EFFECT OF PRUNING DATE ON COLD HARDINESS AND MOISTURE CONTENT OF 'CONCORD' (*VITIS LABRUSCANA* BAILEY) BUD AND CANE TISSUES

By

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ABSTRACT

EFFECT OF PRUNING DATE ON COLD HARDINESS AND MOISTURE CONTENT OF 'CONCORD' (VITIS LABRUSCANA BAILEY) BUD AND CANE TISSUES

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'Concord' grapevines (Vitis labruscana Bailey) were pruned on various dates throughout two dormant seasons, 1974-75 and 1975-76. Cold hardiness and tissue moisture content were measured on each date to determine if time of pruning affected hardiness, and, if so, if differences were related to tissue moisture content. For primary and secondary bud tissues and one-year-old cane tissues, hardiness was greatest and moisture content least in midwinter (Jan). Hardiness decreased and moisture content increased during late winter and spring. Bud tissues of vines pruned early in the dormant season (Dec) tended to be less hardy than those of vines pruned late in the dormant season (Mar). Cane tissues showed no hardiness response to pruning date. Early-pruned vines suffered more spring frost damage than late-pruned vines. Efforts to relate differences in bud hardiness as a function of pruning date to changes in moisture content were inconclusive.

To Angie, for her love and understanding.

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INTRODUCTION

Standard recommendations for grapevines in northern states call for delaying pruning until late winter (Edgerton and Shaulis, 1953) to allow selection of fruiting wood which has overwintered in good condition and adjustment of bud numbers to accommodate losses due to cold. However, Michigan grape growers frequently begin pruning in late fall and continue through late spring in order to completely prune their acreage. I wished to determine if vines pruned early in the dormant season responded differently to low temperatures than unpruned vines and, if differences existed, to assess their magnitude and physiological bases.

LITERATURE REVIEW

Pruning and Winter Injury. Several researchers have observed a relationship between pruning and winter damage. Burkholder (1936) reported that 'Jonathan' and 'Stayman' apple trees suffered substantial damage when pruned prior to several days of sub-zero temperatures, whereas unpruned trees showed no injury. He also noted that damage appeared proportional to the severity of pruning and was cultivar dependent. Anthony et al. (1936) also observed a positive correlation between early pruning and winter injury in Pennsylvania apple orchards. On the other hand, Magoon and Dix (1941) found no effect of pruning date on the yield of several grape varieties in Maryland. In spite of this,

the authors implictly acknowledged a possible relationship between pruning and low temperature stress by concluding that growers in colder areas would be advised "to wait until the danger of heavy freezing is past before beginning the pruning work." They also found no difference in foliation date as a function of pruning date. This contradicts the work of Loomis (1939) who reported that late pruning delayed foliation of the grape cultivar Extra in Mississippi.

Edgerton and Shaulis (1953) used artificial freezing methods to test the effect of fall pruning on the cold hardiness of 'Concord' grapevines. In March unpruned controls were hardier than pruned vines, and apical segments were less hardy than basal segments of canes from pruned vines. They also noted that primary bud mortality was greatest near the tips of canes from pruned vines. Rollins et al. (1962) found that the hardiness of twigs from 'Yellow Transparent' apple trees pruned in January decreased by 2.5^{o}C within seven hours after pruning. After one day, pruned trees were 6^{o} less hardy than controls, but after 44 days they had become more hardy than the controls.

All these reports agree that pruning increases the likelihood of low temperature injury. The only proposed hypothesis (Rollins et al., 1962) postulated that the difference in hardiness between pruned and unpruned trees was due to water from the roots. Because the roots supply a constant volume of water, the increase in tissue water would be greater in pruned trees because their reduced tissue volume. This hypothesis merits investigation in grapevines because pruning by the balanced-pruning concept of Partridge (1925) results in removal of a large volume of tissue.

Tissue Water and Plant Hardiness. The relationship between tissue moisture and hardiness has been investigated for many years. Levitt (1941) did an excellent job of reviewing the early literature. Traub (1927) found that apple twigs declined suddenly in percent moisture content in mid-September when the leaves were still green. This occurred in spite of sufficient rainfall. Moisture content rose again in early April before significant rainfall occurred. Wilner (1952) reported that cultural treatments had no effect on the water content of mature twigs of woody plants, and suggested that water content at maturity was genetically determined. Other workers reported low winter moisture values followed by a spring increase in peach buds (Johnston, 1923) and apple twigs (Hildreth, 1926; Stark, 1936). Stark (1936) suggested that water relationships of tip and basal portions of the same apple shoot were different. Wiegand (1906) found that hardiness in buds of various fruit trees and ornamentals was related to bud cell size and water content. Buds with large cell size and high water content were less hardy than buds with small cell size and low water content. At -18°C the former contained ice crystals while the latter resisted ice formation.

In Juniperus chinensis L. 'Hetzi', water content decreased during acclimation while hardiness increased (Pellet and White, 1969). Gusta and Weiser (1972) found that the greatest reduction in leaf hydration in boxwood, an evergreen, appeared to occur during periods when hardiness was increasing rapidly, but that moisture content remained relatively constant during periods when hardiness fluctuated. McKenzie et al. (1974) recently showed the following relationships between moisture and hardiness in Cornus stolonifera Michx., in which short days induce the first stage of hardening:

1. In plants held under long days (LD), water content increased from the base to the apex of twigs. Short day (SD) plants showed no such gradient.
2. Major water losses occurred at the time of maturation of pith cells.
3. SD plants had a 1.5-fold decrease in stomatal resistance and a 3.5-fold increase in root resistance conpared to LD plants.
4. Clones of C. stolonifera varied in their rates of cold acclimation. In all but one of these, the earlier the decrease in water occurred the earlier the plants acclimated to low temperature stress.

The authors suggested that the SD promotion of acclimation (Fuchigami et al., 1971) was due to the reduction in water content, because no plant hardened to -12°C (the magnitudeof the SD response) without first losing tissue water.

