

Figure 3.3.9 Concentration of soluble carbohydrates in grapevine buds of different sections of the shoot. (DM = dry matter). Top: apical buds; middle: intermediate buds; bottom: basal buds.

3.4.3.3 Anions

The dominant anion was sulfate while chloride was detectable only in trace amounts (fig. 3.3.10). Differences in anion concentrations of buds according to bud position were not significant, but changes during sampling were significant for both anions. It seems that the values of sampling date "25.02.99" seem to be due to a faulty preparation. In general, anion concentration was highest at late December/early January and middle of April with about 15 mg/g dry matter. This pattern of anion concentrations is typical for all bud positions and similar to the pattern of soluble sugars.

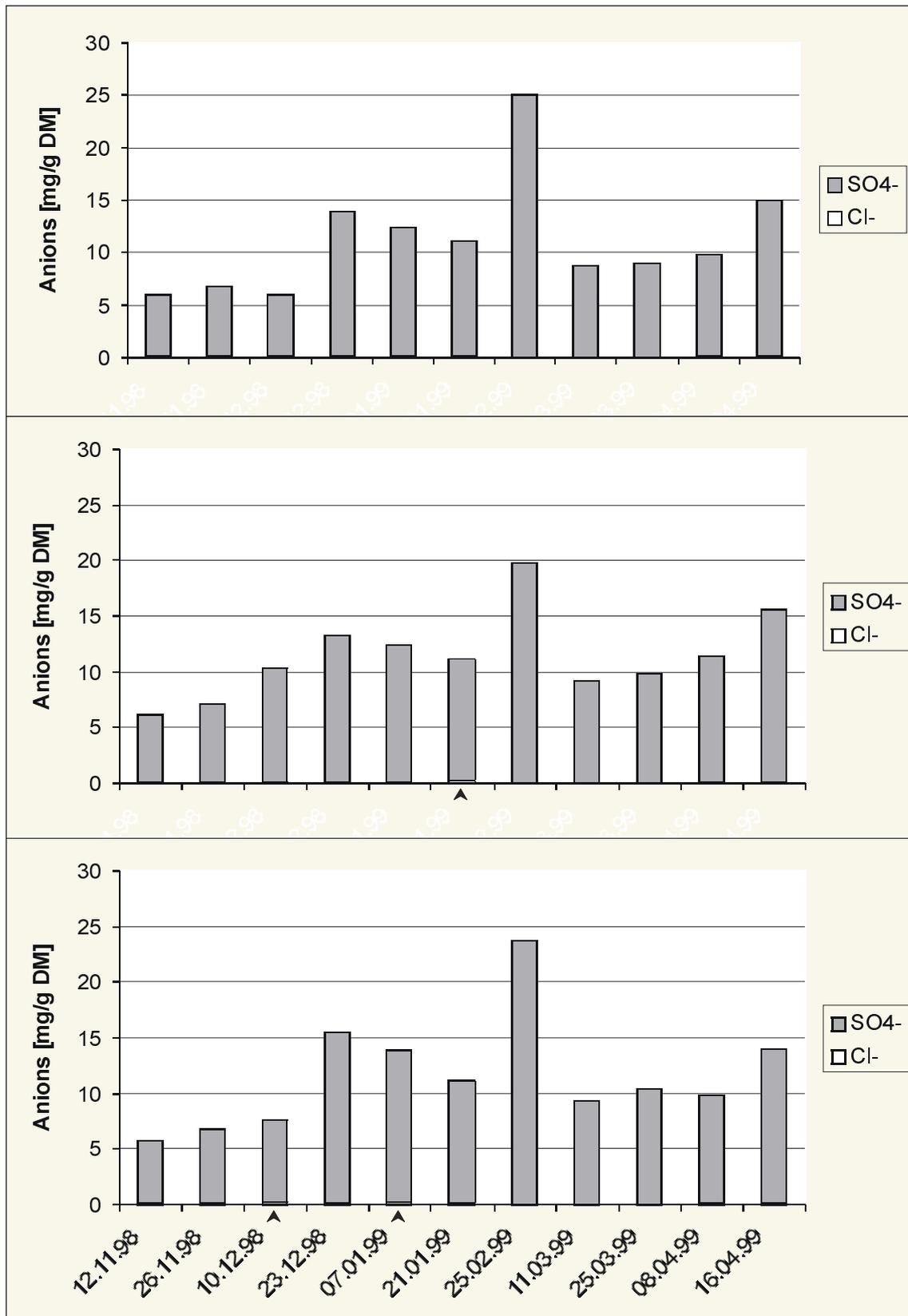


Figure 3.3.10 Concentration of anions in grapevine buds of different sections of the shoot (Cl⁻ was detected only in very low concentrations, see arrowhead ▲). Top: apical buds; middle: intermediate buds; bottom: basal buds. (DM = dry matter)

Table 3: Correlation analysis (Pearson correlation coefficient) between mean air temperature recorded for different time periods (1-9 days, indicated as T1-3 to T1-9) before sampling and sugar concentrations and anion concentrations of grapevine buds.

<i>basal buds</i>		gluc	fruc	suc	raff	stach	Cl ⁻	SO ₄ ⁻
HTE	r =	0.01	-0.01	-0.05	-0.14	0.05	-0.21	-0.04
	p(r) =	0.944	0.953	0.771	0.422	0.782	0.224	0.836
LTE	r =	-0.03	-0.22	-0.54	0.33	0.03	-0.08	-0.42
	p(r) =	0.895	0.342	0.011	0.149	0.891	0.730	0.056
T1-3	r =	-0.54	-0.30	-0.19	-0.65	-0.50	-0.09	-0.02
	p(r) =	0.001	0.051	0.220	0.001	0.001	0.568	0.873
T1-5	r =	-0.57	-0.32	-0.19	-0.75	-0.53	-0.16	0.08
	p(r) =	0.001	0.032	0.228	0.001	0.001	0.310	0.601
T1-7	r =	-0.59	-0.35	-0.20	-0.77	-0.55	-0.20	0.09
	p(r) =	0.001	0.019	0.185	0.001	0.001	0.187	0.571
T1-9	r =	-0.58	-0.33	-0.17	-0.72	-0.55	-0.22	0.07
	p(r) =	0.001	0.027	0.259	0.001	0.001	0.159	0.654
<i>intermediate buds</i>		gluc	fruc	suc	raff	stach	Cl ⁻	SO ₄ ⁻
HTE	r =	0.01	0.02	0.05	0.03	0.12	0.02	0.15
	p(r) =	0.956	0.923	0.765	0.845	0.488	0.908	0.369
LTE	r =	-0.26	-0.22	-0.12	-0.08	-0.07	0.15	-0.22
	p(r) =	0.226	0.308	0.578	0.728	0.759	0.503	0.307
T1-3	r =	-0.65	-0.52	-0.43	-0.70	-0.55	-0.08	-0.03
	p(r) =	0.001	0.001	0.004	0.001	0.001	0.602	0.856
T1-5	r =	-0.67	-0.55	-0.49	-0.77	-0.57	-0.13	0.11
	p(r) =	0.001	0.001	0.001	0.001	0.001	0.417	0.490
T1-7	r =	-0.69	-0.56	-0.51	-0.76	-0.57	-0.15	0.12
	p(r) =	0.001	0.001	0.001	0.001	0.001	0.324	0.444
T1-9	r =	-0.67	-0.57	-0.51	-0.72	-0.56	-0.19	0.10
	p(r) =	0.001	0.001	0.001	0.001	0.001	0.209	0.522
<i>apical buds</i>		gluc	fruc	suc	raff	stach	Cl ⁻	SO ₄ ⁻
HTE	r =	0.05	-0.06	-0.24	0.21	-0.14	-0.18	0.14
	p(r) =	0.782	0.729	0.181	0.247	0.432	0.305	0.440
LTE	r =	-0.25	-0.24	-0.11	-0.11	-0.13	-0.09	0.02
	p(r) =	0.308	0.334	0.693	0.650	0.595	0.730	0.919
T1-3	r =	-0.62	-0.50	-0.43	-0.67	-0.49	-0.05	-0.01
	p(r) =	0.001	0.001	0.004	0.001	0.001	0.754	0.938
T1-5	r =	-0.62	-0.50	-0.45	-0.71	-0.52	-0.05	0.10
	p(r) =	0.001	0.001	0.002	0.001	0.001	0.729	0.509
T1-7	r =	-0.63	-0.52	-0.48	-0.72	-0.54	-0.07	0.12
	p(r) =	0.001	0.001	0.001	0.001	0.001	0.648	0.469
T1-9	r =	-0.63	-0.54	-0.51	-0.71	-0.56	-0.12	0.09
	p(r) =	0.001	0.001	0.001	0.001	0.001	0.453	0.545

3.5 Ice nucleating bacteria

3.5.1 Introduction

Cuttings of well ripened and cold adapted canes were kept at room temperature in the lab to test deacclimatization (see chapter 3.3.5). To avoid drying the lower ends had been immersed in tap water. At the time of swelling the buds usually exhibited HTEs between

– 5 °C and – 7 °C. This was also true for buds at the lower end of the cane which had been immersed inadvertently in the tap water and which were dried superficially prior to testing. However in a few cases exotherms between – 3,8 °C and – 2 °C occurred. This was supposed to be due to ice nucleating bacteria and we tried to isolate and characterize some of them even though this effect was evidently unimportant for freeze damage during the winter.

