Eesti Maaülikool
Estonian University of Life Sciences

EFFECT OF CULTIVATION TECHNIQUES ON GRAPEVINE (*VITIS* SP.) AND STRAWBERRY (*FRAGARIA* ×*ANANASSA* DUCH.) FRUIT QUALITY

KASVATUSTEHNOLOOGILISTE VÕTETE MÕJU VIINAPUU (*VITIS* SP.) JA AEDMAASIKA (*FRAGARIA* × *ANANASSA* DUCH.) VILJADE KVALITEEDILE

REELIKA RÄTSEP

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

Väitekiri
Filosoofiadoktori kraadi taotlemiseks põllumajanduse erialal

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS 7
ABBREVIATIONS 8
1. INTRODUCTION 9
2. GRAPEVINE CULTIVATION 11
   2.1. Grape ripening and quality 11
   2.2. Species and cultivars in cool climate conditions 14
   2.3. Foliar applications 16
   2.4. Canopy management 17
3. STRAWBERRY CULTIVATION 19
   3.1. Factors affecting fruit quality 19
   3.2. Application of humic substances 21
   3.3. Defoliation 22
4. HYPOTHESES AND AIMS OF THE STUDY 25
5. MATERIALS AND METHODS 27
   5.1. Experiments with grapevine (I-III) 27
   5.2. Experiments with strawberry (IV, V) 29
   5.3. Measurements and analysis 30
   5.4. Soil and weather conditions 32
   5.5. Statistical analysis 34
6. RESULTS 35
   6.1. Effect of cultivar properties, glycine-betaine and pruning on grape quality 35
   6.2. Effect of humic substances and flame-defoliation on strawberry fruit quality 37
7. DISCUSSION 39
   7.1. Grape quality depending on cultivar, glycine-betaine and canopy pruning 39
   7.2. Strawberry fruit composition affected by humic substances and flame-defoliation 43
8. CONCLUSIONS 47
REFERENCES 50
SUMMARY IN ESTONIAN 62

ACKNOWLEDGEMENTS..70
ORIGINAL PUBLICATIONS 71
CURRICULUM VITAE...121
ELULOOKIRJELDUS 124
LIST OF PUBLICATIONS 127

LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following research papers, which are referred to by their Roman numerals (**I-V** in the text).

I Vool, E., **Rätsep, R.**, Karp, K. 2015. Effect of genotype on grape quality parameters in cool climate conditions. *Acta Horticulturae* 1082: 353-358. Belgium: ISHS.

II Vool, E., **Rätsep, R.**, Karp, K. 2014. Effect of fertilizing on grapevine fruit maturity in Northern conditions. *Acta Horticulturae* 1017: 231-236. Belgium: ISHS.

III **Rätsep, R**., Karp, K., Vool, E., Tõnutare, T. 2014. Effect of pruning time and method on hybrid grapevine (*Vitis* sp.) 'Hasanski Sladki' berry maturity in a cool climate conditions. *Acta Scientiarum Polonorum - Hortorum Cultus* 13(6): 99-112.

IV **Rätsep, R.**, Vool, E., Karp, K. 2014. Influence of humic fertilizer on the quality of strawberry cultivar 'Darselect'. *Acta Horticulturae* 1049: 911-916. China: ISHS.

V **Rätsep, R.**, Moor, U., Vool, E., Karp, K. 2015. Effect of post-harvest flame-defoliation on strawberry (*Fragaria ×ananassa* Duch.) plant growth and fruit biochemical composition. *Zemdirbyste-Agriculture* 102(4): 403-410.

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The contributions of the authors to the papers:

Paper	Idea and study design	Data collection/ analyses	Data analysis	Manuscript preparation
I	KK	EV, KK, **RR**	EV, KK, **RR**	EV, **RR**, KK
II	KK	EV, KK, **RR**	EV, KK, **RR**	EV, **RR**, KK
III	KK	**RR**, KK, EV	**RR**, KK, EV, TT	**RR**, KK, EV, TT
IV	KK	**RR**, EV, KK	**RR**, EV, KK	**RR**, EV, KK
V	KK	**RR**, KK, TT	**RR**, KK, TT	**RR**, UM, EV, KK

KK – Kadri Karp; EV – Ele Vool; **RR** – Reelika Rätsep; UM – Ulvi Moor; TT – Tõnu Tõnutare.

ABBREVIATIONS

AAC	ascorbic acid content
ACC	total anthocyanin content
BM	grape berry mass
BW	bunch weight of grapes
C	control
CP	grapevine cane pruning
DW	dry weight
FD	flame-defoliation
FD+H	flame-defoliation with humic substances application
FM	strawberry fruit mass
FW	fresh weight
GB	glycine-betaine
H	(liquid) humic substances
$LSD_{0.05}$	least significant difference
MI	maturity index of grapes
mM	millimole (unit)
NS	non-significant
OM	organic apple tree bark mulch
P	pruned
PK	phosphorus-potassium fertilizer
PM	black polyethylene mulch
r	linear correlation coefficient
SP	grapevine spur pruning
SPAD	Soil Plant Analysis Development
SSC	soluble solids content
SS/TA	soluble solids and titratable acids ratio
T	temperature(s)
TAC	titratable acids content
TPC	total phenolic content

1. INTRODUCTION

Increasing demand on the healthy and high quality fruit and berry products leads to the need of adapting to consumer requirements on the market. The objectives of fruit research have changed with the development of cultivation methods to improve quality as a result of consumers' demands for healthy fruit production. Cultivation methods depend on the culture and its plantation age. For example, grapevine (*Vitis* sp.) plantations are productive for decades, while strawberries (*Fragaria ×ananassa* Duch.) can only be grown for three to four years consecutively, demanding different approaches to the cultivation techniques. Fruit quality attributes and good winter hardiness of cultivars grown under Baltic climatic conditions have been the main targets from the beginning of Estonian fruit research and breeding (Kask *et al.*, 2010). In addition to satisfying traditional sensory and chemical quality criteria, fruits should be rich in vitamins and other health-related components, like phenolics (Skrede *et al.,* 2012). The great interest of consumers concerning the health benefits of fruits has also been revealed in Estonia (Moor *et al.,* 2013).

Viticulture in cool climates is of increasing importance for the reason of achieving interesting flavours compared to grapes under more temperate conditions (Dishlers, 2003; Gustafsson and Mårtensson, 2005; Lisek, 2010; Karvonen, 2014). The second reason is related to high pesticide residues found on imported grapes, while in cool climate conditions it is possible to produce healthier fruits. Cooler regions (including Estonia) are expected to be unsuitable for grapevine cultivation due to a short vegetation period and variability of quality parameters regarding insufficient maturation due to variable climatic conditions (Kennedy, 2002; Karvonen, 2014). In Estonia, grapevine research began with investigations of vine physiology in 1950's (Miidla, 1964), and cultivar selection has been active since 2004. Currently, as the number of grape cultivars is increasing rapidly, the focus point of grapevine studies is the production of high quality fruits rich in health promoting compounds.

Areas with different climatic conditions can largely explain the diversity of grapevine cultivars grown and the specific characteristics of the products made from those grapes (Tonietto and Cabonneau, 2004). The most important characteristic in cool climates is the cold tolerance of grapevines. Interspecific hybrids (*V. vinifera* crossings with *V. labrusca*, *V. riparia*, *V.*

rupestris, *V. lincecumii*, *V. amurensis*) have shown variable frost resistance depending on genotype (Lisek, 2007; Lisek, 2010; Karvonen, 2014). In order to improve plant abiotic stress resistance, different foliar applications of biostimulants and growth regulators have been tested (Mickelbart *et al.,* 2006; Ashraf and Foolad, 2007; Mahdi *et al.,* 2014; Kurepin *et al.,* 2015). At the same time, training systems and pruning methods have been investigated in order to affect vegetative and reproductive characteristics of grapevines (Andersen and Simms, 1991), to regulate the yield (Martin and Dunn, 2000; Palliotti *et al.*, 2014) and subsequently also influence the composition of grape products (Nan *et al.*, 2013; Palliotti *et al.*, 2014). Even though grapevine growing is increasing in its popularity, little science-based information exists about grape cultivation techniques and fruit quality attributes in different cultivars in Estonian climatic conditions.

Strawberry is one of the most common fruit crops grown in Estonia. Several investigations related to strawberry defoliation by burning the straw have been carried out, aimed at determining the effect not only on the winter hardiness and pest incidence, but on strawberry yield and fruit quality too (Karp, 2001; Moor *et al.,* 2004). Nevertheless, not all strawberry cultivars are suitable for this purpose, moreover, only cultivars with vigorous growth habit and early fruiting are able to adapt to these changes. In sustainable agriculture, soil composition and plant nutrient availability can be affected by natural biopreparations and/or mulch materials. One possibility is to use preparations containing humic substances which have shown a positive impact on plant physiology by enhancing nutrient uptake due to improved soil structure (Trevisan *et al.,* 2010; Calvo *et al.,* 2014; Tehranifar and Ameri, 2014), equally significantly affecting plant growth, fruit yield and quality (Singh *et al.,* 2010). However, there is still very little data available about the effect of flame-defoliation and humic substances on strawberry plant growth and fruit quality.

2. GRAPEVINE CULTIVATION

2.1. Grape ripening and quality

Estonia is situated beyond the warm temperate climates (annual isotherm of 10°C to 20°C, latitudes 30-50° N and 30-40° S), and therefore included under the cool climate region. Grape biochemical composition, as well as quality, depends on cultivation area and climate (Martin and Dunn, 2000). Cooler regions, including Estonia, are expected to be unsuitable for grapevine cultivation mainly due to a short vegetation period that affects grape maturation and quality due to variable climatic conditions (Kennedy, 2002; Karvonen, 2014). Viticultural practices can be used to control plant response in order to influence berry secondary metabolite concentrations, enhancing grape quality and its nutritive value regarding health benefits (Ferrandino and Lovioso, 2014). There are also certain quality standards for table grapes (Codex stan, 2011), while the berries for wine-making are characterised mostly by maturity parameters (Van Schalkwyk and Archer, 2000), which will be described hereafter.

Grapevine phenological growth stages according to BBCH-identification principles are divided into seven stages which are as follows: (0) sprouting/ bud development; (1) leaf development; (5) inflorescence emerge; (6) flowering; (7) development of fruits; (8) ripening of berries; and (9) senescence (Growth stages of…, 2001). Fruit development and ripening gather a number of sub-stages during which the accumulation of biochemical compounds occurs (Figure 1). From the beginning of fruit set (71; first sub-stage of fruit development stage – 7), the grape berry formation occurs and during this stage mostly acids are accumulated. In addition to other compounds, tartaric acid forms in the outer part of a berry, while malic acid concentration in the berry pulp is highest prior to veraison (Kennedy, 2002). In a period of up to 60 days after flowering, berry size changes, increasing from groat-sized (sub-stage 73) to pea-sized (sub-stage 75) berries, until attaining the size in which the bunch hangs downward and the berries are touching (sub-stage 79) (Growth stages of…, 2001). After about 60 days of berry expansion, the grape ripening starts with veraison (color change; sub-stage 81; 83) involving berry softening (sub-stage 85) until reaching cultivar-specific color and will be ripe for harvesting (sub-stage 89).

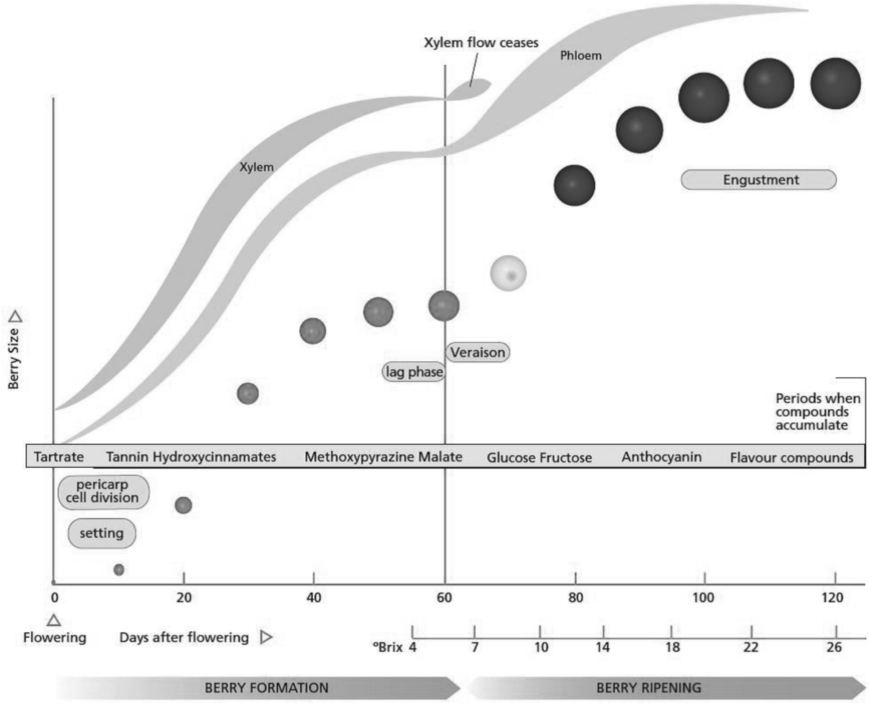

Figure 1. The accumulation of biochemical compounds and color changes in grapes according to Kennedy (2002); and fruit development and ripening stages (Growth stages of…, 2001).

For dark grape cultivars, anthocyanins are mainly accumulated in berry skin simultaneously with veraison (Kuhn *et al.*, 2013). Phenolic compounds accumulate rapidly during the few weeks after veraison and may be variable according to the vine growing conditions (Kennedy, 2002; Topalović and Mikulić-Petkovsék, 2010). Meanwhile, some compounds are diluted due to the increase in berry volume or are reduced on a berry-basis such as malic acid and tannins (Kennedy, 2002). The most extensive increase takes place when glucose and fructose begin to accumulate and after about 120 days of development, the grape berries have obtained sufficient maturity for harvesting.

Assessment of grape technological maturity comprises the measurement of primary quality attributes such as SSC, TAC and pH (Ferrer-Gallego *et al.*, 2012; Pedneault *et al.*, 2013; Nogales-Bueno *et al.*, 2014). High quality wine production with acceptable taste and preservation properties requires juice with pH from 3.2 to 3.6 (Dishlers, 2003). The combination of pH and SSC are used to calculate the values of grape MI which will show the optimum ripeness, the MI should range from 200 to 270 (Van Schalkwyk and Archer, 2000). The reason for adding pH to the formula is due to its important role in wine fermentation processes and wine stability (Van Schalkwyk and Archer, 2000). The lower pH level in grapes ensures less microbial spoilage and organoleptic degradation of the wine (Mira de Orduña, 2010). The recommended SSC should be at least 16 °Brix, while the recommended TAC is from 0.6 to 0.7 g 100 g^{-1} (Van Schalkwyk and Archer, 2000; Codex stan, 2011). In cool climate conditions, insufficient grape maturation tends to lead to increased TAC and decreased SSC (Kennedy, 2002; Gustafsson and Mårtensson, 2005). However, in southern viticultural areas, the climate-change related concern regarding accelerated grape sugar accumulation is increasing (Mira de Orduña, 2010; Palliotti *et al.,* 2014).

The response to abiotic stress at the berry level drives the accumulation of secondary metabolites in different berry parts (Ferrandino and Lovioso, 2014; Palliotti *et al*., 2014). Phenolic maturity predicts the ripeness degree for the grape skin, pulp and seeds taking into account their total polyphenolic composition (Ferrer-Gallego *et al*., 2012; Nogales-Bueno *et al*., 2014), showing both color as well as aroma profiles in different tissues (Palliotti *et al*., 2014). The content of phenolic compounds vary depending on several factors such as temperature (Haselgrove *et al*., 2000; Ferrer-Gallego *et al*., 2012); cultural practices (Peña-Neira *et al*., 2004; Palliotti *et al*., 2012) and grape developmental stage (Haselgrove *et al*., 2000; Kennedy, 2002). Secondary metabolites contribute to grape and wine taste and aroma, expressing the potential antioxidant capacity of both fruit and wines (Ferrandino and Lovioso, 2014).

Fruit ripening and biochemical composition depend on grapevine cultivation area and specific conditions of the growing year (Martin and Dunn, 2000). The major limiting factor for accumulation of biochemical compounds is plants' tolerance to abiotic stressors. Light interception and temperatures influence the final ACC significantly, but extremely high temperatures ($T>35$ °C) inhibit their accumulation (Figure 2) (Haselgrove *et al*., 2000; Kuhn *et al.,* 2013). More phenolic compounds (including anthocyanins) accumulate under drier and warmer conditions (Rio Segade *et al*., 2008). Direct exposure of fruit to sunlight has been associated with improved fruit quality (Nicolosi *et al*., 2012), for example through increased ACC and TPC as a result of canopy leaf removal (Song *et al*., 2015). ACC varies greatly with cultivar and grape maturity (Ryan and Revilla, 2003; Fournand *et al*., 2006; Topalović and Mikulić-Petkovsék, 2010), but the influence of production area, seasonal conditions (Ferrer-Gallego *et al*., 2012) and yield (Haselgrove *et al*., 2000; Hülya Orak, 2007; Falcão *et al*., 2008) should not be underestimated either.

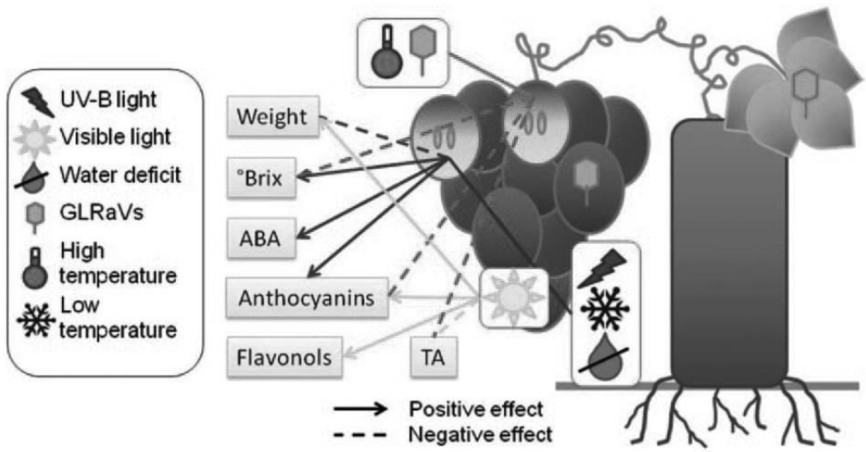

Figure 2. Effect of environmental factors and grape quality parameters according to Kuhn *et al*. (2013)

Because cool and wet weather conditions may influence ripening (Nicholas *et al*., 2011), differences between years in cool climates, can significantly affect ripening and therefore the expected concentrations of berry compounds may not be achieved (Pedneault *et al*., 2013). However, there is not enough scientific-based data concerning the factors affecting grape quality parameters in Estonian conditions.

2.2. Species and cultivars in cool climate conditions

The number of grapevine cultivars is increasing, but the list of winterhardy cultivars for successful viticulture is quite limited. The major restrictive factors are the origin of cultivars, climatic conditions of growth area and the scope of vine growing. Cultivar acclimatization depends on the vitality of buds as environmental and climatic conditions play a major role in budburst, fruit-set, veraison and maturation (Martin and Dunn, 2000). Southern viticultural areas cultivate European grapevine (*Vitis vinifera*) cultivars which are susceptible to downy mildew *(Plasmopara viticola)* and therefore require chemical treatments. Grapevine cultivation in cooler non-traditional vine growing countries enables the use of different hybrids bred from different vine species (*V. vinifera* crossing with other species such as *V. labrusca*, *V. riparia*, *V. rupestris*, *V. lincecumii*, *V. amurensis*) which have demonstrated better survival in extreme climatic conditions and better resistance to fungal diseases (Gustafsson and Mårtensson, 2005; Lisek, 2010; Karvonen, 2014). Most hybrids are described to be winter hardy down to temperatures of -30 °C when dormant, and are thus considered suitable for growing in cool climate conditions. For commercial purposes, mainly *V. vinifera* and the hybrids between *V. vinifera* and *V. labrusca* are cultivated (Lisek, 2010). *V. amurensis* species have been widely used in breeding programs, not only because of their strong cold tolerance and disease resistance, but also high berry ACC (He *et al.,* 2014). Such cultivars are grown in Estonia, cultivated in open field conditions for wine production, but also in protected areas (greenhouses, high tunnels etc.) for growing table grapes.

In a list of recommended fruit and berry cultures for growing in Estonian conditions, the number of grapevine cultivars increased from 3 in 2004 to 14 in 2013 (Eesti puuvilja…, 2013). Presumably, these cultivars suitable for Estonian conditions should be able to ripen below 2100 ºC (incl. T≥10 ºC) effective temperatures (Miidla, 1964). According to Estonian Weather Service, the long-term mean (1981-2010) of the sum of effective temperatures is 1931 ºC which theoretically indicates that Estonian conditions are not suitable for grape cultivation. In cool climate conditions, grape ripening occurs between the last spring frost and the first temperature drop in autumn (Pedneault *et al.*, 2013). The latter indicates the need for a selection of cultivars that attain sufficient maturity in a short vegetation period. The interspecific hybrid 'Hasanski Sladki' (also known as 'Baltica') has shown a good winter hardiness and therefore, is recommended for commercial growing in Estonia (Kivistik *et al.*, 2010) but it is also suitable and widely grown in different climatic conditions in Scandinavia and the United States of America (Gustafsson and Mårtensson, 2005; Hart, 2008; Plocher and Parke, 2008). As discussed previously, these cultivars have probably gained the recommendations due to their adaptability in cool climate conditions, as they were cultivated for many years before approval. Nonetheless, taking the variable climatic conditions of Estonia into consideration, different cultivars and their properties should be investigated more thoroughly.

2.3. Foliar applications

Grapevine is adapted to different climate conditions, however, exposure to various environmental stress factors leads to reduced fruit yield and quality (Mickelbart *et al.*, 2006). One possibility to improve plants' tolerance to abiotic stress factors is exogenous application of GB which enhances osmoregulation in the plants by adjusting the osmotic balance inside the plant cells and tissues (Mahdi *et al.*, 2014; Kurepin *et al.*, 2015). GB is one of the major organic osmolytes that accumulates in a variety of plant species in response to environmental stresses such as drought, extreme temperatures and UV radiation (Mickelbart *et al.*, 2006; Ashraf and Foolad, 2007). Exogenous application of GB to low-accumulating or non-accumulating plants may help to reduce unfavourable effects of environmental stresses (Ashraf and Foolad, 2007). Grapevine itself synthesizes very low concentrations of GB, but is able to take up and accumulate applied GB (Mickelbart *et al.*, 2006). Application of GB to grapevines at critical periods may defend plants from, for example, spring frosts damage and maintain yields, but it was also reported that GB has no effect on physiological parameters of non-stressed plants (Mickelbart *et al.*, 2006). Several studies have been conducted to determine the effectiveness of varying concentrations of GB in different plant species. However, it has been found that grapevine leaves take up GB in proportion to the applied concentration, up to 50 mM; higher concentrations resulted in severe phytotoxicity (Mickelbart *et al.*, 2006).

Foliar fertilization is mainly used to avoid deficiencies and to improve yield quality. K is essential for vine growth and yield, as grapes are a strong sink for K, particularly during ripening (Mpelasoka *et al.,* 2003). Topalović *et al.,* (2011) used PK foliar application starting at 15 days before veraison to show the significant role of P and K on the synthesis of carbohydrates, and indirectly on phenolic compounds. Differences in grape quality in relation to spraying with PK fertilizer became evident about 21-22 days after the treatment (Topalović *et al.,* 2011). It was found that foliar fertilization can enhance the accumulation of grape SSC and ACC, although climatic factors and yearly differences can affect the SSC, TAC and TPC in general (Topalović *et al.,* 2011). Excessive K levels in grapes may impact wine quality negatively, mainly because it decreases free tartaric acid leading to an increase in the pH of grape juice, must and wine (Mpelasoka *et al.,* 2003). Foliar nutrition does not ultimately replace soil fertilization, but it may improve the uptake and the efficiency of the nutrients (Garde-Cerdán *et* *al.,* 2015). Therefore, a demand for investigations regarding to the effects of foliar applications of biopreparations in terms of enhancing the stress tolerance of selected grape cultivars has risen.

2.4. Canopy management

The effects of limited climatic conditions can be mitigated by growing grape cultivars adapted to ripening under cool climate conditions during short seasons, by proper vine training and pruning in order to minimize the light interception and optimize the temperatures for ripening (Gustafsson and Mårtensson, 2005; Song *et al.*, 2015). Different trellis systems, pruning methods and times, including leaf removal, have been used in grape canopy management to influence vine growth and development stages (Martin and Dunn, 2000; Song *et al.*, 2015). In Scandinavian countries mainly the long cane, low cordon, low head training and mini J-style vine training systems are used (Gustafsson and Mårtensson, 2005). Low cordon spur and cane pruning are also widespread in Estonia.

Pruning technologies have a significant part in canopy management practices. The major objectives are to control vigorous grapevine growth, and to enable the canes to be bent to the ground for mulching in case of lacking snow cover protection. Reducing node number per vine and the selection of cane vigour are the main techniques used to manipulate plant yield alongside changes in bunch per vine (Greven *et al.*, 2014). Cane pruning can be used for cultivars with low natural fruitfulness. This entails cutting an adequate number of over-wintered fruit-bearing canes back to five to eight buds. Spur pruning has shown to affect the development of buds on basal nodes resulting in the suppression of apical dominance (Friend and Trought, 2007). Spur pruning is used to enhance the fruitfulness of basal buds and means cutting a number of overwintered fruit-bearing canes back to short two-bud spurs. Investigations related to effects of pruning time on budburst have revealed the hierarchy of buds bursting at different times according to their type and position on the vine (Martin and Dunn, 2000). Although budburst is sensitive to temperature fluctuations, the number of buds that are actually available to burst is limited.

In cool climate conditions, late spring and early autumn frosts in combination with cold summers result in insufficient ripening and low grape quality (Gustafsson and Mårtensson, 2005; Friend *et al.,* 2011). It has been verified that manipulation of bud break can reduce spring frost damage to shoots (Dami *et al.,* 2000) and enhance yield stability (Intrieri and Poni, 1998). On the other hand, long springs increase the duration of xylem sap flow which limits the period of vine pruning and has a significant influence on plant vitality (Keller and Mills, 2007). The vitality of buds depends on the cultivar and its acclimatization ability, as environmental and climatic conditions play an important role in different stages of grape development and ripening (Martin and Dunn, 2000; Karvonen, 2014). Delayed spur pruning has been proven to delay grapevine budburst and therefore, reduce the risk of frost damage early in spring (Friend *et al.,* 2011). Delayed pruning can affect other phenological events related to vine growth and grape quality as well, however variations in pruning times may delay ripening which in turn may result in grapes attaining insufficient maturity for harvesting (Martin and Dunn, 2000; Friend and Trought, 2007). Therefore, the effect of pruning methods in combination with different treatment times on selected cultivars would give more precise knowledge for the selection of suitable canopy management techniques.

3. STRAWBERRY CULTIVATION

3.1. Factors affecting fruit quality

The generation of inflorescence primordia in the strawberry crown starts in autumn in short day and cool temperature conditions, and continues depending on environmental conditions in spring (Hancock, 1999). In addition, genetic background and variations across genotypes play an important role in plant growth and determination of phytochemical composition of strawberries, which also affects fruit nutritional quality (Kafkas *et al.,* 2007; Tulipani *et al.,* 2011; Hasing and Whitaker, 2014; Mezzetti, 2014; Gasperotti *et al.,* 2015). The annual climatic and environmental conditions have shown significant influence on strawberry quality parameters and biochemical composition of fruits (Moor *et al.*, 2004; Crespo *et al.*, 2010; Tulipani *et al.,* 2011), and changes during the different ripening stages of the fruits may only partly explain differences in accumulation of biochemical compounds in strawberries (Khanizadeh *et al.*, 2014). Moreover, Tulipani *et al.,* (2011) have found that nutritional properties of strawberries vary greatly depending on several pre- and post-harvest factors, while Crecente-Campo *et al.*, (2012) reported that the effect of cultivation systems on the accumulation of bioactive compounds is still not clear.

