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Carbohydrate Storage in Buds of 'Kerner', 'Solaris', 'Regent' and 'Rondo' Grapevines

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Abstract

The purpose of this study was; to compare sugar content and composition in buds of four in Sweden commercially grown grapevine cultivars (‘Kerner’, ‘Regent’, ‘Rondo’ and ‘Solaris’), at three different vineyards in Scania and try to investigate the relationship between sugar content and cold hardiness. The concentration of fructose, glucose, sucrose, raffinose and stachyose was determined in the lower positioned buds, 2-8 and upper positioned buds, 9-14 of the grapevine shoots with high performance liquid chromatography (HPLC).

The cultivar ‘Solaris’ seems to be more cold tender than ‘Kerner’, ‘Regent’ and ‘Rondo’ when comparing the ratio of fructose and glucose to sucrose. This ratio is strongly related to cold hardiness in *Vitis vinifera* grapevine cultivars. The ratio increases as the two monosaccharides fructose and glucose accumulates. No clear difference in the ratio could be seen between the other cultivars.

Lower positioned buds showed a higher ratio than upper positioned buds, significantly in more than half of the observations (53.3 %).

Differences between vineyards showed no clear patterns when comparing the ratio. This is probably because of differences in many cultural practices that may influence cold hardiness such as choice of rootstock, fertilisation strategy, temperature in the vineyard, and occurrence of diseases.

No correlation was found between the temperature on the sampling day and the mean temperature five days preceding sampling and sugar concentration.

Sammanfattning

Syftet med den här studien var att jämföra sockerkoncentration och sammansättning i knoppar hos fyra kommersiellt odlade vindruvssorter (’Kerner’, ’Solaris’, ’Regent’ och ’Rondo’) vid tre olika vingårdar i Skåne och att försöka förstå om det finns en tydlig korrelation mellan sockerkoncentrationen och vinterhärdigheten i de övervintrande knopparna. Koncentrationen av fruktos, glukos, sukros, raffinos och stackyos bestämdes i knoppar från skottets nedre del och skottets övre del med hjälp av high performance liquid chromatography (HPLC).

Sorten ’Solaris’ verkar mindre vinterhärdig än ’Kerner’, ’Regent’ och ’Rondo’ när man jämför kvoten fruktos + glukos / sukros. Den här kvoten är kopplad till vinterhärdighet i *Vitis vinifera*. Ingen tydlig skillnad kunde ses i kvoten mellan de andra sorterna.

Knoppar från skottets nedre del hade en högre kvot än knopparna från skottets övre del, i mer än hälften av observationerna (53,3 %).

Inget tydligt mönster kunde ses när man jämförde kvoten mellan vingårdarna. Troligen kan ingen tydligen skillnad ses på grund av olika odlingsförhållanden och metoder som praktiseras vid de olika vingårdarna. Klimatet, sjukdomstrycket och användning av grundstammar och gödsel skiljer sig mellan de olika vingårdarna.

Ingen korrelation kunde upptäckas mellan temperaturen i luften samma dag som provtagning eller lufttemperaturen 1-5 dagar före provtagningen och sockerkoncentrationen.

Index

1. Introduction

1.1. Purpose of the study

The purpose of this thesis was to compare sugar content and composition in buds of four in Sweden commercially grown grapevine cultivars (‘Kerner’, ‘Regent’, ‘Rondo’ and ‘Solaris’), at three different vineyards in Scania and try to investigate the relationship between sugar content and cold hardiness. The method of choice was to determine the concentration of sugars (fructose, glucose, sucrose, raffinose and stachyose) in the lower positioned buds, 2-8 and upper positioned buds, 9-14 of the grapevine shoots. Apart from the experimental investigation, a literature study about correlation between sugar content and cold hardiness has also been conducted with focus on woody plants and especially on grapevine.

1.2. Hypothesis

1) *V. vinifera* cultivar ‘Kerner’ shows clear differences in sugar content and profile compared to the three hybrid cultivars ‘Rondo’, ‘Regent’ and ‘Solaris’.
2) Buds positioned at the upper part of the shoot differ in sugar content from buds positioned at the lower part of the shoot indicating that the upper positioned buds are less cold resistant.

2. Background

2.1. Grapevine

In the 2007 statistics from the Food and Agriculture Organisation of the United Nation (FAO), grapevine is listed as the number one fruit crop in the world when it comes to area utilized for production. In terms of million tonnes produced, only banana (including plantain) exceeds it. In value, grapevine exceeds all other fruit crops due to its multiple use for wine, rasin, juice, jelly and fresh fruit.

Grapevine belongs to the genus *Vitis* and the family Vitaceae. Vitaceae is primarily distributed in tropical or subtropical parts of the world but *Vitis* is mainly a temperate zone genus, occurring sexual reproductive only in the Northern hemisphere (Jackson, 2000). *Vitis* is divided into two subgenera, *Euvitis* and *Muscadinia* (Gray et al., 2005). *Vitis vinfera* is the most important commercial species and belongs to the subgenera *Euvitis*, which is divided into three groups; an American group, an Asian group and a European or central Asian group which only contains one species, *V. vinifera*. The *V. vinifera* species is believed to originate from Caucasus, between the Black Sea and the Caspian Sea (Gustafsson & Mårtensson, 2005).

Most cultivated grapevine cultivars around the world are bred from pure *V. vinifera* or are hybrids between *V. vinifera* and other *Vitis* species. Species that have been used in breeding of grapevine cultivars because of desired traits as disease, pest and abiotic stress resistance can be seen in table 1.

Table 1: Germplasm used in grapevine breeding, according to Allewelldt & Possingham, (1988). In bold species from the Asian group, underlined species from the European group and in italic species from the American group.

Abiotic stress factor	
Winter hardiness	***V. amurensis***, *V. riparia*
Chlorosis	*V. vinifera, V. berlandieri*
Fungus diseases	
Plasmopara viticola	*V. riparia, V. rupestris, V. lincecumii, V. aestivalis, V. cinerea, V. berlandieri, V. labrusca*
Uncinula necator	*V. aestivalis, V. cinerea, V. berlandieri, V. labrusca*
Botrytis cinerea	American species, *V. vinifera*
Pseudopeziza tracheiphila	*V. vinifera*
Bacteria	
Agrobacterium tumefaciens	***V. amurensis***, *V. labrusca*
Nematodes	
Meloidogyne sp.	*V. champini*
Xiphinema sp.	*V. rufotomentosa*
Insects	
Phylloxera vastatrix	*V. riparia, V. rupestris, V. berlandieri, V. cinerea, V. champini*

2.2. Grapevine cultivation in Sweden

In the year of 2006 there were four companies in Sweden selling own produced wine on the market (SJV, 2009). About 20 growers had established vineyards for future possibilities to produce wine commercially. The total production area for grapevines was 10 hectares.

2.3. Cold hardiness

The ability to survive adverse winter conditions is an important characteristic of woody perennial plants living in the temperate zone (Ashworth et al., 1998). Low mid-winter temperatures generally are limiting to successful growth of different species. In addition, selection of favourable sites to reduce the likelihood of freezing injury is a primary consideration affecting the location of commercial fruit plantings in temperate regions. Poor winter hardiness results in substantial economic losses in these kinds of plantings.

Plants that are “cold hardy” have a genetic potential to withstand low temperatures. Also environmental signals play an important role in cold hardiness by inducing cold acclimation. Acclimation (hardening off) is essential for many woody plants to be able to survive low temperatures. Decreasing photoperiod and low non freezing temperatures are two important inducing environmental signals.

2.3.1. Mechanism of freezing

The mechanism that allows plant to withstand temperatures below 0ºC is classified in two main categories: (1) avoidance of freezing through supercooling and (2) tolerance of freezing by extracellular ice formation (Banuelos et al., 2008). The freezing point of a plant is always below that of pure water (Levitt, 1980). When the plant is cooled, its temperature normally drops below its freezing point without ice formation, known as supercooling. Extracellular freezing is a process in which ice forms on the surface of a cell or between the cell wall and the protoplast. It occurs in all plants in temperate climates during winter freezing temperatures after slow freezing and minor supercooling and can lead to injury depending on the freezing temperature (Levitt, 1978).

Water freezes in different ways in moderately winter hardy and extreme freeze tolerant woody plants (Ashworth et al., 1998). Species that exhibit deep supercooling are moderately cold hardy. Species such as grapevine, apple, cheery and pear among others belong to this group. They are limited to where the minimum winter temperature stays above -40 ºC. The most cold hardy woody plants do not exhibit deep supercooling, such as aspen, whose cells may survive temperatures as low as -196 ºC (Banuelos et al., 2008). The level of freezing tolerance in these species reflects cellular tolerance to the stresses accompanying extracellular ice formation and cell dehydration.

2.3.2. Physiological factors

The onset of cold hardiness has been associated with increase in total protoplasm, total sugars, proteins, nucleic acids and starch hydrolyses (Stushnoff et al., 1998). These compounds increase cell sap concentration which increases freezing tolerance (Levitt,1980). The increase in cell sap concentration is however unable by itself to account for the whole effect of the increasing in freezing tolerance that accompanies it.