3.5.2 Isolation of bacteria and freeze tests

A total of 172 samples were taken from tap water which had contained buds with high exotherms and put on different culture media. The best results were obtained with King's B-medium (Difco). Six different bacterial strains were isolated and determined by the Institute of Food Technology, Department of Microbiology of the University of Hohenheim where the BBL® CRYSTAL™ ID System (Enteric/Nonfermenter and Rapid Stool/Enteric ID Kit, Becton Dickinson) was used.

Isolated bacteria colonies were then suspended in a few milliliters of culture medium. Cut buds from the field were put on filter paper soaked with the bacterial suspension, incubated over night at room temperatures and then measured with the Kryoscan. Buds incubated on filter paper soaked with tap water were used as controls. The cut surfaces had been protected by aluminum foil to avoid contamination of the measuring device.

3.5.3 Results

It was found that 2 from 6 isolated bacteria strains promoted early freezing of the buds, one acting at $-2\text{ }^{\circ}\text{C}$ and one at $-3.8\text{ }^{\circ}\text{C}$. The latter turned out to be *Pseudomonas fluorescens* the first one could not be identified.

4 Concluding Discussion

4.1 The growth of ice crystals and morphological structures

In our tests the growth of ice crystals in leaf segments, once initiated, was never stopped. However it was at least slowed down as compared to the growth speed of ice on filter paper where the exotherms appear as needle sharp peaks. This could be demonstrated particularly in older leaves where the exotherms appearing during a second freezing cycle after thawing are considerably sharper and higher indicating the disruption of the cell walls during the initial cycle (fig 3.2.6). From the different forms of the exotherms the slowing effect of intact cell walls as compared to free solution might be estimated 50 – 70 % depending on the age of the leaf and hence on the mechanical resistance of its cell walls.

One of the factors to explain the mechanisms of frost resistance in buds is the existence of a barrier between the bud base and the bud cones (JONES *et al.*, 2000) which would stop the growth of ice crystals completely.

Although, neither JONES nor we ourselves were able to find cytological evidence for barriers in the bud and our experiments to stop ice crystal growth by leaf veins gave no conclusive results, there is no doubt that morphological structures can contribute to supercooling of buds or parts of them. These barriers seem to be present not only during winter but they seem to persist even in deacclimated buds. HAMED *et al.* (2000) found that freezing in germinating buds always started from the stem but that ice spread ($\sim 2 - 5$ mm per second) was stopped for some minutes by structures in the bud base which, however, could not be localized exactly due to the poor resolution of the imaging technique. QUAMME *et al.* (1995) describe anatomical features

which facilitate supercooling of the flower within dormant peach flower buds. The possible existence of barriers can also be demonstrated on grapevine leaves. Fig 4.1.1 shows a leaf after a frost night which destroyed large areas at the tip but only small fields in the basal parts delimited by small leaf veins.



Fig. 4.1.1 Partly frozen grapevine leaf with lesions delimited by leaf veins

It is evident that these veins stopped the growth of ice crystals. However, the freezing tests with small leaf pieces separated in two halves by a leaf vein revealed that this seems not to work under all conditions. We never obtained two exotherms: neither in *Phaseolus vulgaris* nor in *Vitis vinifera* veins in leaf pieces were able to delimit the growth of ice crystals to one segment of the piece. On the other hand freezing of flower petioles and other small cane segments frequently generated several exotherms which could be even reproduced after thawing in a second freezing cycle (fig. 3.2.7). This indicates the presence of separately freezing areas separated by structures which are able to block the growth of the ice crystals.

The ice crystals on leaf segments might bypass the blocking vein by growing along the edges which would not occur on an uninjured leaf. On the other hand it is well known that prevention of deep supercooling in some cereals is one method to control frost damage because ice crystals growing at higher temperatures seem to be less deleterious (for literature see ASHWORTH, 1992) and the freezing conditions in the copper wells of our test system are certainly more severe than in the air (due to an accelerated thermal conductivity).

In this context the question arises why the highest exotherms in our leaf tests were around -7°C whereas spring frost damage is expected already at higher temperatures. Actually most reports in this field indicate air temperatures. We could find no data on leaf temperatures although it is well known that, due to heat radiation, in clear nights the leaves are colder than the surrounding air. On the other hand few “very high temperature” sites on the leaf are sufficient to start ice growth which would then continue over larger areas. In this context it is interesting to note that whole leaves of corn seedlings immersed in 15 ml of sterile distilled water in test tubes froze at -7°C (MITTELSTÄDT, 1995). This temperature seems to be characteristic for the most active intrinsic ice nuclei of supercooling plants.

4.2 Factors influencing susceptibility and resistance to frost

Although a large number of data have been accumulated we were not able to define single factors of frost resistance which would yield the basis for the identification of responsible genes. However in the light of these results it is worth while to rediscuss old explanations for frost resistance derived from experiences of vine growers.

4.2.1 Acclimatation and deacclimatation

There are many publications on the influence of fluctuations in winter temperatures on the buds' cold hardiness. HUBACKOVA (1994, 1996) found correlations between LT_{50} of chilled buds and the maximum and mean temperatures but not of the minimum temperatures of the cold season but no fast response. It is, however, difficult to prove a causal relationship.

According to our observations it is at the best unclear if acclimatation and deacclimation are controlled exclusively by temperature or whether there are intrinsic mechanisms (e.g. hormone influence). WOLPERT and HOWELL (1984, 1985a, 1985b, 1986) describe quite a number of phenomena whose effects on cold acclimatation cannot easily be attributed to temperature influences, such as the effect of night interruption on frost resistance of potted 'Concord' grapevines or the effects of cane length and pruning date. According to DURNER and GIANFAGNA (1991) ethephon treatment prolongs dormancy and enhances supercooling in peach flower buds. HOWELL and SHAULIS (1980) showed that the cold resistance both of buds and canes on the same grapevine varied up to 12 °C depending on periderm development, cane diameter, persistence of the summer laterals and leaf exposure to the sky during the growing season and, last not least, on the variety. LEDDET *et al.* (1993) and DEREUDDRE *et al.* (1993) describe positive effects of a controlled chilling on buds prior to or after bud-burst, respectively.

Our exotherm measurements revealed no defined conditions for cold acclimatation of the buds because the date where the LTEs decreased could not be determined exactly due to the late start of the measurements or variations in the beginning of the measurements (fig. 3.3.2 and 3.3.3). DÜRING (1997) used temperature-induced hardening of buds prior to tests for frost resistance of cuttings; this hardening was, however, achieved by temperatures below – 12 °C within a short time and might thus be due to

water migration (see chapter 4.5) rather than to metabolic activities of the buds.

Vine growers know that grapevine buds lose frost resistance in mid-March. According to DAMBORSKA (1978) 2-3 days of + 12 °C were sometimes sufficient to kill 10-40 % of the buds by immediately following frost of – 19 °C. This may be true in March, but according to our results this is not sufficient in midwinter. Well adapted canes transferred into the laboratory lost their adaptation at room temperature only after about 2 weeks. DAMBORSKA states that “at the end of the winter when budding is initiated, the buds lose resistance much more quickly than in the preceding months”. WOLF and COOK (1992) in tests with three grape cultivars found deacclimation after 16 days at 23 °C and “deacclimation rate tended to increase as the winter progressed”. SCHNABEL and WAMPLE (1987) found that photoperiod and temperature influenced cold hardiness of ‘Riesling’. LIPE *et al.* (1992) could show that evaporative cooling seemed to slow down deacclimation whereas ORFFER and GOUSSARD (1980) showed that hot water treatment used to accelerate bud burst implied deacclimation.

4.2.2 Humidity and water content

Although it is generally assumed by viticulturists that humid or water soaked buds are more susceptible to sudden frosts this could not be corroborated by our tests where the water content was not correlated to the LTEs. It is true that sometimes a certain chain reaction could be observed: The HTE1 starting in the free water of the bud scales was followed immediately by the HTE2 caused by the freezing of the apoplastic solution of the bud base. In autumn or spring this process might continue even into the shoot primordia, however in cold adapted buds this was not the case.

4.2.2.1 Surface humidity

It is true that humidity on and between the bud scales leads to a rise of the HTEs from below 10 °C to around 4 – 6 °C. An explanation would be that HT ice nucleators present on the scales which are meaningless under dry conditions are now contacted by capillary water. According to many authors (for literature see LINDOW, 1983) every frost damage of frost-sensitive supercooling plants which occurs above – 7 °C is caused by epiphytic bacteria such as *Pseudomonas*. Actually we could isolate these bacteria from some wet buds. Particularly in soaked buds the freezing temperature frequently rose to values up to – 2 °C.

These bacterial effects, however, seem to be unimportant for the frost resistance of compound buds in the winter since the LTEs which indicate frost damage of buds are independent from the HTEs and therefore a direct influence of surface humidity seems not very probable. Sunny, cold weather in mid-winter frequently leads to hoar-frost on the scales which, after thawing in the sunshine would wet the bud scales. This effect has been obtained inadvertently by harvesting frozen canes (fig. 3.2.9) and will be discussed later in the context of our freeze drying model.

