Freezing tests with small leaf segments separated in two halves by a leaf vein revealed that under our chilling conditions neither veins of *Phaseolus vulgaris* leaves nor of *Vitis vinifera* leaves were able to stop the growth of ice crystals: as a rule only one single exotherm was obtained indicating the complete freezing of both leaf halves. On the other hand the test of flower petioles and other small cane segments frequently revealed the presence of separatly freezing areas which remain separated even after repeated freezing (fig. 3.2.7).

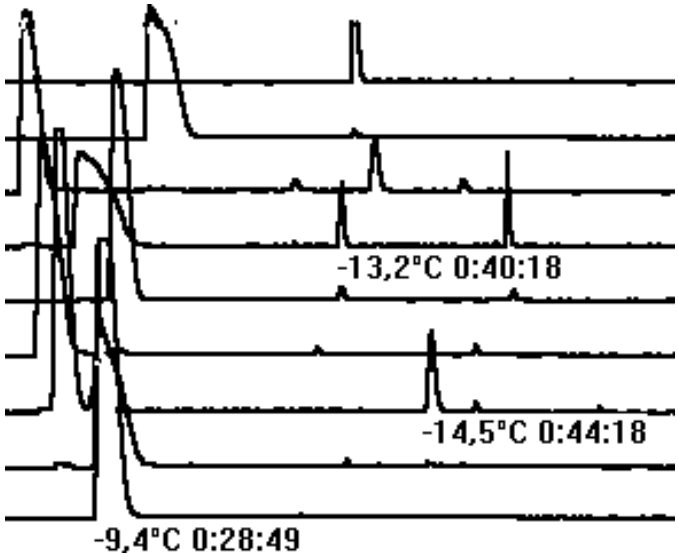

Fig. 3.2.7 Freezing of flower petioles of *Phaseolus*; sometimes two to three exotherms appear whose values are reproducible after thawing and refreezing

Cooling rate: Within the range tested in our experiments (1 – 12 °C/h) no significant differences of the average freezing points of different specimens could be found. This again shows that freezing is not simply a matter of probability depending on time.

3.2.2 Sensitivity of the Kryoscan Apparatus

To test the sensitivity of the measuring device water droplets were put on 5 mm filter paper confettis which were stuck with water free silica on the temperature sensors. Of course a direct contact of the droplets to the sensor plates would have resulted in a much higher sensitivity but this arrangement would not have represented the real measuring conditions for buds.

Under these conditions the smallest amount of water which could be detected on filter paper by Kryoscan was around 0.2 µl. This would correspond to around 0.3 – 0.5 mg of leaf or bud tissue.

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. This poses the problem whether in some experiments the disappearance of the LTE of the primary bud is caused by lowering of their freezing point below the temperature range of the device (– 30 °C) or by the decrease of their water content below the sensitivity of the device. This will be discussed later.

3.2.3 Quantification of exotherms

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.

There were, however, no good correlations in practical experiments – not even if measurements were carried out with small droplets of water put directly on the temperature sensors. This was due to the fact that the exothermal heat is distributed between the freezing solution and the sensor, which in the case of the Kryoscan device is a small ceramic plate protecting the copper latches soldered to the semiconductors of the PELTIER microelements and which has a relatively high mass as compared to the freezing sample.

In our model experiments we used small filter paper confettis as solution carriers since the cotton fibers might be considered a crude model of a plant tissue. The paper was fixed to the sensor plates with small amounts of water free silica grease to facilitate heat transfer. Although this system was complicated by the different heat conductivity of the dry and wet parts of the filter paper, of the grease and of the ceramic plate, tests with different amounts of water allowed to construct a kind of calibration curve. This was also possible using small leaf pieces. The usefulness of this calibration curve in interpreting the results of bud measurements was, however, rather limited due to the complicated structure of the buds. The HTEs are produced by freezing of apoplastic water in the bud pad. Now the relative amount of this tissue could not never be kept constant because it was virtually impossible to cut "standardized" buds from the cane; their overall size, as well as the relative size of their components and their water content, were rather variable. In addition the surface of the cut was not always flat and different amounts of heat conducting grease had to be used to fix the bud. This would result in variations of the contact of the bud to the sensor, the

heat conductivity of the contact zone and the distribution of the latent heat between sensor, bud parts and grease. All these effects would influence size and shape of the exotherms.

It is evident that exotherms obtained under such conditions cannot be used for quantitative measurements but only to get preliminary indications on the water content.

3.2.4 Location of Freezing Events in Grapevine Buds

It is not always easy to evaluate the exotherms occuring during freezing of complicated structures such as the compound bud of a grapevine. In their basic paper of 1987 WOLF and POOL discuss these issues but in 1994 WOLF and COOK still state that "some judgement is required to distinguish the LTEs of primary and secondary buds if this is of interest to the researcher".

3.2.4.1 Interpretation of exotherms

Temperature measurements on complete cold adapted latent buds during freezing usually revealed one or two high temperature exotherms (HTE1, HTE2) at temperatures between – 5 and – 10 °C and one to three low temperature exotherms (LTE1,2,3) at temperatures down to – 25 °C, depending on the frost adaptation of the buds (fig. 3.2.8).

In many cases exotherms at very low temperatures (VLTEs) were observed which formed no definite peak but a slow rise and fall of the temperature (fig 3.2.9).

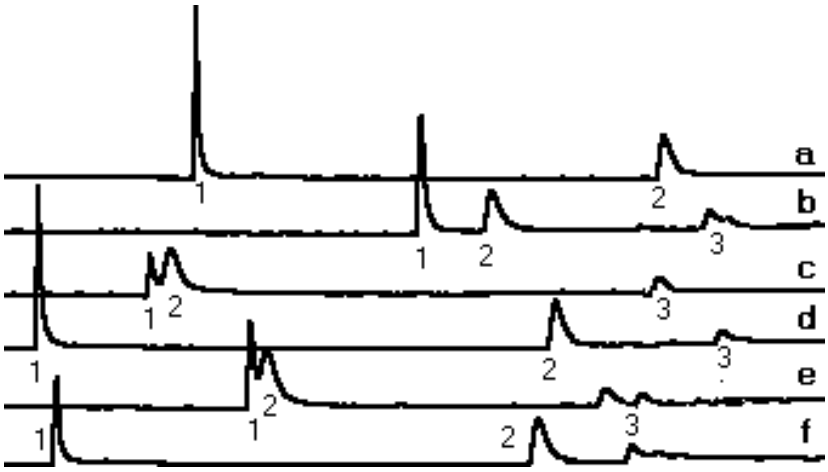

Fig. 3.2.8 Example for the freezing of buds with humid scales in March. Two exotherm types HTE1 and HTE2 (1 and 2). The highest start near 4 °C. It seems that HTE2 is either triggered by HTE1 (c, e) or occurs at a more or less lower temperature (down to - 14 °C); the LTE exotherms (3) around -15 °C are less variable and evidently not influenced by the HTEs. Sometimes two LTEs are visible.

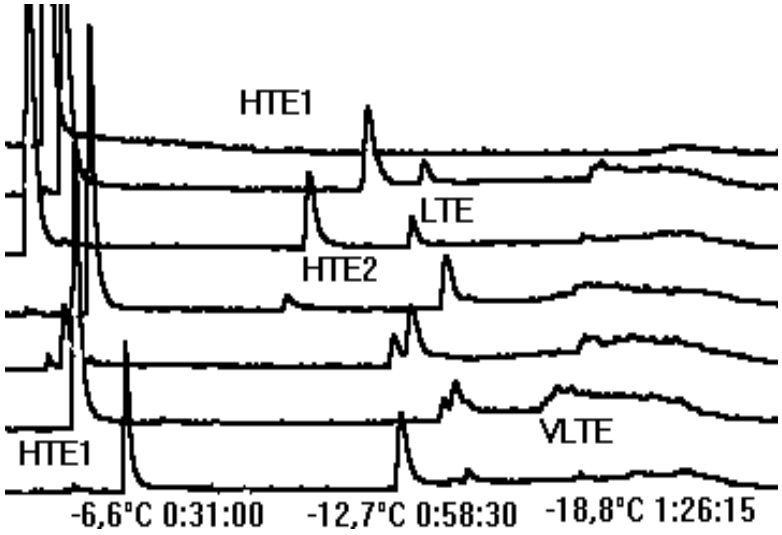

Fig. 3.2.9 VLTEs showing up as slow rise and fall of the temperature difference between bud and container; in this case they occurred after bud swelling at relatively high temperatures (-18.8 °C and lower); in winter they could be observed around -30 °C and were usually not recorded.

