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Changes of temperature exotherms and soluble sugars in grapevine (*Vitis vinifera* L.) buds during winter

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Summary

Nonstructural carbohydrates (NSC), including water soluble carbohydrates (WSC) are thought to serve an important role in freezing tolerance of many plants. Raffinose family oligosaccharides (RFOs) are α-galactosyl derivatives of sucrose. The most common RFOs are the trisaccharide raffinose, the tetrasaccharide stachyose, and the pentasaccharide verbascose. RFOs are nearly ubiquitous in the plant kingdom and are found in a large variety of seeds from many different families.

Severely cold winter temperatures can significantly impact grapevine productivity through tissue and organ destruction caused by freeze injury. Crop loss and the need to retrain vines after bud, cane, and trunk injury mean financial loss, often for one or more years.

Buds of grapevine (*Vitis vinifera* L.), grown at the vineyard of the Institute of Fruit Science, Vegetable Science and Viticulture, University of Hohenheim, Germany, were sampled during winter and analyzed for their concentration of soluble sugars (i.e. glucose, fructose, sucrose, raffinose and stachyose) and thermal analysis was performed to determine their freezing points. Freezing of extracellular water was recorded from -5 to -16 °C with a minimum at the beginning of January; freezing of intra-cellular water was recorded from -11 to -24 °C. Apical buds are very important organs as they determine further growth and development of tree species. Bud physiological state, including saccharide metabolism, determines their growth activity. The concentration of soluble sugars was highest by the end of December. Sugar concentrations in basal buds were significant higher than in buds from intermediate and apical shoot sections. A significant correlation could be proofed between sugar concentrations (i.e. raffinose and stachyose) and air temperature before sampling. But there was no correlation between freezing temperature of extra-cellular or intra-cellular water and soluble sugars in bud tissues.

Introduction

Plants have evolved a variety of mechanisms for resisting cold temperatures (a review is given by e.g. BURKE et al., 1976). Temperature is expected to affect all plant processes. Consistent with this, expression of a large number of specific mRNAs and proteins is up-regulated during cold-acclimation. Their possible functions are outlined, encompassing a wide range of processes, some possibly related to other winter stresses besides cold. Some of the cold-responsive sequences are associated with constitutive or stress-related metabolism, others are probably protective, some may influence freezing, and many are of as yet unknown function (PEARCE, 1999). Soluble carbohydrates are strongly correlated with freezing tolerance of plants. Sucrose is often the main sugar associated with cold hardiness, but not for all species (GUSTA et al., 1996). Fructans accumulate in grasses (SMITH, 1968) and raffinose is the main sugar in e.g. *Ajuga reptans* (BACHMANN et al., 1994). Sugars are known to protect cells and membranes (e.g. LEVITT, 1980) and soluble carbohydrates in the apoplast interfere with ice crystal growth and reduced mechanical injury associated with freezing (OLIEN, 1967).

The resistance of dormant grapevine buds against cold temperatures is based on supercooling (BURKE et al., 1976; ANDREWS et al., 1984). The freezing temperature of supercooled tissues can be monitored by the detection of evolved (exothermic) heat caused by freezing. In grapevine buds two temperature exotherms are normally registered. The high-temperature exotherm (HTE) indicates freezing of extracellular water and the low-temperature exotherm (LTE) indicates freezing of intra-cellular water (WOLF and COOK, 1994). The exotherms showing up as temperature peaks during freezing of supercooled water are generated by the dissipation of latent heat. The peak area should thus correspond to the amount of freezing water, whereas the form of the peak is influenced by the degree of supercooling and by the freezing speed which depends on the structure of the tissue.

The following investigations were conducted to characterize freezing events and changes in soluble carbohydrates of grapevine (*Vitis vinifera* L.) buds during winter months.

Materials and methods

Plant material

Vitis vinifera L. (cv. *Bacchus*) was grown at the vineyard of Hohenheim University, Germany. Bud samples were collected from basal, intermediate, and apical sections of the shoots. Sampling took place after natural defoliation at the following dates: 1998/11/12, 1998/11/26, 1998/12/10, 1998/12/23, 1999/01/07, 1999/01/21, 1999/02/25, 1999/03/11, 1999/03/25, 1999/04/08 and 1999/04/16. Sampling was repeated in the following winter season (1999/2000).

