009, 2.4 g N plant⁻¹.

Annually, at the end of the vegetation period (the end of September), the diameter (cm) and height (cm) of the blueberry bushes were measured. The plant diameter was measured across and along the row, and its average was calculated. The number of shoots longer than 15 cm per plant was counted. Ten plants were measured in each replication.

Soil samples were taken prior to establishing the experiments in the spring of 2006 and in the autumn of 2006 and 2009. Samples were taken close to the plants from the soil layer at 20 cm depth. The pH was measured from a soil suspension with 1 M KCl (1:5 w/v) using the Evikon pH meter.

Chlorophyll content, a key indicator of plant health, was evaluated using a portable chlorophyll meter SPAD-500 (Minolta Camera Co. Ltd. Japan). Leaves of the same age and position (from the central part of one-year-old, non-fruit-bearing shoots) on the plant were used; young leaves with uneven colour were left aside. Each reading consisted of measurements from 10 different plants, and one sample consisted of 30 leaf measurements on average. SPAD readings were performed in the beginning of August (blueberry harvesting time in Estonia), 2009.

The nutrient (nitrogen – N%; phosphorus – P%; potassium – K%; calcium – Ca%; magnesium – Mg%) content in the blueberry leaves was determined in the Laboratory of Plant Biotechnology at the Estonian University of Life Sciences. Leaf samples were collected in the beginning of August, 2009. 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 Kjeltac 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 air-acetylene flame. P was determined at the wavelength 720 nm by the stannous chloride method and Ca at the wavelength 570 nm using the *o*-cresolphthalein complexone method; and 8-Hydroxyquinoline was used to remove magnesium interference and 2-amino-2-methylpropan-1-ol was used as a buffer. Mg was determined using Titan Yellow at the wavelength 540 nm. All nutrient concentrations were expressed on a dry weight basis (% DW). Four sample replicate in every fertilization variant were analysed, and one sample consisted of leaves from ten plants.

The experimental design was a randomized complete block with four replications and 10 plants per plot. One-way analysis of variance (ANOVA) was used to compare all significant differences among fertilizers. Mean values are shown in figures and tables. Different letters indicate significant ($p < 0.05$) differences between fertilizers.

RESULTS AND DISCUSSION

When blueberry plants were measured in juvenile growth stage in the fourth growing year (2009), the highest growth (62.5 cm) was obtained with the synthetic fertilizer Cropcare (Figure 1). Among the organic fertilizers, Kemira Bio had the most positive effect on the plant growth. During the first two experimental years (2006 and 2007), Biolan and chicken manure compost allowed for vigorous plant growth. However, by the end of the experimental period the plant height was not as high that obtained with the synthetic fertilizer. The minimum plant growth was observed with the natural fertilizer Algomin, for which the plant height was only 15.8 cm in the fourth (2009) growth year (Figure 1). Blueberry plants also had small diameters (20.1 cm) (Figure 1) in the Algomin treatment when compared with the other treatments used in the trial. The highest diameter was reached with Cropcare, Kemira Bio and chicken manure composts. Nevertheless, there was no significant difference between the last two mentioned fertilizers and Biolan.



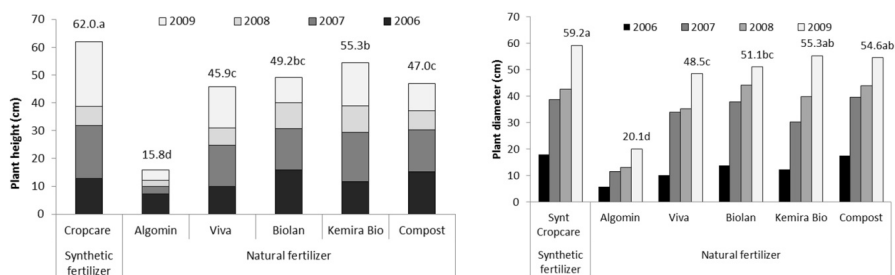


Figure 1. Plant height (cm) and plant diameter (cm) of half-highbush blueberry 'Northblue' plants (2006, 2007, 2008 and 2009) receiving different fertilization treatments. (a, b, c ... significant differences in 2009; Cropcare (6-6-19), Algomin (2.5-0.2-0.2), Viva (5-1.7-6.2), Biolan (2.0-1.2-2.0), Kemira Bio (4-2-3); Compost - chicken manure compost (5-3-16)).

Luby et al. (1986) found that when half-highbush blueberry 'Northblue' plants reached maturity in their tenth growing year, their average plant height was 65 cm. The blueberry plants in our trial were much younger, and their height varied from 46 to 55 cm when organic fertilizers were used. Taking into consideration the northern climatic conditions of Estonia, it may be concluded that blueberries grew well when the organic fertilizers Kemira Bio, Biolan, Viva or chicken manure compost were used. In a previous trial conducted in Estonia on mineral soil with 'Northblue', the plant height was close to 40 cm in the seventh growing year (Starast et al., 2005). Similar growth parameters in our trial were monitored in a commercial experiment with half-highbush blueberry 'Northblue' plants on peat soil (Tasa et al., 2012).

In the fourth year (2009), the highest number of new shoots was obtained with Viva and Cropcare, followed by Kemira Bio, Biolan and chicken manure compost (Table 1). As observed for the plant height and diameter, Algomin fertilizer had only minimal effect on shoot formation. Starast et al. (2005) have found that the establishment of new shoots in organic conditions is 2.2 shoots plant⁻¹. In our trial, the number of new shoots was a slightly higher 3.2 shoots plant⁻¹, considering the means of all organic fertilizers.

Table 1. Number of shoots (2009) and SPAD readings (2009) of half-highbush blueberry 'Northblue' plants and soil pH_{KCl} values (2006 and 2009) receiving different fertilization treatments.

	Shoots number	SPAD readings		Soil pH _{KCl}
	2009	2009	2006	2009
Cropcare	4.6a	34.6a	3.3E	3.1d
Algomin	2.0c	31.3bc	4.9A	5.2a
Viva	4.4a	30.4c	4.3B	4.0b
Biolan	3.3b	32.2b	4.3B	3.5c
Kemira Bio	3.5b	31.2c	3.7D	3.7c
Chicken manure compost	3.0b	31.1c	4.0C	3.5c

A, B, C ... significant differences in 2006; a, b, c ... significant differences in 2009.

All the fertilizers used in the experiment affected soil pH (Table 1). The soil pH measured before the experiment in the spring of 2006 was 3.5, and it increased with the addition of all organic fertilizers by the end of the season 2006, especially with Algomin fertilizer (pH=4.9). The smallest pH increase was observed with Kemira Bio (pH=3.7), while the synthetic fertilizer Cropcare decreased the soil pH to 3.3. Afterwards, the soil pH decreased or levelled off during all the experimental years with all fertilizers, except

Algomin, where the pH continued to increase up to pH 5.2 measured in the autumn, 2009. With the fertilizers Biolan and chicken manure compost, the soil pH decreased to the initial value pH 3.5. The soil acidity was stable with Kemira Bio (pH=3.7) during the whole experimental period. The organic fertilizer Viva increased soil acidity up to 4.0, while synthetic fertilizer Cropcare had the lowest pH (pH=3.1). Comparing different parameters of the experiment, we may suggest that low soil pH (Cropcare) (Table 1) is greatly correlated with vigorous plant growth (Figure 1) and shoot formation (Table 1). As soil pH increased (Table 1) too high (Algomin), it also suppressed plant growth (Figure 1, Table 1). Blueberry is a calcifuge; its production is strongly influenced by soil pH, and the optimal soil pH for blueberries lies around pH 4.8-5.5 (Korsac, 1988). However, our trial showed that plants grown on peat soil prefer even stronger soil acidity. The same conclusion has been drawn by Paal et al. (2011), who found that there was no need to increase pH of acid soils (pH=2.6) with liming. In contrast, raising the pH could have a negative effect on the blueberry growth instead. They also concluded that the fertilization of peat soil, rich in organic matter, was very necessary.

Similar to our results, Warman and Shanmugam (2008) have stated that synthetic fertilizers suitable for blueberry production decrease the soil pH and organic fertilizers increase the soil acidity. Three natural fertilizers in our trial – Biolan, Kemira Bio and chicken manure compost – contain a significant proportion of chicken manure. Chicken manure has a neutral reaction (Dikinya and Mufwanzala, 2010). In addition, López-Mosquera et al. (2008) have shown that the drying and pelletizing process increases pH as well. These results indicate that using the mentioned fertilizers, the soil pH will increase. Nevertheless, in our trial soil, pH increased significantly in the first year, but later decreased and stabilized. Previous studies have shown that chicken manure does not always influence the soil pH similarly, and its activity is dependent on the soil type where the fertilizer is used (Dikinya and Mufwanzala, 2010).

The SPAD chlorophyll meter read highest when the synthetic fertilizer Cropcare (Table 1) was used. The plants with lowest SPAD readings were measured in the Viva, Kemira Bio and chicken manure compost treatments. Thus, there was no significant difference compared to the Algomin treatment.

Analysis of element accumulation in the leaves showed that Ca content stayed within limits recommended by Trevett (1972) (Table 2). Mg content was higher than the recommended range. However, K content was in the recommended range only with the Biolan fertiliser treatment. A similar result was observed with P content, which stayed in the suitable range only with Biolan and with Algomin fertiliser. N content was in the recommended range only with Algomin treatment. Leaf analyses showed insufficient element uptake in all the fertilizer treatments applied in the trial, which indicates that it might be necessary to increase the fertilizer amount. The sufficiency levels, which were worked out by Trevett (1972), are for lowbush blueberry, and therefore these ranges may not be directly extrapolated to half-highbush blueberry. Moreover, sufficient levels of leaf nutrition uptake have not yet been established for half-highbush blueberry, and other researchers have previously noted that evaluating half-highbush blueberry nutrition uptake using nutrient levels established for other blueberry species is complicated (Warman and Shanmugam, 2008). The mentioned authors compared half-highbush blueberry mineral content in leaves with the nutrient ranges for *Vaccinium corymbosum*. Although Korsac (1988) has indicated all blueberries have relatively low nutrient requirements, there is still a need, for effective and environmentally friendly production, to work out critical nutrient levels for half-highbush blueberry (Starast et al., 2007a; Routray and Orsat, 2011).

The SPAD chlorophyll meter is a functional portable tool for evaluation of plant nutrition. Previous experiments have investigated the correlation between SPAD readings and nitrogen content of leaf tissue in various plant species (Porro et al., 2001) including lowbush blueberry (Starast et al., 2007b). In our trial, no strong correlation between SPAD measurements and leaf nitrogen content was noticed. On the contrary, there was a significant correlation between SPAD reading and Mg content in plant leaves. A similar result was found by Shaahan et al. (1999) who have stated that SPAD chlorophyll meter can be



used to assess Mg nutrition status for some fruit trees. The results in our experiment might be due to the low nitrogen content in the leaves after fertilizing, while the magnesium content stayed high.

Table 2. Nutrient content (% DW) in leaf tissue of half-highbush blueberry 'Northblue' (2009) receiving different fertilization treatments. Sufficiency levels of nutrients in leaves of lowbush blueberry from Trevett (1972).

	N%	P%	K%	Ca%	Mg%
Cropcare	1.28	0.12	0.38	0.49	0.41
Algomin	1.66	0.06	0.32	0.54	0.45
Viva	1.25	0.11	0.37	0.48	0.42
Biolan	1.24	0.12	0.40	0.51	0.37
Kemira Bio	1.19	0.10	0.34	0.49	0.40
Chicken manure compost	1.23	0.09	0.32	0.48	0.39
Sufficiency level (Trevett, 1972)	1.60-2.38	0.12-0.22	0.4-0.9	0.27-0.52	0.12-0.25

CONCLUSIONS

In conclusion, Algomin fertilizer, with high inorganic content, is not suitable for half-highbush blueberry plant cultivation. Blueberry is a calcifuge, and Algomin fertilizer increases the soil pH and suppresses blueberry growth. Other natural manufactured fertilizers, which were used in our trial, are suitable for blueberry growing on peat soil. In organic farming conditions, the most effective fertilizers were Biolan (2.0-1.2-2.0) and Kemira Bio (2.0-1.2-2.0). Macronutrient (N, P, K) uptake by the leaves indicates insufficiency of nutrients. Therefore, higher fertilizer amounts per plant require further investigation.

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Article

Sustainable Fertilizer Strategies for *Vaccinium corymbosum* x *V. angustifolium* under Abandoned Peatland Conditions

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Abstract: Revegetating abandoned peatlands plays an important role in reducing the CO₂ footprint. One possibility for carbon reduction is cultivating blueberries as calcifuge plants in acidic peat soil. The aim of the experiment was to find out the effect of different fertilizers on half-highbush blueberry cultivar ‘Northblue’ growth and biochemical parameters in peatland conditions. The experiment was carried out in 2011–2015 with four organic and one mineral fertilizer, where three were composted chicken manure- and one maltose-based organic fertilizer. The soil of the experimental area belongs to the soil subgroup *Fibri-Dystric Histosol* with the peat layer 1.0–1.5 m deep. Organic fertilizer 4–1–2, which contained seaweed but had low phosphorus and potassium content, resulted in high yields in 2011 and 2013, with similar vegetative growth and comparable biochemical parameters as mineral fertilizer 6–14–23. The principal component analysis showed that the experimental year was more important in determining fruit parameters than the fertilizer type. However, our results indicated that the organic fertilizers are alternatives to mineral fertilizer for organic production.

Keywords: anthocyanin; total phenol content; growth parameters; peatland revegetation; organic fertilizers; yield

1. Introduction

In the European horticulture industry, Estonia is a remarkable peat exporter [1]. Peat is mostly used as an organic amendment for soil improvement and as a substrate for ornamental plants and vegetable production, but is also used as fuel for household heating. Since abandoned peatland constitutes substantial CO₂ emissions—ca. 8 million metric tons annually, which is more than all Estonian cars and trucks produce combined [2,3]—restoring or revegetating these areas is vital for sustainable environmental management. In Estonia, peatlands with a depth greater than 30 cm cover an area of about 915,000 ha [4], of which about 5000 ha are abandoned peat mining areas, while on another 11,000 ha, mining is still in progress [2]. About 25% of European peatlands are located in the Baltic Sea basin, making up at least 14% of the basin area [5]. Many of these (41%) are located in the northern sub-basin—in the Bothnian Bay area, which is the northern part of Baltic Sea, where Estonia sits. After Finland and Sweden, Estonia is third in Europe in terms of area of peatland [6].

