Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Magister project in Biology

Department of Biosystems and Technology

Frost hardiness of grapevine cultivars as affected by ground cover under Scandinavian conditions

30 credits

Magdalena Jansson

Alnarp, 2013

Självständigt arbete vid LTJ-fakulteten, SLU

Independent work at LTJ Faculty

Department of Biosystems and Technology

Faculty of Landscape Planning, Horticulture and Agricultural Sciences, LTJ

SLU, Swedish University of Agricultural Sciences

Frost hardiness of grapevine cultivars as affected by ground cover under Scandinavian conditions

Härdighet för olika vitvinsorter beroende på markbehandling under skandinaviska förhållanden

Course Title: Paper in biology - master's thesis
Course code: EX0717
Credits: 30 hec
Level and depth: Advanced A1E
Place of publication: Alnarp
Year of publication: 2013
Author: Magdalena Jansson
Supervisor: Beatrix Alsanius, Horticulture, SLU, Alnarp
Examiner: Helene Larsson Jönsson , Horticulture, SLU, Alnarp
Online publication: http://stud.epsilon.slu.se
Picture cover: Grapevine, Bjärehalvön, by Magdalena Jansson
Title of series: Självständigt arbete vid LTJ-fakulteten, SLU

Keywords: frost hardiness, grapevine, ground cover, Scandinavia

Contents

Contents 2
Abstract 3
Sammanfattning 3
1 Introduction 4
1.1 Cold hardiness 4
1.1.1 Root 5
1.1.2 Buds, leaves and canes 6
1.2 Climate 8
1.2.1 Temperature 8
1.2.2 Wind 8
1.2.3 Light 9
1.2.4 Precipitation 9
1.2.5 Soil 9
2 Aim 11
2.1 Hypothesis 11
3 Materials and methods 11
3.1 Terroir of the vineyard in Broddarp 11
3.2 Plant material 12
3.3 Experimental design 12
3.4 Analyses 13
3.4.1 Microclimate and soil temperature registrations 13
3.4.2 Plant analyses 13
3.4.3 Frost hardiness test 13
3.4.6 Statistical analyses 16
4 Results 17
4.1 Growth of cultivars in relation to ground cover 17
4.2 Monitored microclimate 18
4.3 Frost hardiness 22
5 Discussion 24
6 References 26
7 Appendix 29

Abstract

Cultivation of grapevine is novel under Scandinavian conditions. We studied the impact of ground cover on frost hardiness of grapevine cultivars in Northwestern Scania in a two-factorial trial with four cultivars Solaris, Pinot Gris, Siegerrebe, Ortega and three kinds of ground cover (open soil, plastic cover and gravel). The study was conducted in a grapevine orchard, planted in May 2009, in Broddarp on Bjärepenisula Sweden (N latitude 56°; longitude E 12°). Soil and air temperature were monitored continuously.

Frost hardiness measurements were performed every second week from late September to early December 2009. Frost resistance, LT_{50}, was determined by electrolyte leakage measurement of the upper stems after artificial freezing at -15 °C. The results showed that Solaris is the significantly most frost hardy of the cultivars under Scandinavian conditions. Ground covers showed no significant difference with respect to frost hardiness in general.

Buds were visually assessed based on BBCH scale in the beginning of the following season (May 2010). Swollen buds were counted. State of development of buds dependent on ground cover do not differ noticeably. At the time Solaris have the biggest bud growth.

Sammanfattning

Odling av vin under Skandinaviska förhållanden är en relativt ny företeelse. I den här studien undersöktes det hur olika vinsorters härdighet påverkades beroende på olika mark-behandlingar. Studien utfördes i en nyanlagd vinodling som planterades i maj 2009 i nordvästra Skåne i byn Broddarp på Bjärehalvön (N latitud 56°; longitud E 12°). Studien bygger på ett två-faktoriellt försök med fyra vitvinssorter (Solaris, Pinot Gris, Siegerrebe, Ortega) och tre typer av markbehandlingar (öppen mark, plastfolie och singel). Mark- och lufttemperatur mättes kontinuerligt.

Härdighetstester utfördes varannan vecka från slutet av september till början av december 2009. Härdighet (LT_{50}) fastställdes för de övre delarna av plantan genom exponering av artificiell frysning vid -15°C, mätt genom elektrolytläckagemätningar. Resultaten visar att Solaris är signifikant härdigare än de andra sorterna. Markbehandlingarna visar inga signifikanta skillnader för att märkbart påverka härdigheten.

En visuell bedömning av knoppar gjordes i början av maj. Svällande knoppar räknades och bedömdes utifrån BBCH-skalan. Resultatet visar att Solaris hade flest svällande knoppar vid räkningstillfället. De olika markbehandlingarna påverkar inte knoppsprickningen märkbart.

1 Introduction

Cultivation of grapevine is novel under Scandinavian conditions. During recent years, however cultivation of grapevines has increased in the Southern part of Sweden. In 2010, there were 50 wine growers in Sweden who produced wine to National Systembolaget (Systembolaget, 2010).

The climate is the main limiting factor for producing grapevines with high quality and therefore the choice of cultivar, rootstock and habitat with a favourable micro-climate is very important. In this study cold hardiness of four different grapevine cultivars as related to three different kind of ground cover were examined during the plant establishment.

1.1 Cold hardiness

Cold hardiness is influenced by genetic, environmental and viticulture conditions (Jackson, 2000). It is the survival capacity of a specific tissue of the grapevine to withstand freezing temperature stress (Howell, 2000; Zabadal *et al*., 2007). The capacity to acclimate early in the autumn, to become resistant during the winter, to have a slow response to temperature changes in the winter and to have delayed deacclimation during the spring is necessary for the survival (Howell, 2000). Cultural practices like inadequate nutrient uptake, heavily shaded canopies, high levels of pests and disease as well as stressors that decrease or delay cork cambium (periderm) formation inhibit cold acclimation (Dami, 2007).

Cold acclimation in grapevines occurs in two stages. The first stage takes place in late summer/early fall. It is induced by low temperatures, but above 0 °C. During the first stage grapevine does not reach maximum cold hardiness. The second stage is induced by temperatures below freezing and cold hardiness increase. During midwinter, grapevine reaches its maximum hardiness (Zabadal *et al*., 2007).

Freezing avoidance and freezing tolerance are mechanisms to avoid freezing stress. Bud tissues survive by avoiding formation of ice crystals with supercooling. Supercooling means that the liquid in the cells temporarily does not freeze at temperatures well below 0 °C. An exothermic reaction occurs when ice crystals are formed intracellular releasing heat which lowers the freezing point (Taiz & Zeiger, 2002). Deep supercooling has been shown in parenchyma tissue in *V. riparia* at -40 °C (Howell, 2000).

Freezing tolerance is the capacity to tolerate ice formation in intracellular tissue and concentrations of solutes in the cell, by increasing the concentration of solutes in the cell the freezing point will be depressed (Taiz & Zeiger, 2002; Larcher, 2003). The depression of the freezing point provides a moderate and reliable protection (Larcher, 2003). Ice is usually formed in the intercellular spaces in the xylem vessels. Intracellular freezing destroys the membrane structure and causes leakage of vacuole and cytoplasm contents, causing cell death. With continued cold temperatures the growth of extracellular ice crystals result movement of liquid water from the protoplasm to the extracellular ice. The movement causes excessive dehydration (Taiz & Zeiger, 2002; Dami, 2007). When temperature increases, the process is reversed. This may cause several lethal processes, by dehydration stress, ice crystals forming in the cell, cell freezing and impaired function of the membrane which lead to local tissue mortality (Howell, 2000).