Methods

Thermal analysis was performed by using an exotherm-measuring-instrument (Kryoscan 4.0, manufactured by R. Blaich, University of Hohenheim, Germany). The computerized system is capable of controlling the freezing rate and collecting, storing, and analyzing data to determine their freezing point using temperature exotherm analysis. The principle of this analysis is described by WAMPLE et al. (1990). With the multi-channel data registration 16 buds were measured simultaneously with a cooling rate of 8 °K/h and a temperature precision of 0.2 °K. Temperature measurements on complete cold adapted latent buds during freezing usually revealed one or two high temperature exotherms (HTEs) at temperatures between -5 and -10°C and one to three low temperature exotherms at temperatures (LTEs) down to -25°C, depending on the frost adaptation of the buds.

Dry matter (DM) content from buds and according shoot sections was determined. For carbohydrate analysis, the lyophilized samples were ground. 60 mg of dried tissue was extracted twice for 15 min in 7 ml boiling H_2O_{dest}. The two extracts were centrifuged, and the combined supernatant (14 ml) was passed through a 0.22 µm membrane filter.

Soluble sugars were quantified using high-performance anion exchange chromatography (HPAE) combined with pulsed amperometric detection (PAD) in a Dionex® DX300 ion chromatograph fitted with a CarboPac® PA-1 column (CHATTERTON et al., 1989). Flow rate was 1 ml*min^{-1} at about 1900 psi. Carbohydrate elution was effected under alkaline conditions (150 mM NaOH). The high pH (12-13) of the eluant converts hydroxyl groups of the oligosaccharides into oxyanions. The degree of oxyanion interaction with the anion exchange resin determines carbohydrate retention times. Adding a competing ion such as acetate (0-500 mM NaOAc) to the eluant reduces the retention times. Sugars in the extract were identified by comparing their HPLC-PAD retention times on various gradients with those of known sugars (i.e. glucose, fructose, sucrose, raffinose, stachyose) and by spiking the extracts with known pure sugars. Known pure sugars were used for calculation of the sugar concentration in extracts.

Statistical analysis was undertaken with SAS 6.12 (SAS Institute Inc., Cary, NC/USA) using PROC ANOVA/GLM procedures and Tukey's studentized range test for analysis of variance and PROC CORR procedures and Pearson's correlation coefficient for analysis of correlation.

Results

Temperature exotherms

The average weight of buds was around 30 mg, about 40-60 % were contributed by the bud scales and the wool. The greatest part of living tissue consists of a woody bud pad (subtending nodal tissue) which carries the shoot primordia. Histologically it belongs to the cane and its size depends among others on the experience and skill of the person removing the bud. The freezing of the apoplastic water of the bud always produces a relatively large HTE. Only in 60 % of all buds at least one distinct LTE of the primary shoot primordium (primary bud) was obtained and only in few cases the LTEs of the small secondary buds could be detected. Evidently the water content of these shoot primordia, in particular of the secondary and tertiary buds is at the limit of the resolution of the measuring device which is further diminished by the (then frozen) apoplastic water of the bud pad which separates the freezing shoot primordia from the sensor plate.

High-temperature exotherms (HTEs) indicating the freezing of extracellular water were recorded from -5 to -16 °C with the minimum at the beginning of January (Fig. 1). Low-temperature exotherms (LTE) indicating the freezing of intra-cellular water were recorded from -11 to -24 °C. Low-temperature exotherms of basal buds were significant higher than exotherms of intermediate and apical buds. Temperature exotherms were neither correlated to air temperature measured during the days before sampling nor to any of the soluble sugar concentrations (Tab. 1).

In our case it could be clearly shown, that an HTE caused no cell damage in the buds (Fig. 3), while after the appearence of the LTE(s) a coagulation of protoplasts in primary and secondary buds could be found (Fig. 4).

Soluble sugars

Soluble sugars were detected as glucose, fructose, sucrose, raffinose and stachyose with highest concentrations in basal buds and lowest concentrations in apical buds (Fig. 2). Sucrose was the dominant soluble carbohydrate and the reducing sugars (i.e. glucose, fructose) reached also rather high concentrations while raffinose and stachyose was detectable only in small concentrations. From November to late December/early January total soluble sugars increased from about