The content of available mineral elements in peat soil is small, because organic matter mineralization after decomposition is a time-consuming process [7,8]. Therefore, plant growth on peat soil is highly fertilizer dependent [9,10]. Growing calcifuge plants with fertilizers on abandoned peatlands is one of the options to reduce carbon emission. It was concluded from an earlier study

in Estonia that blueberry (*Vaccinium* spp.) cultivation on peatlands is economically profitable [10]. The peculiarity of Estonia is that most of the blueberry cultivation follows organic farming principles, as diseases and pests have not yet caused economically important yield losses, thus making organic technologies easily adoptable. In addition, it has been stated that the blueberry fields located on peatlands have lower disease indices [11]. However, there is little information about appropriate organic fertilizers for half-highbush blueberry cultivation on peat soil.

The suitable blueberry species for growing under the northern climatic conditions is a half-highbush blueberry *Vaccinium corymbosum* × *V. angustifolium* [12]. Half-highbush blueberry is an interspecific hybrid of lowbush and highbush (*V. corymbosum* L.) blueberries that has inherited the ability to survive the harsh winters of northern areas [13]. However, winter damage, especially fluctuating temperatures in January and February, is among the major causes of blueberry yield loss [12]. A previous study showed that half-highbush cultivar (cv.) ‘Northblue’ is winter-hardy and performs well under cold climatic conditions. This cultivar has big berries, but contains lower amounts of anthocyanins and other polyphenolic compounds compared to commonly cultivated half-highbush blueberry cv. ‘Northcountry’ [14]. Anthocyanins are bioactive flavonoid compounds that are beneficial against many chronic diseases, and therefore blueberry is one of the fruits that is popular for its taste and richness in anthocyanins [15]. These compounds are blue, red, or purple pigments that are found in plants, especially flowers, fruits, and tubers. In acidic conditions, anthocyanin appears as a red pigment, while a blue pigment occurs in alkaline conditions. Although the polyphenolic compound content is species-specific, the weather conditions during the vegetative period also have an important effect on biochemical and growth parameters [16]. Previous studies of blueberries on peat and mineral soils have shown that yield and other plant parameters are strongly related to weather conditions, i.e., temperatures, precipitation and sunshine hours [9,17].

Since blueberry forms symbiosis with ericoid mycorrhizal fungi, which decompose the organic matter in the soil [18,19], organic amendments, i.e., organic fertilizers, are important for the soil microorganism and mycorrhizal symbiosis. Blueberry plants need acidic soil, of pH_{KCl} 4.0–5.5, which is also rich in organic matter [20]. Previous studies have shown that blueberries grow well in highly acidic peat soil $\text{pH}_{\text{KCl}} > 4.0$ without the need of liming [17]. Growing blueberries in peat soil provides better nutrition supply compared to mineral soil, resulting improved growth and higher yield [12]. An experiment with different half-highbush blueberry cultivars evaluated the effect of cultural practices on mycorrhizal colonization and stated that the mycorrhizal colonization was higher on the roots of half-highbush cv. ‘Northblue’, with a positive correlation between mycorrhizal colonization and growth and yield [21]. When managed organically, research with a number of crops suggested a consistent reduction in soil-borne diseases [18], increased defense mechanisms of plants such as antioxidant production [22], and an increase in both microorganism diversity and biological activity in the soil [23]. It was confirmed in a previous study that the organic fertilizer increased the soil biota activity, mycorrhizal colonization, and leaf antioxidant content relative to conventional N source, and improved tolerance to soil pathogens [19]. Experiments conducted in peatland have shown that the lowbush blueberry cultivation could be an option for the vegetation restoration of abandoned peatlands as plant cover provides a suitable habitat for various arthropods [24].

Revegetating abandoned peatlands with blueberries could reduce the negative impacts of these environmentally sensitive areas, and therefore, more information on fertilizer strategies needs to be gathered. The hypothesis was that organic fertilizers are suitable for organic production, have a positive effect on the vegetative and biochemical parameters of half-highbush blueberry cv. ‘Northblue’, and are feasible alternatives to mineral fertilizer on peatlands after intensive peat production. The aim of the study was to determine the effect of different fertilizers on half-highbush blueberry cv. ‘Northblue’ plant growth and fruit biochemical parameters in peatland conditions.

2. Materials and Methods

2.1. Site Description

The experiment was carried out on an abandoned peat field in South Estonia in Tartu county (58° 23' N, 26° 31' E '7'45', H: 33 m). Data were collected in 2011–2013 and in 2015. In 2014, the blueberry plants had severe frost damage, and due to lack of yield, the biochemical analysis were not taken and are not presented in this study.

The experiment was established in the spring of 2006 with one-year-old half-highbush blueberry (*V. corymbosum* × *V. angustifolium*) cv. 'Northblue'. Plants were propagated with hardwood cuttings. The experiment was a randomized complete block design with three replicates with 10 plants per plot, with plant spacing of 1.0 × 1.5 m. Weeding was maintained manually and pesticides were not used. Fertilization was carried out every year once a year, at the beginning of May, which is also the beginning of the growing season in Estonia. The fertilization rate with organic fertilizers was 70 kg/ha N, an additional 50 kg/ha N was given with feather meal 14–1–0. The total N fertilization rate in a year was 120 kg/ha in each fertilizer treatment. All the fertilizers, organic and mineral, used in the experiment were granulated. Fertilizers were selected based on their composition suitability for blueberry cultivation.

One mineral and four organic fertilizers were used in the experiment:

- Min: mineral fertilizer 6–14–23 (plus Mg 3%, S 11%, B 0.05%, Cu 0.1%, Fe 0.1%, Mn 0.7%, Mo 0.01%, Zn 0.01%). Fertilizer 6–14–23 has been commonly used in conventional berry production in Estonia. Mineral fertilizer was considered as the control.
- Org 1: Organic fertilizer 3–1–7 contains mainly composted chicken manure and vinasse extract (9%, potassium rich by-product of the sugar industry) and molasses.
- Org 2: Organic fertilizer 4–1–2 contains chicken manure compost and seaweed meal (plus Cu 0.01%, Fe 0.1%, Mn 0.04%, Zn 0.02%).
- Org 3: Organic fertilizer 5–3–16 is a chicken manure compost.
- Org 4: Organic fertilizer 9–1–4 is maltose based organic fertilizer (plus Mg 0.3%, S 3.0%, B 0.015%, Cu 0.1%, Fe 0.1%, Mn 0.7%, Mo 0.01%, Zn 0.01%).

2.2. Soil and Plant Description

The soil of the experimental area belongs to the soil subgroup Fibri–Dystric Histosol [25], with a peat layer 1.0–1.5 m deep and a flat field area. According to the classification of Estonian vegetation site types [26], this area is classified as an Oligotrophic (ombrotrophic), heavily drained bog of the raised bog site type; until the 1980s, it was used for industrial peat production—peat milling specifically. Soil samples were taken separately at 0 to 20 cm depth from each treatment replicate in the first week of August in 2013 and in 2014 (before the first harvest). To determine nutrient (P, K, Ca, Mg) concentration, pH_{KCl} and organic matter content, five soil samples were collected from each plot, then air-dried and analyzed at the Plant Biochemistry Laboratory of the Estonian University of Life Sciences. The contents of available P, K, Ca and Mg were determined by the aluminium lactate method [27]. The mean nutrient contents of the experimental plots in 2013 and 2014 are shown in Table 1. Soil pH_{KCl} was 3.2–3.6 and organic matter content ranged from 80% to 83% in 2013. Soil pH_{KCl} was not analyzed in 2014—the organic matter content ranged from 82% to 83%.

Table 1. Nutrient (mg kg⁻¹) and organic matter (%) content of the experimental treatments in 2013 and 2014.

Year	Treatment	pH	P	K	Ca	Mg	Org.
2013	Min	3.2	258	1530	1924	958	82
	Org 1	3.5	83	1280	2732	843	83
	Org 2	3.5	103	222	2630	812	82
	Org 3	3.6	56	151	2978	777	82
	Org 4	3.6	112	234	2720	799	80
2014	Min	<i>n.a.</i>	447	1680	2546	761	83
	Org 1	<i>n.a.</i>	87	1723	4677	743	82
	Org 2	<i>n.a.</i>	83	254	4378	690	83
	Org 3	<i>n.a.</i>	74	172	4291	661	83
	Org 4	<i>n.a.</i>	37	216	4649	677	82

Org. = organic matter%; *n.a.* = not analyzed.

For the leaf tissue analysis, 150 uniform, uninjured and fully expanded mature leaves were hand-picked in August 2013 and 2014 from each treatment replicate. Analysis were performed in the Plant Biochemistry Laboratory of the Estonian University of Life Sciences, and results are given in g 100 g⁻¹ of dry matter (DM). Based on values recommended by Hart et al. [28], N content in blueberry leaves was deficient in all treatments, and P, Ca and Mg content was optimal in all treatments; K was optimal in the Min in both years and in Org 1 in 2013, and deficient/near optimal in other treatments (Table 2).

Table 2. Nutrient content of the half-highbush blueberry cv. ‘Northblue’ leaves (g 100 g⁻¹ DM) in 2013 and 2014 depending on the fertilizer compared to recommended tissue nutrient contents.

Year	Treatment	N	P	K	Ca	Mg
2013	Min	1.27	0.13	0.50	0.46	0.19
	Org 1	1.24	0.09	0.42	0.48	0.19
	Org 2	1.42	0.10	0.37	0.59	0.26
	Org 3	1.34	0.08	0.32	0.60	0.22
	Org 4	1.33	0.08	0.36	0.53	0.19
2014	Min	1.18	0.13	0.47	0.43	0.17
	Org 1	1.18	0.09	0.37	0.51	0.17
	Org 2	1.19	0.10	0.31	0.62	0.19
	Org 3	1.18	0.09	0.28	0.68	0.19
	Org 4	1.20	0.08	0.34	0.55	0.20
Recommended levels		1.76–2.0	0.10–0.40	0.41–0.70	0.41–0.80	0.13–0.25

Recommended = Recommended tissue nutrient content% [28].

2.3. Weather Conditions

Estonia is situated in the northern part of Europe in the temperate climate zone, where the climate is between continental and maritime, with four seasons. The country is located in a humid zone, where the mean annual precipitation is 672 mm [29]. The vegetation period in Estonia is from May to September with a mean temperature of 13.4 °C. Based on long term (1981–2010) meteorological data, the mean annual temperature is 6.0 °C. The coldest month of the year is February with a mean temperature of −4.5 °C. The warmest month of the year is July with a mean temperature of 17.4 °C. Mean sunshine duration is 1766 h, where December has mean sunshine hours of 20.7 h (lowest) and July of 288 h (highest). Snow cover is usually from the middle of December to the end of March. Based on Estonian Weather Service reports [29], monthly (May–September) temperatures, precipitation and total sunshine hours of the experimental area from 2011–2015 (except 2014) compared to the mean of 1981–2010 are shown in the Table 3. In 2011, the temperatures were slightly higher compared to the 30-year mean values, especially in June and July (17.7 and 20.5, respectively), and had less precipitation.

2013 was also slightly warmer compared to the 30-year mean values and had less precipitation and more sunshine hours from June to September. In 2012, there was more precipitation in May and August—76 and 103 mm, respectively. In 2015, there was less precipitation in June, July and August; however, there were more sunshine hours in August compared to the 30-years mean.

Table 3. Mean monthly temperatures, precipitation and total duration of sunshine hours from 2011–2013 and 2015 compared to the mean of thirty years (1981–2010).

Year	Month	Temp. (°C)	Precip. (mm)	Sun. (h)	1981–2010		
					Temp. (°C)	Precip. (mm)	Sun. (h)
2011	May	11.6	47	280	11.5	55	257
	June	17.7	38	318	15.0	84	251
	July	20.5	59	262	17.6	72	269
	August	16.6	61	216	16.2	86	220
	September	12.9	61	154	11.0	61	136
2012	May	12.0	76	271	11.5	55	257
	June	13.8	89	252	15.0	84	251
	July	18.3	69	281	17.6	72	269
	August	15.2	103	171	16.2	86	220
	September	12.4	57	127	11.0	61	136
2013	May	14.9	73	286	11.5	55	257
	June	18.2	35	269	15.0	84	251
	July	17.9	59	272	17.6	72	269
	August	17.2	79	253	16.2	86	220
	September	11.3	23	186	11.0	61	136
2015	May	10.6	61	224	11.5	55	257
	June	14.6	66	251	15.0	84	25
	July	16.1	68	215	17.6	72	269
	August	17.0	47	305	16.2	86	220
	September	12.8	67	133	11.0	61	136

Temp. (°C) = temperature (°C); Precip. (mm) = precipitation (mm); Sun. (h) = total duration of sunshine hours (h).

2.4. Determination of Vegetative and Yield Parameters

For bush height and diameter (cm) measurements, plants were measured from each treatment replicate with a ruler each year when the vegetative growth of the plants was complete. For bush height, the plants were measured from the base of the plant (soil level) to the top of the plant (highest point). For bush diameter, the plants were measured across the line, within the line and diagonally; from those measurements, the mean bush diameter was calculated.

For the berry mass (g) measurements, uniform and uninjured berries from the first harvest were weighed with scale Scaltec SAC 51. Berry mass was calculated as a mean of 30 fresh fruits from each treatment replicate. For the yield calculation (g plant^{-1}), each year plants were hand-harvested three times or until all berries were ripened, with ca. one-week intervals. The criteria determining the stage of maturity was the fruit's full blue coloration. The yield of each bush from each harvest was weighed with a scale.