When the cold hardiness process starts, starch is converted to soluble sugars and the amount of free amino acids, proteins, nucleic acids and lipids increases (Karmer & Kozlowski, 1979). The carbohydrates status in the plant is important, as they are needed for the transition from non-hardy to hardy condition (Howell, 2000). Reduced carbohydrates contents in the plant can limit cold hardiness properties (Jackson, 2000). Diseased grapevine plants are too weak to produce enough carbohydrates for hardening wood (Stafne, 2007). Cryoprotectives are specific sugars and cold induced proteins which prevent denaturation of proteins, stabilize membranes and bind water during the dehydration (Taiz & Zeiger, 2002; Dami, 2007).

1.1.1 Root

The root has an important function, it takes up and transports water and nutrients, synthesizing hormone (ABA, cytokinines and gibberellins), interacts with mycorrhiza and anchors the plant into the ground. A plant with a well developed root system is more likely to cope with stress. Root growth depends on the cultivar, rootstock and environmental factors. The roots are affected by soil composition, groundwater, mineral and salt content, pH, soil preparation, irrigation and planting density. Generally root density is highest between the surface and down to about 1 meter. Root growth begins during bud swelling and increases after flowering and is greatest during the fruit setting. An airy soil will warm up more quickly in the spring while a water-saturated soil takes longer to heat, which affects root growth and nutrient availability (Jackson, 2000).

Generally, a hardy rootstock has less bark tissue, small phloem and ray cells and narrow xylem vessels. It seems to increase the resistance of the cane because of factors like restrained cane vigor, changed contents of growth regulators and limitation of water availability (Jackson, 2000).

1.1.2 Buds, leaves and canes

As temperature rises in spring, both leaf and cane growth increases (Jackson, 2000). Vegetative growth slows down during the season, but the canes grow throughout the summer and will continue grow as long as the weather allows it. Grapevine does not set any terminal buds like other woody plants, as an indicator of growth cessation or initiation of cold acclimation (Winkler *et al.,* 1974; Dami, 2007; Zabadad *et al*., 2007). If growth is high in the autumn, the canes will not be able to cold acclimate before the frost (Winkler *et al*., 1974). The reason of late-season growth may depend of growth-regulators or nutrient influences (Jackson, 2000). Highly vigorous canes are less cold hardy than less vigorous canes. It depends partly on canopy density causing shade and large cane diameters which are less hardy than canes with moderate diameter. To manage the cane vigor training system, pruning methods, crop control, row-middle management, irrigation, rootstock and nutrition can be adjusted (Zabadal *et al*., 2007).

During late summer and fall cold acclimation starts. A gradual dehydration begins which eliminates free water in the cells. If there is a lot of free water in the cells when the temperature gets low it will form ice crystals in the cells which destroys the membranes. Free water must therefore be bound or eliminated during the acclimation period (Zabadal *et al*., 2007). The degree of dehydration depends mainly on the osmotic potential of the cell. Cold hardiness is affected of increased levels of soluble sugars, oligosaccharides and dehydration and also decrease of the osmotic potential and the freezing point in the sap. Low osmotic potential depress the freezing point in cytoplasm so supercooling occurs instead of crystallization (Jackson, 2000).

Some varieties can withstand low temperatures better than other. The reason for this is that the membranes of the plant are different. Membranes consist of a lipid layer, proteins, sterols and pores. There are differences between the membranes of cold sensitive and cold tolerant plants. In cold sensitive plants, the lipid layer has greater proportion of saturated fatty acid chains than unsaturated fatty acid. This solidifies the membrane at temperatures below 0° C which in turn results in impaired transport. This inhibits the fluid transport, energy distribution and the dependence of enzyme reaction. The cold resistant plants have a greater proportion of unsaturated fatty acid chains in the lipid layer. More unsaturated lipids in membranes results in higher tolerance to cold, sustaining the membrane function despite of the cold (Jackson, 2000; Taiz & Zeiger, 2002).

As the canes mature, cork cambium is formed, preventing water and nutrients to from getting in to the outer tissue. The outer tissue dies and brown staining indicates that the canes are matured. Cold acclimation starts from the base and goes to the top (Goffinet, 2007; Zabadal *et al*., 2007). When canes are growing, the new tissue cannot acclimate because of the high content of free water (Zabdal *et al*., 2007). The upper part of the cane does not develop resistance to cold (Salzman *et al*., 1996). During the fall sieve elements are plugged by polysaccharides which prevent the redistribution of nutrients, and the leaves die and fall off (Jackson, 2000).

Most cells are living cells in grapevine, except for dead water-conducting vessels in wood and waterproof cork in the external bark. Cells have different functions, depending on the location in the plant (Goffinet, 2004). When cells are injured by freezing, intracellular ice formation destroys the structure and cell cytoplasm and vacuole contents leak out which is resulting in cell death (Zabadal *et al*., 2007). If the functions are disturbed and the cells get seriously injured, it will never get repaired, however through isolation of dead cells, renewed cell division and differentiation by nearby living cells (callus), the plant may survive (Goffinet, 2004). Tissues which survive the winter show a light green color when cut. Injured tissue with destroyed cell membranes looks water-soaked (Zabadal *et al*., 2007).

The vascular system development is important for the cold acclimation of the plant. Its development goes from the bottom to the top (Zabadal *et al*., 2007). Vascular cambium produces the secondary phloem and xylem (Raven *et al*., 2005). Phloem distributes organic compounds in the plant, sugars from the photosynthesizing parts transports and redistributes between sink and sources in the plant (Howell, 2000; Raven *et al*., 2005). Phloem are located beneath the bark. It is therefore more affected by temperature fluctuations (Goffinet, 2007). Xylem transports water and nutrients from base to apex (Howell, 2000; Raven et al, 2005). If the transport system is injured during the winter the transport of water, minerals, and photosynthetic products to buds burst and canes will get disturbed, stunted or collapsed. Xylem is seldom injured or damaged, injury to phloem and cambium is more frequent and harmful to the plant. If the phloem is injured and dies it creates a girdle response. Cambium can reestablish the phloem, the survival of the cambium and the extent of the injury are therefore important (Howell, 2000). If cambium in the trunk is killed (and xylem is not impaired) the plant may start to grow and even set flowers and fruit before the root dies because of lack of carbohydrates. The plant will wilt, and collapse may occur at any time during the growing season (Howell, 2000).

1.2 Climate

1.2.1 Temperature

Temperature is the key for sustainable vineyards (Howell, 2000; Zabadal *et al*., 2007). Lethal temperatures limit the survival of the plants (Zabadal *et al*., 2007). Rising temperatures due to global warming cause new opportunities for cultivars that normally are grown in Southern Europe regions to be grown in Northern Europe (Schultz, 2008). The development of frost hardier varieties enables the expansion of viticulture (Howell, 2000).

Orchards situated close to water, i.e. lake or sea, are affected by the heat buffering properties of water. The water is balancing and reducing temperature fluctuations in spring and autumn. The spring become colder which delay bud break. A delayed bud break leads to reduced risk of frostbite of late frost nights. Cold acclimation during the autumn is improved when the temperature is kept stable (Jackson, 2000; Striegler, 2007).

The growth cycle of *Vitis vinifera* is mainly influenced by temperature (Jackson, 2000). Cold acclimation of *Vitis labrusca* and *Vitis riparia* are affected by photoperiod (short day) and cold acclimation, *V. vinifera* respond primarily on temperature but also photoperiod (short day) (Stafne, 2007; Zabadal *et al*., 2007). If the temperature fluctuates it can delay the grapevines from reaching full cold hardiness (Stafne, 2007). Rapid temperature changes are more damaging than the lowest yearly temperature, as destructive intracellular ice is formed (Jackson, 2000). Cold hardiness is dynamic and not constant during the dormant season (Dami, 2007). In the spring when temperature increases, it starts to loose hardiness (deacclimation). Deacclimation occurs more rapidly than acclimation in the fall (Zabadal *et al*., 2007).