Artifically increasing and decreasing blueberry bud moisture content respectively decreased and increased cold hardiness (Bittenbender and Howell, 1975). Li and Weiser (1971) increased the cold hardiness of dogwood 3 to 12°C when they removed 4 to 10% of the stem water by freeze-drying, and the increase was proportional to the amount of water removed. Lumis et al. (1972) explained differences in survival of two species of Rhododendron on the basis of water percentage alone. When tissue water content of the less hardy R. poukahense (54% of dry weight) was artificially decreased to the level of hardy R. cv. Maryann (46%), the hardiness differences were eliminated. Cabbage (Brassica oleracea L. cv. Dittmar) kept under growth chamber conditions known to induce hardiness had less tissue moisture than plants held under warm, non-acclimating

conditions (Kacperska-Palacz et al., 1969). Rice seeds with a water content of 15% of dry weight all germinated after immersion in liquid N_2, but with 21% moisture, no germination occurred (Sakai and Noshiro, 1975).

Water content can affect the nature of the freezing stress and thus the killing temperature (Olien, 1974; Lumis and Mecklenburg, 1974). Metcalf et al. (1970) found that a small change in crown moisture content of wheat and barley resulted in a very large difference in survival at a given temperature. Gullord (1974) reported that differences in leaf moisture content explained 69-72% of the variation in hardiness among selected wheat and rye cultivars.

Plants contain a significant amount of "bound" water which does not freeze (Levitt, 1941; Mazur, 1969). This is associated with macromolecular surfaces and differs from bulk water in several properties, including freezing point (Cooke and Kuntz, 1974). Nuclear magnetic resonance (NMR) spectroscopy has been used to quantify bound water (Toledo et al., 1968; Sussman and Chin, 1966). Cook and Kuntz (1974) concluded that water exists in hydration shells and remains liquid more because of suppressed freezing point than because of supercooling.

Changes in the bound water to bulk water ratio have been proposed as a mechanism of cold hardening (Pellett and White, 1969; Vasil'yev et al., 1975) in spite of earlier rejection of this hypothesis (Stark, 1936; Levitt, 1941, 1956). Recently, Gusta et al. (1975) found no simple relationship between hardiness and bound water content of wheat cultivars. They concluded that the difference between tender and hardy cultivars was the ability of the hardier crown to tolerate diminishing quantities of liquid water.

Recently, deep supercooling has been shown to be a mechanism of ice avoidance (see Levitt, 1972) in azalea flower buds (Graham, 1971; George et al., 1974), apple xylem elements (Quamme et al., 1972) and grape buds and stems (Pierquet et al., 1977). Changes in ability to supercool during acclimation may involve the reduction or elimination of nucleation centers for ice formation or development of barriers to nucleation (Burke et al., 1976). The lower limit of deep supercooling in the homogeneous nucleation temperature of water (approx. -40°C) (Rasmussen and MacKenzie, 1974).

STATEMENT OF PROBLEM

Based on observations in the literature, the problem to be researched in this thesis can be outlined as follows:

1. To determine if 'Concord' grapevines pruned early in the dormant season respond differently to low temperature stress than do unpruned vines.
2. To determine if fluctuations in hardiness due to treatment or time of year can be explained by changes in tissue moisture content.

MATERIALS AND METHODS

Experiment 1 - Effect of pruning date on hardiness and moisture content, 1974-75. In mid-winter, 1974-75, an experiment was initiated at the Michigan State University Horticultural Research Farm using 10-year-old 'Concord' (Vitis labruscana Bailey) grapevines. Vines were divided into six treatment groups and each was assigned to one of six pruning dates (14 Jan., 13 Feb., 27 Feb., 27 Mar., 17 Apr., and 8 May). Treatments were arranged in a completely randomized design with four replicates. Each treatment group was balanced pruned by means of a 30+10 formula (i.e., 30 buds retained for the first pound of cane prunings and 10 buds for each additional pound) and trained to a 4-arm Kniffen system. On each date canes were collected from the vines pruned and from all previously pruned vines for determinations of moisture content and cold hardiness. Thus, on the first pruning date one group was sampled, while on the sixth date all six treatments were sampled. The number of buds required for sampling was calculated in advance, and during pruning these were left in addition to nodes retained by the pruning formula. Thus, changes in bud and cane hardiness and moisture content could be followed subsequent to pruning and compared with that of control (unpruned) vines.

Experiment 2 - Effect of pruning date on hardiness and moisture content, and field freeze injury, 1975-76. In winter 1975-76 the experimental area was increased to include a block of four-year-old vines on

the same site. A randomized block design was used to partition out varability due to vine age. Five pruning dates were used (5 Dec., 8 Jan., 13 Feb., 29 Feb., and 30 Mar.), vines being sampled as in Experiment 1.

Warm air temperatures in early and mid-April (Table A12) resulted in rapid bud development. A severe freeze ($-4^{o}C$) on 26 April caused extensive damage to swelling buds. Canes bearing 12 to 16 nodes were examined on 16 and 18 May to determine whether the extent of injury was related to pruning date. Observations included 1) the number of buds which swelled prior to the freeze and were subsequently killed, and 2) the number of buds swelling at the time of observation. Data were taken on 6 to 10 nodes per replicate.

Experiment 3 - Effect of sampling date on hardiness and moisture content of non-pruned vines, 1975-76. The experimental design for Experiments 1 and 2 allowed for comparisons only within sampling dates. Experiment 3 was initiated at the Rogers Concord vineyard in Lawton, Michigan to provide information on the relationship between tissue moisture and cold hardiness from late fall to early spring. The vines had been balanced pruned for five previous years but were not pruned during the sampling period.

Experiment 4 - Effect of node position on moisture content. On 11 April, 1976 canes 14 to 18 nodes in length were gathered from unpruned Concord grapevines at the MSU Horticultural Research Farm. Node numbers 2, 4, 6, 8, 10, and 12 were collected individually. Tissue moisture content was determined as a function of node position.

Sampling procedure. In all experiments sample material consisted ofone-year-old, well-exposed, mature cone pieces (10-12cm) of uniform diameter (6-7mm) with one bud located in the middle. Exposure and

maturity were assessed visually and only canes with reddish-brown periderm were used. Samples were divided into two lots for separate determinations of cold hardiness and moisture content of buds and canes.

Cold hardiness evaluation. Freezing technique for canes and buds was essentially that used by Howell and Weiser (1970) as modified by Stergios and Howell (1973). Cane sections were inserted into vacuum flasks and placed in a Revco chest freezer. The temperature was reduced at a consistent rate (3-5^{o}C/hr in side the flasks). Cane temperatures were monitored with a 24-gauge copper-constantan thermocouple inserted into the pith of a representative cane in each flask. Test temperatures varied with the time of year and expected hardiness of the material. A control (unfrozen) sample was included for each treatment to estimate field mortality. A temperature range was chosen such that the warmest temperature produced no injury and the coldest was lethal for all tissues. At regular temperature intervals flasks were removed and allowed to warm to room temperature overnight. Canes were then placed in a humid chamber for 7-10 days to allow injured tissues to turn brown (Stergios and Howell, 1973) after which they were sectioned transversely with a razor blade, observed through a binocular microscope, and rated as alive or dead. Buds were considered dead when any part of the primordium was brown, while twigs were arbitrarily recorded as dead when more than half of the phloem-cambium area was brown.