2.5. Fruit Biochemical Analysis

Blueberries were harvested in the first weeks of August in every experimental year. Analysis were conducted from the first harvest. For preparing the laboratory samples, 250 g of berries were taken from each of the treatment replicates and pureed. Analysis were conducted on fresh berries one day after harvesting and expressed by fresh weight (FW). All the analysis and measurements were performed in three replicates.

The total phenol content (TPC) was determined using the Folin–Ciocalteu method [30] with a Shimadzu UV Visible Spectrophotometer UVmini-1240. Ethanol-acetone (7:3) solution was used as the

solvent to extract the phenolic compounds. In the experiment, 0.3 mL of the plant extract was mixed with 7.7 mL of distilled water and 0.5 mL of Folin–Ciocalteu reagent (1:1 solution with water). After 1 min, 1.5 mL of a 2% sodium carbonate solution was added. Samples were held for 2 h at 22 °C and the absorbance was read at 765 nm. The total phenol content was expressed as mg of gallic acid per 100 g of fresh berries.

The total anthocyanin content (ACC) was estimated using the pH differential method [30]. Absorbance was measured with a Shimadzu UV Visible Spectrophotometer UVmini-1240 at 510 nm and at 700 nm in buffers at pH 1.0 (HCl 0.1 N) and pH 4.5 (citrate buffer). The extraction solution contained hydrochloric acid (0.1 M) and ethanol (96%) in *v:v* ratio of 15:85. The results were expressed as mg of cyaniding–3–glycoside per 100 g of fresh berries.

A Pocket Pal–1 digital hand-held refractometer (Atago) was used for soluble solids content (SSC) measurements. SSC was estimated as °Brix. Titratable acid content (TAC) was analyzed using a standard acid–base titration method [30]. An aliquot of sample (40 mL) was titrated with 0.1 M NaOH solution to a phenolphthalein endpoint (pH 8.2). EasyPlus Tritation (Mettler Toledo) was used for measuring (with electrode DG 111-SC for endpoint detections). Titratable acids content was expressed as mg citric acid per 100 g of fruit fresh weight (FW), as citric acid was the dominant organic acid in blueberries, using the milliequivalent factor of 0.064 for the citric acid. The Brix/acid ratio (SSC/TAC) was calculated by dividing soluble solids by titratable acids content. For the determination of ascorbic acid content (ASC), hydrochloric and acetic acids were immediately added to the fruit puree to avoid ascorbic acid breakdown in the air. ASC was titrated with the solution of 2,6–dichlorophenolindophenol [31] using an automatic titrator (EasyPlus, Mettler-Toledo International Inc.), and expressed as mg 100 g⁻¹ FW.

2.6. Statistical Analysis

All measurements were carried out on three parallel samples for each variable and data were expressed in tables as the mean value ± standard deviation (SD). The data were evaluated by one-way analysis of variance (ANOVA), and the means were compared using a Fisher’s least significant difference (LSD) test at a 5% probability level. A principal component analysis (PCA) was performed to describe the structure of all the analyzed parameters in relation to the fertilizers and experimental years. A principal component analysis (PCA) was applied to describe the structure of all the analyzed parameters in relation to the fertilizers and experimental years. The PCA is a dimensional modelling method that helps to visualize correlations between data points and give an interpretable overview of the main information from multidimensional data, in which the results are estimated and summarized into a few underlying variables. Analyses were performed using standardized mean data. All analyses were performed using Statistica for Windows version 12.0 (StatSoft, Inc., Tulsa, OK, USA).

3. Results

3.1. Vegetative and Yield Parameters

Bush diameter ranged from 75 to 118 cm in the experiment (Table 4). Fertilizers did not have an effect on bush diameter or height in 2013 and 2015. In 2011, the bush diameter was statistically smaller with the use of Org 4 fertilizer compared to with the other treatments. In 2012, a similar trend continued, where the Org 4 treatment resulted in a smaller bush diameter compared to the Org 1 and Org 2 treatments. The use of the Org 4 treatment also resulted in lower bush height in 2011 and 2012.

Table 4. The effect of fertilizing on the half-highbush blueberry cv. ‘Northblue’ berry mass, bush diameter, bush height and yield in 2011–2013 and 2015.

Year	Treatment	Bush Diameter	Bush Height	Berry Mass	Yield
2011	Min	100 ± 9 ^a	83 ± 4 ^a	2.3 ± 0.2 ^a	568 ± 8 ^d
	Org 1	94 ± 4 ^a	78 ± 5 ^a	2.1 ± 0.3 ^a	412 ± 32 ^e
	Org 2	96 ± 5 ^a	82 ± 7 ^a	2.1 ± 0.1 ^a	1222 ± 33 ^a
	Org 3	96 ± 6 ^a	82 ± 4 ^a	2.3 ± 0.1 ^a	878 ± 13 ^b
2012	Org 4	75 ± 8 ^b	59 ± 3 ^b	2.0 ± 0.1 ^a	827 ± 28 ^c
	Min	109 ± 3 ^{a,b}	81 ± 1 ^a	2.5 ± 0.1 ^{a,b}	2043 ± 41 ^a
	Org 1	116 ± 3 ^a	82 ± 1.8 ^a	2.7 ± 0.1 ^a	1986 ± 78 ^a
	Org 2	112 ± 11 ^a	79 ± 2 ^{a,b}	2.7 ± 0.2 ^a	2014 ± 59 ^a
2013	Org 3	107 ± 5 ^{a,b}	77 ± 4 ^{b,c}	2.4 ± 0.2 ^b	1508 ± 62 ^b
	Org 4	99 ± 2 ^b	73 ± 1 ^c	2.7 ± 0.2 ^a	1126 ± 82 ^c
	Min	103 ± 10 ^a	87 ± 7 ^a	1.6 ± 0.4 ^a	328 ± 28 ^c
	Org 1	100 ± 8 ^a	85 ± 8 ^a	1.4 ± 0.1 ^a	335 ± 38 ^c
2015	Org 2	101 ± 4 ^a	89 ± 6 ^a	1.3 ± 0.0 ^a	649 ± 13 ^a
	Org 3	96 ± 6 ^a	86 ± 6 ^a	1.4 ± 0.1 ^a	285 ± 38 ^c
	Org 4	93 ± 3 ^a	78 ± 6 ^a	1.4 ± 0.1 ^a	579 ± 42 ^b
	Min	118 ± 10 ^a	100 ± 4 ^a	1.9 ± 0.1 ^a	1046 ± 360 ^a
2015	Org 1	117 ± 4 ^a	105 ± 5 ^a	1.9 ± 0.1 ^{a,b}	796 ± 29 ^a
	Org 2	111 ± 9 ^a	100 ± 7 ^a	1.7 ± 0.1 ^b	859 ± 309 ^a
	Org 3	108 ± 3 ^a	98 ± 4 ^a	2.0 ± 0.1 ^a	723 ± 110 ^a
	Org 4	109 ± 3 ^a	100 ± 2 ^a	1.7 ± 0.2 ^b	741 ± 143 ^a

Bush diameter (cm); bush height (cm); yield (g plant⁻¹); berry mass (g); ± SD. Means for each parameter followed by the different letter within each year in each column are significantly different ($p \leq 0.05$).

Berry mass did not differ significantly in 2011 and 2013 between the treatment types—ranging from 2.0 to 2.3 g in 2011, and 1.3–1.6 g in 2013 (Table 4). In 2012 and 2015, there were statistical differences, but no obvious trend was noticed. In 2012, the use of the Org 1, Org 2 and Org 4 fertilizers had a positive effect on berry mass compared to the use of Org 3. In 2015, the use of Min and Org 3 had a positive effect on berry mass compared to the results for the use of Org 2 and Org 4.

The fertilizers had a significant effect on the yield, which ranged from 285 to 2043 g plant⁻¹ (Table 4). The use of the Org 2 treatment resulted the highest yield in 2011 and 2013. In 2011, the yield from the Org 2 treatment was more than double than those of Min and Org 1 treatments, and more than one third higher than for those of Org 3 and Org 4 treatments. In 2012, a higher yield was obtained from the Min, Org 2 and Org 1 treatments compared to that of the Org 3 and Org 4 treatments. In 2015, the yields ranged from 723 to 1046 g plant⁻¹, and no effect of fertilizing was observed.

3.2. Effect of the Fertilizer and Experimental Year on the Biochemical and Vegetative Parameters of Blueberries as Characterized on PCA

The PCA showed that the first principal component (PC1) explained 44% of the total variance in the data, and the second principal component (PC2) explained 23% (Figure 1). The PC1 and PC2 explained 68% of the variance in the data for both Figure 1a,b. PC3 explained 12% of the variance in the data in Figure 1a and described the negative correlation of ACC (data not presented in figure). PC1 was more related to the SSC, TAC, SSC/TAC, berry mass, dry matter and yield, whereas total PC2 was more related to the TPC, bush height and bush diameter in Figure 1a. TPC was positively correlated with 2011 in PC2; while yield, berry mass and TAC were positively correlated in PC1 and were highest in 2012.

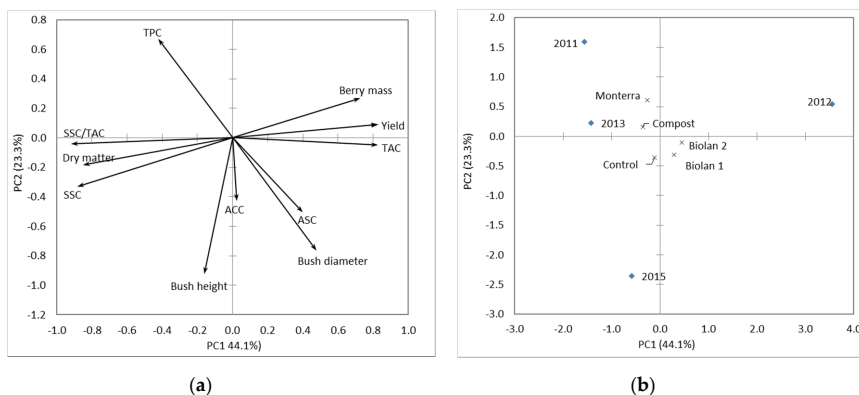


Figure 1. Principal component analysis (PCA) of the structure of biochemical and vegetative parameters in relation to the fertilizer type and experimental year's climate: (a) biochemical parameters in relation to the vegetative parameters; (b) fertilizer type in relation to the experimental year.

PCA demonstrated that experimental years distinguished more clearly than different fertilizers, and that their relative importance in determining fruit characteristics was larger (Figure 1). For instance, high yield and large fruits were characteristic of 2012, and high total polyphenol content was characteristic of 2011. The PCA also showed a close positive relationship between the berry mass and TAC of fruits and negative relationship between berry mass and SSC of the fruit.

3.3. Fruit Biochemical Parameters

The TPC ranged from 134 to 220 mg 100 g⁻¹ FW, and the effect of the fertilizer was significant (Table 5). Fruits produced under the Min treatment had a higher TPC in 2012, 2013 and 2015 compared to those produced under the Org 1 treatment. The Org 2 treatment resulted in statistically lower fruit TPC in 2012 and in 2015 compared to that of the fruits produced under the Min treatment. The ACC ranged from 48 to 136 mg 100 g⁻¹ FW. The use of Min resulted in statistically lower ACC in fruits in 2012 and in 2013 compared to the use of the Org 4 treatment. Treatment with Org 4 resulted in higher ACC in 2012, 2013 and 2015 compared to treatment with Org 1.

The SSC ranged from 9.6 to 11.9 Brix° in the experiment, with a significant effect of the fertilizers being observed (Table 5). In 2011, the SSC was statistically lower with the use of the Org 1 and Org 2 fertilizers, where Org 4 treatment resulted in the highest SSC. The Min treatment resulted in the highest SSC in 2012 compared to the Org 2 and Org 4 treatments. In 2013, fertilizing with Org 1 and Org 2 resulted in the lowest SSC compared to the other treatments. In 2015, a similar trend continued, where Org 1 resulted in the lowest SSC compared to the Org 3 and Org 4 treatments. TAC did not have any statistical differences between the treatments in 2011, 2013 and 2015; however, in 2012, the TAC was statistically different with the use of Min fertilizer compared to the organic fertilizers. SSC/TAC varied between the treatments in 2012, where the highest SSC/TAC was achieved with Min fertilizer.

ASC had high variance in the experiment, ranging from 4.5 to 18.3 g 100 g⁻¹ FW (Table 5). In 2011, the Org 4 treatment resulted in statistically higher ASC compared to the other treatments, where the lowest results were obtained with the use of Org 2. In 2012, there was an opposite effect, where the Org 4 treatment resulted in relatively low ASC compared to the Min treatment. In 2013, a similar trend continued, where Min treatment resulted in higher ASC compared to the Org 4 treatment, and to the other organic fertilizers' ASC. In 2015, Min resulted in a higher ASC compared to the Org 4 treatment.

Table 5. The effect of the fertilizer on the half-highbush blueberry cv. ‘Northblue’ biochemical parameters in 2011–2013 and 2015.