Concerning strawberry fruit quality, the content of biochemical compounds determines the inner quality which is also more important to consumers nowadays. Strawberry fruits possess a considerable amount of essential nutrients and beneficial phytochemicals, which may have relevant biological activity in human health (Giampieri *et al.,* 2012). Sugars and acids are responsible for the strawberry taste (Cordenunsi *et al.,* 2002; Crespo *et al.,* 2010), as the SS/TA determines the sour-sweetness of strawberry fruits. However, metabolomic analyses have revealed significant changes in primary metabolites between strawberry cultivars grown under different conditions, including sugars and organic acids (Akhatou *et al.,* 2016). Strawberries contain a considerable amount of phenolic compounds including flavonols and anthocyanins, and possess high antioxidant activity (Määttä-Riihinen *et al.*, 2004; Fernandes *et al.,* 2012). There is an increasing demand for fresh fruits and berries with high content of health-related compounds and antioxidants. Strawberries grown under organic farming systems were found to contain higher levels of anthocyanins compared to strawberries grown under integrated management systems (Fernandes *et al.,* 2012). In addition to phenolic compounds, ascorbic acid is also known to be one of the effective antioxidants.

The strawberry inflorescence can be divided according to blossom position from which fruits will later develop in the same order. Primary fruits develop first and are greatest in size, followed by the secondary fruits, while tertiary strawberries are the smallest in size. Fruit grading has been also used for strawberry yield division as first grade (fruits more than 2 cm in diameter) and second grade (fruits less than 2 cm in diameter) (Moor *et al.,* 2005). The concentration of strawberry secondary metabolic compounds has been described to differ even according to fruit position on the cluster (Anttonen *et al.,* 2006; Tsormpatsidis *et al.,* 2011). Picking by color is generally used to determine strawberry harvest date. This however, does not coincide with the developmental time and fruit position on the cluster, leaving the effects of these parameters on fruit chemical composition unclear (Tsormpatsidis *et al.,* 2011).

Strawberry fruit order has been shown to significantly affect the SSC (Tsormpatsidis *et al.,* 2011), and TPC in fruits (Anttonen *et al.,* 2006). The ACC has not shown consistency in the theories of being affected by dilution effect or planting-date in primary fruits (Anttonen *et al.,* 2006). According to literature, the anthocyanin profile appears to be mainly genetically inherited rather than affected by abiotic factors (Crespo *et al.,* 2010). The main anthocyanin in strawberries is pelargonidin-3-glucoside regardless of genetic and environmental factors (Fernandes *et al.,* 2012). Nonetheless, higher light and temperature conditions have been shown to increase the accumulation of fruit secondary metabolites (Anttonen *et al.,* 2006; Tsormpatsidis *et al.,* 2011). For example, UV radiation has been proven to enhance strawberry color development by increasing fruit ACC by up to 31% and TPC up to 20% in 'Elsanta' strawberries (Tsormpatsidis *et al.,* 2011). The selection of cultivars with the desired composition of these compounds is the ultimate approach to manipulate fruits' TPC (Anttonen *et al.,* 2006). On the other hand, the end use of strawberry fruits according to fruit order could be adjusted by their different content of biochemical compounds.

3.2. Application of humic substances

In sustainable cultivation methods, soil composition and content of plant nutrients can be affected by natural preparations and biostimulants. EBIC (European Biostimulants Industry Council, 2013) has declared the definition for biostimulants as follows: "Plant biostimulants contain substance(s) and/or micro-organisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality."

In June-bearing strawberries, soil nutrient uptake has two major peaks, firstly during fall and secondly in spring growth (Neri *et al.,* 2002). During afore-mentioned periods, one possibility is to use preparations containing humic substances. Humic substances have been described as refractory, dark-colored heterogeneous organic compounds produced as byproducts of microbial metabolism (Stevenson, 1994; Calvo *et al.,* 2014). It has been mentioned that humin should be described preferably as a humic-containing substance, rather than a humic substance, because it consists of humic and non-humic materials (Rice and MacCarthy, 1990; Nardi *et al.,* 2009). The composition and specific structural characteristics of humic acids differ according to the source of organic material and the time of its transformation (Berbara and García, 2014; Calvo *et al.,* 2014). For example, vermicompost leachates contain high amounts of humic acids which have been used to improve strawberry growth parameters and thereby fruit yield too (Singh *et al.*, 2010).

Humic substances are widely used in sustainable horticulture, because of their positive effect on plant physiology by improving soil structure and nutrient availability, therefore also influencing nutrient uptake and root architecture (Trevisan *et al.,* 2010; Calvo *et al.*, 2014; Tehranifar and Ameri, 2014; Canellas *et al.,* 2015). Humic substances may promote plant growth through the induction of carbon and nitrogen metabolism and may have significant impact on secondary metabolism (Canellas *et al.,* 2015). The positive effect of the humic acid foliar applications on fruit quality is likely due to an indirect positive physiological effect (Neri *et al.,* 2002). Nutrient application near blooming period enhances the number of crowns thereby increasing the number of inflorescences (Khalid *et al.*, 2013). A positive effect on organic strawberry fruit quality has been obtained with repetitive humic acid foliar applications from flowering to harvest (Hosseini Farahi *et al.*, 2013). Organic amendments such as farmyard manure and vermicomposting-based substances have shown beneficial effects on growth-related and yield qualitative parameters (Khalid *et al.*, 2013).

Composts and fertilization have revealed a positive impact not only on strawberry growth (Singh *et al.*, 2010) and yield (Sønsteby *et al.,* 2004) but on the biochemical composition also (Wang and Lin, 2003; Moor *et al.,* 2004; Moor *et al.*, 2009). Arancon *et al.,* (2004) have reported that positive effects of vermicomposts on plant growth and yield were due to the nutrient availability of plant growth-influencing materials, such as plant growth regulators and humic acids, produced by the greatly increased microbial populations resulting from earthworm activity. Vermicompost leachates also contribute to plant development due to presence of humic acids (Arancon *et al.,* 2004), which regulate many processes of plant development including macro- and micronutrients absorption (Singh *et al.,* 2010). The growth responses appear either due to the ability of humic acids to act as plant growth regulators and promote growth or because the humates may have hormonal plant growth regulators adsorbed onto them (Arancon *et al.,* 2004).

Organic fertilizers are hydrophilic in nature, absorbing moisture and acting as a bond between soil and plant rendering nutrients, thus improving the soil structure and indirectly enhancing fruit quality (Khalid *et al.*, 2013). Organic amendments improve the moisture-holding capacity of soils and increase crop yields (Arancon *et al.,* 2004). Different preparations, such as compost or fertilizers as soil supplements have been reported to have a significant influence on phenolic compounds (Wang and Lin, 2003). On the other hand, the effect of additional humic acid amendments depends on the cultivar, because the role of cultivar properties have been confirmed as the main sources of variation in biochemical composition and post-harvest quality (Crespo *et al.*, 2010; Martinez *et al.*, 2015).

3.3. Defoliation

Strawberry plantations are mainly defoliated for renovation and disease control purposes (Wildung, 2000). Earlier investigations regarding strawberry defoliation aimed to find the effect of defoliation on yield, winter hardiness and pest incidence by burning straw mulch (Karp, 2001). Several studies abroad have also reported the results of postharvest defoliation, showing differences according to the extent of defoliation (Albregts *et al.*, 1992); cultivar and cropping year and the development phase of the crop (Daugaard *et al.*, 2003).

Experiments with post-harvest defoliation showed a reduction in the incidence of *Botrytis* rot in only one year out of three (Daugaard *et al.*, 2003). Partial leaf removal after planting affects plant leaf development rate and photosynthesis related to the physiological changes caused by defoliation (Casierra-Posada *et al.*, 2012). In some cases, post-harvest defoliation treatment has increased the yield in some cultivars (Nestby, 1985), but yields can be affected tremendously by changing the extent of defoliation and plant development phase (Albregts *et al.*, 1992; Casierra-Posada *et al.*, 2012). Strawberry plants respond to the post-harvest defoliation differently, depending on the cultivar and cropping year (Daugaard *et al.*, 2003; Whitehouse *et al.*, 2009). The means chosen to execute the treatment are also of essential importance, for example if a tractor driven mechanical cutting machine is used, or if the whole plantation area is burnt with straw mulch. Doing the latter in large areas is now prohibited, and it can cause serious losses concerning not only pests, but their natural enemies living in the plantation too. Different defoliation methods, such as mowing, burning of the whole plantation area or directed flaming, affect the plants differently. Some research has been done using the post-harvest straw mulch burning in relation to plant protection; this practice was shown to decrease the occurrence of spider mites (*Tetranycus urticae*) significantly (Metspalu *et al.*, 2000). Flame-defoliation could be used to remove the old strawberry foliage, but this may damage the strawberry crown (Wildung, 2000). Changes in plant biomass production (such as the number or weight of leaves) may change the nutritional allocation patterns in fruits, which are directly related to yield quality (Correia *et al.*, 2011). Fertilized plants were shown to be affected by defoliation showing decreased content of AAC in strawberries (Moor *et al.*, 2004). Therefore, defoliation can reduce strawberry plant growth; decrease productivity, as well as yield quality, including changes in concentrations of health beneficial compounds (Albregts and Howard 1972; Albregts *et al.*, 1992; Casierra-Posada *et al.*, 2012).

The timing of the defoliation is also important, since strawberry flower bud induction is sensitive to thermo-photoperiod and to some agronomic and nutritional factors (Savini *et al.*, 2005). In the northern hemisphere, the June-bearing strawberries initiate flower buds from late summer to autumn under short-day conditions. The delay in defoliation time can cause changes in strawberry plant growth processes, reducing leaf area and plant size which in turn have an impact on strawberry inflorescence and fruit formation (Casierra-Posada *et al.*, 2012). Plant leaf area is responsible for plant health and vitality due to photosynthesis processes and their influence on the leaf-root ratio. The extent of defoliation treatments was found to affect strawberry flower initiation significantly due to differences in the inductive capacity of leaves of differing maturity (Thompson and Guttridge, 1960). Defoliation can be put into practice in case of early-fruiting cultivars with vigorous growth habit, which can withstand the treatment. The growth of new leaves occurs within a week after post-harvest flaming and cultivars with vigorous growth habit are able to recover by the time of flower bud initiation and before dormancy period. Leaves also provide the winter cover in order to protect the buds initiated in the strawberry crown.

4. HYPOTHESES AND AIMS OF THE STUDY

Grape quality is significanty affected by cultivar properties and cultivation technology. There are substantial variations in the accumulation patterns of biochemical compounds in different European grapevine cultivars, but there is little information about interspecific hybrid grapevine cultivars. Yet it could be suspected that interspecific crossings between species with high cold tolerance achieve sufficient maturity requirements in Estonian climatic conditions **(I)**. In cool climate conditions, it is important to improve the plants' susceptibility in terms of cool spring and summer conditions. Glycine-betaine should increase the stress tolerance of interspecific hybrid cultivars in cool climate conditions and thus also affect the maturity parameters of grapes **(II)**. In addition, grape maturity parameters can be affected by canopy pruning methods and by the timing of pruning as well. Spring pruning is suitable for bud burst manipulation, and delayed growth start will help to reduce night frost damage **(III)**. On the other hand, it may have a negative effect on grape maturation due to the shorter period for ripening.

Humic substances enhance the rooting after planting of strawberry frigo-plants and therefore plant growth in general, while the effect on strawberry fruit composition is less studied **(IV)**. Strawberry defoliation is suitable for using in organic cultivation as a means of plant protection, but it affects plant vitality – the post-harvest flaming can damage the strawberry plant crown and decrease the number of leaves, also influencing the fruit biochemical composition. The directed row flamer originally designed for weed management purposes in vegetable fields could be used for strawberry post-harvest defoliation due to its adjustable burners causing less damage to the plants **(V)**. For plant recovery purposes, application of humic substances after the defoliation may improve plant growth and fruit quality.

The main aim of the current thesis was to find out of the effects of cultivation techniques on the fruit quality of two different horticultural crops. On one hand, grapevine, which is a woody plant and on the other hand, strawberry, which is an herbaceous plant.

Based on the hypotheses the following aims were formulated, to determine the effect of:

- cultivar on the hybrid grapevine fruit quality **(I)**;
- foliar treatments of glycine-betaine on the maturity parameters of grapes of interspecific hybrid cultivars 'Hasanski Sladki' and 'Rondo' **(II)**;
- pruning methods and timing on the hybrid grapevine 'Hasanski Sladki' maturity parameters **(III)**;
- treatments with humic substances on the biochemical composition of strawberry 'Darselect' fruits during the first year after planting **(IV)**;
- post-harvest defoliation with directed propane flamer on strawberry 'Darselect' fruit composition **(V)**.

5. MATERIALS AND METHODS

5.1. Experiments with grapevine (I-III)

Experiments site and maintenance

The vine studies were carried out in experimental outdoor vineyards of the Estonian University of Life Sciences (58°35'N, 26°52'E), where the vineyard was established in 2007 (Table 1) (**I-III**). The plantation was established on 2×2 m spaces with *in vitro* propagated own-rooted plants. The experimental design was a randomized block with four replications. The training system was low double trunk 25 cm high, and the total height of the canopy was approximately 1.6 m. Double trunk means that two horizontally curved woody branches were left per plant, each of which had up to five fruit-bearing canes.

Table 1. Overview of the conducted experiments

Paper	Culture	Impact factor	Measurements and analysis	Years of experiment	Location of plantations
I	Grapevine	Cultivar properties	pH, SSC, TAC, SS/TA, TPC	2011-2012	Rõhu Experimental Station , Tartu County
II	Grapevine	Foliar treatments (GB, PK)	pH, SSC, TAC, SS/TA, MI, ACC, TPC	2009	Rõhu Experimental Station, Tartu County
III	Grapevine	Canopy management (SP, CP)	SPAD, FM, BW, pH, SSC, TAC, SS/TA, MI, ACC, TPC	2011-2012	Rõhu Experimental Station, Tartu County
IV	Strawberry	H amendment	SPAD, FM, SSC, TAC, SS/TA, AAC, ACC, TPC	2010	Research Centre of Organic Farming, Tartu County
V	Strawberry	FD and FD+H amendment	SPAD, FM, SSC, TAC, SS/TA, AAC, ACC, TPC	2010-2013	Research Centre of Organic Farming, Tartu County

Suckers were cut and removed throughout the summer. In August, the cane top was cut back. Leaf removal adjacent to bunches was implemented at the beginning of veraison when removing the leaves from the east side of the canopy to allow morning sun exposure due to the occurrence of dew. In October, all the fruit bearing canes were cut back to three-bud spurs (**I, II**). The rows were covered with 0.04 mm thick black polyethylene mulch. Between the rows there was sown grass, which was mown regularly during the vegetation period.

Experimental treatments

Properties of interspecific hybrid dark-colored grape 'Hasanski Sladki', 'Rondo', 'Zilga' and 'Kuzminski Sinii' were investigated in 2011 and 2012, and of white grape 'Jubilei Novgoroda', 'Korinka Russkaja', 'Severnõi Rannii' and 'Supaga' in 2012 (Table 2) (**I**).

Table 2. Grape cultivars under investigation

Cultivar	Country of origin	Pedigree
'Hasanski Sladki' (syn. 'Hasaine Sladki', 'Varajane Sinine', 'Baltica')	Russia	*V. amurensis, V. labrusca*, *V. riparia* and *V. vinifera*
'Rondo'	Germany	'Zarya Severa' × 'Saint Laurent' (incl. *V. vinifera*, *V. amurensis*)
'Zilga'	Latvia	('Smuglanka' × 'Dvietes Zila') × 'Jubileinaja Novgoroda' (*V. amurensis*, *V. labrusca*, *V. vinifera*)
'Kuzminski Sinii'	Russia	Suspectedly bred from *V. vinifera* and *V. amurensis*
'Jubilei Novgoroda' (syn. 'Jubileinaja Novgoroda')	Russia	'Malingre Precoce' × 'Ruskii Konkord'
'Korinka Russkaja' (syn. 'Russkaja Korinka')	Russia	'Zaria Severa' × 'Kishmish Chernyi'
'Severnõi Rannii'	Russia	*V. vinifera*
'Supaga'	Latvia	'Madeleine Angevine' × 'Dvietes Zila'

The grapevine cultivars were selected for the experiments based on the list of recommended cultivars which are already widely cultivated in Estonian climatic conditions as well (**I-III**).

GB foliar applications were tested on grape cultivars 'Hasanski Sladki' and 'Rondo' in comparison with PK-fertilizer that is used in conventional cultivation (**II**). The treatments were as follows:

- C – control without any spraying treatment;

- PK – water soluble fertilizer (PK 1-18-38) was used as a foliar treatment at a concentration of 20 g of fertilizer per 10 L of water, and applied once at the beginning of August in 2010;
- GB – as natural product derived from sugar beet molasses foliar treatments at a concentration of 62.5 g (40 mM) of GB / 10 L of water, applied twice in a season: at the beginning of June (during the third leaf phase) and August;
- GB+PK – applied once at the beginning of August.

The grapevine pruning experiment was carried out with cultivar 'Hasanski Sladki' (Table 1 and 2) (**III**). Vine treatments were executed in autumn after leaf fall (2010 and 2011), and in spring at two-leaf phase (2011 and 2012). The pruning treatments were as follows:

- SP – four over-wintered fruit-bearing canes were pruned back to short two-bud spurs and new shoots were directed vertically;
- CP – two over-wintered fruit-bearing canes were pruned back to eight buds and bent horizontally.

5.2. Experiments with strawberry (IV, V)

Experimental site and plant material

The strawberry experimental plantation was established in 2010 at the Research Centre of Organic Farming of the Estonian University of Life Sciences (58°21' N, 26°40' E) (Table 1). As a pre-planting procedure weeds were destroyed when the plantation area was covered with black plastic from spring 2009 to the next spring 2010 until ploughing and establishment were carried out; and an ecological fertilizer (NPK 4.5-2.5-8) – produced from at least 30% malt germs – was used as pre-establishment soil supplement.

Strawberry cultivar 'Darselect' frigo-plants were planted in May 2010 in a one-row system with 50 cm spacing. After planting, half of the strawberry plantation area (including rows) was covered with a 3-5 cm thick OM layer made of leftover branches of organic apple tree canopy pruning, the second half with black 0.04 mm thick PM. The experimental layout was a randomized block design with three replicates (12 plants per replication).

The H is an organic preparation containing 15% total humic extract, 12% humic acid and 3% fulvic acid obtained from the process of decomposition and transformation of organic matter originating from leonardite. Application of H is described in Table 3 (**IV**, **V**). FD treatments were executed in 2011–2012, and the data was collected in 2012-2013 (**V**).

Table 3. Experimental treatments in strawberry plantation

Paper	Year	Treatment abbrev.	Pre-harvest treatments*	First/last harvest date	Post-harvest treatments
IV	Planting 2010	C	0.5 l water	10.07. 19.07.	0.5 l water
		H	0.5 l H		0.5 l H
V	2011	C	1 l water	27.06. 15.07.	1 l water
		FD	1 l water		FD + 1 l water
		FD+H	1 l H		FD + 1 l H
	2012	C	1 l water	25.06. 16.07.	1 l water
		FD	1 l water		FD + 1 l water
		FD+H	1 l H		FD + 1 l H
	2013	C	1 l water	20.06. 03.07.	Plants were dug out
		FD	1 l water		
		FD+H	1 l H		

Note: *Pre-harvest treatments were executed at the beginning of plant growth and at full bloom. Treatments abbreviations: C – control; FD – flame-defoliation; H – humic substances; FD+H – flame-defoliation + humic substances.

The flaming-machine is a product of Elomestari Company from Finland (www.elomestari.fi). The machine works with propane and it has two 20 cm wide burners with covers controlling the heating of the row flamer including mounting for selective (directed) flaming.

5.3. Measurements and analysis

Grapes were harvested in mid September before the permanent autumn frosts (**I-III**). The number of berries per bunch was recorded and grape berry weight was calculated as the mean of 100 berries (**I**). BW of ten randomly selected grape bunches of a vine was determined in each replication (**II, III**).

Strawberry fruits were picked according to surface color and fruit order (primary, secondary, tertiary) in clusters (Figure 3), and strawberry FM and total yield per plant were weighed during fruit harvest during the period from 20 June to 20 July (**IV, V**). The number of leaves and inflorescences were counted during flowering (in May) in both experimental years (**IV, V**). DW of strawberry roots and crowns was determined in 2013; the leaves were cut off and after cleaning the soil off, plant crowns and roots were dried until a constant weight was recorded (**V**).

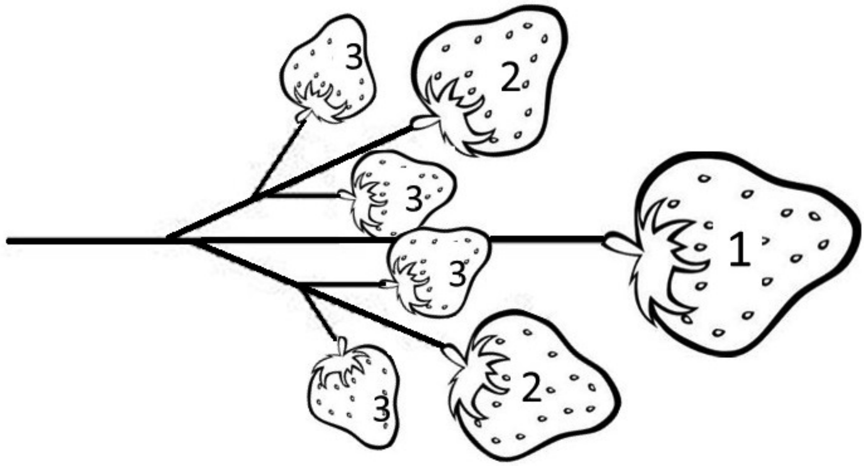

Figure 3. Strawberry fruiting order in clusters: (1) – primary fruit, (2) – secondary fruit, (3) – tertiary fruit.

SPAD values were measured for 10 fully expanded leaves of strawberry plants in May, and 30 from grapevines in July from the middle of shoots in order to estimate the chlorophyll content of leaves using a portable chlorophyll meter (SPAD-502, Minolta, Japan) (**III, V**). This permits a rapid and non-destructive determination of leaf chlorophyll content by measuring leaf transmittance.

SSC (°Brix) was measured from an average of 10 to 30 fresh fruits (strawberries and grapes, respectively) in three replications by refractometer (Atago Pocket Refractometer Pal-1, Tokyo, Japan) (**I-V**). All the other biochemical parameters were determined from frozen fruits, whereas collected fruit samples were stored at −20°C until the analysis (**I-V**). Strawberry fruits were analysed separately according to fruit order (**IV, V**). TAC was determined manually by the titration method with aqueous 0.1 M NaOH solution (buret Mettler Toledo DL 50 Randolino), using Bromothymol blue as an end point indicator (Chone *et al.*, 2001). The TAC of strawberries was expressed as citric acid g 100 g^{-1} FW (**IV, V**) and grapes' TAC as tartaric acid g 100 g^{-1} FW (**I-III**). SS/TA was calculated based on SSC and TAC for all cultivars under investigation. Grape juice pH was measured with a pH/conductivity meter (HD 2156.1, Delta OHM). Grape MI was calculated according to the formula determined by Coombe *et al.* (1980): MI=°Brix × pH^2.

Strawberry AAC was determined iodometrically with the modified Tillman's method, and expressed as mg 100 g^{-1} of strawberry FW (**IV, V**). TPC was determined from grape skin and a whole straberry fruits with the Folin-Ciocalteau phenol reagent method (Slinkard and Singleton, 1977), using a spectrophotometer (UVmini-1240 Shimadzu, Kyoto, Japan) at 765 nm. The TPC was expressed as mg of gallic acid equivalents 100 g^{-1} FW. ACC was estimated by a pH differential method from the skin of grapes (**II**, **III**; Cheng and Breen, 1991), and the whole strawberry fruits (**IV**, **V**), where absorbance was measured with spectrometrically at 510 and at 700 nm (UVmini-1240 Shimadzu, Kyoto, Japan) in buffers at pH=1.0 (HCl 0.1N) and pH=4.5 (citrate buffer). Strawberry ACC was expressed as mg of pelargonidin-3-glucoside equivalent 100 g^{-1} FW, and grape ACC as mg of cyanidin-3-glucoside equivalent 100 g^{-1} FW.

5.4. Soil and weather conditions

Soil conditions

The soil of Rõhu Experimental Station vineyard was Stagnic Luvisol (FAO soil classification) (**I-III**). Vineyards soil pH_{KCl} was 5.8 and soil organic matter by loss on ignition method was 2.5%. Humus layer thickness was 50 cm. The concentrations of plant-available phosphorous (P 324 mg kg^{-1}), potassium (K 241 mg kg^{-1}), calcium (Ca 2050 mg kg^{-1}) and magnesium (Mg 222 mg kg^{-1}) were high in the soil and hence, no fertilizers were used. Also no irrigation systems were used.

The soil of the Research Centre of Organic Farming experimental area was Stagnic Luvisol (FAO soil classification) with soil pH_{KCl} 6.6 and soil organic matter by loss on ignition method was 2.3% at the time of plantation establishment (**VI, V**). The concentrations of plant-available phosphorous (P 256 mg kg^{-1}), potassium (K 306 mg kg^{-1}), calcium (Ca 3930 mg kg^{-1}) and magnesium (Mg 600 mg kg^{-1}) were high in the soil.

Temperatures

In 2009, June was up to 1.4 °C cooler, and September warmer when compared to the many years' mean temperatures (Table 4). In 2010, the warmest month was July with 21.7 °C surpassing all the other experimental years and the long-term mean (17.6 °C). In the summer of 2011, mean temperatures in June, July and September were up to 3.3 °C higher than the long-term mean. In 2012, the mean temperature in August was the coolest (14.8 °C) compared to other years under investigation and the mean of 1981–2010 (16.2 °C). In 2013, April was the coolest with an average temperature of only 4 °C, but May and June were warmer than in the other experimental years, and up to 2.8 °C warmer than the long-term mean.

The sum of effective temperatures (T≥10 °C) from April to September was above 2100° in three of the five experimental years (Table 4). The sum was highest (2498°) in 2011 and lowest (1838°) in 2010. In all experimental years, the sum was higher (from 1967° to 2498°) than the long-term mean (1931°), except in 2010 (as mentioned previously).

Table 4. Average air temperature (°C) and total monthly precipitation (mm) at the experimental site during the years of studies and long-term average (1981–2010)

Month	Air temperature (°C)					
	2009[a,b]	2010[a,b]	2011[a,b]	2012[a,b]	2013[a,b]	1981–2010 [b]
April	5.3	5.7	5.7	4.6	4.0	5.5
May	11.3	12.2	11.0	11.4	15.5	11.5
June	13.6	14.3	17.2	13.3	17.8	15.0
July	16.8	21.7	20.0	17.7	17.5	17.6
August	14.9	17.8	15.9	14.8	16.6	16.2
September	12.4	10.7	12.3	11.9	10.8	11.0
October	3.7	3.8	6.8	5.3	6.6	6.1
T (≥10 °C)	2156°	1838°	2498°	1967°	2263°	1931°
	Precipitation (mm)					
April	3	25	1	45	36	30
May	18	97	58	78	65	55
June	151	98	35	98	29	84
July	97	38	48	80	67	72
August	85	148	55	80	73	86
September	58	99	80	61	38	61
October	132	59	48	72	45	69
Sum of precipit.	444	564	325	474	353	457

[a]Data was collected from an automatic weather station at the experimental farm.

[b]Data according to Estonian Weather Service (www.ilmateenistus.ee) database. T – sum of effective temperatures (T≥10 °C).

From all the experimental years, June of 2009 (151 mm) and August of 2010 (148 mm) were the rainiest (Table 4), while in June of 2011 and 2013 there was less precipitation with totals of 35 and 29 mm, respectively. In 2011, there was almost no rain in April and 31 mm less precipitation in August compared to long-term mean (86 mm).

5.5. Statistical analysis

One-way ANOVA was applied in order to test the effect of grapevine cultivar properties, glycine-betaine treatment and canopy pruning on grape quality (**I-III)** and to test the effect of the treatments with humic substances and flame-defoliation on strawberry quality parameters according to the fruit orders (**IV, V**). To evaluate significances of differences among treatments the least significant difference ($LSD_{0.05}$) was calculated at the $P \leq 0.05$ level of confidence. Standard deviations of the mean values are also presented (±SD) (**V**).