High temperature exotherms: It is generally accepted that HTEs are due to the freezing of extracellular water (either apoplastic or in the xylem) which involves no damage of the bud. Dry buds exhibit only one HTE at around – 10 °C (HTE2) whereas buds moistened by rain, dew or condensed water show a very sharp exotherm between – 4 to – 6 °C (HTE1, fig. 3.2.10 and 3.2.11). Evidently the growth of the ice crystals is not impeded by cell walls.

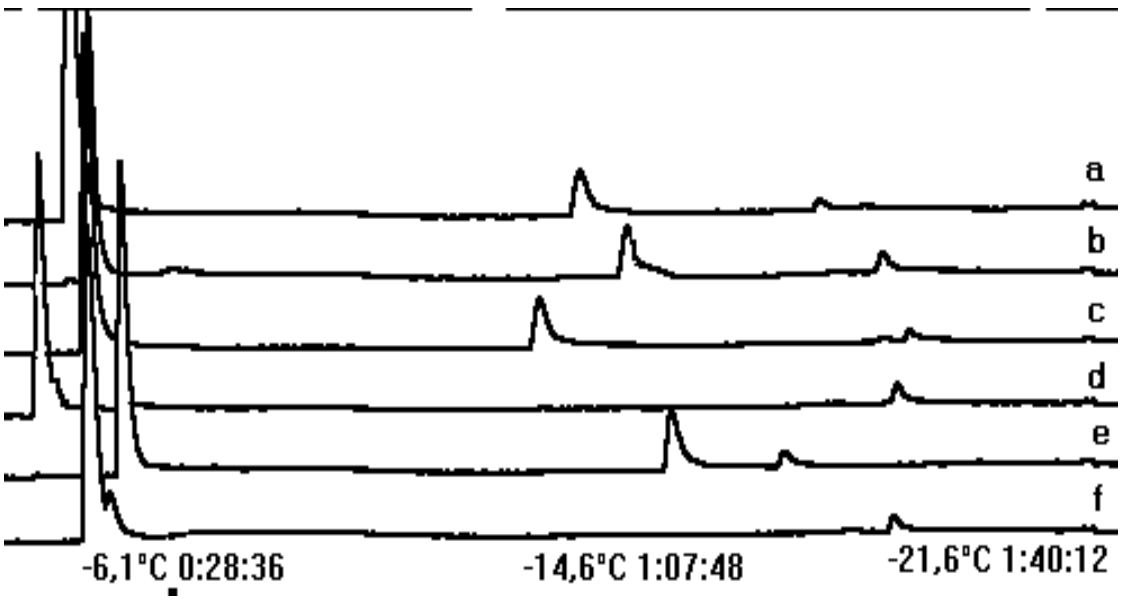

Fig. 3.2.10 Example for the freezing of buds with wet bud scales in February. HTE1 (around - 6 °C) is always very large and (in d and f) seems to trigger HTE2, which in the other cases appears between - 14 and - 17 °C. LTEs around - 21 °C are not influenced by HTE2.

We therefore assume that this exotherm indicates freezing of capillary water on or between the scale buds since its size depends on weather conditions leading to water deposits on the bud either directly by rain or indirectly by condensated water on frozen buds which thaw in the laboratory.

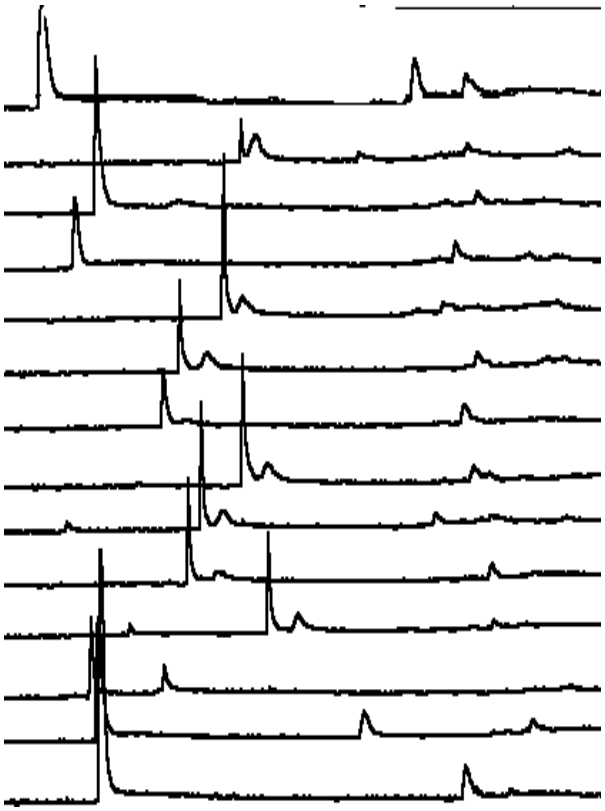

Fig. 3.2.11 Example of a test run with 14 humid buds in February, showing large variations of HTE1 and HTE2 but only small variations of the LTEs

HTE2 usually is less sharp but longer and we assign it to the freezing of apoplastic water of the bud base. Frequently HTE2 seems to be triggered by HTE1 which leads to a rise of the HTE2 values as compared to dry buds (figs. 3.2.8 and 3.2.10). This causes problems with the statistical evaluation of the data: can HTE2 values obtained from wet buds (possibly triggered by the HTE1) and from dry buds (without HTE1) be merged? In terms of winter frost damage this was not really important since the rise of the HTE values caused by surface humidity of the bud would not influence the LTEs which are responsible for frost resistance (fig. 3.2.11).

It is interesting to note that a considerable shift of the HTEs towards lower temperatures occurred if cut buds were allowed to dry for an hour or so (fig. 3.2.12). This seemed to lead to a superficial drying of the cut surface but (as indicated by the size of the exotherms) not to an important loss of water of the bud. This observation complements the results of WOLF and POOL (1987) and

JONES *et al.* (2000) who could rise the HTE above - 5 °C by soaking the cut surface with buffer or pectinase solution.

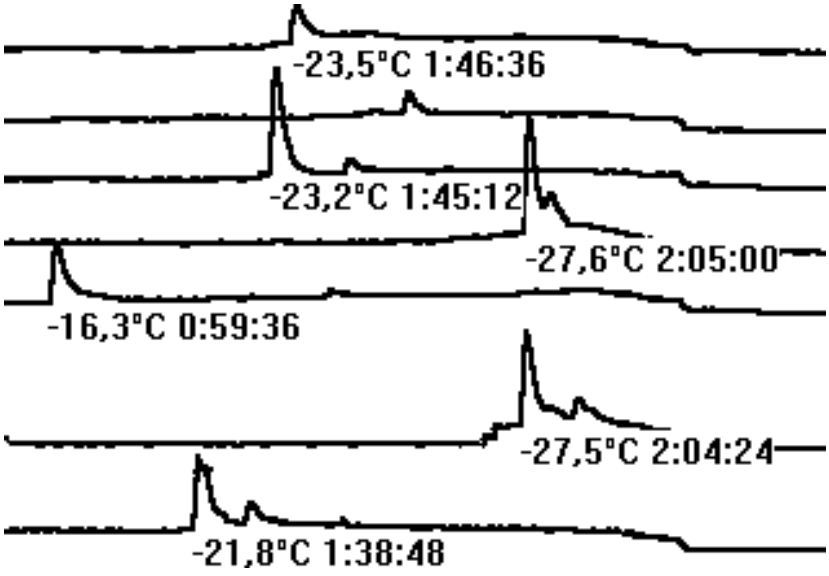

Fig. 3.2.12 Exotherms from buds dried after cutting, According to the size of the HTEs, there was not much loss of water, however they appear only after supercooling to very low values normally reached only by LTEs.

In this context the question arises where the water freezing during the HTE is located. HAMED *et al.* (2000) used a thermal imaging system to follow the spread of ice in canes and buds. Since the rate of spread was comparable to that of pure supercooled water they suggest that the ice is travelling in the vascular tissues. Yet most authors assume that HTE water is located in the apoplast and there are a number of observations which support this opinion. It is by no means clear wether the the grapevine xylem in winter is empty or not. The different forms of our HTE1 (free water in the bud scales) and HTE2 (fig. 3.2.10 and 3.2.11) indicate that growth of ice during HTE2 is slowed down by the tissue. LEDDET et al. (1993) found that the HTE2 causes a moderate shrinking of the protoplasts of the subtending nodal tissue which is evidently caused by a water migration from the vacuole to the apoplast during freezing.