Year	Treatment	TPC	ACC	SSC	TAC	SSC/TAC	ASC
2011	Min	207 ± 10 ^{a,b}	113 ± 7 ^a	11.2 ± 0.1 ^b	0.8 ± 0.1 ^a	14.4 ± 1.5 ^a	9.5 ± 0.4 ^b
	Org 1	195 ± 10 ^{b,c}	97 ± 6 ^b	10.8 ± 0.1 ^c	0.8 ± 0.1 ^a	13.6 ± 1.6 ^a	6.6 ± 0.4 ^c
	Org 2	206 ± 10 ^{a,b,c}	113 ± 12 ^a	10.8 ± 0.1 ^c	0.8 ± 0.1 ^a	13.6 ± 1.4 ^a	4.5 ± 0.1 ^d
	Org 3	191 ± 5 ^c	100 ± 11 ^{a,b}	11.2 ± 0.1 ^b	0.7 ± 0.1 ^a	16.2 ± 2.3 ^a	6.3 ± 0.2 ^c
2012	Org 4	220 ± 10 ^a	78 ± 4 ^c	11.7 ± 0.1 ^a	0.8 ± 0.1 ^a	14.7 ± 1.3 ^a	10.4 ± 0.8 ^a
	Min	182 ± 10 ^a	75 ± 8 ^c	9.9 ± 0.1 ^a	0.9 ± 0.1 ^b	11.1 ± 0.7 ^a	18.3 ± 0.7 ^a
	Org 1	147 ± 10 ^b	65 ± 7 ^c	9.6 ± 0.1 ^b	1.1 ± 0.1 ^a	8.7 ± 0.6 ^b	13.5 ± 0.2 ^c
	Org 2	148 ± 11 ^b	79 ± 4 ^c	9.1 ± 0.1 ^c	1.1 ± 0.1 ^a	8.1 ± 0.6 ^b	14.5 ± 0.2 ^b
2013	Org 3	191 ± 13 ^a	103 ± 13 ^b	9.6 ± 0.1 ^b	1.1 ± 0.1 ^a	8.7 ± 0.7 ^b	12.5 ± 0.5 ^d
	Org 4	157 ± 10 ^b	130 ± 16 ^a	9.1 ± 0.1 ^c	1.1 ± 0.1 ^a	8.0 ± 0.7 ^b	13.0 ± 0.0 ^{c,d}
	Min	204 ± 19 ^a	60 ± 6 ^b	11.9 ± 0.1 ^a	0.8 ± 0.1 ^a	14.3 ± 2.0 ^a	16.4 ± 0.3 ^a
	Org 1	149 ± 17 ^b	48 ± 8 ^c	11.6 ± 0.1 ^b	0.9 ± 0.1 ^a	13.4 ± 1.4 ^a	11.1 ± 1.1 ^b
2015	Org 2	193 ± 20 ^a	73 ± 7 ^a	11.6 ± 0.1 ^b	0.9 ± 0.1 ^a	13.0 ± 1.5 ^a	8.3 ± 0.4 ^c
	Org 3	198 ± 10 ^a	58 ± 6 ^{b,c}	11.8 ± 0.1 ^a	0.9 ± 0.1 ^a	13.4 ± 1.3 ^a	12.6 ± 1.7 ^b
	Org 4	194 ± 11 ^a	75 ± 5 ^a	11.9 ± 0.2 ^a	0.9 ± 0.1 ^a	12.9 ± 1.2 ^a	13.2 ± 1.7 ^b
	Min	170 ± 5 ^a	136 ± 8 ^a	11.8 ± 0.1 ^{a,b}	0.9 ± 0.1 ^a	13.3 ± 0.9 ^a	15.5 ± 1.0 ^a
2015	Org 1	146 ± 5 ^{b,c}	108 ± 16 ^b	11.6 ± 0.1 ^b	0.9 ± 0.1 ^a	13.4 ± 0.9 ^a	14.4 ± 1.0 ^{a,b}
	Org 2	134 ± 10 ^c	131 ± 5 ^a	11.7 ± 0.1 ^{a,b}	0.9 ± 0.1 ^a	13.3 ± 0.6 ^a	14.1 ± 0.8 ^{a,b}
	Org 3	158 ± 10 ^{a,b}	116 ± 12 ^{a,b}	11.9 ± 0.2 ^a	0.9 ± 0.1 ^a	13.7 ± 1.1 ^a	14.8 ± 0.8 ^a
	Org 4	158 ± 10 ^{a,b}	135 ± 10 ^a	11.8 ± 0.1 ^a	0.9 ± 0.1 ^a	13.0 ± 1.2 ^a	13.3 ± 0.3 ^b

TPC = total phenol content (mg 100 g⁻¹ FW); ACC = total anthocyanin content (mg 100 g⁻¹ FW); SSC = soluble solids content (Brix°); TAC = titratable acid content (g 100 g⁻¹ FW); SSC/TA = soluble solids and titratable acid content ratio; ASC = ascorbic acid content (mg 100 g⁻¹ FW); ±SD. Means for each parameter followed by the different letter within each year in each column are significantly different ($p \leq 0.05$).

4. Discussion

4.1. Vegetative Parameters

Blueberry plants had reached the mature stage in the experiment. In the first two years, the bush diameter and height were smaller with the use of Org 4; however, growth stabilized by the end of the experiment, where different treatment types did not have a significant effect on the vegetative parameters. Growth parameters in our study were affected by winter damage, and due to that, the results were fluctuating; however, the plants reached a height specific to their species. An earlier study stated that blueberries are more susceptible to winter damage on peat soil compared to mineral soil, although in peat soil the cv. ‘Northblue’ performed better than the other half-highbush cultivars [12]. Bush height varied in 2011 from 59 cm to 83 cm, and increased from 98 to 105 cm by the end of the experiment. In a previously mentioned study conducted on peat soil with cv. ‘Northblue’, the plant height was 48 cm, which was lower than in our study [12].

Although fertilizer had a significant effect on yield, the experimental year had a stronger impact, resulting in over sevenfold differences in yield. Yearly differences were caused by weather conditions. The effect of the experimental year was demonstrated by PCA, showing that the experimental year’s weather had a strong effect on fruit parameters, while the fertilizer type did not have a strong correlation with the variables. Previous studies have suggested that different environmental conditions affect blueberry mineral nutrition [31], but so do the year, site and cultivar interaction [12]. Once the plants reach full maturity, the yield fluctuates from year to year as a result of weather conditions and pruning [16]. As the abandoned peatlands in Estonia have similar organic matter content and soil acidity, the field to field variation of these areas is more modest than on mineral soil, and the yearly weather has a more significant impact on the fertilizer’s effects on yield [12,17]. Previous studies on abandoned peatlands showed that blueberry cultivation without fertilizer produces yields that are near negligible; therefore, fertilization is necessary to provide adequate productivity [9]. In our

study, in 2011 and 2013, the temperatures were slightly higher and there were less precipitation and more sunshine hours from June to September in 2013 compared to the 30-year mean values. In both years, the highest yield was achieved with the Org 2 treatment. However, in 2013, the yield was rather low compared to other experimental years. An earlier study conducted with lowbush blueberry on peat soil also concluded that fertilization had an effect and high variance on yield where yield varied from 14 to 393 g plant⁻¹ in a 5-year-old plantation; however, the effect of the fertilizer was weather-dependent [9]. Despite of the weather conditions in our study, the use of Org 2 fertilizer gave a stable yield performance in many experimental years. All of the fertilizers used in our experiment were granulated, but differed in composition; the Org 1, Org 2 and Org 3 contained chicken manure, and the Org 4 contained maltose, but also differed in phosphorus and potassium content. With reference to the different fertilizer compositions, the timescale of fertilizer decomposition may also have been different, as well as weather dependent. In 2011, there was less precipitation from May to August, which may have had a negative effect on nutrient uptake and plant growth.

Organic fertilizers used in the study showed positive results compared to the mineral fertilizer. Although organic fertilizer Org 2 had lower potassium and phosphorus content compared to the mineral fertilizer, it resulted in similar vegetative growth and a high yield. As described, the Org 2 fertilizer contains seaweed. Many studies have stated a wide range of beneficial effects of seaweed extract application on plants, such as early seed germination and establishment, improved crop performance and yield, increased resistance to biotic and abiotic stress, and the improved postharvest shelf-life of perishable commodities [32–35]. Seaweed contains macro- and micro-elements, amino acids, vitamins and growth hormones like cytokinins, auxins, and abscisic acid, that have an effect on cellular metabolism in plants resulting in improved growth and yield [36–38]. Seaweed also affects the chemical, physical and biological properties of soil, which all influence plant growth. Its extracts improve soil health by enhancing the moisture-holding capacity, increasing the growth of beneficial soil microbes, and encouraging the growth of beneficial fungi to stimulate mycorrhizal development [35,39]. A study found that seaweed oligosaccharides, which are produced by the enzymatic degradation of alginic acid, significantly improved the hyphal growth and elongation of arbuscular mycorrhizal fungi, but also activated their infectivity on trifoliolate orange seedlings [40]. Although the organic fertilizer Org 2 had a lower P and K content, the positive performance could be related to the seaweed concentration, which may have a beneficial effect on soil biota, mycorrhizal development and plant growth, although this needs further research.

4.2. Biochemical Parameters

The biochemical composition was affected by the fertilizer type and the year. The use of the Min treatment had higher TPC in 2012, 2013 and 2015 compared to the organic fertilizer Org 1. Valuable antioxidants in blueberries include phenolic compounds, the major role of which is to protect organisms against the oxidative stress induced by free radicals [41]. Previous studies have stated that the organically grown blueberry cultivar 'Powderblue' had a higher TPC compared to conventionally grown cultivars [41]. Another study conducted with different high-bush cultivars compared TPC results from conventional and organic farms and found that there were no statistical differences between the cultivation types, except that there were significantly higher tannin levels in plants grown under organic cultivation [42]. Moreover, a study by Wang et al. [43] showed that blueberry fruit grown from organic culture yielded significantly higher TPC, malic acid, total anthocyanin content, antioxidant activity and sugars (fructose and glucose), than fruit from the conventional culture. Oppositely, in our study, the use of Min fertilizer increased TPC during some of the experimental years compared to some organic fertilizers. A study established in Korea with different half-highbush cultivars had a TPC of cv. 'Northblue' of 247.8 [44], which indicates species-specific TPC results from our experiment.

There was high variability in ACC caused by the treatments. The use of the organic fertilizer Org 4 resulted in higher ACC in most of the experimental years compared to Org 1 and Min. Cultivation practices are the main factors that affect the concentration of anthocyanins in fruits depending on the

genotype [15]. A study conducted by You et al. [41] found that the total anthocyanin content and total phenol content accumulated in different blueberry cultivars was either more or comparable in the case of organically grown cultivars compared to in conventionally grown rabbiteye blueberries. A similar study that focused on the effect of different cultivation practices on highbush blueberries, stated that the total anthocyanin content was significantly higher in organically cultivated blueberries [43].

ASC was more affected by the experimental year than by the fertilization method. A previous study with cv. 'Northblue' stated an ASC of ca. 15 mg 100 g⁻¹ FW [14], which is similar to the 2015 results. An earlier study concluded that climatic factors like light intensity and temperature are the most important in determining the final ASC [45]. It has been reported that the cooler climate and higher light intensity tend to increase the content of ASC [9]. In our study, the temperatures were slightly higher with less precipitation in June and July 2011 compared to the 30-year mean; however, the ASC was lower compared to other experimental years. Overall, the use on Min fertilizer tended to result in a higher ASC. In 2012 and 2015, the Min treatment resulted in a higher ASC in fruits compared to the Org 4. In 2013, the use of Min resulted in highest ASC compared to organic fertilizers.

The fertilizers used in our experiment had a different effect on biochemical composition due to the different composition. The Min fertilizer had higher potassium (19%) content compared to organic fertilizers. Previous studies suggest that adequate potassium nutrition greatly influences the synthesis of sucrose and starch in different fruits, berries and vegetables, for example in apple [46,47] and in strawberry [48]. Potassium levels have different effects on organic acid metabolism depending on the plant species [49] and also strengthen photosynthesis at the source to supply the sink with enough carbohydrates [50]. In an experiment with apples, K fertilization promoted higher berry mass, higher Ca²⁺, soluble solid content, and lower TAC [47]. In our study, the K content in the leaves was optimal with the use of mineral fertilizer and the Org 1. Org 1 fertilizer also had a higher K content in the fertilizer (7%) compared to the other fertilizers used in the study. According to Hart et al. [28] the optimum K range in blueberry leaves is 0.41%–0.70%. Despite the higher K content in the Min and Org 1 fertilizer, no clear trends in the effect on biochemical content were observed. Although, in some experimental years, the use of Min treatment resulted in higher TPC and ASC, decreased TAC and increased SSC/TAC content, but this effect was not observed in every experimental year.

5. Conclusions

As the abandoned peatlands are environmentally sensitive areas, we conclude that organic fertilizers are feasible alternatives to mineral fertilizers for half-highbush blueberry cv. 'Northblue' organic cultivation under abandoned peatland conditions; thus, the hypothesis was confirmed. Since blueberry producers are primarily interested in high yield, the low nutrient fertilizer Org 2 could be recommended for organic production in peatland areas due to its more stable yield performance. Based on results, it may be concluded that fertilizer composition had an effect on plant vegetative and fruit biochemical parameters; however, the experimental year had more significant impact.

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Article

Comparison of Regular Atmospheric Storage versus Modified Atmospheric Packaging on Postharvest Quality of Organically Grown Lowbush and Half-Highbush Blueberries

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Abstract: The aim of the study was to determine the effect of modified atmosphere (MA) packages on the external quality of organically grown lowbush blueberry and half-highbush blueberry ('Northblue') and the nutritional value of the fruits. Fruits were divided into plastic punnets and stored as follows: regular atmosphere (RA), punnets without packing; punnets sealed in a low-density polyethylene (LDPE, Estiko) bag; punnets sealed in an Xtend[®] blueberry bag (Stepac). Fruits were stored at 3 ± 1 °C. Compared to RA conditions, the Xtend[®] package prolonged the postharvest life for 15 days for lowbush and 9 days for half-highbush blueberries. Fruit dry matter (DM) and titratable acidity (TA) were higher in the Xtend[®] package. Fruit SSC decreased in the LDPE packages and increased in the Xtend[®] packages during storage. Based on the decreased soluble solids content (SSC) and titratable acidity (TA) ratio (SSC:TA) values during storage, it can be concluded that the taste of the fruits became sourer in all packages. Anthocyanin biosynthesis of lowbush blueberries was suppressed in MA, but this effect was not noticed for 'Northblue'. Regarding fruit firmness, shrivelling, and decay, there were significant differences between the MA packages, but the genetic differences were more important: half-highbush blueberry fruits were firmer and less shrivelled.