Two-way ANOVA was executed to test the effect of pruning treatments and time on grape biochemical composition (**III**) and the effects of strawberry fruit order and treatments (**V**). The mean effect was evaluated at the confidence levels of $P \leq 0.05$*, 0.01** or 0.001*** or marked as NS.

Pearson's correlation coefficients (*r*) were calculated in the grapevine pruning experiment (**III**) to study the statistical relationship between the leaf SPAD readings, yield components (BM; BW; berries per bunch) and grape quality parameters (SSC; TAC; SS/TA; TPC; ACC; MI; pH); and between strawberry plant growth attributes (**V**) (number of leaves; number of inflorescences; SPAD values; yield; crown and root mass) and fruit quality parameters (SSC; TAC; SS/TA; AAC; TPC; ACC). The strength of a relationship was estimated as $r \leq 0.3$ (weak), $0.3 \leq r \leq 0.7$ (moderate) and $r \leq 0.7$ (strong). All the results in this case were considered statistically significant at $P \leq 0.05$* (**III**, **V**) and $P \leq 0.01$** (**V**). All the data analysed in the experiments met the assumptions of normality and no additional transformations were made.

6. RESULTS

6.1. Effect of cultivar properties, glycine-betaine and pruning on grape quality

Technological maturity

SSC ranged from 12.7 to 19.9 in dark grapes and from 15.1 to 17.0 °Brix in white grapes depending on cultivar properties (Figure 1 in **I**). 'Hasanski Sladki' obtained the highest SSC among dark grapes in both experimental years (2011 and 2012). Among white grapes, 'Jubilei Novgoroda' and 'Severnõi Rannii' showed the highest SSC. In the foliar applications experiment the highest variations in SSC were recorded in 'Rondo' grapes: from 13.7 to 16.0 °Brix in 2009 (Figure 1A in **II**). GB had no effect on the SSC of neither of the two experimental cultivars. On the contrary to the expectations, 'Rondo' was affected positively by PK-treatment instead. In the grapevine pruning experiment with 'Hasanski Sladki', the SSC ranged from 17.1 to 19.8 °Brix and pruning time caused significant differences in grapes of SP-vines (Figure 1 in **III**). Autumn pruning increased the SSC compared to spring treatment in both experimental years and in the two years' mean (Table 2 in **III**). The average effects of pruning time and treatment were significant for SSC at $P \leq 0.01$.

TAC was different among dark cultivars, being significantly highest in 'Zilga' in 2011, but highest in 'Hasanski Sladki' in 2012 (Figure 2 in **I**). Among white cultivars the content was highest in 'Supaga'. The foliar GB decreased the TAC in both cultivars (Figure 1B in **II**). In 'Rondo' TAC was significantly lower in all treatments. In the pruning experiment 'Hasanski Sladki' TAC ranged from 1.3 to 2.1 g 100 g^{-1} and pruning time had a significant effect only on CP in 2012 (Figure 2 in **III**). Autumn CP decreased the TAC in year 2012 and in the two-year average (Table 2 in **III**). The overall effects of pruning times ($P \leq 0.05$) and treatments ($P \leq 0.001$) were statistically significant for TAC.

The grape pH ranged depending on cultivar from 2.9 to 3.8 in dark and from 2.9 to 3.3 in white grape cultivars (Figure 4 in **I**). In dark grapes, juice pH was significantly higher in 'Kuzminski Sinii' and in white grapes in 'Severnõi Rannii'. In experiments with foliar applications, the grape juice pH varied from 3.1 to 3.2 in 'Hasanski Sladki', and from 2.8 to 2.9 in 'Rondo' (Figure 1D in **II**). In 'Hasanski Sladki', juice pH was significantly decreased the most by GB when compared to control, while there was no effect of GB on 'Rondo's pH.

None of the foliar treatments affected the MI of 'Hasanski Sladki', while the MI of 'Rondo' was significantly increased by PK instead of GB. 'Hasanski Sladki' grapes had higher MI ranging from 202 to 209 (Figure 1E in **II**). In 'Rondo', the index varied from 114 to 136 and did not reach the optimum of 200. In the pruning experiment 'Hasanski Sladki' MI varied from 132 to 213, and the variations were caused by pruning time (Figure 4 in **III**). For SP, the effect of pruning time differed in the two years; spring pruning in 2011 decreased the MI, but the effect was on the opposite in 2012. In CP, the autumn treatment increased the MI compared to spring in 2011. Spring SP had a positive influence on the MI for the average of two years, and the average effect of pruning time and treatment was also significant ($P \leq 0.01$) (Table 2 in **III**).

Phenolic quality

TPC was affected by grapevine cultivar properties and the results ranged from 214 to 540 in dark grapes, and from 102 to 168 mg 100 g^{-1} in white grapes (Figure 5 in **I**). In dark grapes, the positive effect of genotype on TPC were observed in 'Rondo' in both years, and in 'Kuzminski Sinii' in 2011. Among white grapes, the TPC in 'Supaga' berries was significantly higher. In both pruning treatments, pruning time did not result in statistically significant differences in TPC (Figure 5 in **III**). In the average of two years, spring pruning decreased the content in both treatments (Table 2 in **III**).

In the experiment with foliar treatments, grape ACC in 'Hasanski Sladki' ranged from 84 to 99, and in 'Rondo' from 160 to 219 mg 100 g^{-1} (Figure 1F in **II**). Foliar treatments did not affect 'Hasanski Sladki' ACC but GB and PK-treatments significantly increased the ACC of 'Rondo' fruits. High variability among pruning treatments was found for ACC, ranging from 73 to 113 mg 100 g^{-1} in SP and 31 to 77 mg 100 g^{-1} in CP (Figure 6 in **III**). Spring pruning caused an increase of ACC of up to 60% in 2012, and in the two–year average also. The effect of pruning times and methods were statistically significant ($P \leq 0.001$; Table 2 in **III**).

Correlations between grapevine yield components and fruit quality parameters

According to the pruning treatment, in SP, a strong positive correlation was found between BM and SSC, SS/TA, TPC, MI and pH (Table 3 in

III). Strong negative correlations were found between BM and TAC and ACC. BW correlated moderately positively with SSC and strongly with TPC, and the correlations were negative and strong with MI and pH. There was strong positive correlation between the number of berries per bunch and TAC, while strong negative correlations were evident with SS/TA and ACC.

In CP, a strong positive correlation appeared between BM and SSC and pH, a moderate positive correlation appeared between BM and SS/TA, TPC and MI (Table 3 in **III**). A negative moderate correlation was observed with TAC. BW correlated moderately negatively with SSC. Number of berries per bunch was positively moderately correlated with TAC and negatively with SS/TA.

6.2. Effect of humic substances and flame-defoliation on strawberry fruit quality

Taste-related parameters

In the post-planting H application experiment strawberry SSC ranged from 8.0 to 12.8 °Brix (Figure 1A in **IV**). H had a significant positive effect on SSC in tertiary fruits in OM, and negative in PM. In the FD experiment FD increased the SSC in primary fruits in 2012, but decreased it in the next experimental year (Table 4 in **V**). FD+H decreased SSC by 19% in primary and tertiary fruits in 2012, but increased the content by 50% in 2013. In the average of two years, the effect of fruit order on SSC was NS, and the influence of treatment was statistically significant at $P \leq 0.001$.

TAC varied in the H application experiment from 0.71 to 0.89 g 100 g^{-1} (Figure 1B in **IV**). Differences appeared in primary fruits in the OM and in secondary fruits in the PM, where the TAC was increased by H application. FD treatment increased TAC by up to 32% in all fruit orders in 2012 and decreased it in primary fruits in 2013, while FD+H affected it inversely in both years (Table 4 in **V**). The average effect of fruit order on TAC was statistically significant at $P \leq 0.05$ and treatments at $P \leq 0.001$.

H application caused SS/TA ratio variation from 10.7 to 14.4 (Figure 1C in **IV**). H had a statistically significant effect only on tertiary fruits in the OM, but at the same time a negative effect on the PM. SS/TA decreased due to FD in primary and tertiary fruit order, while FD+H increased the ratio compared to FD in 2012 (Table 4 in **V**). In 2013, the FD+H increased the ratio in primary and tertiary fruits by up to 54% compared to C. The average effect of fruit order was significant for the SS/TA at $P \leq 0.01$ and treatments at $P \leq 0.001$.

Antioxidants

In the first growth year, AAC values ranged from 79 to 94 mg 100 g^{-1} depending on H treatment (Figure 1E in **IV**). H had a positive effect on AAC in secondary fruits in OM. ACC varied from 113 to 215 mg 100 g^{-1} (Figure 1D in **IV**). A negative effect was noted in tertiary fruits in OM and a positive effect on secondary fruits' ACC in PM (increase up to 18%). FD and FD+H decreased the AAC by 11% in primary and by 36% in tertiary fruits in 2012 (Table 4 in **V**). In the next experimental year, ACC was increased by FD by 38% in primary and by 62% by FD+H in tertiary fruits (from 9.5 to 15.4 mg 100 g^{-1} FW). The average effect of fruit orders and treatments on ACC was statistically significant at $P \leq 0.001$.

H significantly influenced TPC in both mulches, and differences were related to fruit order. TPC varied from 158 to 226 mg 100 g^{-1} (Figure 1F in **IV**). In OM, the TPC was increased by 16% in secondary and tertiary fruits, in the PM by 13% in primary and secondary fruits. TPC decreased by about 7% in tertiary fruits in PM. TPC was also significantly affected by the FD treatments in all fruit orders (Table 4 in **V**). FD+H increased TPC in primary and tertiary fruits but decreased it in secondary fruits in 2012. In 2013, FD increased the TPC by 12% in primary fruits but decreased it significantly in tertiary fruits. Mean effect of fruit order and treatments on TPC was significant at $P \leq 0.001$.

Correlations between strawberry plant growth, yield components and fruit quality parameters

Pearson's correlation coefficients revealed a moderate positive correlation between TAC and the number of leaves and inflorescences; a moderate negative correlation was found between TAC and SPAD, and TAC and yield (Table 5 in **V**). A moderate positive relationship was found between SS/TA and SPAD value. TPC correlated moderately negatively with SPAD and yield. A strong negative correlation was observed between ACC and number of leaves, while ACC had a positive moderate correlation with SPAD and with yield. No correlation was found between the mass of roots and crowns and fruit quality parameters.

7. DISCUSSION

7.1. Grape quality depending on cultivar, glycine-betaine and canopy pruning

Technological maturity

Results of the cultivar experiment revealed the significant effect of genotype on grape quality and not all the cultivars achieved sufficient maturity (Figure 1 in **I**). The lowest SSC was in ’Rondo’ grapes. ’Rondo’ has been described as a cultivar susceptible to frost damage (Lisek, 2007), which means that the buds of the cultivar will freeze entirely in winter and the vines will start their growth quite late in spring, affecting fruit ripening significantly. The highest SSC among dark-colored grape cultivars was recorded in ’Hasanski Sladki’ in both experimental years (2011–2012), reaching the optimum value of °Brix for winemaking recommended by Van Schalkwyk and Archer, (2000). Sugar accumulation in berries starts during the ripening period and depends on the weather conditions; temperature effects on final SSC are reported to be relatively small (Coombe, 1987). According to Gustafsson and Mårtensson (2005) insufficient grape maturation is a common problem in cold climate conditions, leading to low SSC. Cultivar-derived differences in SSC are described to be significant, although variations in SSC may even appear in the same cultivar in different vineyards (Pedneault *et al.*, 2013). In our experiments, SSC tended to vary more due to cultivar properties. Foliar GB treatment did not influence the SSC of ‘Hasanski Sladki’, and even so, the grapes reached up to 21.5 °Brix, the recommended values as mentioned previously (Figure 1.A in **II**). On the other hand, SSC of ’Rondo’ reached up to 16.0 °Brix, which also may be acceptable in combination with low TAC. It may be assumed that as the grapevine plants were considerably young (planted in 2007) and only just at the beginning of their fruit-bearing years (in 2009), the impact of stress factors remained minimal. Moreover, Mickelbart *et al.*, (2006) reported that GB had NS effect on physiological parameters of non-stressed plants, which may partly explain the minimal effect of GB on grapevine fruit quality parameters. PK treatment of vine leaves raised only the SSC of ’Rondo’. As ’Hasanski Sladki’ is an earlier ripening cultivar compared to ’Rondo’, one possibility for this phenomenon may be related to the position of berries in the clusters and their proportion, as ’Rondo’ clusters are larger with densely growing berries. The more sparsely spread out the berries in the cluster, the better are the light conditions, and nutrient distribution occurs among a smaller number of berries. The latter affects the ripening of grapes, because berry weight also can influence the berry sugar concentration significantly (Zsófi *et al.,* 2011). In our experiment, a positive correlation between grape BM and SSC was also found (Table 3 in **III**). However, GB application may have NS effect on the SSC due to loamy nutrient-rich soil with minimal plant stressors. Results of the grapevine pruning experiment showed that autumn SP increased the SSC of 'Hasanski Sladki' (Table 2 and Figure 1 in **III**). In 2011, the increase was similar to the recommended level (19.8 °Brix) probably due to a longer ripening period related to warmer seasonal conditions showing higher temperatures in June and in July (Table 4). On the other hand, Greven *et al.,* (2015) proved that SSC accumulation from ripening time onwards was generally faster for vines pruned to a lower node number. Our results correspond partly with the finding, as SP treatment was more intensive, fewer nodes were left on the vines and higher SSC was determined, but only in the case of autumn pruning. Also, a shorter ripening time is suggested for the vines pruned to a lower node number (Greven *et al.,* 2015), which may explain the positive effect of autumn SP. The decrease of SSC in 2012 could be due to a cooler and rainier season, though autumn SP still affected the content significantly due to pruning time.

SSC tended to remain at lower levels, but at the same time TAC values were considerably high throughout the experimental years. TAC differed between the years among dark grapes showing extremely high contents in both experimental years (Figure 2 in **I**). The climatic conditions have a significant influence on TAC in grapes (Topalović and Mikulić-Petkovsék, 2010); and in cool climate conditions, insufficient grape maturation leads to increased TAC and decreased SSC (Kennedy, 2002; Gustafsson and Mårtensson, 2005). The results of the present experiment conform to the previously mentioned, as higher TAC contents were obtained in 2012 which was cooler, with a considerable precipitation rate (Table 4). Although foliar GB treatment decreased TAC in grapes, the levels still remained high (Figure 1.B in **II**). It has been reported that GB application to grapevines at critical times may help the plants to overcome stressful periods (Mickelbart *et al.*, 2006). In the present experiment, as June was the coolest and most rainy month in 2009, compared to the long-term mean (Table 4), it may have slowed down the flowering and fruit formation processes, thereby affecting the TAC (Figure 1.B in **II**). The results of the grapevine pruning experiment also showed that TAC was high in both treatments and pruning times (up to 2.1 g 100 g^{-1} FW), surpassing the recommended values (up to 0.7 g 100 g^{-1}) (Figure 2 in **III**). Increased TAC's in cooler conditions have also been reported by other authors, reaching up to 2.0 g 100 g^{-1} in a single year, even in grapes of European vines (Iyer *et al.,* 2012). Therefore, the influence of CP is considered to be positive when decreasing TAC.

The grape juice pH ranged from 2.9 to 3.8 in dark, and from 2.9 to 3.3 in white grape cultivars (Figure 4 in **I**), which shows higher variations between the pH values of different genotypes below and above the recommended range. In order to produce quality wine with acceptable taste and preservation properties, juice pH should range from 3.2 to 3.6 (Dishlers, 2003). pH value is also important for consumers' healthy diet and from the standpoint of grape juice biological stability (Mira de Orduña, 2010). Though, GB decreased the juice pH of 'Hasanski Sladki', but the values reached still to the recommended level (Figure 1.D in **II**). At the same time, 'Rondo' did not achieve the optimum quality level of pH, showing values of pH 2.9 only. The decreasing effect of GB on grape pH may be caused due to reduced plant stress symptoms which created favourable conditions for vegetative plant growth. Simultaneously, when the temperatures during veraison in August were considerably lower compared to the mean of many years, the plant growth slowed down and affected fruit quality also.

Although the MI of both cultivars was not significantly affected by GB treatment, 'Hasanski Sladki' grapes still achieved the minimum recommended value. MI of 'Hasanski Sladki' grapes reached 209, while in 'Rondo' it was 136 (Figure 1.E in **II**). For quality wine grapes it should be in the range from 200 to 270 (Van Schalkwyk and Archer, 2000); the high SSC and pH values according to cultivar play a major role determining the maturity of grapes. In general, both cultivars investigated responded to the GB treatments differently, showing more variations in terms of cultivar properties (Figure 1.E in **II**). 'Hasanski Sladki' as an early ripening cultivar produced higher SSC, considerably high TAC and optimal pH, which leads to sufficient MI in relation to its genotype and was not significanlty influenced by GB. Autumn pruning also increased the MI values of grapes in both treatments in the warmer year 2011 (from 206 to 213), reaching the minimum of recommended level (Figure

4 in **III**). This could be caused due to fact that in the case of autumn pruning, the vines are able to start their growth as soon as the favourable weather conditions occur. Moreover, when vines are spring-pruned, the bud hierarchy will be changed after the treatment and it will take longer to start the growth, resulting in a shorter ripening period. This corresponds with Martin and Dunn (2000) who confirmed that different types of buds burst at different times according to a hierarchy, depending on different types of shoots and their bud positions. In cool climates, as cool and wet weather conditions may influence ripening (Nicholas *et al.*, 2011), differences between the years can significantly affect ripening and therefore the expected concentrations of berry compounds may not be achieved (Pedneault *et al.*, 2013).

Phenolic quality

In dark grapes, the highest TPC was obtained in 'Rondo' in both years, and in 'Kuzminski Sinii' in 2011 (Figure 5 in **I**). Higher values of ACC were determined in the cooler summer conditions in 2012 (Table 4). The temperatures during berry maturation in August 2012 were somewhat lower than in 2011. ACC has found to vary greatly with cultivar and grape maturity (Ryan and Revilla, 2003; Fournand *et al.,* 2006), but also production area and seasonal conditions (Ferrer-Gallego *et al.,* 2012). Foliar treatments did not affect the ACC of 'Hasanski Sladki', whereas GB increased the ACC of 'Rondo' grapes (Figure 1.F in **II**). Topalović *et al.,* (2011) found that foliar fertilization can enhance the accumulation of grape ACC, although climatic factors and yearly differences may affect TPC in general. In the present study, the second GB treatment executed in August may have played an important role, as the accumulation of ACC starts with berry veraison. It may also have enhanced the increase of ACC in 'Rondo' grapes. In the pruning treatments, autumn pruning significantly increased TPC in the two years' mean up to 311 mg 100 g^{-1} showing relatively high concentrations (Table 2 in **III**). The reason for differences in TPC and ACC between the years could be related to the weather conditions, as discussed earlier, and hence enhanced grape ripening. It has also been demonstrated by other authors that TPC varies depending on several factors including temperature during ripening (Haselgrove *et al.,* 2000; Ferrer-Gallego *et al.,* 2012) and cultural practices used (Peña-Neira *et al.,* 2004; Palliotti *et al.,* 2012). The differences in the present experiment may be related to variations in compound bud position, and whether the shoots develop from the large central primary bud, from a smaller secondary bud or from both at the same time. Reducing node number per vine and the selection of cane vigour are the main techniques used to influence vine growth and development (Martin and Dunn, 2000; Song *et al*., 2015), and to manipulate plant yield (Greven *et al*., 2014).

Pruning technologies play an important role in canopy aeration and light interception conditions. Differences in ACC (Figure 6 in **III**) were probably affected by lower night temperatures accompanied by major day-night temperature fluctuations. The temperature fluctuations during ACC accumulation caused stress (Figure 2) and therefore the increase in ACC occurred. In addition to temperatures, light conditions are also important. As in the present experiment, the height of trellis system and row spacing were taken into account to provide maximum light exposure, which is especially important in cooler climatic conditions. Nicolosi *et al.* (2012) indicated that direct sunlight interception by fruit has been associated with improved fruit quality and is generally desirable in most vineyards. The angle of sunshine in northern areas differs from the southern parts of the world; in cool climate conditions the sun shines obliquely rather than directly overhead and therefore the shading effect of the leaves is minor. The effect was especially evident in the case of SP, because of the particular treatment being more extensive, and therefore more stressful to vines compared to CP. In the case of SP, the fruit-bearing shoots developed from the two proximal buds that were left after pruning. According to Andersen and Sims (1991), the lower the pruning severity, the greater the proportion of highly productive shoots derived from primary buds. Although budburst is sensitive to temperature fluctuations, the number of buds that are actually available to burst is limited. In CP, the reproductive buds were in the middle of the cane and hence, the basal buds did not burst at all because of apical dominance.

7.2. Strawberry fruit composition affected by humic substances and flame-defoliation

Taste-related parameters

The results of post-planting H application indicated that fruit order affected strawberry taste-related parameters and the sweetest were tertiary fruits (Figure 1.A-C in **IV**). This corresponds with other authors who described the differences in strawberry metabolic compounds in relation to fruit order/position on the cluster (Anttonen *et al.,* 2006; Tsormpatsidis *et al.,* 2011). Strawberry fruit order has been shown to affect significantly the SSC (Tsormpatsidis *et al.,* 2011). In the first experimental year, H only increased the SS/TA of tertiary strawberry fruits (Figure 1.C in **IV**). According to literature, the differences are more pronounced in mineral fertilizing experiments, as additional NPK fertilization in the first growth year was shown to reduce the SSC and SS/TA ratio in strawberry fruits changing the flavour to more acidic (Moor *et al.,* 2009). On the other hand, SSC also depends on weather conditions (Leskinen *et al.,* 2002). In our experiment, SSC was most likely influenced by warmer weather conditions with up to 4.1 °C higher temperatures in July 2010 at the time when the tertiary fruits were picked (Table 4). The influence of H was expected to appear during the next experimental years along with increasing plant nutrient uptake.

In the next experimental years, the influence of defoliation on strawberry biochemical characteristics was significantly related to plant growth (Table 5 in **V**). TAC had a positive correlation with the number of leaves, which shows that higher number of leaves led to the increased titratable acids. This is in an agreement with Correia *et al.* (2011), who reported that TAC was positively related to the fresh weight of above-ground biomass and number of leaves in some strawberry cultivars. On the other hand, SS/TA showed a positive correlation with SPAD; consequently higher chlorophyll content in leaves affected fruit taste, enhancing strawberry sweetness. In general, FD treatment had a significant influence on strawberry fruit biochemical composition, but the treatment-induced variations were yearly different in direction (Table 4 in **V**). Earlier findings have declared that the effect of cultivation techniques on fruit quality parameters depend significantly on environmental conditions (Moor *et al.*, 2004; Crespo *et al.*, 2010). Our results refer also to multiple reasons – it could have been due to differences in weather conditions, but also to the age of strawberry plants. In 2013, the temperatures in June were up to 4.5 °C warmer, and rainfall significantly lower in June and July during flowering and fruiting than previous year and long-term mean (Table 4). According to literature, plants need to adapt to changing environmental conditions for survival in terms of increasing both biotic and abiotic stressors (Van den Ende and El-Esawe, 2014). Therefore, in addition to the effect of FD, seasonal temperature fluctuations and variable precipitation rates could also have caused differences in plant response and hence influenced the effect of defoliation.

Moreover, the interaction in combined FD+H treatment became evident, resulting in an increase of up to 41% in SSC in the last experimental year (Table 4 in **V**). In our experiment, application of H probably enhanced the nutrient availability by promoting rooting structure and soil microbial activity as also described by other authors (Trevisan *et al.,* 2010; Calvo *et al*., 2014; Tehranifar and Ameri, 2014; Canellas *et al.,* 2015). Availability of plant nutrients has been reported to be a significant factor for influencing SSC and SS/TA, especially in the case of higher absorption of nitrogen and/or additional fertilization (Hargreaves *et al*., 2008; Dadashpour and Jouki, 2012). On the other hand, fertilization of defoliated plants was found to have no effect on the SSC, and variations in the results were assumed to be due to cultivar and plant age (Moor *et al*., 2004), which also corresponds with our results as it was the last productive year of the plantation and strawberry plants were aged.

Antioxidants

In the first strawberry growth year, H had a positive effect on AAC in secondary fruits in OM (Figure 1.E in **IV**). This is contrary to the results of Moor *et al.* (2004; 2005), who found that fertilization decreased AAC with organic straw mulch. Humic substances may promote plant growth through the induction of carbon and nitrogen metabolism and may have a strong impact on secondary metabolism (Khalid *et al*., 2013; Canellas *et al.,* 2015). However, in our experiment no significant correlations were found between AAC and plant growth parameters (Table 5 in **V**). Moreover, the results of the current experiment show high variations in the AAC in all years under investigation, and differences even between fruit orders. This allows the suggestion that from preharvest factors, light intensity and temperature are more important in determining the final AAC than cultural practices, as also described by other authors (Lee and Kader, 2000; Moor *et al.,* 2005; Tulipani *et al.,* 2011).

H application significantly increased strawberry ACC in the PM in planting year (Figure 1.D in **IV**). H amendments may affect the content of antioxidants significantly, but it depends on several factors such as mulching and fruit order. Anttonen *et al.* (2006) found that strawberry fruit order affected the TPC, ACC and antioxidant activity, which was equally evident in the present experiment. Moor *et al.* (2005) also observed higher ACC in strawberry fruits grown on plastic mulch; moreover, fertilization in combination with straw mulch had a significant negative influence compared to plastic mulch. At the same time, strawberry plants grown in soil with additional fertilization and compost bore fruits with significantly enhanced ACC (Wang and Lin, 2003).

In the next experimental years, the effect of fruit order on strawberry biochemical composition remained significant, as FD+H had a positive influence on the TPC and ACC in all fruit orders (Table 4 in **V**). These findings correspond with Wang and Lin (2003) who reported increased accumulation of TPC in strawberries due to improved plant nutrient uptake. Several authors have indicated the positive effect of H on plant physiology and soil structure due to better nutrient availability (Trevisan *et al.,* 2010; Calvo *et al.*, 2014; Tehranifar and Ameri, 2014; Canellas *et al.,* 2015). Additional treatment with H advanced the defoliated plants' recovery by improving the root system, increasing the weight of strawberry roots and crowns. This in turn increased nutrient uptake and therefore, TPC and ACC in both experimental years also. In addition, interactions between treatments may have occurred due to plant age, as also found by Tõnutare *et al.* (2009) – the content of anthocyanins showed a tendency to increase in three-year-old plantations. Post-harvest FD increased TPC in primary fruits and decreased the content in tertiary fruits in 2013, which was the last year of yielding. Anttonen *et al.* (2006) have also found fruit size-related differences up to 2-fold in accumulation of phenolic compounds and anthocyanins between fruit orders. In the present experiment, the results can be also related to FM and fruit maturation conditions. In 2012, primary fruits were picked three weeks earlier than tertiary fruits, and in 2013 the interval was two weeks.

In correlation analysis, the TPC was found to be lower in the case of higher yield and predictably higher chlorophyll content (SPAD) due to the increased plant nutrient availability and therefore enhanced plant growth (Table 5 in **V**). According to Anttonen *et al.* (2006) the shading effect of vigorous leaves may decrease the ACC. In the present experiment, differences in leaf growth did not affect TPC but a greater number of leaves decreased the ACC significantly. Therefore it can be assumed that in the present experiment, the higher number of leaves affected the ACC negatively, while the higher SPAD had a positive effect.

8. CONCLUSIONS

In the present thesis, the effect of cultivar properties, foliar treatments with glycine-betaine, and different pruning methods and times on hybrid grapevine maturity and quality parameters; as well as the influence of application of humic substances and post-harvest flame-defoliation on strawberry fruit taste-related parameters and antioxidants were investigated and discussed.

Based on the established hypotheses and aims the conclusions are the following:

- Grapevine cultivars had significant effect on the hybrid grapevine fruit composition and quality (**I**). The hypothesis was only partly confirmed as not all the cultivars investigated achieved the sufficient maturity requirements in Estonian climatic conditions. Most of the cultivars achieved the recommended soluble solids content according to table grape standard (16 °Brix); only 'Hasanski Sladki' reached the optimum necessary for winemaking (20 °Brix). The content of titratable acids was above the recommended level in all cultivars, the lowest contents were determined from dark grapes in 'Kuzminski Sinii', and from white grapes in 'Severnõi Rannii'. High phenolic quality was determined in dark grapes of 'Rondo' and 'Kuzminski Sinii'.

