Fruit Quality Variation and Freezing Resistance Mechanisms in Northern Clones of *Vitis riparia* Michx.

A THESIS

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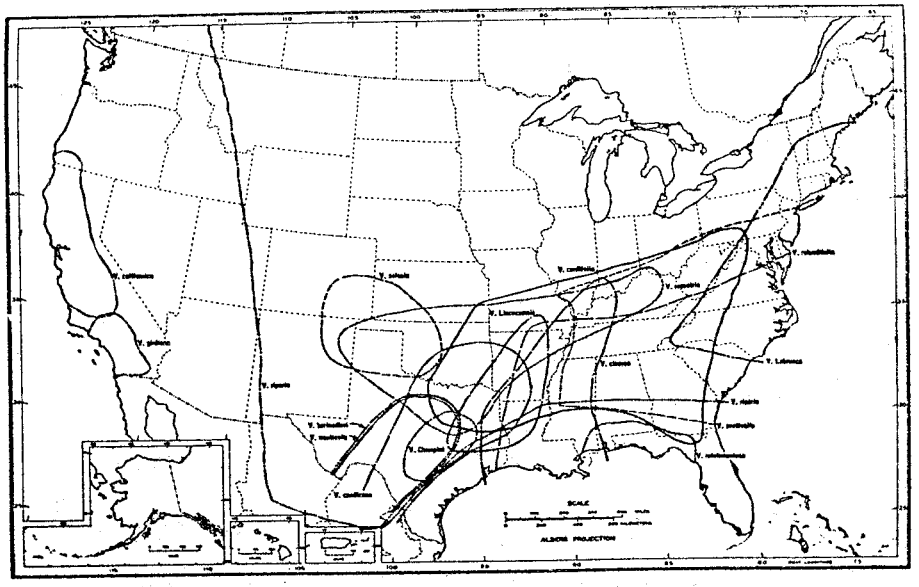
INTRODUCTION

Compared to other areas of the world, North America is a vast storehouse of diversity for the genus Vitis. Taxonomists are not in full agreement concerning the systematics of this genus, but the two most authoritative texts, Bailey(10) and Munson(104), list the number of North American species at 30 and 26, respectively. In contrast, Europe and Asia combined have 10-15 species of Vitis(43). It was in fact the grapes of the North American continent, and not the Indians or wild game, that most impressed the first explorers. The Norse explorer, Lief the Lucky, son of Eric the Red, is said to have landed in New England in about 1000 A.D., and was much impressed by the rich growth of native grapes(64,134). He dubbed the New World "Wineland the Good."

After approximately 200 unsuccessful years of attempts to grow the Old World grape, Vitis vinifera L., growers turned their attention to the native North American species(64, 174). Bailey (10) relates that numerous botanical classifications of the genus Vitis were published in the early and mid-1800's. However, it wasn't until 1899 that an accurate systematic evaluation of the qualities of the North American Vitis species was published(103).

In spite of the great diversity in North America, the only grape species present in the upper Midwest is the Riverbank or Frost grape, Vitis riparia Michx (V. vulpina L.)(Fig. 1). This

Figure 1. Range of some North American species of Vitis. From Winkler(181). (Note that he shows no northern boundary for the species V. riparia. This is incorrect; the northern boundary is as shown in Fig. 2)



The distribution of the important and interesting native species of *Vitis* in the United States.

species has long been considered of great promise for breeding cold hardy cultivars(64,103). Munson(104) wrote in reference to V. riparia: "There is all the great Northwest region, consisting of Iowa, Wisconsin, Minnesota, Nebraska, Dakota and parts of Wyoming and Montana, that has a superior climate for extra early grapes, if they can be obtained hardy enough to endure the winters. It is a region free from mildew and rot. There is nothing to prevent it from becoming a great grape country, for the material certainly exists which can be developed into very excellent varieties. All the streams of that territory have growing along their borders an abundance of wild V. vulpina . . ."

Historically, the most hardy grape cultivars have been of V. riparia parentage. Hedrick(64) lists as among the most hardy cultivars 'Beta,' 'Bacchus,' 'Clinton,' 'Elvira,' 'Ironclad,' and 'Janesville,' all of which are partially or pure V. riparia. 'Beta,' 'Clinton,' and 'Elvira' are still regarded as among the most hardy cultivars in New York(148).

In addition to cold tolerance, V. riparia has other desirable characteristics: the species has great resistance to the phylloxera root louse(Dactylasphaera vitifoliae Shimer), is easy to root and graft, and ripens early(64,104); the fruit has a good flavor(Munson(104) describes the fruit as "juicy, pure and vinous.").

The author is interested in V. riparia primarily as a parent for breeding cold hardy wine grapes. Cultivars of V. riparia

parentage, such as 'Clinton' and 'Elvira,' have also been regarded as having acceptable wine quality(64). Olmo(109) regards V. riparia as one of the best North American grape species for breeding wine grapes, because the characteristic flavor is easily ameliorated in breeding.

Two quite successful wine cultivars of V. riparia parentage are 'Baco Noir' and 'Foch.' They have recently been planted in large acreage in eastern grape growing regions such as New York (106). Wines from these cultivars have scored well in a number of wine research programs(16,30,39,50,71,123), and they are recommended for planting over a wide area of North America (6,8,11,20,42,45,174)[1]. In 1973, a 'Foch' wine tied for first place in the First National Amateur Winemaker's Competition, in competition with many wines made from V. vinifera cultivars(102). In 1975, prices paid for these cultivars by eastern wineries were as much as four times as great as the price paid for 'Concord' grapes(7).

The present situation in Minnesota

In his treatise on the climate of Minnesota, Amdur(2) found that the lack of a sufficiently long and/or warm growing season

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1. Brooks and Olmo(23) give the parentage of these cultivars as follows: 'Baco Noir' = V. vinifera cv. Folle blanche X V. riparia; 'Foch' = (V. riparia X V. rupestris) X V. vinifera cv. Goldriesling. While neither is hardy in Minnesota(65), they illustrate the point that wine from grapes of V. riparia origin can be widely accepted. These hybrids were developed in France, and are part of the group of grape culitvars commonly referred to as "French hybrids"(174).

has not been the limiting factor in viticultural expansion in this region. He cited the very cold winters as being the limiting factor. Indeed, the only cultivar recommended as fully hardy in the state is 'Beta'(65). This cultivar is of parentage V. riparia X V. labrusca cv. Concord(116), and is in the author's experience of poor quality for wine.

Recently in Minnesota there has been a surge of interest among amateurs in the French hybrid wine grapes(48), but growers must bury these grapes for winter protection. Hansen(61) labelled the growing of fruits which require winter protection "horticulture on crutches," an apt description. Labor requirements for growing grapes in the eastern U.S. have been calculated to be between 52 and 84 hr/acre/yr(73,107,152). Covering grapes for winter protection requires approximately 35 hr/acre/yr(24), thereby increasing labor costs by 42 to 67 percent. Even in Russia, where machines have been developed that can bury 12-15 acrea of vines per day, the concensus of opinion among research workers is that winter protection will never be truly economic; developing winter hardy cultivars is regarded as the only long term solution(40). It appears highly unlikely that wine grape growing in Minnesota will ever be economically competitive unless high quality cultivars are developed which are fully hardy.

The intent of this thesis is to report on initial studies serving as a basis for the development of cold hardy wine grapes for the upper Midwest. Two areas of research were explored:

fruit quality variation in wild populations of V. riparia, and mechanisms of cold hardiness of this species. Accordingly, the remainder of the thesis is divided into two sections.

Part A:

FRUIT QUALITY VARIATION

LITERATURE REVIEW

The Riverbank Grape, *Vitis riparia*, has the largest range of any of the native species of *Vitis*(Fig. 1,2). Intraspecific variation is quite common in species with such wide ranges, and particularly in species which are cross-pollinated and therefore highly heterozygous(154).(All native grape species are dioecious (10,64,104) and therefore cross-pollinated.) It seems likely that *V. riparia* should also exhibit variation. However, documentation of intraspecific variation in North American *Vitis* species is quite sparse.

In Hedrick's descriptions of the native grape species(64), most are described as being variable in at least one trait. It is interesting to note that *V. riparia* is described by Hedrick as the most variable of species.

Munson(104) describes the North American *Vitis* species in much greater detail than does Hedrick, but the characters exhibiting significant variation are essentially the same. Munson, however, ascribes some of the observed variations to introgression with other species. With the exception of *V. rotundifolia*, which has 40 chromosomes, all *Vitis* species have 38 chromosomes and are completely interfertile(43). The species are apparently separated by differences in time of blooming(104); there is some overlap in this character and this would explain Munson's observation of natural hybrids.

Munson(104) also noted that species having wide distributions "vary greatly among their individual vines from different regions in nearly all their characters, hence vines for experimental purposes taken at random can promise little in development toward improvement of varieties for cultivation."

Bailey(10) also felt that there was much intraspecific variability, though, like Munson and Hedrick, he offered no documentation for this. He felt that most of the so-called hybrids which earlier taxonomists referred to could be explained on the basis of intraspecific variation. He stated that because of this variation, he found it difficult to construct an identification key for the genus. Bailey remarked on the wide distribution of V. riparia and said that because of this the species "presents many aspects," though he did not elaborate.

In his detailed work with V. cinerea, Barrett(13) noted great variation in cluster size and shape, vine productivity and vigor, and sugar and acid content of the fruit. In another study(12) he noted intraspecific variations in black rot resistance in nine North American grape species. V. riparia was found to be the most variable of the species, some vines being entirely free of infection and others being heavily infected in both foliage and fruit. Clones of this species derived from northern sources were more susceptible than were clones from southern areas.

Kliewer(80) measured the concentrations of tartrates,

malates, glucose and fructose in 26 species of Vitis growing at Davis, California. Of those species which were represented by more than one clone, intraspecific variation was evident for the characteristics measured. V. riparia was represented by three clones: total acidity varied from .58% to 1.62%, pH from 3.54 to 3.87 and percent soluble solids from 22.7% to 31.5%. V. riparia was, relative to the other species, very high in both percent soluble solids and total acidity.

METHODS AND MATERIALS

Fruit collection: Fruit was collected from vines of V. riparia growing in their native habitat(Fig. 3). The principal area of collection was along the Minnesota River between Belle Plain, Minnesota, and Shakopee, Minnesota(Fig. 4). Fruit was collected only from vines growing on the north bank(ie. facing south), so as to minimize variations in sunlight exposure. Selection of particular vines was randomized as much as possible so as to represent a cross-section of the population. However, vines which were heavily diseased were not sampled.

Fruit was also collected from vines growing at the Canadian Department of Agriculture Research Station at Morden, Manitoba (Fig. 5). These vines had originally been grown from seed collected in the Riding Mountains area of Manitoba(86), which is said to be the northernmost population of this species(35,104).

It is known that plants on the periphery of a species' range often represent extremes of types(38,66,87,154), and this is why the Morden population was sampled.

From six to fifteen representative clusters were harvested from each vine, and placed in sealed plastic bags until the juice was extracted (2-48 hours). The clusters were sized visually into three classes: small(less than 5 cm. in length), medium(5-10 cm.), and large(greater than 10 cm.).