4.2.2.2 Water content

The loss of water in canes and buds by the end of autumn is very often explained in terms of cold adaptation via concentration of the cell sap (POGOSYAN *et al.*, 1975). Seasonal changes in water content were correlated significantly with seasonal changes in the supercooling point of overwintering flower buds of peach (QUAMME, 1983), and JOHNSTON (1923) found a close relationship between peach flower bud hardiness and water content of the flower buds throughout the winter season. In *Rhododendron*, both water content of the flower buds and degree of supercooling were shown to change seasonally with air temperature (GRAHAM and MULLIN, 1976a). However

WOLPERT and HOWELL (1984) state that “although water content of primary buds was inversely related to cold hardiness in a general way, specific hardiness differences could not be correlated with water content”. One year later these authors find a correlation between tissue moisture decline in canes and buds and acclimation (WOLPERT and HOWELL, 1985). However their curves show that the water loss might precede frost hardening considerably.

Our analyses, too, have shown that the main reduction of the water content occurred from October till December. Vinegrowers in moderate climates know that by that time the grapevine buds are far from frost hardened – a fact that is corroborated by the LTE analyses which indicate maximal hardening by the end of January. In the experiments of SALZMANN *et al.* (1996) ‘Concord’ buds reached their minimum water content in late November, before reaching their maximum acclimation level. HAMMAN *et al.* (1990) found that, although buds dehardened rather quickly there was only a small increase in water content. Certainly the lowering of the freezing point by the loss of water might contribute to frost resistance but there seems to be no direct effect. Drying of cut buds resulted in a considerable lowering of the HTEs (fig. 3.2.12). This is no natural effect, since on the one hand these buds lose their water by evaporation through the cutting plane which is not the case under field conditions and on the other hand HTE values do not indicate the frost resistance of the shoot primordia. However it corroborates the experience that water loss is able to lower ice nucleation in general. This is, however, not due to a concentration of osmotically active substances, but to the decreasing probability that the water might contact a HT nucleation site. The induction of LEA-like proteins by bud desiccation might also play a role (SALZMANN *et al.*, 1996) if it is true that these proteins are involved in cold acclimation of the grape bud, although FENNEL and HOOVER (1991) found that buds can be programmed for endodormancy with accompanying desiccation without cold acclimation.

According to BALÓ and BALÓ (1975, cited after CURRLE, 1983) and HAMMAN *et al.* (1990) the water content of the cane basis is lower than at its top, in our analyses significant differences could only be found in autumn.

MISIK (1997) has studied the relation of frost resistance and of “bound water” in the canes by measuring their dielectric constant (which is correlated with the water content) with microwaves but his results were somewhat inconsistent.

4.2.3 Osmotically active substances

4.2.3.1 Carbohydrates

It is common knowledge that many roots and tubers such as potatoes exhibit low temperature sweetening (JANICK, 1995). In grapevine relations of sugar content of the cane and frost resistance have been postulated as early as 1882 by MÜLLER-THURGAU. It is generally known that cold hardiness can be hampered by insufficient carbohydrate reserves in the cane due to overloading of the plant. WAMPLE and BARY (1992) report on the harvest date as a factor in carbohydrate storage and hence cold hardiness. Many authors report varietal differences in carbohydrate contents which seem to be correlated with the frost resistance of the varieties (RYABCHUN and ARESTOVA, 1987; REUTHER, 1975, JONES *et al.*, 2000).

In addition to the loss of water the conversion of starch into mono- or disaccharides is thought to rise the osmotic value of the cell sap and thus to lower its freezing point. In addition soluble carbohydrates, in particular of the fructosan type, are supposed to act as cryoprotectants. (KOUSSA *et al.*, 1998).

WINKLER and WILLIAMS (1945), EIFERT *et al.* (1961), KLIEWER, 1965; REUTHER (1975), WAMPLE and BARRY (1992) and KOUSSA *et al.* (1998) stated that the conversion of starch to soluble sugars seems to be controlled by low temperatures and that the maxima of frost hardiness of the buds and of

sugar concentration in the cane appear at the same time. Enzymes related to that process have been studied by ORTOIDZE (1987), and WIENHAUS (1996) who found an activation of phosphatases. AIT BARKA and AUDRAN (1996) and BERBEZY *et al.*, (1997) describe the activation by frost of amylases and the change of amylase isoform patterns in the internodes during the winter.

This is corroborated by our bud analyses (fig. 3.3.7) which for the first time combine sugar analysis and freezing point measurement in a great number of buds. We found the same sugar concentrations in buds as EIFERT in shoots – unlike KOUSSA *et al.* (1998) who report that internodes contained lower amounts of sugar than buds. According to REUTHER (1975) the sugar content of the basal parts of the canes are lower than at its top, but our analyses have shown either the opposite or no significant differences. Analyses by paper chromatography (PANCZEL, 1962) which were confirmed by KLEWER (1965) had already revealed the presence of glucose, fructose, maltose, saccharose, melibiose, raffinose and stachyose in grapevine shoots. WAMPLE and BARY (1992) reported that sucrose, glucose and fructose constitute also the major sugars in buds. In our analyses we found also raffinose and stachyose. Insofar there seem to exist no particular differences between canes and buds. For cryoprotection, the ratio between particular sugars might prove to be crucial (LIU *et al.* 1997, OBENDORF 1997). Sucrose is known to be able to retain the liquid-crystalline state of membranes under osmotic stress caused by cold, draught, or salinity. Raffinose is supposed to improve the protective quality of sucrose and also to inhibit the tendency of sucrose to crystalline and hence to lose its protective effect under stress conditions (CAFFREY *et al.*, 1988). IMANISHI *et al.* (1997) found in their experiments, that the raffinose family of the oligosaccharides are involved in the freezing tolerance of plants, they are potential cryoprotectants in cold-acclimated plant cells. 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 WAMPLE and BARY (1992), too, found a relation between soluble carbohydrates and air temperature. This is confirmed by our results, however, the correlation coefficient of glucose, fructose and stachyose with air temperature before sampling 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.

While we did no quantitative analyses of the starch, it could be easily seen that it disappeared by the end of march (prior to bud swelling) like this has been described several times for the starch of the cane (EIFERT *et al.*, 1961; WIENHAUS, 1969) and of the bud during bud burst (AIT BARKA and AUDRAN, 1996; BERBEZY *et al.*, 1997). Our results show that this mobilization occurs well before that time. There are no principal differences between the starch in the cane and in the shoot primordia of the bud. Later on the starch accumulates again in the shoot apex prior to inflorescence induction (BOTTI and SANDOVAL, 1990).

There are indications for the indirect effect of carbohydrates on frost hardiness. WAMPLE *et al.* (1993) could influence both by adequate nitrogen fertilization of 'Riesling' grapes. KORKAS (1994) demonstrated the influences of varying nitrogen supply on the soluble carbohydrates.

4.2.3.2 Anions

MALAKHOVA and SMURYGIN (1977) reported a positive effect of spraying with trace element solutions. In this context our anion analyses gave no indication of such a role in frost resistance. Certainly the concentration of anions cannot contribute much to the osmotic value of the bud tissue. The

influence of mineral nutrition on frost resistance as described by WILHELM (1964) for sodium and by POGOSYAN (1998) seems to act via different mechanisms.

4.3 Other factors

4.3.1 Substances

AIT BARKA and AUDRAN (1997) found changes of proline concentrations in shoots and buds in response to low temperatures and could correlate them to frost resistance, however only after bud burst (stage 2 and 3) and not during the winter. ZILKAH *et al.* (1996) found that urea treatment of leaves improved freezing protection in avocados and peach, an effect that could be interpreted in terms of nitrogen nutrition.

4.3.2 Ice nucleating bacteria

Pseudomonas bacteria might play a certain role in terms of frost damage in *Vitis*. GAINARD and LUISETTI (1992, 1993) and GARDEA *et al.* (1993) found them on leaves where they might cause damage during spring frosts. We could isolate them from very wet buds which often showed a HTE at -3.8 °C. This could be important in cases where very wet weather is followed by a sudden frost. However, since the HTE would seldom trigger the premature appearance of the LTE (see fig. 3.3 and 3.5) the influence of *Pseudomonas* bacteria on freeze damage seems negligible and shall not be discussed here.

4.3.4 Effect of latent heat

KROG *et al.* (1979) reported that the flowers of the African alpine plant *Lobelia telekii* contain a water reservoir with ice nucleation sites which starts freezing already at temperatures around $-1\text{ }^{\circ}\text{C}$. The fusion heat is sufficient to protect the generative parts of the flowers from the night frosts. According to SCHNELL *et al.* (1991) this ice nucleation is caused by *Pseudomonas chloraphis*.

It has never been discussed that the latent heat of the freezing water in the canes might contribute to the survival of buds in a cold night. However small this influence may be: frost resistance of buds is a very complex phenomenon where the sum of single factors is important although their individual contribution might be very small.

4.4 The freeze drying model

The frost resistance of new grapevine varieties is usually tested by storing cold hardened woody cuttings for some weeks at different constant temperatures between -12 and $-25\text{ }^{\circ}\text{C}$. At this occasion DÜRING *et al.* (1990 and unpublished) observed that buds which had been preincubated for some days at $-12\text{ }^{\circ}\text{C}$ (a temperature which would not damage the buds) survived even $-25\text{ }^{\circ}\text{C}$, a temperature which would usually kill the buds of all varieties. It is difficult to explain this effect by biochemical activities such as sugar accumulation; neither can it be attributed to a general loss of water, since the cuttings were sealed in plastic bags.