Keywords: *Vaccinium angustifolium*; *V. corymbosum* x *V. angustifolium*; anthocyanins; soluble solids; titratable acidity; colour

1. Introduction

Interest in blueberries (*Vaccinium* spp.) is rising because of their health-promoting constituents, including flavonols, tannins, phenolic acids, and anthocyanins, which help to prevent cardiovascular diseases, cancer, and inflammation [1]. Internationally blueberries are sold in fresh, frozen, and processed forms, and additional research is needed to extend the postharvest life of fresh blueberries to further extend the marketing season and to reduce waste.

The postharvest quality of blueberries is affected by diverse physical, physiological, and pathological processes and aspects of blueberry deterioration including decay, shrivelling, and softening [2]. Major causes of postharvest spoilage for blueberries are fungal decay and physiological changes [3]. Though, storing blueberries in a CO₂-enriched atmosphere is an effective way of extending the postharvest life and inhibiting postharvest decay without fungicidal treatments [4].

Modified-atmosphere packaging has the potential to provide low O₂/high CO₂ regimes, but the packaging must maintain the appropriate atmospheric composition over a range of temperatures commonly encountered between harvest and consumption [5].

Modified atmosphere packages combined with an optimal storing temperature extend the storability of fresh produce by maintaining the sensory and nutritional quality [6,7]. Although several studies have found no significant differences in storing blueberries in the range of 0–5 °C [2,8,9], the most popular storage temperature is close to 0 °C [10,11]. At these temperatures, the blueberry may be stored in a regular atmospheric storage for a maximum of 2 weeks. By raising the humidity to 85–89%, the postharvest life can be extended for up to 6 weeks [12,13].

In Estonia, blueberries are sold mostly as fresh market fruit. Since a truly temperate climate is prevalent for the region [14], winter hardiness is the most important requirement for cultivar selection. Previous studies have suggested lowbush (*V. angustifolium* Ait.) and half-highbush blueberries (*V. corymbosum* × *V. angustifolium*) ('Northblue') as being suitable for cultivating under these climatic conditions [15–17].

A large volume of postharvest work has been performed with highbush blueberry cultivars (*Vaccinium corymbosum* L.), with particular reference to controlled atmosphere storage [5]. However, the current novel study was initiated as information concerning the modified atmosphere storage of lowbush and half-highbush blueberries is not well known. Furthermore, there are very few postharvest studies concerning organically grown blueberries.

Our hypothesis was that postharvest life of lowbush blueberries and half-highbush blueberries may be extended using modified atmosphere packaging without affecting the fruit quality. The aim of the study was to determine the effect of modified atmosphere (MA) packages on organically grown lowbush blueberry and half-highbush blueberry ('Northblue') external quality and the nutritional value of the fruits.

2. Materials and Methods

2.1. Plant Material and Storage Conditions

Two species of blueberry were investigated: the lowbush blueberry (*Vaccinium angustifolium* Ait.) and the half-highbush blueberry (*Vaccinium corymbosum* × *V. angustifolium*) cultivar 'Northblue'. The fruits were collected from a commercial farm in the Tartu county, South Estonia (58°12' N, 26°41' E). Bushes were grown organically in the soil subgroup *Fibri-Dystric Histosol* [18] with a residual peat layer that was 1.0–1.5 m deep. Uniform, disease-free blueberries at commercial maturity (beginning of August) were hand-picked into regular-atmosphere 250-g perforated polyethylene terephthalate "plastic" punnets (Infia TR80/58 mm, Produce Packaging, HL Hutchinson Ltd., Cambridgeshire, UK). Punnets were designed for soft and highly perishable fruits such as cherries and tomatoes [19]. Mass of the perforated punnets was 8 g and the dimensions were 143 × 96 × 58 mm. Punnets had four circular perforations (diameter 8 mm) at the bottom and the lid had eight oval perforations (20 × 5 mm). Treatments included:

1. a control, consisting of four regular atmospheric storage (RA) punnets only;
2. four regular atmosphere punnets sealed in a 30 µm thick low-density polyethylene (LDPE) modified atmosphere bag (product of Estiko, Estonia);
3. four regular atmosphere punnets sealed in an Xtend[®] modified atmosphere blueberry bag (Stepac, Israel).

There were 6 replicates per treatment and all treatments, including the control, were stored at 3 ± 1 °C for six weeks. Relative humidity ranged from 96 to 98%. The fruits were analysed on the day of harvest and then 1 replication of four boxes of blueberries was destructively sampled each week. Postharvest shelf-life was considered terminated when either the berries were too soft (firmness below 6.0 points), when shrivelling was ≥10%, or when the decay was ≥5%. For each treatment shelf-life was

considered terminated at a different time: in regular atmosphere punnets it was 22 days for lowbush blueberry and 28 days for half-highbush 'Northblue'; in LDPE modified packaging, it was 22 days for lowbush and 37 days for the half-highbush blueberry 'Northblue'. For Xtend[®] modified atmospheric packaging it was 37 days for both species. In this manuscript, the results at harvest (in the tables named Pre-storage) and at the end (in the tables named After storage) of each treatment are presented.

2.2. Gas Measurements

During storage, O₂ and CO₂ concentrations were measured using a hand-held gas analyser OXYBABY V (WITT-Gasetechnik GmbH & Co. KG, Witten, Germany). O₂ and CO₂ concentrations (%) were measured nine times from within each closed system. The integrity of each bagged system was maintained as an impermeable rubber septum was placed on the outside of each modified atmospheric bag and, through the septum, a gas analyser needle was inserted, and an aliquot of air was drawn out for both O₂ and CO₂ concentrations. O₂ and CO₂ concentrations in the natural atmosphere were measured above the berries from the headspace immediately above the perforated holes in the punnets.

2.3. Subjective Quality Measurements

Fruit firmness, shrivelling, and decay were determined after storage at 3 °C. Firmness was evaluated on a sub-sample of 10 berries by hand rolling, using a 1–9 scale (1 = berry ruptures on touch, 4.5 = berry surface depressed on touch, 9 = berry is firm, not yielding to touch). Shrivelling of the fruits was determined visually and was expressed as a percentage of all fruits in a punnet. Fruit decay was visually evaluated and it was expressed as a percentage of all fruits in a punnet. Any berries with visible mould growth were considered decayed. Pathogens were not identified in the experiment.

A trained sensory panel of 10 assessors was used for the sensory descriptive analysis. Prior to the sensory evaluation, assessors attended a discussion and training session, in which they were introduced to the experiment-specific criteria for sensory analyses. Evaluation criteria were conducted with modifications per the Schotsmans et al. [20] study.

2.4. Fruit Quality Analyses

Fruit quality characteristics were determined for each of the four punnets per replicate. All diseased berries were counted and removed. For chemical analyses, 100 g of the remaining healthy berries from each replication were pureed using a hand-held blender (Turbo MR 5550 M FP, Braun GmbH, Barcelona, Spain). Measurements were repeated four times. Dry matter (DM) was determined using a 10 ± 1 g sample and drying in a thermostat (Modell 400, Memmert GmbH + Co. KG Co., Schwabach, Germany) at 105 °C to a constant weight. Fruit dry matter content (%) was calculated on a dry weight and fresh weight basis.

Soluble solids content (SSC) was analysed using a digital Pocket Pal-1 refractometer (Atago Co., Ltd., Tokyo, Japan). The instrument was calibrated with distilled water and the lens was carefully rinsed and wiped dry between samples. Results are expressed as % of the fresh weight (FW) basis.

Titrateable acidity (TA) was measured by neutralizing (to pH 8.2) 0.1 M of NaOH solution (automatic titrator, Mettler Toledo DL 50 Randolino). Titrateable acidity was expressed as % of citric acid (% FW), as citric acid was the dominant organic acid in blueberries, using the milliequivalent factor of 0.064 for the citric acid. From these data, the SSC:TA ratio was calculated.

For the determination of anthocyanins (ACY), 50 whole fruits were crushed, and 10 g of the crushed fruit was soaked in an extracting solution containing HCl (0.1 M):C₂H₅OH (96%) = 15:85 (v/v). Solutions were shaken and held at 5 °C for 24 h. Total anthocyanin content was determined spectrophotometrically using a Thermo Spectronic Helios β spectrophotometer (Thermo Scientific Inc., Loughborough, UK) by determining the difference in the absorbance between solutions of pH 1.0 and pH 4.5 at emissions of 510 and 700 nm [21]. Values are expressed as mg cyanidin-3-glucoside equivalents per 100 g FW using a molar extinction coefficient of 26,900 L mol⁻¹ cm⁻¹.

2.5. Colour Measurements

Both the external (fruit surface/exocarp) and internal (flesh/mesocarp) colour were recorded using a reflectance colourimeter (Model CR-400, Minolta Co., Ltd., Tokyo, Japan). Two readings per fruit were taken on opposite sides of each of the 10 fruits from each replicate (from four punnets). In order to measure the colour of the fruit surface, the natural wax coating was removed mechanically (the method by Kalt et al. [22]), and the same fruits were measured with a wax and without a wax coating. For fruit flesh colour measurements, each fruit was bisected and measurements were taken immediately to avoid discolouration. The colour 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), and h^* (hue angle). A white plate was used for calibration (Illuminants C: $Y = 92.6x + 0.3134y = 0.3196$; illuminants D65: $Y = 92.6x + 0.3160y = 0.3324$).

2.6. Statistical Analysis

Statistical evaluation of the experimental results was performed by one-way analysis of variance (ANOVA, Fisher LSD Test). In figures and tables, all data are presented as means of the replications. Different letters indicate significant differences at $p < 0.05$, similar letters do not indicate significant differences ($p > 0.05$). Data of O_2 and CO_2 from two blueberry species that were measured from different modified atmosphere packages (LDPE film and Xtend[®] film) were statistically analysed at the end of the storage period. Depending on the storage conditions (RA, LDPE and Xtend[®] film) firmness, shrivelling, and decay were compared at the end of the storage period as well. Dry matter, total anthocyanins, titratable acids, total soluble solids, SSC:TA, and the colour parameters ($L^*a^*b^*$, C^* and h^*) were statistically compared in pre-storage and after storage depending on the storage conditions (RA, LDPE, Xtend[®] film).

3. Results and Discussion

3.1. Storage Time

Regular atmospheric (RA) storage punnets at 3 ± 1 °C and a relative humidity over 90% resulted in a postharvest life of 22 days for lowbush blueberries and up to 28 days for 'Northblue', but no longer (Figure 1). There was a significant improvement when the RA punnets were kept in modified atmosphere packages. Indeed, both the LDPE and Xtend[®] packages had a positive effect on the postharvest life for 'Northblue' for up to 37 days. However, for the lowbush blueberry LDPE, the packaging extended the postharvest life for only 22 days, which was similar to the regular atmosphere storage in punnets, whereas the Xtend[®] packaging resulted in a shelf-life of up to 37 days. The postharvest shelf-life of blueberries is strongly correlated with genetics [23]. In our study, the half-highbush blueberry ('Northblue') had a longer postharvest life compared to the lowbush blueberry in the LDPE film, which refers to the genetic difference of these two taxa and may suggest that the suitability of the film is species-specific. Peano et al. [24] described that increasing the quantities of CO_2 within the high-density polyethylene pallet and decreasing the quantities of O_2 so as to reach values in a range of 10–12% for both gases made it possible to induce a slowdown of the respiratory metabolism of the fruits, which increased the conservation period up to 45 days. In the mentioned study, the blueberries were kept for another 15 days under in RA conditions, achieving an overall period of conservation of 60 days.

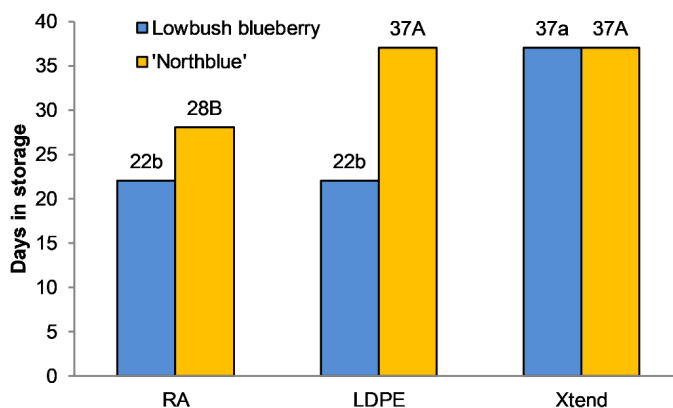


Figure 1. The postharvest life of the lowbush blueberry and half-highbush blueberry cultivar 'Northblue' at 3 ± 1 °C in regular atmosphere (RA) or modified atmosphere packages (LDPE film and Xtend® film). The means with different letters are significantly different ($p < 0.05$): a, b, c for lowbush blueberry and A, B, C for 'Northblue'. LDPE (30 μ m low-density polyethylene bag, product of Estiko, Estonia. Xtend® blueberry bag, product of Stepac, Israel).

3.2. O₂ and CO₂ Changes during Storage

The modified atmosphere packaging consists of sealing a certain quantity of fruit or vegetables using plastic films; then the respiration of commodities increases the CO₂ concentration and decreases the O₂ concentration inside the packages, while the transpiration rate increases the vapour pressure [25]. Even different cultivars of the same species can exhibit different respiration rates [13,23,26].

This trend was also observed in our study, although the O₂ and CO₂ concentrations did not reach the recommended levels suggested by Kader [27] and Mattos et al. [13]. They suggested that the optimal storage conditions of blueberries ranged from 0–5 °C with O₂ concentrations between 2–5% and CO₂ concentrations between 12–20%. In our trial, the lowest O₂ concentration recorded was 13% and the maximum CO₂ concentration was ca. 9.3%. The O₂ concentration in the modified atmosphere packages did not have a significant difference: the O₂ concentration dropped by 15.7% in the LDPE film bag and to 13.9% in the Xtend® bag for lowbush blueberries (Figure 2). For the half-highbush blueberry, the O₂ concentration at the end of the trial decreased to 14.0% in the LDPE film bag and to 13.0% in the Xtend® film bag.