Low temperature exotherms: LTEs are assumed to indicate symplastic freezing of the primary, secondary and (rarely) tertiary buds (shoot primordia) causing their irreversible damage. This isssue will be discussed further in the following paragraphs. In the rare cases where two or three LTEs were produced during freezing (fig. 3.2.8) the measuring conditions for each LTE were identical. Thus their different relative size which corresponds to the relative size of the primary and secondary shoot primordia corroborates the conclusion of freezing experiments with separated bud parts (see below, chapter 3.2.4.2): LTEs should be caused by freezing of the shoot primordia and not by other compounds such as symplastic cell contents of the subtending nodal tissue which remained unfrozen during the HTE. If, in our experiments, 2 or 3 LTEs appeared one larger exotherm was followed by one or two very small exotherms, although in two or three cases the small LTEs occurred first.

WOLF and POOL (1987) and WOLF and COOK (1992) also used the different size of LTEs as an indicator for their provenience because LTEs originating from primary buds are in the order of two to four times larger than those from subsidiary buds. This corresponds to practical experiences: Very often frost damage is limited to the primary bud while secondary and tertiary shoot primordia are still able to leaf out. The latest data on this subject can be found in HAJDU and GABÓR (1997).

Very low temperature exotherms: VLTEs in buds are due to the freezing of the living cells of the nodal tissue subtending the shoot primordia within the ice structure formed previously by the apoplastic water (as indicated by the appearance of the HTE). They occur during the freezing of woody stem tissues where they appear between – 15 °C and – 30 °C and are due to the freezing of xylem ray parenchyma cells (e.g. GRAHAM and MULLIN, 1976b; KURODA *et al.*, 1997). Actually the bud pad (subtending nodal tissue) is part of the woody cane, therefore VLTEs of buds were not further analyzed: They indicate the death of wood cells and our studies were focused on the survival of shoot primordia.

After bud swelling the living wood cells lose their high frost resistance and we often observed VLTEs at temperatures well below – 20 °C (fig. 3.2.9).

WOLF and POOL (1987) and WOLF and COOK (1992) avoided mixing up HTEs and LTEs by a wick system which led growing ice crystals to the bud pad and thus avoided a substancial supercooling because comparatively large amounts of water on the wick rose the HTE to over – 5 °C. This was not necessary with our device because we could record the curves for each bud separately and we did not want to lose additional information on the HTEs.

3.2.4.2 Microscopical analyses

On the basis of microscopical analyses LEDDET *et al.* (1993) stated that the first exotherm at – 12.9 °C (sic!) caused histological alterations in the pith of the basal part of the bud, the second exotherm at – 17 °C induced some ruptures of the cell wall and an extension of the contraction of the cytoplasm through the basal third of the pith and the third exotherm at -21 °C produced irreversible alterations throughout the main bud while the secondary buds remained intact. This is somewhat contradictory to the interpretations of the exotherms by all other authors. Therefore we tried to reproduce the results of LEDDET *et al.* In addition we wanted to look for the permeability barrier demonstrated by JONES *et al.* (2000).

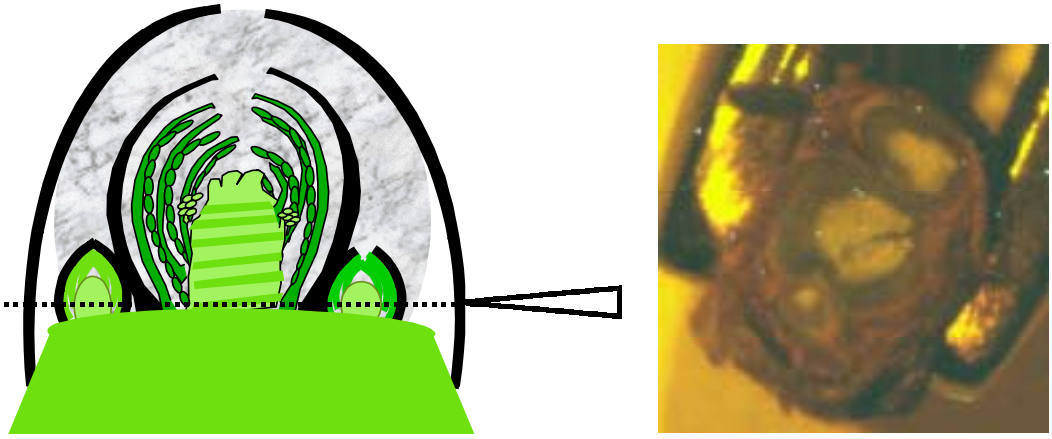

Fig. 3.2.13 *Left:* Cutting plane in a latent bud prepared for HPLC analyses and separate exotherm measurements of the bud pad and the apical region with shoot primordia. *Right:* Upper half of such a bud kept between tweezers

In a preliminary experiment basal and apical parts of latent buds (cut according to fig. 3.2.13) were frozen separately to get indications on the location of the freezing events. This had been done already by LEDDET *et al.* (1993) who found a HTE and several LTEs associated with the dissected primordium and gave

many sophisticated details on the exotherms appearing. However, they presented exotherm curves drawn by hand and gave only insufficient details on the methods used.

In our experiments only the lower half of the bud with the nodal tissue produced HTEs. LTEs were restricted to the apical part containing only primary and secondary shoot primordia which, however, never showed a HTE und thus seem to contain only small amounts of apoplastic water. This effect was also described by KANG *et al.* (1998) who state that the HTE of grapevine buds dissappeared after removal of the subtending tissue. It must be pointed out, though, that for all our following measurements complete latent buds were used to avoid structural damages of the shoot primordia which might influence the temperature of their LTEs.

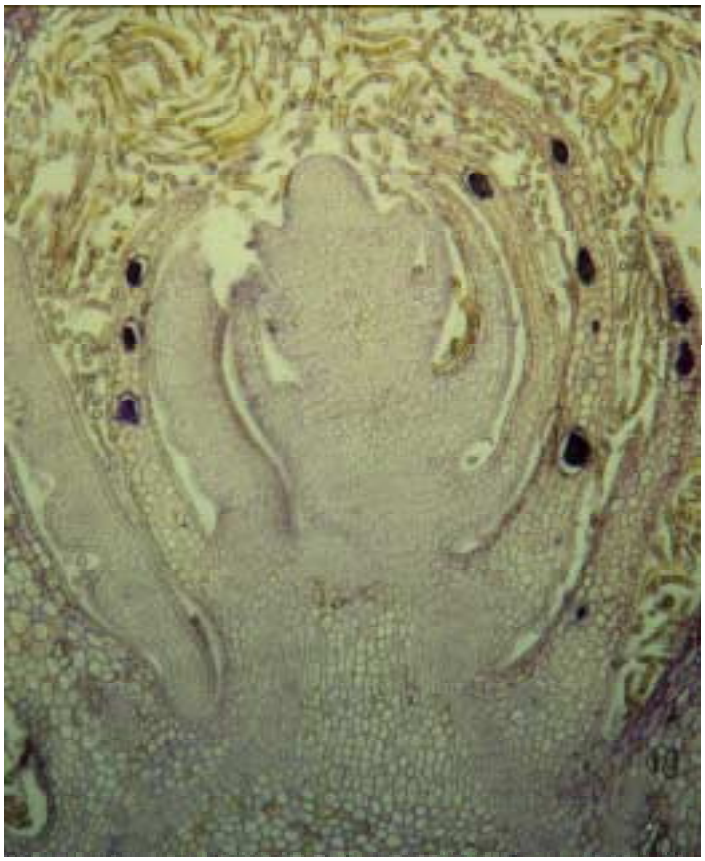

Fig. 3.2.14 Section through primary vegetation cone of a compound bud after occurrence of a HTE. No damage is visible (haematoxylin).