It is, however, interesting to discuss this observation in the context of effects which occurred in our freezing experiments and which had been already observed PIERQUET *et al.* in buds of *V. riparia* harvested at $-24\text{ }^{\circ}\text{C}$

and by WOLF and POOLE (1987) and WOLF and COOK (1992) who state that the “inability to detect LTEs in buds that have been subjected to prolonged subfreezing temperatures is a recognized limitation of thermal analysis”. Tissue dehydration and accompanying disappearance of LTEs was reported also for *Prunus* flower buds by KADER and PROEBSTING (1992).

We observed the same effects if canes were stored for some days at temperatures of -12 °C for cold adaptation experiments. In addition interrupting of the supercooling cycle after appearance of the HTE and holding the temperature for several hours before further lowering was sufficient for the disappearance of the LTEs. This was unexpected and bothering because some scheduled experiments could not be carried out. However, together with the effects described by other authors it yields further evidence for a model explaining parts of the frost hardening process by migration of water from the primordia to the subtending plant tissue. This could explain both the disappearance of the LTEs and the uncommon frost hardening of the buds. WOLF and POOL (1987) state that unreliable detection of LTEs might be due to water migration from bud primordia to non-lethal sites of freezing, as described earlier in some other species by GRAHAM and MULLIN (1976a), ISHIKAWA and SAKAI (1982) and QUAMME (1983).

This migration can be explained by energy differences between the water in the supercooled – but not yet frozen – shoot primordia and the ice present in the cane tissue. Even if the ice and the supercooled liquid water have the same temperature, the water potential of ice is lower due to the energy loss during freezing. That means that the vapor pressure over the ice is lower than over the liquid phase, in other words: Ice crystals grow at the expense of the liquid phase even if there is no direct contact. This leads to a very gentle and slow desiccation of the shoot primordia which thus can reach a very high frost resistance. This effect may last very long in the field since the ice in canes and bud pads, although frozen only after supercooling to below – 10 °C

(average), will remain frozen until the air temperature surpasses 0 °C and that might take quite a while. There are the complementary observations of PIERQUET *et al.* (1977) that water moves from the stem tissue into the bud tissue again during thawing and that the dry bud tissues require a significant amount of time (around 48 h) to become fully rehydrated.

There are two observations which might support this hypothesis

(1) The measurement of sugars and anions revealed an increase of the anion concentration in the buds on February 25 (fig. 3.3.10) and also of the sugars of the basal buds (fig. 3.3.9). Although an erroneous analysis cannot be completely excluded it is striking that a strong frost period preceded this date.

(2) As discussed in chapter 3.4.2 buds from canes cut at low temperatures (where it could be assumed that the extracellular water was frozen) usually accumulated condensed water on or between the scales. The freezing of this capillary water resulted in sharp and large high temperature exotherms (HTE1). Sometimes this led to the disappearance of LTEs. First it was assumed that the strong HTE1 had triggered the subsequent freezing of all bud parts. This should, however, not be the case since wet buds harvested in rainy mornings at temperatures above 0 °C usually exhibited HTE1, HTE2 and LTE. This could be explained as follows: A cane, harvested at – 8 °C in the morning and hence frozen during the night is brought into room temperature. The temperature of the shoot primordia will rise much faster than the temperature of the nodal tissue of the frozen cane, since the primordia are small and are supercooled although not frozen and hence need no additional fusion energy. Their water potential is thus much higher than that of the subtending nodal tissue and therefore a further water loss of the primordia will occur which either leads to a further lowering of the supercooling temperature of the bud or to a small exotherm which is below the sensitivity of the thermosensor. The first hypothesis seems more probable

in view of the considerable lowering of the HTE by superficial drying of the cutting plane of the nodal tissue (fig. 3.2.12).

KANG *et al.* (1998) state that unlike the buds of apple, peach and pears, grapevine and persimmon buds show no significant interdependence of cooling rate and LTE temperature which could be attributed to water migration during cooling. Like previous authors (e.g. ISHIKAWA and SAKAI, 1982, 1985; GRAHAM and MULLIN, 1976 a,b) they differentiate between freezing tolerance caused by extra-organ freezing involving water migration and “real supercooling”. However, the study of their tables reveals a consistent (though not significant) lowering of the freezing resistance with a decreasing cooling rate. We presume that this mechanism is operative in all buds, although there might be considerable differences of the speed and the direction of water migration depending on the bud anatomy. In grapevine the migration from the primordia to the subtending tissues would be slower since it is supposed to go via the vapour phase whereas in extra-organ freezing the water migrates to spaces immediately adjacent to the vacuole.

5 Literature Cited

- Ait Barka E, Audran JC (1996)** Response of Champagne vines to freezing temperatures: effect of controlled cooling on sugar reserves in bud complexes before and during budbreak. *Can. J. Bot.* 74, 492-505
- Ait Barka E, Audran JC (1997)** Response of Champenoise Grapevine to Low Temperatures: Changes of Shoot and Bud Proline Concentrations in Response to Low Temperatures and Correlations with Freezing Tolerance. *J. Hort. Sci.* 72, 577-582
- Allegretto B (1984)** Anwendung von Polymeren zum Frostschutz in der Landwirtschaft. *Chemtechn.* 3, 152-155
- Anderson JA, Ashworth EN (1985)** Ice Nucleation in Tomato Plants. *J. Amer. Soc. Hort. Sci.* 110, 291-296
- Anderson JA, Whitworth J (1993)** Supercooling Strawberry Plants Inoculated with Ice-nucleation-active Bacteria and Treated with Frostgard. *Hort. Sci.* 28, 828-830
- Anderson JA, Buchanan DW, Stall RE, Hall CB (1982)** Frost Injury of Tender Plants Increased by *Pseudomonas syringae* van Hall. *J. Amer. Soc. Hort. Sci.* 107, 123-125
- Andrews CJ (1996)** How Do Plants Survive Ice? *Ann. Bot.* 78, 529-536
- Andrews PK, Sandidge CR III, Toyama TK (1984)** Deep Supercooling of Dormant and Deacclimating *Vitis* Buds. *Amer. J. Enol. Vitic.* 35, 175-177
- Andrews PK, Proebsting Jr EL (1987)** Effects of Temperature on the Deep Supercooling Characteristics of Dormant and Deacclimating Sweet Cherry Flower Buds. *J. Amer. Soc. Hort. Sci.* 112, 334-340

- Andrews PK, Proebsting EL, Campbell GS (1983)** An Exotherm Sensor for Measuring the Cold Hardiness of Deep-supercooled Flower Buds by Differential Thermal Analysis. Hort. Sci. 18, 77-78
- Andrews PK, Proebsting Jr EL, Gross DC (1986)** Ice Nucleation and Supercooling in Freeze-sensitive Peach and Sweet Cherry Tissues. J. Amer. Soc. Hort. Sci. 111, 232-236
- Andrews PK, Sandidge III CR, Toyama TK (1984)** Deep Supercooling of Dormant and Deacclimating *Vitis* Buds. Amer. J. Enol. Vitic. 35, 175-177
- Andrews PK, Proebsting EL, Gross DC (1986)** Ice nucleation and supercooling in freeze sensitive peach and sweet cherry tissues. J. Amer. Soc. Hort. Sci. 111, 232-236
- Army DC, Lindow SE, Upper GD (1976)** Frost Sensitivity of *Zea mays* Increased by application of *Pseudomonas syringae*. Nature 262, 282-284
- Ashworth EN, Davis GA (1987)** Influence of Ice Nucleation Temperature on the Freezing of Peach Flower Buds. Hort. Sci. 22, 923-925
- Ashworth EN (1989)** Freezing Injury in Deciduous Fruit Crops: Opportunities for Chemical Manipulation. Acta Hort. 239, 175-185
- Ashworth EN (1992)** Formation and Spread of Ice in Plant Tissues. Hort. Rev. 13, 215-255
- Ashworth EN, Wisniewski ME (1991)** Response of Fruit Tree Tissues to Freezing Temperatures. Hort. Sci. 26, 501-504
- Ashworth EN, Davis GA, Anderson JA (1985)** Factors affecting ice nucleation in plant tissues. Plant Physiol. 79, 1033-1037
- Ashworth EN, Davis GA (1984)** Ice Nucleation Within Peach Trees. J. Amer. Soc. Hort. Sci. 109, 198-201