Tissue moisture evaluation. For Experiment 1 primary and secondary buds were excised, separated and weighed singly on a Mettler H31 single-pan balance. Tertiary buds were excluded because their small size precluded accurate measurement on the Mettler balance. Cane segments (2-4cm), cut from the middle of the sample piece, but just proximal to

the node, were used for cane moisture measurements. All tissues were oven-dried overnight at 70°C and reweighed. From these data the amount of water was calculated by difference and expressed as grams of water per gram of tissue dry weight.

The moisture content procedure for Experiment 2 was changed because the data for the previous year were very variable (coeff. var. = 15-30%). Bud tissues weighed directly on the balance gained and lost moisture too quickly, especially when they were dried. On the suggestion of Olien (personal communication), three primary and secondary buds per replicate were excised, separated and placed in air-tight glass vials (7.5 x 15mm) with ground-glass stoppers. These containers greatly reduced water loss during weighing and decreased variation (coeff. var = 2-10%). Three cane sections per replicate were weighed together directly on the balance with acceptable results. All tissues were oven-dried for 36 hours at 70°C and vial weights were taken after drying.

The only difference in procedure for Experiment 3 was that three cane peices per replicate were placed in large glass vials (25 x 50mm) with ground-glass stoppers. Bud tissues were handled as in Experiment 2. The moisture content procedure for Experiment 4 was identical with that for Experiment 2.

RESULTS

Experiments 1 and 2 - Effects of pruning date on hardiness and moisture content, 1974-75 and 1975-76, and field freeze injury, 1976. Complete data for the hardiness portion of both experiments are presented in the Appendix, Tables A1-A11. Hardiness values for Experiment 1 were averaged over several test temperatures and are presented as percent kill in Tables 1-3 for primary buds, secondary buds and canes, respectively. Data were not analyzed statistically because of the small number of observations (4) per temperature, but some trends were evident.

Tertiary bud hardiness data for individual dates appear only in the Appendix for several reasons: 1) tertiary buds produce little crop, even in years when primary and secondary buds are killed; 2) they present no physiological information which differs from that of other tissues; 3) no moisture content data are available for comparison.

Primary buds of vines pruned early in the dormant season tended to be less hardy than those pruned late in the dormant season (Table 1). The same is true of secondary buds (Table 2) and canes (Table 3) but to a lesser extent. The data also suggest that vines pruned on the sample date (i.e., treatment 2 on date 2 through treatment 6 on date 6) tended to be less hardy than vines pruned 2 to 4 weeks earlier. This effect can also be seen for secondary buds and canes.

Differences in moisture content of primary buds (Table 1) were not statistically significant until the last two sampling dates. On 17 April,

1975, the highest primary bud moisture content was associated with the greatest percent kill (treatment 1). However, among the remaining treatments, percent kill ranged from 0 to 42 percent while moisture content did not differ. For 8 May, the reverse was true, the greatest percent kill being associated with the lowest moisture content.

Hardiness data for Experiment 2 are presented both in main effects tables (Tables 4 and 5) and together with tissue moisture content in Tables 6-8. Percent kill values were combined across replicates and statistically analyzed with test temperatures as blocks.

Unfrozen controls for each treatment provided an estimate of percent field kill. Values were corrected by subtraction of field injury. Experimental values which were less than values for field injury were assumed to be zero. All corrected percent kill values were transformed by arcsine transformation (Bartlett, 1947) prior to statistical analysis, and significant differences were determined with transformed data.

Vines pruned early in the dormant season suffered more injury than those pruned later (Table 4). Generally, hardiness differences between tissues (Table 5) were not as marked as previously reported (Stergios and Howell, 1976; Pogosyan and Sarkaisova, 1967; Pierquet et al., 1977). For primary buds, differences in percent kill were significant on only two dates (13 Feb. and 30 Mar.), and in both cases the vines pruned earlier were less hardy than those pruned later (Table 6). The 29 Feb. sample date showed the same trend although the values were not statistically significant.

Pruning date had no significant effect on hardiness of secondary buds and hardiness of canes was affected only on 30 Mar., the earliest pruned vines showing the greatest injury (Tables 7 and 8). In contrast

with the data obtained in Experiment 1, vines sampled at the time of pruning were never significantly less hardy than other treatments.

Moisture content in Experiment 2 was not related to tissue hardiness (Table 6-8).

Generally, spring freeze injury to both primary and secondary buds was greater near the distal end than near the basal end of the cane (Table 13), but differences were significant in only one case for each tissue. There was no effect of pruning date on injury to primary buds. In secondary buds pruning tended to increase injury but only for nodes 7-9 was the effect significant. After the freeze a greater percentage of primary and secondary buds were alive near the base of the canes and pruning tended to decrease the number of live buds (Table 14).

Experiment 3 - Effect of sampling date on hardiness and moisture content of non-pruned vines, 1975-76. Data for Experiment 3 are expressed as T_{50} (the theoretical temperature at which 50% of the tissues die) calculated by means of the Spearman-Karber equation as modified by Bittenbender and Howell (1975). The T_{50} is an absolute hardiness measure which allows comparison across dates.

Maximum hardiness for all tissues was achieved in mid-winter (Table 9). Primary buds appeared to harden slower and deharden faster than either secondary buds or canes. Canes were hardier than buds in mid-winter and secondary buds were hardier than primary buds. In early March the hardiness difference among tissues disappeared, but canes were again hardier than bud tissues in April.

For the first four sample dates, canes contained significantly more water than bud tissues. For the next three sample dates differences among tissues were nonsignificant. On the last sample date moisture

content differed significantly in all three tissues; primary buds contained the most water and canes the least. Water content declined from December to early February in all tissues, then rose during the dehardening period (March-April) in bud tissues, with the primary bud increasing more dramatically than the secondary. Canes showed no such increase in moisture content through 5 April.