Plant parts differ in sensitivity to cold temperatures. Buds in the top are more sensitive to cold than the buds at the bottom of the plant. Primary buds are less hardy then secondary and tertiary buds. The roots are also sensitive to cold, which is due to the low content of soluble sugars (Jackson, 2000). Woody tissues generally have greater cold hardiness than dormant buds and roots (Howell, 2000).

1.2.2 Wind

Strong winds may limit the cultivation of grapevine. It increases evaporation and erosion which leads to reduced cane height and leaf size, and the number of stomata. Bunches become smaller, fewer and with delayed maturation. Many of these factors depend on the stomata closure due to increased water loss. Stomata closure reduce photosynthetic activity which leads to reduced growth. The physiological damage persists long after the wind has subsided.

To reduce wind speed, shelters can be set up or the plant can be tied up. However, there are also risks with shelters, the air can become stationary which lead to increased risk of spring and autumn frost during clear nights and also an improved risk of increased humidity. High humidity factors risk of fungal attack. Stationary air can be counteracted by topography and slope (Jackson, 2000).

1.2.3 Light

Light activates photosynthesis, providing energy for the growth (Jackson, 2000). Light during the growing season is important for maximum cold hardiness. Shade during the season inhibits the reproductive growth, but stimulate the vegetative growth. Too vigorous vegetative growth in the autumn inhibits adaption to cold (Stafne, 2007).

Sun exposure improves the cane maturation. It might depend on the sunlight's influence on heating and drying of the canes surface (Jackson, 2000).

1.2.4 Precipitation

Most of the water absorbed in the plant is used for cooling. If the plant becomes overheated, the metabolism will be inhibited. The most common sign of water stress is displayed by stunted cane growth. When water is available again, the physiological activities quickly return to normal. If water shortages persist for a long period it leads to dead cells. Well-developed vine plants usually have a large root system and therefore, rarely suffer from water stress. Heavy rainfall and high humidity are bigger problems. When warm, moist air is cooled, dew on the leaves is formed, which provide ideal conditions for fungal attack (Jackson, 2000).

1.2.5 Soil

Soil properties affect the plant in different ways. Texture, bedrock, structure, drainage, depth, nutrient and organic content are important factors for plant development, growth, fruit setting and product quality. Grapevine is grown in most soils, from igneous (granite), sedimentary (limestone) to the metamorphic rocks (Jackson, 2000). Soil texture, mineral composition and humus content affects soil structure, water and air content (Eriksson *et al.*, 2005). A soil with a high proportion of material with small particle size (e.g. clay) has higher water retention properties than soil with a larger particle size (e.g. sand). This is due to the strong capillary retention of water in the small pores in clayey soils. The water available for roots, however, is low in clayey soils because water molecules are so tightly bound to clay particles. In a loamy soil, pores are very small which makes it difficult for oxygen to diffuse in to the soil. Soils with a greater texture have larger pores and the oxygen can easily diffuse, in contrast, water storage capacity is low. Soil texture also affects the heat capacity. When the sun shines on a soil with fine texture and high water content solar energy is used for the evaporation. Stony and airy soils absorb the heat and reflect it back at night, which accelerates the ripening and reduces the risk of frost in the autumn (Jackson, 2000). A wet soil will take longer to warm up in spring than a dry soil (Eriksson *et al.*, 2005). Moist soils conduct heat better then dry soils and the frost hazard is lower (Striegler, 2007).

The color of the soil surface affects the soil temperature. Dark soils absorb and light soils reflect heat. It takes more energy to heat wet and dark soils. Reflective ground cover can be used to reduce the rapid rise of temperature in the ground in spring and provide greater insulation of the crop during the season. Increased insulation affects the sugar concentration, anthocyanins, tannin content and photosynthesis. Warm soils positively affect nitrification, increase potassium uptake, but decrease magnesium and iron uptake. The organic content of the soil improves soil structure and water holding capacity and increases the nutrients availability. If soil aggregate structure is crumbly and grainy, it is oxygen-rich and has a good water storage capacity which makes it easy for the root to penetrate and grow. It provides for a well-developed root system that makes the plant resistant to drought stress and nutrient deficiency. Restricted root growth affects nutrient uptake. However in long-time crops as grapevine, the mobility of nutrients in the soil is an essential factor. Potassium and phosphorus content are highest at the soil surface and in the upper horizon whereas magnesium and calcium disperse in the profile. The nutrient content of soil and nutrient uptake by the plant depend on several factors such as pH, water content, oxygen content, temperature, soil particles, root surfaces and mycorhiza (Jackson, 2000).

Infiltration into the soil is affected by its texture and structure. In sandy soils water is filtered quickly deeper into the ground, while it moves slower in clayey soils. However, the horizontal spread of water is greater in a loamy soil than in a sandy soil. In general, grapevine is more sensitive to too much water than to drought. Too much water and consistently too little oxygen, affecting plant growth and increases the risk of root pathogen attack. For cultivation in arid areas, salinity in the root zone becomes a problem. In poorly drained soils the salt stays in the ground while the water evaporates (Jackson, 2000).

2 Aim

The aim of this project is to study the development of frost hardiness in different cultivars in relation to different ground cover.

2.1 Hypothesis

1. The four white grapevine cultivars may differ in growth habits, abilities for frost hardiness acclimation, diverse frost hardiness and bud break.
2. The ground cover (open soil, plastic cover and gravel) may affect soil thermal properties and the microclimate depending on water infiltration, thermal conductivity, heat radiation, evaporation and chilling effects which in turn affect growth, frost acclimation and bud break for the different cultivars.
    a. Plastic cover is expected to provide a higher soil temperature and higher day temperature in the crop than the open soil treatment.
    b. Gravel is expected to lower the soil temperature and higher the stock temperature in the evening than the open soil treatment.

3 Materials and methods

3.1 Terroir of the vineyard in Broddarp

The vineyard is located in Broddarp (N latitude 56°; longitude E 12°), in the south of Sweden on Bjäre peninsula (figure 1). The landscape on Bjäre peninsula consists of slightly rolling hills, open grazing and cultivation areas. The microclimate varies within the area, due to the shifting landscape. Altitude is 0-150 meters. Shrubbery, stone walls, groves and roche moutonnée, are around the plots and act as windbreaks (Länstyrelsen, 2006). The distance to Kattegatt is 3 km. The area has a flat underlying bedrock layer with different kinds of gneiss (Wikman & Bergström, 1987). Gneiss is a metamorphic rock and has good draining properties. The dominating soil type is moraine. The most common

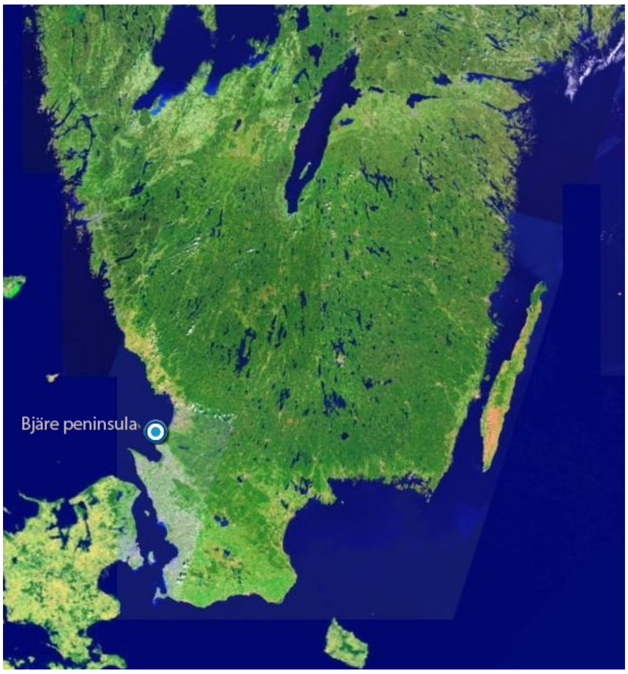

Figure 1. Map of southern Sweden, Bjäre peninsula is located in the lower left corner.
http://www.hitta.se/LargeMap.aspx?var=bj%e4rehalv%f6n

composition of the soil is sandy-silty-moraine. Soil depth of the area is approximate 5-15 meters in some case even more shallow (Ringberg, 1995).