- Ashworth EN, Stirm VE, Volenec JJ (1993)** Seasonal variations in soluble sugars and starch within woody stems of *Cornus sericea* L. *Tree Physiol.* 13, 379-388
- Audran JC, Leddet C, Dereuddre J, Ait Barka E, Brun O (1993)** Réponse de la Vigne (*Vitis vinifera* L) aux Températures Inférieures à 0° C. I. Effets d'un Refroidissement Contrôlé sur des Sarments Aoûtés. *Agronomie* 13, 491-498
- Babo AW, Mach E (1923)** Handbuch des Weinbaus und der Kellerwirtschaft. 1. Halbband. Paul Parey, Berlin, Germany
- Bachmann M, Matile P, Keller F (1994)** Metabolism of the raffinose family oligosaccharides in leaves of *Ajuga reptans*. *Annu. Rev. Plant Physiol.* 27, 507-528
- Bálo E, Bálo S (1968)** Veränderungen im Wassergehalt der Rebhölzer vom Herbst bis Frühjahr. *Szölö és Gyümölcsterm (Budapest)* 4, 157-173
- Beall PT (1981)** The water of life. *The Sciences*, 6-29
- Beall PT (1983)** States of water in biological systems. *Cryobiology* 20, 324-334
- Berbezy P, Legendre L, Maujean A (1997)** Alpha-amylase Isoform Pattern Changes During the Winter Season in the Winter-resting Stem Internodes of *Vitis vinifera*. *Plant Physiol. Biochem.* 35, 685-691
- Biermann J, Stushnoff C, Burke MJ (1979)** Differential Thermal Analysis and Freezing Injury in Cold Hardy Blueberry Flower Buds. *J. Amer. Soc. Hort. Sci.* 104, 444-449
- Bossis E, Lemanceau P, Latour X, Gardan L (2000)** The taxonomy of *Pseudomonas fluorescens* and *Pseudomonas putida*: current status and need for revision. *Agronomie* 20, 51-63

- Botti C, Sandoval E (1990)** Inflorescence Bud Induction in *Vitis vinifera* L. cv. Thompson Seedless: Cytohistological Events and Starch Accumulation in the Shoot apex. *Vitis* 29, 123-131
- Brush RA, Griffith M, Mlynarz A (1994)** Characterization and Quantification of Intrinsic Ice Nucleators in Winter Rye (*Secale cereale*) Leaves. *Plant Physiol.* 104 725-735
- Burke MJ, Gusta LV, Quamme HA, Weiser CJ, Li PH (1976)** Freezing and injury in plants. *Annu. Rev. Plant Physiol.* 27, 507-528
- Buttrose M S (1969)** The dissolution and accumulation of starch granules in grape vine cane. *Austr. J. Biol. Sci.* 22, 1297-1303
- Callan NW (1990)** Dormancy Effects on Supercooling in Deacclimated 'Meteor' Tart Cherry Flower Buds. *J. Amer. Soc. Hort. Sci.* 115, 982-986
- Chatterton NJ, Harrison PA, Thornley WR, Bennett JH (1989)** Purification and quantification of kestoses (fructosylsucroses) by gel permeation and anion exchange chromatography. *Plant Physiol. Biochem.* 27, 289-295
- Clore WJ, Wallace MA, Fay RD (1974)** Bud Survival of Grape Varieties at Sub-zero Temperatures in Washington. *Amer. J. Enol. Vitic.* 25, 24-29
- Currle O, Bauer O, Hofäcker W, Schumann F, Frisch W (1982)** *Biologie der Rebe*. D. Meininger Verlag Neustadt an der Weinstraße.
- Damborska M (1978)** The effect of higher winter temperatures on changes of the frost resistance of grapevine buds. *Vitis* 17, 341-349
- Dereuddre J, Audran JC, Leddet C, Ait Barka E, Brun O (1993)** Réponse de la Vigne (*Vitis vinifera* L) aux Températures inférieures à 0°C. III. Effets d'un Refroidissement contrôlé sur des Bourgeons au Cours du Débourrement. *Agronomie* 13, 509-514

- Dubrovsky M, Petera V, Sikyta B, Hegerová H. (1989)** Measurement of the Ice Nucleation Activity of *Pseudomonas syringae* CCM 4073. Biotechnology Techniques 3, 173-178
- Düring H, Lang A, Oggionni F (1987)** Patterns of water flow in Riesling berries in relation to developmental changes in their xylem morphology. Vitis 26, 123-131
- Düring H, Ortoidze TV, Bushnell B (1990)** Effects of Subzero Temperatures on Chlorophyll Fluorescence of Grapevine Buds. J. Plant Physiol. 136, 758-760
- Düring H. (1997)** Potential frost resistance of grape: Kinetics of temperature-induced hardening of Riesling and Silvaner buds. Vitis 36, 213-214
- Durner EF, Gianfagna TJ (1991)** Ethephon Prolongs Dormancy and Enhances Supercooling in Peach Flower Buds. J. Amer. Soc. Hort. Sci. 116, 500-506
- Eifert A (1975)** Einige Aspekte der Frosthärteprüfung bei Modellversuchen in Klimakammern. Vitis 13, 297-302
- Elvira-Ruenco M, van Vuurde JWJL (2000)** Natural incidence of endophytic bacteria in pea cultivars under field conditions. Can. J. Microbiol. 46, 1036-1041
- Ernst J (1947)** Introduction to Biophysics. Academic Press, Budapest
- Ernst J (1977)** Bound water is real meaning. Acta Biochim. Biophys. Acad. Sci. Hung. 12, 275-277
- Ernst M, Chatterton NJ, Harrison PA (1996)** Purification and Characterization of a New Fructan Series from Species of *Asteraceae*. New Phytol. 132, 63-66

- Fabbi A, Esposito P, Lambardi M (1987)** Aspetti anatomici dei danni da freddo in barbatelle di portinnesti di vite in vivaio. *Rivista di Viticoltura e di Enologia* 40, 61-69
- Fennel A, Hoover E (1991)** Photoperiod influences growth, bud dormancy and cold acclimation in *V. labruscana* and *V. Riparia*. *J. Amer. Soc. Hort. Sci.* 116, 270-273
- Ferreira RB, Monteiro S, Picarra-Pereira MA, Tanganho MC, Loureiro VB, Teixeira AR (2000)** Characterization of the Proteins From Grapes and Wines by Immunological Methods. *Amer. J. Enol. Vitic.* 51, 22-28
- Flinn CL, Ashworth EN (1994)** Blueberry Flower-bud Hardiness Is Not Estimated by Differential Thermal Analysis. *J. Amer. Soc. Hort. Sci.* 119, 295-298
- Flinn CL, Ashworth EN (1994)** Seasonal Changes in Ice Distribution and Xylem Development in Blueberry Flower Buds. *J. Amer. Soc. Hort. Sci.* 119, 1176-1184
- Flinn CL, Ashworth EN (1995)** The relationship between carbohydrates and flower bud hardiness among three *Forsythia taxa*. *J. Amer. Soc. Hort. Sci.* 120, 607-613
- Franks F (1983)** Solute-water interactions: Do polyhydroxy compounds alter the properties of water? *Cryobiology* 20, 335-345
- Fu P, Wilen RW, Wu GH, Robertson AJ, Gusta LV (2000)** Dehydrin Gene Expression and Leaf Water Potential Differs Between Spring and Winter Cereals During Cold Acclimation. *J. Plant Physiol.* 156, 394-400
- Fuller MP, White GG, Charman A (1994)** The Freezing Characteristics of Cauliflower Curd. *Annu. Appl. Biol.* 125, 179-188
- Fuller MP, Telli G (1999)** An investigation of the frost hardiness of grapevine (*Vitis vinifera*) during bud break. *Annu. Appl. Biol.* 135, 589-595

- Gaignard JL, Luisetti J (1992)** Criblage de Molécules Chimiques Actives Contre *Pseudomonas syringae pv syringae* Sur Plants de Vigne Cultivés in vitro et in situ. *Agronomie* 12, 401-409
- Gaignard JL, Luisetti J (1993)** *Pseudomonas syringae*, Bactérie Épiphyte, Glacogène et Pathogène. *Agronomie* 13, 333-370
- Gardea AA, Lombard PB, Crisosto CH, Moore LW, Fuchigami LH. Gusta LV (1993)** Evaluation of Frostgard as an Antifreeze, Inhibitor of Ice Nucleators, and Cryoprotectant on Pinot noir Leaf Tissue. *Amer. J. Enol. Vitic.* 44, 232-235
- Gibb KS, Constable FE, Moran JR, Padovan AC (1999)** Phytoplasmas in Australian grapevines- detection, differentiation and associated diseases. *Vitis* 38, 107-114
- Glasser O (1951)** Colloid Chemistry. *Med Phys Year Book*, Publ. Inc. Chicago, Vol. 1
- Goldstein G, Nobel PS (1991)** Changes in Osmotic Pressure and Mucilage During Low-Temperature Acclimation of *Opuntia ficus-indica*. *Plant Physiol.* 97, 954-961
- Goldstein G, Nobel (1994)** Water Relations and Low-Temperature Acclimation for Cactus Species Varying in Freezing Tolerance. *Plant Physiol.* 104, 675-681
- Golodriga PY, Kireeva LK (1964)** Water forms and frost hardiness of different grape varieties. *Agrobiologia* 6, 943-945
- Graham PR, Mullin R (1976a)** A study of flower bud hardiness in *Azalea*. *J. Amer. Soc. Hort. Sci.* 101, 7-10
- Graham PR, Mullin R (1976b)** The Determination of Lethal Freezing Temperatures in Buds and Stems of Deciduous *Azalea* by a Freezing Curve Method. *J. Amer. Soc. Hort. Sci.* 101, 3-7