The berries were stripped from the clusters, then crushed and squeezed by hand through several layers of cheesecloth. This seemed to be effective since there appeared to be very little juice left in the pulp after squeezing. The juice was strained once more through cheesecloth, placed in labelled test tubes, sealed with a cork and stored in a freezer at approximately -23^{o}C until analyzed(1-10 days).

Analysis: The frozen juice samples were thawed quickly in a hot water bath, decanted and analyzed. Percent soluble solids was determined using a hand refractometer(American Optical Model No. 10430). Total acidity was determined by titrating 5 ml. of juice to an endpoint of pH 8.2, using a 0.1 N sodium hydroxide solution(3). Juice pH was determined with a glass electrode pH meter.

RESULTS AND DISCUSSION

General observations: The values for sugar content(soluble solids), pH, total acidity and cluster size are shown in Table 1. In general, there appears to be considerable variability in fruit quality of V. riparia(Fig. 6,7). There appeared to be little variability in berry size among clones sampled, all berries being quite small(approximately 5 mm. in diameter or less). The total acidity ranged from 2.23% to 4.35%. These acid contents are considerably higher than those reported for other species of Vitis(29,80,92,137), and for interspecific hybrids(6,50,71,136). This agrees with the taxonomic literature which describe V. riparia as being a species of high acidity(10,64,104).

Soluble solids content ranged from 13.8° Brix to 26.5°, with the best clones exceeding that reported fro most other species (29,92,137), and for interspecific hybrids(6,39,50,71,123,136). These data agree with Kliewer(80), who reported that the clone which exhibited the highest sugar content (31.5° Brix) was a vine of V. riparia.

Figure 8 illustrates the frequency of three cluster size classes in clones sampled. Although it would have been better to have determined the numerical average cluster size of each clone, rather than assign each to one of three classes, the data show that, in general, V. riparia has moderately small to small clusters. This agrees with taxonomic descriptions of the species

(10,64,104). However, some clones have clusters noticeably larger than average(Fig. 9,10). These clones would be the most desirable for breeding purposes, providing their other characteristics(sugar and acid content, productivity, disease resistance) were satisfactory.

It is interesting to note that, within a population, there is considerable variation in pH at any given level of total acidity(Fig. 11,12). This is due to variations in buffering capacity of the juice, and is a characteristic of other species as well as V. riparia(5). In selecting clones of V. riparia for breeding wine grapes, it would probably be best to avoid those which have a high pH. A low pH in wines is generally desirable because it inhibits autolysis of yeast cells(enzymatic self-destruction), aids in extracting pigments from the skins of red grapes, and inhibits spoilage bacteria(4,5). The cultivar Foch, for example, often has too high a pH, even though its acid level is generally high. In a number of studies, spoilage of, and low sensory scores given to, 'Foch' wines have been attributed to its high pH(15,16,30,39,78).

Significance of 1976 Morden selections: In 1976, the number of clones which fruited at Morden was far fewer than in 1975. This is most likely due to the severe frost experienced on the night of May 17(Fig. 13). Weather data from the Morden station show a noticeable warming trend beginning about May 8, 1976. Prior to the hard frost, there were 9 consecutive days when the

mean temperature was above 50° F.

This temperature has significance in that grapevines begin growth when mean daily temperatures reach this level(181). Amdur(2) has shown that five to seven days of mean temperatures above 50° were required to produce active bud growth of 'Beta' vines. Since there were nine days of mean temperatures in excess of this threshold level(Fig. 13), and since pure V. riparia vines are known to begin growth earlier than any other native Vitis species(10,104), the Morden vines were most likely in active growth by May 16.

Damage to growing vine tissue begins when the temperature drops to about 30°F(181,184). A temperature of 26° or lower for a few hours will kill all green shoots, flower clusters and even partially opened buds(181). Temperature minima recorded on the night of May 17 were 30° (shelter value) and 26° at ground level. These minima are indicative of a radiation type frost, during which the foliage temperature may be considerably colder than the air temperature(2).

Therefore it seems quite likely that the damage at Morden in 1976 was due to the late frost which followed the period of nine days during which the mean temperature was above 50°F. Those clones which did bear fruit in 1976 must have some mechanism for avoiding or tolerating late frosts. The characteristic of late budding would allow avoidance of late frosts, and the characteristic of fruitful secondary buds(buds which will grow if the

primary bud or shoot is killed) would be a frost tolerance mechanism(Fig. 14). Either character would be desirable for breeding grapes for northern areas.

Minnesota and Manitoba populations compared: A comparison of fruit quality characteristics of the two populations reveals an interesting relationship. In Table 2 are shown the mean values of percent soluble solids and total acidity of the two populations. It is apparent that at the time of harvest, the fruit from the two populations was at approximately the same stage of maturity. However, the Manitoba population required substantially fewer degree days and a shorter growing season to reach this state of maturity.

Such a result is not surprising; the growing season at the site of origin of the Manitoba clones is much shorter than the growing season in Minnesota(Fig. 15,16). The selection pressure for early ripening in Manitoba is much stronger than in Minnesota.

One complicating factor in this comparison is that the two populations were grown at different sites. The daylength during the growing season at Morden is longer than in Minnesota, and it is unclear how this would affect the relationship. However, the evidence does seem to indicate that more northerly clones of V. riparia ripen earlier than do local Minnesota clones.

Such variation in photoperiodic response is not uncommon in woody plant species. Timing of growth cessation has been shown to be inversely correlated with latitude of origin and/or length

of growing season in Populus balsamifera L. and P. trichorcarpa (113), P. deltoides Marsh.(79), Acer rubrum L.(162), P. tremuloides Michx.(168), Picea mariana(Mill.) BSP(166), and Juglans nigra L.(183). It has been shown that cessation of active growth is necessary for cold acclimation in the fall(72,90,97,153,177). Therefore, it may be expected that plants from northern areas, or from areas with a short growing season, will be somewhat more hardy than those of the same species of more southern origin or from areas with a longer growing season(This would be particularly true if critical temperatures occur early in the acclimation process.). This relationship has been shown in a number of woody plants. In Acer rubrum L.(162), Gleditsia triacanthos L.(32), Fraxinus americana L.(182), Pinus palustris(1), Pinus strobus(95), P. resinosa Ait.(14), P. taeda L.(101), Quercus rubra L.(47), and in Pseudotsuga menziesii, Thuja plicata, and Tsuga heterophylla(145), hardiness has been shown to be inversely correlated to length of growing season at site of origin. Quamme, et al(131) showed that native blueberry, Vaccinium angustifolium Ait., from northern Minnesota acclimated earlier than clones from Michigan, although both clones were equally hardy in midwinter. Smithberg and Weiser(153) found that when clones of Cornus stolonifera Michx. from widely divergent areas were grown at one location, acclimation occurred earliest in those clones originating in areas of shortest growing seasons. However, as in the case of blueberry, all clones were equally hardy by midwinter.

More direct evidence exists that latitudinal variation in acclimation occurs in V. riparia. Dorsey(41) reported that the foliage of V. riparia clones collected in central Iowa and northward was more resistant to fall frosts than clones of more southern origin. In his table-grape breeding work, Peterson(115) obtained earlier ripening progeny, and progeny of slightly greater hardiness, when V. riparia clones from northern North Dakota were used as the hardy parent, as compared to South Dakota V. riparia parents. Potapenko and Kostriki(124) obtained similar results using their native species V. amurensis as the hardy parent. As grown in southern Quebec, V. riparia clones of Manitoba origin were earlier ripening than clones of local origin(170). Also, the leaves of the Manitoba clones naturally senesced before the first fall frost in nearly all seasons; those of local origin did not.

The genetic potential for early acclimation is a very important character for developing cold hardy cultivars(77,82,153,160, 169). Therefore, the earlier ripening Manitoba V. riparia ecotype would probably be the most valuable source of hardiness in developing cold hardy grape cultivars.

Mechanism and significance of ecotypic variation: The mechanism of variation between ecotypes of a species has been most clearly demonstrated by the group at Stanford. In general, differences between races of a species are determined by a system of genes, each having minor but additive effects(37,38,67). The

multiple gene nature of these characteristics allows flexibility in adapting to many small variations in habitat(37).

When different races of a species are crossed, "ecotypic heterosis" often occurs. Clausen and Hiesey(37) explained that "genes having additive, subtractive, and complementary effects on a character are frequently carried by separate races of the species and cause transgressive segregation in interracial crosses. In the case of physiological differences, such segregation may far exceed the limits found in the parental races." For example, some F_2 progeny of the Potentilla glandulosa cross of "Coastal X Alpine" ecotypes, surpassed the Alpine ecotype in both vigor and frost hardiness(38).

This phenomenon has horticultural as well as ecological significance. It has ecological significance because "in nature there is a backlog of unutilized evolutionary resources from which races capable of fitting into many new environments could be synthesized. Hybrids from such(interracial) crossings are also often superior to their parents in their ability to succeed in a wide range of environments."(38).

Interracial crosses have horticultural significance because the variability expressed by the progeny of such crosses may far surpass that in wild populations of the species(37,38,66). Thus many new recombinants not found in either ecotype may be selected.

Therefore it would seem that, rather than simply selecting for breeding purposes the best individual clones in wild popula-

tions of V. riparia, crossing of ecotypes might yield some combinations superior to the best individuals in each population. This procedure, sometimes referred to as "semi-domestication" of the wild species, is thought to have been an early method of improvement in many of our crop plants(9). The interracial crosses occurred accidentally as early Man moved from camp to camp, taking seeds of his crop with him.

Limitations of this study: When studying variations in particular traits among individuals in any species, a commonly employed technique is the uniform environmental plot(14,38,47, 67,101,153,162). In this procedure, individuals from throughout a species' range are grown at one site, thereby minimizing environmental effects on the traits measured.

Due to time and space constraints, the uniform environmental plot was not employed in this study. Therefore, the variability due to environment and technique was not measured. The variation due to technique is most likely minimal since the refractometer has an accuracy of $\pm.2^{o}$, and the pH meter $\pm.02$, but the inability to separate environmental variability from genetic variability is a real limitation in this type of study.

Those clones which appeared poor in fruit quality cannot necessarily be discarded as genetically inferior, since the environmental variability could not be measured. However, some of those which appeared superior in fruit quality for their time of harvest(eg. clones 14,35,74,89) may be expected to perform at

least as well, or better, when grown under standard vineyard conditions.

CONCLUSIONS

1. The Vitis riparia populations sampled can be characterized as moderately high to high in soluble solids and total acidity, with generally small clusters and berries.

2. There was considerable variation in percent soluble solids, total acidity, pH and cluster size in the populations sampled. However, because of the sampling methods employed, the contribution of technique and environmental variability to the total variability could not be determined. Nevertheless, those selections which were superior in their fruit quality characteristics while growing in the wild may be expected to perform at least as well under standard vineyard conditions.

3. In using V. riparia for breeding hardy wine grapes, juice pH should be considered an important fruit quality characteristic. Low pH clones are probably the most desirable.

4. In the V. riparia populations studied, many clones showed soluble solids sufficiently high for wine, but in all cases, the total acidity was too high. Therefore, it is necessary to breed for lower total acidity, either by interspecific hybridization with low acid types, or by making intraspecific crosses and selecting for lower acidity.