In contrast, the CO₂ concentrations showed significantly different results compared to the O₂, where CO₂ increased to 3.4% in the LDPE film and to 8.5% in the Xtend® for lowbush blueberries (Figure 3). The difference between CO₂ concentrations for lowbush blueberries may have been caused by the shorter storing time in the LDPE package. However, the same phenomenon was noticed with 'Northblue', where the CO₂ content in the LDPE film was significantly lower (4.8%) compared to Xtend® (9.4%). In the previous modified atmosphere study with raspberries, the O₂ concentration was also somewhat higher in the Xtend® film compared to LDPE: 15.7 and 14.9%, respectively [28].

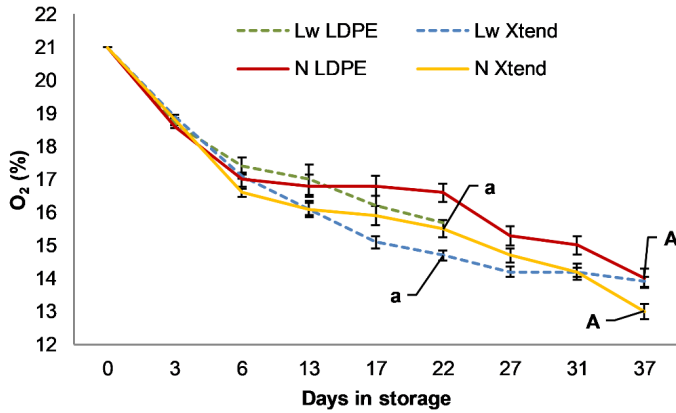


Figure 2. The changes in the O₂ concentration in the modified atmosphere packages (LDPE film and Xtend® film) of the lowbush blueberry and half-highbush blueberry cultivar ‘Northblue’ during storage at 3 ± 1 °C. (LW: lowbush blueberry. N: half-highbush blueberry (‘Northblue’)). Means for each parameter followed by a different letter are significantly different ($p < 0.05$): a, b, c for lowbush blueberries and A, B, C for ‘Northblue’. LDPE (30 µm low-density polyethylene bag, product of Estiko, Estonia. Error bars indicate the standard error. Xtend® blueberry bag, product of Stepac, Israel.)

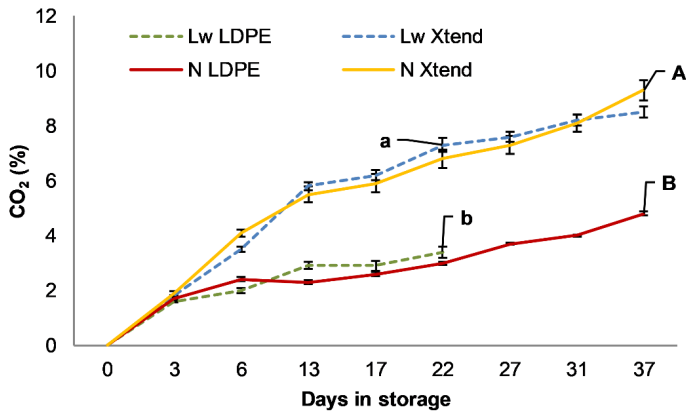


Figure 3. The changes in the CO₂ concentration (%) in the modified atmosphere packages (LDPE film and Xtend® film) of lowbush blueberry and half-highbush blueberry cultivar ‘Northblue’ during storage at 3 ± 1 °C. (LW: lowbush blueberry. N: half-highbush blueberry (‘Northblue’)). The means for each parameter followed by a different letter are significantly different ($p < 0.05$): a, b, c for lowbush blueberry and A, B, C for ‘Northblue’. Error bars indicate the standard error. LDPE (30 µm low-density polyethylene bag, product of Estiko, Estonia. Xtend® blueberry bag, product of Stepac, Israel.)

At the end of the raspberry experiment, the O₂ content reached 15.1% in the LDPE and 13.1% in the Xtend® bags. In that study, the CO₂ content increased to 5.1% in the LDPE film and to 6.1% in the Xtend® bags during the first 24 h, at the end of the experiment, it became 5.9% in the LDPE and to 7.3% in the Xtend®. In another previous modified atmosphere experiment with strawberries, the O₂ decrease and the CO₂ increase was more rapid in the LDPE packages compared to the Xtend® packages, which suggests that that LDPE bags are less permeable to respiration gases [29]. Our experiment showed similar trends, where the LDPE bag’s CO₂ concentration did not reach the desired

levels, which may have resulted in a poorer storability for the lowbush blueberry. Beaudry et al. [5] observed that the atmospheric partial pressures of O₂ within the low-density polyethylene packages containing same mass of blueberries decreased with the increasing temperature and vice versa, which indicates that the activation energy of the film O₂ permeation was less than the activation energy of the fruit. Previous studies have also reported that the steady-state O₂ and CO₂ levels depend on film permeability and the product respiration rate and that the temperature dependence is determined by the film type and commodity physiology [5,23]. Different varieties of the same product exhibit specific respiration rates [13] and the success of the modified atmosphere packaging greatly depend on the accuracy of the predictive respiration rate [27]. The MAP storage with the starch films by Giuggioli et al. [30] helped to control the changes in post-harvest physicochemical properties, such as the pH and TA, but also maintained the antioxidant and nutritional values of fruits after 15 days of storage. In another experiment with three highbush blueberry cultivars 'Coville', 'Blueray', and 'Jersey', the fruit respiration rates in the modified atmosphere decreased with the increasing CO₂, but were little affected by changes in O₂ [23]. On the contrary, Beaudry et al. [5] suggested that the respiration is minimally affected by levels of CO₂ below the approximate 20 kPa that accumulated in the packages under hypoxic conditions. In their work with highbush cultivar 'Bluecrop', the oxygen consumption decreased in response to the decreasing temperature and decreasing steady-state O₂, where the shape of the O₂-dependent respiratory curves changed with temperature. The phenomenon was that, at the higher temperatures, the O₂ uptake did not appear to approach saturation even at the highest levels of steady-state O₂ generated and, as a result, the fruits were found to be more sensitive to restricted O₂ availability as temperatures increased. Hall and Forsyth [31], on the other hand, indicated that the longer the fruits were left on the bushes, the lower their rate of respiration would be. In our experiment, berries from both taxa were picked at the same time and there were no differences in the berry ripening stages. During the trial, the O₂ consumption and CO₂ production were similar with both taxa at a temperature of 3 ± 1 °C but they were influenced by the modified atmosphere packages. At the same time, the content of CO₂ did not increase up to the critical level in any of the used modified atmosphere packages. For the half-highbush blueberry, the limits had not been worked out till now, but earlier studies have mentioned that for highbush and lowbush blueberries, the suitable CO₂ content in storage ranges from 5% to 15% when kept at 5 °C or below [5,12]. Analogous parameters for O₂ content are between 1 and 10%. In our study, the oxygen content was higher in each package in case of both taxa during the experimental period. It can be concluded that the LDPE package is not suitable for lowbush blueberry because it did not extend the postharvest life and the gas concentration did not exceed to desired levels. Although O₂ did not decrease and CO₂ did not increase as expected, especially in LDPE package, the positive side was that the anaerobic respiration did not take place during storage in both packages.

3.3. Fruit Firmness

Early works have stated that blueberries undergo chemical and physical modification during storage [7], which includes fruit firmness, shrivelling, senescence, and the development of decay organisms.

In our study, the lowbush blueberry fruit in the Xtend[®] film stayed firmer compared to the regular atmosphere, scoring 6.0 and 5.0 points, respectively (Table 1). This result is an expected consequence, which allows us to suggest that the respiration rate is higher in the regular atmosphere and that an atmosphere has a major role in accelerating the moisture loss, although the loss of firmness may have been affected by the subsequent compression of the berries in the box during the storage. Prange et al. [32] showed that the firmness of the lowbush blueberry decreased over time, especially after 42 days (1.6–3.4 points in the 0–5-point scale). However, they suggested that an O₂ concentration of 1–5% may also improve the firmness retention with a storage time >28 days. Paniagua et al. [2] reported that the storage atmosphere influenced the firmness of the blueberries, however, its effect varied among the cultivars and storage temperature.

Table 1. The fruit firmness (points), shrivelling (%), and decay (%) of the lowbush blueberry and half-highbush blueberry cultivar ‘Northblue’ after storage at 3 ± 1 °C in a regular atmosphere (RA) or modified atmosphere packages (LDPE film and Xtend® film).

	Lowbush Blueberry			‘Northblue’		
	RA	LDPE	Xtend®	RA	LDPE	Xtend®
Firmness (points)	5.0b	5.3ab	6.0a	7.3A	7.0A	7.0A
Shrivelling (%)	13.0a	3.0c	5.0b	0.1B	0.1B	2.0A
Decay (%)	0.1b	0.4a	0.1b	3.0C	15.0A	7.0B

Means for each parameter followed by the same letter within each row are significantly different ($p < 0.05$): a, b, c for lowbush blueberry and A, B, C for ‘Northblue’. LDPE (30 µm low-density polyethylene bag, product of Estiko, Estonia). Xtend® blueberry bag, product of Stepac, Israel).

For instance, the cultivar ‘Brigitta’ had firmer fruits in CO₂ of 10% in both 2.5% or 20% of oxygen in comparison to air at 4 °C. In our study, the CO₂ content was higher in the Xtend® packages for both taxa, but only the lowbush blueberry stayed firmer in the Xtend® package (6.0 points) compared to the regular atmosphere (5.0 points). For the ‘Northblue’, there was no significant difference between the firmness of fruits stored in the modified atmosphere packages, nor with the regular atmosphere. Correspondingly, the firmness of the ‘Northblue’ fruit was slightly higher compared to the lowbush fruit, where the firmness was 7.3 for the regular atmosphere and 7.0 in both modified atmosphere packages. ‘Northblue’ had a longer postharvest life compared to the lowbush blueberry, which could also be correlated to a better firmness performance. As mentioned, the lowbush blueberry had a softer fruit compared to the half-highbush blueberry, which is a function of the genetic difference of these two taxa and agrees with earlier studies claiming that the firmness is determined on the genetics of the cultivar [2,8]. Ballington et al. [33] stated that when the blueberries are grown in a single location and year, the genetic factors are more important than the environmental differences within the field. Vicente et al. [34] claimed that the firmness/softening depends mainly on hemicellulosic depolymerization, however, Fava et al. [35] stated that the elasticity/turgidity is more related to the internal turgor pressure regulated largely by the cuticular wax properties, because wax is a very good barrier to the excessive water loss [36]. Based on this knowledge, the interaction between wax and the gradient was also observed in our study, where the half-highbush cultivar ‘Northblue’ with a high epidermal wax concentration, had less shrivelling, but the fruits were also firmer.

Anatomical differences between the blueberry cultivars could influence CO₂ and O₂ diffusion into the blueberry tissue, affecting the internal gas concentration and, hence, contributing to the genetic variability in response to the atmospheric change [2]. This trend was also observed in our trial, where the genetical variation had a significant influence on the firmness. The parental phenotype in the blueberry often determines progeny firmness characteristics [37]. The lowbush blueberry produces a soft-fruited progeny [38] and has also been shown to be less firm than a highbush blueberry [33]. The mentioned species are both ancestors of the half-highbush blueberry in our trial, thus, concerning the firmness, the half-highbush blueberry (‘Northblue’) performed similarly to the highbush blueberry. Ehlenfeldt and Martin [39] found that the half-highbush cultivar ‘Polaris’ produced berries as firm as the highbush cultivar ‘Duke’. The other two half-highbush blueberry cultivars in the trial, ‘St. Cloud’ and ‘Friendship’, had lower firmness values. Consequently, we conclude that the half-highbush blueberry cultivars that possess significant amounts of *V. angustifolium* ancestry seem to show a propensity for producing softer fruit. Giongo et al. [40] claimed that after the harvest, berry turgidity becomes more important than firmness. In the aforementioned study, the hybrid ‘Northblue’ was placed in a group which was characterized by a low texture performance with a high elasticity and deformable structure, which may lead to the perception of gumminess by consumers. The storage index for the texture dynamics of 27 cultivars (both highbush and hybrid) was employed, where ‘Northblue’ ranked slightly below average. Although the half-highbush blueberry ‘Northblue’ did not have good postharvest properties in this particular study, we concluded that it has better texture dynamics than the lowbush cultivar in our trial. As reported, the half-highbush blueberry ‘Northblue’

performed better than the lowbush blueberry, having a firmness around 7.0–7.3 points, while the lowbush blueberry firmness range was within 5.0–6.0 points.

3.4. Fruit Shrivelling

Berries are very susceptible to water loss, which results in a loss of gloss, fruit shrivelling, and an increase of firmness [12]. Weight losses of 5% lead to wilting and poor texture, and the taste is considered critical for blueberry marketability [41]. In our trial, the lowbush blueberry had a higher shrivelling percentage than the half-highbush blueberry 'Northblue', which can be correlated to a higher water loss of lowbush compared to the half-highbush 'Northblue' (Table 1).

There was a significant interaction between the modified atmosphere packages and fruit shrivelling (Table 1). The highest percentage of shrivelling for the lowbush blueberry was in the regular atmospheric storage (13.0%) compared to Xtend[®] (5.0%) and to LDPE films (3.0%). Contrastingly, the half-highbush blueberry had more shrivelled fruits in the Xtend[®] film bags (2.0) compared to the regular atmospheric storage (0.1%) and LDPE film bag treatment (0.1%).

High water loss from the fruit is correlated with the high transpiration intensity [13], which can lead to extensive shrivelling and loss in marketable berries. For both taxa, the CO₂ content was higher in the Xtend[®] package compared to the LDPE package. Correspondingly, the fruits in the Xtend[®] film had a higher percentage of shrivelling, 5.0% for lowbush blueberry and 2.0% for 'Northblue'. When comparing the overall postharvest life performance, we conclude that the genetic factors are again more important than the O₂ and CO₂ content in the packages, although the packaging had significant effects, the half-highbush blueberry 'Northblue' had remarkably less shrivelled berries compared to the lowbush blueberry, and the half-highbush performed better with both modified atmosphere packages.