2.3.2.1. Sugars

It is believed that the major change in cell sap concentration is due to changes of concentration of sugars. The sugar concentration usually increases in the fall when plants harden, and decreases in the spring as plants deharden. An increase of total soluble sugars (TSS) and a decrease in starch content has been observed to be synchronized with an increase in freezing tolerance in many plant species. Sugars may increase freezing tolerance in two ways, by the osmotic effect and by the metabolic effect. Through accumulating in the vacuole, sugar can decrease the amount of ice formed, and thereby increase the avoidance of freeze-induced dehydration. Secondly, by being metabolised in the protoplasm at low, hardening temperatures, they produce unknown protective changes. Many hardy plants can survive much lower temperatures than those that might be expected regarding to the sugar concentration theory, which may favour the metabolic explanation.

The sugars raffinose and stachyose belongs to the raffinose family of oligosaccharides (RFO). They increase in concentration in woody cold hardy plants during the winter months (Stushnoff et al., 1998). The RFO are significantly correlated with cold hardiness in many species e.g. ‘Red delicious’ apple (*Malus domestica*), ‘Red Lake’ current (*Ribes rubrum*), western sandcherry (*Prunus besseyi*) and ‘Valiant’ grapevine (*V. riparia x V. labrusca*). In less hardy woody plants as *V. vinifera* the metabolism differs. The RFO do not increase as much in *V. vinifera* during the winter months as in *V. riparia*, *V. labrusca* and other more cold hardy woody species. Hamman et al. (1996) concluded that the ratio of fructose plus glucose to sucrose is strongly related to cold hardiness in *V. vinifera* grapevine cultivars. This ratio increases as the two monosaccharide’s fructose and glucose accumulate. Probably the monosaccaride synthesis pathway is interfering with the RFO pathway, which uses sucrose as a substrate for synthesis of raffinose and stachyose. In some other cold hardy taxa, fructose and glucose are sometimes together with the disaccharide sucrose physiologically high during the dormant season, but fluctuate from month to month and are not significantly correlated with cold hardiness (Stushnoff et al., 1993).

2.3.3. Cold acclimation and dormancy

In many temperate woody plants, growth cessation and the induction of dormancy are necessary for acclimation and a decreasing photoperiod is the environmental signal promoting the inductive process (Fenell & Mathiason, 2002). Two types of cold acclimation can be distinguished (Sakai & Larcher, 1987). One is categorized by species that respond directly to low winter temperatures. Plants growing in areas with a mild winter usually belong to this group. As temperature drops resistance is enhanced. But if these plants are kept in the warm the resistance will remain low. The second category of cold acclimation involves species with inherent periodicity in resistance. Woody plants of the temperate zone belong to this category. In autumn they begin a gradual transition to developmental arrest and in spring they resume activity again. This development of dormancy will take place even though temperature remains high. Freeze-induced dehydration is an important step in cold acclimation to avoid freezing of dormant parts as buds (Stushnoff et al., 1998).

2.3.4. Cold hardiness in grapevine

The effect of winter injury can be extensive, complex and devastating for vineyard business (Zabadal et al., 2007). The European *V. vinifera* cultivars are generally cold tender and more susceptible to winter injury than hybrids of American species. Among commercially grown species, *V. riparia* appears to be most cold hardy.

Cold hardiness and frost resistance in grapevines varies over time (Seyedbagheri & Fallahi, 1994). One factor controlling the resistance at a given time is the temperature that the grapevine has previously been exposed to. Hamman et al. (1996) showed that sugar concentration in buds and bud cold hardiness, measured by lowest survival temperature (LST_{100}) determined by visual browning were correlated to mean minimum temperature 2- 15 days before sampling. Badulescu & Ernst (2006) showed that the mean temperature 1-3 days before sampling date is highly correlated to the sugar concentration in grapevine buds and the correlation increases when including up to 7 days before sampling. When considering a longer time period than 7 days before sampling, no correlation could be found.

Frost resistance in one cultivar varies during the year, between years, and between sites (Eifert, 1975). However, the difference between cultivars shows patterns associated to their degrees of resistance.

2.3.5. Cold acclimation and dormancy in grapevine

Usually production regions are recommended on the basis of midwinter minimum temperature. However, injury during unseasonable low temperatures in the fall when the canes are maturing and acclimating can be as damaging as low midwinter temperatures (Fenell & Mathiason, 2002). Acclimation and freezing tolerance characteristics are very important in grapevine as many production regions incur damaging freezes in fall, winter and spring. The response to decreasing photoperiod or short days (SD) varies between different grapevine species (Fenell & Hoover, 1991). *Vitis labrusca* exposed to SD shows greater periderm development, reduction in growth, and onset of bud dormancy. The same responses can be seen in *V. riparia* which exhibit a classic two stage acclimation response with induction of dormancy and acclimation occurring in response to short photoperiod at high temperatures (Fenell & Mathiason, 2002). Under short photoperiod growth ceases, node tissue mature, dormancy is induced, freezing tolerance of the bud increases, and the ability to supercool is enhanced by changes in the stem tissue adjacent to the bud. Subsequent exposure to subzero temperatures induces a rapid increase in freezing tolerance in *V. riparia*. In contrast, the cultivar 'Seyval Blanc', a hybrid of *V. vinifera* and most truly *V. lincecumii* and *V. rupestris*, does not acclimate in response to decreasing photoperiod (Smiley, 2008). Under SD, growth continues and dormancy and acclimation is not induced. Most *V. vinifera* cultivars are relatively insensitive to photoperiod, and they are instead influenced by temperature changes (Jackson, 2000).

2.3.6. Cultural practises affecting cold hardiness in grapevine

2.3.6.1. Vineyard site selection

Site selection might be the most important choice a new grower can make (Zabadal et al., 2007). Topography has a strong influence of the mesoclimate in the vineyard. A slope reduces the vineyards potential for winter injuries by drawing cold air away. Slopes adjacent to lakes and rivers are beneficial due to the air circulation created by the temperature differential between land and water, which can diminish the development of temperature inversions and radiative frost development (Jackson, 2000). There are two weather conditions that produce freezing temperatures; (1) rapid radiational cooling at night and (2) introduction of cold air mass with temperatures below freezing, which occurs when it is cloudy and windy. In a radiation freeze, plant tissue and thermal radiation from the warm air at the ground loose their heat to the night sky, when it is very clear and there is no wind (Cornell University, 2009). The lowest temperatures are found at the ground level and increases with elevation. Cold air is denser than warm air and is therefore found at the ground level, with usually a layer of warmer air above it. This makes cold air flow down hill. As long as nothing interrupts this flow cold air will move out from a vineyard located on a slope. Any barriers of movement of cold air should be eliminated. A sunny south turning slope might seem beneficial for location of a vineyard but it might also cause a problem. A lot of sun in the winter can cause the grapevine to deharden, but this might be a major problem at more southern latitudes.

2.3.6.2. Training system

The training system may influence the bud survival (Seyedbagheri & Fallahi, 1994). A low training system with ridged plants might be the best way to protect the grapevine during the winter. But it is also extremely laborious and if not uncovered in time the grapevine may rot. A high training system as a high positioned cordon benefits on the other hand over a low positioned cordon when it is not covered due to the avoidance of low cold air. Kubecka (1968) measured the decline in temperature with decreasing height above the soil surface as 1 ºC per 50 cm change in height. When early fall frost is a concern for a vineyard located on a slope, low trained wines may be an option since heat radiation from the soil combined with the cold air flow may minimize the drop in air temperature and thereby frost damage (Jackson, 2000).

2.3.6.2. Cultivar

More cold hardy cultivars are becoming available and breeders continue to develop more cold hardy cultivars with good wine quality (Zabadal et al., 2007). Unfortunately the most popular choices of cultivars by growers and consumers are often the most cold tender ones. One way to reduce the risk of losses due to winter injuries is to diversify a cultivar mix.

2.3.6.3. Fertilization

Nitrogen fertilization in late fall can reduce cold hardiness, since it promotes growth of the grapevine and delay acclimation (Seyedbagheri & Fallahi, 1994). Plant nutrients should be applied in a proper amount and at the right time to increase the yield and to provide an adequate degree of hardening. Poor fertilization is also a factor that can contribute to reduced cold hardiness affecting photosynthesis and carbohydrate storage.

2.3.6.4. Healthy foliage

A healthy foliage is important to keep up as good photosynthetic capacity as possible. Therefore it is important to control diseases and insects that may reduce leaf area. Accumulated carbohydrates produced during the season increases the cold hardiness by improving cane maturation. A powdery mildew infection that reduces the photosynthetic capacity of the grapevine can reduce cold hardiness due to poorly matured canes. Reduced carbohydrate accumulation could also limit the supercooling of cellular fluids (Jackson, 2000). Also defoliation cause reduced cold hardiness (Mansfield and Howell, 1981).