- Gross DC, Proebsting Jr EL, Andrews PK (1984)** The Effects of Ice Nucleation-active Bacteria on Temperatures of Ice Nucleation and Freeze Injury of Prunus Flower Buds at Various Stages of Development. *J. Amer. Soc. Hort. Sci.* 109, 375-380
- Guerrier-Julien J, Deloire A, Coudret A (1996)** Water relations in grapevine micro-cuttings grown in vitro. *Biologia Plantarum* 38, 149-152
- Gusta LV, Wilen RW, Fu P (1996)** Low-temperature stress tolerance: the role of abscisic acid, sugars, and heat-stable proteins. *Hort. Sci.* 31, 39-46
- Hajdu E, Gábor G (1997)** Winter Tolerance of Grape Varieties in the Winter of 1995/96. *Horticultural Science-Kertészeti Tudomány* 29, 43-47
- Hamed F, Fuller MP, Telli G (2000)** The Pattern of Freezing of Grapevine Shoots during Early Bud Growth. *CryoLetters* 21, 255-260
- Hamman Jr RA, Renquist AR, Hughes HG (1990)** Pruning Effects on Cold Hardiness and Water Content During Deacclimation of Merlot Bud and Cane Tissues. *Amer. J. Enol. Vitic.* 41, 251-260
- Hamman Jr RA, Dami IE, Walsh TM, Stushnoff C (1996)** Seasonal Carbohydrate Changes and Cold Hardiness of Chardonnay and Riesling Grapevines. *Amer. J. Enol. Vitic.* 47, 31-36
- Himelrick DG, Pool RM, McInnis PJ. (1991)** Cryoprotectants Influence Freezing Resistance of Grapevine Bud and Leaf Tissue. *Hort. Sci.* 26, 406-407
- Hirano SS, Baker LS, Upper CD (1985)** Ice Nucleation Temperature of Individual Leaves in Relation to Population Sizes of Ice Nucleation Active Bacteria and Frost Injury. *Plant Physiol.* 77, 259-265
- Hon-WaiChing, Griffith M, Mlynarz A, Kwok YC, Yang DSC, Hon WC (1995)** Antifreeze proteins in winter rye are similar to pathogenesis-related proteins. *Plant Physiol.* 109, 879-889

- Howell GS, Shaulis N (1980)** Factors Influencing Within-Vine Variation in the Cold Resistance of Cane and Primary Bud Tissues. *Amer. J. Enol. Vitic.* 31, 158-161
- Hubackova M (1994)** The grapevine buds cold hardiness in juvenile plants. *Ochrana Rostlin* 30, 305-309
- Hubackova M (1996)** Dependence of grapevine bud cold hardiness on fluctuations in winter temperatures. *Amer. J. Enol. Vitic.* 47, 100-102
- Hummel RL, Moore PP (1997)** Freeze Resistance of Pacific Northwest Strawberry Flowers. *J. Amer. Soc. Hort. Sci.* 122, 179-182
- Ide H, Price WS, Arata Y, Ishikawa M (1998)** Freezing behaviors in leaf buds of cold-hardy conifers visualized by NMR microscopy. *Tree Physiol.* 18, 451-458
- Imanishi HT, Suzuki T, Masuda K, Harada T (1998)** Accumulation of raffinose and stachyose in shoot apices of *Lonicera coerulea* L. during cold acclimation. *Scientia Horticulturae* 72, 255-263
- Irving RM, Lanphear FO (1967)** Environmental Control of Cold Hardiness in Woody Plants. *Plant Physiol.* 42, 1191-1196
- Itier B, Flura D, Brun O, Luisetti J, Gaignard JL, Choisy C, Lemoine G (1991)**: Analyse de la Gélivité des Bourgeons de Vigne. Expérimentation in situ sur le Vignoble Champenois. *Agronomie* 11, 169-174
- Ishikawa M, Sakai A (1982)** Characteristics of freezing avoidance in comparison with freezing tolerance: a demonstration of extraorgan freezing. In: *Plant Cold Hardiness and Freezing Stress. Mechanisms and Crop Implications*. Vol 2. Li PH, Sakai A (Eds). Academic Press NY 325-340
- Ishikawa M, Sakai A (1985)** Extraorgan freezing in wintering flower buds of *Cornus officinalis* Sieb. et Zucc. *Plant Cell and Environment* 8, 333-338

- Janick J (Ed.) (1995)** Low Temperature Sweetening in Roots and Tubers.
Horticultural Reviews
- Johansen DA (1940)** Plant Microtechnique. McGraw-Hill, New York
- Johnson DE, Howell GS (1981)** The Effect of Cane Morphology and Cultivar on the Phenological Development and Critical Temperatures of Primary Buds on Grape Canes. J. Amer. Soc. Hort. Sci. 106, 545-549
- Johnston ES (1923)** Moisture relation of peach buds during winter and spring. Md. Agr. Expt. Sta. Bul. 255, 59-86
- Jones KS, Paroschy J, McKersie BD, Bowley SR (1999)** Carbohydrate Composition and Freezing Tolerance of Canes and Buds in *Vitis vinifera*. J. Plant Physiol. 155, 101-106
- Jones KS, McKersie BD, Paroschy J (2000)** Prevention of Ice Propagation by Permeability Barriers in Bud Axes of *Vitis vinifera*. Can. J. Bot. 78, 3-9
- Kader SA, Proebsting EL (1992)** Freezing behavior of *Prunus*, subgenus Padus, flower buds. J. Amer. Soc. Hort. Sci. 117, 955-960
- Kadir SA, Proebsting EL (1994)** Screening sweet cherry selections for dormant floral bud hardiness. Hort. Sci. 29, 104-106
- Kaku S, Iwaya M, Jeon KB (1981)** Supercooling ability, water content and hardiness of *Rhododendron* flower buds during cold acclimation and deacclimation *Rhododendron kiusianum*, *Rhododendron X akebono*. Plant Cell Physiol. 22, 1561-1569
- Kang SK, Motosugi H, Yonemori K, Sugiura A (1997)** Exothermic Characteristics of Dormant Buds of Persimmon (*Diospyros kaki* Thunb.) in Relation to Cold Hardiness. HortScience 32, 840-843
- Kang SK, Motosugi H, Yonemori K, Sugiura A (1998)** Supercooling Characteristics of Some Deciduous Fruit Trees as Related to Water Movement within the Bud. J. Hort. Sci. Biotech. 73,165-172

- King, EO, Ward MK, Raney DE (1954)** Two Simple Media For The Demonstration of Pyocyanin And Fluorescein. J. Lab & Clin. Med. 34, 301-307
- Kliwer WM (1965)** The Sugars of Grapevines. II. Identification and Seasonal Changes in the Concentration of Several Trace Sugars in *Vitis vinifera*. Amer. J. Enol. Vitic. 16, 168-178
- Koleda I (1975)** Ergebnisse von Kreuzungen zwischen *Vitis amurensis* und *Vitis vinifera* in der Züchtung frostwiderstandsfähiger Reben. Vitis 14, 1-5
- Korkas E (1994)** Die Dynamik "nicht-struktureller" Kohlenhydrate in Reben (*Vitis vinifera* L. cv. Riesling) im Verlauf zweier Vegetationsperioden unter dem Einfluss einer langjährig variierten Stickstoffdüngung. Rheinische Friedrich-Wilhelms-Universität Bonn, Dissertation. Als Manuskript gedruckt.
- Koussa T, Cherrad M, Bertrand A, Broquedis M (1998)** Comparaison de la Teneur en Amidon, en Glucides Solubles et en Acide Abscissique des Bourgeons Latents et des Entre-noeuds au Cours du Cycle Végétatif de la vigne. Vitis 37, 5-10
- Krog JO, Zachariassen KE, Larsen B, Smidsrod O (1979)** Thermal buffering in Afro-alpine plants due to nucleating agent-induced water freezing. Nature 282, 300-301
- Kuroda K, Ohtani J, Fujikawa S (1997)** Supercooling of Xylem Ray Parenchyma Cells in Tropical and Subtropical hardwood Species. Trees 12, 97-106
- Larcher W (1985)** 2.4 Schädigung der Pflanzen durch Frost. In: Die nichtparasitären Pflanzenkrankheiten. 5. Teil Handbuch für Pflanzenkrankheiten. Paul Parey, Berlin Hamburg

- Lee R E jr, Warren G J, Gusta L V (eds.) (1995)** Biological Ice Nucleation and its Applications. APS Press St Paul
- Leddet C, Audran JC, Dereuddre J, Ait Barka E, Brun O (1993)** Réponse de la Vigne (*Vitis vinifera* L) aux Températures inférieures à 0°C. II. Effets d'un Refroidissement Contrôlé sur des Bourgeons Latents Avant le Débourrement. 13, 499-508
- Levitt J (1980)** Response of plants to environmental stress, chilling, freezing and high temperature. Academic Press New York
- Li PH (1984)** Subzero Temperature Stress Physiology of Herbaceous Plants. Horticultural Reviews 6, 373-416
- Lindemann J, Suslow TV (1987)** Competition Between Ice Nucleation-Active Wild Type and Ice Nucleation-Deficient Deletion Mutant Strains of *Pseudomonas syringae* and *P. fluorescens* Biovar I and Biological Control of Frost Injury on Strawberry Blossoms. Phytopathology 77, 882-886.
- Lindow SE (1983)** The Role of Bacterial Ice Nucleation in Frost Injury to Plants. Annu. Rev. Phytopathol. 21, 363-384
- Lindow SE, Arny DC, Upper CD (1978)** *Erwinia herbicola*: A Bacterial Ice Nucleus Active in Increasing Frost Injury to Corn. Phytopathology 68, 523-527
- Lindow SE, Arny DC, Upper CD (1982)** Bacterial Ice Nucleation: A Factor in Frost Injury to Plants. Plant Physiol. 70, 1084-1089
- Lindow SE, Hirano SS, Barchet WR, Arny DC (1982)** Relationship between Ice Nucleation Frequency of Bacteria and Frost Injury. Plant Physiol. 70, 1090-1093