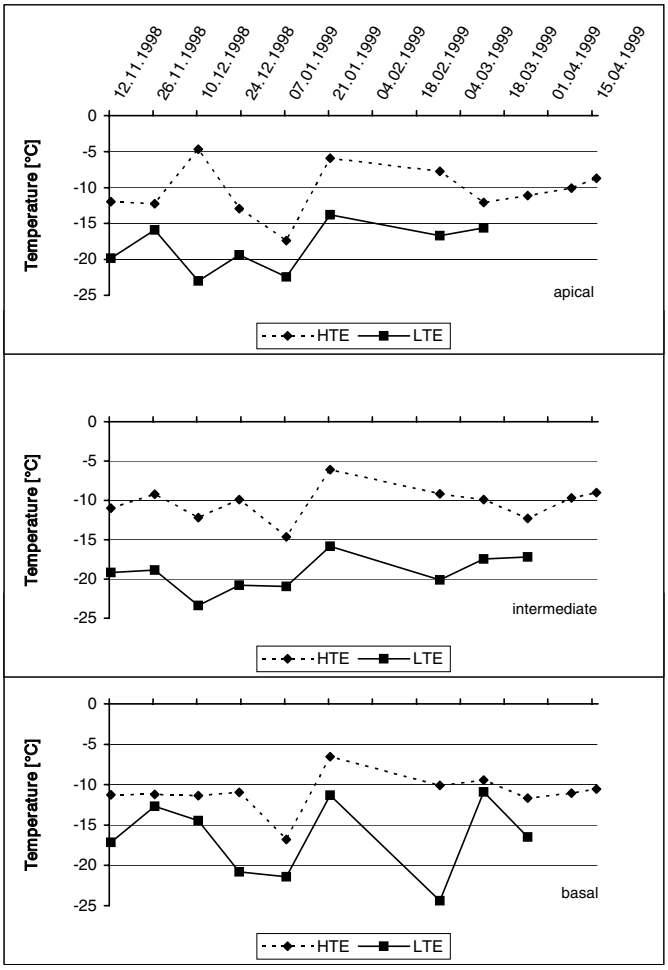

Fig. 1: High-temperature exotherms (HTE) and low-temperature exotherms (LTE) of grapevine buds of different sections of the shoot.

80 mg/g DM to almost 150 mg/g DM. The lowest concentration of total soluble sugars was registered at the end of March with about 35 mg/g DM followed by a slow increase of the sugar concentration in the bud tissue.

Differences in sugar concentrations of buds according to bud position were significant between basal/intermediate and basal/apical for the following fractions: total soluble sugars, fructose and sucrose. The concentration of glucose was significantly different only for basal and terminal buds. Differences in sugar concentrations between intermediate and apical buds were never significant. For apical and intermediate buds a similar pattern of significant changes during sampling in total soluble sugars was notable.

Between sugar concentrations and air temperature of the days before sampling (3, 5, 7, 9 days before sampling) exists a significant correlation (Tab. 1). The Pearson's correlation coefficient is regularly higher for intermediate and apical buds than for basal buds. The correlation coefficient was highest for raffinose and also stachyose showed a relatively high correlation coefficient. From the dominating sugars, the correlation coefficient from sucrose concentration with air temperature before sampling was lowest while the correlation coefficient of glucose concentration with air temperature before sampling was highest. Generally the correlation coefficient between sugar concentrations and air temperature before sampling increased from three days before sampling to seven days before sampling and decreased with longer time periods (Tab. 1).

Tab. 1: Correlation analysis (Pearson's correlation coefficient) between mean air temperature recorded for different time periods (1-9 days, indicated as T_{1-3} to T_{1-9}) before sampling, temperature exothermes (HTE = high-temperature exotherme, LTE = low-temperature exotherme) and sugar concentrations of grapevine buds.

Basal buds		Glucose	Fructose	Sucrose	Raffinose	Stachyose
HTE	r =	0.01	-0.01	-0.05	-0.14	0.05
	p(r) =	0.944	0.953	0.771	0.422	0.782
LTE	r =	-0.03	-0.22	-0.54	0.33	0.03
	p(r) =	0.895	0.342	0.011	0.149	0.891
T_{1-3}	r =	-0.54	-0.30	-0.19	-0.65	-0.50
	p(r) =	0.001	0.051	0.220	0.001	0.001
T_{1-5}	r =	-0.57	-0.32	-0.19	-0.75	-0.53
	p(r) =	0.001	0.032	0.228	0.001	0.001
T_{1-7}	r =	-0.59	-0.35	-0.20	-0.77	-0.55
	p(r) =	0.001	0.019	0.185	0.001	0.001
T_{1-9}	r =	-0.58	-0.33	-0.17	-0.72	-0.55
	p(r) =	0.001	0.027	0.259	0.001	0.001