- Foliar treatments with glycine-betaine had a significant effect on the quality parameters of interspecific hybrid cultivar 'Rondo', while earlier-fruiting 'Hasanski Sladki' showed high results for fruit technological maturity despite the treatments (**II**). Spraying with glycine-betaine decreased the titratable acidity in grapes of both cultivars, but the levels still remained high. The content of anthocyanins was also significantly affected by glycine-betaine and was increased in 'Rondo' grapes. It can be concluded that the hypothesis was proved only in case of one experimental cultivar.

- Autumn pruning affected positively the maturity of 'Hasanski Sladki' grapes, thereby increasing the content of total phenolics up to 30% compared to spring pruning (**III**). Spring cane pruning increased the content of soluble solids, total phenolics and anthocyanins, while the maturity parameters did not reach recommended level in case of spring spur pruning. The hypothesis was confirmed that spring pruning caused changes in bud hierarcy which led to the delay in grapevine growth and therefore ripening period was shortened, thus affecting significantly grape maturity. Though cane pruning decreased the titratable acidity towards the recommended level, still the contents were high in both treatments and pruning times. Spring pruning due to its impact on bud burst could be recommended in areas where late spring frosts occur.

- Application of humic substances to strawberry plants increased the soluble solids content of fruits grown on organic mulch in all fruit orders, while the effect on titratable acidity was revealed only in primary fruits on organic and in secondary fruits on polyethylene mulch (**IV**). Additional amendments of humic substances increased the total phenolics content of strawberries on both mulches, and affected positively ascorbic acid content in fruits grown on organic mulch. Based on the results obtained, it can be concluded that the hypothesis found confirmation – post-planting application of humic substances affected significantly the taste-related compounds and antioxidants in strawberries.

- Strawberry anthocyanins were reduced by flame-defoliation in all fruit orders in the succeeding year after the treatment, while total phenolics' were increased in primary, and decreased in tertiary fruits in the second experimental year (**V**). Flame-defoliation in combination with humic substances had a positive influence on the total phenolic and anthocyanin contents in all fruit orders. This confirms the hypothesis that flaming machine designed for vegetable weed management is suitable also for directed flaming in strawberry plantations. Flame-defoliation had significant effect on plant growth by decreasing the number of leaves, and root and crown mass, which had impact on strawberry fruit composition according to fruit order. On the basis of the results obtained, the post-harvest flame-defoliation could be recommended for use in organic strawberry plantations for a single treatment in the second growth year.

Further study:

Regarding viticultural technologies, the results presented in the current thesis are the first science-based investigations in open field conditions. It is essential to work on the list of cultivars that are suitable for table grape cultivation and for winemaking as well. On the assumption of cultivar properties, appropriate cultivation technologies should be developed and recommendations for Estonian grape growers should be made.

As different plant species and even different cultivars of a species have diverse genetics, the need to investigate the endogenous production and exogenous application of biostimulant that increase plant stress tolerance and delay grapevine budbreak is of great importance. Due to high variations in the results of foliar applications of glycine-betaine, the studies need to be continued in order to find out whether the foliar treatments may be required for older vines.

Future studies are needed to explore ways of achieving optimum technological maturity and phenolic quality parameters with different cultivation technologies.

Different technologies are developed for organic strawberry cultivation, however these technologies affect plant growth according to cultivar properties and therefore, fruit biochemical composition and healthiness are also influenced. Further experiments may be required to investigate the influence of flaming on different early-ripening strawberry cultivars and their quality parameters.

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SUMMARY IN ESTONIAN

KASVATUSTEHNOLOOGILISTE VÕTETE MÕJU VIINAPUU (*Vitis* sp.) JA AEDMAASIKA (*Fragaria ×ananassa* Duch.) VILJADE KVALITEEDILE

Sissejuhatus

Nõudlus kodumaiste aiasaaduste järele aina suureneb ning järjest rohkem pannakse rõhku puuviljade ja marjade sisemisele kvaliteedile. Seoses pidevalt laieneva sortimendiga uuenevad vastavalt ka kasvatustehnoloogilised võtted ja muutuvad teadusuuringute suunad. Viinapuid haarava maa-ala suurus ei ole täpselt teada, kuid tegemist on tootmises uue ja perspektiivse kultuuriga. 2016. aastal asutasid tootjad Eesti Viinamarjakasvatajate ja Veinivalmistajate Liidu. Eestis kasvatatakse erinevaid külmakindlaid viinapuu sorte, millest 14 on ka Eesti Aiandusliidu poolt kodu- või äriaeda soovitatud sortide nimekirjas. Viinapuu liikide vahelised hübriidid (*V. vinifera* ristatud liikidega *V. labrusca*, *V. riparia*, *V. rupestris*, *V. lincecumii*, *V. amurensis*) on mitme teadusuuringu põhjal näidanud erinevat külma- ja talvekindlust (Lisek, 2007; Lisek, 2010; Karvonen, 2014). Uuritud on ka lehekaudsete bioloogiliste preparaatide mõju taimede abiootilise stressi vähendamise eesmärgil (Mickelbart jt, 2006; Ashraf ja Foolad, 2007; Mahdi jt, 2014; Kurepin jt, 2015), võrakujunduse võimalusi (Andersen ja Simms, 1991) ja selle kaudu saagi (Martin ja Dunn, 2000; Palliotti jt, 2014) ning viinamarjade biokeemiliste ühendite sisalduse mõjutamist (Nan jt, 2013; Palliotti jt, 2014). Viinamarjade küpsus mõjutab eelkõige lauamarjade, aga ka viinamarjadest valmistatavate toodete (vein, mahl jne) kvaliteeti. Eristatakse tehnoloogilist küpsust (mahla kuivaine, orgaanilised ehk tiitritavad happed ja pH) ja fenoolset küpsust (üldfenoolid ja antotsüaanid). Tehnoloogilise küpsuse hindamine toimub soovitatavate normide alusel, millest mahla kuivaine peab lauaviinamarjades olema vähemalt 16 ºBrix ja veini valmistamiseks vähemalt 20 ºBrix (van Schalkwyk ja Archer, 2000; Codex stan, 2011). pH ja happed mõjutavad marjamahla/veini säilivust ja mikrobioloogilist stabiilsust (van Schalkwyk ja Archer, 2000; Mira de Orduña, 2010). Fenoolsete ühendite sisalduse järgi on võimalik hinnata viinamarjakestade küpsust seoses värvuse ja aroomiga (Ferrer-Gallego jt, 2012; Nogales-Bueno jt, 2014; Palliotti jt, 2014). Samas mõjutavad kõiki eespool nimetatud küpsusparameetreid sordi omadused ja kasvatustehnoloogiad, mis põhjustavad olulisi erinevusi marjade biokeemiliste ühendite sisalduses. Eelneva põhjal püstitati hüpotees, et viinapuu liikide vahelised hübriidsordid saavutavad Eesti kliima tingimustes soovitud küpsuse (**I**). Teisalt võib viinapuulehtede pritsimine glütsiini-betaiini lahusega suurendada taimede stressitaluvust, mõjutades sellega omakorda viljade kvaliteeti (**II**). Järelikult peaks nimetatud preparaadiga pritsimine suurendama hübriidsortide stressitaluvust kevadel ja soodustama seeläbi saagi valmimist. Taimede vastupidavust kevadistele öökülmadele võivad mõjutada ka erinevad võralõikusviisid ja lõikamise aeg (**III**). Kuigi viinamarjakasvatus muutub Eestis üha populaarsemaks, on teaduspõhist infot viinapuu erinevate kasvatustehnoloogiate, sortide omaduste ja viljade kvaliteedi parameetrite kohta siiski piiratud koguses.

Marjakultuuridest on Eestis enam levinud aedmaasikas, mille viljade kvaliteeti hinnatakse maitsega seotud parameetrite (mahla kuivaine, orgaanilised happed ja nende suhe) alusel. Aedmaasikad sisaldavad märkimisväärses koguses askorbiinhapet ehk vitamiin C ja fenoolseid ühendeid (sh antotsüaane), mis on tuntud oma antioksüdatiivsete omaduste poolest (Määttä-Riihinen jt, 2004; Fernandes jt*,* 2012). Kirjanduse andmetel sõltub maasikate sisemine kvaliteet mitmest saagikoristuseelsest ja -järgsest tegurist (Tulipani jt*,* 2011). Samas on väidetud, et kasvatustehnoloogiate mõju bioaktiivsete ühendite sisaldusele on veel ebaselge (Crecente-Campo jt*,* 2012). Maasikataimede lehtede hävitamist põhumultši põletamisel on uuritud eesmärgiga selgitada välja selle mõju nii talvekindlusele, kahjurite levikule kui ka saagi- ja viljade kvaliteediparameetritele (Karp, 2001; Moor jt*,* 2004). Kuid kõik aedmaasika sordid ei sobi selleks. Defoliatsioon ehk lehtede eemaldamine sobib sortidele, mis on tugeva kasvuga ja mille viljad valmivad vara. Saagikoristusjärgne defoliatsioon võib kahjustada juurekaela, vähendades uute lehtede ja õisikute teket ning mõjutades seeläbi viljade biokeemiliste ühendite sisaldust (**V**). Köögiviljapõldude umbrohutõrjeks välja töötatud propaaniga töötav leegitaja võib sobida ka aedmaasikataimede lehtede põletamiseks, sest masina reguleeritavaid põleteid on võimalik suunata üksnes lehtedele, mis minimeerib mõju ülejäänud taimedele. Lehtede põletamisega eemaldame aga oluliselt loomulikul viisil taimejäänuste lagunemisega mulda sattuvate toiteelementide hulka. Maheviljeluses on erinevate biopreparaatidega võimalik taimede toiteelementide omastamist parandada. Üks võimalus on kasutada humiinaineid sisaldavaid preparaate, mis mõjutavad mulla struktuursust ja soodustavad seeläbi toiteelementide omastamist ning taimes toimuvaid füsioloogilisi protsesse (Trevisan jt*,* 2010; Calvo jt, 2014; Tehranifar ja Ameri, 2014). Eelneva põhjal püstitati hüpotees, et pärast leegitamismasinaga lehtede hävitamist soodustab humiinaineid sisaldava lahusega kastmine taimede taastumist (**IV**). Kuigi biopreparaatidega on läbi viidud erinevaid katseid, ei ole lehtede leegitamise ja humiinpreparaatide koosmõju maasikate maitseparameetritele ega tervistavate ühendite sisaldusele uuritud.

Doktoritöö üldiseks eesmärgiks oli selgitada välja mahetehnoloogiliste võtete mõju kahe erineva aiakultuuri viljade kvaliteedile, milleks valiti üks puittaim – viinapuu, ja teine rohttaimede esindaja – aedmaasikas. Käesoleva doktoritöö eesmärgiks oli selgitada välja:

- sordiomaduste mõju viinapuu hübriidsortide viljade kvaliteedile (**I**);
- glütsiini-betaiini lahusega pritsimise mõju sortide ’Hasanski Sladki’ ja ’Rondo’ saagi küpsusparameetritele (**II**);
- viinapuu võralõikusviisi ja -aja mõju sordi ’Hasanski Sladki’ saagi küpsusparameetritele (**III**);
- humiinhappeid sisaldava preparaadiga kastmise mõju aedmaasika sordi ’Darselect’ viljade biokeemiliste ühendite sisaldusele istutusjärgsel aastal (**IV**);
- saagikoristusjärgse defoliatsiooni mõju aedmaasika sordi ’Darselect’ viljade kvaliteedile (**V**).

Katsematerjal ja metoodika

Doktoritöös on kasutatud ajavahemikul 2009–2013 Eesti Maaülikooli Rõhu katsejaama ja Mahekeskuse aia katseistandikest kogutud andmeid (tabel 5). Sordiomaduste mõju viinapuu liikidevaheliste hübriidsortide marjade küpsemisele uuriti tumedaviljalistel sortidel ’Hasanski Sladki’, ’Rondo’, ’Zilga’, ’Kuzminski Sinii’ aastatel 2011–2012 ja heledaviljalistel sortidel ’Jubilei Novgoroda’, ’Korinka Russkaja’, ’Severnõi Rannii’ ning ’Supaga’ 2012. aastal (**I**). Sordid valiti katsesse lähtuvalt Eesti kliimas kasvatamiseks soovitatud viinapuu sortide nimekirjast ja asjaolust, et need sordid on juba taimekasvatajate seas levinud.

Tabel 5. Ülevaade doktoritöö aluseks olnud katsetest

Artikkel	Kultuur	Uuritud mõjutegur	Mõõtmised ja analüüsid	Katseaastad	Katseaedade asukoht
I	Viinapuu	Sort	pH, SSC, TAC, SS/TA, TPC	2011–2012	EMÜ Rõhu katseaed, Tartumaa
II	Viinapuu	Glütsiin-betaiin, PK-vedelväetis	pH, SSC, TAC, SS/ TA, MI, ACC, TPC	2009	EMÜ Rõhu katseaed, Tartumaa
III	Viinapuu	Võralõikusviisid ja -aeg	SPAD, FM, BW, pH, SSC, TAC, SS/ TA, MI, ACC, TPC	2011–2012	EMÜ Rõhu katseaed, Tartumaa
IV	Maasikas	Humiinhappeid sisaldav preparaat	SPAD, FM, SSC, TAC, SS/TA, AAC, ACC, TPC	2010	EMÜ Mahekeskus, Tartumaa
V	Maasikas	Defoliatsioon ja humiinhappeid sisaldav preparaat	SPAD, FM, SSC, TAC, SS/TA, AAC, ACC, TPC	2010–2013	EMÜ Mahekeskus, Tartumaa

Viinapuulehtede pritsimise mõju loodusliku taimekasvuregulaatori glütsiini-betaiini, PK-vedelväetise ja nende kahe preparaadi kooskasutamisel uuriti sortide 'Hasanski Sladki' ning 'Rondo' saagi kvaliteedile (**II**). Katsevariantideks olid (i) kontrollvariant, (ii) lehtede pritsimine PK-vedelväetisega 2010. aasta augusti alguses tootja soovitatud koguses (20 g väetist 10 l vee kohta), (iii) glütsiini-betaiiniga pritsimine juuni alguses viinapuu kolme lehe faasis ja teist korda augusti alguses, (iv) glütsiini-betaiini ja PK-väetisega pritsimine augustis.

Viinapuu võralõikusviiside ja lõikusaja mõju viinamarjade küpsemisele ning saagi kvaliteedile katsetati sordil 'Hasanski Sladki' (**III**). Nii pikk kui ka lühike lõikus teostati nii sügisel (oktoobris) kui ka kevadel (mais) viinapuu kahe lehe faasis (2011–2012).

Katseid varajase aedmaasika sordiga 'Darselect' alustati 2010. aastal. Frigotaimed istutati ühte ritta reavahedega 50 cm (**IV**, **V**). Katses oli kaks multši – õunapuu hakkepuit ja must kilemultš. Kõik variandid olid kolmes korduses ja igas korduses 12 maasikataime. Aedmaasikataimi kasteti humiinaineid sisaldava preparaadiga istutamise aastal. Kastmiseks kasutatud preparaat sisaldas 15% humiinekstrakte, 12% humiinhappeid ja 3% fulvohappeid, mis on saadud pruunsüsi oksüdatsioonil tekkinud poolvedelast leonardiidist. Aedmaasika lehed eemaldati leegitamisega, kasutades köögiviljaaedades umbrohutõrje tegemiseks välja töötatud Soome ettevõte Elomestari masinat (**V**). Propaaniga töötaval masinal on kaks 20 cm laiust põletit koos leegi suunamise kontrolleritega, mis tagavad soovitud leegitamise suuna ja asendi. Sealjuures uuriti leegitamise ja humiinaineid sisaldava lahusega kastmise mõju taimede kasvule ja viljade kvaliteedile.

Teostatud mõõtmised ja analüüsid

Marjamahla kuivaine (°Brix) määrati maasikate puhul 10 külmutatud ja viinamarjade puhul 30 värske marja keskmisena kolmes korduses portatiivse refraktomeetriga (Atago Pocket Refractometer Pal-1, Tokyo, Jaapan) (**I-V**). Ülejäänud biokeemiliste parameetrite analüüsiks kasutati temperatuuril –20 °C sügavkülmutatud marjaproove. Aedmaasikavilju analüüsiti vastavate järkude (esimene, teine, kolmas) kaupa kobaras. Orgaanilised happed määrati tiitrimise teel 0,1 M NaOH lahusega (bürett Mettler Toledo DL 50 Randolino), kasutades tiitrimise lõpp-punkti indikaatorina tümoolsinist (Chone jt, 2001). Viinamarjade tiitritavate hapete sisaldus arvutati viinhappele g 100 g^{-1} (**I-III**) ja aedmaasika viljade puhul sidrunhappele g 100 g^{-1} (**IV**, **V**) värske viljamassi kohta. Mahla kuivaine ja hapete suhe arvutati eelnimetatud näitajate põhjal ja väljendati suhtarvuna. Viinamarjamahla pH mõõdeti pH-meetriga (HD 2156.1, Delta OHM) (**I-III**). Viinamarjade küpsusindeks arvutati Coombe'i jt (1980) välja toodud valemi järgi: $MI = °Brix \times pH^2$.

Maasika viljade askorbiinhappesisaldus määrati jodomeetriliselt. Kasutati Tillmansi meetodit väljendatuna mg 100 g^{-1} värske viljamassi kohta (**IV**, **V**). Üldfenoolide sisaldus määrati homogeniseeritud viinamarjakestadest ja maasikapüreest spektrofotomeetriliselt lainepikkusel 765 nm (UVmini-1240 Shimadzu, Kyoto, Jaapan) Folin-Ciocalteau meetodil (Slinkard ja Singleton, 1977) väljendatuna gallushappe sisaldusena mg 100 g^{-1} värske viljamassi kohta. Antotsüaanide sisaldus määrati pH-diferentsiaalmeetodil tumedate viinamarjade kestast (**II**, **III**) (Cheng ja Breen, 1991) ja kogu maasika viljadest. Proovide neelduvust mõõdeti spektrofotomeetriliselt lainepikkustel 510 ja 700 nm (UVmini-1240 Shimadzu, Kyoto, Jaapan). Antotsüaanide kogusisaldus väljendus viinamarja puhul tsüanidiin-3-glükosiidi sisaldusena mg 100 g^{-1} ja maasika puhul pelargonidiin-3-glükosiidi sisaldusena värske viljamassi kohta.

Statistilisteks analüüsideks kasutati ühe- ja kahefaktorilist dispersioonanalüüsi. Taime kasvu ja saagi kvaliteediparameetrite vaheliste seoste hindamiseks arvutati Pearsoni korrelatsioonikoefitsient (*r*).

Tulemused

Viinapuu katsetulemustest selgus, et enamik katses olnud sortidest saavutas lauaviinamarjade mahla soovitatava kuivainesisalduse (16 °Brix) (**I**). Sort 'Hasanski Sladki' saavutas aga ka veinitegemiseks vajaliku küpsuse (20 °Brix). Happesisaldus oli kõikide sortide viljades liiga kõrge (katses 0,8–1,6 g 100 g^{-1}; soovitatav sisaldus 0,6–0,7 g 100 g^{-1}). Fenoolne kvaliteet oli kõrgem tumedaviljaliste sortide 'Rondo' ja 'Kuzminzki Sinii' viinamarjadel. Sordi mõju kohta püstitatud hüpotees leidis osaliselt kinnitust – kõik hübriidsordid ei saavutanud soovitatavat küpsust.

Erinevused nii tehnoloogilise kui ka fenoolse küpsuse osas tulenesid sordi omadustest. 'Hasanski Sladki' saagi küpsusparameetrid saavutasid soovitatava taseme sõltumata sellest, kas taimede lehti pritsiti või mitte (**II**). Glütsiini-betaiiniga töötlemine vähendas mõlema katsesordi happesisaldust (kuni 1,2 g 100 g^{-1}-ni), kuid tulemused ületasid siiski soovitatava vahemiku. Sordi 'Rondo' antotsüaanide sisaldus glütsiini-betaiini lahusega pritsimisel aga suurenes. Lähtuvalt tulemustest võib öelda, et hüpotees leidis kinnitust ja glütsiini-betaiiniga pritsimine mõjutab viljade kvaliteeti, kuid mitte kõigi kvaliteediparameetrite osas.

Sügisene lõikusaeg mõjutas positiivselt viinamarjade küpsemist, sealhulgas suurenes enim polüfenoolide sisaldus (**III**). Kevadine pikk lõikusviis avaldas positiivset mõju viinamarjade mahla kuivaine, üldfenoolide ja antotsüaanide sisaldusele. Kevadise lühikese lõikuse puhul jäid aga küpsusparameetrid soovitatavast vahemikust madalamaks. Kevadine lõikusaeg mõjutas viinapuu pungade puhkemist, mille puhul jäi taimede kasvu algus hilisemaks, mis omakorda vähendas saagi kvaliteeti. Kuigi pikk lõikusviis vähendas viinamarjade happesisaldust, oli see olenemata lõikusajast siiski liiga kõrge võrreldes soovitatava sisaldusega. Järelikult leidis püstitatud hüpoteesi kinnitust ning erinevad võralõikusviisid ja aeg mõjutavad oluliselt viinapuu viljade kvaliteeti. Kevadist lõikusaega, mis lükkab viinapuu pungade puhkemist edasi, võib soovitada piirkondades, kus esineb hiliseid öökülmasid.

Aedmaasikataimede istutusjärgne kastmine humiinaineid sisaldava lahusega suurendas viljamahla kuivainesisaldust orgaanilise multši puhul kõigis vilja järkudes, samas aga mõju tiitritavate hapete sisaldusele avaldus vaid orgaanilisel multšil esimese ja kilemultšil teise järgu viljadele (**IV**). Kastmine humiinaineid sisaldava lahusega mõjutas oluliselt ka viljade antioksüdantsete ühendite sisaldust, suurendades askorbiinhappe ja fenoolsete ühendite sisaldust mõlema multši puhul. Tulemustest lähtuvalt võib öelda, et hüpotees leidis kinnitust, sest aedmaasikataimede istutusjärgne humiinainetega väetamine mõjutas oluliselt viljade biokeemilist koostist.

Aedmaasika antotsüaanide sisaldus vähenes leegitamisele järgneval katseaastal kõikides viljajärkudes, samas kui üldfenoolide sisaldus suurenes esimese ja vähenes kolmanda järgu viljades (**V**). Leegitamisele järgnenud humiinpreparaadi lahusega kastmine mõjutas positiivselt üldfenoolide ja antotsüaanide sisaldust kogu saagis. Leegitamine mõjutas oluliselt taimede kasvu, vähendades lehtede arvu ja juurte massi, mis omakorda mõjutas maasikate biokeemiliste ühendite sisaldust sõltuvalt vilja järgust. Järelikult püstitatud hüpotees leidis kinnitust – propaaniga töötav leegitaja sobib aedmaasikalehtede lokaalseks hävitamiseks ja saagikoristusjärgne defoliatsioon mõjutab oluliselt viljade kvaliteeti. Tuginedes saadud tulemustele, võib soovitada lehtede leegitamist aedmaasika istandikes ainult teisel kasvuaastal. Kahel järjestikusel aastal mõjutab leegitamine taimede kasvu ja saagi kvaliteeti negatiivselt.

Kokkuvõtteks võib öelda, et kasvatustehnoloogiliste võtete rakendamisega bioaktiivsete ühendite sisalduse mõjutamise eesmärgil on võimalik tõsta kodumaiste puuviljade ja marjade kvaliteeti.

Edasist uurimist vajavad teemad

Käesolevas doktoritöös kajastatud tulemused on esimesed seoses viinapuude kasvatustehnoloogiaid puudutavate katsetega Eestis avamaa tingimustes. Edasistes uuringutes on vajalik katsetada pikemat aega toimivate lehtede kaudu lisatavate biostimulantide mõju erineva vanusega viinapuude stressitaluvusele. Teisalt nõuavad lai viinamarja sortide valik ja üha lisanduvad uued sordid täpsemaid ning pikaajalisemaid uuringuid nende sobivuse kohta jaheda kliimaga piirkondadesse. Selleks on vaja välja töötada soovitatavate sortide nimekiri ja nende kasvatusjuhised nii laua- kui ka veiniviinamarjade kasvatamiseks.

Maasikakasvatuses laieneb sortiment pidevalt uute saagikamate sortidega ja seetõttu on oluline jätkata katseid leegitamise mõju uurimiseks teiste varavalmivate sortide puhul. Keskkonnasäästlik taimekasvatus täieneb pidevalt erinevate biopreparaatidega, kuid nende sobivus ja efektiivsus aedmaasikate kasvule ja saagile Eesti kliima tingimustes ei ole teada.

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I

ORIGINAL PUBLICATIONS

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Effect of Genotype on Grape Quality Parameters in Cool Climate Conditions

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Abstract

Traditional vine-producing regions grow *Vitis vinifera* L. cultivars, which are sensitive to downy mildew (*Plasmopara viticola*). Large amounts of pesticide residues found in imported grapes has raised the question if it would be possible in the Nordic countries – as non-traditional vine growing countries – to grow grapes without chemicals, and thereby to get a healthier yield. Nowadays most common cultivars grown in Estonia are obtained by crossing *V. vinifera*, *V. amurensis* and *V. labrusca*. The aim of the present experiment was to find out the effect of genotype on grape quality in cool climate conditions. The research was conducted with red (‘Hasanski Sladki’, ‘Rondo’, ‘Zilga’, ‘Kuzminski Sinii’) and white (‘Jubilei Novgoroda’, ‘Korinka Russkaja’, ‘Severnõi Rannii’, ‘Supaga’) grape cultivars. The plantation was established with double trunk training at the experimental vineyard (northern latitude 57-59°) of the Estonian University of Life Sciences. The experiment was carried out with red grapes in 2011 and 2012, and with white grapes in 2012. The results of the study indicated, that fruits of all cultivars achieved the minimum content of soluble solids required for table grapes by mid-September. The highest soluble solids content among red grape cultivars was found in ‘Hasanski Sladki’ (18.9°Brix) and among white cultivars in ‘Jubilei Novgoroda’ (16.5°Brix) and in Severnõi Rannii (17°Brix). The sweetest red grape was ‘Kuzminski Sinii’ (Brix/acids ratio 19) and the white grape was ‘Severnõi Rannii’ (22). Among red grapes ‘Kuzminski Sinii’ and among white ones ‘Supaga’ had the highest total phenolic content (540 and 168 mg 100 g^{-1}, respectively).

INTRODUCTION

The number of grapevine cultivars is large, but for successful growing the list is quite limited. The first restrictive factor is the cultivar’s origin, the second is the growing area climatic conditions and the third is the grapes’ purpose of use. For commercial purposes, *Vitis vinifera* and the hybrids between *V. vinifera* and *V. labrusca* are mainly cultivated (Lisek, 2010). According to the heavily regulated EU wine market, it is forbidden to use ‘hybrid’ grapes for “Quality wine” production (Meloni and Swinnen, 2012). This statement excludes wine production in northern countries, so gives a possibility to produce table wine and table grapes from hybrid vines. Nowadays the most common cultivars grown in Estonia were obtained by crossing *V. vinifera*, *V. amurensis* and *V. labrusca*. The hybrids have important advantages, like disease resistance in high rainfall conditions, tolerance to harsh winter conditions, and a higher quality of grapes in the cool climate (Gustafsson and Mårtensson, 2005). Traditional grape-producing regions grow *V. vinifera* cultivars, which are sensitive to downy mildew (*Plasmopara viticola*). Large amounts of pesticide residues found in imported grapes has raised the question if it would be possible in the Nordic countries – as non-traditional vine growing countries – to grow grapes without chemicals, and thereby to get a healthier yield. The growers are facing the question, and want to know which cultivars are suitable for cool climate and give high quality yield. The berry biochemical composition, as well as quality, depends

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on the area of cultivation and the climatic conditions (Martin and Dunn, 2000). The most common quality parameters measured in grapes are soluble solids, titratable acids, Brix/acids ratio, pH and total phenolic content, but a single indicator is not enough to ensure good quality or maturity. In northern conditions, differences related to the temperature and precipitation variability can significantly affect grape quality. The aim of the present experiment was to determine the effect of genotype on grape quality in cool climate conditions.