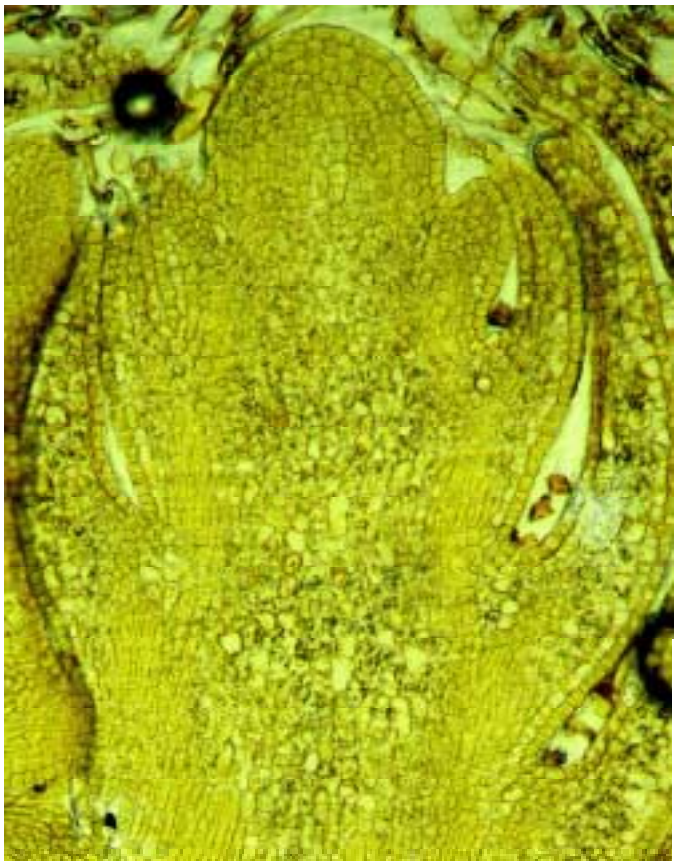

Fig. 3.2.15 Semithin section through vegetation cone of primary bud.

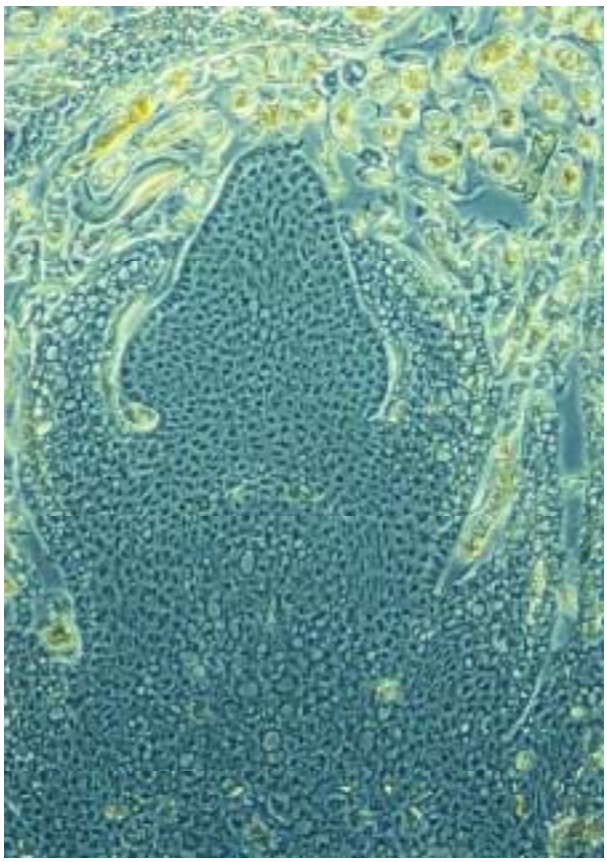

Fig. 3.2.16 Vegetation cone after appearance of LTE : Due to freezing the cytoplasm has coagulated and forms small clots within the cell

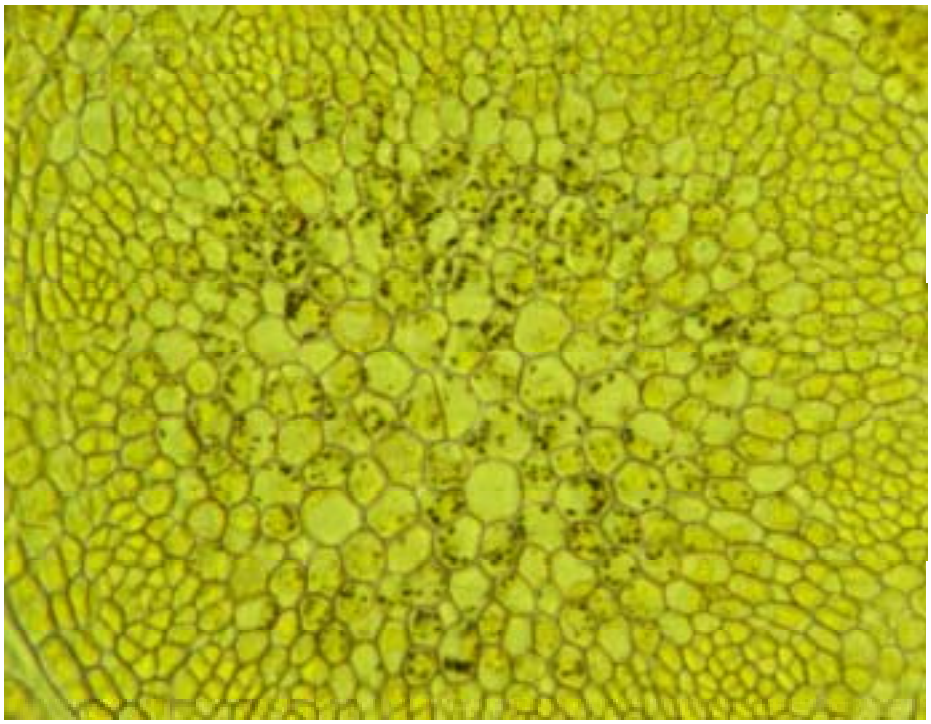

Fig. 3.2.17 Cross section through basis of vegetation cone of a bud after occurrence of a HTE; no damage visible; dark dots are starch granula

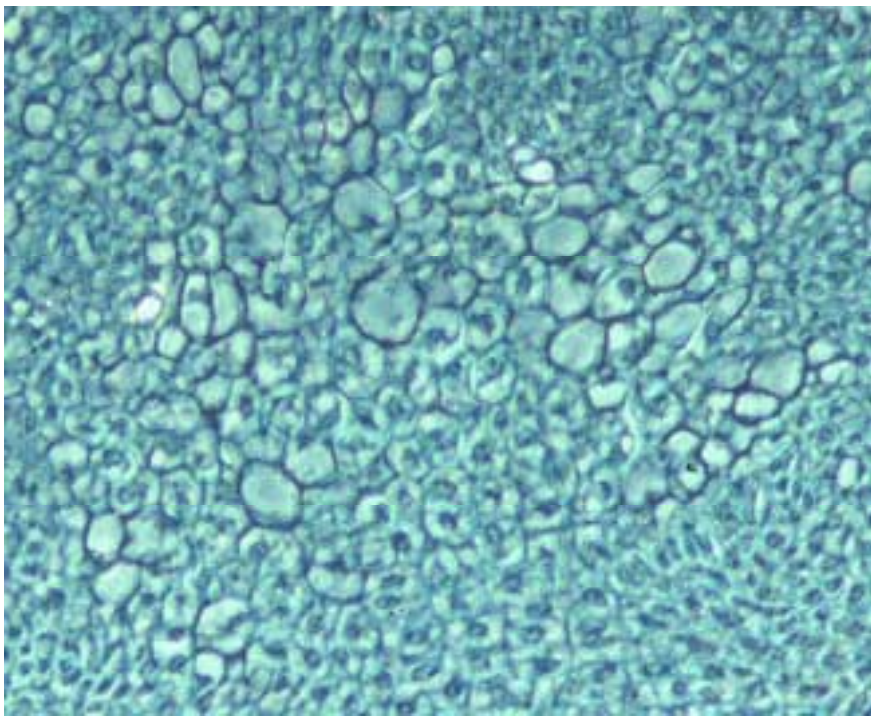

Fig. 3.2.18 Cross section through basis of vegetation cone of a bud after occurrence of a LTE; cytoplasm has coagulated.

In our case it could be clearly shown, that a HTE caused no cell damage in the buds (fig. 3.2.14, 3.12.15 and 3.2.17), while after the appearence of the LTE(s) a coagulation of protoplasts in primary and secondary buds could be found (fig. 3.2.16 and 3.2.18). Insofar we could reproduce the results of LEDDET *et al.* (1993). Sometimes we found similar effects as described by these authors.

However, the grapevine bud is a very difficult object in terms of histological preparation and possible artifacts (as also stated by KANG *et al.,* 1998) so we didn't want to carry our interpretations further since it is always easy to look for regions in microscopical sections whose appearance might support a preconceived hypothesis. The results of HAJDU and GÁBOR (1997) who analyzed survival rates of primary and secondary buds of different varieties after a severe winter frost in Hungary indicate that the secondary buds are not so much hardier as the results of LEDDET *et al.* would indicate, their survival rate is only between 10 and 20 % higher than that of the main buds.