5. There apparently exist in the Morden V. riparia population, genes for tolerance or avoidance of late spring frosts, or for productive secondary buds. Any of these characters would be valuable for breeding grapes for northern areas.

6. Evidence indicates that in V. riparia there may be a correlation between length of growing season at the site of origin and fruit ripening date. This variation may be useful in two ways: a) Because the northern clones ripen earliest, they would be the most desirable V. riparia clones to use when hybridizing with the long-season European, V. vinifera, wine grapes. b) Based on work done with other species(37,38,66,67), it may be expected that useful recombinant types, not found in either parental population, will be obtained by crossing ecotypes of V. riparia. Since transgressive segregation of characters is frequent when making such crosses(37,38), it should be possible to obtain progeny with better combinations of fruit quality characters and earliness than are found in natural populations.

Table 1. Fruit quality of *V. riparia* clones sampled in 1975 and 1976.

Clone+	Origin	Date sampled	Soluble solids (°Brix)	Total acidity (% as tartaric)	pH	Cluster size*
1	Minn.	8/21/75	20.4°	3.15%	2.65	S
2	"	9/7/75	19.5	4.30	2.61	S
3	"	8/27/75	15.4	--	--	S
4	"	"	13.8	--	--	M
5	"	9/8/75	18.8	3.54	2.71	M
6	"	"	21.0	3.57	2.85	M
7	"	9/4/75	19.8	3.33	2.71	M
8	"	"	18.5	3.44	2.93	-
9	"	"	--	2.48	2.99	-
10	"	"	--	--	2.94	-
11	"	"	--	--	3.02	-
12	"	"	16.4	3.02	3.08	-
13	"	"	18.8	3.82	2.77	-
14	"	"	19.5	3.10	2.88	L
"	"	9/23/75	25.0	2.76	2.89	L
15	"	9/12/75	16.5	2.95	2.73	S
16	"	"	20.4	4.16	2.89	S
17	"	"	--	--	2.72	S
18	"	"	15.8	2.98	2.76	M
19	"	"	18.2	3.92	2.63	M
20	"	"	18.7	2.39	2.85	M
21	"	"	16.2	3.75	2.71	S
22	"	"	15.2	3.55	2.60	M
23	"	"	17.6	3.51	2.52	M
24	"	"	19.0	3.20	2.69	M
25	"	"	22.2	2.24	2.81	M
26	"	"	18.8	3.41	2.67	S
27	"	"	16.2	--	2.98	S
28	"	"	18.0	4.23	2.48	L
29	"	9/13/75	21.4	3.92	2.66	S
30	"	"	16.8	2.48	2.81	M

Table 1(Con't.)

31	Minn.	9/13/75	19.7	2.51	2.65	S
32	"	"	21.6	4.13	2.55	M
33	"	"	19.5	3.89	2.51	S
34	"	"	18.1	4.16	2.57	S
35	"	"	23.0	2.23	3.03	L
36	"	"	20.2	3.10	2.77	M
37	Manitoba	9/15/75	20.8	3.98	2.75	M
38	"	"	18.5	3.83	2.94	M
39	"	"	21.0	4.00	2.82	M
40	"	"	16.0	3.62	2.70	M
41	"	"	18.2	2.79	3.05	M
42	"	"	17.1	2.53	3.36	M
43	"	"	18.0	2.79	3.10	M
44	"	"	17.5	3.45	3.11	S
45	"	"	18.9	3.59	2.84	S
46	"	"	17.8	3.17	2.71	S
47	"	"	15.2	4.15	2.75	M
48	"	"	19.6	3.58	2.85	S
49	"	"	18.2	3.20	2.93	S
50	"	"	16.0	3.03	2.97	S
51	"	"	18.3	3.97	2.80	M
52	"	"	23.0	3.86	2.75	S
53	"	"	18.6	3.31	2.91	M
54	"	"	15.2	3.59	2.78	M
55	"	"	19.0	4.14	2.70	M
56	"	"	17.6	3.07	2.98	S
57	"	"	17.0	3.17	2.67	S
58	"	"	18.0	3.21	2.59	M
59	"	"	19.5	3.27	2.75	S
60	"	"	18.2	4.04	2.60	M
61	"	"	16.5	3.21	2.63	M
62	"	"	15.6	3.28	2.94	M
63	"	"	18.8	3.94	2.65	M
64	"	"	19.0	3.07	2.77	S
65	"	"	18.8	3.97	2.70	S
66	"	"	17.0	4.35	2.46	M
67	"	"	20.8	3.45	2.65	S
68	"	"	18.2	3.73	2.58	S

Table 1(Con't.)

69	Manitoba	9/15/75	20.8	3.80	2.75	M
70	Minn.	9/19/75	19.0	3.26	2.86	M
71	"	"	18.8	2.99	2.89	M
72	"	"	20.0	2.75	2.80	M
73	"	"	23.4	2.83	2.94	M
74	"	"	24.2	3.00	3.09	L
75	"	"	17.0	4.24	2.75	S
76	"	"	22.6	3.48	2.80	M
77	"	"	17.2	4.03	2.76	M
78	"	"	19.8	3.27	2.69	M
79	"	"	17.0	3.75	2.67	M
80	"	"	23.8	2.89	2.83	M
81	"	"	23.6	3.37	2.84	M
82	"	"	23.8	--	2.84	S
83	"	"	23.4	2.97	2.97	M
84	"	"	19.6	4.31	2.61	M
85	"	"	24.0	3.42	2.85	M
86	"	"	20.8	--	3.18	S
87	"	"	22.1	--	2.71	S
88	"	9/26/75	20.6	3.90	2.62	S
89	"	"	26.5	2.64	3.24	L
90	"	"	20.5	3.11	2.64	L
91	"	"	20.6	3.00	3.68	M
92	"	"	18.8	2.66	2.88	M
93	"	"	23.5	2.76	2.84	M
94	"	"	16.5	2.93	2.79	M
95	"	"	22.3	2.93	2.79	M
96	"	"	19.0	3.14	2.85	M
97	Manitoba	9/3/76	20.8	2.80	3.11	S
98	"	"	22.2	4.37	2.88	S
99	"	"	20.8	4.01	2.55	M
100	"	"	23.8	3.17	2.52	-
101	"	"	20.2	2.00	2.74	-
102	"	"	22.2	3.57	2.69	S
103	"	"	19.8	2.97	2.79	-
41	"	"	21.2	2.36	3.26	M
42	"	"	21.4	2.27	3.27	M

Table 1(Con't.)

61	Manitoba	9/3/76	23.2	3.21	2.78	M
66	"	"	21.0	3.62	2.60	M

+ The reader should note that, when sampled, these clones were not necessarily at maximum quality. Therefore, comparisons between clones harvested on different dates are invalid.

* Small = less than 5 cm. in length; Medium = 5 to 10 cm.; Large = greater than 10 cm.

Table 2. Mean values of % soluble solids and total acidity for Minnesota* and Manitoba *V. riparia* populations.

Number of clones sampled	Source	Latitude	% Soluble solids	% Acid	#Days since+ last 0°C.	Degree days+ (Base 50°F)
33	Manitoba	49° N	18.3 ± 1.18	3.52 ± .57	123	1792
21	Minnesota	45° N	18.7 ± 2.2	3.34 ± .66	145	2341

* The Minnesota population is comprised of clones collected September 12 and 13, 1975.

+ Manitoba climate data taken from records of the Morden, Manitoba, research station; Minnesota climate data from U.S. Dept. Commerce, Environmental Data Service, Monthly Climatological Data.

Figure 2. Range of Vitis riparia Michx.(dotted line). Determined by descriptions from Bailey(10), Munson(64), Winkler(181), and from personal correspondence with herbarium curators in Maine, Quebec, Ontario and Manitoba.

Figure 3. Examples of typical growth habit of V. riparia in its natural habitat along the Minnesota River.

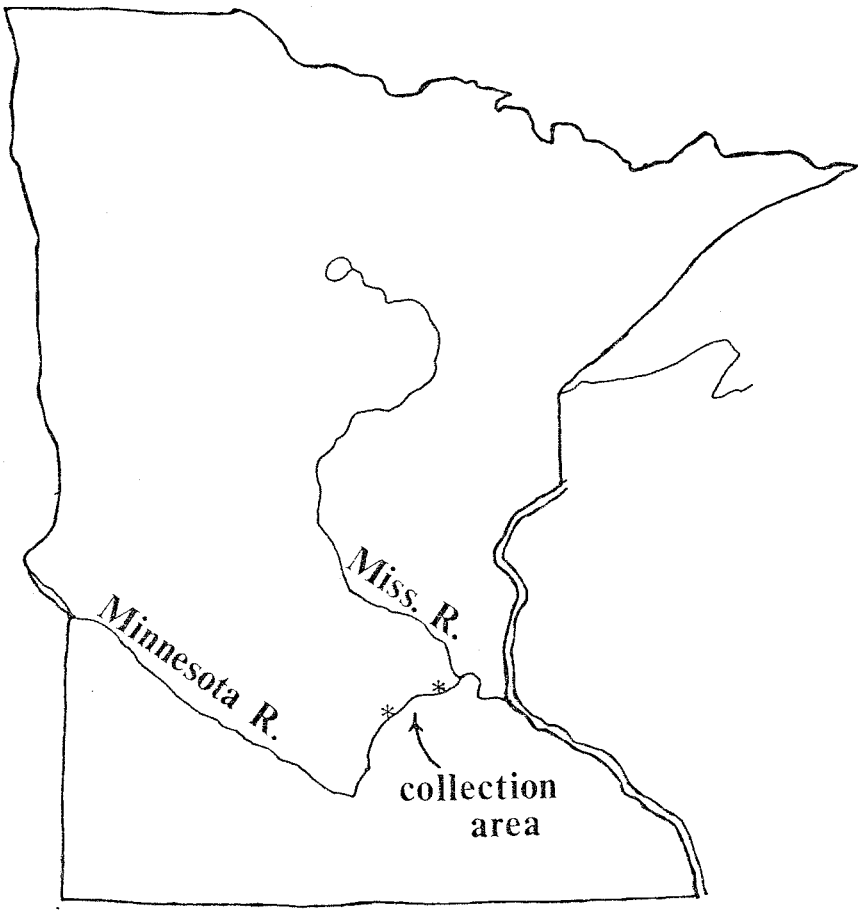


Figure 4. Collection area of Minnesota Vitis riparia clones.

Figure 5. Origin of Manitoba Vitis riparia clones.