MATERIALS AND METHODS

The vineyard was established in 2007 at the Estonian University of Life Sciences experimental station with interspecific red hybrid grape cultivars 'Hasanski Sladki', 'Rondo', 'Zilga', 'Kuzminski Sinii' and with white grape cultivars 'Jubilei Novgoroda', 'Korinka Russkaja', 'Severnõi Rannii' and 'Supaga'. The cultivars 'Hasanski Sladki', 'Kuzminski Sinii', 'Jubilei Novgoroda', 'Korinka Russkaja', 'Severnõi Rannii' were bred in Russia; the cultivars 'Zilga' and 'Supaga' were from Latvia, and 'Rondo' was from Germany. The experiment was carried out with red grapes in 2011 and 2012, and with white grapes in 2012. The plantation was established on 2×2 m spacings, using in vitro propagated own-rooted plants, trained in a low double trunk trellis. The experimental design was a randomized block with 4 replicates. The experimental area soil was sandy loam with pH_{KCl} 5.8, and a 4.4% humus content. The content of P, K, Ca and Mg was sufficient in the soil. No fertilizers, irrigation or winter cover were used.

The period of active plant growth temperatures started (both years, 2011 and 2012) at the beginning of May and ended at the beginning of October (respectively October 8 and 6). The sum of active temperatures (>10°C) was 2498°C in 2011 and 1967°C in 2012. The length of active plant growth period was 155 days in 2011 and 150 days in 2012. The summer of 2011 was warmer compared to 2012 and the long term (1971-2000) mean. The precipitation level was lower than the long term mean in 2011 and higher in 2012.

Fruit quality characteristics like titratable acids (mg 100 g^{-1}), Brix/acids ratio, pH, total phenolics (mg 100 g^{-1}) content were measured from frozen grapes. The soluble solids (SS, °Brix) content was measured from fresh berries by refractometer (Atago Pocket Refractometer Pal-1). Titratable acids (TA) content was determined by the titration method with aqueous 0.1 M NaOH solution. Soluble solids and titratable acids (SS:TA) ratio was calculated. pH was evaluated from grape juice with a pH/conductivity-meter. Total phenolics (TP) content was determined by spectrophotometer (at 765 nm) from grape skin with the Folin-Ciocalteau phenol reagent method.

The data was tested by a one-way analysis of variance (ANOVA). To evaluate significant influence, the least significant difference ($LSD_{0.05}$) was calculated. In the figures, the mean values to be compared are followed by the same letter if they are not significantly different at $P<0.05$.

RESULTS AND DISCUSSION

SS content ranged from 12.7 to 19.9°Brix in red grapes and from 15.1 to 17.0°Brix in white grapes (Fig. 1). The highest SS content among red grapes was in 'Hasanski Sladki' in both years, and the lowest in 'Rondo' and 'Zilga'. Among white grapes the highest SS content were in 'Jubilei Novgoroda' and 'Severnõi Rannii'. Low temperature and water stress have been reported to prolong the ripening period and decrease fruit SS content (Nicholas et al., 2011; Zsofia et al., 2011). The SS content was lower among red grapes in 2012, when the summer temperatures were lower and precipitation level higher, as compared with 2011. Sugar concentration often depends on the origin of the species used for breeding. Liu et al. (2006) found that among 98 grape cultivars, *V. labrusca* and *V. vinifera* hybrids were in the top 10 having the highest sugar content. In this study only hybrid cultivars were used. The average SS content in the German red cultivar 'Rondo' was 19.7 in Poland (Lisek, 2010). In our experiment, SS content was lower (the two years average in this study was 14.8°Brix). This confirmed that berry SS content was related to climate and in cooler conditions it was lower. For the purpose of use, hybrid cultivars may contain more sugars. The recommended °Brix value for wine grapes (from 20 to 23°Brix; Schalkwyk and Archer, 2000) and table grapes (at least 16°Brix; Codex stan., 2011) is different. In this experiment the red cultivar 'Hasanski Sladki' was more suitable for wine production and the other cultivars for fresh consumption, based on Brix value.

TA content was different for the two years among red grapes (Fig. 2). In 2011, TA content was significantly higher in 'Zilga', but in 2012 it was in 'Hasanski Sladki' (there was no difference between 'Rondo'). Among white cultivars the content was significantly higher in 'Supaga' and lowest in 'Severnõi Rannii'. TA content in grapes was highly related to climatic conditions (Topalovic and Mikulic-Petkovsek, 2010). In southern Europe the traditional grape growing countries have a quite mild climate, but in northern regions the temperature during ripening is significantly lower. Because of that the TA content in berries was high (the average content 1.2 mg 100 g^{-1}) compared to the suggested optimal value from 0.6 to 0.7 mg 100 g^{-1} for wine grapes (Schalkwyk and Archer, 2000). Also the higher contents were obtained in the cooler year (2012). In China a study indicated that among the same climatic conditions *V. vinifera* grape cultivars have significantly higher total acid content (Liu et al., 2006). Based on the present data, hybrid cultivars could be more acceptable for consumers in the Nordic countries. However, the perception of sweetness is greatly dependent on the sugar-acid ratio and the single value of TA is not sufficient to characterize a cultivar's flavor.

The SS:TA ratio ranged from 8.8 to 22.1 in red and from 10.5 to 21.7 in white grape cultivars (Fig. 3). In red grapes, the ratio was significantly higher in the Russian cultivar 'Kuzminski Sinii' both years, and among white grapes 'Severnõi Rannii' was higher. The white grapes seem to have been sweeter than red but cultivar characteristics and climate play an important role. Consumers mostly prefer grapes with higher sugar-acid ratio (Jayasena and Cameron, 2008). Based on this study, the Russian hybrid cultivars tasted sweeter than the Latvian and German ones.

The grape juice pH ranged from 2.94 to 3.79 in red and from 2.97 to 3.31 in white grape cultivars (Fig. 4). In red grapes, juice pH was significantly higher in 'Kuzminski Sinii' and in white grapes 'Severnõi Rannii' was higher. The pH value is important for the consumers' healthy diet and from the berry juice biological stability standpoint. The pH value stayed in the range of 3.2 to 3.6 in mature grapes (Dokoozlian, 2000), with the upper value equal to that in the present study. A lower pH level in grapes provide less microbial spoilage and organoleptic degradation of wine (de Orduńa, 2010). Since pH is correlated with acid concentration, one could assume that higher TA content leads to higher pH value. This study did not confirm that statement, and pH value was more influenced by climate than cultivar. Schalkwyk and Archer (2000) who have detected correlation between cool climates and grape pH value were of the same opinion. Latvian cultivars had a lower juice pH value in our study.

The grape TP content ranged from 214 to 540 mg 100 g^{-1} in red grapes, and from 102 to 168 mg 100 g^{-1} in white grapes (Fig. 5). In red grapes, the highest content was obtained with 'Rondo' both years, and with 'Kuzminski Sinii' in 2011. Among white grapes, the TA content in 'Supaga' berries was significantly higher. There was no relation between the cultivar's origin and TP content for red grapes, because the German cultivar 'Rondo' and the Russian cultivar 'Kuzminski Sinii' had similarly higher TP content in both years. Important differences among the cultivars were also found by Orak (2007). Additionally, temperature and precipitation influenced TP content. As in this study, others (Rio Segade et al., 2008) have also found that dryer and warmer weather promotes phenolic compounds accumulation.

CONCLUSIONS

The results indicate that grape quality is more influenced by weather than the country of origin of cultivars. According to the sweetness (based on SS/TA ratio), we recommend growing the Russian cultivars 'Kuzminski Sinii' among red and 'Severnõi Rannii' among white grapes in the Nordic countries. From the healthiness point of view, the best were the red German cultivar ‘Rondo’ and the white Latvian cultivar ‘Supaga’.

We conclude that Russian hybrid vine cultivars performed well in a cool climate and showed good grape quality. Future studies are needed to observe different cultivar’s sustainability and change of berry quality in relation to long-term weather patterns.

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Figures

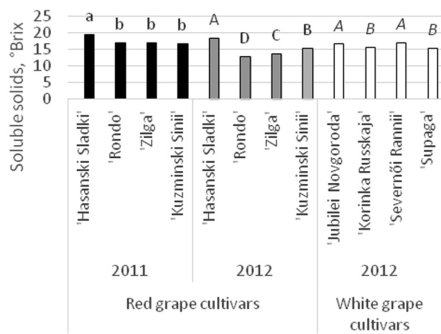

Fig. 1. Soluble solids content of red (2011-2012) and white (2012) interspecific hybrid grape cultivars.

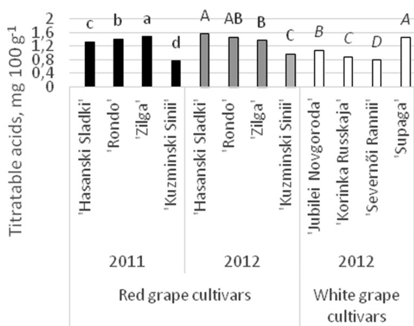

Fig. 2. Titratable acids content of red (2011-2012) and white (2012) interspecific hybrid grape cultivars.

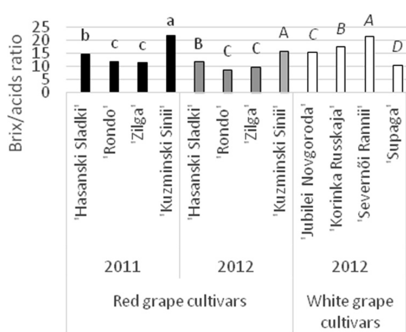

Fig. 3. Soluble solids/titratable acids ratio of red (2011-2012) and white (2012) interspecific hybrid grape cultivars.

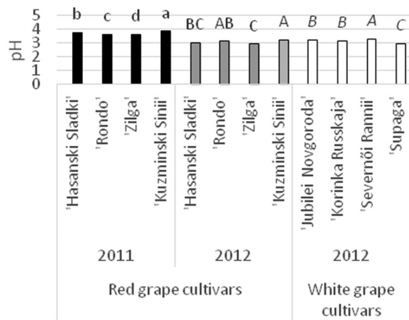

Fig. 4. Berry juice pH value of red (2011-2012) and white (2012) interspecific hybrid grape cultivars.

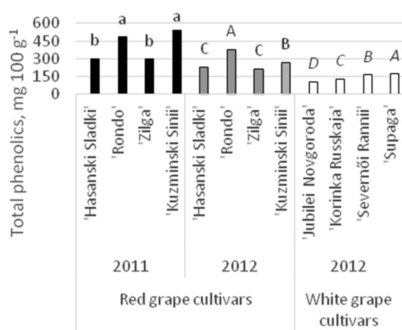

Fig. 5. Total phenolic content of red (2011-2012) and white (2012) interspecific hybrid grape cultivars.

II

Vool, E., **Rätsep, R.**, Karp, K. 2014. Effect of fertilizing on grapevine fruit maturity in Northern conditions. *Acta Horticulturae* 1017: 231-236. Belgium: ISHS.

Effect of Fertilizing on Grapevine Fruit Maturity in Northern Conditions

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Keywords: soluble solids, titratable acids, sugars and acids ration, pH, anthocyanins

Abstract

The aim of the present experiment was to evaluate the influence of foliar treatments (PK fertilizer and glycinebetaine) on interspecific hybrid grape cultivars 'Hasanski Sladki' (HS) and 'Rondo' (R) maturity parameters in northern conditions. The plantation was established in 2007 and, in 2009, the experiment with foliar treatments: PK fertilizer, glycinebetaine, and glycinebetaine plus PK fertilizer was carried out.

The grape soluble solids content ranged from 13.7 to 21.5°Brix, foliar treatment had no influence and results variation was caused by cultivar properties. All foliar treatments increased soluble solids and titratable acids ration on R. The berries pH was lower on HS in all fertilizing treatments. Maturity index (soluble solids × pH^2) depended barely on cultivar and ranged from 116 to 135 in R and from 203 to 209 in HS. Total anthocyanins content was higher in R fruits and the content increased with PK-fertilizing. The study showed that foliar treatment had no influence on grape maturity and the latter was more affected by cultivar properties. Based on the study, cultivar HS is more suitable for wine production in northern conditions, whereas, R needs to be further investigated.

INTRODUCTION

Grapevine growing is not new in northern countries but has becoming popular among farmers in the last years. The yield development is mostly influenced by early autumn and late spring frost in Northland. The air temperature during the grapes ripening has a decisive influence on wine quality (Jackson and Lombard, 1993; Yamane et al., 2006). The selection of grapevine cultivars and growth technology give a possibility to produce healthy fruits in a cold climate zone. In short a summer condition, the choice of cultivar short ripening period is important. At the same time, however, a cool climate affects the quality of wines as well; the best French wines come from the cooler regions of the country (Jones and Davis, 2000). The importance of species genetic and environmental factors on grape and wine quality is also reported by Pereira et al. (2005). According to the Tonietto and Carbonneau (2004) classification, the suitable vine for cooler climate is obtained as a result of interbreeding among species. The most common species for breeding are *Vitis vinifera* (L.), *V. amurensis* (Rupr.), and *V. labrusca* (L.). According to Lisek (2007) *V. labruscana* group has the best cold resistance. The hybrid cultivars of *V. labrusca* and *V. vinifera* are characterised by high sugars, especially sucrose, and low acids content (Liu et al., 2006). In addition, frost resistance depends also on the buds sugars content (Hamman et al., 1996).

Since the synthesis of bioactive compounds in the plant is related to environmental conditions, wine produced in Nordic climate can contain more health-enhancing compounds. However, agro-ecological factor, for instance the choice of cultivars, solar intensity, temperature and soil conditions additionally affect antioxidant anthocyanins content (Yokotsuka et al., 1999; Pomar et al., 2005; Teszlak et al., 2005; Fournand et al., 2006; Yamane et al., 2006; Mulero et al., 2010).

Unfavorable growth conditions cause a stress in plants, resulting in reduced plant vitality, fruit quality and yield. One possibility to enhance plants tolerance to low temperatures is application of glycinebetaine (GB), which is quaternary ammonium compound and acts as an osmoprotectant in the plants by adjusting the osmotic balance inside the plant cells and tissues. Although, on the one hand, it is found, that GB has no effect on physiological parameters of non-stressed plants (Mickelbart et al., 2006). On the other hand, it was found that the vine stress tolerance can significantly increase with plants GB application, resulting in an increase of fruit polyphenols compounds and anthocyanins content (Teszlak et al., 2005).

Based on the previous, a hypothesis may be posed: spraying the vines with GB and PK fertilizer solution increases plants stress tolerance in Nordic conditions, and thus also affects the maturity parameters of the grapes. This study is the first one to find out cultivars suitability for wine production in Nordic conditions in Estonia. The aim of the present experiment was to evaluate the influence of foliar treatments (PK fertilizer and glycinebetaine) on grape interspecific hybrid cultivars 'Hasanski Sladki' and 'Rondo' maturity parameters in northern conditions.

MATERIAL AND METHODS

The study was carried out in experimental outdoor vineyards of the Estonian University of Life Sciences (58°35'N; 26°52'E). In vitro propagated vines were planted in June 2007 with spacing of 2 m between the plants and 2.5 m between the rows and cultivated on their own roots. Vineyards soil pH_{KCl} was 5.8 and humus content was 4.4%. The content of P, K and Mg was high in the soil and Ca content was sufficient. The last night frost was on 24 April in 2009, with a temperature drop below -1.4°C. The average temperature was +5.3°C in April and +14.4°C from June to September. Thereby, June was the coolest month and September – the warmest compared to the many years' average temperature. The first autumn frost was registered on 3 October, when the average daily air temperature was -0.4°C. The sum of active temperatures in 2009 was 2156.

Two red grape hybrid cultivars, such as Russian Primorsky Krai breed 'Hasanski Sladki' (HS; interspecific hybrid of *V. amurensis*, *V. labrusca*, *V. riparia* and *V. vinifera*) and former Czechoslovakia breed 'Rondo' (R; crossing by *V. amurensis* cultivars) were under investigation. The rows were covered with 0.04 mm thick black polyethylene mulch. Between the rows there was sawn grass, which was mown regularly during the vegetation period. A training system, where 2 horizontally curved woody branches were left per plant, each of which had up to 5 fertile canes, was used for the experiment. The plants were cut back in autumn. R hibernated under a textile covering, whereas, HS was without any covering. Every week in July axillary shoots emerging from the leaf axils were removed. In August, the cane top was cut back and basal leaves in the area of the clusters were removed. PK water soluble fertilizer 1-18-38 (in concentration of 20 g of fertilizer per 10 L of water) was used as a foliar treatment. The foliar-applied glycinebetaine as natural product derived from sugar beet molasses was used in concentration of 62.5 g of fertilizer per 10 L of water. In the experiment there were 4 replications of each foliar treatment and each replication was conducted on 9 plants. The treatments were following:

1. control (C);
2. PK fertilizer (PK): applied once at the beginning of August;
3. glycinebetaine (GB): applied twice at the beginning of June (during the third leaf phase) and August;
4. glycinebetaine + PK fertilizer (GB+PK): applied once at the beginning of August.

The frozen fruit from the first harvest of November 2009 were used for biochemical analyses. Soluble solids (SS) content in fruit juice was measured by refractometer (Atago Pocket Refractometer Pal-1). Analyses were made once a week in September and once in early October. Titratable acids (TA) content was determined by titration method with aqueous 0.1 M NaOH solution. The TA content was expressed as tartaric acid mg per 100 g of fresh fruit. Soluble solids and titratable acids ratio (SS:TA) was calculated based on the content of SS and TA. Fruit juice pH was measured with pH-meter. Maturity index was calculated with the following formula: soluble solids content × pH^2. The content of total anthocyanins (TAC) was estimated by a pH-differential method. The pH values of diluted grape extracts were 1.0 and 4.5. Absorbance was measured by Jenway 6300 spectrophotometer at 510 and 700 nm. The total anthocyanins content was calculated in milligrams of cyanidin-3-glucoside equivalent per 100 g of fresh weight.

A one-way analysis of variance was used for maturity parameters. To evaluate significances of differences among treatments, the least significant difference ($LSD_{0.05}$) was calculated. Different letters in the figures marked significant differences.

RESULTS

Grape SS content in HS ranged from 21.0 to 21.5 and in R from 13.7 to 16.0°Brix (Fig. 1A). Fertilizing had no influence on HS but increased R's SS content in PK-treatment. TA content was influenced by the fertilizer; thereby PK and GB treatments decreased the content in HS (Fig. 1B). In R TA content was significantly lower in all treatments compared with control. Grape SS:TA ration varied in HS from 6.1 to 14.6 and in R from 9.7 to 13.1 (Fig. 1C). PK and GB application increased SS:TA ration in HS. In R, the ration was significantly higher in PK treatment. The grape juice pH ranged from 3.09 to 3.15 in HS and from 2.88 to 2.91 in R (Fig. 1D). In HS, juice pH was significantly higher in control variant and in R – in case of GB+PK treatment. Higher maturity index from 202 to 209 was in HS grapes (Fig. 1E). In R, it ranged from 114 to 136. Fertilization significantly influenced only R cultivar increasing its maturity index in case of PK treatment and lowering it in GB+PK treatment. The grape TAC content in HS berries ranged from 84 to 99 and, in R berries – from 160 to 219 mg per 100 g^{-1} (Fig. 1F). Fertilization did not affect HS TAC content. PK treatment significantly increased TAC content of R berries.

DISCUSSION

The eligible SS content of berries used in wine production is °Brix 20 (Schalkwyk and Archer, 2000) and acid content is 0.7 mg 100 g^{-1} (Dishlers, 2003). As it is sugar content that determines the alcohol content of wines, the main problem in northern conditions is particularly low sugar and excessively high acid contents. In the present experiment the same problem revealed in R cultivars. Still, it was found that even in case of low °Brix value it is possible to make wine with good taste properties by combining various cultivars. For example with *Vitis labrusca* type of grapes SS content ranging from 16 to 18 is necessary for ideal combination, and any increase of this value may lower wine quality (Khanizadeh et al., 2008). However, the present experiment showed that, in northern conditions, grape SS content in HS cultivars may be high. Albeit sugar accumulation in the berries starts during the ripening period and depends on the weather conditions, temperature effects on final sugar accumulation are reported to be relatively small (Coombe, 1987). Liu et al. (2006) has found that total soluble sugars are significantly higher in hybrids between *V. labrusca* and *V. vinifera* than that in *V. vinifera* cultivars. Both hybrids were used in the present experiment but SS content varied significantly due to variation in primary cultivars. PK treatment of the leaves only raised RO SS content. We may, thus, hypothesise that difference in the effect is related to cultivar properties. HS is an early ripening cultivar. Therefore, the berries in the clusters are sparse; R clusters are larger with densely growing berries. The latter affects the ripening of the berries. The more sparsely are the berries on the cluster, the better are the light conditions, and nutrients distribution occurs among a smaller number of berries. Similarly, GB application may not have any effect on the SS content due to loamy nutrient-rich soil with no plant stressors. Equally, hybrids may be more resistant to cold springs. Furthermore, in case of R it was confirmed that P fertilization could increase the content of soluble solids (Xuede et al., 2012).

In order to produce quality wine with positive taste and preservation properties, juice pH should be from 3.2 to 3.6 (Dishlers, 2003) and ripening index should range from 200 to 270 (Schalkwyk and Archer, 2000). pH content is in close correlation with titratable acids – long ripening periods characteristic of cool climates may cause organic acid content increase and, therefore, increase of grape pH (Schalkwyk and Archer, 2000). Lower pH level is more beneficial for wine production as, in that case, juice fermentation is cleaner and wine is less liable to microbial spoilage. Since pH linearly correlates with sugar concentration, one of the expected results of the present study was the fact that pH in R was lower compared to HS. Only HS cultivar maturity index remained within the eligible range, which confirms the hypothesis, that early ripening vine cultivars are more suitable for northern conditions. Fertilization influence on grape pH may be caused by potassium accumulation, which is temperature dependent. Potassium levels increase significantly in grape clusters, specifically, during grape maturation, because of potassium redistribution from other above-ground vegetative vine organs. Likewise, insufficient effect of PK and GB treatments may be caused by the fact that GB involving treatment reduces plant stress symptoms and create favourable conditions for plant vegetative growth.

Torchio et al. (2010), has found, that the anthocyanins content increased with the level of soluble solids. This fact was confirmed during the present experiment in case of cultivar R. TAC content increase induced by PK-fertilizer treatment in R, may be related to higher SS and P content of the fertilizer (Xuede et al., 2012). Thus P-rich fertilizer fosters both TAC and SS content increase. Therefore, TA content depended on fruit physiological state and cultivar. Grape anthocyanins content increase is both light and temperature dependent. Light is of decisive importance for anthocyanins content rise, particularly at the beginning of the maturation period. The experiment revealed that anthocyanins content increase linearly correlated with the amount of solar radiation (Cortell et al., 2007). The colour break stage of the cultivars used in the experiment starts at different times and varies in duration. In case of HS, it starts in August when temperature fluctuation is smaller than in September, when R cultivar colour breaks stage begins. Significant temperature fluctuation may be the reason for variation in TAC content. Additionally, numerous environmental factors, cultivar properties, and cultivation technologies influence grape TAC content (Teszlak et al., 2005).

CONCLUSIONS

The study showed that foliar application of glycinebetaine had no influence on grape maturity and the latter was more affected by cultivar properties. Foliar PK treatment increased ‘Rondo’ SS and TAC content as well SS:TA ration. The study showed that ‘Hasanski Sladki’ is more suitable for wine production in northern conditions based on its fruit maturity parameters. ‘Rondo’ needs to be further investigated.

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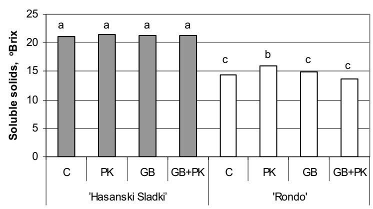
Figures

A



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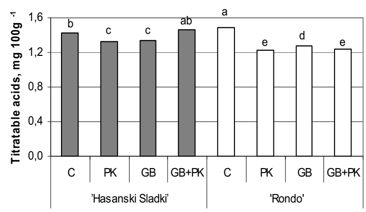

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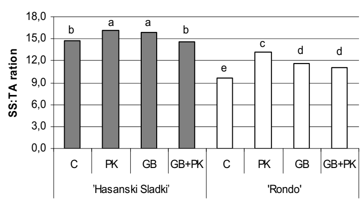

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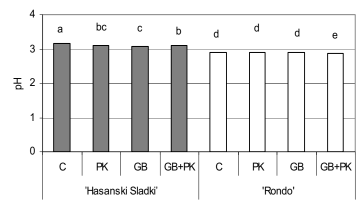

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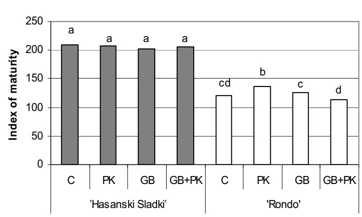

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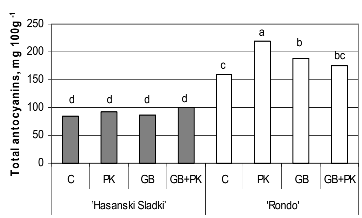

Fig. 1. Effect of fertilizing on grapes 'Hasanski Sladki' and 'Rondo' content of soluble solids (A), titritable acids (B), SS:TA ration (C), fruit pH (D), index of maturity (E) and total anthocyanins (F). Treatments: C – control; PK – PK-fertilizer, GB – glycinebetaine, GB+PK – glycinebetaine + PK fertilizer.

236

III

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EFFECT OF PRUNING TIME AND METHOD ON HYBRID GRAPEVINE (*Vitis* sp.) 'HASANSKI SLADKI' BERRY MATURITY IN A COOL CLIMATE CONDITIONS

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Abstract. Climate and weather conditions are important factors influencing grapevine growth and fruit quality. Cooler regions are expected to be unsuitable for grape growing due to insufficient maturation and variability of quality parameters. Therefore, a field trial was conducted, aimed to determine the effect of pruning time on low cordon cane (CP) and spur pruned (SP) grapevines of the hybrid cultivar Hasanski Sladki in a cool climate conditions. A vineyard, with the low double trunk (25 cm in height) training system, was established at the experimental station of the Estonian University of Life Sciences (58°23'17'' N, 26°41'50'' E) in June 2007. The treatments were carried out in autumn after leaf fall and in spring at the two leaf phase in 2010/2011 and 2011/2012. Pruning time affected grape maturity parameters depending on pruning method. Autumn SP increased the soluble solids content from 18.5 to 19.8 °Brix in 2011 and from 17.1 to 18.0 in 2012. Titratable acids content was high in both experimental years ranging from 1.3 to 2.1 g 100 g^{-1}, and only autumn CP decreased it. Pruning in spring significantly decreased the soluble solids/ titratable acids for both pruning methods. The timing of SP affected the maturity index (MI = °Brix × pH^2) variably; in 2011, spring pruning decreased the index whereas; the index was increased in 2012. Spring pruning decreased the total phenolics up to 22% in both treatments in the two years mean. In CP, spring pruning increased anthocyanins content from 31 to 77 mg 100 g^{-1} in 2012.

Key words: soluble solids, titratable acids, total phenolics, anthocyanins, maturity index

INTRODUCTION

Viticulture in cool climates (10 to 20°C annual isotherm, latitudes 30–50° N and 30–40° S) is of increasing interest as cool weather conditions favour flavours not achieved under more temperate conditions [Gustafsson and Mårtensson 2005]. Climate

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has a significant influence on grape quality [Falcão et al. 2008, Ferrer-Gallego et al. 2012] but the suitability of cooler regions, especially for high quality grape production, has not been fully investigated and berry maturation can be problematic [Gustafsson and Mårtensson 2005, Iyer et al. 2012]. Acclimatization is cultivar-dependent determining the vitality of buds as environmental and climatic conditions play a major role in budburst, fruit-set, veraison and maturation [Martin and Dunn 2000]. According to Gustafsson and Mårtensson [2005], hybrids of *Vitis labrusca*, *V. riparia* and *V. amurensis*, are winter hardy down to temperatures of -30°C when dormant, and are therefore suitable for growing in cool climate areas. For instance in Poland, the best tolerance to extremely low temperatures (no damage to shoots or buds) was shown by an American hybrid 'Alwood' and 'Zilga' of the *V. labruscana* group [Lisek 2007]. The hybrid cultivar Hasanski Sladki has shown a good winter hardiness and therefore, is recommended for commercial growing in Estonia [Kivistik et al. 2010] but it is also suitable and widely grown in different climatic conditions in Scandinavia and the United States of America [Gustafsson and Mårtensson 2005, Hart 2008, Plocher and Parke 2008]. In addition to cultivar selection, the training system is also important. Various vine training systems are used in Scandinavian countries (long cane, low cordon, low head training and mini J-style) [Gustafsson and Mårtensson 2005]. Low cordon spur and long cane pruning are used widely in Estonia so that canes can be bent to the ground and covered by the snow layer in winter.