Correlation coefficients relating moisture content vs. hardiness were significant for bud tissues but not for canes (Table 10).

Experiment 4 - Effect of node position on moisture content. Bud water content was generally greater at more distal nodes (Table 11) and buds generally contained more water than cane tissues. Although analysis of variance indicated no significant effect of node position, the linear component was significant at 1% (Table 12).

DISCUSSION

Data from Experiments 1 and 2 support the hypothesis that vines pruned before mid-February are less hardy in the spring than unpruned vines (Edgerton and Shaulis, 1953), and suggest that the relationship may be quantitative, i.e., the earlier the pruning date the less the hardiness. These effects are greatest in the primary bud which is most productive (Stergios and Howell, 1974) and least hardy (Stergios and Howell, 1977). Primary buds were injured more by spring frost than secondary buds, and early-pruned vines suffered more damage than late-pruned vines. No data were taken on foliation date *per se*, but observations in vineyards show that vines which suffered more damage began to develop early (Byrne, 1976; Howell and Wolpert, unpublished).

The relationship between moisture and hardiness (Table 9) is in total agreement with the literature (Pellett and White, 1969; Lumis *et al.*, 1972; McKenzie *et al.*, 1974; Burke *et al.*, 1976). There is an inverse relationship between the two factors: more moisture/ less hardy (fall and spring) and less moisture/ more hardy (winter). However, in instances where pruning date affected hardiness, no concomitant differences in moisture content could be found.

Several explanations may be offered for the lack of a close relationship between moisture content and small changes in hardiness. First, perhaps moisture content has no effect on hardiness. Hardiness fluctuations may be due to some other factor(s), physical or physiological,

other than gross water content (Gusta and Weiser, 1972; Bittenbender and Howell, 1975).

Secondly, variability may have been large enough to mask the relationship. The experimental area at the Research Farm is a marginal Concord grape site. Extremely low mid-winter temperatures (-28°C, 18 Jan., 1976, Table 15) killed a large number of buds, which affected the hardiness evaluation. In addition, error may have been involved in the sampling procedure. Because of limited plant material in Experiments 1 and 2, whole canes were collected and sections were randomly allocated to test temperatures. Experiment 4 showed that a gradient of water does exist from base to apex in a grape cane. This variation in water content may have affected hardiness, if the water/hardiness relationship is valid.

Thirdly, during the preparation of twigs for freezing they may have thawed long enough to alter their hardiness. Pierquet et al., (1977) have shown that wild grape (Vitis riparia Michx.) twigs deharden when thawed for 24 hours; wood exotherm occurs at a warmer temperature and bud exotherms appear where none were present during freezing of non-thawed material. The authors speculate that the change is due to water entering the bud from the thawed cane, but they present no data on bud water content.

Fourthly, variation may have existed in techniques. The freezing process within several vacuum flasks may have been different enough (e.g., amount of supercooling) to affect the percent kill (Olien, personal communication). Also, the method of determining water content of buds measured gross bud moisture i.e., water content of both the primordia and fleshy bud scales. Water could have moved between the

primordia and bud scales during hardiness fluctuations without any apparent change in total bud moisture.

Thus, the question: Is water content directly related to small hardiness fluctuations and differences due to pruning date? has not been adequately answered. The data presented here neither support the hypothesis nor refute it. Further research is needed, with emphasis on eliminating sources of variation, before the question can be answered.

Several other questions are raised by this research: 1) If the pruning date effect is quantitative (i.e., the earlier the pruning takes place the greater the deleterious effect) as the data suggest, what changes take place and how does pruning effect them? 2) Why do primary buds respond more than secondary buds? 3) Why do early-pruned vines suffer more damage in a spring frost? 4) Why do apical buds develop earlier than basal buds? Answers to these questions will not only improve our understanding of vine hardiness physiology, they will provide a basis for cultural modification of grapevines to reduce the impact of cold stress.

Table 1. Effect of pruning date on hardiness (percent kill, %K)[z] and moisture content[y] of primary buds of 'Concord' grapevines, 1974-75.

Date of pruning	Date sampled, 1975											
	14 Jan		13 Feb		27 Feb		27 Mar		17 Apr		8 May	
	%K	H_2O	%K	H_2O	%K	H_2O	%K	H_2O	%K	H_2O	%K	H_2O
14 Jan	42	0.56	31	0.57a[x]	67	0.70a	17	0.70a	50	0.91a	44	1.37c
13 Feb			50	0.54a	33	0.72a	25	0.64a	33	0.74b	31	1.68ab
27 Feb					58	0.74a	0	0.67a	8	0.71b	25	1.61b
27 Mar							8	0.65a	0	0.62b	19	1.67ab
17 Apr									42	0.68b	19	1.86a
8 May											25	1.66ab

[z]Percent kill is averaged for several test temperatures.

[y]Moisture content is expressed as g H_2O/ g tissue dry wt.

[x]Mean comparison by Duncan's Multiple Range Test. Within columns, means followed by the same letter are not significantly different at p=.05.

Table 2. Effect of pruning date on hardiness (percent kill, %K)[z] and moisture content[y] of secondary buds of 'Concord' grapevines, 1974-75.

Date of pruning	Date sampled, 1975											
	14 Jan		13 Feb		27 Feb		27 Mar		17 Apr		8 May	
	%K	H_2O	%K	H_2O	%K	H_2O	%K	H_2O	%K	H_2O	%K	H_2O
14 Jan	42	0.59	25	0.56a[x]	42	0.89a	0	0.75a	33	0.85a	31	1.16b
13 Feb			50	0.44b	25	0.62c	8	0.66ab	25	0.68b	25	1.40a
27 Feb					42	0.77b	0	0.62b	8	0.72ab	19	1.17b
27 Mar							17	0.62b	17	0.46c	17	1.29ab
17 Apr									42	0.36c	17	1.18b
8 May											25	1.12b

[z]Percent kill is averaged for several test temperatures.

[y]Moisture content is expressed as g H_2O/ g tissue dry wt.

[x]Mean separation by Duncan's Multiple Range Test. Within columns, means followed by the same letter are not significantly different at p=.05.