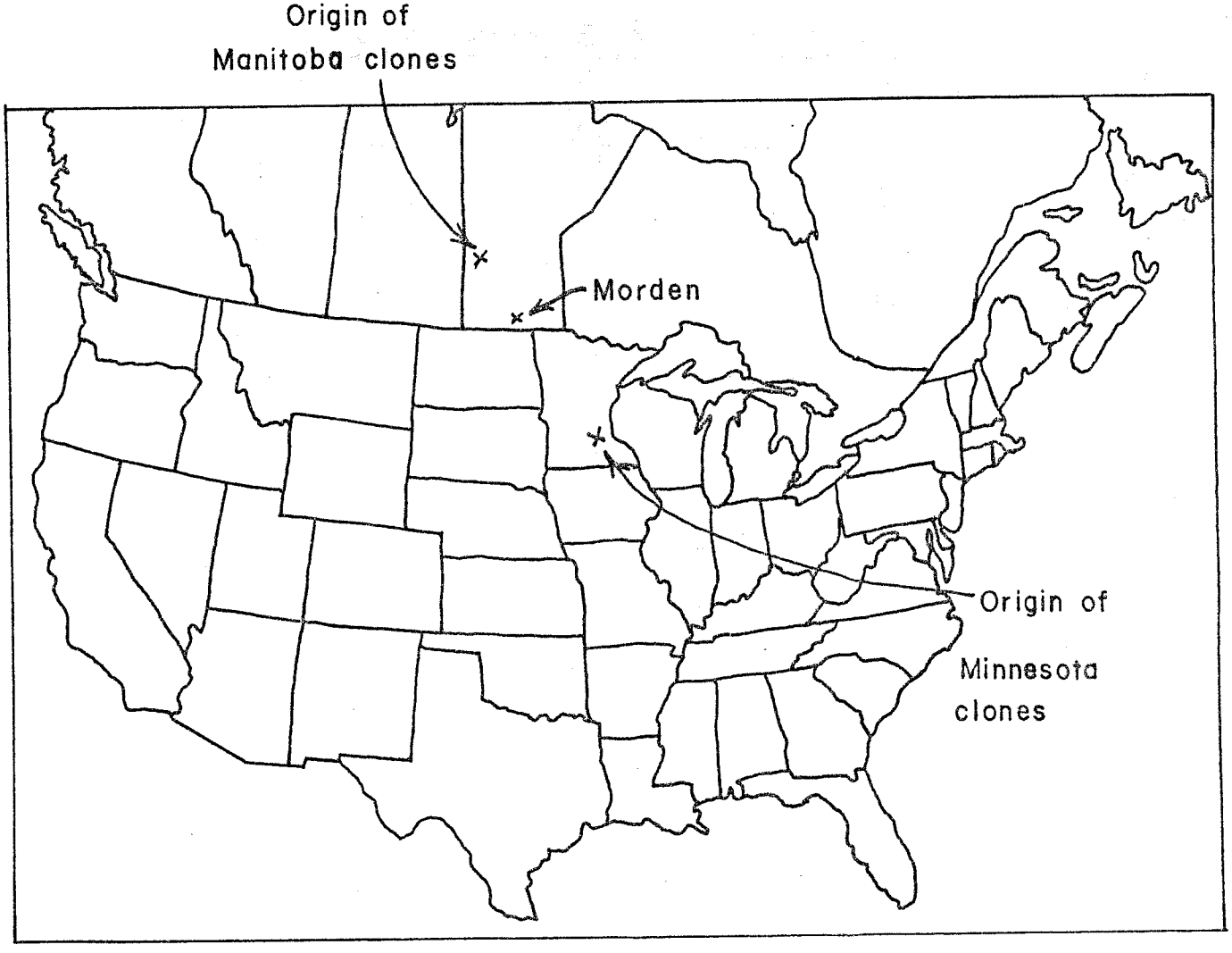

Origin of
Manitoba clones
Morden
Origin of
Minnesota
clones

Figure 6. Fruit quality variation for soluble solids in two populations of Vitis riparia.(Minnesota population comprised of clones collected September 12 & 13, 1975.)

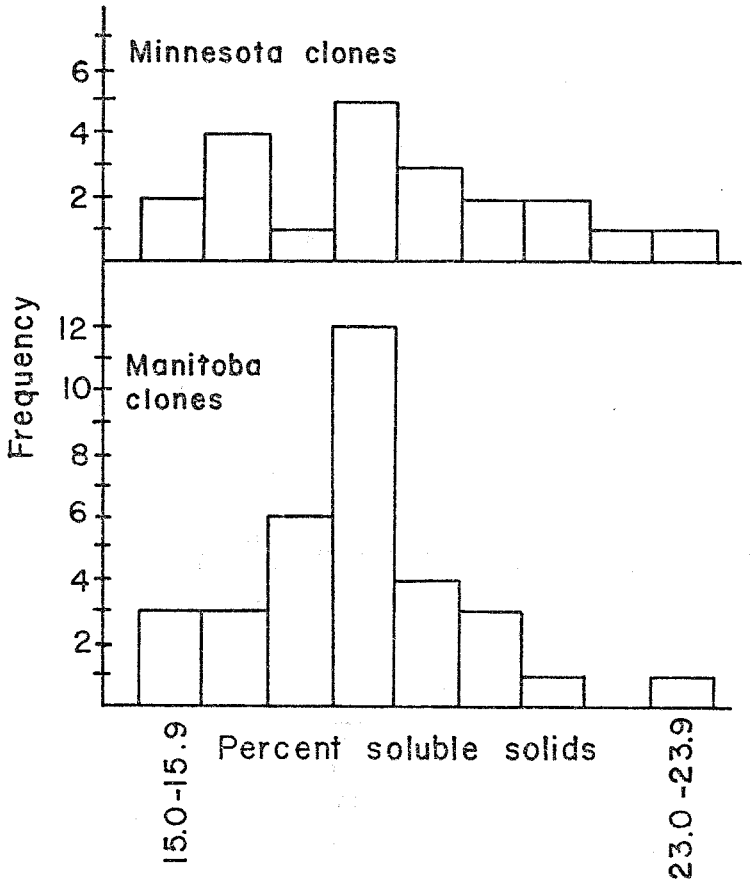
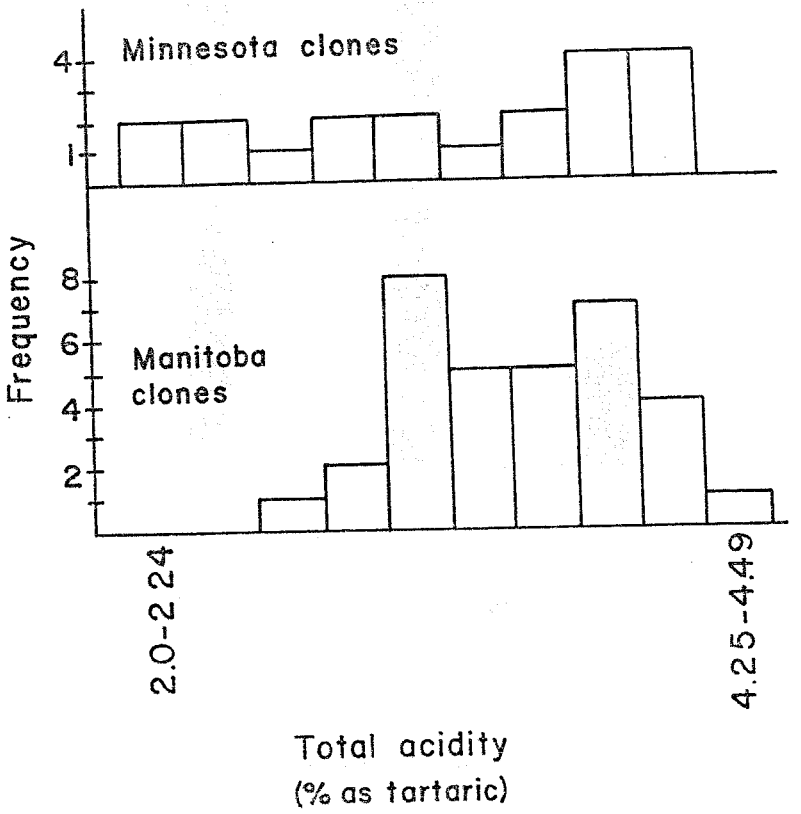


Figure 7. Fruit quality variation for total acidity in two populations of Vitis riparia.(Minnesota population comprised of clones collected September 12 & 13, 1975.)



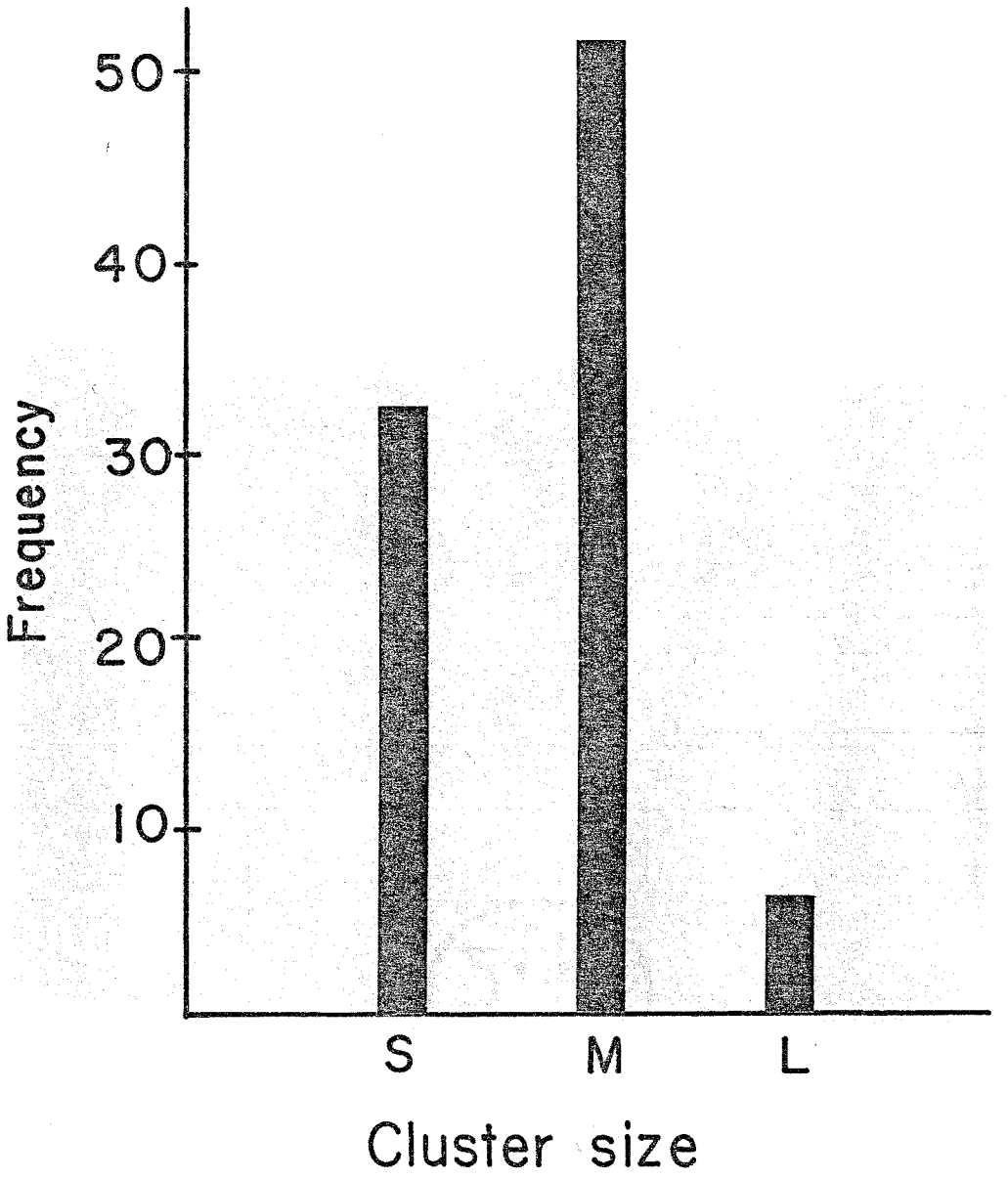


Figure 8. Frequency of three size classes of Vitis riparia clones collected in 1975. Includes both Minnesota and Manitoba populations.