Although some cultivars are extremely cold hardy, susceptibility to spring frosts can still be a problem. Manipulation of bud break can reduce spring frost damage to shoots [Dami et al., 2000] and enhance yield stability [Intrieri and Poni, 1998]; delayed pruning can also influence other phenological events related to vine growth and grape quality [Martin and Dunn 2000, Friend and Trought 2007], but can also result in later ripening which may result in vines being unable to attain sufficient maturity for harvesting [Friend and Trought 2007]. The influence of pruning time on grape quality parameters has been little investigated in Nordic areas, where it is important to take many factors into account, such as the duration of xylem sap flow because of the long-lasting spring [Keller and Mills 2007].

The measurement of total soluble solids is a well-established parameter for basic grape maturity assessment [Ferrer-Gallego et al. 2012], although alone is not enough to ensure the maturity of grapes [Ferrer-Gallego et al. 2012, Iyer et al. 2012]. Combinations of soluble solids, titratable acids and pH values are generally used to determine the optimum ripeness of grapes for making red wine [Coombe et al. 1980, Hunter et al. 1991, Iyer et al. 2012]. According to these measurements, the recommended range of values for wine are at maturity index (MI = °Brix × pH^2) from 200 to 270 [Schalkwyk and Archer 2000]. Phenolics content varies depending on several factors such as temperature [Haselgrove et al. 2000, Ferrer-Gallego et al. 2012]; cultural practices [Peña-Neira et al. 2004, Palliotti et al. 2012] and grape developmental stage [Haselgrove et al. 2000]. For red grapes, the content of anthocyanins varies greatly with cultivar and grape maturity [Ryan and Revilla 2003, Fournand et al. 2006], production area, seasonal conditions [Ferrer-Gallego et al. 2012] and yield [Haselgrove et al. 2000, Hülya Orak 2007, Falcão et al. 2008]. The complexity of factors affecting grapevine yields and their composition vary significantly from one place to another and one year to the next [Martin and Dunn 2000].

Thus grape maturity parameters can be affected by pruning time. Delayed pruning is suitable for bud burst manipulation, but it may have a negative effect on grape maturation due to the shorter period for ripening. The aim of the present experiment was to evaluate the effect of pruning time on maturity parameters in the hybrid grapevine 'Hasanski Sladki' that were either low cordon cane or spur pruned and growing in cool climate conditions.

MATERIAL AND METHODS

Site and plant material. The vineyard was established at the experimental station of the Estonian University of Life Sciences (58°23'17'' N, 26°41'50'' E) in June 2007. Plantation soil was sandy loam with pH_{KCl} 5.8, with 4.4% humus content and a 50 cm thick humus layer. The content of P, K, Ca and Mg was sufficient in the soil and hence, no fertilizers were used. The grapevines were propagated *in vitro* and grown as own-rooted. They were spaced 2 × 2 m apart and planted in single rows with 0.04 mm thick and 1 m wide black polyethylene mulch with turf between the mulched beds. The experiment was conducted using a randomized block design with 4 replications and 8 vines in each. Rows were oriented from north to south. Vines were not irrigated or covered for winter.

The interspecific *V. amurensis* hybrid cv. Hasanski Sladki (synonyms 'Baltica', 'Hasansky Sladky', 'Hasan (Xasan) Sweet', 'Varajane Sinine') originates from Hasan (Xasan), Primorsky Krai, Russia [Gustafsson and Mårtensson 2005, Hart 2008, Plocher and Parke 2008, Smiley et al. 2008]. It was developed by the breeder, A. K. Bous and released in the late 1950's or early 1960's [Hart 2008, Plocher and Parke 2008]. This early season cultivar is rather disease resistant, having strong but not excessive vigour and a procumbent growth habit. Clusters are of medium size, long and slightly loose, with an average weight of 90 g. The berries are blue-coloured, small to medium-sized (average weight 2 g). When dormant, the cultivar is winter hardy down to -20°C.

Pruning technology. The training system was low double trunk (25 cm in height). The vine treatments were spur pruning (SP) and cane pruning (CP) in autumn (2010 and 2011) after leaf fall and at two-leaf phase in spring (2011 and 2012). With SP, four overwintered fruit-bearing canes were pruned to short two-bud spurs and new shoots were directed vertically. With CP, an over-wintered fruit-bearing cane was pruned to 8 buds and bent horizontally. In summer, any shoots growing from summer buds were cut and removed from all vines. All fruiting shoots were cut off to 10 leaves after clusters. The height of the canopy was approximately 1.6 m. Suckers were cut and removed throughout the summer. Leaf removal adjacent to berry clusters was implemented at the beginning of veraison when removing the leaves from the east side of the canopy to allow morning sun exposure due to the occurrence of dew.

Weather conditions. By the end of November 2010, temperatures were already down to -25°C. On 15 April 2011, temperatures were above 5°C and attained 20°C by the end of the month. The period of active plant growth temperatures in 2011 was from

7 May to 8 October. Summer of 2011 was warm; the mean temperature in July was 3.3°C higher than the long term mean (tab. 1). First night frosts occurred on 20 October. In 2011, the sum of active temperatures (> 10°C) was 2498°C, and the length of active plant growth period was 155 days.

Table. 1. Weather conditions in 2011–2012: monthly mean air temperature and total monthly precipitation compared to the long term mean (1971–2000)

Month	Air temperature (°C)			Precipitation (mm)		
	2011[a]	2012[a]	1971–2000[b]	2011[a]	2012[a]	1971–2000[b]
April	5.7	4.6	4.7	1	45	33
May	11.0	11.4	11.1	58	78	53
June	17.2	13.3	15.1	35	98	69
July	20.0	17.7	16.9	48	80	76
August	15.9	14.8	15.6	55	80	80
September	12.3	11.9	10.4	80	61	67

[a] Data was collected from automatic weather station of the experimental station
[b] Data according to the Estonian Hydrological and Meteorological Institute (www.emhi.ee) database

In 2012, temperatures were above 10°C from the beginning of May until 6 October. The active plant growth period was 150 days. Late spring frosts stopped in mid-May and temperature remained > 0°C until 20 October. Mean temperatures in June and July were cooler than in 2011; respectively 3.9°C and 2.3°C lower (tab. 1). The sum of active temperatures from 1 April until 31 October 2012 was 1967°C.

In 2011, there was almost no rain in April but 27 mm more rain than the long term mean in September (tab. 1). In 2012, the precipitation level was significantly higher than the long term mean in April, May and June but similar to it in August and September.

Sample preparation and determination. All the analyses and measurements were carried out in 2011 and 2012. Fully expanded leaves were selected in July from the middle of shoots to determine chlorophyll content of the vine leaves using a portable SPAD-502 (Soil Plant Analysis Development), chlorophyll meter (Minolta). This permits a rapid and non-destructive determination of leaf chlorophyll content by measuring leaf transmittance. One replication comprised measurement of 30 leaves.

The weight of ten randomly selected bunches of a vine was determined in each replication. The number of berries per bunch was recorded. Grape weight was calculated as the mean of 100 berries. The soluble solids content (SSC) was measured from fresh berries by refractometer (Atago Pocket Refractometer Pal-1). For °Brix measurement, 30 grapes in 3 replications from the different parts of a cluster were picked and analyzed.

All the other biochemical parameters were determined from frozen (-20°C) grapes. Titratable acids content (TAC) was determined manually by the titration method with 0.1 M NaOH solution, using Bromothymol blue as an end point indicator [Chone et al. 2001]. The TAC was expressed as grams of tartaric acid per 100 g of fresh weight

(FW). Soluble solids and titratable acids ratio (SSC/TAC) was calculated based on the content of SSC and TAC. pH was evaluated from grape juice with a pH/conductivity meter (HD 2156.1, Delta OHM). Maturity index (MI) was calculated according to the formula determined by Coombe et al. [1980]: MI = °Brix × pH^2. The content of total anthocyanins (ACC) was estimated by a pH differential method from grape skin [Cheng and Breen 1991]. Absorbance was measured with a UVmini-1240 Shimadzu spectrophotometer at 510 and at 700 nm in buffers at pH 1.0 (HCl 0.1N) and pH 4.5 (citrate buffer). The results were expressed as mg of cyanidin-3-glucoside equivalent per 100 g of FW. Total phenolics content (TPC) was determined from grape skin with the Folin-Ciocalteau phenol reagent method [Slinkard and Singleton 1977], using a spectrophotometer (UVmini-1240 Shimadzu) at 765 nm. The TPC was expressed as mg of gallic acid equivalents per 100 g of FW.

Statistical analysis. The results of grape chemical composition were tested by one-way analysis of variance and the two years mean were tested by two-way analysis of variance (factors were pruning treatment and pruning time). To evaluate the effect of treatments, the least significant difference ($LSD_{0.05}$) was calculated. Different letters on figures and tables mark significant differences at $P \leq 0.05$. Linear correlation coefficients were calculated between variables with the significance of coefficients being $P \leq 0.01$*. The strength of the relationships was estimated as $r \leq 0.3$ (weak), $0.3 \leq r \leq 0.7$ (moderate) and $r \leq 0.7$ (strong).

RESULTS

Soluble solids, titratable acids and maturity index. SSC ranged from 17.1 to 19.8 °Brix and pruning time caused significant differences in SP (fig. 1). Autumn pruning increased the SSC compared to spring treatment in both experimental years and in two years mean (tab. 2). The mean effect of pruning time and treatment was significant at $P \leq 0.01$. TAC ranged from 1.3 to 2.1 g 100 g^{-1} (fig. 2). Pruning time had no effect in

Table. 2. The effect of pruning time and treatment on 'Hasanski Sladki' grape biochemical composition (2011–2012)

Treatment	Time	Soluble solids, °Brix	Titratable acids, g 100 g^{-1} FW	Soluble solids/ titratable acids	Maturity index	Total phenolics, mg 100 g^{-1} FW	Anthocyanins, mg 100 g^{-1} FW
SP	autumn	18.9a	1.7b	11.3c	172b	311a	92a
	spring	18.9a	1.4d	13.3a	188a	261b	94a
CP	autumn	17.8b	1.8a	10.1d	169b	309a	42c
	spring	18.9a	1.6c	12.4b	172b	240b	70b
Mean effect of time		**	*	**	**	NS	***
Mean effect of pruning		**	***	***	**	**	***

NS, *, **, *** – non-significant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively
Different letters mark significant differences at $P \leq 0.05$.

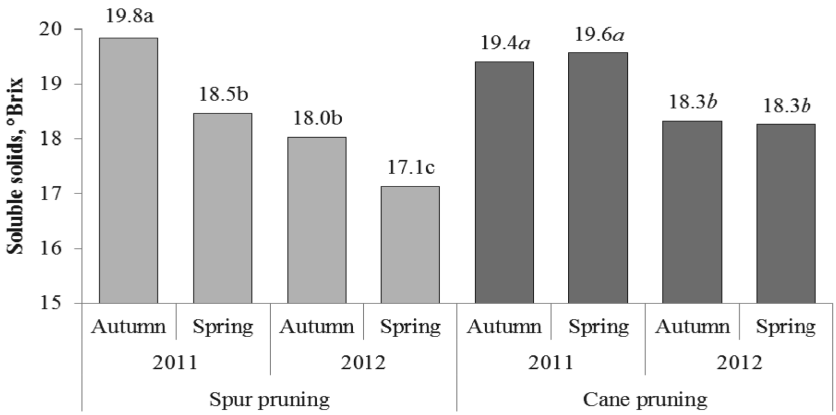

Fig. 1. Effect of spur and cane pruning times (autumn, spring) on 'Hasanski Sladki' soluble solids content in 2011 and 2012. Values followed by the same letter are not significantly different at P≤ 0.05

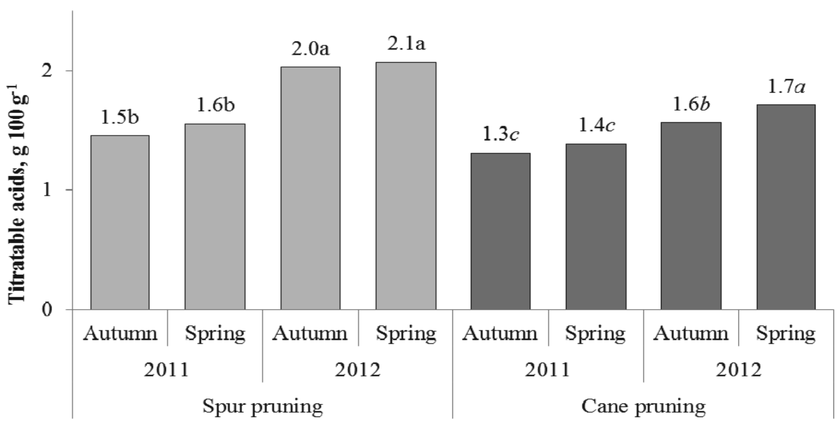

Fig. 2. Effect of spur and cane pruning times (autumn, spring) on 'Hasanski Sladki' titratable acids content in 2011 and 2012. Values followed by the same letter are not significantly different at P≤ 0.05

SP vines. Autumn CP had a negative effect in year 2012 and in the two years means (tab. 2). The mean effect of time ($P \leq 0.05$) and treatment ($P \leq 0.001$) was significant. SSC/TAC varied from 8.3 to 14.8 (fig. 3). Spring SP decreased the ratio in year 2011, with 12.5% lower results. Spring CP decreased the ratio significantly in 2012. In the two years mean, SSC/TAC was significantly affected by pruning time; spring pruning caused an increase for both pruning methods (tab. 2). The experimental mean effect of pruning time ($P \leq 0.01$) and treatment ($P \leq 0.001$) was also significant.

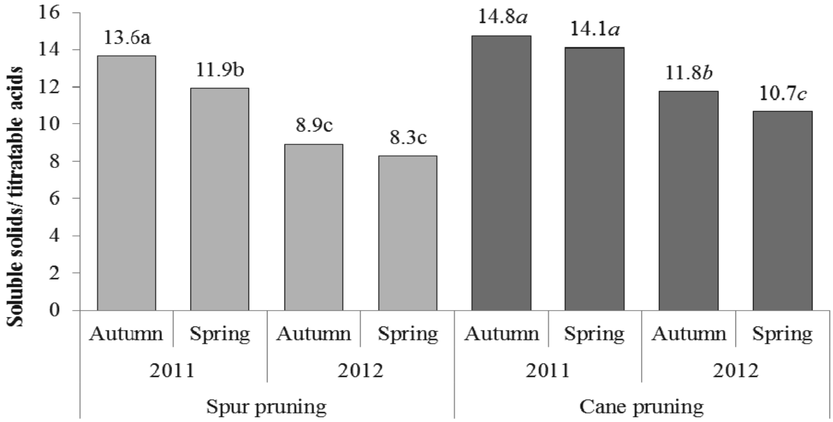

Fig. 3. Effect of spur and cane pruning times (autumn, spring) on 'Hasanski Sladki' soluble solids and titratable acids ratio in 2011 and 2012. Values followed by the same letter are not significantly different at $P \leq 0.05$

Grape MI varied from 132 to 213, and the variations were caused by pruning time (fig. 4). For SP, the effect of pruning time differed in the two years; in 2011, spring pruning decreased the MI, but in 2012 increased it. In CP, the autumn treatment increased the MI compared to spring in 2011. Spring SP increased the MI in the two years mean, and the mean effect of the pruning time and treatment was ($P \leq 0.01$) also significant (tab. 2).

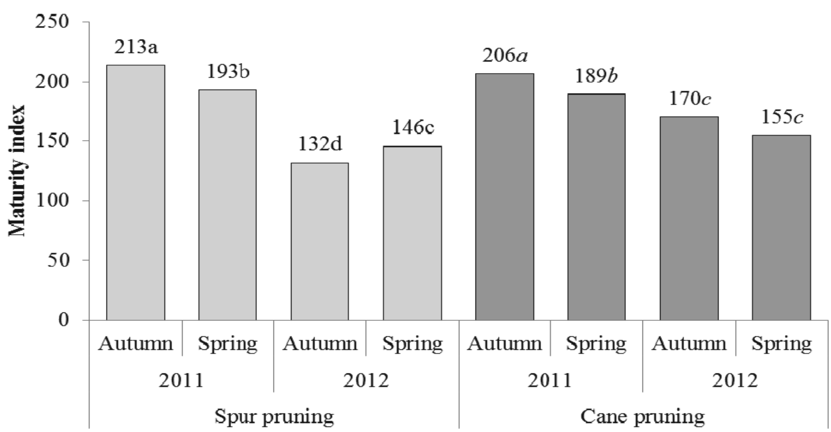

Fig. 4. Effect of spur and cane pruning times (autumn, spring) on 'Hasanski Sladki' maturity index in 2011 and 2012. Values followed by the same letter are not significantly different at $P \leq 0.05$

Total phenolics and anthocyanins content. TPC ranged from 224 to 393 mg 100 g^{-1} in SP and from 192 to 298 mg 100 g^{-1} in CP (fig. 5). In both pruning treatments, pruning time did not cause significant differences. In two years mean, spring pruning decreased the TPC in both treatments (tab. 2). The pruning methods mean effect was significant ($P \leq 0.01$).

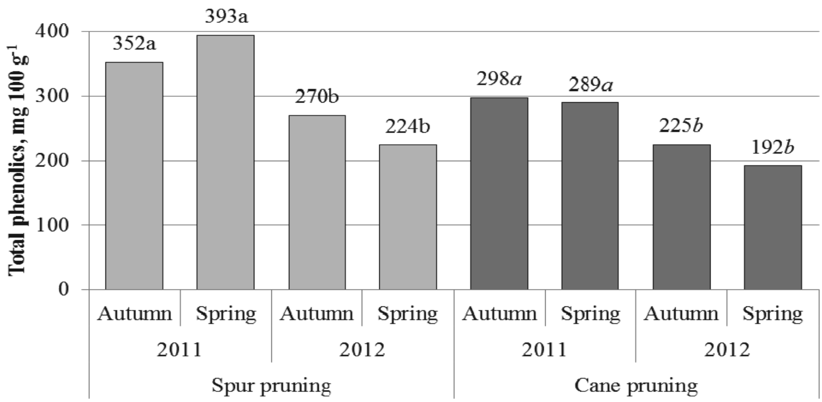

Fig. 5. Effect of spur and cane pruning times (autumn, spring) on 'Hasanski Sladki' total phenolics content in 2011 and 2012. Values followed by the same letter are not significantly different at P≤ 0.05

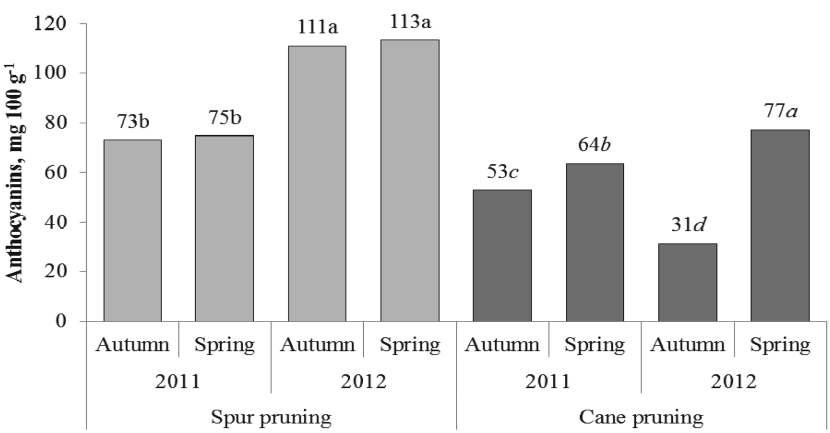

Fig. 6. Effect of spur and cane pruning times (autumn, spring) on 'Hasanski Sladki' anthocyanins content in 2011 and 2012. Values followed by the same letter are not significantly different at P≤ 0.05

High variability among treatments was found for ACC, ranging from 73 to 113 mg 100 g^{-1} in SP and 31 to 77 mg 100 g^{-1} in CP (fig. 6). The time of SP had no significant effect. The influence of pruning time was evident in CP; spring pruning caused an increase of up to 60% in 2012. In two years mean, spring pruning time increased the ACC, and the effect of pruning time and method was significant ($P \leq 0.001$, tab. 2).

Correlations. No significant correlations were found between SPAD readings and grape maturity parameters for either pruning method. In SP, berry weight was strongly positively correlated with SSC (r = 0.729), SSC/TAC (r = 0.878), TPC (r = 0.939), MI (r = 0.885) and pH (r = 0.923) (tab. 3). Strong negative correlations were found between berry weight and TAC (r = -0.922) and ACC (r = -0.900). Bunch weight correlated moderately positively with SSC (r = 0.610) and strongly with TPC (r = 0.963), and the correlations were negative and strong with MI (r = -0.942) and pH (r = -0.914). There was strong positive correlation between the number of berries per bunch and TAC (r = 0.883), while strong negative correlations were evident with SSC/TAC (r = -0.820) and ACC (r = -0.866).

Table. 3. The correlation coefficients (r) between leaf SPAD readings, yield components and fruit maturity parameters of 'Hasanski Sladki'

	Parameters	Soluble solids, °Brix	Titratable acids, g 100 g^{-1} FW	Soluble solids × titratable acids	Total phenolics, mg 100 g^{-1} FW	Anthocyanins, mg 100 g^{-1} FW	Maturity index	pH
Spur pruning	SPAD	-0.054	-0.003	-0.035	-0.344	0.234	-0.114	-0.142
	Berry weight, g	0.729*	-0.922*	0.878*	0.939*	-0.900*	0.885*	0.923*
	Bunch weight,g	0.610*	0.050	0.295	0.963*	-0.489	-0.942*	-0.914*
	Berries × bunch	-0.554	0.883*	-0.820*	-0.034	-0.866*	-0.259	0.045
Cane pruning	SPAD	-0.375	0.281	-0.330	-0.541	-0.512	-0.270	-0.382
	Berry weight, g	0.756*	-0.620*	0.691*	0.684*	0.256	0.590*	0.806*
	Bunch weight,g	-0.537*	0.004	-0.198	0.161	-0.127	-0.113	0.066
	Berries × bunch	-0.163	0.627*	-0.623*	-0.546	0.397	-0.182	-0.154

* Indicates significance at $P \leq 0.01$

In CP, a strong positive correlation was found between berry weight and SSC (r = 0.756) and pH (r = 0.806), moderate positive correlation appeared between berry weight and SSC/TAC (r = 0.691), TPC (r = 0.684) and MI (r = 0.590). Negative moderate correlation was with TAC (r = -0.620). Bunch weight correlated moderately negatively with SSC (r = -0.537). Number of berries per bunch was positively moderately correlated with TAC (r = 0.627) and negatively with SSC/TAC (r = -0.623).

DISCUSSION

Pruning time effects on grape maturity parameters differed for cane and spur pruning. The differences may be related to variations in compound bud position, and whether the shoots develop from the large central primary bud, from smaller secondary bud or from both at the same time. Martin and Dunn [2000] investigated the effect of pruning time on budburst and found that different types of buds burst at different times according to a hierarchy of bursting which depends on different types of shoots and their bud positions. In our experiment, in the case of SP, the fruit bearing shoots developed from the two proximal buds that were left after pruning. This treatment stimulated the vines to produce shoots with bursting primary and secondary buds. The impact of CP was less stressful to the vines – fewer shoots were formed, because the secondary buds did not burst at all. According to Andersen and Sims [1991], the lower the pruning severity, the greater the proportion of highly productive shoots derived from primary buds. In CP, the reproductive buds were in the middle of the cane and hence, the basal buds did not burst at all because of apical dominance.

In cold climate conditions, a common problem of insufficient grape maturation is because of TAC being too high and SSC too low [Gustafsson and Mårtensson 2005]. The recommended °Brix for red wine grapes according to Schalkwyk and Archer [2000] is from 20 to 23. In the present experiment, the SSC achieved almost the recommended minimum (being 19.8°Brix) in 2011, but did not reach optimum in 2012. The decrease of SSC in 2012 may be related to the lower temperatures and higher precipitation rate of this season. The TAC achieved in the present experiment was significantly higher (from 1.3 to 2.1 g 100 g^{-1}) compared to the recommended values for red wine grapes. According to Schalkwyk and Archer [2000], the recommended acid concentration should be from 0.6 to 0.7 g 100 g^{-1}. Iyer et al. [2012] determined TAC values in *V. vinifera* grapes from 0.6 to 2 g 100 g^{-1} in a single year in a cooler vine growing area in U.S.A. In our case, the higher contents were also obtained in the cooler year. Therefore, the influence of CP is considered to be positive when decreasing TAC towards the recommended values for wine production, though, contents still remained high. Autumn pruning also increased the MI values of grapes in both treatments in the warmer year 2011, reaching (from 206 to 213) almost to the recommended level, which is, according to Schalkwyk and Archer [2000] from 200 to 270.

In the present study, significantly increased TPC in case of autumn pruning in two years mean up to 311 mg 100 g^{-1} show quite high concentrations. The reason for differences in TPC and ACC between the years could be related to the weather being warmer in 2011 than in 2012 and hence more favorable for grape ripening. Higher values of ACC were determined in the cooler summer conditions in 2012. The temperatures during berry maturation in August 2012 were somewhat lower than in 2011 and the long-term mean (tab. 1). Differences in ACC were caused by lower night temperatures which caused major day-night temperature fluctuations. The temperature fluctuations during ACC accumulation caused stress and therefore the increase of ACC occurred. The effect was especially evident in the case of SP, because of the particular treatment being more extensive compared to CP, as discussed previously. In addition to temperatures, light conditions are also important. Nicolosi et al. [2012] indicate that direct sunlight interception by fruit has been associated with improved fruit quality and is generally desirable to some degree in most vineyards. In the present experiment, the height of the trellis system and row spacing were taken into account to provide maximum light exposure that is available in cooler climatic conditions. The angle of sunshine in northern areas differs from the southern parts of the world; in Nordic conditions the sun shines obliquely rather than directly overhead and therefore the shading effect of the leaves is minor.

Correlation results indicate that the leaf function parameter SPAD (chlorophyll content estimated non-destructively) had no influence on grape maturity. Pruning treatment and time had no effect on leaf functioning during yield formation and therefore, no influence on berry maturation. Most of the maturity parameters determined in the present experiment were affected by berry weight due to the higher soluble solids and lower acids concentration in heavier berries. In SP, TPC was increased in heavier berries and bunches. At the same time, an increase of ACC was determined in lighter berries and bunches with fewer grapes. These results suggest that thinner bunches provide better light conditions for berries which influenced the ACC. Light is a limiting factor in the accumulation of anthocyanins during the early stages of ripening [Haselgrove et al. 2000]. In CP, yield parameters had no effect on the anthocyanins accumulation. Meanwhile in SP, the positive correlation between the number of berries per bunch and TAC show that significantly increased number of grapes in a bunch resulted in higher concentration of acids.

CONCLUSION

The results indicate that pruning time had a significant influence on 'Hasanski Sladki' grape maturation and this varied with pruning method. Autumn SP increased the SSC, but the time had no effect on CP. Though, autumn CP decreased the TAC, the concentrations remained high compared to the recommended level. In neither pruning treatment, did pruning time cause significant differences in TPC. Pruning time increased the anthocyanin accumulation in spring CP in the cooler year, but had no significant influence in SP.

In conclusion, pruning in spring is suitable for CP grapevines, because it did not decrease the SSC, TPC and ACC, but in SP vines, soluble solids may stay below the recommended level with spring pruning. Future studies are needed to find ways of achieving the optimum concentration of acids.