3.5. Fruit Decay

Postharvest diseases of blueberries are usually caused by fungi, with anthracnose (*Colletotrichum acutatum*) being the most common fungal disease, followed by alternaria rot (*Alternaria* spp.) and grey mould (*Botrytis cinerea*) [10,42]. In an experiment with highbush blueberry, Echeverría et al. [43] found that there were no differences in the postharvest behaviour between fruits from organic or from conventional fertilization. Larger pathogen damage occurred in the fruits from organic fertilization treatments and were more decayed. Previous studies claim that lowbush blueberries show little decay during the storage, which is defined as the presence of visible mould [11]. This trend was also noticed in our trial, where the lowbush blueberry had less decayed berries compared to the half-highbush blueberry 'Northblue' (Table 1). In the modified atmospheric storage, the berries continue to respire the trapped air until the CO₂ concentration rapidly approached the critical 10–15% level necessary to inhibit the *Botrytis* growth [12]. In our study, the CO₂ concentration was much lower for the lowbush, peaking at only 3.4% in the LDPE film bag and 8.5% in the Xtend[®] package. For the half-highbush blueberry 'Northblue', the CO₂ content in the LDPE was 4.8% and 9.4% in the Xtend[®] film. These results indicate that for the half-highbush blueberry 'Northblue', CO₂ concentration in the modified atmospheric packaging must be much higher to inhibit fungal growth. Paniagua et al. [2] observed that after 6 weeks of storage, the low oxygen concentrations (2.5% O₂ + 10% CO₂) significantly reduced the decay for the cultivar 'Maru' in comparison to the air storage and both the controlled atmospheres decreased the decay for 'Brigitta'. High CO₂ concentrations suppress decay, weight loss, and softening [32]. This was also observed in our study, where the CO₂ concentration was higher in the Xtend[®] film compared to the LDPE film and, correspondingly, there were less decayed berries. Prange et al. [32] noticed that when increasing CO₂, the visible decay decreased for both highbush varieties 'Fundy' and 'Blomidon', and at 15% CO₂, it was virtually absent (0.1%). In their study, the increase of unmarketable berries was more related to a loss of firmness than due to the visible decay level. A similar trend was observed in our trial with the lowbush blueberry, where the increase of the unmarketable berries was more related to a loss of shrivelling than due to visible decay.

A low O₂ concentration during the blueberry storage has very little effect on the decay organism activity or survival at levels above the fermentation threshold of most commodities [13], thus, low O₂ and elevated CO₂ concentrations can remarkably reduce the rates of ripening and senescence, primarily reducing the synthesis and perception of ethylene [44]. Zheng et al. [45] found out that the modification of storage atmospheres induces plant defensive responses and increase disease resistance in postharvest commodities. A negative plant response to the modified atmosphere packaging is seen when the respiration is reduced as O₂ becomes limiting and the lower O₂ limit is frequently considered to be the level of O₂ that induces fermentation [13]. A high ethylene content can influence the quality characteristics of blueberries like firmness, shrivelling, and decay. In our study, the O₂ level did not have significant differences between the taxa, nor between the modified atmosphere packages. As mentioned before, for the lowbush blueberry, there were less decayed berries compared to the half-highbush blueberry 'Northblue' (Table 1). For lowbush, there was only a 0.1% of decayed berries observed in the regular atmospheric storage and in the Xtend[®] film, which is significantly less compared to the LDPE film (0.4%). The percentage of decayed fruits for the half-highbush blueberry 'Northblue' was 15.0% in the LDPE film, 7.0% in the Xtend[®] film, and 3.0% in the regular atmospheric storage. The fungal growth of the half-highbush blueberry 'Northblue' could have been suppressed by lowering the storing temperature since the previous studies have demonstrated that storing them at 0 °C has the great benefit of maintaining the quality. In the study by Paniagua et al. [2], a higher temperature (4 °C in comparison to 0 °C) resulted in more rot incidence from 5 weeks onwards for 'Brigitta' and after 4 weeks onwards for 'Maru'. Earlier works have also suggested that minimal mechanical damage and storage at 0 °C gives the advantage of maintaining the quality of the highbush [10] and lowbush blueberries [11,46]. The reported enhanced temperature conditions during blueberry storage could also be beneficial for the half-highbush blueberry 'Northblue' storage since the decay percentage was high when the fruit was stored at 3 ± 1 °C.

3.6. Chemical Composition

Fruit sensory quality, which is based on the chemical composition of the fruit, is strongly influenced by the storing techniques. At the end of the experiment, fruit dry matter content in both blueberry taxa was significantly higher in fruits stored in Xtend[®] packages compared to the RA and LDPE packages lowbush blueberry (Table 2). It showed that water loss was higher in Xtend[®] packages. Kalt and McDonald [47] have described the chemical composition of several lowbush blueberries ('Blomidon', 'Cumberland', 'Fundy') and these cultivars had a higher average dry matter content (15.4%) compared to the blueberries in our study (the average pre-storage dry matter content was 13.6%). A lower fruit dry matter content in our study may have been caused by the climate conditions, cultivation techniques, but may also be due to the genetic differences of the lowbush species.

The most important group of phenolics in blueberries is the flavonoids. The high antioxidant activity of fruits is attributed to anthocyanins. According to Chiabrando and Giacalone [6], the high antioxidant capacity of phenolic compounds has been maintained in blueberry fruits stored at 4 °C. For the lowbush blueberry, the anthocyanin content was significantly higher in the regular atmosphere (151 mg 100 g⁻¹) compared to the modified atmosphere packages (96 mg 100 g⁻¹ in LDPE and 100 mg 100 g⁻¹ in Xtend[®]), which indicates that the modified atmosphere inhibited the anthocyanin biosynthesis. It could be suggested that the anthocyanin inhibition in LDPE is species-specific for lowbush blueberries, and may have been caused by the high shrivelling percentage. In the current study 'Northblue' had no significant difference between the anthocyanin content and the storage conditions and in contrast to the lowbush blueberry, neither of the packages influenced the anthocyanin biosynthesis. The same packaging materials as in our trial were used in modified atmosphere experiments with raspberry 'Polka' [28] and three different strawberry cultivars [29], where it was found that raspberries held in LDPE packages had significantly lower anthocyanin contents compared to the regular-atmosphere-stored fruits; however, for strawberries in LDPE packages, anthocyanin biosynthesis was inhibited only for 'Sonata', not in other cultivars.

Table 2. The fruit dry matter (DM), anthocyanins (ACY), and soluble solids (SSC) content, titratable acidity (TA), soluble solids/titratable acidity ratio (TSS:TA) of lowbush blueberry and half-highbush blueberry cultivar ‘Northblue’ at harvest (Pre-storage) and after storage at 3 ± 1 °C in regular atmosphere (RA) or modified atmosphere packages (LDPE film and Xtend[®] film).

	Lowbush Blueberry				‘Northblue’			
	Pre-storage	RA	After storage LDPE	Xtend [®]	Pre-storage	RA	After storage LDPE	Xtend [®]
DM (%)	13.6b	13.6b	13.6b	15.1a	13.1B	13.9AB	13.2B	14.5A
ACY (mg/100 g)	53c	151a	96b	110b	41B	103A	101A	103A
SSC (%)	13.1b	12.5c	12.3c	14.0a	13.4C	14.4A	12.4D	13.9B
TA (%)	0.15b	0.14b	0.18b	0.27a	0.66D	0.73C	0.82B	0.89A
SSC:TA	88b	94a	67c	52d	20A	20A	15B	16B

Means for each parameter followed by the same letter within each row are significantly different ($p < 0.05$): a, b, c ($p < 0.05$) for lowbush blueberry and A, B, C ($p < 0.05$) for ‘Northblue’. LDPE (30 μ m low-density polyethylene bag, product of Estiko, Estonia). Xtend[®] blueberry bag, product of Stepac, Israel).

The Xtend[®] package had a positive effect on the content of the soluble solids of both blueberry taxa: it was the only package which caused an increase of the soluble solids content during storage (Table 2). The LDPE film caused a significant decrease of the SSC in both blueberry taxa compared to the initial value, whereas the effect of the regular atmosphere storage depended on the blueberry taxa. The content of soluble solids of ‘Northblue’ fruits increased in the regular atmosphere and it resulted in significantly higher soluble solid contents compared to the fruits stored in either modified atmosphere packages after storage. Contrarily, the content of the soluble solids of the lowbush blueberry decreased in the regular atmosphere. Chiabrando and Giacalone [6] compared four different modified atmosphere packages with a highbush blueberry cultivar ‘Lateblue’ storage and found out that the microperforated (1 mm \varnothing) film and non-perforated film affected the total soluble solids content positively, having a total soluble solids content of 11.7% and 11.5%, respectively.

The Xtend[®] package caused an increase in the titratable acids content in both blueberry taxa (Table 2), which may have been influenced by the dry matter content decrease during storage. Titratable acidity of ‘Northblue’ fruits increased in all packages (up by 0.73% in the regular atmosphere, 0.82% in LDPE, and 0.89% in Xtend[®], compared to the pre-storage (0.66%)). Lowbush blueberry titratable acidity remained unchanged in the regular atmosphere and LDPE packages during storage. On the contrary to our experiment, Zheng et al. [45] studied the impact of high oxygen (40%, 60%, 80%, and 100%) in modified atmospheres on highbush blueberry cultivar ‘Duke’ and reported significantly lower titratable acidity (0.41–0.45%) within all the O₂ types, but also with storage at 5 °C for 9, 14, or 35 days compared to the initial value (0.82%). Chiabrando and Giacalone [6] reported that the highbush blueberry cultivar ‘Lateblue’ had the highest titratable acidity (147.89 meq L⁻¹) with the microperforated (1 mm \varnothing) film, compared to other modified atmosphere packages (38.89–59.58 meq L⁻¹) and to the control (baskets without film): 60.25 meq L⁻¹. These results indicate that the half-highbush blueberry ‘Northblue’ also has a high titratable acidity compared to the lowbush blueberry and is similar to the highbush cultivar ‘Lateblue’. Since ‘Northblue’ had significantly higher titratable acidity values compared to the lowbush blueberry, it indicates the genetic differences of these taxa, but also lets us conclude that the storage conditions had a smaller effect on the titratable acidity. Duan et al. [48] claimed that during postharvest storage, acid metabolism converted to starch and acid to sugar, thus, resulting in a decrease of the titratable acidity and an increase of soluble solids. However, in our study, this was not obvious, in fact, the titratable acids content increased in all storage conditions for ‘Northblue’ and in the Xtend[®] film for the lowbush blueberry. Gonçalves et al. [49] mentioned that the blueberries produced in organic farming had lower levels of titratable acids and the same situation continued after 7 and 14 days of storage.

The increase of the titratable acidity during regular and modified atmosphere storage might have also influenced the taste of the berries in our trial. Beaudry [50] reported in his study that blueberries

should contain >10% soluble solids, 0.3–1.3% titratable acidity, and have an SSC:TA ratio between 10 and 33. A 0.1% decrease in the acid concentration is known to be equivalent to a 1% increase in the perceived sweetness in the blueberry fruit. In our study, the lowbush blueberry had a decrease of both soluble solids and an increase in the titratable acidity in the LDPE film (Table 2), which indicates, that the taste of fruits probably became more acidic. Changes in the taste are very well shown with the SSC:TA ratio, where the berry fruit in the LDPE film had a significantly lower SSC:TA ratio, 67, while the initial value was 88. The lowbush blueberry fruits in the Xtend® package had an increase of both soluble solids and titratable acidity, resulting in the lowest SSC:TA ratio (among the package).

For 'Northblue', both the modified atmosphere packages caused a decrease in the SSC:TA ratio compared to the initial value. However, the differences in the SSC:TA between the regular atmosphere stored and modified atmosphere stored fruits were 4 to 5 units compared to the lowbush blueberries, where the difference between the regular atmosphere stored and the modified atmosphere stored fruits in the Xtend® package was 42 units (Table 2). Comparing these two taxa, the soluble solids and titratable acidity ratio had remarkable differences, where 'Northblue' had a more than four times lower SSC:TA ratio compared to the lowbush blueberry's initial value, which again indicates the genetic impact and its association to the flavour. The genetic impact is obvious, and the influence of the modified atmosphere package was also demonstrated. Thus, our results indicated that the taste-related properties of lowbush blueberries are more easily affected by the storage atmosphere than those of 'Northblue'.

3.7. Fruit Colour Changes during Storage

Blueberry colour is a complex quality characteristic affected by the quantity and structure of the surface waxes [51] and the anthocyanin content [52]. The colour of the fruit is also an important quality factor influencing fresh-market value and acceptability by the consumers [53–55].

The L* axis represents the lightness changes from 0, which has no lightness (absolute black) to 100, which is the maximum lightness (absolute white) [56]. It is likely that the surface wax affects the L* value (i.e., higher amounts of surface wax might lighten the fruit), as well as the visual perception of the fruit colour (e.g., blue chroma) [57]. However, in our study, this phenomenon was not apparent. The lowbush fruit surface with wax was lighter in colour before pre-storage (L* 29.3) compared to after storage in the regular atmosphere (L* 29.7) and in the LDPE film (L* 29.5) compared to the Xtend® (L* 25.6) (Table 3).

The 'Northblue' fruit surface with wax was lighter in colour in pre-storage (L* 29.0) and in the LDPE film (L* 28.9) compared to the regular atmosphere (L* 26.0) and to the Xtend® film (L* 27.0). Surface colour without wax was not different with respect to the L* values for both taxa. In the study with two highbush blueberry cultivars 'Bluetta' and 'Duke', where the blueberries were stored at room temperature or at 10 °C, results were similar concerning the L* value [58]. In the mentioned study was no significant difference between both blueberry cultivars during the storage when the fruits were stored at 10 °C, where, at the end of the trial (at the 16th day), the L* value for 'Bluetta' was 26.5 and for 'Duke' 25.9. It was interesting that the cultivar 'Duke' had an increase in L* value, while 'Bluetta' had a slight decrease in lightness during storage. When comparing these results to our study, it can be suggested that the lowbush blueberry and half-highbush blueberry 'Northblue' have a higher L* value compared to the highbush cultivars 'Bluetta' and 'Duke', which again reflect the genetic differences of these three taxa. In our study, the lowbush blueberry fruit flesh was lighter in colour with pre-storage (L* 39.1) and in the regular atmosphere (L* 41.3) compared to the fruit stored in the LDPE (L* 36.8) and in the Xtend® film (L* 36.3). For 'Northblue', the fruit flesh was lighter in colour in the LDPE film (L* 55.7) compared to the initial value (L* 48.9) and to the regular atmosphere (L* 48.0), but there was no difference in the Xtend® film (L* 52.3).