2.3.6.5. Irrigation

Water sprinklers in a vineyard can protect against frost damage in the spring. As water is added and freezes it releases heat. If this method is used in the fall, it might stimulate growth and thereby cause increased frost damage instead. Water sprinklers are most efficient against radiative frost (Jackson, 2000).

2.3.6.6. Cover crop

A cover crop between the rows takes up excess water and nitrogen that might delay acclimation (Mansfield and Howell, 1981). Weeds within the row should be controlled so that the competition will not be too high. Maintaining the soil immediately under the grapevines free of vegetation can also be good since bare soil both absorb more heat during the day and releases more heat during the night than covered soil does (Jackson, 2000).

2.3.6.7. Rootstocks

A major increase in use of rootstocks came with the phylloxera epidemic around 1860. Before that rootstocks in grapevine production were only used in special cases. Since then grafting onto American *Vitis* species, that are resistant to phylloxera became a must. But a rootstock can also pass on other wanted characteristics, such as cold hardiness.

A cold hardy rootstock appears to increase the resistance of the scion to cold damage (Jackson, 2000). Hardy rootstock generally have less bark tissue, contain smaller phloem and ray cells, and possess woody tissue with narrow xylem vessels. The cold hardy rootstocks may hold back scion vigour, modify the content of growth regulators and may earlier limit water availability. A strong relationship between declining cane water content and early acclimation has been described by Miller et al. (1988). Tissue dehydration protects against ice crystal formation that rupture cell membranes in no-acclimated plants, causing freezing injury. It could be expected that any rootstock that affects biochemical changes and tissue dehydration may alter cold hardiness. Water content is also important during deacclimation since tissue needs to rehydrate to resume growth. The rootstocks SO4 (Selection Oppenheim No. 4) and Kober 125AA are used by Swedish growers. SO4 has *V. riparia* and *V. berlandieri* in its parentage. As mentioned before, *V. riparia* is very cold hardy and *V. berlandieri* was mainly chosen because of its tolerance to high pH. Kober 125 AA has the same parentage as SO4 (Shaffer et al., 2004). Some differences between SO4 and 125 AA are demonstrated in table 2.

Table 2: Rootstock characteristics, according to Shaffer et al. (2004). 1 = lowest, shortest, susceptible, 4 = highest, longest, resistant

Characteristics	**SO4**	**125AA**
Adaptability to shallow, dry, clay soil	1	3
Adaptability to deep slit or loam	1	2
Adaptability to deep dry sandy soil	1	1
Tolerance of water logged soil	3	-
Tolerance of lime	2	4
Tolerance of acid soil	3	3
Nitrogen uptake	4	4
Vigour conveyed to grapevine	3	4
Drought tolerance	2	4
Resistance to ring nematode	2	-
Tendency to overbear	1	2
Time to ripening	2	4

3. Material and Methods

3.1. Plant material

During the winter of 2008/2009, samples of the grapevine cultivars ‘Kerner’, ‘Solaris’, ‘Regent’ and ‘Rondo’ (table 3) were collected from three different vineyards in the south of

Sweden. ‘Kerner’ was only collected at Domän Sånana for all the three sampling dates. ‘Solaris’, ‘Regent’ and ‘ Rondo’ were chosen because they are standard cultivars grown in Sweden. ‘Kerner’ was chosen since it is a pure *V. vinifera* cultivar with no other species in its pedigree and therefore could be expected to have a different sugar concentration and a fluctuating pattern compared to the other cultivars used in this study that are hybrids.

Table 3: Cultivar information from VIVC, Vitis International Variety Catalog.

Cultivar	**Colour of berry**	**Breeding year**	**Institution**	**Cross**	**Species**
‘Solaris’	Green	1975	Staatliches Weinbauinstitut Freiburg, Germany	‘Merzling’ X ‘Geisenheim 6493’	*V.vinifera,* *V.amurensis*
‘Rondo’	Blue	1964	Forschungsanstalt Geisenheim, Germany	‘Zarya Severa’ X ‘St. Laurent’	*V.vinifera,* *V.amurensis*
‘Kerner’	Green	1929	Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau, Lauffen, Würrtemberg, Germany	‘Riesling’ X ‘Trollinger’	*V.vinifera*
‘Regent’	Blue	1967	Julius Kühn-Institut Bundesforschungsinstitut für Kulturpflanzen (JKI), Geilweilerhof, Germany	‘Diana’ X ‘Chambourcin’	*V.aestivalis,* *V.berlandieri,* *V.cinerea,* *V.labrusca,* *V.lincecumii,* *V.riparia* *V.rupestris*, *V.vinifera*

3.2. Vineyards

3.2.1. Domän Sånana, Skillinge (latitude,55°27'N; longitude,14º16'E)

The vineyard covers a planting of 700 grapevines on an area of 0.5 ha. Samples were collected on December 4, January 21 and March 4 from grapevines planted between 2002-

2006. ‘Rondo’ was grafted on SO4 rootstocks. From ‘Solaris’, the samples were taken from grapevines grown on own roots as well as grafted on SO4. ‘Kerner’ and ‘Regent’ was grown on own roots. The soil type was sandy with a pH of 4.9. The grapevines were spaced 1.35 m apart with 2 m between rows. The rows were planted in northwest –southeast direction. The spaces between the rows were covered with grass and trimmed regularly. Within the rows weeding was mainly handled manually by hand hoeing, though Roundup was applied once in the autumn and then the soil within the row was tilled. The grapevines were trained to double guyot. In february a soil amendment, calcium magnesium carbonate ($CaMg(CO_3)_2$) was added within the rows, in an amount of 3000 kg/ha. Pesticide applications were uniform across the vineyard. The plant protection products Kumulus (BASF, USA), Candit (BASF, USA) and Teldor (Bayer CropScience, Germany) were used against powdery mildew and grey mold. No fertilization practises were undertaken during the last season. The field was not irrigated. The harvest dates the season previous to this study, in 2008 were for ‘Solaris’ September 25, ‘Regent’ October 13, ‘Rondo’ October 26 and for ‘Kerner’ October 27. The field was protected from the north by houses and deciduous trees, in east and south by a pine forest and in west newly planted wind protection is organised.

3.2.2. The vineyard in Åhus (latitude,55º54'N, longitude,14º15'E)

This vineyard covers about 1 ha with a total of 3000 grapevines. Samples were collected on December 2, January 20 and March 2 from vines planted in 2005 except ‘Kerner’ that was planted in 2007. ‘Kerner’ grapevines were grown on own roots. ‘Solaris’, ‘Regent’ and ‘Rondo’ were grafted on SO4 rootstocks. The soil type is sandy with small parts of clay. The south part of the field contains more clay. The pH in the field varies between 6.5 – 8.2. The grapevines were spaced 1.25 m apart with 2.25 m between rows. The rows were planted in north-south direction. The spaces between the rows were tilled during the winter. Within the rows the weeding was handled by hand hoeing. During the summer the grass was allowed to take over and it was then kept trimmed. The grapevines were trained to a simple guyot. Fertilizer, and pesticide application were uniform across the vineyard. Once in the spring, fertilization was applied in the form of NPK 11-5-19 (Lantmännen, Sweden), in an amount of 100kg/ha. Sulfur was sprayed a couple of times during the season as leaf nutrition, thereby also giving a protection against fungi. Plant protection products used are Teldor (Bayer CropScience, Germany) and Candit (BASF, USA). No irrigation is used in the field during the season. The harvest dates the season previous to this study, in 2008 were for ‘Solaris’ September 27, ‘Rondo’ October 11 and ‘Regent’ October 18. A forest of spruce in the west allows wind protection. In 2006 wind protection in form of different trees was planted in the north part of the field, but they were not yet old enough to provide sufficient wind protection.

3.2.3. Nangijala vineyard, Klagshamn (latitude,55º32'N, longitude,12º55'E)

The vineyard covers 1 ha, with 4000 grapevines. Samples were collected on, December 3, January 19 and March 3 from ‘Rondo’ and ‘Regent’ planted in 2001 and from ‘Solaris’ planted in 2003. ‘Rondo’ was grafted on SO4 rootstock, ‘Solaris’ on AA125, and ‘Regent’ grapevines were grown on own roots. The soil type was mainly consisting of clay with parts of sand, calcium and flintstone with a pH between 7.4-7.8. ‘Rondo’ was spaced 1.66 m apart and ‘Solaris’ and ‘Regent’ were spaced 1.25 m apart, but all were having the same row distance (2 m). Grass was kept between the rows and trimmed regularly. Within the rows weeding was handled manually by hand hoeing. The rows were planted in north – south direction. The grapevines were trained to simple guyot, except ‘Rondo’ that was trained to double guyot. No irrigation was conducted the season previous to this study, instead roots were pruned to allow them to seek downwards for water. Fertilizer, application in the form of magnesium sulphate was applied 7-8 times per season and sulphur was sprayed at the same frequency. Ash and ground wood was also applied within the rows. The start of harvest, the season previous to this study, in 2008 were for ‘Solaris’ between September 26 and 27, for ‘Rondo’ and ‘Regent’ between October 8 and 18. Poplar trees provided wind protection in the directions west, east and south.