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WPŁYW CZASU I METODY CIĘCIA NA DOJRZAŁOŚĆ OWOCÓW MIESZAŃCÓW ZŁOŻONYCH WINOROŚLI (*Vitis* sp.) 'HASANSKI SLADKI' W WARUNKACH CHŁODNEGO KLIMATU

Streszczenie. Warunki klimatyczne i pogodowe są ważnymi czynnikami wpływającymi na wzrost winorośli i jakość owoców. Uważa się, że chłodniejsze regiony są nieodpowiednie dla wzrostu winorośli ze względu na niedostateczne dojrzewanie oraz różnorodność parametrów jakościowych. Przeprowadzono więc próbę polową, mającą na celu określenie wpływu czasu przycinania na niskie cięcie (CP) i krótkie cięcie (SP) winorośli hybrydowej odmiany Hasanski Sladki w warunkach chłodnego klimatu. Winnica z systemem uprawy dwuramiennego pnia (25 cm wysokości) została założona w stacji eksperymentalnej Estońskiego Uniwersytetu Przyrodniczego (58°23'17''N, 26°41'50''E) w czerwcu 2007. Zabiegi przeprowadzono jesienią, po opadnięciu liści, oraz wiosną, w fazie dwóch liści, w latach 2010/2011 oraz 2011/2012. Czas przycinania wpływał na parametry dojrzałości winogron w zależności od metody przycinania. Jesienne samooczyszczanie zwiększało zawartość związków rozpuszczalnych z 18,5 do 19,8 °Brix w 2011 r. oraz z 17,1 do 18,0 w 2012 r. Kwasowość była wysoka w obydwu latach i wynosiła od 1,3 do 2,1 g 100 g^{-1} i zmniejszało ją tylko CP. Cięcie wiosenne istotnie zmniejszało stosunek: rozpuszczalne substancje/kwasowość w przypadku obydwu metod. Czas SP miał wpływ na zmienność wskaźnika dojrzałości (MI = °Brix × pH^2). W roku 2011 wiosenne cięcie zmniejszało ten wskaźnik, natomiast w roku 2012 wskaźnik zwiększył się. Wiosenne cięcie zmniejszało całkowitą zawartość fenoli do 22% w obu zabiegach w średniej dwuletniej. W przypadku CP, wiosenne cięcie zwiększało zawartość antocyjanów z 31 do 77 mg 100 g^{-1} w roku 2012.

Słowa kluczowe: rozpuszczalne substancje, kwasowość, całkowita zawartość fenoli, antocyjany, wskaźnik dojrzałości

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IV

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Influence of Humic Fertilizer on the Quality of Strawberry Cultivar 'Darselect'

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Keywords: *Fragaria* × *ananassa* Duch., soluble solids, anthocyanins, total phenolics, ascorbic acid

Abstract

The aim of the present experiment was to evaluate the influence of liquid humic fertilizer treatment according to different mulch materials (tree bark, plastic) on the biochemical composition of 'Darselect' fruits. The experiment was carried out at the Research Centre of Organic Farming of Estonian University of Life Sciences in 2010, in the first year after planting. The results showed that the effect of humic fertilizing on strawberry soluble solids and titratable acid ratio was not significant in primary and secondary order fruit. Ascorbic acid values ranged from 79 to 94 mg 100g^{-1} and fertilization had a positive effect on secondary fruits in plants growing in apple tree bark mulch. Also, fertilizing positively influenced the content of total phenolics and anthocyanin in fruits; the increase was 18%, and from 12 to 16% respectively. The results indicate that the cultivation technology had a minor effect on the taste parameters (soluble solids, acids and their ratio) of strawberry, but significantly affected strawberry fruit antioxidants (ascorbic acid, total phenolics and anthocyanins) content according to mulch material and fruit order.

INTRODUCTION

Species and cultivars play an important role in determining fruit antioxidant potential (Scalzo et al., 2005; Crespo et al., 2010). Strawberry fruits contain a wide array of phenolic compounds including flavonols and anthocyanins, and compared with other fruits strawberries possess high antioxidant activity (Määttä-Rihinen et al., 2004). Strawberry biochemical composition is affected by different factors. Compost and fertilizers as soil supplements have a significant influence e.g. phenolic compounds (Wang and Lin, 2003). Anttonen et al. (2006) found that fruit order caused 1.5 to 2.0-fold differences in phenolics contents. Moor et al. (2004, 2009) concluded that mineral fertilizers had a significant influence on the content of ascorbic acid and soluble solids, but the influence depended on the mulch material. In the case of plastic mulch, fertilization had a positive influence on ascorbic acid content. In contrast, for straw mulch, fertilization decreased the content of ascorbic acid in fruits.

In organic cultivation methods, soil composition and content of plant nutrients can be affected by natural fertilizers e.g., liquid humic substances or mulch materials. Alvarez and Grigera (2005) have noted that humic acids increase the number of roots and therefore stimulate nutrient absorption, plant development and growth. Singh et al. (2010) found that foliar application of vermicompost leachates containing a high amount of humic acid remarkably improved growth parameters and thereby also total fruit yield. Studies conducted in Norway showed a significant negative effect of tree bark mulch on total fruit yield and the number of fruits compared to bare soil (Sønsteby et al., 2004).

The objective of this work was to evaluate the influence of liquid humic fertilizer treatment used in association with different mulch materials (tree bark, plastic) on the biochemical composition of the strawberry cultivar 'Darselect' during the first year after planting.

MATERIAL AND METHODS

The experiment was carried out in 2010 in a strawberry experimental plantation located at the Research Centre of Organic Farming of Estonian University of Life Sciences (58°21'N, 26°40'E, 68 m a.s.l.). In 2009, weeds were destroyed as the plantation area was completely covered with black plastic from summer to the next spring until cultivation and plant establishment were carried out. Monterra Malt NPK 4.5-2.5-8 "V", which contains the main plant nutrients and 30% malt, stimulating the growth of plant roots and promoting nutrient absorption, was used as a pre-establishment fertilizer. 'Darselect' frigo plants were planted at the end of May with 50 cm spacing. The liquid humic substance used was black colored organic fertilizer containing 12% humic acid and 3% fulvic acid. The application of humic fertilizer (HF) at a concentration of 50 ml of substance per 10 L of water applied at 0.5 L per plant was executed one week after planting, during flowering and fruiting, and in the middle of August. Control plants (C) received water only. Organic apple tree bark mulch (OM) was made from the leftover branches of canopy pruning. The synthetic cover was 0.04 mm thick black polyethylene mulch (PM). The experimental design was a randomized block design with four replicates and 12 plants per replication.

The soil in the plantation was stagnic luvisols (FAO soil classification). Soil pH_{KCl} was 6.8 and humus content was 4.0%, which were suitable for strawberry growth. The soil content of P, K and Mg was high and Ca content was sufficient. In 2010, average air temperatures were 12.2°C in May, 14.3°C in June, and 21.7°C in July, whereas average temperatures in Estonia in the period from 1966 to 1998 were respectively 11.0°C, 15.1°C and 16.7°C. It appears that in July 2010 the temperature was 5°C higher than usual. May and June were quite rainy, with precipitation respectively 97.4 and 98 mm. In contrast, in July 33,6 mm less rain fell compared to the average of many years, which might have influenced the results of the experiment.

The data were collected in summer 2010. Fruits were picked according to surface color and fruit order in clusters, and stored at -20°C until analysis. Fruit orders were analyzed separately. Soluble solids content (SSC) was measured by refractometer (Atago Pocket Refractometer Pal-1). Titratable acids content (TAC) was determined by a titration method (Mettler Toledo DL 50 Randolino) with aqueous 0.1 M NaOH solution, using phenolphthalein as an endpoint indicator. The TAC was expressed as citric acid mg per 100 g of fresh fruit. Soluble solids and titratable acids ratio (SS:TA) was calculated based on the content of SS and TA. Ascorbic acid content (AAC) was determined iodometrically with the modified Tillman's method. Fruits were cut into pieces, and then crushed quickly and 10 g of pulp was taken for each analysis. For analysis, 60 ml of a mixture of metaphosphoric and acetic acid (3% HPO_3 + 8% CH_3COOH) was added instantly to the pulp to avoid vitamin C breakdown in the air (Paim and Reis, 2000). The content of total anthocyanins (TAC) was estimated by a pH differential method (Cheng and Breen, 1991). Absorbance was measured with a Jenway 6300 spectrophotometer at 510 and at 700 nm in buffers at pH 1.0 (HCl 0.1N) and pH 4.5 (citrate buffer). The results were expressed as milligrams of pelargonidin-3-glucoside equivalent per 100 g of fresh weight. Total phenolics content (TPC) was determined with the Folin-Ciocalteau phenol reagent method, using a spectrophotometer at 765 nm. The total phenolic content was expressed as gallic acid equivalents in mg 100 g^{-1} fresh weight of pulp.

In the study, strawberry chemical composition results were tested by a one-way analysis of variance.

RESULTS AND DISCUSSION

Taste-Related Parameters

SSC ranged from 8.0 to 12.8°Brix (Fig. 1A). The application of fertilizer positively and significantly influenced the SSC in tertiary fruits in OM, and negatively in PM treatment. TAC value varied from 0.71 to 0.89 g 100 g^{-1} (Fig. 1B). Differences appeared in primary fruits in the OM and in secondary fruits in the PM treatment, where the HF increased TAC. SS:TA ratio values varied from 10.7 to 14.4 (Fig. 1C). HF had a statistically significant effect only on tertiary fruits in the OM, but at the same time a negative influence in the PM treatment.

Sugars and acids are responsible for strawberry taste; furthermore, acids play a great role in the soluble solids pool (Cordenunsi et al., 2002; Crespo et al., 2010). SS:TA ratio determines the bitter-sweetness of strawberry fruits. The results of the present experiment indicate that fruit order had an effect on taste parameters and the sweetest were tertiary fruits. HF had a positive influence only on SSC, affecting the taste of tertiary strawberries only. Differences have been more pronounced in previous mineral fertilizing experiments. According to Moor et al. (2009), fertilization treatments conducted during the first year reduced the SSC and SS:TA ratio in strawberry fruits and strawberry flavor was influenced towards being more acidic and less sweet by additional fertilization with NPK fertilizer. In older plantations, the influence of liquid fertilizer on SSC was not significant in either organic or synthetic mulch (Moor et al., 2004).

Sugar content is dependent on weather conditions (Leskinen et al., 2002), as well as fertilizer treatment. In the present study the soluble solids content might have been influenced by higher temperatures in July when the tertiary fruits were picked. Therefore, it is necessary to continue the experiments to investigate the influence of supplementary fertilization. On average in the first year, the influence of liquid humic fertilizer on taste parameters was insignificant, probably because of the high humus content, and for that reason the additional humic substances had no effect. The influence could appear from this time onward as strawberry plants are growing and mineral plant nutrient uptake is increasing.

Antioxidants

AAC values ranged from 79 to 94 mg 100 g^{-1} (Fig. 1D). HF had a positive effect on AAC in secondary fruits in OM treatment. ACYC was from 113 to 215 mg 100 g^{-1} (Fig. 1E). HF had a positive effect on ACYC in the PM treatment and the increase was 18% in secondary fruits. TPC varied from 158 to 226 mg 100 g^{-1} (Fig. 1F). HF influenced significantly TPC in both mulch treatments, and differences were related to fruit order. In the OM treatment the TPC increase was from 13 to 16% in secondary and tertiary fruits, in the PM from 12 to 13% in primary and secondary fruits and, in the PM tertiary fruits TPC decreased by about 7% compared to the control.

We may suspect that fertilizing has an effect on the content of antioxidants, but the effect depends on several conditions such as mulching and fruit order. Moor et al. (2004) found that fertilization had a significant positive influence on ascorbic acid content (increase by 3%) with plastic and was negative (decrease by 10%) with organic straw mulch. Singh et al. (2010) indicated that foliar application of vermicompost leachates significantly influenced some quality parameters e.g. total soluble solids, acidity and ascorbic acid content.

Strawberries contain a wide range of phenolic compounds including anthocyanins (Määttä-Riihinen et al., 2004). Anttonen et al. (2006) found that strawberry fruit order affected the content of total phenolics, anthocyanins and antioxidant activity, which was equally evident in the present experiment. In our experiment the HF increased significantly the ACYC in the PM. Moor et al. (2005) observed higher anthocyanin content in strawberry fruits grown on plastic mulch; however, fertilization and the use of straw mulch compared to plastic mulch, had a significant negative influence. At the same time, strawberry plants grown in soil supplemented with fertilizer and compost had significantly enhanced anthocyanin content in the fruit (Wang and Lin, 2003). Fan et al. (2011) studied the influence of production systems on the antioxidant capacity and phenolic content concentrations in strawberry fruits. Comparing the plastic mulch and additional white row covers with the commonly used matted-row system, the strawberries grown on plastic mulch had a significantly higher amount of phenolic compounds than others.

The content of antioxidants can be affected by various weather conditions such as light and temperature. A shading effect on anthocyanin content was observed by Anttonen et al. (2006) between two light levels, and the difference was smaller in strawberries planted later, probably due to the increased levels of natural light. In the present study the positive effect of fertilization on ACYC could be affected by the first cropping year. Presumably, due to sunny weather in summer 2010 and smaller plant size, the reduced leaf shading had an effect on ACYC. Differences might appear after fertilizing when plants grow bigger and the influence of shading increases. Wang and Camp (2000) stated that strawberries grown at the highest temperature (30/22°C) were the darkest red, and had the greatest fruit surface and flesh pigment intensity. Probably the temperature and its fluctuations are higher under the plastic mulch than under the tree bark mulch. In the present experiment, the results were influenced by mulch material – the positive effect on ACYC was evident in plastic mulch. Marais et al. (2001) indicated that fluctuating temperature increased the anthocyanin content in apple fruits harvested in different areas. It could be similar in strawberries.

CONCLUSIONS

The results of this experiment indicate that humic fertilizer does not produce significantly larger strawberry fruits, but it affects taste parameters positively in smaller fruits with organic mulch treatment. At the same time, fertilizing increases the total phenolics content in both organic and synthetic mulch, and positively affects ascorbic acid content in organic mulch treatment. These results are important when choosing cultivation technology in order to influence yield quality during the first year after planting.

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Figures

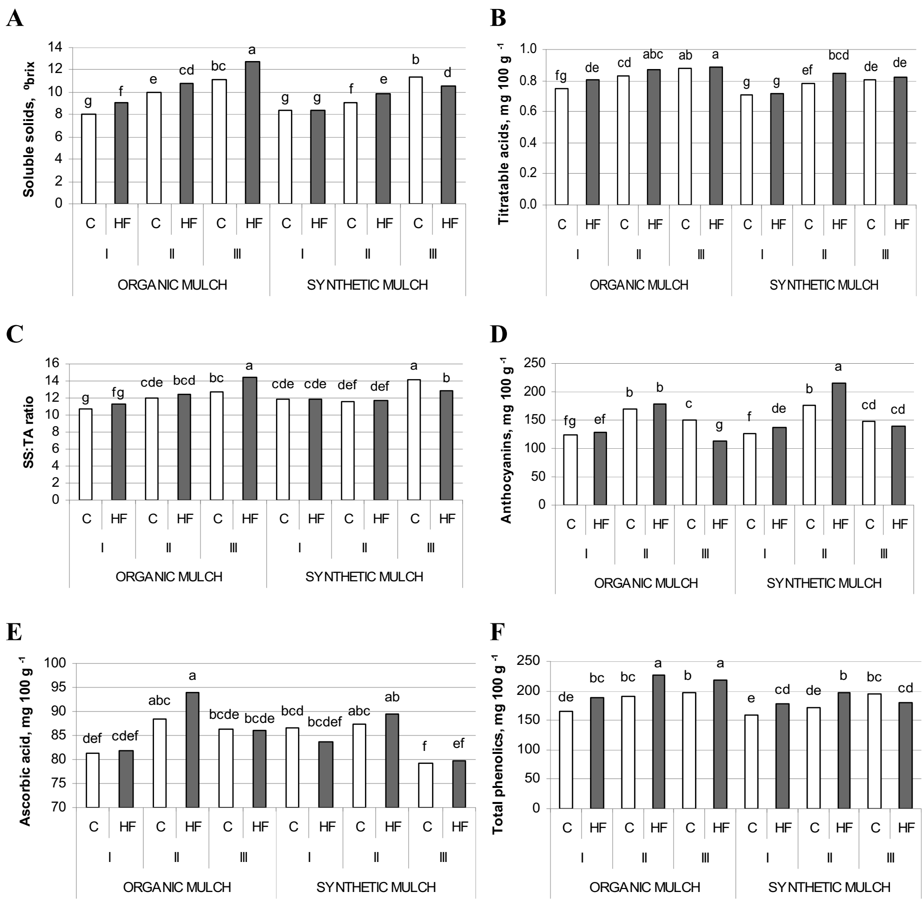

Fig. 1. Effect of fertilizing on strawberry 'Darselect' content of soluble solids (A), titratable acids (B), total anthocyanins (D), ascorbic acid (E), total phenolics (F) and SS:TA ratio (C). Fruit order: primary (I), secondary (II), tertiary (III). C – control; HF – humic fertilizer; organic mulch – tree bark; synthetic mulch – plastic. Different letters on peaks mark significant differences at P≤0.05.

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Effect of post-harvest flame-defoliation on strawberry (*Fragaria* × *ananassa* Duch.) growth and fruit biochemical composition

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Abstract

The experiment with strawberry (*Fragaria* × *ananassa* Duch.) cultivar 'Darselect' was carried out at the Research Centre of Organic Farming of Estonian University of Life Sciences (58º21′ N, 26º40′ E). The objective of the present research was to determine the influence of post-harvest defoliation with a directed propane flamer and the combination of flaming and additional application of humic substances on strawberry growth and fruit biochemical composition in two successive years. Defoliation decreased the number of leaves and inflorescences, and crown and root mass. Post-harvest flame-defoliation of two-year-old plants increased the yield in the next year (in 2012), but the succeeding flame-defoliation decreased the yield in the following year (in 2013). The effect of defoliation on fruit biochemical composition was significant, but differed between years and fruit orders. The defoliation treatment increased soluble solids content in primary fruits from 8.9 to 9.3 °Brix, but decreased anthocyanins content in all fruit orders in 2012. Flaming treatment increased the content of phenolics from 181 to 206 mg 100 g^{-1} in primary, but decreased from 268 to 254 mg 100 g^{-1} in tertiary fruits in 2013. Application of humic substance to the defoliated plants increased the number of leaves in 2012, and crown and root mass in 2013, but decreased the yield in both years. The influence of the treatment differed yearly for soluble solids – decreasing the content in 2012, but increasing in 2013. Humic substances had positive influence on the total phenolics and anthocyanins content in both years.

Keywords: anthocyanins, ascorbic acid, humic acids, soluble solids, titratable acids, total phenolics.

Introduction

The tendency towards environmentally friendly cultivation methods producing healthy fruits is constantly increasing. Organically grown strawberries tended to have higher soluble solids/titratable acids ratio indicating somewhat sweeter taste compared to conventional ones (Tõnutare et al., 2009), containing up to 20% more ascorbic acid and phenolics, and anthocyanins (Fernandes et al., 2012). There is data claiming that cultivation systems may have no clear effect on the accumulation of phenolic compounds (Crecente-Campo et al., 2012). Contrary to previous, it has been revealed that environmental conditions affect significantly strawberry quality parameters (Moor et al., 2004; Crespo et al., 2010), and biochemical composition of fruits also (Khanizadeh et al., 2014).

Strawberry plantations are mainly defoliated for renovation and disease control purposes (Wildung, 2000). Experiments with post-harvest defoliation have shown the reducing effect on the incidence of *Botrytis* rot only in one year out of three (Daugaard et al., 2003). Partial leaf removal after planting affects plant leaf rate and photosynthesis related to the physiological changes caused by defoliation (Casierra-Posada et al., 2012). In some cases, post-harvest defoliation treatment has increased the yield in some cultivars (Nestby, 1985), but yields can be affected by changing the extent of defoliation (Albregts et al., 1992). Clearly, it has been found that strawberry plants respond to the post-harvest defoliation differently depending on the cultivar and cropping year (Daugaard et al., 2003; Whitehouse et al., 2009). Some research has been done using the post-harvest straw mulch burning in relation to plant protection which was shown to decrease the occurrence of spider mites (*Tetranycus urticae*) significantly (Metspalu et al., 2000). Flame-defoliation could be used to remove the old strawberry foliage, but this may damage the strawberry crown (Wildung, 2000).

The timing of the defoliation is also important, because strawberry flower bud induction is sensitive to thermo-photoperiod and to some agronomic and nutritional factors (Savini et al., 2005). Plant leaf area is responsible for plant health and vitality due to photosynthesis processes and their influence on the leaf-root ratio. The extent of defoliation treatments was found to affect strawberry flower initiation significantly due to differences in the inductive capacity of leaves of differing maturity (Thompson, Guttridge, 1960). In northern hemisphere, the June-bearing strawberries initiate flower buds form late summer to autumn under short-day conditions. Therefore in cool climate conditions, the defoliation of strawberry plants should be executed at the end of harvest. The growth of new leaves occurs in a week after post-harvest flaming and cultivars with vigorous growth habit are able to recover by the time of flower bud initiation and before dormancy period (Metspalu et al., 2000). Leaves also provide the winter cover in order to protect the buds initiated in the strawberry crown. Flame-defoliation treatment can be an alternative method for mowing, though its effect on strawberry plant growth and fruit quality has been less studied.

For the reason of strawberry plant health and recovery from the defoliation treatment, several studies related to plant nutrition have been conducted. Defoliated plants were found to be affected by fertilization showing decreased content of vitamin C in strawberries (Moor et al., 2004). The utilization of plant bio-stimulants is quite a common practice in organic cultivation. Application of humic acid-containing substances has been used due to their ability to improve plant nutrient availability and therefore plant growth and yield quality (Calvo et al., 2014). Organic amendments such as farm yard manure and vermicomposting-based substances have shown the improving effect on growth-related and yield qualitative parameters (Khalid et al., 2013). Positive effect on organic strawberry fruit quality has been obtained with repetitive humic acid foliar applications from flowering to harvest (Hosseini Farahi et al., 2013). Humic acid treatments have shown a positive effect on assimilation and increased plant nutrient (N and P) uptake (Tehranifar, Ameri, 2014). Any changes in the plant biomass production (such as the number of leaves) may change the nutrient allocation patterns in fruits, which are directly related to yield quality (Correia et al., 2011). Application of liquid humic substances was found to increase strawberry total phenolics, anthocyanins and ascorbic acid contents in strawberry cv. 'Darselect' fruits in the following year after planting (Rätsep et al., 2014). On the other hand, the effect of additional humic acid amendments depends on the cultivar, because the role of cultivar properties has been confirmed as the main source of variation in biochemical composition and post-harvest quality (Crespo et al., 2010; Martinez et al., 2015).

In conclusion of the previous, several research problems were pointed out: a directed row flamer could be useful in strawberry plantations in order to remove old leaves causing minor plant damage, but affecting strawberry plant parameters and fruit quality. Moreover, besides environmentally friendly fruit production, under organic cultivation conditions it is possible to obtain considerably high contents of biochemical compounds, in particular total phenolics (Crecente-Campo et al., 2012). The problematic point could be the effect of flame-defoliation on the plant vitality – the post-harvest treatment may damage strawberry plant crowns, therefore affecting strawberry growth, and yield and fruit biochemical composition. For that reason, the application of liquid humic substances was additionally tested on the defoliated plants. There is little data about the effect of defoliation on fruit quality. The objective of the present research was to determine the influence of post-harvest defoliation with a directed propane flamer and the combination of flaming and application of humic substances on strawberry growth and fruit biochemical composition.

Material and methods

Experimental area and treatments. The strawberry experimental plantation was established at the Estonian University of Life Sciences (58º21′ N, 26º40′ E, 68 m a.s.l.). The pre-planting procedures were as follows: weeds were destroyed when the plantation area was covered with black plastic from spring 2009 to the next spring 2010 until ploughing and establishing were carried out; an ecological fertilizer (NPK 4.5-2.5-8) – produced from at least 30% malt germs – was used as pre-establishment soil supplement. Strawberry cultivar 'Darselect' frigo-plants were planted in May, 2010 in a one-row system with 50 cm spacing. Strawberry plantation area (including rows) was mulched after planting with 3–5 cm thick organic apple tree bark mulch layer made of leftover branches of organic apple tree canopy pruning. The experimental layout was a randomized block design with three replicates (12 plants per replication).

Flaming was executed in 2011 and 2012, and the following treatments were implemented: control (C), flame-defoliation (FD) and flame-defoliation with humic acid (FD + H) treatment. Control plants were neither defoliated nor fertilized, but water was applied to the control plants at the same time when the other plants received humic substances. Post-harvest FD was executed with a directed row flamer by burning all the leaves of treated plants after fruit harvesting in the second (2011) and third (2012) growth year. The flamer is a product of "Elomestari" (Finland). The machine is working with propane and it has two 20 cm wide burners with covers controlling the heating of the row flamer including mounting for selective (directed) flaming.

Soil and weather conditions. The plantation soil is *Stagnic Luvisol* (FAO soil classification). The soil pHKCl was 6.6 at the time of plantation establishment and humus content was 4.9%. The content of P, K and Mg was high and Ca content was high in the soil (Table 1).

Table 1. The content of soil nutrients, carbon and humus, pH and carbon content in biomass in 2012

Treatment	pH_{KCl}	P mg kg^{-1}	K mg kg^{-1}	Ca mg kg^{-1}	Mg mg kg^{-1}	C %	Humus %	C in biomass C mg^{-1} DW
Control	6.6	256	306	3930	600	3.2	4.9	0.525
Fertilized	6.7	286	347	5131	492	3.0	5.2	0.766

DW – dry weight

Table 2. Average temperatures and total monthly precipitation of 2011–2013 and of long-term average (1971–2000)

Month	Air temperature °C				Precipitation mm			
	2011[1]	2012[1]	2013[1]	1971–2000[2]	2011[1,2]	2012[1]	2013[1,2]	1971–2000[2]
April	5.7	4.6	4.0	4.7	11	45	36	33
May	11.0	11.4	15.5	11.1	58	78	65	53
June	17.2	13.3	17.8	15.1	35	98	29	69
July	20.0	17.7	17.5	16.9	48	80	67	76
August	15.9	14.8	16.6	15.6	55	80	73	80
September	12.3	11.9	10.8	10.4	80	61	38	67

Note. [1] – data was collected from automatic weather station of experimental garden, [1] – data according to the Estonian Weather Service (www.ilmateenistus.ee) database.

In 2011, the temperatures from March to May were 0.5°C warmer than in 1971–2000. The meteorological summer months (from June to August) were remarkably warm exceeding the long-term temperatures up to 2.4°C compared to. Monthly average temperatures in July, August and September were higher than long-term mean, up to 3.1, 0.3 and 1.9 °C, respectively (Table 2). The sum of active temperatures was 1828°C. Precipitation sum was 292 mm which was only 38 mm lower than the long-term mean (330 mm). Rainfall in June, July and August was somewhat lower – 34, 28 and 25 mm, respectively, than many years mean precipitation.

In 2012, the period of active temperatures (above 10°C) started at the beginning of May and ended on the 6th of October. Compared to long-term average, monthly average temperatures were warmer in May, July and September, but the temperatures in June were 1.8°C lower than in 1971–2000 (Table 2). The sum of active temperatures in 2012 was 1967°C. The amount of precipitation was somewhat higher in April, May and June (up to 12, 25 and 29 mm more), but quite at the same level in July, August and September in comparison with many years mean.

In 2013, the active plant growth period started on the 7th of May and ended on the 23rd of September. The sum of active temperatures was 2263°C which is 332°C more than many years average (1936°C). Monthly average temperatures were up to 4.4°C warmer, except April being 0.7°C cooler than long-term mean (Table 2). The precipitation sum from 1 April to 31 October was 352 mm, which was 152 mm less than in 2012 and 86 mm less than the mean (438) of 1971–2000. Rainfall was a bit higher only in April and May compared to long-term mean, while all the other months had lower precipitation rate and in June and September it was even up to 40 and 29 mm lower, respectively.