Table 3. The fruit surface (with wax and without wax) and flesh instrumental colour ($L^*a^*b^*$, C^* and h^*) of the lowbush blueberry and half-highbush blueberry cultivar ‘Northblue’ at harvest (Pre-storage) and after storage at $3 \pm 1^\circ\text{C}$ in the regular atmosphere (RA) or modified atmosphere packages (LDPE film and Xtend® film).

Colour Measurement	Lowbush Blueberry				‘Northblue’			
	Pre-storage	RA	After Storage LDPE	Xtend®	Pre-storage	RA	After Storage LDPE	Xtend®
Surface (with wax) colour								
L^*	29.3a	29.7a	29.5a	25.6b	29.0A	26.0B	28.9A	27.0B
a^*	1.07b	0.7c	0.8bc	2.0a	0.4C	1.0B	0.8B	1.5A
b^*	−5.6b	−6.3b	−6.0b	−4.4a	−4.7B	−4.0A	−4.3A	−4.0A
C^*	5.7ab	6.3a	6.1ab	5.5b	4.7A	4.1B	4.4AB	4.4AB
h^*	281b	277b	280b	297a	275B	284AB	282AB	291A
Surface (without wax) colour								
L^*	23.5a	22.9a	22.7a	23.4a	24.1A	24.1A	24.0A	24.2A
a^*	1.1a	0.9a	0.9a	1.1a	0.8B	1.3A	1.6A	1.6A
b^*	−0.8bc	−0.6a	−1.0c	−1.9d	0.0A	−1.5C	−1.0B	−0.9B
C^*	1.4b	1.2b	1.4b	2.3a	0.8B	2.1A	2.0A	1.9A
h^*	325a	327a	314b	306b	116D	313C	324B	330A
Flesh colour								
L^*	39.1a	41.3a	36.8b	36.3b	48.9B	48.0B	55.7A	52.3AB
a^*	2.4c	4.3b	5.3a	5.8a	−2.9C	−0.9A	−2.9C	−2.3B
b^*	3.6a	3.9a	2.9b	2.8b	9.3C	8.8C	11.2B	13.3A
C^*	5.0c	6.1b	6.5b	7.4a	9.8C	8.9C	11.7B	13.5A
h^*	90b	44d	68c	200a	105A	94B	103A	99AB

Means for each parameter followed by the same letter within each row are significantly different ($p < 0.05$): a, b, c for lowbush blueberry and A, B, C for ‘Northblue’. LDPE (30 μm low-density polyethylene bag, product of Estiko, Estonia). Xtend® blueberry bag, product of Stepac, Israel).

For both taxa, the lowbush and half-highbush blueberry, the fruit surface with wax had a higher degree in redness in the Xtend® film compared to the pre-storage and the LDPE film (Table 3). The lowest degree in redness was in the regular atmosphere ($a^* 0.7$) for the lowbush blueberry. For ‘Northblue’, the lowest degree in redness was in the pre-storage ($a^* 0.4$) compared to the Xtend® package. Fruit surface colour without the wax was not different between the treatments in the lowbush trial, but for the ‘Northblue’ there was, where the initial value ($a^* 0.8$) was different for all the storage types, which also indicates that the fruits became redder during storage. The lowbush blueberry’s fruit flesh had the highest degree in redness in the LDPE ($a^* 5.3$) and in the Xtend® films ($a^* 5.8$) compared to the initial value ($a^* 2.4$). The flesh colour was remarkably different between the lowbush and the hybrid, where ‘the Northblue’ fruits were greener in colour, especially before storage ($a^* −2.9$) and in the LDPE film ($a^* −2.9$) compared to the fruits in the regular atmosphere ($a^* −0.9$) and in the Xtend® film ($a^* −2.3$).

The lowbush blueberry fruit surface colour with wax was bluer in colour in the pre-storage ($b^* −5.6$), in the regular atmosphere ($b^* −6.3$), and in the LDPE film ($b^* −6.0$) (Table 3) compared to the Xtend® film ($b^* −4.4$). For ‘Northblue’, the fruit surface colour with wax was bluer in colour to the pre-storage ($b^* −4.7$) compared to all other storage types. Lowbush fruit without wax was bluer in colour in the Xtend® film ($b^* −1.9$) compared to the pre-storage and other storage types. For ‘Northblue’, the fruit colour without the wax was bluer in the regular atmosphere ($b^* −1.5$) compared to the initial value ($b^* 0.0$). The fruit flesh had the highest degree of yellowness in the pre-storage ($b^* 3.6$) and in the regular atmosphere ($b^* 3.9$) conditions for the lowbush blueberry. For ‘Northblue’, the fruit was more yellow in colour in the Xtend® package ($b^* 13.3$) compared to the pre-storage ($b^* 9.3$), to the regular atmosphere ($b^* 8.8$), and to the LDPE ($b^* 11.2$) (Table 3). There was a pronounced effect of the taxa concerning fruit flesh b^* values, where ‘Northblue’ had significantly higher b^* values in a ratio of 8.8–13.3, when compared to the lowbush, which had b^* values in a ratio between 2.8–3.9.

The lowbush blueberry's flesh colour was higher in RA (b^* 3.9) compared to LDPE (b^* 2.9) and to Xtend[®] (b^* 2.8), which may be due to the high shrivelling percentage that also had an impact on the anthocyanin inhibition during storage.

The chroma value was affected by the storage conditions, indicating that the tonality of the fruit colour changed after the harvest and was differentiated significantly ($p > 0.05$) (Table 3). The packaging material did not affect the C^* values of the exocarp and mesocarp. The lowbush fruit colour with wax had the highest C^* value in the regular atmosphere (C^* 6.3) compared to the Xtend[®] film (C^* 5.5). For the half-highbush blueberry 'Northblue', the C^* was highest in the pre-storage (C^* 4.7) compared to the regular atmosphere (C^* 4.1). The Xtend[®] had a significant effect on the chroma when the chroma measurements were taken without the wax from the mesocarp. Chroma values for lowbush were higher in the Xtend[®] film (C^* 2.3) when compared to the pre-storage (C^* 1.4) and to the other storage types (for example, C^* 1.2 in a regular atmosphere and C^* 1.4 in the LDPE film). For 'Northblue', the surface colour without wax was higher in chroma values in all the other storage conditions when compared to the pre-storage. There were also higher C^* values in the mesocarp compared to the exocarp of the half-highbush blueberry 'Northblue' fruit. Chroma measured from fruit flesh was higher in the Xtend[®] film for both taxa compared to all other storage types.

Hue angle is a good measure of blueberry colour and the blue colour of the fruits has been suggested as the best criteria of fruit maturity and decision-making regarding harvesting time [58,59]. The higher hue angle values indicate bluer colours. For the lowbush blueberry, the h^* from the fruit with wax was higher in the Xtend[®] package (h^* 297) compared to the pre-storage (h^* 281), to the regular atmosphere (h^* 277), and to the LDPE bag (h^* 280) (Table 3). For 'Northblue', the hue angle increased in the Xtend[®] film (h^* 291) compared to the initial value (h^* 275). In the Eum et al. study [58], the hue angle was similar for highbush cultivars 'Bluetta' and 'Duke', when compared to the h^* values we got in our study. The highbush blueberry cultivar 'Bluetta' had an h^* angle of 289.6 at 10 °C, while 'Duke' had a hue angle of 298.1. Although there was a significant difference between the two highbush cultivars at the beginning of the trial, these two cultivars had similar values during the rest of the storage. Compared to our study, the highbush blueberry cultivars had similar h^* values to the lowbush blueberry and half-highbush blueberry 'Northblue', when the hue angle was measured from the surface with wax, which indicates that these taxa have similar blue colours. The h^* value, which was measured from the fruit without wax, was higher both in the pre-storage (h^* 325) and in the regular atmosphere (h^* 327) compared to the modified atmosphere packages, where, in the LDPE, it was 314 and in the Xtend[®], it was 306. For half-highbush, the h^* values without wax were approximately three times higher in the Xtend[®] (h^* 330) compared to the initial value (h^* 116). For the lowbush blueberry, the hue angle measured from the fruit flesh decreased in the regular atmosphere (h^* 44) and in the LDPE film (h^* 68), while it increased in the Xtend[®] film (h^* 200) compared with the pre-storage (h^* 90). For 'Northblue', the h^* decreased in the regular atmosphere (h^* 94) compared to the pre-storage (h^* 105) and to the modified atmosphere package LDPE (h^* 103).

There was a pronounced effect on the genetic difference when measuring the fruit flesh a^* values, indicating that the lowbush fruit flesh is redder in colour and that the 'Northblue' fruit is greener in colour. In addition, there was a significant difference between these two taxa concerning fruit flesh b^* values, where 'Northblue' had a significantly higher b^* value compared to the lowbush blueberry. Earlier studies have reported that the blueberry surface colour correlates well with the measurements of soluble solids, anthocyanins content, and titratable acid content [22,52]. Earlier research from Ballinger et al. [60] indicated that the surface colour is well correlated within the cultivar. For lowbush, the titratable acidity and the soluble solids content were higher in the Xtend[®] film compared to the initial value. That phenomenon was also observed for the half-highbush blueberry, where, in the Xtend[®] film, the titratable acidity and soluble solids values were higher compared to the pre-storage. These results had a significant interaction with the colour values, where the fruit surface colour with wax had a higher degree of redness in the Xtend[®] film compared to the pre-storage for both taxa. A similar trend was applicable for the hue angle, where, in Xtend[®] film, the h^* values were higher

compared with the initial value. For 'Northblue', the hue angle of the fruit without wax was also higher in the Xtend[®] package compared to the pre-storage. For lowbush, the same interaction occurred with the fruit flesh h^* measurements. For both blueberry taxa, the Xtend[®] film had a pronounced effect on the fruit surface colour concerning without wax C^* and on fruit flesh C^* values.

4. Conclusions

Compared to regular atmosphere conditions, the Xtend[®] package prolonged the postharvest life of lowbush blueberries for 15 days and half-highbush blueberries for 9 days. LDPE package did not prolong the postharvest life of lowbush but extended the postharvest life of 'Northblue' for 9 days. The CO₂ content was significantly higher in Xtend[®] film compared to the LDPE at the end of the storage. It can be concluded that lowbush blueberries need a higher CO₂ content for retaining postharvest quality compared to half-highbush blueberry ('Northblue').

Both modified atmosphere packages had a negative effect on blueberry taste-related properties irrespectively of the taxa. The SSC:TA ratio of the blueberries in the modified atmosphere decreased both compared to the initial value and compared to the RA-stored blueberries by the end of storage.

The content of anthocyanins increased significantly with all storage conditions irrespectively of the taxa. However, for lowbush blueberries, both the modified atmosphere packages suppressed anthocyanin biosynthesis, whereas the 'Northblue' anthocyanins in MA were no different from RA.

The genetic differences were more important concerning fruit firmness, shrivelling, and decay. Both MA packages had an impact on the firmness and the shrivelling, but the half-highbush blueberry ('Northblue') fruits were firmer and less shrivelled compared to the lowbush.

Conclusively, both MA packages extended the postharvest life of 'Northblue' considerably, which is valuable information for producers. For lowbush blueberries, a higher CO₂ than the LDPE could provide in the current study was needed in order to retain the external postharvest quality.

Further studies should be conducted to find out the metabolic differences of blueberry taxa and to match the respiration of the product with the permeation rates of the packages. The use of biodegradable films should be considered in order to ensure sustainability.

Author Contributions: Conceptualization, M.S., U.M.; methodology, M.S., U.M.; software, P.P.; formal analysis, M.S., A.K.; investigation, A.K.; resources, U.M.; data curation, M.S.; writing—original draft preparation, A.K., C.K., M.S.; writing—review and editing, U.M., P.P., M.S., C.K., A.K.; visualization, M.S., P.P.; supervision, M.S., U.M.; project administration, U.M.; funding acquisition, U.M.

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LIST OF ORIGINAL PUBLICATIONS

1.1. Articles indexed by Thomson Reuters Web of Science or Scopus:

Koort, A.; Starast, M.; Põldma, P.; Moor, U.; Mainla, L.; Maante-Kuljus, M.; Karp, K. (2020). Sustainable Fertilizer Strategies for *Vaccinium corymbosum* x *V. angustifolium* under Abandoned Peatland, *Agriculture*, 10 (4), 121

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3.1. Articles published in conference proceedings indexed by Thomson Reuters or Scopus:

Põldma, P.; Mainla, L.; Karp, K.; Maante-Kuljus, M.; **Koort, A.**; Moor, U. (2021). Effect of irrigation regime on yield and plant growth of ‘Sonsation’ strawberry grown in an open field in Estonia. *Acta Horticulturae*, 1309, 671–676.

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3.4. Articles/ presentations published in conference proceedings not indexed by Thomson Reuters or Scopus:

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3.5. Articles published in local conference proceedings

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5.2. Conference abstracts

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6.3. Popular science articles

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VIIS VIIMAST KAITSMIST

HANNES TAMME

PUIDU KUIVATUSE KONTROLI JA OPTIMEERIMISE MEETODITE
ARENDAMINE

DEVELOPMENT OF CONTROL AND OPTIMIZATION METHODS FOR WOOD
DRYING

Professor Emeritus **Peeter Muiste**, PhD, teadur **Valdek Tamme**

30. august 2023

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