3.3. Experimental design

Buds of grapevine shoots divided into lower positioned buds and upper positioned buds of three to four different cultivars grown at three different vineyards and collected at three different dates (December-March) were divided into 60 different treatments replicated in four blocks giving 240 observations in total (table 4). The blocks in the different vineyards were placed differently depending on vineyard design.

Table 4: Treatments

Collected Samples, no.	Vineyard	Cultivar	Position	Sampled month	Collected Samples, no.	Vineyard	Cultivar	Position	Sampled month
1	Sånana	Kerner	Lower	Dec	31	Åhus	Rondo	Lower	Jan
2	Sånana	Kerner	Upper	Dec	32	Åhus	Rondo	Upper	Jan
3	Sånana	Solaris	Lower	Dec	33	Åhus	Regent	Lower	Jan
4	Sånana	Solaris	Upper	Dec	34	Åhus	Regent	Upper	Jan
5	Sånana	Rondo	Lower	Dec	35	Nangijala	Solaris	Lower	Jan
6	Sånana	Rondo	Upper	Dec	36	Nangijala	Solaris	Upper	Jan
7	Sånana	Regent	Lower	Dec	37	Nangijala	Rondo	Lower	Jan
8	Sånana	Regent	Upper	Dec	38	Nangijala	Rondo	Upper	Jan
9	Åhus	Solaris	Lower	Dec	39	Nangijala	Regent	Lower	Jan
10	Åhus	Solaris	Upper	Dec	40	Nangijala	Regent	Upper	Jan
11	Åhus	Rondo	Lower	Dec	41	Sånana	Kerner	Lower	March
12	Åhus	Rondo	Upper	Dec	42	Sånana	Kerner	Upper	March
12	Åhus	Rondo	Upper	Dec	43	Sånana	Solaris	Lower	March
13	Åhus	Regent	Lower	Dec	44	Sånana	Solaris	Upper	March
14	Åhus	Regent	Upper	Dec	45	Sånana	Rondo	Lower	March
15	Nangijala	Solaris	Lower	Dec	46	Sånana	Rondo	Upper	March
16	Nangijala	Solaris	Upper	Dec	47	Sånana	Regent	Lower	March
17	Nangijala	Rondo	Lower	Dec	48	Sånana	Regent	Upper	March
18	Nangijala	Rondo	Upper	Dec	49	Åhus	Solaris	Lower	March
19	Nangijala	Regent	Lower	Dec	50	Åhus	Solaris	Upper	March
20	Nangijala	Regent	Upper	Dec	51	Åhus	Rondo	Lower	March
21	Sånana	Kerner	Lower	Jan	52	Åhus	Rondo	Upper	March
22	Sånana	Kerner	Upper	Jan	53	Åhus	Regent	Lower	March
23	Sånana	Solaris	Lower	Jan	54	Åhus	Regent	Upper	March
24	Sånana	Solaris	Upper	Jan	55	Nangijala	Solaris	Lower	March
25	Sånana	Rondo	Lower	Jan	56	Nangijala	Solaris	Upper	March
26	Sånana	Rondo	Upper	Jan	57	Nangijala	Rondo	Lower	March
27	Sånana	Regent	Lower	Jan	58	Nangijala	Rondo	Upper	March
28	Sånana	Regent	Upper	Jan	59	Nangijala	Regent	Lower	March
29	Åhus	Solaris	Lower	Jan	60	Nangijala	Regent	Upper	March
30	Åhus	Solaris	Upper	Jan					

3.4. Carbohydrate analyses

Buds collected from the three different sample periods in December 2008, January 2009 and March 2009 were used in the carbohydrate analyses. Samples were taken as 7-10 shoots from each of four different blocks in the different fields. Shoots were collected between 8.30 am and 11.30 am to avoid daily fluctuations and then transported by car to the Swedish Agriculture University (SLU) in Alnarp. The shoots were separated in two parts; the lower with the 2-8 buds counting from the base of the shoot and the upper part with the buds 9-14. The buds from the different positions were removed and stored in -80ºC until further used. Later the buds were freeze dried for 3-4 days. Then they were grounded in a mill (Yellow line, Kika Werke, Germany) and stored in -20 ºC. Soluble carbohydrates were extracted from dried tissue (70 mg) with 1 ml of 70 % ethanol in the -20 ºC freezer for 14 days. Later the mixtures were centrifuged at 13000 rpm for 5 minutes. From the supernatant, 100 µl were placed in 300 µl vials. Soluble sugars were quantified using high performance liquid chromatography (HPLC). Samples of 20 µl were injected automatically with an Autosampler (HPLC Autosampler 465, Kontron Instruments, USA) onto a NH_2 column at 25 ºC. The mobile phase (65:35 acetonnitrile/water) was supplied by an isocratic pump (515 HPLC Pump,Waters, USA) at 1 ml/min. After leaving the column, the separated sugars were detected using a refractive index detector (RefractoMonitor IV, LDC- Analytical, USA) whose output were linked to a chart recorder (Analog-Digital, HPLC Technology, UK). Standards of pure sugars (fructose, glucose, sucrose, raffinose and stacyhose) were used for calculation of the sugar concentration in the extracts.

3.5. Temperature data

Temperature data was obtained from the Swedish Meteorological and Hydrological Institute (SMHI). Three temperature measuring stations located as close to the vineyards as possible were chosen. The stations were Skillinge A (5429) latitude, 55º29'N, longitude, 14º19'E, located 4.6 km north east of Domän Sånana, Malmö A (5235) latitude, 55º34'N, longitude, 13º04'E, located 10 km north east of Nangijala Vineyard and Kristianstad (6403) latitude, 56º00'N, longitude,14º17'E, located 12 km north of the Vineyard in Åhus.

3.6. Statistical analyses

Statistical analyses were undertaken with Minitab 15. Data were analysed using ANOVA with least significant differences (0.05), using the application general linear model. Pearsons's correlation coefficient was used for analysis of correlation.

4. Results

4.1. Bud position

Putting all treatments and replicates together for comparing bud position, no significant differences could be find for fructose, glucose, sucrose, raffinose, stachyose or TSS. For the ratio (fructose+glucose)/ sucrose, a significant difference between lower (bud 2-8) and upper (bud 9-14) positioned buds was found (p = 0.007 (**)). Further investigations were conducted by comparing the ratio in buds positioned at the lower and the upper part of the shoots for all the 60 treatments individually (figure 1). All pair-wise comparison except one show a higher ratio in the lower positioned buds when only considering the mean value of the four replicates, though only sixteen of the comparisons (32 treatments) show a significantly higher ratio in the lower positioned buds.