Intermediate buds		Glucose	Fructose	Sucrose	Raffinose	Stachyose
HTE	r =	0.01	0.02	0.05	0.03	0.12
	p(r) =	0.956	0.923	0.765	0.845	0.488
LTE	r =	-0.26	-0.22	-0.12	-0.08	-0.07
	p(r) =	0.226	0.308	0.578	0.728	0.759
T_{1-3}	r =	-0.65	-0.52	-0.43	-0.70	-0.55
	p(r) =	0.001	0.001	0.004	0.001	0.001
T_{1-5}	r =	-0.67	-0.55	-0.49	-0.77	-0.57
	p(r) =	0.001	0.001	0.001	0.001	0.001
T_{1-7}	r =	-0.69	-0.56	-0.51	-0.76	-0.57
	p(r) =	0.001	0.001	0.001	0.001	0.001
T_{1-9}	r =	-0.67	-0.57	-0.51	-0.72	-0.156
	p(r) =	0.001	0.001	0.001	0.001	0.001

Apical buds		Glucose	Fructose	Sucrose	Raffinose	Stachyose
HTE	r =	0.05	-0.06	-0.24	-0.21	-0.14
	p(r) =	0.782	0.729	0.181	0.247	0.432
LTE	r =	-0.25	-0.24	-0.11	-0.11	-0.13
	p(r) =	0.308	0.334	0.693	0.650	0.595
T_{1-3}	r =	-0.62	-0.50	-0.43	-0.67	-0.49
	p(r) =	0.001	0.001	0.004	0.001	0.001
T_{1-5}	r =	-0.62	0.50	-0.45	-0.71	-0.52
	p(r) =	0.001	-0.001	0.002	0.001	0.001
T_{1-7}	r =	-0.63	-0.52	-0.48	-0.72	-0.54
	p(r) =	0.001	0.001	0.001	0.001	0.001
T_{1-9}	r =	-0.63	-0.54	-0.51	-0.71	-0.56
	p(r) =	0.001	0.001	0.001	0.001	0.001

Discussion

Overwintering canes of woody plants may freeze at temperatures below -10 °C without damage. It is generally assumed that in this case ice crystals are formed in the xylem or in the apoplast but not within the cells of pith rays or meristems which freeze only at considerable lower temperatures (generally below -30°C) if the plants are cold adapted. A special case are winter buds. While the base of the bud behaves like the cane tissue, the eyes of frost hardened woody plants freeze at -20°C or below. Therefore superchilling of such buds usually produces so called high temperature exotherms (HTEs) caused by freezing of apoplastic water and one ore more low temperature exotherms (LTE) resulting from the freezing of cells. The number of LTEs depends on the complexity of the bud. There is a large number of papers on these phenomena in different plants: *Vitis* (Andrews et al., 1984), tomato (Anderson and Ashworth, 1985), *Prunus* spp. (Quamme, 1974), cherry (Andrews and Proebsting, 1987; Callan, 1990), peach (Quamme, 1978, 1983; Andrews et al., 1986; Ashworth and Davis, 1984, 1987; Rajashekar, 1989), apple (Quamme, 1976), persimmon (Kang et al., 1997), blackberry