We were not able to find cytological evidence for the permeability barrier demonstrated by JONES *et al.* (2000). The images published by these authors show that during the winter the penetration into the shoot primordium of 3000 MW dextran labelled by rhodamine green is inhibited whereas a nonacclimated shoot primordium is penetrated. Small wonder, since the figure in the publication shows well differentiated vessels in the primordium near bud burst (compared figs. 3.1.6 and 3.1.7). KANG *et al.* (1998) suppose that in apple, peach and pear provascular cells provide a barrier to the spread of ice crystals into primordia whereas they observed no such barriers in grape an persimmon buds. HAMED *et al.* (2000) could, however, demonstrate at least a temporary (2-26 min) inhibition of ice spread from the grapevine cane into the bud primordia at the time of bud burst and attributes that to procambial cells.

3.3 Exotherm measurements in buds

3.3.1 Introductory remarks

The initial idea had been to use wood cuttings kept under controlled conditions so that a well defined and readily reproducible cold adaptation might be obtained. This did not work because it was virtually impossible to control the water content of the buds on large amounts of harvested wood. While the humidity of buds harvested in the field prior to analysis was always sufficient to allow exotherm measurements this was not the case with buds taken from wood cuttings after prolonged storage at low temperatures (wrapped in plastic bags and stored either in the field shaded near the grapevines or in a cold room). While these buds were still viable and would burst at room temperature if the cuttings were watered, the water content of the shoot primordia was too low to measure LTEs, an effect which will be discussed later in detail.

One problem was water or ice condensation on the inside of the plastic wrappings, one other might be the translocation of water within the cane. Although some of these effects might be reversible by changes of temperature and humidity this would at the same time influence cold adaptation which would make the data worthless.

Therefore most of the results presented in the following chapters were obtained with buds taken in the field during the winters 98/99 and 99/00, where the climatic conditions where recorded but could evidently not be influenced.

3.3.2 Exotherms and the course of frost resistance

The (single) HTE2 of dry buds shows no significant changes through the year, its average varies around 11 °C both in summer and in winter. The effect of humidity on or between the bud scales which causes the appearance of a very sharp HTE1 (usually between – 6 ° and – 4 ° C, even up to – 3 °C) was already discussed in the previous chapters.

LTEs are rarely found during the summer, during this time the HTE seems to initiate a chain reaction involving the freezing of all bud components. LTEs appear only after the autumnal loss of water in canes and buds (fig. 3.3.7) and they probably indicate the compartimentalization of water so that ice crystal growth starting during the HTE cannot continue into the shoot primordia. Figures 3.3.1, 3.3.2 and 3.3.3 show that the LTE values reached their minimum (below – 20 °C) during January/February and rose again in March.
No differences could be found between the exotherm values of basal, medial or topmost buds.

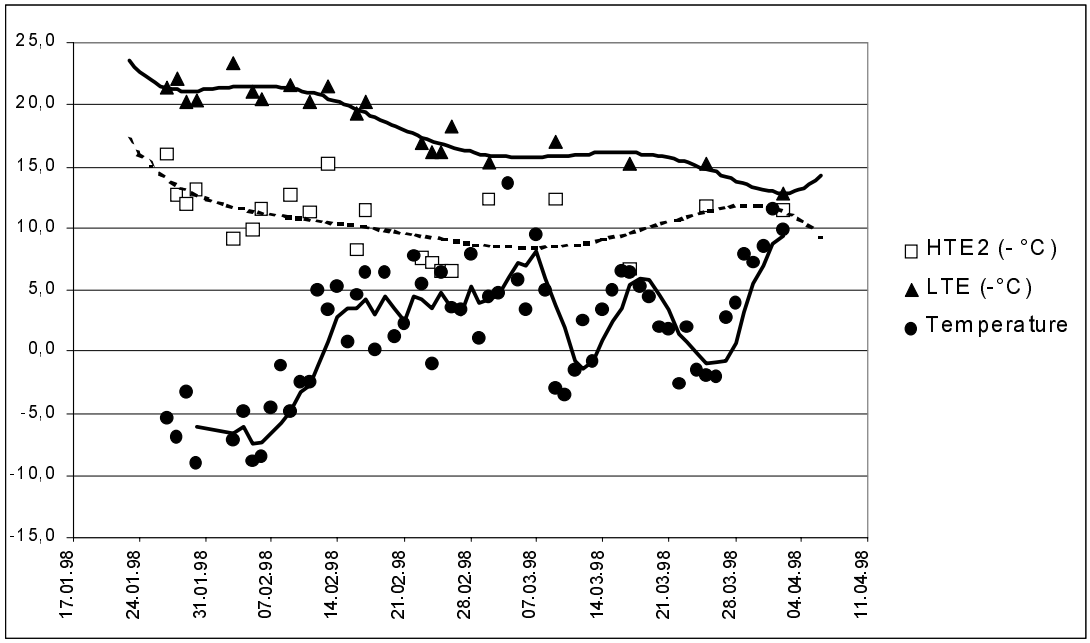

Fig. 3.3.1 HTEs, LTEs of grapevine buds (cv. Bacchus) and temperature January-March 1998. Exotherm temperatures must be read negative!

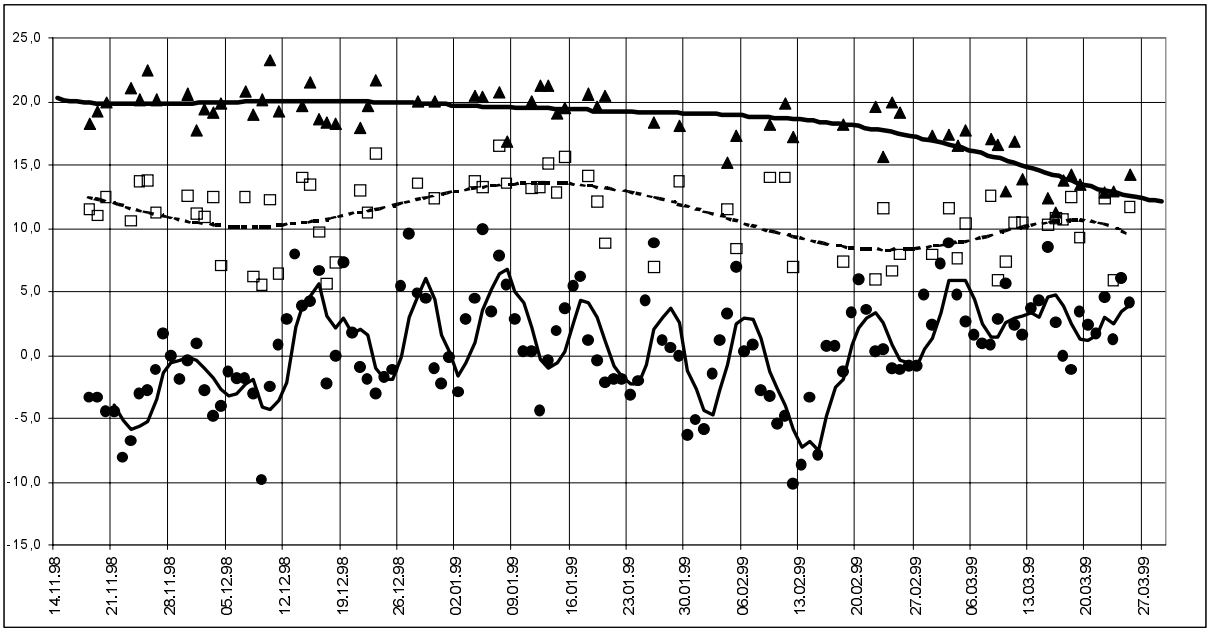

Fig. 3.3.2 HTEs, LTEs of grapevine buds (cv. Bacchus) and temperature during winter 1998/1999. Exotherm temperatures must be read negative!