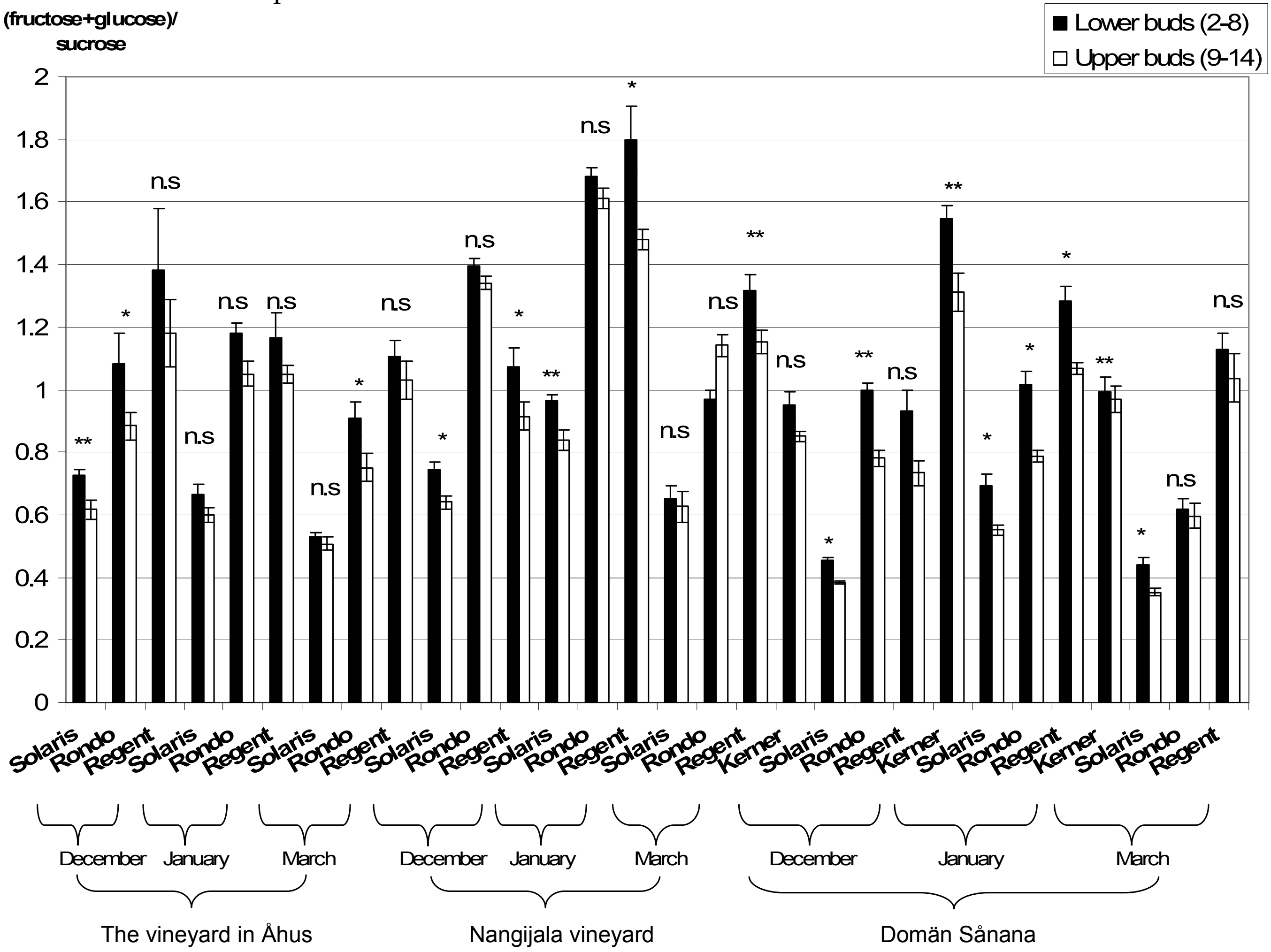

Figure 1: Ratio, (fructose + glucose) / sucrose between lower and upper positioned buds. Data are means ± SE from four replicates.

4.2. Cultivar differences

When comparing the different cultivars the ratio was once more considered. The ratio of ‘Solaris’ is in general lower than the ratio for the other three cultivars (table 5). In 15 of the 18 comparisons of cultivars, ‘Solaris’ shows a significantly lower ratio than the other three cultivars. More often can a higher ratio be found in ‘Regent’ compared to ‘Rondo’. But no trend of significantly difference could be seen between ‘Kerner’, ‘Regent’ and ‘Rondo’. One exception at Domän Sånana is illustrated in figure 2.

Table 5: Ratio (fructose+glucose) / sucrose calculated as mean value of four replicates.

Vineyard	**Sampled, month**	**Bud position**	**’Kerner’**	**’Regent’**	**’Rondo’**	**’Solaris’**
Åhus	December	Lower		1.38A[1]	1.08 AC	0.72 BC
Åhus	December	Upper		1.18A	0.88AC	0.62BC
Nangijala	December	Lower		1.07A	1.40B	0.74C
Nangijala	December	Upper		0.92A	1.34B	0.64C
Sånana	December	Lower	0.95A	0.93A	1.00A	0.46B
Sånana	December	Upper	0.85A	0.73BC	0.78AC	0.38D
Åhus	**January**	**Lower**		**1.17A**[2]	**1.18A**	**0.67B**
Åhus	January	Upper		1.05A	1.05A	0.60B
Nangijala	**January**	**Lower**		**1.80A**	**1.68A**	**0.96B**
Nangijala	January	Upper		1.48A	1.61A	0.84B
Sånana	**January**	**Lower**	**1.55A**	**1.28B**	**1.02C**	**0.70D**
Sånana	January	Upper	1.31A	1.07B	0.79C	0.55D
Åhus	March	Lower		1.11A	0.91B	0.53C
Åhus	March	Upper		1.03A	0.75B	0.51C
Nangijala	March	Lower		1.31A	0.97B	0.65C
Nangijala	March	Upper		1.15A	1.14A	0.63B
Sånana	March	Lower	0.99A	1.13A	0.62B	0.44B
Sånana	March	Upper	0.97A	1.04A	0.60B	0.35C

[1] Different letters within the row show significant difference at the 5 % level.

[2] Data in bold is illustrated in figure 2.

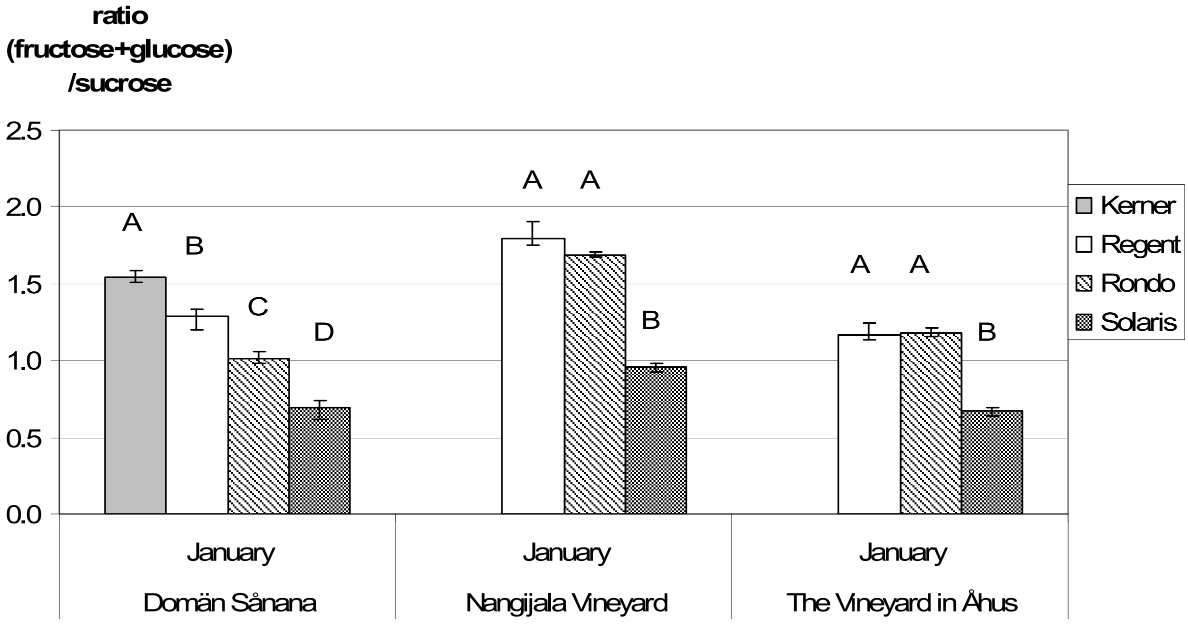

Figure 2: Ratio (fructose+glucose)/sucrose from lower positioned buds sampled in January at the three different vineyards. The ratios have been calculated as means of four replicates. Error bars indicates SE±. Different letters indicate a difference at the 5 % level.

When adding all vineyards and lower and upper buds together (N=80) one can still see a lower ratio in 'Solaris' compared to the other three cultivars (table 6). Considering fructose, glucose, sucrose, raffinose and total soluble sugar sugars (fructose+glucose+sucrose+raffinose+stachyose) content separately no clear difference can be seen between the different cultivars. Though for stachyose a significantly higher amount can be seen in the cultivar 'Kerner'.

Table 6: Fructose, glucose, sucrose, raffinose, stachyose, total soluble sugars (fructose+glucose+sucrose+raffinose+stachyose) as mg/g dw and ratio (fructose+glucose)/sucrose calculated as means of 24 replicates for 'Regent', 'Rondo' and 'Solaris' and as means of 8 replicates for 'Kerner'.

Sampled, month	Cultivar	Fructose	Glucose	Sucrose	Raffinose	Stachyose	TSS	Ratio
December	'Kerner'	13.5A[1]	11.8AB	27.9AB	2.5A	2.2A	58.0A	0.9A
December	'Regent'	14.4A	13.9A	27.4A	2.5A	1.0B	59.2A	1.0A
December	'Rondo'	21.3B	20.8C	39.6CD	1.2B	1.3B	84.1B	1.1A
December	'Solaris'	11.3A	9.6B	36.6BD	1.1B	1.4B	60.0A	0.6B
January	'Kerner'	28.5A	20.6A	34.4A	3.9A	3.2A	90.5A	1.4A
January	'Regent'	18.9B	15.8B	26.6B	3.1B	1.2B	65.6B	1.3AB
January	'Rondo'	18.6B	14.0B	27.4B	1.1C	1.1B	62.2B	1.2B
January	'Solaris'	16.6B	9.9C	36.3A	1.7D	1.4C	65.9B	0.7C
March	'Kerner'	22.2A	11.6A	34.4A	4.1A	3.0A	75.2A	1.0AB
March	'Regent'	20.2A	12.6A	29.4A	3.4A	1.1B	66.8A	1.1A
March	'Rondo'	15.6B	5.6B	26.4A	1.7B	1.2B	50.6B	0.8B
March	'Solaris'	10.4C	3.6C	27.5A	2.3C	1.0B	44.7B	0.5C

[1] Different letters within each column for each month, indicate a difference at the 5 % level

4.3. Temperature

Putting all treatments and replicates together (N=240) for comparing the sugar concentration at the different sampling dates, a significant difference could be found for the content of glucose, raffinose and total soluble sugars (fructose+glucose+sucrose+raffinose+stachyose) (table 7). Glucose content goes down in March as well as TSS, but raffinose content increase from December to March

Table 7: Difference in glucose, raffinose and total soluble sugars (fructose+glucose+sucrose+raffinose+stachyose) concentration in mg/g dw from December to March. Data are means of the 240 observations at each sampling dates.