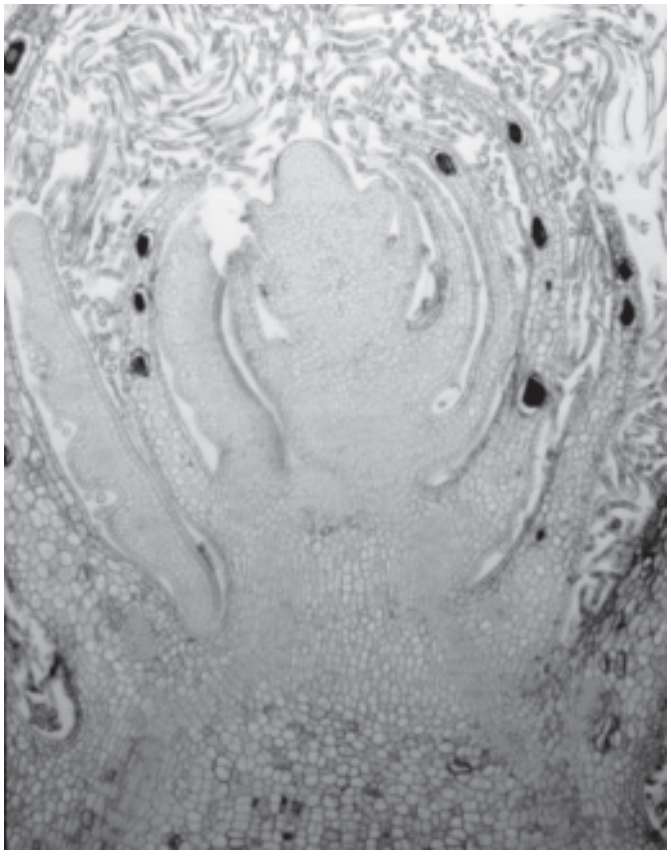

Fig. 3: Section through primary vegetation cone of a compound bud after occurrence of a HTE. No damage is visible.

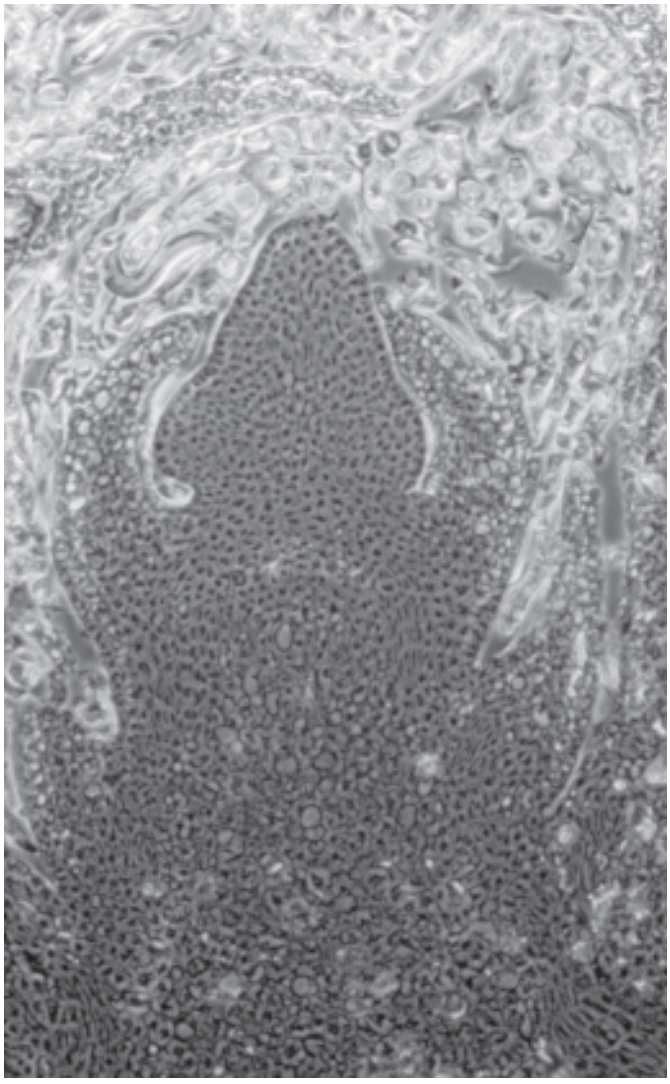

Fig. 4: Vegetation cone after appearance of LTE: Due to freezing the cytoplasm has coagulated and forms small clots within the cell.

(WARMUND et al., 1992), blueberry (BIERMANN et al., 1979; FLINN and ASHWORTH, 1994 a, b), strawberry (HUMMEL and MOORE, 1997) *Forsythia* (FLINN and ASHWORTH, 1995), azalea (GRAHAM and MULLIN, 1976 a, b), Rhododendron (KAKU et al., 1981), conifers (IDE et al., 1998). It is generally assumed that a high frost resistence depends on a gradual cold acclimatisation, the mechanisms of which are, however, far from being known. Important factors are desiccation, change of cell wall structures (in wood: WISNIEWSKY, 1993), biochemical processes such as deposition of specific proteins (MARENTES et al., 1993; HON et al., 1995 in graminees; SALZMANN et al., 1996 in grapevine), proline (AIT BARKA and AUDRAN, 1997) or the conversion of polysaccharides to monosaccharides (KLIEWER, 1965).