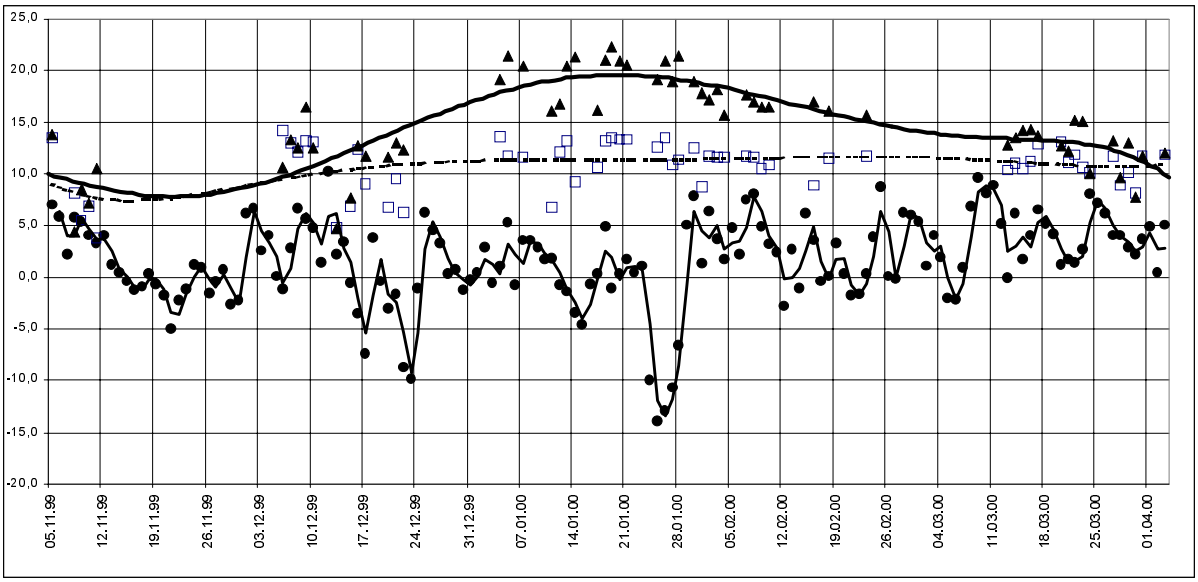

Fig. 3.3.3 HTEs, LTEs of grapevine buds (cv. Bacchus) and temperature during winter 1999/2000. Exotherm temperatures must be read negative!

3.3.3 Modifications of the freezing cycle

Some preliminary experiments revealed no significant differences of the exotherm values obtained at different cooling rates. Since this had already been found by KANG *et al.* (1998) we used a constant rate of 9 °C per hour for all further experiments.

To test wether LTEs could be provoked by prolonged influence of temperatures slightly below the HTE2, the freezing cycle was stopped after the HTE2 had appeared. Part of these buds were later used for microscopical analyses, the rest was kept for 12 – 24 h at a constant temperature. The result was unequivocal: No LTEs appeared – neither during 24 h of constant temperature nor after the following further lowering of the temperature to – 30°C.

3.3.4 Relations to humidity and water contents of buds and cane

3.3.4.1 Introduction

The effect of humid or wet bud surfaces has been discussed already in chapter 3.2 and this shall not be repeated here.

Water content of overwintering perennial plant tissues, e.g. flower buds (QUAMME, 1978, 1983) and stem tissue (WOLPERT *et al.*, 1984) nearly always decreases with increasing hardiness and increases as plants deacclimate (BURKE *et al.*, 1976; LEVITT, 1980). This phenomenon has been reported for a variety of perennial plants, including grape (WOLPERT, 1984).

Grapevines have a relatively stable moisture content in the tissues in autumn and winter. It is susceptible to intensive dehydration at sub-zero temperatures (100 % damage), so that disruption of the cells' water balance can considerably lower the frost resistance (POGOSYAN *et al.*, 1975).

In grapevine several studies have shown a relationship between tissue moisture content and the spread of ice. In the absence of ice formation, tissues are generally not injured by freezing temperatures (ASHWORTH, 1992).

According to MISIK (1997) “biological bound water” plays a great role in the resistance to the unfavorable environmental conditions and the water state of the cane is suitable to characterize the frost hardiness of grape. Even today, different authors do not agree on the structure of pure liquid water particularly in living cells and tissue. Its characteristics and structure has not yet been modelled in such a way that would satisfyingly answer all the questions. There are at least two fractions of water inside the cell, and these fractions differ from each other significantly. The fraction which is similar to extracellular water is called free water, while the fraction forming links inside the cell through hydration, swelling, adsorption, solution, bound, etc. is called bound water (ERNST, 1977; BEALL, 1981, 1983; PISSIS, 1990) which plays an important role in the resistance to unfavorable environmental (frost, drought) conditions (ERNST, 1947; GLASSER, 1951; FRANKS, 1983).

The correlation between the water state (bound water) and frost hardiness of a vine cane has already been demonstrated, but the exact description of such a correlation has met with difficulties partly because of the interpretation of the parameters obtained and partly because of the inaccuracy of the methodologies applied (GOLODRIGA-KIREEVA, 1964). MISIK (1997) states that the frost hardiness of cane depends on its water content, however only "inside" the variety and not generally (comparing different varieties). According to this author the actual frost hardiness of a cane is determined partly by its genetic bases, partly by external effects, and influence of both factors are manifested in the water state of cane.

Since water seems to play an important role for cold hardiness and for the freezing process in the cell tissues of grapevine, the determination of the

changes of the water content over the year was a prerequisite for further analysis.

3.3.4.2 Air humidity

Week correlations between air humidy and HTE-values could be shown (fig. 3.3.4, 3.3.5 and 3.3.6). This is to be expected since high air humidity mostly means wet weather conditions resulting in humid or wet bud scales. This would occur also after very cold weather with low air humidity due to condensating water in the laboratory prior to exotherm measurements. By tentatively eliminating such values the correlation could be slightly improved but we didn't do that since this could not be done without some arbitrariness.