Table 3. Effect of pruning date on hardiness (percent kill, %K)[z] and moisture content[y] of canes of 'Concord' grapevines, 1974-75.[x]

Date of pruning	Date sampled, 1975											
	14 Jan		13 Feb		27 Feb		27 Mar		17 Apr		8 May	
	%K	H_2O	%K	H_2O	%K	H_2O	%K	H_2O	%K	H_2O	%K	H_2O
14 Jan	33	0.84	56	0.80	50	0.84	42	0.81	25	0.83	25	1.02
13 Feb			44	0.80	17	0.86	8	0.81	25	0.82	31	1.04
27 Feb					50	0.82	0	0.80	0	0.83	12	1.06
27 Mar							11	0.78	17	0.81	12	1.02
17 Apr									33	0.79	6	1.03
8 May											12	1.03

[z]Percent kill is averaged for several test temperatures.

[y]Moisture content is expressed as g H_2O/ g tissue dry wt.

[x]No differences in moisture content were significant at p=.05 by Duncan's Multiple Range Test.

Table 4. Main effect of pruning date on hardiness of 'Concord' grape buds and canes in Experiment 2, 1975-76. Values are percentage kill averaged for several test temperatures and all tissues. Test temperatures varied with time of year so that mean comparisons can be made only within one sampling date.

Date of pruning	Sampling date				
	5 Dec	8 Jan	13 Feb	29 Feb	30 Mar
5 Dec	- -[z]	34a[y]	26a	38a	49a
8 Jan		30a	14b	32b	43ab
13 Feb			13b	28b	35bc
29 Feb				34ab	36bc
30 Mar					34c

[z]No comparison possible.

[y]Mean separation by Duncan's Multiple Range Test. Within columns, means followed by the same letter are not significantly different at p=.05.

Table 5. Main effect of tissues on hardiness of 'Concord' grape buds and canes in Experiment 2, 1976-76. Values are percentage kill averaged for several test temperatures and all pruning dates. Test temperatures varied with time of year so that mean comparisons can be made only within one sampling date.

	Sampling date				
Tissue	5 Dec	8 Jan	13 Feb	29 Feb	30 Mar
Primary bud	26b[z]	44a	22a	36a	45a
Secondary bud	13b	36ab	17a	31a	43a
Tertiary bud	12b	20b	18a	30a	36b
Cane	77a	28b	13a	35a	33b

[z]Mean separation by Duncan's Multiple Range Test. Within columns, means followed by the same letter are not significantly different at p=.05.

Table 6. Effect of pruning date on hardiness (percent kill, %K)[z] and moisture content[y] of primary buds of 'Concord' grapevines, 1975-76.

Date of pruning	Sampling date									
	5 Dec		8 Jan		13 Feb		29 Feb		30 Mar	
	%K	H_2O	%K	H_2O	%K	H_2O	%K	H_2O	%K	H_2O
5 Dec	26	0.72	36a	0.55a	38a	0.64a	48a	0.75a	51a	0.91a
8 Jan			51a	0.59a	16b	0.72a	30a	0.78a	55a	0.88ab
13 Feb					14b	0.71a	27a	0.77a	36b	0.96a
29 Feb							41a	0.74a	33b	0.89a
30 Mar									42ab	0.77b

[z]Percent kill is an average for several test temperatures. Test temperatures varied with time of year so that mean comparisons can be made only within one sampling date.

[y]Moisture content is expressed as g H_2O/ g tissue dry wt.

[x]Mean separation by Duncan's Multiple Range Test. Within columns, means followed by the same letter are not significantly different at p=.05.

Table 7. Effect of pruning date on hardiness (percent kill, %K)[z] and moisture content of secondary buds of 'Concord' grapevines, 1975-76.[x]

Date of pruning	Sampling date: 5 Dec		8 Jan		13 Feb		29 Feb		30 Mar	
	%K	H_2O	%K	H_2O	%K	H_2O	%K	H_2O	%K	H_2O
5 Dec	13	0.76	37	0.55	22	0.66	34	0.78	53	0.89
8 Jan			37	0.58	18	0.76	31	0.73	44	0.90
13 Feb					11	0.70	21	0.80	41	0.89
29 Feb							37	0.80	45	0.88
30 Mar									34	0.91

[z]Percent kill is an average for several test temperatures. Test temperatures varied with time of year so that mean comparisons can be made only within one sampling date.

[y]Moisture content is expressed as g H_2O/ g tissue dry wt.

[x]No differences in hardiness or moisture content were significant at p=.05 by Duncan's Multiple Range Test.

Table 8. Effect of pruning date on hardiness (percent kill, %K)[z] and moisture content[y] of canes of 'Concord' grapevines, 1975-76.

Date of pruning	Sampling date: 5 Dec		8 Jan		13 Feb		29 Feb		30 Mar	
	%K	H_2O	%K	H_2O	%K	H_2O	%K	H_2O	%K	H_2O
5 Dec	77	0.78	39a[x]	0.80a	19a	0.76a	39a	- -[w]	42a	0.78a
8 Jan			17a	0.73b	4a	0.78a	36a	- -	33b	0.76a
13 Feb					15a	0.74a	36a	- -	30b	0.79a
29 Feb							28a	- -	32b	0.77a
30 Mar									28b	0.70a

[z]Percent kill is averaged for several test temperatures. Test temperatures varied with time of year so that mean comparisons can be made only within one sampling date.

[y]Moisture content is expressed as g H_2O/ g tissue dry wt.

[x]Mean separation by Duncan's Multiple Range Test. Within columns, means followed by the same letter are not significantly different at p=.05.

[w]Missing data.

Table 9. Hardiness (T_{50})[z] and moisture content[y] of buds and canes of 'Concord' grapevines sampled on various dates throughout the dormant season, 1975-76, in Lawton, Michigan.

		Sampling date, 1975-76							
	Tissue	18 Nov	10 Dec	29 Dec	5 Feb	18 Feb	3 Mar	21 Mar	5 Apr
Hardiness (T_{50})	Primary bud	-17.0 a[x] h	-23.5 b f	-25.0 b e	-26.0 c d	-23.0 c f	-21.5 ab g	-17.0 a h	-13.0 b i
	Secondary bud	-17.5 a g	-26.0 a e	-26.0 ab e	-27.5 b d	-26.0 b e	-22.5 a f	-18.2 a g	-13.0 b h
	Cane	-13.0 b k	-26.0 a f	-27.0 a ef	-29.0 a d	-28.0 a de	-21.0 b g	-18.0 a h	-16.0 a i
Moisture content	Primary bud	0.70 b f	0.82 b e	0.61 b g	0.57 b h	0.78 a ef	0.73 a f	0.86 a e	1.07 a d
	Secondary bud	0.71 b f	0.78 b ef	0.62 b g	0.58 b g	0.80 a def	0.81 a de	0.84 a de	0.89 b d
	Cane	0.84 a f	0.93 a d	0.77 a fg	0.75 a fg	0.72 a g	0.74 a g	0.76 a fg	0.74 c g

[z] T_{50} (temperature at which 50% of specimens are killed) was calculated by means of Spearman-Karber equation (Bittenbender and Howell, 1974).