ANDREWS et al. (1983, 1984, 1986), WOLF and POOL (1986, 1987), and WOLF and COOK (1994) compared varieties on the basis of exotherm measurements since it had been shown by PIERQUET and STUSHNOFF (1977), PIERQUET et al. (1980) and QUAMME (1986) that LTEs were reliable indicators for the death of grapevine buds. However, FLINN and ASHWORTH (1994) concluded from experiments with blueberries and *Forsythia* that this is not necessarily true for flower buds of other plants which are anatomically different although KADIR and PROEBSTING (1994) had used exotherm measurements to test sweet cherry cultivars for dormant bud hardiness.

Temperature exotherms in our investigations showed a systematic pattern for apical, intermediate and basal buds. With the exception of one measuring date, freezing of extra- and intra-cellular water was at the lowest temperature at the end of December and the beginning of January. There are several factors affecting the thermal analysis of grapevine buds (WOLF and COOK, 1994).

Research on soluble sugars in grapevine is focussed on berries. Glucose, fructose and sucrose constitute the major sugars in the grape plant and also in buds (WAMPLE and BARY, 1992), according to our findings. The presence of raffinose and stachyose is also reported in grape plants (not specifically for buds), detected with means of paper chromatography (KLIEWER, 1965). Our results show, that raffinose and stachyose were present in grapevine buds throughout the whole sampling period, but only in low concentrations. In grapevine buds, KOUSSA et al. (1998) found generally lower concentrations of total soluble sugars compared to internodes. In their experiments, the accumulation of raffinose and sucrose, starting in October, was related to the decrease of daily average temperatures and also WAMPLE and BARY (1992) and HAMMAN et al. (1996) found a relation between soluble carbohydrates and air temperature. KOUSSA et al. (1998) see the role of soluble carbohydrates as cryoprotectors during winter confirmed and the presence of carbohydrate mediated frost protection of buds of Norway spruce (LIPAVSKÁ, et al., 2000). IMANISHI et al. (1997) found in their experiments, that the raffinose family of the oligosaccharides is involved in the freezing tolerance of plants, they are potential cryoprotectants in cold-acclimated plant cells. This is confirmed by our results, however, the correlation coefficient of glucose, fructose and stachyose with air temperature before samp-

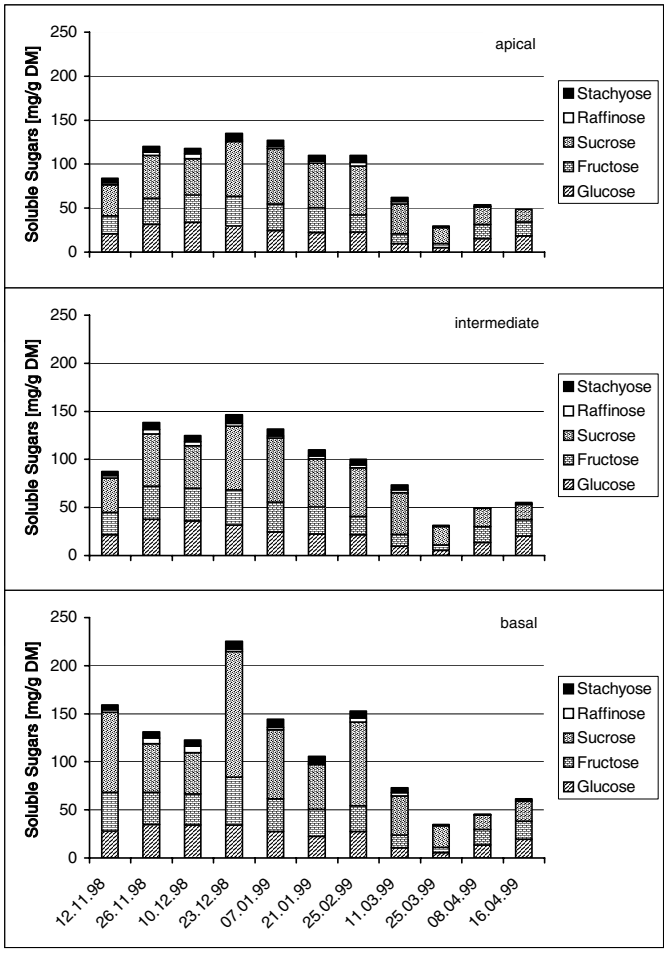

Fig. 2: Concentration of soluble carbohydrates in grapevine buds of different sections of the shoot. (DM = dry matter)

ling is higher than the correlation coefficient of sucrose with air temperature. But in both investigations, concentration of raffinose in grapevine buds is well correlated to air temperature before sampling. We anticipate that further investigations will better define the role of soluble carbohydrates as cryoprotectors.

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