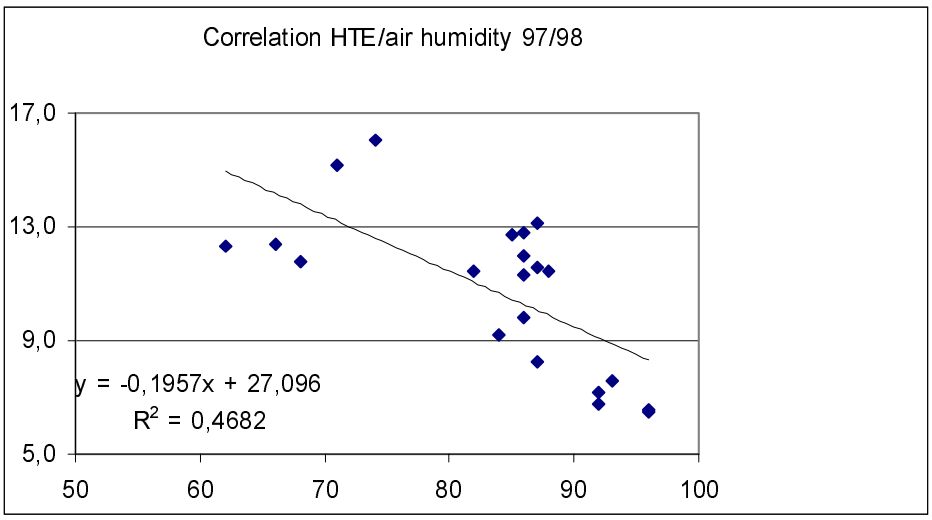

Abb. 3.3.4 Weak correlation between air humidity and HTE during winter 1997/1998

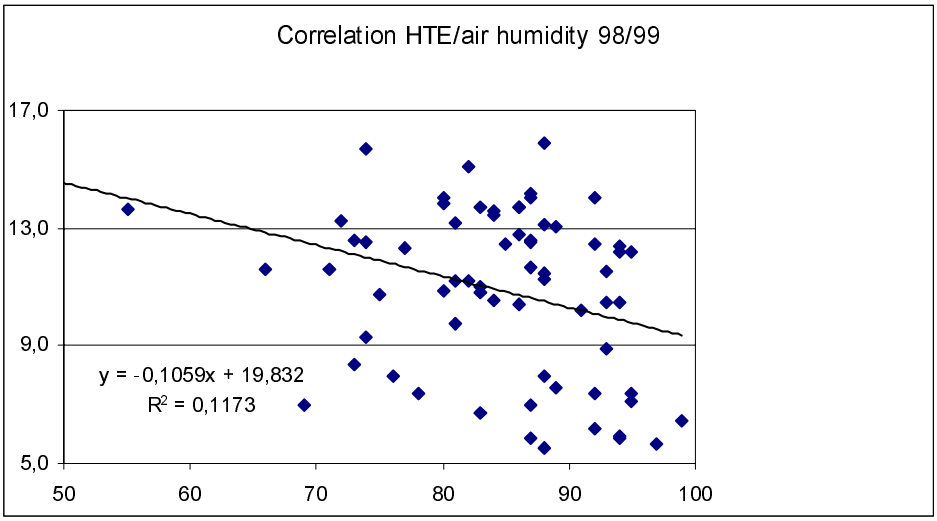

Abb. 3.3.5 Weak correlation between air humidity and HTE during winter 1998/1999

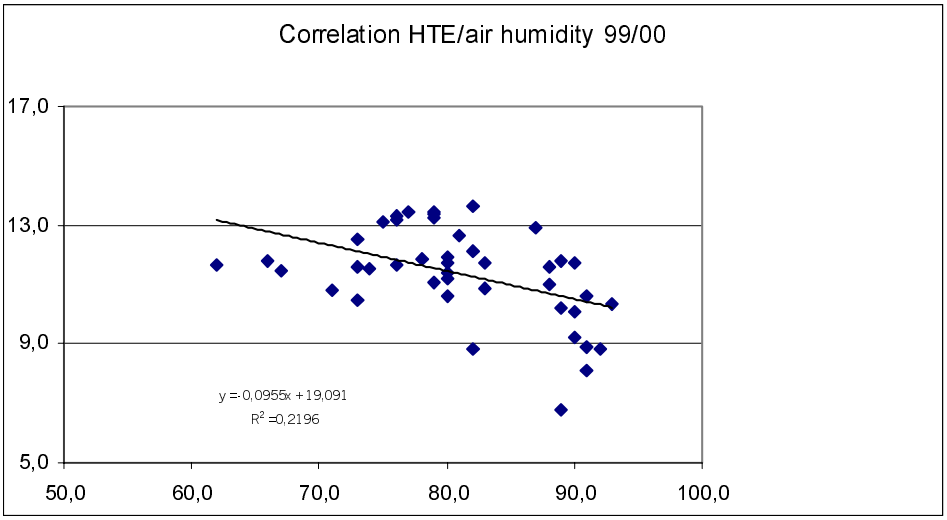

Abb. 3.3.6 Weak correlation between air humidity and HTE during winter 1999/2000

3.3.4.3 Water content of wood and buds

This was determined regularly every 2 weeks from March 1998 until Mai 2000. The water content of the buds varied between 40 % and 82%, in the canes between 46-76%. BALÓ and BALÓ (1968) found in their experiments with different vines that the average water level of wintered woods was 48-56%. The lowest water content in wood and buds (about 40 %) was found between November and February (Tables 1 and 2, fig. 3.3.7). In general shoot sections and buds from the apical shoot area contained more water than in the basal area. The water content of the cane increased with distance from the trunk and was on the average 13.3% higher than in the buds which, particularly in the first two months lose water faster than the cane. It is, however, interesting to note that during the coldest period in January and February the water content in all buds rose transiently. The early stages of bud swelling, which can easily be shown by the growing size of the exotherms, shows no correspondance in the overall water content of the buds.

The water content of the canes began to increase in march, the buds followed later surpassing the cane rapidly (fig. 3.3.7). Only after bud ripening (mid August) the bud water content decreased again belowe that of the cane. In 1999 the apical position showed a higher increase in water content in April than the basal position which always has lower water content than all other positions.

These results are consistent with work of WOLPERT (1985) and HAMMAN *et al.* (1990) who stated that the bud water content is relatively independent from the content of the cane. In this respect there seem to be considerable differences between the years (compare table 1 and table 2, p. 35 ff.). In 1998 an early rise of the water content of the cane at March 13 after a comparatively warm period between February 14 and March 7 is associated with drying of the intermediate and apical buds. This might be due to the frost immediately before the water determination and could be tentatively explained by water migration which will be discussed later.

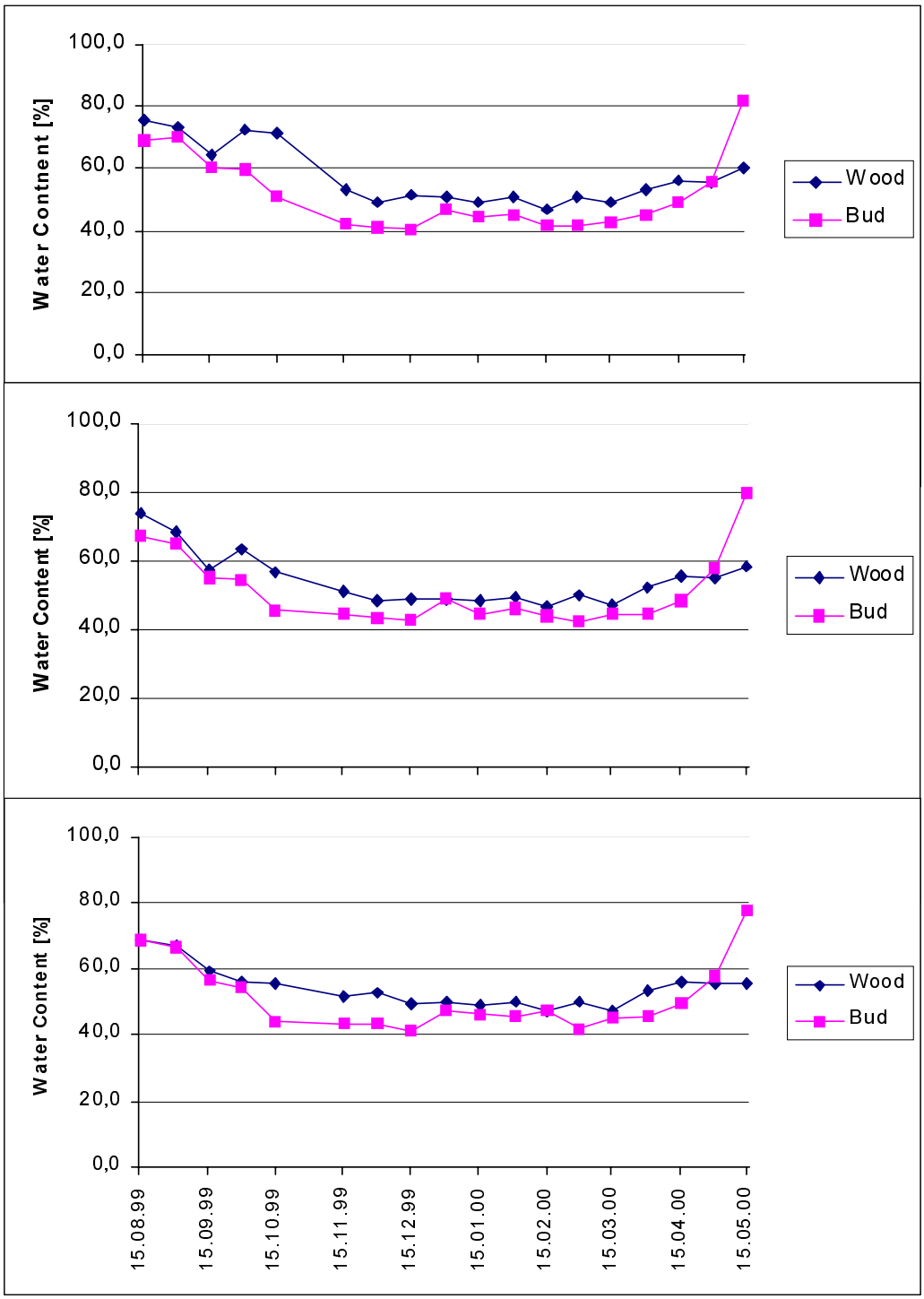

Figure 3.3.7 Water content of grapevine buds of different shoot sections in 1999/2000; top: apical buds; middle: intermediate buds; bottom: basal buds.

3.3.5 Deacclimatation

Well ripened canes, harvested by the end of February 1998 and 1999 when a good cold adaptation had been reached, were kept in the laboratory at room temperature to test for deacclimatation (deadaptation). If the canes were kept in sealed plastic bags the LTEs dissappeared rapdily under these conditions which was probably due to water migration phenomena which will be discussed later. Therefore the lower ends of the canes were put in tap water. In this case good LTEs were obtained which revealed that the cold adaptation got lost after some weeks when the buds began to swell. By this time only HTEs around – 6 °C occurred but no more lower exotherms. These experiments were, however, not continued since such conditions did not reflect the true conditions in the field and would thus give no trustworthy information on real deacclimatation conditions.

Table 1. Water content of wood and buds in 1998.