[y] Moisture content expressed as g H_2O/ g tissue dry wt.

[x] Mean separation by Duncan's Multiple Range Test. Letters following values indicate significance within columns and letters below values indicate significance within rows. The same letter within a column or row indicates that respective values are not significantly different at p=.05.

Table 10. Correlation coefficients for moisture content vs. hardiness (T_{50}) for Experiment 3, 1975-76.

Tissue	Correlation coefficient
Primary bud	0.802 **
Secondary bud	0.638 **
Cane	0.048

** Significant at p=.01.

Table 11. Moisture content[z] of buds and canes of 'Concord' grapevines on 17 April, 1976 as affected by node position.

	Node position					
Tissue	2	4	6	8	10	12
Primary bud	0.92 ab[y] d	0.99 a cd	1.06 a cd	0.97 a cd	1.10 a c	1.03 a c
Secondary bud	0.93 a c	0.94 ab c	0.96 a c	0.97 a c	1.04 a c	1.06 a c
Cane	0.79 b c	0.83 b c	0.80 b c	0.78 b c	0.79 b c	0.79 b c

[z]Moisture content expressed as g H_2O/ g dry wt.

[y]Mean separation by Tukey's HSD at p=.05. Letters following values indicate significance within columns and letters below values indicate significance within rows. The same letter within a row or column indicates that respective values are not significantly different

Table 12. Analysis of variance of effects of node position on moisture content of buds and canes of 'Concord' grapevines. (Data presented in Table 11.)

Source	df	SS	MS	F value
Total	53	7737.05	- -	- -
Block	2	133.60	66.80	1.40 n.s.
Tissue (T)	2	4877.83	2438.91	51.02 **
Pri + Sec vs. Cane	1	4797.07	4797.07	100.03 **
Pri vs. Sec	1	80.76	80.76	1.69 n.s.
Node (N)	5	553.24	110.65	2.31 n.s.
Linear	1	345.90	345.90	7.23 **
Quadratic	1	7.04	7.04	0.16 n.s.
Cubic	1	4.74	4.74	0.09 n.s.
N x T	10	547.33	54.73	1.14 n.s.
Error	34	1625.05	47.80	- -

Table 13. Effect of pruning date and node position on the cane on percent of buds killed by freeze on 26 April, 1976. Injury evaluated 16 and 18 May.

Bud	Node no.	Pruning date, 1975-76 5 Dec	8 Jan	13 Feb	29 Feb	30 Mar	Unpruned
Primary	1-3	45 a[zy]	32 a	46 a	53 a	36 a	44 ab
		c	c	c	c	c	c
	4-6	65 a	49 a	69 a	62 a	66 a	38 b
		c	c	c	c	c	c
	7-9	63 a	51 a	59 a	62 a	54 a	65 ab
		c	c	c	c	c	c
	10-12	72 a	71 a	67 a	77 a	62 a	79 a
		c	c	c	c	c	c
Secondary	1-3	19 b	30 a	26 a	15 a	12 a	16 a
		c	c	c	c	c	c
	4-6	44 ab	51 a	42 a	38 a	20 a	24 a
		c	c	c	c	c	c
	7-9	63 a	64 a	54 a	56 a	40 a	19 a
		c	c	cd	cd	cd	d
	10-12	66 a	61 a	58 a	50 a	30 a	35 a
		c	c	c	c	c	c

[z]Values represent means of 2 replicates each comprising 20-30 observations.

[y]Mean separation by Duncan's Multiple Range Test. Letters following values indicate significance within columns and letters below values indicate significance within rows. The same letter within a row or column indicates that respective values are not significantly different at p=.05.

Table 14. Effect of pruning date in 1975-76 on percent buds swelling on 16 or 18 May following a freeze on 26 April.

Bud	Node no.	Pruning date, 1975-76					
		5 Dec	8 Jan	13 Feb	29 Feb	30 Mar	Unpruned
Primary	1-3	15 a[zy]	17 a	10 a	19 a	32 a	10 a
		cd	cd	d	c	c	d
	4-6	5 a	0 a	5 a	7 a	14 ab	15 a
		c	c	c	c	c	c
	7-9	3 a	4 a	2 a	4 a	12 b	7 a
		c	c	c	c	c	c
	10-12	3 a	4 a	0 a	3 a	16 ab	9 a
		c	c	c	c	c	c
Secondary	1-3	30 a	30 a	29 a	38 a	22 a	37 a
		c	c	c	c	c	c
	4-6	5 a	0 b	5 a	7 a	14 a	15 a
		c	c	c	c	c	c
	7-9	10 a	13 ab	12 a	9 a	34 a	35 a
		c	c	c	c	c	c
	10-12	4 a	15 ab	8 a	5 a	15 a	20 a
		c	c	c	c	c	c

[z]Values represent means of 2 replicates each comprising 20-30 observations.

[y]Mean separation by Duncan's Multiple Range Test. Letters following values indicate significance within columns and letters below values indicate significance within rows. The same letter within a row or column indicates that repsective values are not significantly different at p=.05.

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APPENDIX

Table A1. Number of buds and canes killed at all test temperatures for sample date 1, 14 Jan, 1975. Values are number killed of four observations, unless otherwise indicated. Only the data for temperatures with asterisks were used in computations.

		Tissue			
Pruning date	Test temperature	Primary bud	Secondary bud	Tertiary bud	Cane
14 Jan, 1975	Control	2	0	0	0
	-15*	1	1	1	0
	-20*	0	0	0	0
	-25*	4	4	4	4
	-30	4	4	4	4
	-35	4	4	4	4

Table A2. Number of buds and canes killed at all test temperatures for sample date 2, 13 Feb, 1975. Unless otherwise indicated, values are number killed of four observations. Only the data for temperatures with asterisks were used in computations.