3.2 Plant material

This study is based on a two-factorial field trial, performed in the autumn 2009. Factor 1 represents different cultivars; factor 2 are three types of ground cover. Four grapevine cultivars, Solaris, Pinot Gris, Siegerrebe and Ortega, were planted in May 2009 with a split-plot design, on four locations. Solaris served as a control. All cultivars are grafted on the same rootstock, SO4. Solaris is developed in Freiburg im Breisgau, Germany and is a hybrid between Merzling x (Zarya x Muskat Ottonel). Bud burst and flowering time and maturation is early (Torstensson & Pappinen, 2002). Pinot Gris is probably a mutant of Pinot Noir originally from Burgundy region in France. Bud burst and flowering time is late and maturation is medium to late (Winegrowers, 2010). Siegerrebe is bred in Alzey, Rheinhessen Germany and it is a Vitis *vinifera* cultivar crossing between Madeleine d'Angevine x Gewürztraminer. Bud burst and flowering time is medium late to late and maturation is early. Ortega is developed in Würzburg, Germany and it is a Vitis *vinifera* cultivar crossing between Müller-Thurgau x Sigerrebe. Bud burst and flowering time is late and maturation is in September-October (Torstensson & Pappinen, 2002).

3.3 Experimental design

This trial was performed in two adjacent fields (Appendix 2). Samples are primarily taken from plot 2 and the remain ones were captured in plot number 1 (Siegerrebe-open soil and Ortega-gravel). The plants are planted in rows with 26 plants per row. The outer plants in each row are not included in the experiment. The lanes between the rows are covered with grass and clover mixture. The plant distance was 1 m. The rows are situated in north-south direction.

Three kinds of ground cover are included in to the experiment; open soil, plastic cover (Don and Low MyPex ® Groundcover) and gravel. The plastic cover was black and water-permeable. Depth of the gravel layer is 0.15 m and its particle size was ∅ 30-120 mm. The row width was 1 m.

3.4 Analyses

3.4.1 Microclimate and soil temperature registrations

A weather station (16.99 Automatic weather station, 8 channels, Eijkelkamp Agrisearch Equipment, Netherlands) and temperature loggers (Tinytag Aquatic 2, 80 to -40°C, Gemini Data Loggers) were installed on 25 September 2009. The following meteorological data were recorded every 30 minutes; temperature, precipitation, wind speed, wind direction, air pressure, radiation and humidity. The soil temperature loggers are buried at a depth of 15 cm close to plant roots, with a density of two temperature loggers per ground cover and cultivar. They were randomly spread over the plot. Registrations were made every 30 minutes.

3.4.2 Plant analyses

Between 28 September and 9 October, main cane length and internodes were measured and number of nodes counted to check the growth of cultivars and the effect of ground cover .

3.4.3 Frost hardiness test

Frost hardiness measurements for cane tissue were performed every second week from late September to early December 2009.

At each sampling three plants from each treatment and cultivar were randomly selected at the fields. Samples were collected from one of the main canes. Nodes 6-9, from the top, were sampled and put in a enclosable plastic bag with a small amount of distilled water (about 10 ml) and stored in a cooler bag until arrival at the laboratory at Skogforsk, Ekebo, Svalöv (appendix 1). Samples were cooled to 2°C (at a rate of 10° C per hour) and then to -15°C (at the rate of -2°C per hour). -15°C was held for 3 hours, before the temperature was increased at a rate of 10°C per hour (Bengtsson, 2007). The controls were stored in a refrigerator at approximate 7° C.

In the end of January a special test was done. This test, however, differs from the other, no artificial freezing has been done and it is only for checking the survival and frost hardiness development depending on natural conditions.

Cooling stress injury was observed by electrolyte leakage. The leakage of electrolytes is correlated with the degree of injury. Electrolyte leakage occurs at plasmolysis, i.e. the cell membrane breaks and the cell dies (Raven et al., 2005). A high amount of released electrolytes indicates stronger injured and killed cells then low electrolyte content (Prášil & Zámečník, 1997). Release in electrolyte may be measured by change in electrical conductivity CEC (mS cm^{-1}).

After the freezing session one centimeter from each internodes (including the control) were cut out and placed in individual plastic test tubes with a lid. The test tubes containing 10 ml of ultra pure water. Test tubes were shaken on a horizontal shaker for 24 h before electrical conductivity was measured (Eco scan CON5 with ATC, Eutech instrument). All the samples were autoclaved at 120° C and 120 kPa for 20 minutes, cooled and shaken for another 24 hours followed by a second electrical conductivity measurement. A small volume, estimated 5%, of water evaporates during autoclaving, which was compensated for in the calculations (Bengtsson, 2007). $RC_{control}$ is the average amount of the controls, representing undamaged tissue and an Index of Injury of 0 % (Prášil & Zámečník, 1997). Index of injury at a temperature of -15° C was calculated.

$$I_t = \frac{100\,(RC_{frozen} - RC_{control})}{1 - RC_{control}} \qquad \text{(I)}$$

$$RC_{control} = \frac{EC_{unfrozen}}{EC_{unfrozenkilled}} \qquad \text{(II a)}$$

$$RC_{frozen} = \frac{EC_{frozen}}{EC_{frozenkilled}} \qquad \text{(II b)}$$

$$EC = electric\ conductivity$$

3.4.5 Visual assessment

On May 5, 2010 a visual assessment were made (figure 2). Buds on each plant were counted according BBCH development scale (figure 3), to get an idea how far the plants have come in its development at the time. Each plant was pruned to four buds. Buds who reached BBCH 05 or more (figure 2A and B) were counted.