- Lipavska H, Svoboda H, Albrechtova J (2000)** Annual dynamics of the content of non-structural saccharides in the context of structural development of vegetative buds of Norway spruce. *J. Plant Physiol.* 157, 365-373
- Lipe WN, Baumhardt L, Wendt CW, Rayburn DJ (1992)** Differential Thermal Analysis of Deacclimating Chardonnay and Cabernet Sauvignon Grape Buds as Affected by Evaporative Cooling. *Amer. J. Enol. Vitic.* 43, 355-361
- Lipp CC, Goldstein G, Meinzer FC, Niemczura W (1994)** Freezing Tolerance and Avoidance in High-elevation Hawaiian plants. *Plant Cell Environment* 17, 1035-1044
- Lück W (1964)** Feuchtigkeit. Oldenbourg Verlag, München
- Malakhova NP Smurygin AS (1977)** Einfluss von Spurenelementen auf die Lebensfähigkeit von Winterknospen der Rebe. *Sadovod. Vinogradar. I Vinodel. Moldavii (Kishiniev)* 32, 23-24
- Maki LR, Galyan EL, Chang-Chien,MM, Caldwell DR. (1974)** Ice Nucleation Induced by *Pseudomonas syringae*. *Applied Microbiology* 28, 456-459
- Marentes E, Griffith M, Mlynarz A, Brush RA (1993)** Proteins accumulate in the apoplast of winter rye leaves during cold acclimation. *Physiologia Plantarum* 87, 499-507
- Matthews MA, Anderson MM, Schultz HR (1987)** Phenolic and growth response to early and late season water deficits in Cabernet franc. *Vitis* 26, 147-160

- Misik S (1997)** Role of Bound Water in Frost Hardiness of Vine Cane Studied by Microwave Method Proceedings 4th Internat Sympos on cool climate viticulture & enology, Rochester, New York, USA, 16-20 July 1996, II-105-II-111
- Mittelstädt H. (1994)** Maßnahmen zum Schutz von Spätfrösten. Teil II. Erwerbsobstbau 36, 1-10
- Mittelstädt H (1995)** The ice nucleation activity of *Pseudomonas fluorescens* Migula and its inhibition by various chemicals. J. Phytopathol. 143, 311-317
- Morrison JC, Iodi M (1990)** The development of primary bud necrosis in Thompson Seedless and Flame Seedless grapevines. Vitis 29, 133-144
- Müller-Thurgau H (1882)** Über Zuckerrücklagen in Pflanzenteilen infolge niedriger Temperatur. Landw. Jahrb. 11, 751-828
- O'Connor RE, Hayward AC, Ewings KN, Symons MH (1988)** Application of Multidishes to the Characterization of Proteolytic Psychrotrophs from Raw Milk. Journal of Microbiological Methods 8, 199-207
- Olien CR (1967)** Freezing stress and survival. Annu. Rev. Plant Physiol. 18, 387-408
- Orffer CJ, Goussard PG (1980)** Effect of hot-water treatments on budburst and rooting of grapevine cuttings. Vitis 19, 1-3
- Ortoidze TV (1987)** Enzyme system activity and the dynamics of carbohydrates at low temperatures in grapevine plants. Soobshcheniya Akademii Nauk Gruzinskoi SSR 126, 401-404
- Palacios VM, Nebot Sanz E, Pérez Rodríguez L (1997)** Use of Factor Analysis for the Characterization and Modelling of Maturation of Palomino Fino Grapes in the Jerez Region. Amer. J. Enol. Vitic. 48, 317-322

- Panczel M (1962)** Papierchromatographische Untersuchungen des Zuckergehaltes der Rebe. Mitteilungen Klosterneuburg Serie A 12, 124-129
- Panopoulos NJ (1995)** Ice nucleation genes as reporters. In Lee et al (1995) 271-282
- Patakas A. (2000)** Changes in the solutes contributing to osmotic potential during leaf ontogeny in grapevine leaves. Amer. J. Enol. Vitic. 51, 223-226.
- Pierquet P, Stushnoff C, Burke MJ (1977)** Low Temperature Exotherms in Stem and Bud Tissues of *Vitis riparia* Michx. J. Amer. Soc. Hort. Sci. 102, 54-55
- Pierquet, P., Stushnoff, C. (1980)** Relationship of low temperature exotherms to cold injury in *Vitis riparia* Michx. Amer. J. Enol. Vitic. 31, 1-6
- Pissis P (1990)** The dielectric relaxation of water in plant tissue. J. Exp. Bot. 41, 677-684
- Poghosyan KS (1998)** Influence of Mineral Nutrition on the Vine Frost-Resistance. VIIème Symposium International sur la Génétique et l'Amélioration de la Vigne, Montpellier
- Pogosyan KS, Sklyarova IA, Karapetyan ZG (1975)** Relation between vine frost-resistance and tissue water content. Biol. Zh. Armenii 28, 23-28
- Pool RM, McInnis PJ (1991)** Cryoprotectants Influence Freezing Resistance of Grapevine Bud and Leaf Tissue. HortScience 26, 406-407
- Possingham JV (1994)** New Concepts in Pruning Grapevines. Horticultural Reviews, 16, 235-254
- Pratt C (1974)** Vegetative Anatomy of Cultivated Grapes - a Review. Amer. J. Enol. Vitic. 25, 131-150

- Pratt C (1978)** Shoot Nodes of *Vitis labruscana* Bailey Cv. Concord. *Vitis* 17, 329-334
- Pratt C (1959)** Radiation Damage in Shoot Apices of Concord Grape. *Amer. J. Bot.* 46, 103-109
- Proebsting Jr EL (1979)** Determining T50 of Peach Flower Buds with Exotherm Analysis. *HortScience* 14, 597-598
- Proebsting Jr EL, Ahmedulla M, Brummund V P (1980)** Seasonal changes in low temperature resistance of grapevine buds. *Amer. J. Enol. Vitic.* 31, 329-336
- Quamme HA. (1974)** An Exothermic Process Involved in the Freezing Injury to Flower Buds of Several *Prunus* Species. *J. Amer. Soc. Hort. Sci.* 99, 315-318
- Quamme HA (1976)** Relationship of the Low Temperature Exotherm to Apple and Pear Production in North America. *Can. J. Plant. Sci.* 6, 493-500
- Quamme HA (1978)** Mechanism of Supercooling in Overwintering Peach Flower Buds. *J. Amer. Soc. Hort. Sci.* 103, 57-61
- Quamme HA (1983)** Relationship of Air Temperature to Water Content and Supercooling of Overwintering Peach Flower Buds. *J. Amer. Soc. Hort. Sci.* 108, 697-701
- Quamme HA (1986)** Use of thermal analysis to measure the freezing resistance of grape buds. *Can. J. Plant Sci.* 66, 945-952
- Quamme HA (1995)** Deep Supercooling in Buds of Woody Plants. In Lee et al. (1995), 183-199
- Quamme H, Stushnoff C, Weiser CJ (1972)** The Relationship of Exotherms to Cold Injury in Apple Stem Tissues. *J. Amer. Soc. Hort. Sci.* 97, 608-613.

- Quamme HA, Su WA, Veto LJ (1995)** Anatomical features facilitating supercooling of the flower within the dormant peach flower. *J. Amer. Soc. Hort. Sci.* 120, 814-822
- Rajashekar CB (1989)** Supercooling characteristics of isolated peach flower bud primordial. *Plant Physiol.* 89, 1031-1034
- Rajashekar CB, Burke MJ (1996):** Freezing Characteristics of Rigid Plant Tissues. *Plant Physiol.* 111, 597-603
- Reuther G (1975)** Physiologische Kriterien der Klimaresistenz als sortenspezifische Merkmale. *Angew. Bot.* 49, 75-91
- Ribéreau-Gayon J, Peynaud E (eds) (1980)** *Traité d'ampélogie. Sciences et techniques de la vigne. Tome 1 – Biologie de la vigne. Sols de vignobles*
- Rieger M (1989)** Freeze Protection for Horticultural Crops. *Hort. Rev.* 11, 45-109
- Ryabchun RT, Arestova NO (1987)** Starch accumulation and hydrolysis in grapevine cultivars differing in frost resistance. *Povyshenie Effeektivnosti Proizvodstva Vinograda i Produktov ego Pererabotki* 45, 59-66
- Salzman RA, Bressan RA, Hasegawa PM, Ashworth EN, Bordelon BP (1996)** Programmed Accumulation of LEA-like Proteins During Desiccation and Cold Acclimation of Overwintering Grape Buds. *Plant, Cell and Environment* 19, 713-720
- Sauter JJ, Van Cleve B (1991)** Biochemical and Ultrastructural Results during Starch-Sugar-Conversion in Ray Parenchyma Cells of *Populus* during Cold Adaptation. *J. Plant Physiol.* 139, 19-26
- Schnabel BJ, Wample RL (1987)** Dormancy and Cold Hardiness in *Vitis vinifera* L. cv. White Riesling as Influenced by Photoperiod and Temperature. *Amer. J. Enol. Vitic.* 38, 265-272