	December	January	March
Glucose	12.871A[1]	12.008A	7.728B
Raffinose	1.266A	2.056B	2.637B
TSS	66.80A	67.16A	56.12B

[1] Different letters within the row indicate a difference at the 5 % level.

Temperature sum measured at 6 am each day for the different weather stations close by the vineyards were compared from the first of September until the three different sampling dates (table 8). It can be seen that the temperatures have been lowest at the weather station in Kristianstad located 12 km north of the vineyard in Åhus and that it also have been lower temperatures between the sampling dates there than at the other weather stations in Malmö and Skillinge. The temperature has been highest at Skillinge weather station located 4.6 km north east of Domän Sånana.

Table 8: Sum of temperature from each day at 6 am from September 1 until sampling date and mean (sum of temperatures/days (1 September until sampled date)).

Vineyard	**Weather station**	**Sampled, date**	**No. of days**	**Sum of temperature**	**Mean**
Domän Sånana	Skillinge	04-dec	95	843.7	8.9
Domän Sånana	Skillinge	21-jan	143	917.7	6.4
Domän Sånana	Skillinge	04-mar	185	924.8	5
The Vineyard in Åhus	Kristianstad	02-dec	93	671.1	7.2
The Vineyard in Åhus	Kristianstad	20-jan	142	672.2	4.7
The Vineyard in Åhus	Kristianstad	02-mar	183	618.2	3.4
Nangijala Vineyard	Malmö	03-dec	94	788.1	8.4
Nangijala Vineyard	Malmö	19-jan	141	822.1	5.8
Nangijala Vineyard	Malmö	03-mar	94	805.7	4.37

The temperature measured at the different weather stations can be seen in figure 3. The temperature at Skillinge weather station close by Domän Sånana is mostly above the mean temperature of the three weather stations. The temperature at Kristianstad weather station close by the vineyard in Åhus is generally below the mean temperature of the three weather stations. The temperature at Malmö weather station close by Nangijala vineyard is both above and below the mean temperature. The temperature of the different sampling dates within the different vineyards were quite alike.

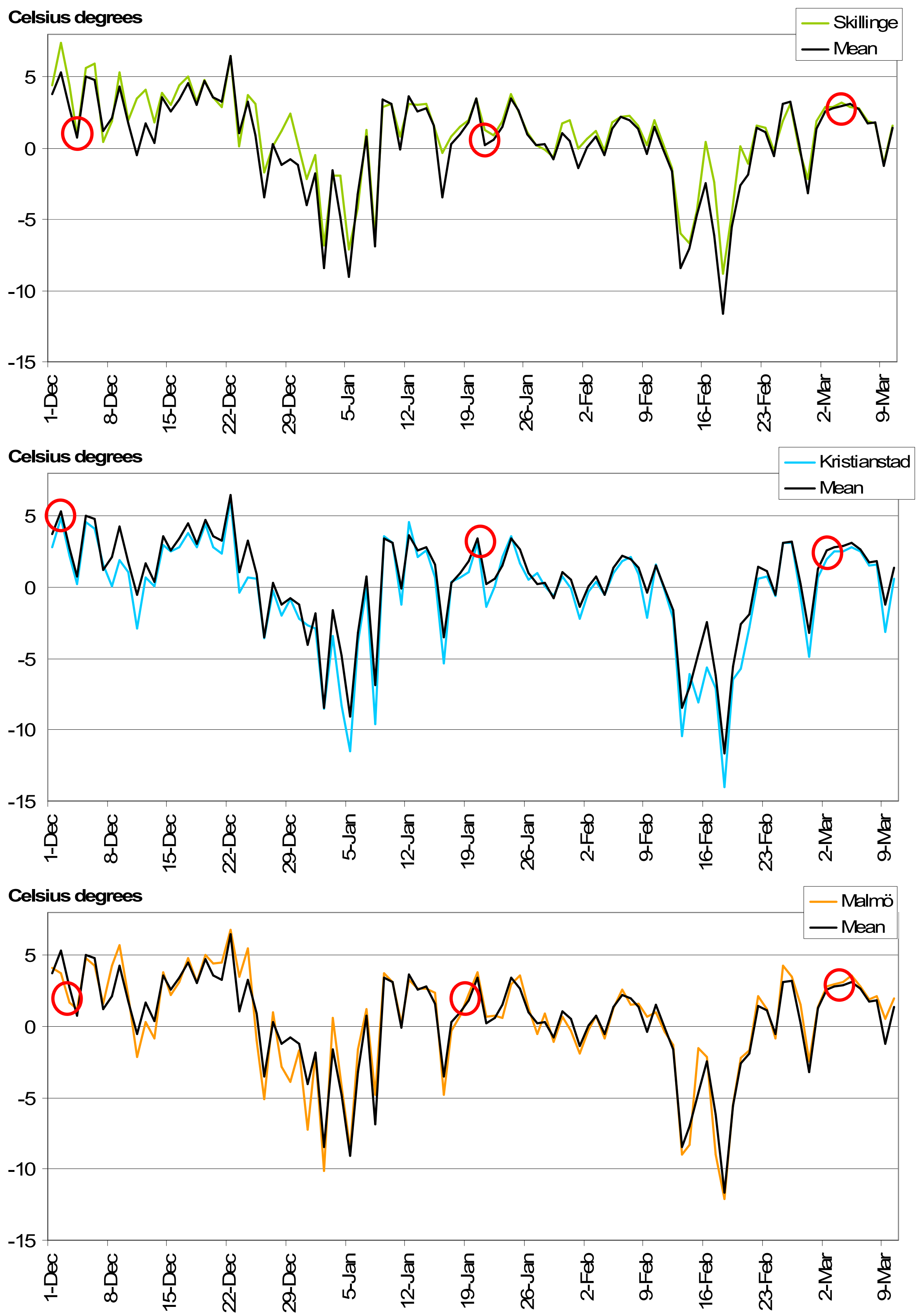

Figure 3: Temperature from December 1 to March 10 at 6 am at the different weather stations and the mean temperature of the three weather stations. Red circles indicate the sampling dates for the different vineyards close by the weather stations.

Correlation analysis was conducted to investigate if there was any correlation between air temperature on the sampling day and the mean temperature of the five days preceding the sampling and sugar concentration for each cultivar and each bud position (lower or upper) at the different vineyards (table 9). No pattern can be seen. Sometimes the correlation coefficient is negative, meaning that the sugar concentration increase with decreasing temperature and sometimes the correlation coefficient is positive meaning the sugar concentration increase with increasing temperature.

Table 9: Correlation analysis (Pearson's correlation coefficient) between air temperatures recorded for different time periods (0 = temperature on the same day as sampling, 5 = mean temperature of day 1-5 preceding sampling) and sugar concentrations of grapevines. r = correlation coefficient, p (r) = probability. Data are based on means of four replicates from each collection date (N=3).

Domän Sånana

	Cultivar	**Bud position**	**Days preceding sampling**	**Fructose**	**Glucose**	**Sucrose**	**Raffinose**	**Stachyose**
r = p(r) =	'Kerner'	Lower	0	0.092 0.941 n.s	-0.392 0.744 n.s	0.405 0.735 n.s	0.523 0.735 n.s	0.296 0.809 n.s
r = p(r) =	'Kerner'	Upper	0	0.482 0.680 n.s	-0.341 0.778 n.s	0.921 0.254 n.s	0.871 0.327 n.s	0.727 0.482 n.s
r = p(r) =	'Kerner'	Lower	5	-0.772 0.438 n.s	-0.378 0.753 n.s	-0.935 0.753 n.s	-0.974 0.146 n.s	-0.887 0.306 n.s
r = p(r) =	'Kerner'	Upper	5	-0.962 0.177 n.s	-0.428 0.718 n.s	-0.925 0.249 n.s	-0.962 0.176 n.s	-0.999 0.022 *
r = p(r) =	'Solaris'	Lower	0	-0.765 0.446 n.s	-0.997 0.046 *	-0.793 0.417 n.s	0.917 0.262 n.s	-0.752 0.459 n.s
r = p(r) =	'Solaris'	Upper	0	-0.812 0.397 n.s	-0.998 0.038 *	-0.920 0.256 n.s	0.999 0.027 *	-0.847 0.357 n.s
r = p(r) =	'Solaris'	Lower	5	0.080 0.949 n.s	0.651 0.549 n.s	0.991 0.086 n.s	-0.361 0.765 n.s	0.998 0.045 *
r = p(r) =	'Solaris'	Upper	5	0.156 0.900 n.s	0.660 0.541 n.s	0.926 0.247 n.s	-0.673 0.530 n.s	0.974 0.146 n.s
r = p(r) =	'Regent'	Lower	0	0.921 0.255 n.s	0.309 0.800 n.s	0.882 0.313 n.s	0.821 0.387 n.s	0.110 0.930 n.s
r = p(r) =	'Regent'	Upper	0	0.888 0.304 n.s	0.682 0.523 n.s	0.969 0.158 n.s	0.881 0.313 n.s	0.664 0.538 n.s
r =	'Regent'	Lower	5	-0.925	-0.893	-0.285	-0.983	-0.784