Figure 9. Clusters representing small, medium and large size classes.

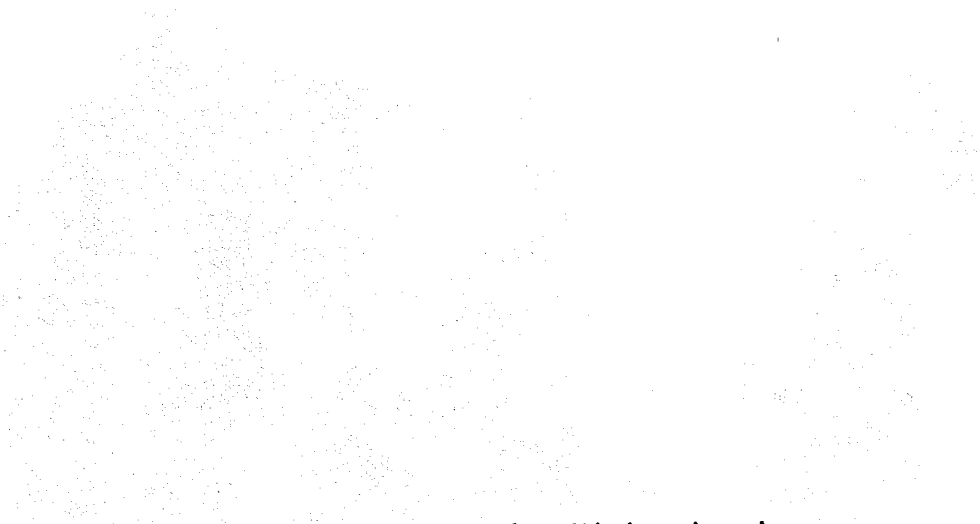
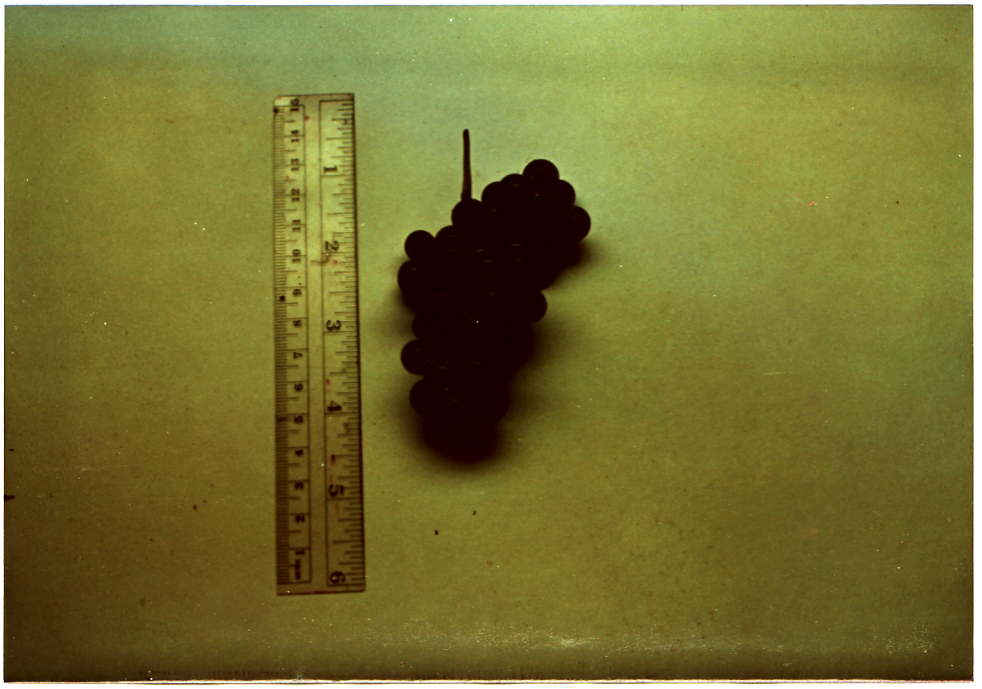


Figure 10. Typical average size Vitis riparia cluster(top) compared to superior clone(bottom).




#74

Figure 11. Correlation of total acidity and pH in the Minnesota Vitis riparia clones.

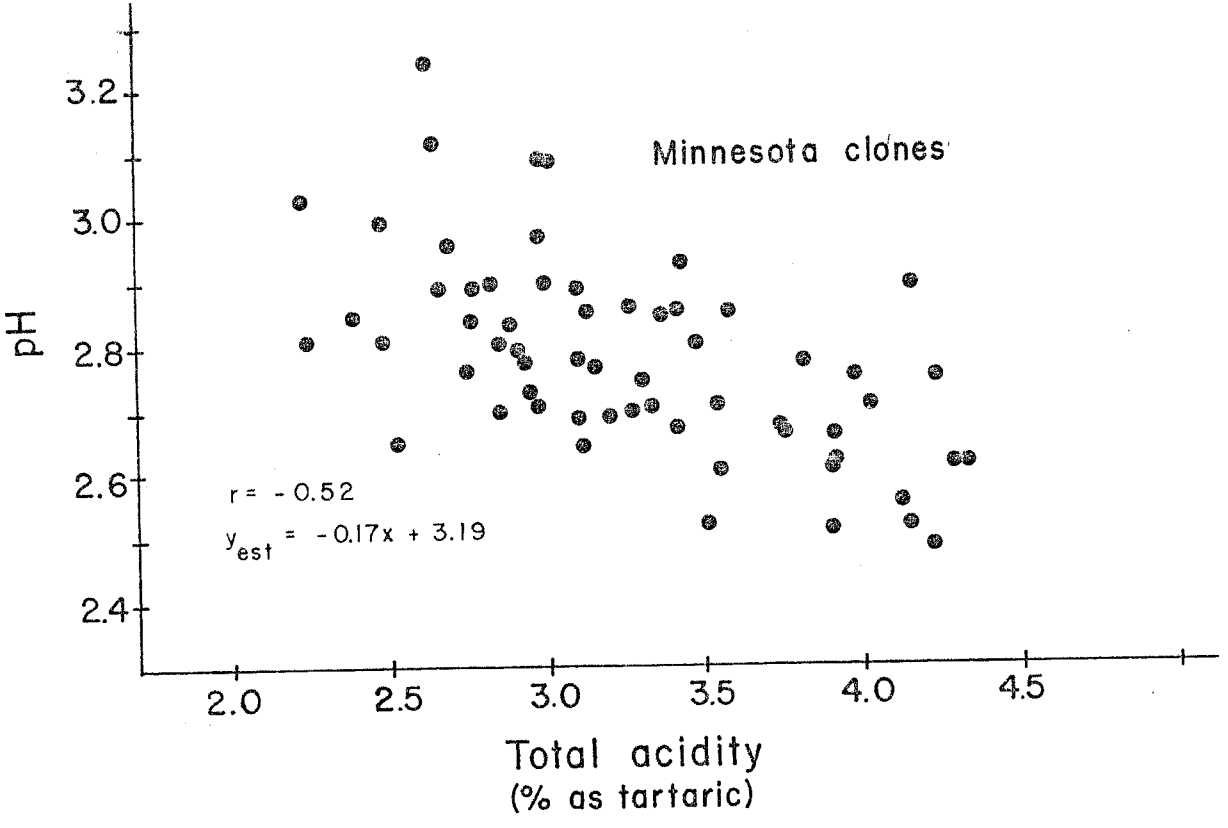

Minnesota clones
r = - 0.52
y_{est} = - 0.17x + 3.19
pH
3.2
3.0
2.8
2.6
2.4
2.0
2.5
3.0
3.5
4.0
4.5
Total acidity
(% as tartaric)

Figure 12. Correlation of total acidity and pH in the Manitoba Vitis riparia clones.