Pruning date	Test temperature	Tissue			
		Primary bud	Secondary bud	Tertiary bud	Cane
14 Jan, 1975	Control	1	1	1	1
	-15*	0	0	1	0
	-20*	2	1	1	1
	-25*	0	0	0	4
	-30*	3	3	3	4
	-35	4	4	4	4
13 Feb, 1975	Control	0	0	0	0
	-15*	1	1	0	0
	-20*	3	2	1	1
	-25*	0	1	0	2
	-30*	4	4	4	4
	-35	4	4	4	4

Table A3. Number of buds and canes killed at all test temperatures for sample date 3, 27 Feb,1975. Unless otherwise indicated, values are number killed of four observations. Only the data for temperatures with asterisks were used in computations.

Pruning date	Test temperature	Tissue			
		Primary bud	Secondary bud	Tertiary bud	Cane
14 Jan, 1975	Control	1	0	1	1
	-15*	1	0	0	0
	-20*	3	1	1	3
	-25*	4	4	4	3
	-30	4	4	4	4
	-35	4	4	4	4
13 Feb, 1975	Control	0	0	0	0
	-15*	0	0	0	0
	-20*	0	1	0	1
	-25*	4	2	0	1
	-30	4	4	4	4
	-35	4	4	4	4
27 Feb, 1975	Control	0	0	0	0
	-15*	2	1	1	0
	-20*	1	0	0	2
	-25*	4	4	4	4
	-30	4	4	4	4
	-35	4	4	4	4

Table A4. Number of buds and canes killed at all test temperatures for sample date 4, 27 Mar, 1975. Unless otherwise indicated, values are number killed of four observations. Only the data for temperatures with asterisks were used in computations.

Pruning date	Test temperature	Tissue			
		Primary bud	Secondary bud	Tertiary bud	Cane
14 Jan, 1975	Control	3	1	0	0
	-10*	1	0	0	0
	-15*	1	0	0	2
	-20*	0	0	0	2
	-25	4	4	4	3
	-30	4	4	4	4
13 Feb, 1975	Control	2	1	1	0
	-10*	1	0	1	0
	-15*	0	0	0	0
	-20*	2	1	1	1
	-25	4	4	4	4
	-30	4	4	4	4
27 Feb, 1975	Control	0	0	0	0
	-10*	0	0	0	0
	-15*	0[z]	0[z]	0[z]	0
	-20*	0[z]	0[z]	0[z]	0
	-25	4	4	4	4
	-30	4	4	4	4
27 Mar, 1975	Control	0	0	0	1
	-10*	0	0	0	0
	-15*	0	1	0	0
	-20*	1	1	1	2
	-25	4	4	4	4
	-30	4	4	4	4

[z]Number killed of three observations.

Table A5. Number of buds and canes killed at all test temperatures for sample date 5, 17 Apr, 1975. Unless otherwise indicates, values are number killed of four observations. Only the data for temperatures with asterisks were used in computations.

Pruning date	Test temperature	Tissue			
		Primary bud	Secondary bud	Tertiary bud	Cane
14 Jan, 1975	Control	0	0	0	0
	-11*	1	0	0	0
	-15*	2	1	1	1
	-17.5*	3	3	3	2
	-20	4	4	4	4
	-25	4	4	4	4
13 Feb, 1975	Control	0	0	0	0
	-11*	1	0	0[z]	1
	-15*	1	1	0	1
	-17.5*	2	2	2	1
	-20	4	4	4	4
	-25	4	4	4	4
27 Feb, 1975	Control	0	0	0	1
	-11*	0	0	0	0
	-15*	0	0	0	0
	-17.5*	1	1	1	1
	-20	4	3	4	4
	-25	4	4	4	4
27 Mar, 1975	Control	0[z]	0[z]	0[z]	0
	-11*	0	1	0	0
	-15*	0	0	0	1
	-17.5*	0	1	0	1
	-20	4	4	4	4
	-25	4	4	4	4
17 Apr, 1975	Control	0	0	0	1
	-11*	1	1	1	0
	-15*	2	2	2	2
	-17.5*	2	2	2	2
	-20	4	4	4	4
	-25	4	4	4	4

[z]Number killed of three observations.

Table A6. Number of buds and canes killed at all test temperatures for sample date 6, 8 May, 1975. Unless otherwise indicated, values are number killed of four observations. Only the data for temperatures with asterisks were used in computations.

Pruning date	Test temperature	Tissue			
		Primary bud	Secondary bud	Tertiary bud	Cane
14 Jan, 1975	Control	0	0	0	0
	0*	1	0	0	0
	-5*	1	0	0	0
	-7.5*	1	1	0	2
	-10*	4	4	3	2
	-15	4	4	4	4
13 Feb, 1975	Control	0	0	0	0
	0*	0	0	0	0
	-5*	1	0	0	1
	-7.5*	0	0	0	0
	-10*	4	4	4	4
	-15	4	4	4	4
27 Feb, 1975	Control	0	0	0	0
	0*	0	0	0	0
	-5*	0	0	0	0
	-7.5*	1	0	0	0
	-10*	3	3	2	2
	-15	4	4	4	4
27 Mar, 1975	Control	0	0	0	0
	0*	0	0	0	0
	-5*	0	0	0	1
	-7.5*	0	0	0	0
	-10*	3	3	3	1
	-15	4	4	4	4
17 Apr, 1975	Control	0	0	0	0
	0*	0	0	0	0
	-5*	0	0	0	0
	-7.5*	0	0	0	0
	-10*	3	3	3	1
	-15	4	4	4	4
8 May, 1975	Control	0	0	0	0
	0*	0	0	0	0
	-5*	0	0	0	0
	-7.5*	1	1	1	1
	-10*	3	3	3	1
	-15	4	4	4	4

Table A7. Number of buds and canes killed at all test temperatures for sample date 1, 5 Dec, 1975. Values are number killed of twelve observations unless otherwise indicated. Only the data for temperatures with asterisks were used in computations.

Pruning date	Test temperature	Tissue			
		Primary bud	Secondary bud	Tertiary bud	Cane
5 Dec, 1975	Control	0	1	0	0
	-10*	0	0	0	0
	-15*	0	0	0	1
	-17.5*	1	2	0	9
	-20*	0	0	0	7
	-25*	8	3	4	12

Table A8. Number of buds and canes killed at all test temperatures for sample date 2, 8 Jan, 1976. Values are number killed of twelve observations unless otherwise indicated. Only the data for temperatures with asterisks were used in computations.