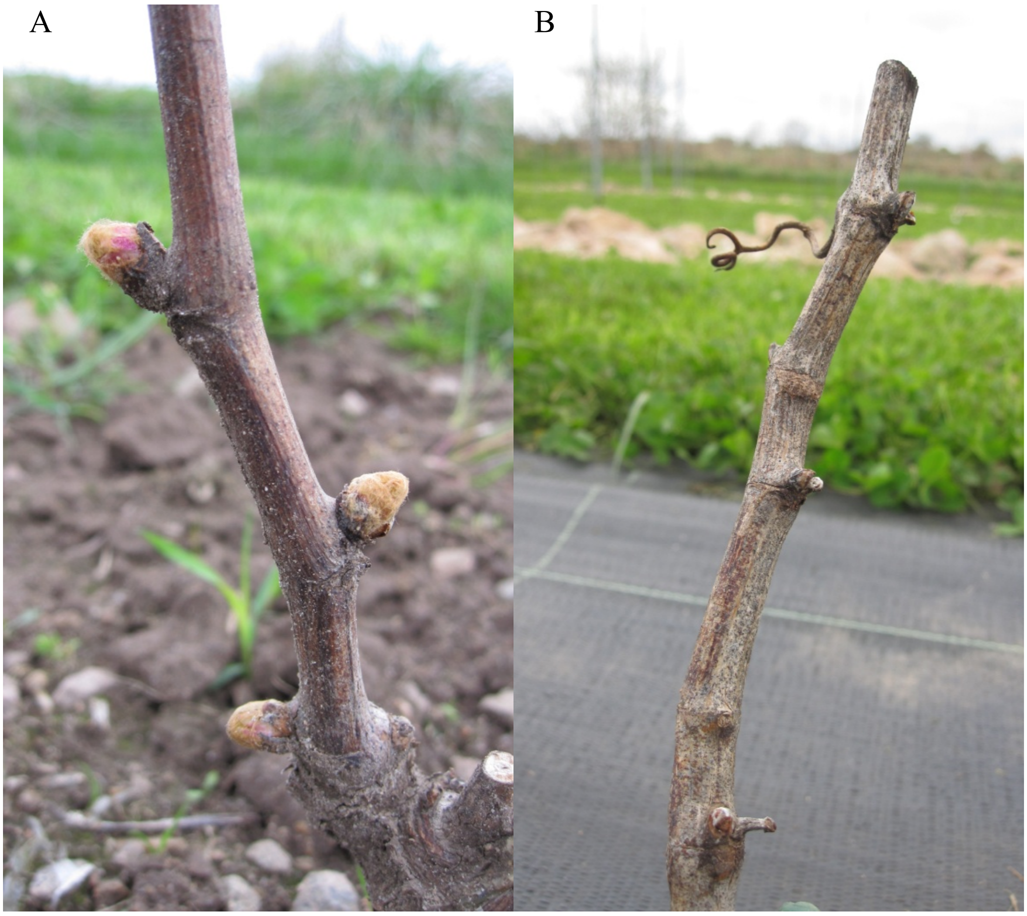

Figure 2. Example of swollen (A) and not swollen (B) buds (it is not the same cultivar in the pictures). The swollen bud correspond to BBCH 05 (figure 3), wool stage (brown wool clearly visible). Non-swollen buds to BBCH 00-0, winterbud-beginning of bud swelling (Lorenz et al., 1994). (Photo: M. Jansson)

Figure 3. BBCH identification key of grapevine (Eichhorn & Lorenz, 1977).

3.4.6 Statistical analyses

The experiment is performed on two factorial trial with a split plot design with four white grape wine varieties (Solaris, Pinot gris, Siegerrebe and Ortega) and their ground covers (open soil, plastic cover and grave). Samples are primarily taken from plot 2 and the remain ones were captured in plot number 1 (Siegerrebe-open soil and Ortega-gravel).One way ANOVA test (Tukey-test $P<0.05$) was used to examine whether the average between the groups differ in vegetative performance, impact of ground cover on growth and frost hardiness effect.

The monitored microclimate registrations (soil temperature and air temperature) where made every 30 minutes. The weather station were located in the middle of the field of plot 2. Two soil temperature loggers were buried per row, the mean temperature of the two loggers were calculated.

A visual assessment were made in May 2010. Buds on each plant were counted according BBCH development scale. Average number of buds was calculated and compared for each variety and soil treatment, a separately statistical study were made for Solaris to reveal probable interactions with the ground cover.

4 Results

4.1 Growth of cultivars in relation to ground cover

Measurements of the vegetative growth (table 1) of the plants showed that Solaris had significantly less number of nodes and the mean length of the longest cane was significantly lower in comparison to the other cultivars. Siegerrebe had the highest mean length of the longest cane and the greatest number of nodes. The internode length did not differ significantly between the varieties. There was a trend to longest internodes in Solaris followed by Pinot gris, Siegerrebe and Ortega (Solaris>Pinot Gris and Siegerrebe>Ortega).

Ground cover did not affect cane length, nor the number of nodes (table 2). However, the internodes were significantly shorter for the grapevine planted in the rows with gravel compared to open soil and plastic cover.

Table 1. Vegetative performance of the four white grapewine varieties. Longest cane (cm), number of nodes and internode length (cm) were measured, in 28 September- 6 october 2009, for the different cultivars (all cultivars are counted regardless of soil treatment) in a two factorial experiment with a split plot design. Factor comprised four white wine cultivars (Solaris, Pinot gris, Siegerrebe and Ortega) and their ground covers (open soil, plastic cover and gravel). Number of plants, Solaris n=72, Pinot gris n=72 Siegerrebe n=72 and Ortega n=72.

Cultivars	Longest cane	Number of nodes	Internode length
Solaris	108.4 a[1]	25.7 a	4.2 a
Pinot gris	127.5 b	31.5 b	4.0 a
Siegerrebe	137.2 bc	34.6 c	4.0 a
Ortega	129.6 b	33.1 bc	3.9 a

[1] Values within the same column followed by different letters are significantly different (Tukey test, P<0.05).

Table 2. Impact of ground cover on growth of white grapewine varieties. Longest cane (cm), number of nodes and internode length (cm) were measured, in 28 September- 6 October 2009 for the different ground cover (all soil treatment are counted regardless of cultivars) in a two factorial experiment with a split plot design. Factor comprised four white wine cultivars (Solaris, Pinot gris, Siegerrebe and Ortega) and their ground covers (open soil, plastic cover and gravel). Number of plants in each treatment, open soil n=96, plastic cover n=96 and gravel n=96.

Ground cover	Longest cane	Number of nodes	Internode lenght
Open soil	129.8 a[1]	31.1 a	4.2 b
Plastic cover	130.0 a	31.4 a	4.2 b
Gravel	117.2 a	31.2 a	3.7 a

[1] Values within the same column followed by different letters are significantly different (Tukey test, P<0.05).

4.2 Monitored microclimate

Soil and air temperature were recorded from May 25 2009 to January 24 2010. Maximal and minimal soil temperature during the period were 15.4°C and -5.0°C, respectively (table 3).The different ground cover did marginally affect the soil temperature. Plastic cover had the highest minimum temperature and mean temperature followed by gravel and open soil. All maximum temperatures where similar. At the end of December the soil temperature reached down to 0°C level for the first time. Then the soil temperature kept below 0°C during the rest of the measurement period, this in conjunction with lower air temperatures.

Air temperatures dropped below 0°C for the first time inthe end of October during the measurement period, but it was steadily below 0°C first at the end of the year (Dec 1) . Maximum and minimum air temperature during the period were 15.44°C and -12.0°C, respectively.