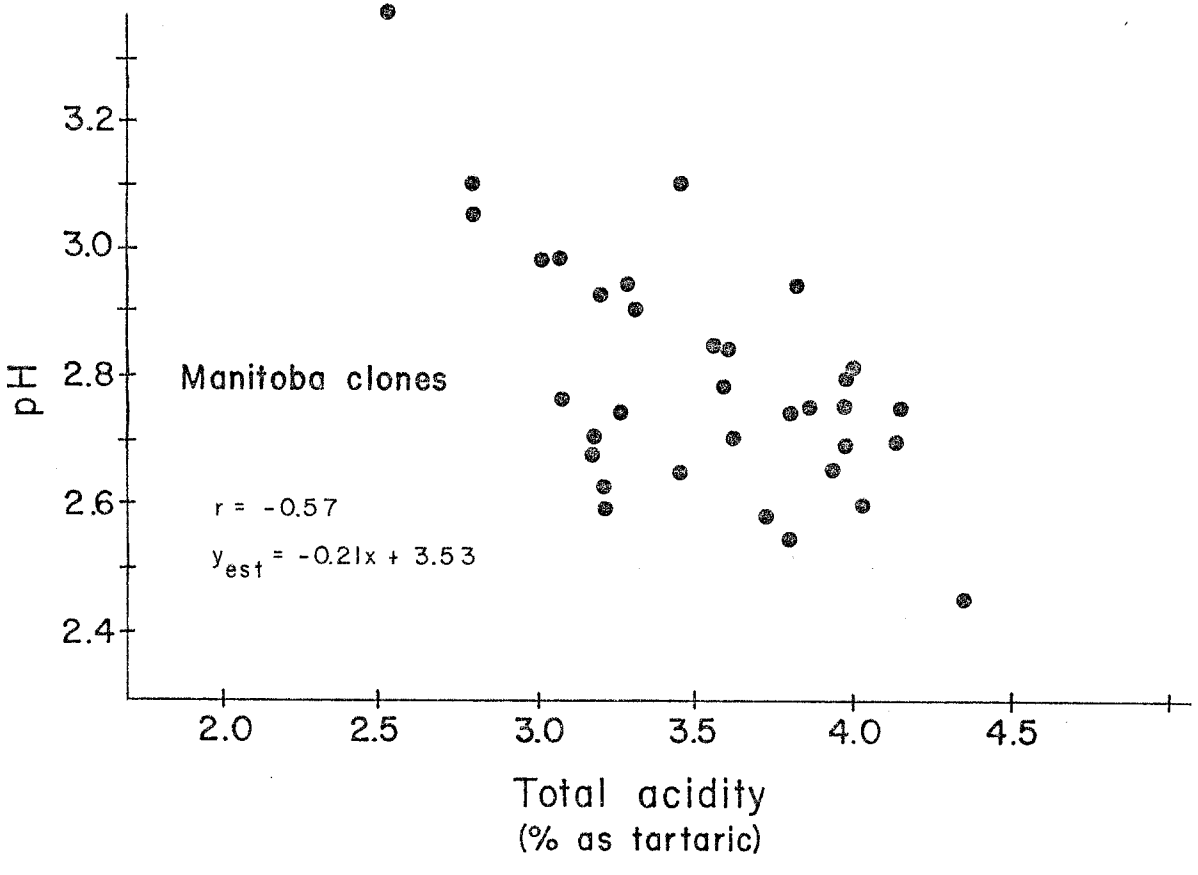
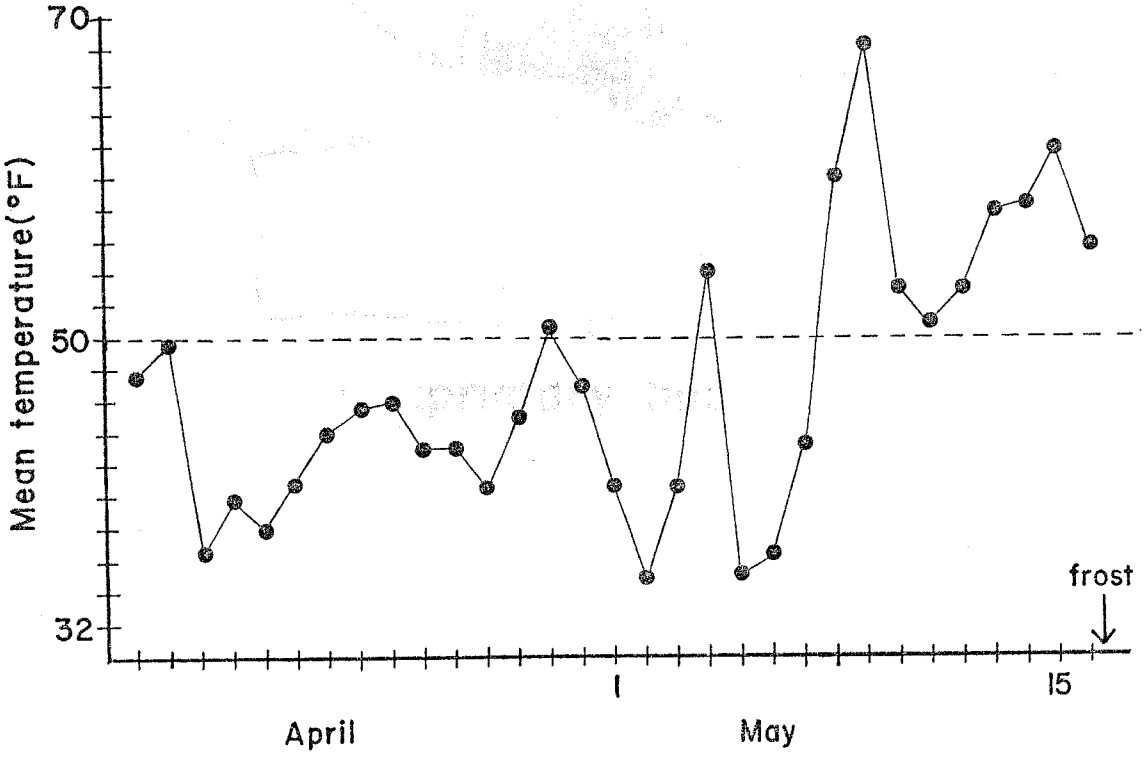

pH
3.2
3.0
2.8
2.6
2.4
Manitoba clones
r = -0.57
y_{est} = -0.21x + 3.53
2.0
2.5
3.0
3.5
4.0
4.5
Total acidity
(% as tartaric)

Figure 13. Mean daily temperatures at the Morden, Manitoba, research station prior to the late frost in spring of 1976.(Data obtained from Morden research station daily weather records.)


70
50
32
Mean temperature(°F)
frost
1
15
April
May

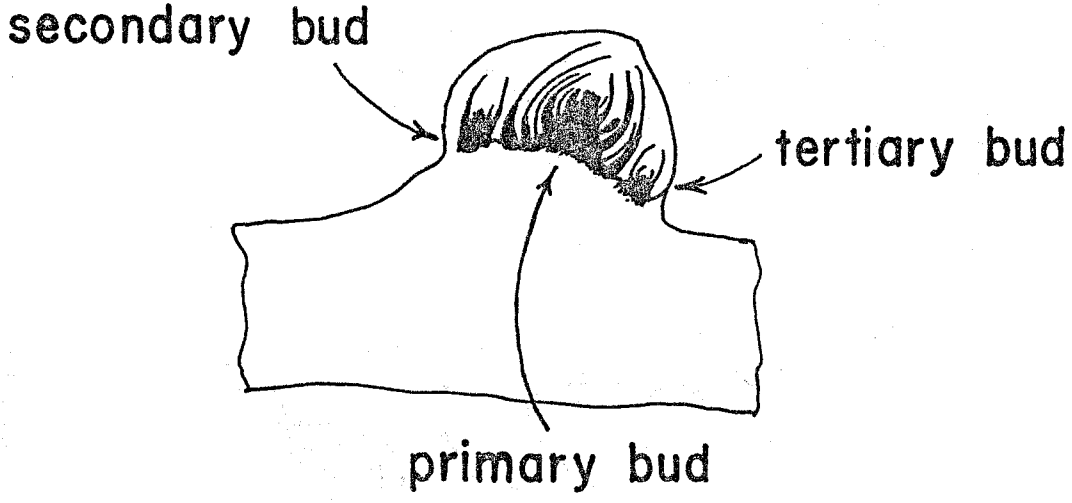


Figure 14. Cross section of grape node. From Winkler(181).

Figure 15. Mean length of growing season in Minnesota(166).

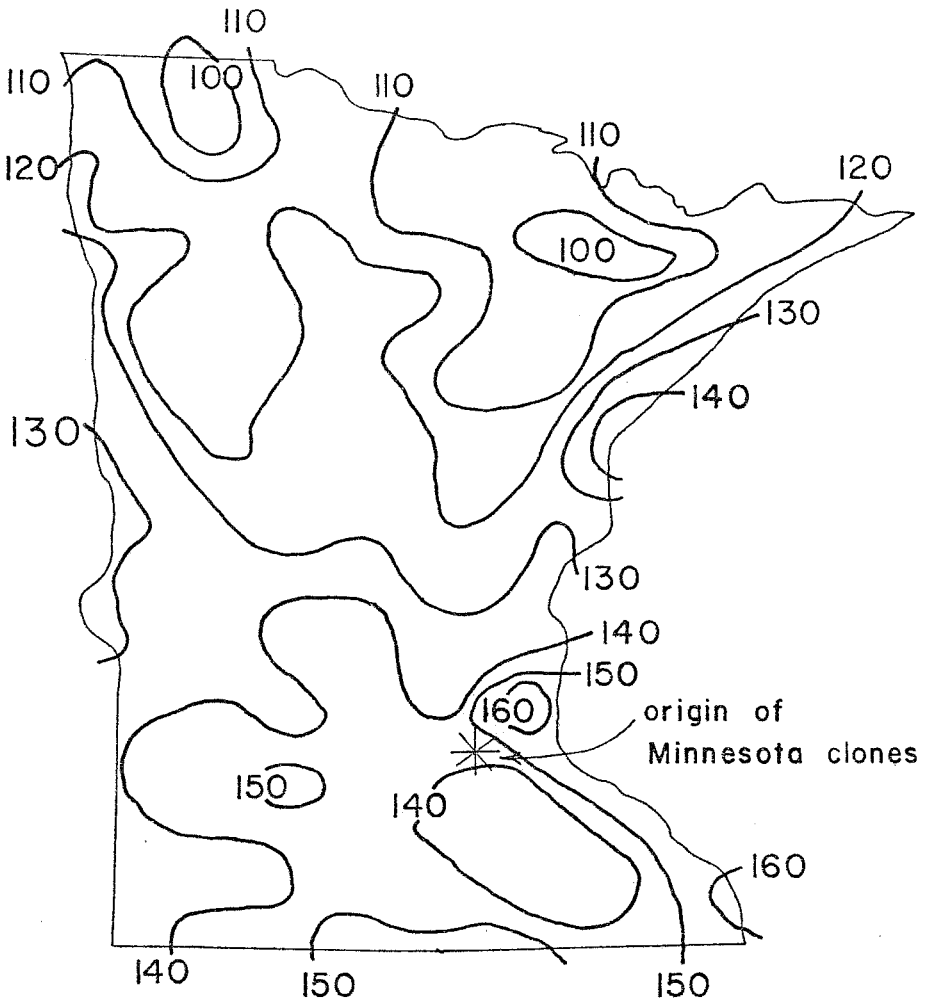


Figure 16. Mean length of growing season in southern Manitoba(149).

Ont.
110
120
100
110
120
110
120
U.S.A.
Origin of
Manitoba
clones
*
100
110
100
Sask.

Part B:

MECHANISMS OF FREEZING RESISTANCE

LITERATURE REVIEW

The acclimation process: In temperate climates, the ability of long-lived perennial plants to tolerate cold temperatures in winter is vital to their survival. Low temperature repeatedly damages economic crops(34,160), and is perhaps the single most important factor limiting distribution of native plants(111). Actively growing plants can withstand temperatures of only a few degrees below $0^{o}C$(27,90,97,98,99,177) but may, in the case of some very hardy species, survive to liquid nitrogen temperature ($-196^{o}C$) only a few weeks later(139,143,153). The process by which plants acquire this hardiness is termed "acclimation."

Acclimation is a very complex process consisting of a number of interrelated physiological phenomena(21,68,90,150,159,177). It has been shown that, in some plants, acclimation is photoperiod induced(72,74,75,99,153,171,180), is phytochrome mediated (180) and usually occurs is two stages(72,141,164,172), the second stage being induced by frost. Acclimation involves a translocatable cold hardiness promoter(s) produced by short-day leaves(49,72,155) and hardiness inhibitor(s) produced by long-day leaves(72,76). Evidence has recently been presented that the hardiness promoter-inhibitor effects may simply be the result of relative concentrations of plant hormones(175). The acclimation process is induced independent of rest period(62,75). An important point to be made here, as noted by Weiser(177), is that many

metabolic changes are taking place in plants when one would expect physiological inactivity due to low temperatures and dormancy.

Theories of cold injury and resistance: Vasil'yev(173) reviewed some of the earliest writings on cold hardiness in plants and noted that, prior to about 1830, investigators were in nearly universal agreement that plants suffer cold injury due to ice rupturing the tissue, much as rocks can be broken by water freezing in cracks.

Göppert(56) thought that plants were killed by cold temperatures when their inherent "vital force" was overcome. He hypothesized that the external and internal changes associated with frost were results, not the causes, of cold injury. Sachs(138) and Treviranus(163) felt that injury was not due to freezing, but to thawing too quickly. Schultz(147) postulated that, just as blood circulation is the foundation of animal life, sap circulation is the foundation of plant life; when the sap froze, death occured. While these theories may seem amusing to us now, they help put into perspective the great amount of cold hardiness research that has been done in recent years.

Modern theories of cold hardiness consider that plants survive by either tolerating freezing or by avoiding it(27,90). Tolerance of freezing means tolerance of extracellular freezing, since intracellular freezing is always fatal(27,88,90,151). Burke, et al(25) explained that freezing curves of plants which

tolerate extracellular ice are very similar to those of ordinary salt solutions, the primary difference between hardy and non-hardy plants being their ability to tolerate cell dehydration.