Pruning date	Test temperature	Tissue			
		Primary bud	Secondary bud	Tertiary bud	Cane
5 Dec, 1975	Control	4[z]	1[z]	0[z]	0[z]
	-22.5*	3	0	0	0
	-25*	5	2	2	5
	-27.5*	12	12	7	9
	-31	12	12	12	12
	-35	12	12	12	12
8 Jan, 1976	Control	1[z]	1[z]	1[z]	0[z]
	-22.5*	4	0	0	0
	-25*	4	3	2	1
	-27.5*	12	11	5	5
	-31	12	12	12	9
	-35	12	12	12	12

[z]Number killed of eleven observations.

Table A9. Number of buds and canes killed at all test temperatures for sample date 3, 13 Feb, 1976. Values are number killed of twelve observations unless otherwise indicated. Only the data for temperatures with asterisks were used in computations.

Pruning date	Test temperature	Tissue			
		Primary bud	Secondary bud	Tertiary bud	Cane
5 Dec, 1975	Control	3[z]	3[z]	0[z]	0[z]
	-15*	5	4	2	0
	-20*	5	5	2[y]	1
	-23*	7	4	2	0
	-25*	10	9	5	8
	-30	12	12	12	12
8 Jan, 1976	Control	1[z]	1[z]	1[z]	0[z]
	-15*	4	0	0	0
	-20*	4	3	2	1
	-23*	12	11	5	5
	-25*	12	12	12	9
	-30	12	12	12	12
13 Feb, 1976	Control	5	1	0	0
	-15*	2	1	0	0
	-20*	3[y]	1[y]	1[y]	1[y]
	-23*	4	3	1	1
	-25*	9	3	4	5
	-30	12	12	12	12

[z]Number killed of 10 observations.

[y]Number killed of 11 observations.

Table A10. Number of buds and canes killed at all test temperatures for sample date 4, 29 Feb, 1976. Values are number killed of 24 observations unless otherwise indicated. Only the data for temperatures with asterisks were used in computations.

Pruning date	Test temperature	Tissue			
		Primary bud	Secondary bud	Tertiary bud	Cane
5 Dec, 1975	Control	3[z]	1[z]	0[z]	0[z]
	-15*	12	2	1	2
	-20*	8	4	2	4
	-25*	22	21	20	22
	-30	24	24	24	24
	-35	24	24	24	24
8 Jan, 1976	Control	10[y]	5[y]	4[y]	0[y]
	-15*	8	4[x]	4	2
	-20*	9[x]	7[x]	5	5
	-25*	23	22	23	19
	-30	24	24	24	24
	-35	24	24	24	24
13 Feb, 1976	Control	7[w]	6[w]	2[w]	0[w]
	-15*	9[x]	4[x]	4[x]	0
	-20*	4[x]	1[x]	2[x]	4
	-25*	20	18	20	22
	-30	24	24	24	24
	-35	24	24	24	24
29 Feb, 1976	Control	9	4[x]	2	0
	-15*	5[x]	0[x]	0	0
	-20*	12[x]	7[x]	3[x]	1
	-25*	24	23	21	19
	-30	24	24	24	24
	-35	24	24	24	24

[z]Number killed of 16 observations.

[y]Number killed of 19 observations.

[x]Number killed of 23 observations.

[w]Number killed of 20 observations.

Table A11. Number of buds and canes killed at all test temperatures for sample date 5, 30 Mar, 1976. Values are number killed of 24 observations unless otherwise indicated. Only the data for temperatures with asterisks were used in computations.

Pruning date	Test temperature	Tissue			
		Primary bud	Secondary bud	Tertiary bud	Cane
5 Dec, 1975	Control	9	1[z]	2	0
	-5*	14	7	3	4
	-10*	11[z]	5[z]	6[z]	3
	-15*	20[z]	16[z]	13[z]	9
	-20	24	24	24	24
	-25	24	24	24	24
8 Jan, 1976	Control	10	4	2	0
	-5*	16	4	3	0
	-10*	14	7	5	3
	-15*	17	16	11	5
	-20	24	24	23	24
	-25	24	24	24	24
13 Feb, 1976	Control	9	3	3	0
	-5*	10	4	0	0
	-10*	12	8	1	1
	-15*	12	10	9	4
	-20	24	24	24	24
	-25	24	24	24	24
29 Feb, 1976	Control	10	3	2	0
	-5*	11[z]	6[z]	2[z]	1
	-10*	9[z]	9[z]	3[z]	1
	-15*	14	10	9	5
	-20	24	24	24	24
	-25	24	24	24	24
30 Mar, 1976	Control	7	5	1	1
	-5*	10	6	1	0
	-10*	8[z]	5	1	0
	-15*	14[z]	10	9	5
	-20	24	24	24	24
	-25	24	24	24	24

[z]Number killed of 23 observations.

Table A12. Daily temperature maxima and minima (^{o}C) for spring 1976 at the Michigan State University Horticultural Research Farm.

	Feb.		Mar.		Apr.		May	
Day	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
1	-3	-5	11	-3	17	1	18	6
2	-2	-25	0	-2	6	1	18	4
3	-9	-21	-1	-1	13	-3	12	1
4	-2	-8	6	4	17	-2	6	0
5	-4	-12	15	3	7	-3	16	11
6	-7	-12	11	-8	14	3	25	3
7	-7	-13	2	-2	16	1	6	0
8	0	-12	2	-9	12	-3	14	-1
9	1	-13	2	-4	7	-6	23	7
10	7	-10	7	-4	12	1	22	10
11	9	0	3	-6	17	-1	23	8
12	3	-4	4	-1	4	-6	14	1
13	9	2	14	-4	9	-3	16	2
14	3	-9	0	-6	17	6	23	14
15	3	-1	6	-4	24	16	23	15
16	16	-1	2	-3	27	15	19	16
17	3	0	2	-11	28	16	21	12
18	2	1	-1	-9	27	14	14	4
19	15	-1	13	9	27	14	16	2
20	4	-3	21	12	21	10	18	8
21	9	-1	19	1	14	8	24	9
22	1	-4	1	-9	21	8	21	4
23	-2	-12	4	-1	18	3	17	5
24	2	-9	12	7	16	5	16	3
25	14	7	21	4	13	5	17	6
26	17	6	16	5	6	-3	18	6
27	14	0	23	12	2	-2	20	6
28	17	1	4	-3	8	-1	23	11
29	11	1	12	2	14	0	22	15
30			13	4	15	2	18	14
31			23	4			23	15