Water content %		
Basal	***Wood***	***Bud***
Date	***mean of 6 values***	***mean of 3 values***
20.02.1998	64,28	72,10
27.02.1998	53,16	65,30
06.03.1998	45,08	42,10
13.03.1998	70,70	45,75
Intermediate	***Wood***	***Bud***
20.02.1998	56,73	49,10
27.02.1998	48,52	33,30
06.03.1998	49,03	41,55
13.03.1998	70,88	28,80
Apical	***Wood***	***Bud***
20.02.1998	57,58	46,40
27.02.1998	49,86	38,00
06.03.1998	50,22	46,10
13.03.1998	65,14	29,30

Table 2. Water content (%) of wood and buds in 1999/2000

Basal	***Wood***	***Bud***
Date	***mean of 6 values***	***mean of 3 values***
19.08.1999	68,28	69,93
31.08.1999	66,14	67,47
13.09.1999	58,84	57,13
27.09.1999	56,24	56,30
08.10.1999	54,60	45,57
02.11.1999	51,92	44,60
25.11.1999	53,40	43,97
02.12.1999	49,56	42,53
15.12.1999	50,08	49,23
03.01.2000	48,92	46,57
17.01.2000	50,12	46,57
01.02.2000	47,24	47,97
14.02.2000	50,42	41,07
13.03.2000	47,57	46,17
22.03.2000	53,46	45,80
01.04.2000	56,56	47,83
13.04.2000	55,42	58,97
27.04.2000	55,32	77,93

Table 2 **continued**

Intermediate	*Wood*	*Bud*
Date	*mean of 6 values*	*mean of 3 values*
19.08.1999	73,28	64,65
31.08.1999	68,85	62,75
13.09.1999	57,78	53,85
27.09.1999	61,88	51,65
08.10.1999	57,07	44,35
02.11.1999	50,98	42,90
25.11.1999	48,27	42,55
02.12.1999	49,20	41,45
15.12.1999	48,90	46,90
03.01.2000	48,38	43,35
17.01.2000	49,20	44,75
01.02.2000	46,45	40,95
14.02.2000	49,90	43,15
13.03.2000	47,40	42,55
22.03.2000	52,13	43,80
01.04.2000	55,20	50,35
13.04.2000	54,87	55,70
27.04.2000	55,56	55,53

Apical	*Wood*	*Bud*
Date	*mean*	*mean*
19.08.1999	75,70	68,93
31.08.1999	72,90	69,97
13.09.1999	64,28	60,23
27.09.1999	72,42	59,40
08.10.1999	71,32	50,80
02.11.1999	53,26	42,20
25.11.1999	48,88	40,67
02.12.1999	51,64	40,33
15.12.1999	50,64	46,53
03.01.2000	49,12	44,27
17.01.2000	50,96	45,07
01.02.2000	46,52	41,53
14.02.2000	50,94	41,47
13.03.2000	49,18	42,53
22.03.2000	53,08	44,87
01.04.2000	55,86	49,13
13.04.2000	55,56	55,53
27.04.2000	60,04	81,77

3.4 Sugars, anions and starch in the buds

3.4.1 Introduction

Among the mechanisms evolved by plants to survive cold temperatures (for a review see e.g. BURKE *et al.* 1976) soluble carbohydrates seem to play a central role. 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). Certain soluble sugars can serve as good cryoprotectant because of their capacity to modify the freezing behavior of aqueous solutions (GOLDSTEIN and NOBEL, 1994)

In trees and shrubs an accumulation of carbohydrates is found during cold acclimation, even though the nature of accumulated sugars can vary from one species to another (FISHER and HÖLL, 1991; STUSHNOFF *et al.*, 1993). 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). SAUTER and VANCLEVE (1991) reported, that the type of carbohydrate accumulated is a more critical factor than the total sugar content. IMANISHI *et al.* (1997) suggest that the high frost resistance of *Lonicera* after acclimatation is due to raffinose and stachyose but not to sucrose. The same seems to be the case in spruce buds (LIPAVSKÁ, 1997).

The accumulation of sugars and dry matter in stems of willow in fall was examined in relation to frost hardening. Starch reserves that built up in stems in early fall were partially mobilized later to support sugar accumulation. Raffinose and sucrose accumulation seemed to be under differential environmental controls. Sucrose accumulation started with the initiation of growth cessation controlled by photoperiod, whereas raffinose accumulation started with falling temperatures later on (ÖGREN, 1999).

Raffinose family oligosaccharides (RFOs) are the most abundant sucrosyl oligosaccharides in plants. The compounds are ά-galactosyl derivatives of sucrose, and the most common are the trisaccharide raffinose, the tetrasaccharide stachyose and the pentasaccharide verbascose. They fulfil important physiological roles such as storage and translocation of carbon and are involved in protection against different types of environmental stresses.

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

3.4.2 Plant Material

Vitis vinifera L. (cv. Bacchus) was grown at the vineyard of the Institute for Fruit-, Vegetable- and Viticulture (370) of the University of Hohenheim, Stuttgart-Germany. Bud samples were collected from basal, intermediate, and apical sections of the shoots. Sampling took place after natural defoliation at the following dates: 12. Nov. 1998, 26. Nov. 1998, 10. Dec. 1998, 23. Dec. 1998, 7. Jan. 1999, 21. Jan. 1999, 25. Feb. 1999, 11. Mar. 1999, 25. Mar. 1999, 8. Apr. 1999 and 16. Apr. 1999. Sampling was repeated in the following winter season (1999/2000).

3.4.3 Results

3.4.3.1 Starch

During autumn and winter the buds contained numerous starch grains which were located mainly in the bud pad, the shoot axis and the stipulae and dissolved at bud burst. Figure 3.3.8a shows stage 1 of bud development (code of EICHHORN and LORENZ, 1977).

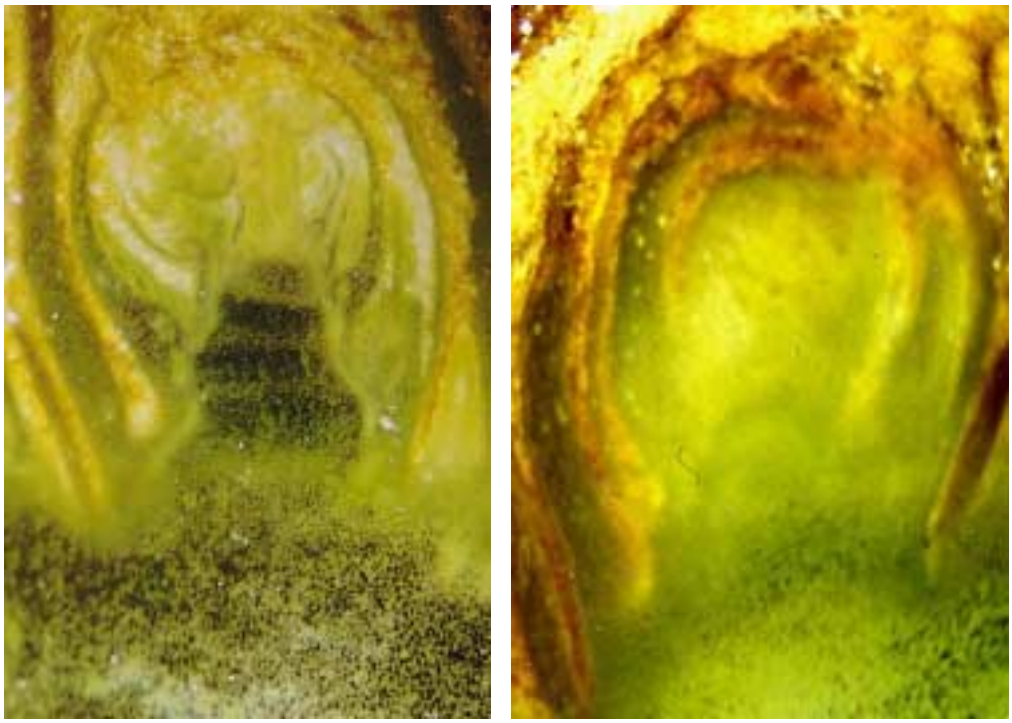

Fig. 3.3.8 Longitudinal section of primary buds stained with iodine solution to show starch granula; buds from 15 (left) and 28 march (right) 2000.

The dissolution of starch prior to bud swelling will not be discussed further here because this is certainly not related to frost resistance: According to BERBEZY *et al.* (1997) there are differences between α-amylase isozyme composition and activity induced by severe drops in temperature and just before bud burst, respectively.

3.4.3.2 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. 3.3.9). Sucrose was the dominant soluble carbohydrate and the reducing sugars (i.e. glucose, fructose) reached also rather high concentrations while raffinose and stachyose were present only in small concentrations. From

November to late December/early January total soluble sugars increased from about 80 mg/g dry matter to almost 150 mg/g dm. The lowest concentration of total soluble sugars was registered by 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 (table 3) 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.

A significant correlation was found between sugar concentrations and air temperature of the days before sampling (3, 5, 7, 9 days before sampling). The PEARSON correlation coefficient is regulary 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.