- Schnell RC, Vali G (1972)** Atmospheric ice nuclei from decomposing vegetation. *Nature* 235, 163-165
- Schnell RC, Fall R, Nemecek-Marshall M, Sweeting K, Laduca R (1991)** A new INA bacterium from high altitude equatorial vegetation. *Int Conf on Biological Ice Nucleation*, 5th Madison, WI.
- Schuetze F (1990)** Microcomputer-controlled Freezing, Data Acquisition and Analysis System for Cold Hardiness Evaluation. *HortScience* 25, 973-976
- Sidebottom C, Bucklel S, Pudney P, Twigg S, Jarman C, Holt C, Telford J, McArthur A, Worrall D, Hubbard R, Lillford P (2000)** Heat-stable Antifreeze Protein from Grass. *Nature* 406, 256
- Sikyta B, Hegerová H, Dubrovský M, Petera V (1989)** Cultivation and Properties of Ice-Nucleation Active Bacteria *Pseudomonas syringae* CCM 4073. *Biotechnology Letters* 11, 307-312
- Sikyta B, Hegerová H, Dubrovský M, Petera V (1990)** Cultivation and Properties of Ice Nucleating Bacterium *Pseudomonas syringae* CCM 4073. *Kem. Ind.* 39, 599-603
- Smith D (1968)** Carbohydrates in grasses. IV Influence of temperature on the sugar and fructosan composition of timothy (*Phleum pratense*) plant parts in anthesis. *Crop Sci.* 8, 331-334
- Steponkus PL (1984)** Role of the Plasma Membrane in Freezing Injury and Cold Acclimation. *Annu. Rev. Plant Physiol.* 35, 543-584
- Stergios BG, Howell Jr GS (1973)** Evaluation of Viability Tests for Cold Stressed Plants. *J. Amer. Soc. Hort. Sci.* 98, 325-330
- Stergios BG, Howell GS (1977)** Effect of Site on Cold Acclimation and Deacclimation of Concord Grapevines. *Amer. . Enol. Vitic* 28, 43-48

- Stergios BG, Howell GS (1977)** Effects of Defoliation, Trellis Height, and Cropping Stress on the Cold Hardiness of Concord Grapevines. *Amer. J. Enol. Vitic.* 28, 34-42
- Turner MA, Arellano F, Kozloff LM (1991)** Components of ice nucleation structures in bacteria. *J. Bacteriol.* 173, 6515-6527
- Upper CD, Vali G (1995)** The Discovery of Bacterial Ice Nucleation and its Role in the Injury of Plants by Frost In: *Biological Ice Nucleation and Its Applications*. R.E. Lee jr et al., ed. APS Press, St. Paul, Minnesota, 29-36
- Vali G (1971)** Quantitative evaluation of experimental results on the heterogenous freezing nucleation of supercooled liquid. *J. Atmos. Sci.* 28, 402-409
- Von Firecks HA (1993)** Exothermic responses of dormant *Salix* stems during exposure to subzero temperatures. *Physiologia Plantarum* 87, 271-278
- Wample RL, Reisenauer G, Bary A (1990)** Microcomputer-controlled Freezing, Data Acquisition and Analysis System for Cold Hardiness Evaluation. *HortScience* 25, 973-976
- Wample RL, Bary A (1992)** Harvest Date as a Factor in Carbohydrate Storage and Cold Hardiness of Cabernet Sauvignon Grapevines. *J. Amer. Soc. Hort. Sci.* 117, 32-36
- Wample RL, Spayd SE, Evans RG, Stevens RG (1993)** Nitrogen Fertilization of White Riesling Grapes in Washington: Nitrogen Seasonal Effects on Bud Cold Hardiness and Carbohydrate Reserves. *Amer. Enol. Vitic.* 44, 159-167
- Warmund MR, Takeda F, Davis GA (1992)** Supercooling and Extracellular Ice Formation in Differentiating Buds of Eastern Thornless Blackberry. *J. Amer. Soc. Hort. Sci.* 117, 941-945

- Warmund MR, English JT (1995)** Freezing Injury and Colonization of 'Redwing' Red Raspberries with Ice-nucleation-active Bacteria. J. Amer. Soc. Hort. Sci. 120, 1041-1044
- Warmund MR, English JT (1998)** Ice Nucleation, Freezing Injury, and Colonization of "Totem" Strawberry Flowers with Ice-nucleation-active (INA) Bacteria. J. Amer. Soc. Hort. 123, 234-238
- Warren G, Corotto L (1989)** The Consensus Sequence of Ice Nucleation Proteins from *Erwinia herbicola*, *Pseudomonas fluorescens* and *Pseudomonas syringae*. Gene 85, 239-242
- Wettlaufer JS, Dash JG (2000)** Melting Below Zero. Scientific American S. 50-53
- Wienhaus H (1969)** Die Aktivität der Phosphatasen im Rebholz und deren Beziehungen zum Kohlenhydratmetabolismus während verschiedener Temperaturbedingungen in der winterlichen Ruheperiode. Vitis 8, 105-510
- Wilhelm A (1964)** Die Kälteresistenz der Rebe in Abhängigkeit von der Kaliumversorgung. Wein Wissenschaft 19, 505-510
- Williams LE, Matthews MA (1945)** Grapevine. Agronomy, 30, 1019-1055
- Winkler AJ, Williams WO, (1945)** Starch and Sugars of *Vitis vinifera*. J. Plant Physiol. 20, 412-432
- Wisniewski M, Lindow SE, Ashworth EN (1997)** Observations of ice nucleation and propagation in plants using infrared video thermography. Plant Physiol. 113, 327-334
- Wisniewski M, Wolf T, Fuchigami L, Henick-Kling T (ed.) (1997)** Wolf, T.E. (ed.) and Harkness, E.M.: Biochemical and biophysical mechanisms of cold hardiness in woody plants. In: Proceedings of the fourth

international symposium on cool climate viticulture & enology,
Rochester, New York, USA, 16-20 July 1996

- Wisniewsky R (1995)** Deep Supercooling in Woody Plants and the Role of Cell Wall Structure. In Lee et al. (1995), 163-181
- Wolf TK, Pool RM (1986)** Microcomputer-based Differential Thermal Analysis of Grapevine Dormant Buds. HortScience 21, 1447-1448
- Wolf T K, Pool R M (1987)** Factors affecting exotherm detection in the differential thermal analysis of grapevine dormant buds. J. Amer. Soc. Hort. Sci. 112, 520-525
- Wolf TK, Cook MK (1992)** Seasonal Deacclimation Patterns of Three Grape Cultivars at Constant, Warm Temperature. Amer. J. Enol. Vitic. 43, 171-179
- Wolf TK, Pool RM (1987)** Factors Affecting Exotherm Detection in the Differential Thermal Analysis of Grapevine Dormant Buds. J. Amer. Soc. Hort. Sci. 112, 520-525
- Wolf TK, Cook MK (1994)** Cold Hardiness of Dormant Buds of Grape Cultivars: Comparison of Thermal Analysis and Field Survival. HortScience 29, 453-1455
- Wolpert JA, Howell GS (1985)** Cold Acclimation of Concord Grapevines. II. Natural Acclimation Pattern and Tissue Moisture Decline in Canes and Primary Buds of Bearing Vines. Amer. J. Enol. Vitic. 36, 189-194
- Wolpert JA, Howell GS (1986)** Effect of Night Interruption on Cold Acclimation of Potted `Concord` Grapevines. J. Amer. Soc. Hort. Sci. 111, 16-20
- Wolpert JA, Howell GS (1984)** Effects of cane length and dormant season pruning date on cold hardiness and water content of concord bud and cane tissues. Amer. J. Enol. Vitic. 35, 237-244

- Wolpert JA, Howell GS (1985)** Cold acclimation of Concord grapevines. I. Variation of cold hardiness within the canopy. *Amer. J. Enol. Vitic.* 36 (3), 185-188
- Wu MT, Hughes HG, Becwar MR (1982)** Low Temperature Exotherms and Cold-hardiness in Bud and Stem Tissue of *Vitis*. *HortScience* 17, 30
- Yankofsky SA, Levin Z, Moshe A (1981)** Association with Citrus of Ice-Nucleating Bacteria and Their Possible Role as Causative Agents of Frost Damage. *Current Microbiology* 5, 213-217
- Yelenosky G (1983)** Ice nucleating active (INA) agents in freezing of young citrus trees. *J. Amer. Soc. Hort. Sci.* 108, 1030-1034
- Yelenosky G (1985)** Cold Hardiness in Citrus. *Horticultural Reviews* 7, 201-238
- Zilkah S, Wiesmann Z, Klein I, David I (1996)** Foliar Applied Urea Improves Freezing Protection to Avocado and Peach. *Scientia Horticulturae* 66, 85-92
- Zoran R, Ashworth EN (1995)** Response of Xylem Ray Parenchyma Cells of Supercooling Wood Tissues to Freezing Stress: Microscopic Study. *Int. J. Plant Sci.* 156, 784-792