p(r) =				0.249 n.s	0.297 n.s	0.816 n.s	0.116 n.s	0.426 n.s
r = p(r) =	‘Regent’	Upper	5	-0.951 0.200 n.s	-1.000 0.019 *	-0.856 0.345 n.s	-0.956 0.190 n.s	-0.999 0.035 *
r = p(r) =	‘Rondo’	Lower	0	-0.638 0.560 n.s	-0.839 0.367 n.s	-0.422 0.723 n.s	0.851 0.352 n.s	0.019 0.988 n.s
r = p(r) =	‘Rondo’	Upper	0	-0.780 0.430 n.s	-0.879 0.316 n.s	-0.744 0.466 n.s	0.708 0.499 n.s	-0.683 0.521 n.s
r = p(r) =	‘Rondo’	Lower	5	0.996 0.056 n.s	0.977 0.136 n.s	0.941 0.220 n.s	-0.226 0.855 n.s	0.697 0.509 n.s
r = p(r) =	‘Rondo’	Upper	5	0.993 0.073 n.s	0.957 0.187 n.s	0.998 0.038 *	0.004 0.997 n.s	1.000 0.018 *

The vineyard in Åhus

	Cultivar	**Bud position**	**Days preceding sampling**	**Fructose**	**Glucose**	**Sucrose**	**Raffinose**	**Stachyose**
r = p(r) =	‘Solaris’	Lower	0	-0.811 0.398 n.s	0.831 0.375 n.s	-0.926 0.246 n.s	-0.981 0.123 n.s	-0.769 0.441 n.s
r = p(r) =	‘Solaris’	Upper	0	-0.833 0.374 n.s	0.726 0.483 n.s	-0.843 0.361 n.s	-0.988 0.098 n.s	-0.820 0.387 n.s
r = p(r) =	‘Solaris’	Lower	5	-0.995 0.066 n.s	0.444 0.707 n.s	-0.991 0.086 n.s	-0.755 0.455 n.s	-0.985 0.109 n.s
r = p(r) =	‘Solaris’	Upper	5	-0.998 0.042 *	0.286 0.815 n.s	-0.999 0.029 *	-0.780 0.430 n.s	-0.996 0.056 n.s
r = p(r) =	‘Regent’	Lower	0	-0.909 0.274 n.s	1.000 0.004 **	-0.996 0.057 n.s	-0.991 0.087 n.s	0.797 0.413 n.s
r = p(r) =	‘Regent’	Upper	0	-0.956 0.189 n.s	0.821 0.387 n.s	-0.851 0.352 n.s	-0.927 0.246 n.s	0.516 0.655 n.s
r = p(r) =	‘Regent’	Lower	5	-0.580 0.606 n.s	0.870 0.328 n.s	-0.819 0.389 n.s	-0.927 0.245 n.s	0.390 0.745 n.s
r = p(r) =	‘Regent’	Upper	5	-0.975 0.143 n.s	0.427 0.719 n.s	-0.999 0.020 *	-0.991 0.086 n.s	0.020 0.987 n.s
r = p(r) =	‘Rondo’	Lower	0	-0.516 0.655 n.s	0.941 0.219 n.s	0.884 0.310 n.s	-0.884 0.310 n.s	-0.763 0.448 n.s
r = p(r) =	‘Rondo’	Upper	0	-0.929 0.241 n.s	0.777 0.433 n.s	-0.676 0.528 n.s	-0.952 0.199 n.s	-0.959 0.182 n.s
r = p(r) =	‘Rondo’	Lower	5	-0.874 0.323 n.s	0.648 0.551 n.s	0.999 0.022 *	-0.534 0.642 n.s	-0.983 0.116 n.s
r = p(r) =	‘Rondo’	Upper	5	-0.990 0.091 n.s	0.360 0.765 n.s	-0.219 0.860 n.s	-0.672 0.531 n.s	-0.972 0.150 n.s

Nangijala vineyard

	Cultivar	Bud position	Days preceding sampling	Fructose	Glucose	Sucrose	Raffinose	Stachyose
r = p(r) =	‘Solaris’	Lower	0	-0.369 0.759 n.s	-0.750 0.460 n.s	-0.742 0.468 n.s	0.762 0.448 n.s	-0.597 0.592 n.s
r = p(r) =	‘Solaris’	Upper	0	-0.403 0.736 n.s	-0.871 0.327 n.s	-0.933 0.234 n.s	0.974 0.146 n.s	-0.801 0.408 n.s
r = p(r) =	‘Solaris’	Lower	5	-0.449 0.704 n.s	0.004 0.997 n.s	-0.008 0.995 n.s	-0.990 0.088 n.s	-0.202 0.871 n.s
r = p(r) =	‘Solaris’	Upper	5	-0.415 0.727 n.s	0.213 0.864 n.s	0.352 0.771 n.s	-0.817 0.391 n.s	0.086 0.945 n.s
r = p(r) =	‘Regent’	Lower	0	0.767 0.444 n.s	-0.690 0.515 n.s	-0.654 0.546 n.s	0.315 0.796 n.s	0.790 0.420 n.s
r = p(r) =	‘Regent’	Upper	0	0.449 0.704 n.s	-0.740 0.470 n.s	-0.889 0.302 n.s	-0.702 0.505 n.s	-0.979 0.130 n.s
r = p(r) =	‘Regent’	Lower	5	0.989 0.093 n.s	-0.081 0.948 n.s	1.000 1.000 n.s	0.499 0.667 n.s	-0.067 0.957 n.s
r = p(r) =	‘Regent’	Upper	5	-0.966 0.167 n.s	-0.011 0.993 n.s	0.933 0.234 n.s	0.999 0.032 *	0.803 0.406 n.s
r = p(r) =	‘Rondo’	Lower	0	-0.830 0.377 n.s	-0.970 0.156 n.s	-0.999 0.021 *	0.270 0.826 n.s	-0.924 0.250 n.s
r = p(r) =	‘Rondo’	Upper	0	-0.990 0.092 n.s	-0.999 0.033 *	-0.914 0.266 n.s	0.618 0.576 n.s	-0.358 0.767 n.s
r = p(r) =	‘Rondo’	Lower	5	0.135 0.913 n.s	0.464 0.693 n.s	0.640 0.558 n.s	0.540 0.637 n.s	0.900 0.287 n.s
r = p(r) =	‘Rondo’	Upper	5	0.766 0.445 n.s	0.703 0.504 n.s	0.911 0.271 n.s	0.177 0.887 n.s	0.935 0.230 n.s

4.4. Vineyard difference

When comparing differences between the vineyards once more the ratio fructose plus glucose to sucrose was chosen for evaluation. ‘Kerner’ was not considered when comparing the vineyards since ‘Kerner’ samples were only taken from Domän Sånana. In December a lower ratio was found at Domän Sånana compared to the Vineyard in Åhus and Nangijala Vineyard (table 10). When looking at ‘Solaris’ at Domän Sånana the ratio is lower both in the lower buds (0.46) and upper positioned buds (0.38) compared to the Vineyard in Åhus (0.72, 0.62) and Nangijala Vineyard (0.74, 0.64). The same pattern can be seen for ‘Regent’ and ‘Rondo’ in December. Looking at January Nangijala Vineyard alone shows higher ratios compared to Domän Sånana and the Vineyard in Åhus that shows very similar ratios. In March Nangijala vineyard stills shows higher ratios compared to the other vineyards (figure 4).

Table 10: Ratio (fructose+glucose) / sucrose calculated as mean value of four replicates.

Cultivar	**Sampled, month**	**Bud position**	**Domän Sånana**	**The Vineyard in Åhus**	**Nangijala Vineyard**
’Solaris’	December	Lower	0.46A[1]	0.72B	0.74B
’Solaris’	December	Upper	0.38A	0.62B	0.64B
‘Regent’	December	Lower	0.93A	1.38A	1.07A
‘Regent’	December	Upper	0.73AC	1.18B	0.92BC
‘Rondo’	December	Lower	1.00A	1.08 A	1.40B
‘Rondo’	December	Upper	0.78A	0.88A	1.34B
’Solaris’	January	Lower	0.70A	0.67A	0.96B
’Solaris’	January	Upper	0.55A	0.60A	0.84B
‘Regent’	January	Lower	1.28A	1.17A	1.80B
‘Regent’	January	Upper	1.07A	1.05A	1.48B
‘Rondo’	January	Lower	1.02A	1.18A	1.68B
‘Rondo’	January	Upper	0.79A	1.05B	1.61C
’Solaris’	March	Lower	0.44AC	0.53BC	0.65B
’Solaris’	March	Upper	**0.35A**[2]	**0.51B**	**0.63B**
‘Regent’	March	Lower	1.13A	1.11A	1.31B
‘Regent’	March	Upper	**1.04A**	**1.03A**	**1.15A**
‘Rondo’	March	Lower	0.62A	0.91B	0.97B
‘Rondo’	March	Upper	**0.60A**	**0.75A**	**1.14B**

[1] Difference in letters shows significance at the 5 % level, reading the table horizontally.