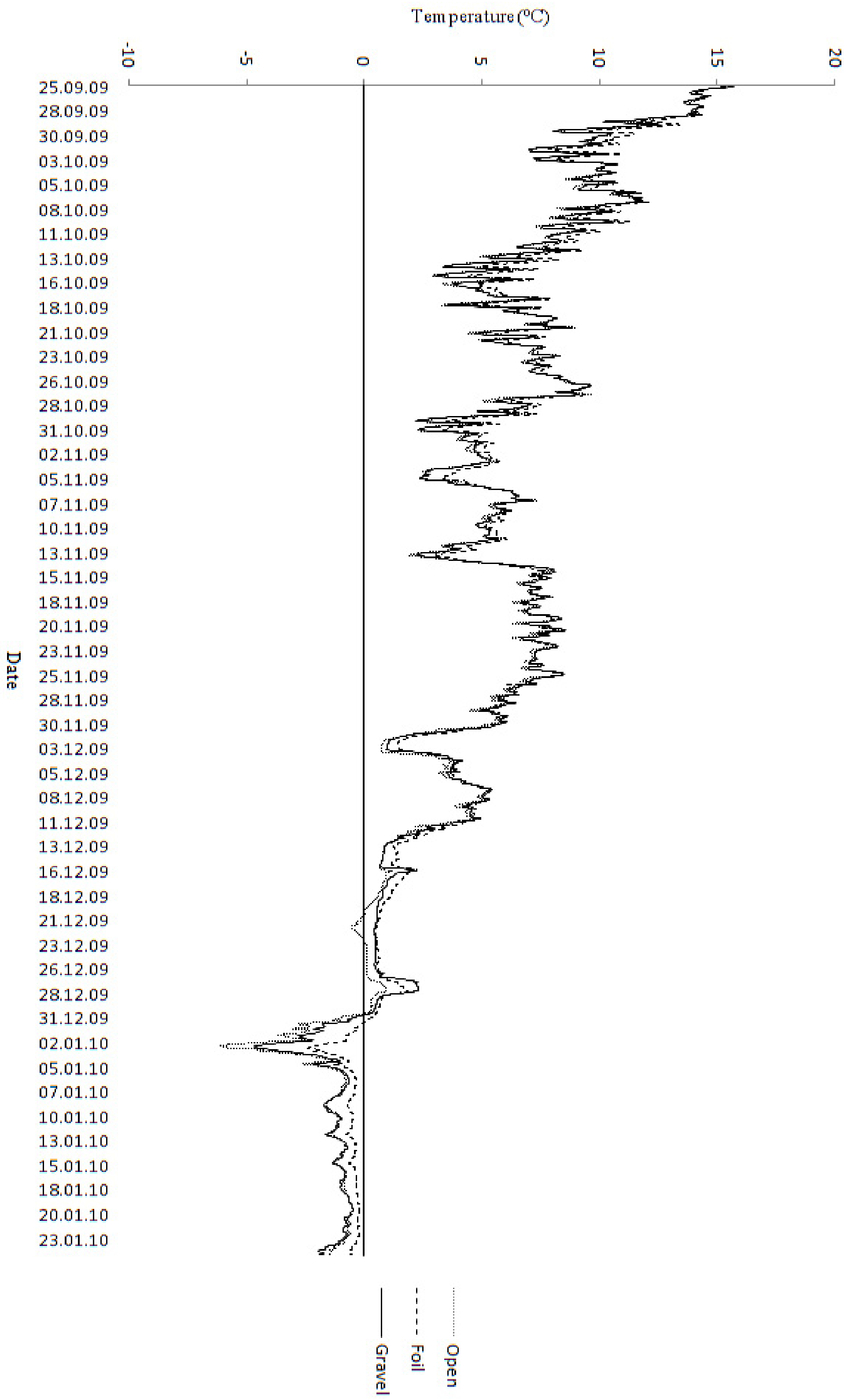

Figure 3. Soil temperature (^{o}C) during September 25, 2009 to January 24, 2010. Temperature measurements were performed using temperature loggers at a density of to two probes for cultivar and soil cover. Registrations were made every 30 minutes.

Table 3. Soil temperature as affected by ground cover. Soil temperature (^{o}C) during the period of September 25, 2009 to January 24, 2010. Temperature measurements were performed using temperature loggers at a density of two probes per cultivar and soil cover. Registrations were made every 30 minutes.

Ground cover	Open soil	Plastic cover	Gravel
Mean temperature	4.1	4.5	4.2
Max. temperature	15.2	15.3	15.4
Min. temperature	-5.0	-2.4	-4.2

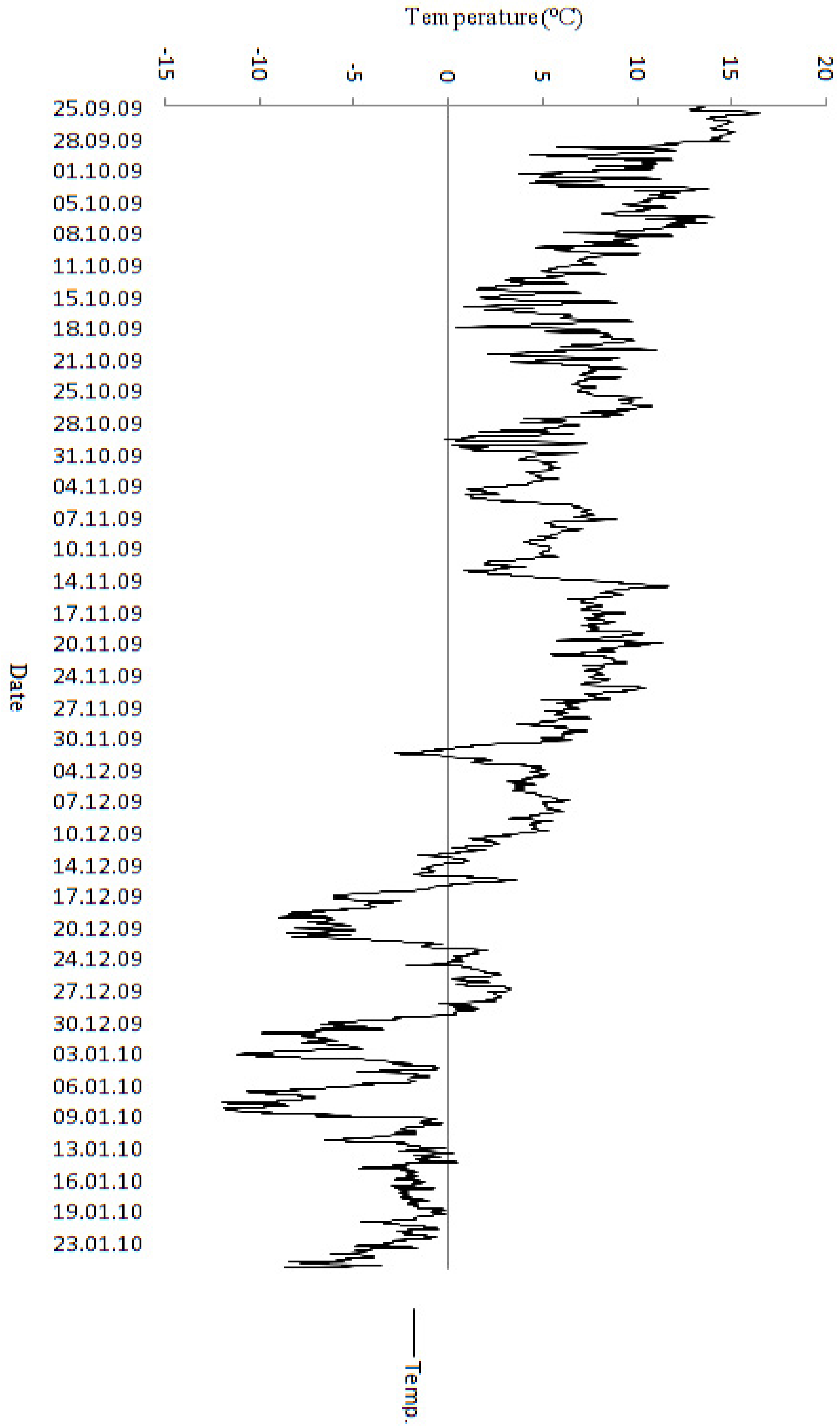

Figure 4. Temperature (^{o}C) data during the period of September 25, 2009 to January 24, 2010. Air temperature were recorded every 30 minutes. Maximal air temperature during the period is 15,44°C and minimum temperature is -12,0°C.

4.3 Frost hardiness

4.3.1 Frost hardiness development

The frost hardiness varie during the experiment (figure 5). At the first sampling event all samples from all cultivars was killed during the artificial freezing. In the second sampling event, frost hardiness increased considerably for all cultivars. At the third sampling frost hardiness was unaffected. In the fourth frost hardiness of Solaris was clearly distinct from the other cultivars. In the fifth sampling, Solaris continued to get hardier, when Pinot gris, Ortega and Siegerrebe remain more or less unaffected. In the sixth run Solaris frost hardiness has increased a lot and became frost hardy. For the other three varieties, frost hardiness was reduced in comparison with previous sampling.