It has been known for many years that some plants can tolerate extracellular freezing. Weigand(176), in 1906, observed that in buds and twigs of many species, ice was found only in the spaces between cells, and that these plants survived. Krasavtsev (83) demonstrated that the amount of ice in some woody plants increases from 0 to -60°C, as water moves out of the cell to external ice nuclei in response to the vapor pressure deficit. He considered this dehydration to increase plant hardiness by preventing intracellular freezing(84). Dehydration has in fact been shown to increase hardiness in dogwood(33,91), willow(139), several coniferous species(143), blueberry(17,19), azalea(93), grape(121), in various species of Populus and Betula(140), and in the root tissues of a number of plants(28,44,70,94,125,179).

This dehydration due to extracellular freezing may, however, be damaging to some plants. Levitt proposed that damage may be due to mechanical disruption of cell or organelle membranes via tension caused by dehydration(90), or due to destructive disulphide linkages that form between sulphydral groups of adjacent protein molecules as they approach one another during freeze dehydration(88,89). Dehydration due to extracellular freezing may also cause irreversible destruction of enzymic processes which are responsible for ATP systhesis(63,146). Hardy plants

supposedly retain "protective substances" which prevent this from occurring. Olien(108) felt that mechanical stress from extracellular ice formation was a major factor in freezing stress. Chandler(31) felt that the concentrated solution within the cell "may become toxic to the protoplasm."

It may seem confusing that there is such a wide variety of explanations for dehydration injury. There need not, however, be any conflict between these theories; different plant species probably are injured via different mechanisms.

The other way by which plants survive low temperatures is by avoidance. One example of freezing avoidance is that of annual plants with little or no frost resistance. They survive by means of very hardy dehydrated seeds. Another example is the low growth stature of some plants, which takes advantage of the insulating effects of snow cover.

Another freezing avoidance mechanism has been termed "deep supercooling" of intracellular water. In a large number of hardy woody plants, water can remain in a liquid state at temperatures far below $0^{o}C$. Supercooling probably occurs because of lack of heterogeneous nucleating substances necessary for ice initiation (27). There is, however, a limit to the stability of supercooled water. This limit is referred to as the homogeneous ice nucleation temperature, where spontaneous change from the liquid to the more stable ice state occurs(51). In a number of laboratory studies, very pure water droplets have been supercooled to low

temperatures, but never below about -40°C(18,46,60,85,133). The presence of solutes or solvents in water can further depress the spontaneous nucleation point(27), so that the predicted homogeneous nucleation temperature in plant tissues is from -38 to -47° (53).

Burke, et al(26) explained that the extent of "deep supercooling" varies with the season and hardiness of the plant, reaching a maximum in midwinter. They speculated that the cold acclimation process in plants which utilize deep supercooling as a means of freezing avoidance, involves reduction or elimination of ice nucleating centers in cells, and development of effective barriers to nucleation by ice in adjacent cells. It has also been speculated that deep supercooling is the only possible mechanism of survival in certain plant tissues, tissues in which the cell walls are too lignified to allow contraction during extracellular freezing(26).

Probably the first to observe deep supercooling(though he did not use this term) was Weigand(176) in 1906. He noted that the vegetative buds of some tree species could be cooled to -26.5°C before ice crystals were observed.

Tumanov and Krasavtsev(164), using differential thermal calorimetric techniques, noted that a low temperature "exotherm" occured when temperatures in oak, birch, fir and pine stems reached the killing point. These exotherms were thought to represent heat released during freezing of supercooled intra-

cellular water. They also observed low temperature exotherms in cherry flower buds(163) at temperatures of -20 to -30°C. These exotherms coincided with tissue injury.

McLeester, et al(99), when comparing freezing curves of dogwood at different times of the year, found no relationship between the temperature of the exotherm and tissue death. What they were most likely observing, however, was not "deep supercooling" but supercooling of "bulk" water, or water in extracellular spaces. The freezing of bulk water is not lethal in hardy species(17,54,57,58,127,130,131,132). They noted that freezing curves could be used as a viability test - dead tissues always produced smooth freezing curves whereas live tissue produced one to three freezing points which deflected the freezing curve.

In another study(98) they found that viability could also be determined by freezing curve studies in plants other than dogwood. Living stem sections of all genera studied revealed two freezing points, while dead tissue exhibited only one. Stergios and Howell(156) found that freezing curves could also be used as a viability test for grape, cherry, raspberry and strawberry.

Quamme, et al(131), observed low temperature exotherms in blueberry stems. These exotherms were associated with injury to the xylem but not to the bark, which was less hardy than xylem. They concluded that differential thermal analysis was not a good technique for determining blueberry stem hardiness, since the

most critical tissue did not exhibit low temperature exotherms. Low temperature exotherms have been observed in flower buds as well as stems of blueberry(17,19). Bittenbender and Howell(19) could not find any correlation between average exotherm temperature(AET) and flower bud hardiness. However, as Biermann, et al(17) demonstrated, this finding is erroneous because hardiness of blueberry flower buds is freezing rate dependent, similar to the results of George, et al(54) with azalea. Bittenbender and Howell lowered the temperature of flower buds at 2-5^{o}C/hr. for the hardiness tests, but froze them at 100-150^{o}/hr. for determining AET. That they found no correlation is therefore not surprising. Biermann, et al, also showed that by artificially dehydrating blueberry flower buds, hardiness could be substantially increased, as determined by AET.

Quamme, et al(130,132) studied hardiness of acclimated winter twigs of 'Haralson' apple, and found that a low temperature exotherm which occurred as low as -42^{o}C was associated with injury to the xylem ray parenchyma. The temperature at which the exotherm occurred was independent of cooling rate. The low temperature exotherms were not observed in twigs freeze-dried to a water content below 8.5%, nor in hydrated twigs ground to a fine powder(132). They concluded that freezing injury to apple xylem is caused by a specific freezing event, that it is different from freezing injury to other stem tissues(132), and that the exotherm was associated with some structrual feature but not

with viability of the tissue(130).

Quamme(127) studied the cold hardiness of overwintering flower buds of several species of Prunus. He found that in apricot, European plum, Japanese plum, peach, and sweet and sour cherry, the temperature at which injury occurred corresponded quite closely to the low temperature exotherm. His conclusion was that flower bud injury in these species results from a discrete freezing event that involves freezing of a "bound" or "supercooled" fraction of water.

The work of George, et al(54) with azalea flower buds was the first to prove that low temperature exotherms represented freezing of supercooled water due to the absence of heterogeneous nucleating agents. Tumanov and Krasevtsev(164) had thought that supercooling was taking place, but that it results instead from a loss of membrane permeability which prevents water from moving out of the cell to extracellular ice nuclei. Quamme(126) agreed with this interpretation. Weiser(177) had postulated that the low temperature exotherm represented a sudden stripping away of tightly bound water from protoplasmic constituents.

George, et al(54) showed by nuclear magnetic resonance spectroscopy techniques that water was in fact supercooled in acclimated azalea flower buds. They also showed that nonliving floral primordia froze at the same temperature as living primordia, implicating a morphological explanation for the existence of supercooled water in buds. At cooling rates faster than

8.5°C/hr., primordia in intact buds froze at higher temperatures. Apparently, at fast freezing rates, the bulk water freezes with such energy that it can "seed" the buds with ice. This is similar to the results of Biermann, et al(17) in their work with blueberries.

Graham and Mullin(57,58) also studied hardiness of azalea. They found that the lethal temperature of azalea stems coincided with a low temperature exotherm, which occurred at between -37° and -42°C in acclimated stems. They also found that hardy cultivars avoided injury by responding to temperature changes more rapidly than tender ones, and that floret hardiness was highly inversely correlated with moisture content(58).

Quamme, et al(129) studied the cold hardiness of buds from peach cultivars which varied widely in field hardiness. They found that in all cases, the low temperature exotherms closely coincided with temperatures at which injury occurred, and concluded that differential thermal analysis is a rapid and accurate method of determining peach flower bud hardiness.

Wiest and Steponkus(178) observed exotherms in leaves and roots of Pyracantha sp., but found no correlation between exotherm temperature and tissue injury. This is similar to the results of McLeester, et al(99), in that in both cases, the exotherms were recorded at -5°C or higher, indicating that non-lethal freezing of "bulk" water was occuring.

Most recently, George and Burke(52), using nuclear magnetic

resonance spectroscopy, determined that the low temperature exotherms which occur in shagbark hickory xylem are the result of freezing of a supercooled fraction of water. Low temperature microscopy revealed that the freezing was occurring intracellularly. They also found that hickory xylem is extremely resistant to dehydration, and felt that this resistance is necessary to allow maintenance of supercooled tissue water at temperatures near -40°C.

The phenomenon of deep supercooling of intracellular water has both horticultural and ecological significance. It has ecological significance because the limit of deep supercooling in plants has been shown to be approximately -47°C(53,133). Therefore, woody plants which have in common supercooling as a means of cold injury avoidance may be expected to have the same northern limit of distribution, as shown by George, et al(53). They studied hardiness of 49 woody species native to North America. Very hardy species with ranges extending into northern Canada and Alaska did not exhibit low temperature exotherms. On the other hand, all those species which did exhibit low temperature exotherms had a common northern boundary which corresponded quite closely to the -40°C average winter minimum isotherm on the plant hardiness zone map(105). These low temperature exotherms occurred between -41° and -47°C, which is within the predicted range for homogeneous nucleation of plant tissue water. The exotherms occurred at the same temperature that resulted in xylem death in

most of the species studied. They concluded that deep supercooling is the mechanism which limits the northern distributions of many North American woody species.

The phenomenon of deep supercooling also has horticultural significance. Quamme(128) showed that the distribution of commercial production of apples and pears in Ontario was closely correlated with the low temperature exotherms of the major commercial cultivars. Apple production is profitable in colder areas than pear production because the major commercial apple cultivars are able to maintain their intracellular water in a supercooled state at lower temperatures than are the major commercial pear cultivars.

Another result of the phenomenon of deep supercooling is that it places a limit on the hardiness which can be achieved through breeding, unless genotypes can be found which do not supercool as a means of freezing avoidance.

It is evident from these findings that deep supercooling is a freezing avoidance mechanism much more common than previously thought. As recently as 1972(90), deep supercooling was thought to play no major role in plant cold resistance.

Freezing resistance mechanisms in grapevines: The first person to speculate on the mechanisms of freezing resistance in grapevines was Gladwin(55), who felt that acclimation was achieved by conversion of starch to sugars, and reduction of shoot water content. However, he did not explain how the starch to

sugar conversion would prevent cold injury. Kondo(81) and Oujan (110) also felt that grapevine cold hardiness was achieved by conversion of starch to sugars, presumably because sugars are considered a "protective" substance. Reuther(135) agreed, adding that grapevines synthesize proteins during hardening, but he did not propose a reason for this.

Marutjan(96) postulated that sugars have a "protective" role in vine hardiness, and even went so far as to list the relative protective values of various sugars(xylose, rhamnose > glucose, fructose, galactose > sorbitol, sucrose, lactose > raffinose, mannitol). By this explanation, the hardiest vines would apparently be those whose sugars are composed of the highest proportion of types with high protective values.