Measurements and analysis. Strawberry plant growth, yield and fruit composition were investigated in 2012 and 2013. Fruits were picked according to surface colour and fruit order (primary, secondary, tertiary) in clusters, fruit mass and total yield per plant were determined at the same time. Leaves and inflorescences were counted during flowering (in May) in both experimental years. Ten normally developed fully expanded leaves per replication were selected for determining chlorophyll content by using a portable SPAD-502 (Soil Plant Analysis Development) chlorophyll meter ("Minolta", Japan). This method permits a rapid and non-destructive determination of leaf chlorophyll content by measuring leaf transmittance. The dry weight of roots and crowns was determined. In 2013, three plants per replication were dug out; the leaves were cut, and after cleaning from soil the plant crowns and roots were dried until a constant weight was recorded.

All the fruits were picked at the same intervals and in one day for every fruit order and for all the treatments in one year. Yield samples were stored at −20ºC until the analysis. Fruits were analysed separately according to fruit orders. Soluble solids content (SSC) was measured by a refractometer Pal-1 ("Atago Pocket", Japan). Titratable acids content (TAC) was determined by titration method (Mettler Toledo DL 50 Randolino) with aqueous 0.1 M NaOH solution, using phenolphthalein as an endpoint indicator. The TAC was expressed as citric acid g per 100 g of fresh fruits. Soluble solids and titratable acids ratio (SSC/TAC) was calculated. Ascorbic acid content (AAC) was determined iodometrically with the modified Tillman's method. For analysis, a mixture of meta-phosphoric and acetic acid (3% HPO_3 + 8% CH_3COOH) was added instantly to the pulp to avoid ascorbic acid breakdown in the air. AAC was expressed as mg 100 g^{-1} of strawberry fresh weight (FW). The content of total anthocyanins (ACC) was estimated by a pH differential method. Absorbency was measured with a spectrophotometer UVmini-1240 ("Shimadzu", Japan) at 510 and at 700 nm in buffers at pH 1.0 (HCl 0.1 N) and pH 4.5 (citrate buffer). The results were expressed as mg of pelargonidin-3-glucoside equivalent to 100 g of FW. Total phenolics content (TPC) was determined with the Folin-Ciocalteau phenol reagent method, using a spectrophotometer UVmini-1240 ("Shimadzu", Japan) at 765 nm. TPC was expressed as mg of gallic acid equivalents to 100 g FW of pulp.

Statistical analysis. Strawberry growth related parameters and the effect of treatments according to fruit order were tested by one-way analysis of variance (*ANOVA*), and to evaluate the effect of treatment, the least significant difference was calculated and different letters in tables mark significant differences at $P \leq 0.05$. To evaluate mean effects of the two factors (fruit order in general and treatment as such in average of two experimental years), the two-way analysis of variance (*ANOVA*) was carried out, and marked as non-significant (ns) or using confidence levels as significant at $P \leq 0.05$*, 0.01** or 0.001***, signifying levels of 95, 90 and 99.9 %, respectively. Standard deviations of the mean values are also presented (±SD). Linear correlation coefficients were calculated between variables (n = 7) with the significance of coefficients being $P \leq 0.05$* and $P \leq 0.01$**. The strength of the relationships was estimated as $r \leq 0.3$ (weak), $0.3 \leq r \leq 0.7$ (moderate) and $r \leq 0.7$ (strong). All the data analysed in the present experiment met the assumptions of normality and no additional transformations were made.

Results

Strawberry growth and yield. Flame-defoliation (FD) decreased the leaf number and fruit mass in both experimental years (Table 3). Flaming increased the yield in 2012, but decreased the yield in 2013. SPAD values were decreased by the FD in 2013 only. FD reduced strawberry crown and root mass by up to 67% decreasing from 447 g to 148 g. Humic amendments had positive effect on the fruit mass of defoliated plants in 2012, but negative effect on the number of inflorescences in 2013, and decreased the yield by up to 22% in both years. Application of humic substances increased the crown and root mass of defoliated plants.

Table 3. The effect of flame-defoliation and humic acid treatments on the strawberry cv. 'Darselect' plant and fruit parameters

Year	Treatment	Leaves No. plant^{-1}	Inflorescences No. plant^{-1}	SPAD values	Fruit mass g	Yield g plant^{-1}	Crown and root mass g plant^{-1}
2012	C	40 ± 2.2 a	11 ± 2.2 a	36 ± 1.2 a	31 ± 1.3 a	503 ± 25 b	–
	FD	25 ± 2.3 b	10 ± 1.3 a	35 ± 2.2 a	26 ± 0.6 b	567 ± 28 a	–
	FD + H	38 ± 2.2 a	11 ± 2.3 a	35 ± 2.5 a	30 ± 0.7 a	444 ± 22 c	–
2013	C	62 ± 8.1 a	19 ± 2.1 a	33 ± 1.5 a	15 ± 0.7 a	227 ± 11 a	447 ± 42 a
	FD	31 ± 9.7 b	15 ± 4.2 a	32 ± 2.0 b	13 ± 0.7 b	215 ± 11 b	148 ± 7 c
	FD + H	42 ± 3.7 b	12 ± 1.2 b	32 ± 1.8 b	13 ± 0.7 b	189 ± 10 c	320 ± 40 b

Notes. C – control, FD – flame-defoliation, FD + H – flame-defoliation + humic acids application; different letters mark significant differences at $P \leq 0.05$ according to treatment and characteristics. Standard deviations of the mean values are also presented (±SD).

Strawberry fruit biochemical composition. The influence of defoliation on soluble solids content (SSC) was significant in both experimental years (Table 4). In primary fruits, FD increased the SSC in 2012, but decreased the content in the next experimental year. FD + H decreased SSC by up to 19% in primary and tertiary fruits in 2012, but increased the content by up to 50% in 2013. In average of two years, the mean effect of fruit order was non-significant, while the significance of treatments was at $P \leq 0.001$.

Table 4. The effect of flame-defoliation and humic acid treatments on the strawberry cv. 'Darselect' fruit biochemical composition according to fruit order and the mean effects of treatment and fruit order (2012–2013)

Year	Fruit order	Treatment	Soluble solids, °Brix	Titratable acids g 100 g^{-1} FW	Soluble solids / titratable acids	Ascorbic acid mg 100 g^{-1} FW	Total phenolics mg 100 g^{-1} FW	Anthocyanins mg 100 g^{-1} FW
2012	Primary	C	8.9 ± 0.2 b	1.03 ± 0.1 b	8.6 ± 0.2 a	116 ± 3.3 a	149 ± 0.2 b	13.2 ± 0.1 b
		FD	9.3 ± 0.1 a	1.36 ± 0.1 a	6.8 ± 0.1 c	103 ± 1.9 b	149 ± 0.2 b	8.5 ± 0.3 c
		FD + H	8.5 ± 0.1 c	1.02 ± 0.1 b	8.4 ± 0.1 b	103 ± 3.2 b	169 ± 0.1 a	14.0 ± 0.2 a
	Secondary	C	8.1 ± 0.1 c	1.02 ± 0.1 b	7.9 ± 0.4 b	92 ± 4.3 a	195 ± 0.2 b	18.2 ± 0.3 b
		FD	8.4 ± 0.2 b	1.14 ± 0.1 a	7.3 ± 0.3 b	99 ± 1.9 a	214 ± 0.4 a	16.4 ± 0.2 c
		FD + H	8.5 ± 0.1 a	0.96 ± 0.1 b	8.9 ± 0.3 a	97 ± 1.6 a	191 ± 0.7 c	19.0 ± 0.2 a
	Tertiary	C	10.9 ± 0.5 a	0.84 ± 0.1 b	13.0 ± 0.9 a	102 ± 5.6 a	195 ± 0.7 b	23.3 ± 0.3 b
		FD	9.6 ± 0.1 b	0.97 ± 0.1 a	10.0 ± 0.5 c	74 ± 3.0 b	178 ± 0.7 c	18.0 ± 0.2 c
		FD + H	8.8 ± 0.1 c	0.80 ± 0.1 b	11.1 ± 0.5 b	65 ± 4.1 c	208 ± 0.4 a	23.6 ± 0.2 a
2013	Primary	C	9.8 ± 0.1 b	1.20 ± 0.1 a	8.2 ± 0.4 b	83 ± 2.1 a	181 ± 0.8 b	6.8 ± 0.3 b
		FD	8.8 ± 0.7 c	1.15 ± 0.1 b	7.6 ± 0.7 b	81 ± 5.4 a	206 ± 2.3 a	9.4 ± 0.5 a
		FD + H	11.0 ± 0.1 a	1.15 ± 0.1 b	9.5 ± 0.3 a	81 ± 1.5 a	183 ± 3.9 b	6.5 ± 0.2 b
	Secondary	C	9.3 ± 0.1 c	1.37 ± 0.1 a	6.8 ± 0.2 a	104 ± 2.0 a	212 ± 1.7 c	10.3 ± 0.4 a
		FD	9.6±0.1 b	1.31 ± 0.1 a	7.3 ± 0.4 a	91 ± 2.9 b	229 ± 1.1 b	10.4 ± 0.3 a
		FD + H	10.0±0.4 a	1.31 ± 0.1 a	7.6 ± 0.4 a	68 ± 21 c	233 ± 3.0 a	10.5 ± 1.2 a
	Tertiary	C	8.3 ± 0.2 b	1.58 ± 0.1 a	5.2 ± 0.1 b	72 ± 2.5 b	268 ± 2.1 b	9.5 ± 0.3 c
		FD	7.8 ± 0.5 c	1.49 ± 0.1 a	5.3 ± 0.5 b	73 ± 2.0 b	254 ± 2.1 c	10.5 ± 0.4 b
		FD + H	11.7 ± 0.4 a	1.47 ± 0.1 a	8.0 ± 0.4 a	87 ± 3.4 a	280 ± 0.5 a	15.4 ± 0.6 a
Mean effect of fruit order			ns	*	**	***	***	***
Mean effect of treatments			***	***	***	***	***	***

Notes. C – control, FD – flame-defoliation, FD + H – flame-defoliation + humic acids application. Different letters mark significant differences at $P \leq 0.05$ among control and different treatments in different fruit orders; ns, *, **, *** – non-significant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively. Standard deviations (±SD) of the mean values are also presented.

FD treatment increased titratable acids content (TAC) by up to 32%, while FD + H decreased it in all fruit orders in 2012 (Table 4). FD and FD + H decreased TAC in primary fruits in 2013. Mean effect of fruit order on TAC was statistically significant at $P \leq 0.05$ and treatments at $P \leq 0.001$. SSC/TAC was influenced due to FD in all fruit orders, while FD + H affected the ratio only in 2012 compared to FD. In 2013, the effect of FD + H was positive in primary and tertiary fruits, increasing the ratio up to 33% compared to C and FD. Mean effect of fruit order was significant for the SSC/TAC at $P \leq 0.01$ and treatments at $P \leq 0.001$.

FD and FD + H decreased the ascorbic acid content (AAC) by up to 11% in primary and 36% in tertiary fruits in 2012 (Table 4). The same effect was noticed in secondary fruits in 2013, while FD + H had positive effect on the tertiary fruits. There was no effect of any experimental treatments on AAC in primary strawberry fruits. Treatments and fruit order had significant mean effect on AAC at $P \leq 0.001$.

Total phenolics content (TPC) was significantly affected by treatments in all fruit orders (Table 4). FD + H increased TPC in primary and tertiary fruits but decreased it in secondary fruits in 2012. In 2013, TPC increased by up to 12% by FD in primary fruits but decreased significantly in tertiary fruits compared to FD + H. Mean effect of fruit order and treatments on TPC was significant at $P \leq 0.001$. ACC was significantly lower in FD for all fruit orders in 2012 (Table 4). Humic acid amendments increased ACC significantly in defoliated plants in all fruit orders in 2012. In the next experimental year, ACC was increased by FD in primary and by FD + H in tertiary fruits (from 10.5 to 15.4 mg 100 g^{-1} FW). The mean effect of fruit order and treatments on ACC was statistically significant at $P \leq 0.001$.

The correlation analysis showed moderate positive correlation between TAC and the number of leaves ($P \leq 0.05$) and inflorescences ($P \leq 0.05$); moderate negative correlation was found between TAC and SPAD ($P \leq 0.01$), and TAC and yield ($P \leq 0.01$) (Table 5). Moderate positive relationship was found between SSC/TAC and SPAD value ($P \leq 0.05$). TPC correlated moderately negatively with SPAD ($P \leq 0.01$) and yield ($P \leq 0.01$). Strong negative correlation was observed between ACC and number of leaves ($P \leq 0.01$), while ACC had positive moderate correlation with SPAD ($P \leq 0.05$) and with yield ($P \leq 0.01$). No correlation was found between the mass of roots and crowns and fruit biochemical parameters.

Table 5. Correlation coefficients (r) between the plant parameters and fruit biochemical parameters of strawberry cv. 'Darselect'

Strawberry plant parameters	Soluble solids, °Brix	Titratable acids g 100 g^{-1}	Soluble solids / titratable acids	Ascorbic acid mg 100 g^{-1}	Total phenolics mg 100 g^{-1}	Anthocyanins mg 100 g^{-1}
Leaves No. plant^{-1}	0.232 ns	0.564 *	−0.404 ns	−0.129 ns	0.310 ns	−0.729 **
Inflorescences No. plant^{-1}	0.379 ns	0.511 *	−0.244 ns	−0.001 ns	0.260 ns	−0.290 ns
SPAD values	−0.182 ns	−0.646 **	0.515 *	−0.065 ns	−0.592 **	0.566 *
Yield g plant^{-1}	−0.26 ns	−0.641 **	0.442 ns	0.259 ns	−0.561 *	0.671 **
Crown and root mass g plant^{-1}	0.277 ns	0.195 ns	0.054 ns	0.336 ns	−0.076 ns	−0.054 ns

ns – correlation coefficients between variables with the significance coefficients being non-significant; significance at $P \leq 0.05$* and $P \leq 0.01$**

Discussion

Strawberry growth and yield. FD treatment decreased the number of leaves in both experimental years and the mass of crowns and roots eventually. Photosynthesis processes after FD treatment were inhibited up to one week as all the leaves were removed. Inhibited processes caused the delay in the growth of new leaves, and due to the growth delay, the competition between foliage development and flower bud initiation occurred. Thompson and Guttridge (1960) have pointed out that strawberry leaves of different maturity are able to reduce flower bud initiation and under some conditions mature leaves can act as inhibitors compared to immature leaves. In the present experiment, the negative tendency in 2013 may be explained not only by repetitive FD treatment but also by the ageing of strawberry plants and their sympodial growth; crowns had arisen above the soil surface, being more influenced by different treatments, especially by flame-defoliation.

Application of humic substances had positive effect on the leaf growth of defoliated plants only in one experimental year out of two. The effect of FD + H on the number of inflorescences became evident only in the next year after the second treatment. Regarding other plant characteristics, the effect of FD + H tended to be decreasing or showing no effect at all. According to literature, humic and fulvic acids can interact with soil nutrients and elicit physiological responses in plants leading to increased plant growth (Calvo et al., 2014). However, in the present experiment additional amendments of humic substances could have contributed to the plant recovery by improving plant nutrient uptake and therefore favoured plant growth. Still, the impact of applying humic substances to the flame-defoliated plants differed yearly as affecting the plant characteristics probably in relation to changed plant physiological processes and soil nutrient level. It has been indicated that strawberry flower bud induction is sensitive to different agronomic and nutritional factors (Savini et al., 2005). Enhanced nutrient uptake probably led to increased nitrogen assimilation which promoted plant growth and inhibited the flower bud initiation. Although additional fertilization can increase flower bud induction successfully only from a low soil nutrient base

(Breen, Martin, 1981), additional fertilization of already fertile soil can inhibit flower bud formation and reduce fruit yield. In the present experiment, high contents of soil nutrients, and additional humic acids could have had negative influence on strawberry plant growth parameters and yielding. Moreover, also the decreased number of inflorescences by FD + H treatment probably affected the strawberry yield.

In the present experiment, the strawberry yields under organic production conditions were in the case of FD + H and FD treatment respectively, in the first year from 444 to 567 g plant^{-1}, but in the second experimental year only from 189 to 215 g plant^{-1}. In conventional strawberry experimental plantations, the average yields obtained using additional fertilization were from 336 to 427 g plant^{-1} (Moor et al., 2009), which agrees with our results. According to Estonian economic analysis, an average strawberry yield, which would be still profitable, is approximately 4400 kg ha^{-1} (Vahejõe et al., 2010). In the present experiment, the average strawberry yield in the first year would have been from 6660 to 8580 kg ha^{-1}, which is higher than the Estonian average, but in the next year from 2835 to 3225 kg ha^{-1} being remarkably lower. The latter indicates that the repeated usage of strawberry flame-defoliation may not be agronomically profitable.

Strawberry fruit biochemical composition. FD had significant influence on strawberry fruit biochemical composition, but the treatment-caused variations in fruit composition differed yearly. This could have been caused by differences in weather conditions, but also by the age of the strawberry plants. Earlier findings showed that the effect of growing methods on fruit quality parameters depended significantly on environmental conditions (Moor et al., 2004; Crespo et al., 2010). In the present experiment, the temperatures in June 2013 were warmer by up to 3.1ºC than in previous years and long-term mean. Moreover, rainfall was higher in May compared to many years mean, but significantly lower in June and July during the flowering and fruiting. According to literature, plants need to adapt to changing environmental conditions for survival in increasing pressure due to biotic and abiotic stressors (Van den Ende, El-Esawe, 2014). Therefore, in addition to the effect of FD as a thermal treatment, also air temperature fluctuations and variable precipitation rates in the present study could have caused differences in plant response and hence influenced the effect of defoliation.

The interaction between flame-defoliation and humic acids application became evident. Combined treatment of FD + H increased the SSC by up to 50% in the last experimental year compared to plants that were defoliated only. Dadashpour and Jouki (2012) concluded the availability of plant nutrients as a significant factor for influencing SSC and SSC/TAC, especially in the case of higher absorption of nitrogen. Moor et al. (2004) have found that fertilization of defoliated plants had no effect on the content of soluble solids and variations in the results depend on the cultivar and plant age. Humic substances had positive influence on the TPC and ACC. The effect may be a result of the additional treatment with humic substances that advanced the defoliated plants' recovery by increasing the mass of strawberry crowns and roots. This, in turn, could have promoted nutrient uptake and therefore, increased the TPC and ACC in both experimental years too. Our findings correspond with those of Wang and Lin (2003) who reported the significant influence of improved plant nutrient uptake on the accumulation of phenolic compounds in strawberries. In addition to previous, interactions between treatments may have occurred due to plant age, as was also found by Tõnutare et al. (2009) – the content of anthocyanins showed the tendency of increasing in three-year-old plantation.

Strawberry fruit biochemical composition was found to be fruit order dependent. Anttonen et al. (2006) have also found up to 2-fold differences in accumulation of phenolic compounds and anthocyanins between fruit orders which have been related to fruit size. In the current study, in the last year of fruiting, the TPC was increased significantly in primary fruit order, but decreased in tertiary fruits due to FD which can be also related to fruit mass and fruit maturation conditions. In 2012, primary fruits were picked three weeks earlier than tertiary fruits, and in 2013 the interval was two weeks. During fruiting in 2012, the temperatures between June and July did not differ significantly, but precipitation was 38 mm higher in July. In 2013, the temperatures in June were up to 4ºC lower compared to July, which shows that tertiary fruits had warmer conditions for maturation than for example in year 2012, while the amounts of rainfall were similar in both experimental years.

The correlation analysis showed that the influence of defoliation on strawberry biochemical characteristics was related to plant growth parameters, and TAC, TPC and ACC were most affected biochemical compounds. TAC had positive correlation with the number of leaves which shows that higher number of leaves led to the increased titratable acids. This is in an agreement with Correia et al. (2011), who reported that TAC was positively related to the fresh weight of above-ground biomass and number of leaves in some strawberry cultivars. On the other hand, SSC/TAC showed positive correlation with SPAD, consequently higher chlorophyll content in leaves affected fruit taste, enhancing strawberry sweetness. Increased nitrogen application rate has been reported to increase the sugar content of strawberries which has also been correlated with increased leaf nitrogen content (Hargreaves et al., 2008). The TPC was found to be related to yield and leaf chlorophyll content (SPAD), but whether it is due to the increased plant nutrient availability and therefore increased nitrogen content in the leaves, it needs further investigations. In the present experiment, differences in leaf growth did not affect TPC but greater number of leaves decreased the ACC significantly. Anttonen et al. (2006) have found that vigorous growth and the shading effect of the leaves can be named as the main factors diminishing ACC. Therefore it can be assumed that in the present experiment, the higher number of leaves affected the ACC negatively, while the higher SPAD had positive effect.

Conclusions

1. Flame-defoliation decreased strawberry plant leaf number and fruit mass. Soluble solids content was increased in primary fruits in the first experimental year, and anthocyanins were reduced in all fruit orders in the succeeding year after the treatment. Total phenolics content was increased in primary, but decreased in tertiary fruits in the second experimental year.

2. The application of humic substances to the defoliated plants increased the number of leaves in the first year, and increased the crown and root mass eventually. The yield was decreased in both years. Soluble solids were decreased in the first experimental year, but increased in the next year. The content of total phenolics and anthocyanins was affected positively in both years.

3. From the previous it can be concluded that post-harvest defoliation, and defoliation in combination with humic substances affected strawberry plant growth parameters and fruit biochemical composition too, but the direction of the influence differed yearly and was also fruit order dependent. On the basis of the yield results obtained, the post-harvest flame-defoliation could be recommended for use in organic strawberry plantations for single treatment in the second growth year. Further experiments may be required to investigate the influence of flame-defoliation on different strawberry cultivars and fruit quality parameters.

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Braškių defoliacijos liepsna po derliaus nuėmimo įtaka augalų augimui ir vaisių biocheminei sudėčiai

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Santrauka

Bandymas su daržinės braškės (*Fragaria* × *ananassa* Duch.) veisle 'Darselect' buvo atliktas Estijos gyvybės mokslų universiteto Ekologinio ūkininkavimo tyrimų centre (58º21′ N, 26º40′ E). Tyrimo tikslas – nustatyti braškių defoliacijos tiesioginės propano liepsnos įrenginiu ir liepsnos bei papildomo huminių medžiagų derinio įtaką augalo augimui ir vaisių biocheminei sudėčiai dvejus metus iš eilės. Defoliacija sumažino lapų bei žiedynų skaičių ir vainikų bei šaknų masę. Dvimečių augalų defoliacija liepsna po braškių derliaus nuėmimo padidino vaisių derlių (2012), tačiau vėlesnė defoliacija sumažino kitų (2013) metų derlių. Defoliacijos poveikis vaisių biocheminei sudėčiai buvo esminis, tačiau skyrėsi tarp metų ir vaisių skynimo eiliškumo. Defoliacija padidino tirpių sausųjų medžiagų kiekį pirmojo skynimo vaisiuose nuo 8,9 iki 9,3 °Brix, bet sumažino antocianinų kiekį visų skynimų vaisiuose 2012 m. Apdorojimas liepsna fenolinių junginių kiekį padidino nuo 181 iki 206 mg 100 g^{-1} pirmojo skynimo vaisiuose, tačiau sumažino jų kiekį nuo 268 iki 254 mg 100 g^{-1} trečiojo skynimo vaisiuose 2013 m. Huminių medžiagų naudojimas defoliuotiems augalams padidino lapų skaičių 2012 m. ir vainikų bei šaknų masę 2013 m., tačiau abiem metais sumažino derlių. Tirpių sausųjų medžiagų kiekis skyrėsi kiekvienais metais – 2012 m. jų kiekis sumažėjo, tačiau 2013 m. padidėjo. Abiem metais huminės medžiagos turėjo teigiamos įtakos bendram fenolinių junginių ir antocianinų kiekiui.

Reikšminiai žodžiai: antocianinai, askorbo rūgštis, bendras fenolių kiekis, huminė rūgštis, tirpios kietosios dalelės, titruojamos rūgštys.

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Kati Bachman, MSc, 2014. Supervisor: Reelika Rätsep. Effect of growing site on the grapevine (*Vitis sp.*) yield quality, Estonian University of Life Sciences.

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Teadustöö põhisuunad

Bio- ja keskkonnateadused, põllumajandusteadus, aiandus

Juhendatud väitekirjad

Kati Bachman, magistrikraad, 2014. (juh) Reelika Rätsep. Kasvukoha mõju viinapuude (*Vitis sp.*) saagi kvaliteedile. Eesti Maaülikool.

Cädy Saar, magistrikraad, 2013. (juh) Ele Vool, Reelika Rätsep, Vanade õunapuude võraharvenduse mõju viljade kvaliteedile ja tervislikkusele, Eesti Maaülikool.

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LIST OF PUBLICATIONS

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

Rätsep, R., Moor, U., Vool, E., Karp, K. 2015. Effect of post-harvest flame-defoliation on strawberry (*Fragaria ×ananassa* Duch.) growth and fruit biochemical composition. *Zemdirbyste-Agriculture* 102(4): 403-410.

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1.2. Articles published in International peer-reviewed journals not indexed by Thomson Reuters or Scopus:

Vool, E., **Rätsep, R.**, Karp, K., Kruus, M., Luik, A., Veromann, E., Mänd, M. 2014. Does Thinning of Old Apple Trees Improve Fruit Quality and Decrease Pest Incidence? *International Journal of Fruit Science*: 1-14.

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

Vool, E., **Rätsep, R**., Karp, K. 2014. Effect of fertilizing on grapevine fruit maturity in Northern conditions. *Acta Horticulturae*: 231-236. Belgium: ISHS.

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

Vool, E., **Rätsep, R.**, Karp, K., Bachman, K., Moor, U. 2014. The quality of grapes in open field and in protected cultivation conditions. *Proceedings of the international conference "Horticulture in quality and culture of life"*: 839-846. Lednice, Czech Republic; 23.-26. September 2014.

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3.5. Articles/ presentations published in local conference proceedings:

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Maante, M., Vool, E., **Rätsep, R.**, Karp, K. 2015. Viinamarjade kvaliteedi mõjutamise võimalused. *Agronoomia 2015*: 185-190. Tartu: Ecoprint, 2015.

5.2. Conference abstracts:

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6.3. Popular science articles

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VIIS VIIMAST KAITSMIST

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SOIL PROPERTIES AFFECTED BY COVER CROPS AND FERTILIZATION IN A CROP ROTATION EXPERIMENT
VAHEKULTUURIDE JA VÄETAMISE MÕJU MULLA OMADUSTELE KÜLVIKORRAKATSES
Dotsent **Endla Reintam**, emeriitprofessor **Anne Luik**
11. veebruar 2016

KRISTI PRAAKLE
CAMPYLOBACTER SPP. AND *LISTERIA MONOCYTOGENES* IN POULTRY PRODUCTS IN ESTONIA
CAMPYLOBACTER SPP. JA *LISTERIA MONOCYTOGENES* LINNULIHATOODETES EESTIS
Professor **Mati Roasto**, professor **Marja-Liisa Hänninen** (Helsingi Ülikool), professor **Hannu Korkeala** (Helsingi Ülikool)
4. märts 2016

TARMO KALL
VERTICAL CRUSTAL MOVEMENTS BASED ON PRECISE LEVELLINGS IN ESTONIA
MAAKOORE VERTIKAALLIIKUMISED EESTIS TÄPPISNIVELLEERIMISTE ANDMETEL
Emeriitprofessor **Jüri Randjärv**, dotsent **Aive Liibusk**
29. aprill 2016

ELSA PUTKU
PREDICTION MODELS OF SOIL ORGANIC CARBON AND BULK DENSITY OF ARABLE MINERAL SOILS
MINERAALSETE PÕLLUMULDADE ORGAANILISE SÜSINIKU JA LASUVUSTIHEDUSE STATISTILISED PROGNOOSIMUDELID
Professor **Alar Astover**, dotsent **Christian Ritz** (Kopenhaageni ülikool)
14. juuni 2016

ALICE AAV
PHENOTYPIC CHARACTERISATION OF POTATO LATE BLIGHT PATHOGEN *PHYTOPHTHORA INFESTANS* IN BALTIC COUNTRIES
KARTULI-LEHEMÄDANIKU TEKITAJA *PHYTOPHTHORA INFESTANS* BALTIKUMI POPULATSIOONIDE FENOTÜÜBILINE ISELOOMUSTAMINE
Dotsent **Eve Runno-Paurson**, professor **Marika Mänd**
20. juuni 2016

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