The seventh sampling which was performed without artificial freezing and ented as a check to compare the data gathered under artificial conditions confirmed Solaris as frost hardy at the event and the three remaining cultivars as non-hardy.

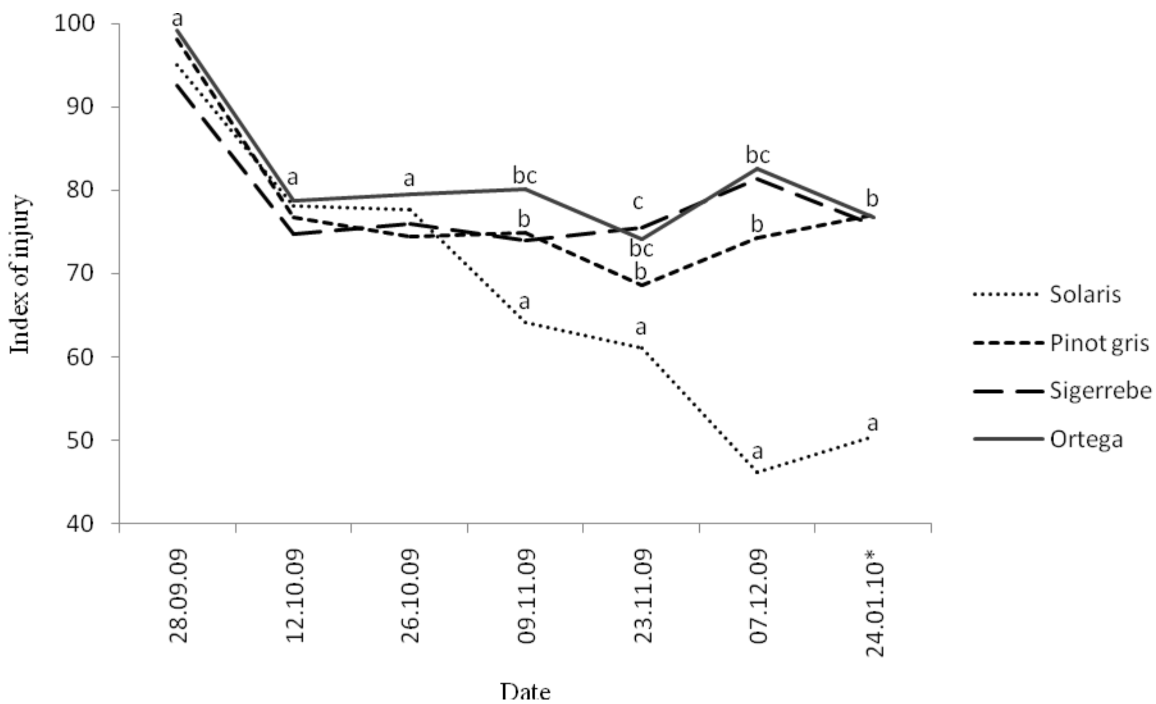

Figure 5. Frost hardiness acclimation during late September to January. Samples were exposed to a temperature of -15 °C. N=27, from each cultivar and occasion. At the different measurement occasions different letters are stated, they indicate the significant differences (Tukey test, P<0.05).
** No artificial freezing.*

4.3.2 Frost hardiness in dependent on cultivars and ground cover

Frost hardiness effect on cultivars (figure 4) showed significant differences. For the first three samplings no significant differences between the cultivars were found but the result from run four to seven showed that Solaris has reached the significantly highest frost hardiness. Solaris was the only cultivar in this experiment that reached frost hardiness at -15 °C.

No ground cover effects on hardiness (table 4) were stated when all cultivars were included into the statistical analysis. As frost hardiness was induced in Solaris, a detailed statistical analysis was performed, indicating an interaction between frost hardiness and ground cover (table 5). Significance for run 5 and 6 is found.

Table 4. Frost hardiness for all cultivars effect in dependent on ground cover (Lt_{50}). Number of plants in each treatment, open soil n=96, plastic cover n=96 and gravel n=96.

	Date	Open	Plastic cover	Gravel
1	28.09.09	96.3a[1]	99.0a	93.6a
2	12.10.09	78.3a	76.5a	76.4a
3	26.10.09	75.9a	75.7a	78.9a
4	09.11.09	71.8a	77.1a	70.9a
5	23.11.09	68.6a	68.3a	72.5a
6	07.12.09	74.5a	72.2a	66.3a
7	24.01.10*	64.6a	71.9a	73.5a
1-6	28.09.09-07.12.09	77.5a	78.1a	76.5a

[1]Values within the same rows followed by different letters are significantly different (Tukey test, P<0.05). No artificial freezing.

Table 5. Frost hardiness effect for Solaris in dependent on ground cover (Lt_{50}).). Number of plants in each treatment, open soil n=96, plastic cover n=96 and gravel n=96.

	Date	Open	Plastic cover	Gravel
1	28.09.09	98.4a[1]	96.1a	90.8a
2	12.10.09	80.7a	75.6a	77.8a
3	26.10.09	78.2a	82.7a	72.2a
4	09.11.09	65.0a	68.2a	59.3a
5	23.11.09	60.7ab	54.0b	68.7b
6	07.12.09	57.7a	47.7ab	32.9b
7	24.01.10*	41.3a	46.5a	63.8a

[1]Values within the same rows followed by different letters are significantly different (Tukey test, P<0,05). *No artificial freezing.

4.3.3 Mean growth of buds in dependent on cultivars and ground cover

The mean growth of buds differed considerably between the cultivars involved in this study. Solaris was the cultivar with the most swollen buds (2.7) when the visual control was made in May 5, 2010, followed by Ortega (1.4), Pinot gris (1.0) and finally Siegerrebe (0.8). Number of plants, Solaris n=72, Pinot gris n=72 Siegerrebe n=72 and Ortega n=72.

No interaction with growth of buds dependent on ground cover was found. Plastic cover and gravel had mean1.5 buds per plant, open soil had mean 1.4. Number of plants in each treatment, open soil n=96, plastic cover n=96 and gravel n=96.

A separate statistical study was done for Solaris to reveal probable interactions with the ground cover; however, these differences were marginal, mean number of buds in open soil(3.1), plastic cover (2.7) and gravel (2.3). Number of plants, n=24 per treatment.

5 Discussion

Among the studied cultivars, Solaris was significantly most frost hardy under southern Sweden conditions.

As previously mentioned , cold acclimation in grapevines occurs in two stages, above 0°C and under 0°C. Temperatures above 0°C do not entail maximum hardiness, it is not until the plant is exposed to temperatures below 0°C as the maximum hardiness can be achieved (Zabadal *et al*., 2007). Our results support this interaction. The temperature measured during autumn 2009 did not go below 0°C until the end of October. Next temperature drop under 0°C was not acted until the end of November, until then the temperature oscillated around 5°C. Solaris reached frost hardiness at -15°C in early December. Ortega, Pinot Gris and Sigerrebe did not achieved frost hardiness at -15°C during the study. High temperature is possibly one of the reasons why the plants did not get hardy at -15°C. Also the fact that the upper parts of the plant even do not get hardy. It depends partly on that cold acclimation development of the plant goes from the bottom to the top (Zabadal *et al*., 2007), those factors probably affect the outcome.

One of the reasons for better cold acclimation might be the less vigorous vegetative growth. Vigorous growth during the autumn prevents cold acclimation before the frost (Winkler, 1974). Highly vigorous canes are therefore less cold hardy than less vigorous canes (Zabadal *et al*., 2007). In this study Solaris had the shortest cane length and minimum number of nodes.

The verification test of survival and frost hardiness development under natural conditions, confirmed that Solaris as the only cultivar that developed hardiness based on the present results.

Ground cover did not affect cane length and number of nodes, but internode length was significantly shorter for the grapevine planted in the rows with gravel compared to open soil and plastic cover.