[2] Data in bold is illustrated in figure 4.

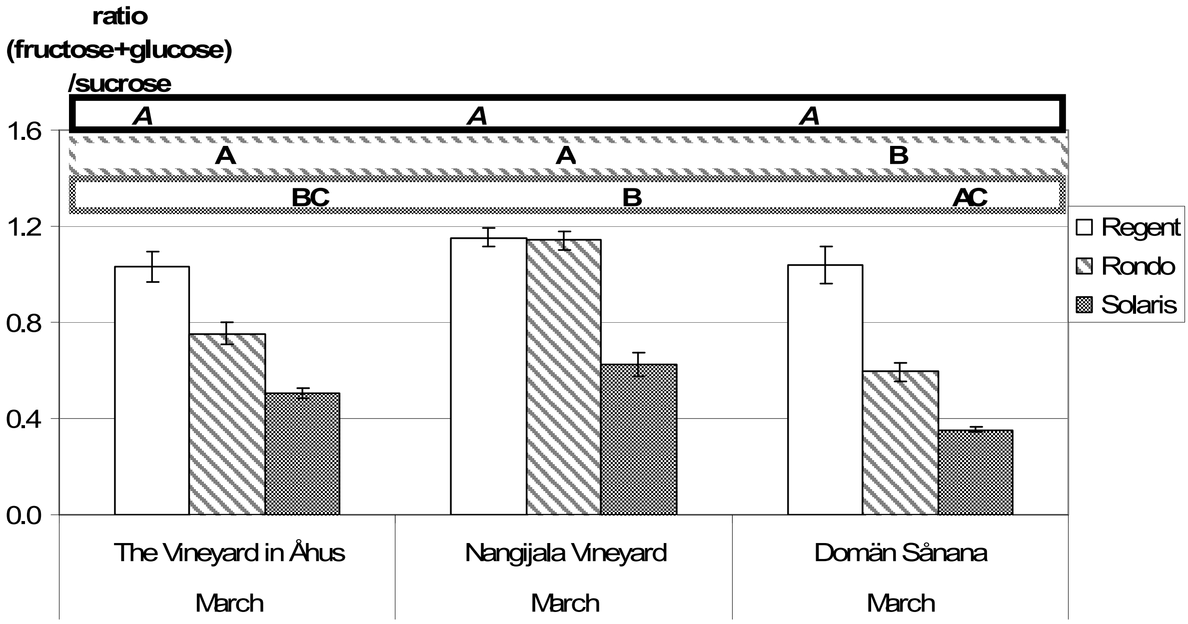

Figure 4: Difference in ratio, (fructose+glucose)/sucrose at the three different vineyards in upper positioned buds in March. The ratios have been calculated as means of four replicates. Error bars indicate SE±. Different letters indicate a difference at the 5 % level, when reading the letters for each cultivar separately.

5. Discussion

5.1. Bud position

Badulescu & Ernst (2006) found a difference in sugar concentration in buds according to position. The difference in bud position was significant for TSS, fructose and sucrose. In this study the only difference between bud positions could be found when comparing the ratio of (fructose + glucose) / sucrose in lower and upper positioned buds. This ratio was suggested by Hamman et al. (1996) as an indicator of cold hardiness in cold tender *V. vinifera* species. The higher the ratio the more cold hardy the cultivar is. It is common to see in a vineyard that the top shoot has dyed away during winter suggesting that the apical buds are more cold tender. When comparing the 60 treatments pair wise, giving 30 observations, 16 of these observations showed a significant difference in the ratio, having a higher ratio in the lower buds than in the upper positioned buds. The lower buds were defined as bud 2-8 and the upper buds were defined as 9-14. A clearer difference in bud position might have been seen if one should have kept to the basal buds up to bud no. 4. One problem though was to come up in a big enough sample to be able to detect the sugars. If more plant material would have been available one could have defined the lower buds as 2-4 and then might have seen a greater difference to buds positioned higher up on the shoot.

5.2. Cultivar differences

The lower ratio of fructose plus glucose to sucrose of 'Solaris' indicates that it might be more cold tender than the other cultivars. 'Kerner' shows a high ratio opposite to what was expected according to the hypothesis. Also the stachysose content of 'Kerner' was higher compared to the other cultivars. Both 'Solaris' and 'Rondo' have some percentage of *V.amurensis* in its pedigree, which is known to be cold hardy. 'Regent' is believed to have a lot of other species in its pedigree, among others also *V. riparia* which is known to be very cold hardy. When comparing the cultivars within each vineyard, the difference in sugars can not be simply explained as cultivar differences. It might also be due to the difference in rootstocks or the microclimate in that part of the field. Also harvest date differs between the cultivars. Hamman et al. (1996) hypothesized that late harvested-fruit might accumulate sugars at the expense of the cane and bud tissue, thereby changing cold hardiness, but could not find a consistent trend in grapevines subjected to late harvest.

5.3. Temperature

Several studies report a relationship between sugars and air temperature (Badulescu & Ernst, 2006; Hamman et al., 1996; Koussa et al., 1998; & Wample & Bary, 1992). No such relationship could be found in this study. There could be several reasons for this. A winter when fluctuation in temperature is greater than the winter of 2008/2009 might show something different. The temperature difference between the three sampling periods was very small. Some winters can have a period of mild temperature that might cause the grapevines to deharden. This might be seen as a change in the concentration of soluble sugars. But the winter of this study, was of quite constant cold, which of course provides better conditions for avoidance of winter damage for the grapevines. The second and third sampling was quite similar when comparing temperatures they have been exposed to 5 days previously. This might be one reason why no difference in sugar concentration could be found here either. In this study samples were only taken 3 times in total, once in December, January and March. A greater difference might have been seen if one could extend the sampling period from November to April. If possible more samples should be collected at different dates, giving more temperatures to correlate with sugar concentration. Further, monitoring the temperature in the actual vineyard would have been better. All the weather stations are located some distance away from the vineyards, which might be one reason for that no correlation could be found. It is also better to monitor the temperature every hour, instead of at 6 am as was done in this study. Then one could correlate the minimum and maximum temperature of 24 hours with the sugar content.

5.4. Vineyard difference

The reasons for the differences in the ratios between the vineyards can be due to multiple factors that differ between the vineyards. Plants health during the season affects the photosynthesis which further influences the accumulation and storage of carbohydrates present during the dormant season. The amount of damage of powdery mildew may also affect the sugar concentration in the buds measured in this study. The availability of nutrients also effects photosynthesis. Since all the vineyards stand on different soils the availability of nutrients may differ. The use of rootstocks differs between the vineyards but also within the vineyards which may affect cold hardiness by allowing an early or late acclimation. The SO4 rootstock was used in all vineyards for ‘Rondo’ but was used inconsistent for the other cultivars at the vineyards. According to Miller et al. (1988) SO4 is intermediate hardy when compared to C-3309 and 5BB. SO4 have the same parentage as 125AA that only was used at Nangijala Vineyard. So one could expect these to show similar degree in hardiness. These should be expected to contribute to earlier acclimation than grapevines grown on own roots. Also root pruning that are practiced at Nangijala vineyard could influence the result and explain some of the difference between the vineyards. Root pruning are often used to control vigour, but root pruning of grapevine varies in degree of controlling vigour (Ferre et al., 2005). Ferree et al., (1999) reported that root pruning decreases net photosynthesis and vigour. One may think a decrease in net photosynthesis may lead to a decrease in carbohydrates available during the dormant period and therefore influence cold hardiness negatively. But a reduced vigour may lead to an early acclimation which on the other hand will be good for the grapevines.

6. Conclusion

The cultivar ‘Solaris’ seems to be more cold tender than ‘Kerner’, ‘Regent’ and ‘Rondo’ when comparing the ratio fructose plus glucose to sucrose. No clear difference could be seen between the other cultivars. Lower positioned buds showed a higher ratio than upper positioned buds, significantly in more than half of the observations (53.3%). Differences between vineyards showed no clear patterns when comparing the ratio of fructose plus glucose to sucrose. This is probably because of the difference in many cultural practises that may influence cold hardiness as, rootstock, fertilisation, temperature, and diseases. No correlation was found between air temperature 0 days before sampling and mean air temperature 5 days preceding sampling and sugar concentration.

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