The temperature in the ground showed small temperature differences between the ground covers, the biggest difference in temperature could be seen for plastic cover, which was two degrees higher than the other ground covers. The energy supply to the soil depends on radiative properties of the mulch and the energy exchange across the mulch (Liakatas *et al,* 1986). Liakates (1986) report showed that black mulches reduced the diurnal temperature and always reduced the radiant heat of the soil. But there are reports contradicting these findings, some tell an increase in temperature and other advocate a decrease in temperature (Liakatas *et al,* 1986). No correlations between increased or decreased frost hardiness and plastic cover were found.

According Nachtergaele (1998) gravel mulch largely affects the temperature, the soil temperature and the soil surface temperatures are higher with gravel than without gravel. This was not supported by the present results.

Differences between growth of buds in dependent on cultivar were found. Solaris was the cultivar with the most swollen buds when the visual control was made; This finding is expected, as Solaris has the earliest bud break of the studied cultivars.
For forthcoming studies on frost hardiness in grapevine, some alterations in the experimental set-up should be considered.

- The stem sample should be taken further down on the cane.
- As the minimum air temperature during the experiment was -12°C, an adaptation of the artificial temperature (-15°C) during frost hardiness tests might be considered in order to receive results relevant for the climate in Southern Sweden. It would be interesting to follow the temperature the plant can withstand during the frost hardiness acclimation at each test occasion, for example 0°C, -5°C, -10°C etc. Alternatively, grapevine should be grown in a climate chamber, for improved temperature control.

6 References

Bengtsson, S. (2007*) Frost hardiness of some New Zealand shrubs*. Department of Horticulture, Swedish University of Agricultural Science, Sweden, Alnarp

Dami, I. (2007) *Freezing survival mechanisms of grapevines*. Horticulture and crop Science, The Ohio State University. Workshop proceedings; Understanding and preventing freeze damage in vineyards. University of Missouri, Columbia

Eichhorn, K.W. and Lorenz, H. (1977) Phaenologische Entwicklungstadiender Rebe. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes (Braunschweig) 29, 119-120

Eriksson, J., Nilsson, I. and Simonsson, M. (2005) Wiklanders marklära. Studentlitteratur, Lund, Sweden

Goffinet M.C. (2004) *Anatomy of grapevine winter injury and recover*. Cornell University Department of Horticultural Sciences, NY State Agricultural Experiment Station, Geneva

Goffinet, M.C. (2007) *Grapevine cold injury and recovery after tissue damage and using cane burial to avoid winter injury.* Department of Horticultural Science, Cornell University, New York, Agricultural Experiment Station. Proceedings of the ASEV 50th anniversary meeting, Washington

Howell, S.G. (2000) *Grapevine cold hardiness: mechanism of cold acclimation, mid-winter hardiness maintenance, and spring deacclimation.* Department of Horticulture, Michigan University, East Lansing, USA. American Society for Enology and Viticulture. Proceedings of the ASEV 50th anniversary meeting, Washington

Hamman, Jr. R.A., Dami I.-E., Walsh T. M., and Stushnoff C. (1996) *Seasonal Carbohydrate Changes and Cold Hardiness of Chardonnay and Riesling Grapevines*. American Society for Enology and Viticulture47(1):31-36

Jackson, R. S. (2000) Wine science principles, practice, perception, 2nd.Academic press

Larcher, W. (2003) Physiological plant ecology -ecophysiology and stress physiology of functional groups. 4^{ed}. Institute of botany. University of Innsbruck, Austria

Liakatas, A., Clark, J.A. and Monteith, J.L. (1986) *Measurements of the heat balance under plastic mulches*. Elsevier Science Publishers B.V. University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, UK

Länstyrelsen (2006) Beskrivning, slutsatser och riktlinjer för karaktärsområdet Bjärehalvön. Det skånska Landsbyggsprogrammet. Tillgänglig på Internet:

http://www.lansstyrelsen.se/skane/Kartor_och_planeringsunderlag/Landsbygdsprogram/24_Bjarehalvon.htm [Hämtad: 2010-04-12]

Nachtergaele, J., Poesen, J. & van Wesemael, B. (1998) *Gravel mulching in vineyards of southern Switzerland.* Soil & Tilllage Research Vol. 46, No. 1 (51-59)

Prášil, I.& Zámečník, J. (1997) *The use of a conductivity measurement method for assessing freezing injury. Influence of leakage time, segment number, size and shape in sample on evaluation of the degree of injury.* Research Institute of Crop Production, Department of Physiology and Molecular Biology, Drnovská, Czech Republic

Raven. P.H., Evert, R.F. and Eichhorn, S.E. (2005) Biology of plants. 7ed W.H. Freeman and Company Publishers

Ringberg, B. (1995) Beskrivning till jordartskartan Halmstad SV, SGU.

Salzman, R.A., Bressan, R.A., Hasegawa, P.M., Ashworth, E.N. and Bordelon, B.P. (1996) *Programmed accumulation of LEA-like proteins during desiccation and cold acclimation of overwintering grape buds.* Plant, Cell Environment, Vol. 19, No. 6 (713-720)

Schultz, H.R. (2008) *Climate change and viticulture: A European perspective on climatology, carbon dioxide and UV-B effects*. Fachgebiet Weinbau, Institut für Rebenzüchtning und Rebenveredlung, Forschnungsanstalt, Geisenheim, Germany

Stafne, E.T. (2007) *Factors affecting cold hardiness in grapevines*. Department of Horticulture and Landscape Archirecture, Oklahoma State University, Stillwater, OK Workshop proceedings; Understanding and preventing freeze damage in vineyards. University of Missouri, Columbia

Striegler, K.R (2007) *Passive freeze prevention methods.* Institute of Continental Climate Viticulture and Enology, University of Missouri-Columbia

Systembolaget (2010) Lokalproducerat nu i de tre närmaste systembutikerna Pressmeddelande. Tillgänglig på Internet: http://www.systembolaget.se/Press/Pressmeddelanden/Arkiv/20101/2010/Lokalproducerat-nu-i-de-tre-narmaste-systembutikerna/ [Hämtad: 2011-06-18]

Taiz, L. and Zeiger, E. (2002) Plant physiology, 3rd ed. Sinauer Associates, Inc., Publishers, Sunderland, Massachsetts

Torstensson, L. and Pappinen, L. (2002) Odla och tillverka vin. Pagina Förlags AB, Sundbyberg

Wikman, H. and Bergstöm, J. (1987) Beskrivning till berggrundskartan Halmstad SV. -SGU Af 133

Winegrowers (2010) Pinot gris. Tillgänglig på Internet: http://www.winegrowers.info/varieties/Vine_varieties/Pinot_gris.htm [Hämtad 2010-11-05]

Winkler, A.J., Cook, J. A., Kliewer, W.M. & Lider, L.A. (1974) General Viticulture. Univ. California Press. Berkeley, Los Angeles, London

Zabadal, T.J., Dami, I.E., Goffinet, M.C., Martinson, T.E.and Chien, M.L. (2007) *Winter injury to grapevines and methods of protection.* Extension Bulletin, E2930, June
A collaboration of; Cornell University, Michigan State University, The Ohio State University, The Pennsylvania State Universitet

Photo sequence describing the sampling process. (Photo: M. Jansson)

1. Plants
2. Sample node 6-9.
3. a, b and c are control sample
4. Collected and cut internodes

5-6.All internodes were sorted and put in to a bag with a small amount of distilled water.

7. Samples were put in the freezer
8. Computer with the freezing program linked to the freezer

9-10. Sample cut in small pieces and put in to test tubes.

Map of cultivation area (http://www.hitta.se/LargeMap.aspx?var=broddarp).