Grinenko(59) and Pogosyan and Krasvtsev(120) felt that grapevine cold hardiness depended on a high water-retaining capacity in the cells. This prevents dehydration and subsequent damage to protoplasm. Krasevtsev(83) disagreed, and felt instead that the movement of water out of the cell (thereby preventing intracellular freezing) was the mechanism of cold hardiness.

Paroschy(112), on the other hand, felt that during certain conditions of freezing and thawing, the major protective tissue (phellem) is ruptured due to differential expansion and contraction in the various stem tissues. Such rupturing is followed by detrimental dehydration and gradual death. He felt that the less hardy species studied, Vitis vinifera, experiences this "stress

cracking" more frequently and to a greater degree than V. labrusca, a hardier species.

There has been some evidence that deep supercooling of intracellular water may be a mechanism of freezing resistance in grapevines. Pogosyan(118) was able to artificially harden interspecific grape hybrids(species not stated) only to about -40°C. This is within the range predicted for homogeneous nucleation of plant tissue water(53).

Pogosyan and Krasavtsev(120) noted that as frost damage occurred in grapevines, "the heat loss curve was not smooth." This was hypothesized to be due to intracellular ice formation.

Paroschy(112) measured differential contraction of grapevine stem tissues. He noted that all tissues contracted upon cooling, then suddenly expanded at temperatures of -30° to -45°C. It is known that woody tissues contract during extracellular freezing as water moves outside the cell to spaces previously occupied by air(31,146). The sudden expansion is therefore most likely due to intracellular freezing of water, and would circumstantially support the existence of supercooled water at these low temperatures. Paroschy concludes, however, that suitable conditions do not occur in nature for intracellular freezing. It is interesting that he found this sudden expansion during cooling to occur in both V. labrusca and V. vinifera culitvars.

Most recently, Pierquet, et al(117), using the technique of differential thermal analysis, recorded low temperature freezing

points (exotherms) in bud and stem tissues of Vitis riparia, but did not attempt to correlate this with tissue death. The intent of the second part of this thesis was therefore to clarify the relationship between the low temperature exotherm and tissue death in V. riparia.

METHODS AND MATERIALS

Three related experiments were performed during the winter of 1976-77. First, stem sections and buds were analyzed periodically by differential thermal analysis(DTA) throughout the winter. Secondly, the relationship between the temperature at which the exotherm occurs and the hardiness of stems and buds was investigated. Finally, since there have been reports that DTA could be used as a test of viability for certain woody species(17,98,99, 156), the relationship between viability of stem and bud, and presence or absence of exotherms was explored.

Periodic DTA of stems and buds: Stem and bud hardiness was evaluated by DTA at 2-4 week intervals. On the morning that DTA was performed, stem sections were collected from a vigorous Vitis riparia vine growing near the St. Paul campus. The sections consisted of one bud and the internodal sections on either side. They were transported to the St. Paul campus in snow-filled plastic bags, when snow was available, or simply in sealed plastic bags, but were always moved and stored at ambient tempera-

tures. During preparation, a section was removed from the bag and brought inside at room temperature. Stem sections 5-7 mm. long, or buds from which most of the adjacent stem tissue was excised, were placed in small aluminum foil cups. The end of a 0.08 mm., chromel-constantan thermocouple was inserted under a bud scale or under the bark of a stem section. The foil was crimped to prevent slipping of the thermocouple.

Samples were then inserted into individual, glass lined wells in an aluminum block. The block acted as a heat sink and temperature stabilizer. Reference thermocouples were wrapped with dry paraffin of a mass approximately equal to the mass of bud or stem. The wells were sealed with wood plugs. Total time of preparation was usually less than ten minutes. The block was then placed in a Revco freezer set at -75^{o}C and allowed to cool from room temperature. Cooling rate with this arrangement was 110-120^{o}/hr.

When water froze within a sample, heat was released and it was recorded as a temperature difference between the sample thermocouple and its corresponding reference thermocouple. This "exotherm" was recorded on a Honeywell Electronik 194 strip chart recorder. One replication consisted of two samples. At least two reps were performed on each date.

To monitor cooling rate of the block, an additional thermocouple was inserted in a small hole drilled into the middle of the aluminum block.

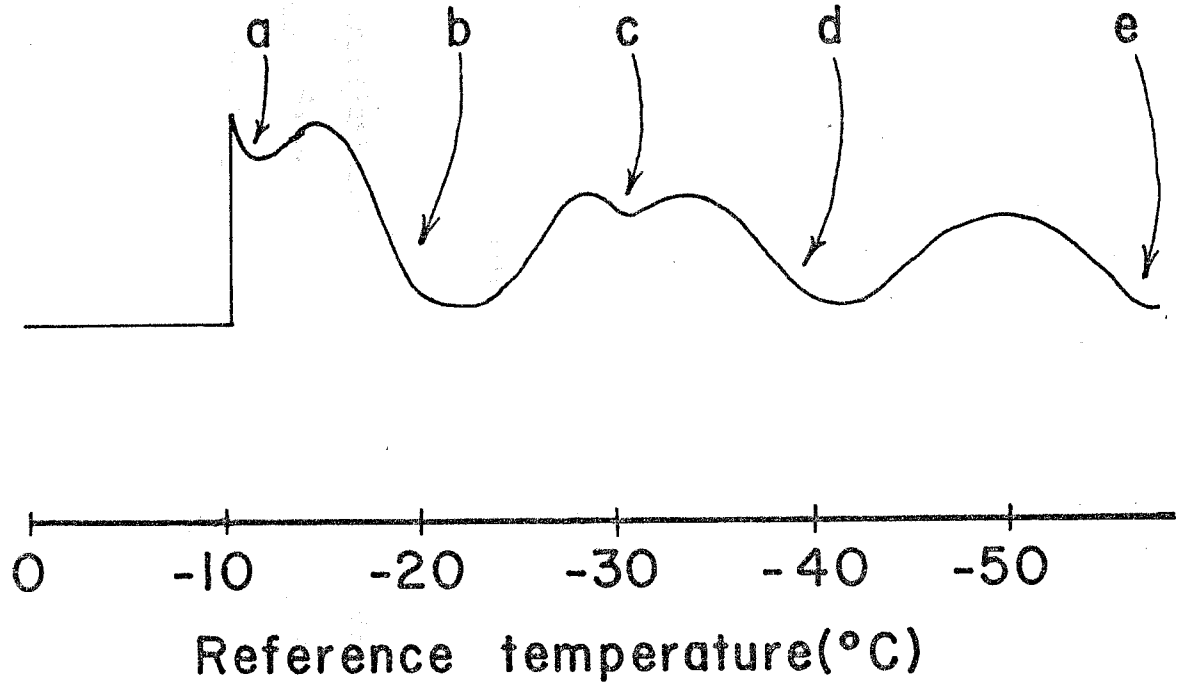
Relationship between low temperature exotherms and viability of stems and buds: Figure 17 shows a typical DTA profile of cold acclimated V. riparia stem tissue. On February 12, 1977, stem sections were collected, transported and subjected to DTA as described above. Three reps of two samples each were removed at each point "a" through "e" as shown in Fig. 17. The samples were thawed at room temperature, placed in a water-saturated atmosphere at approximately 12°C for 10-14 days, then sectioned for viability determinations. A tissue was judged dead if it was brown or off-color, live if it was green.

To test bud viability, five reps of two buds each were cooled as described above. The buds were removed immediately after exotherms were recorded. They were thawed and rated for viability as described above.

The average exotherm temperature(AET) was computed, and another five reps were cooled to within 2°C of the AET. They were then removed, thawed and rated for viability as previously described. A control sample of 15 buds was also rated for viability.

In a previous study(117) it was shown that fully acclimated V. riparia buds exhibited no low temperature exotherms, indicating that the buds had apparently lost all freezable water. If this were so, it should be possible to cool such "dehydrated" buds to very low temperatures without sustaining injury. Therefore, on January 30, 1977, buds were collected, prepared and

Figure 17. Differential Thermal Analysis profile of a cold-acclimated Vitis riparia stem section. Inflections from base line represent heat released during freezing events.(Points a through e are referred to in text.)


a
b
c
d
e
0
-10
-20
-30
-40
-50
Reference temperature(°C)

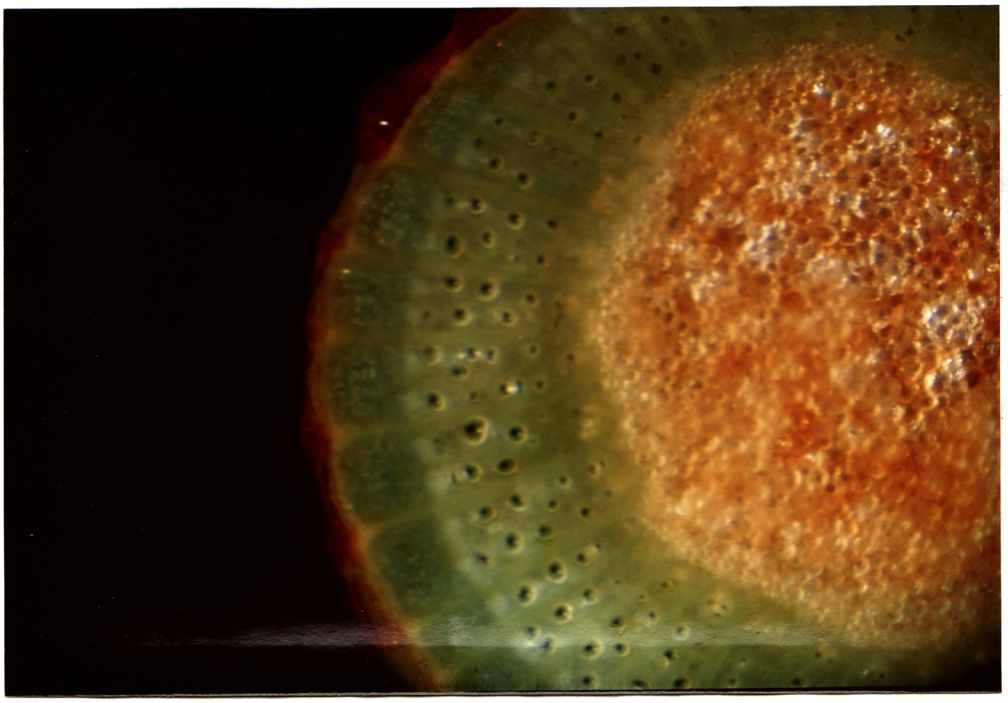
cooled as described above. Six reps of two buds each were cooled to -46.5°C, which is within 0.5° of the predicted homogeneous nucleation temperature for plant tissue water(53). No exotherms were recorded in any of the samples. These buds were thawed, incubated and rated for viability as described previously. A control sample of 12 buds was also rated for viability.

DTA as a viability test: To test the hypothesis that DTA can be used as a viability test for V. riparia, on February 7, buds and stem sections were collected, prepared and cooled to approximately -50°C as described above. The samples were then thawed in air at room temperature, and re-frozen either immediately, 24 hours later or 48 hours later. One rep of two samples was used for each treatment. The samples which were re-frozen 24 and 48 hours later were stored in a water-saturated atmosphere at +3° until re-freezing.

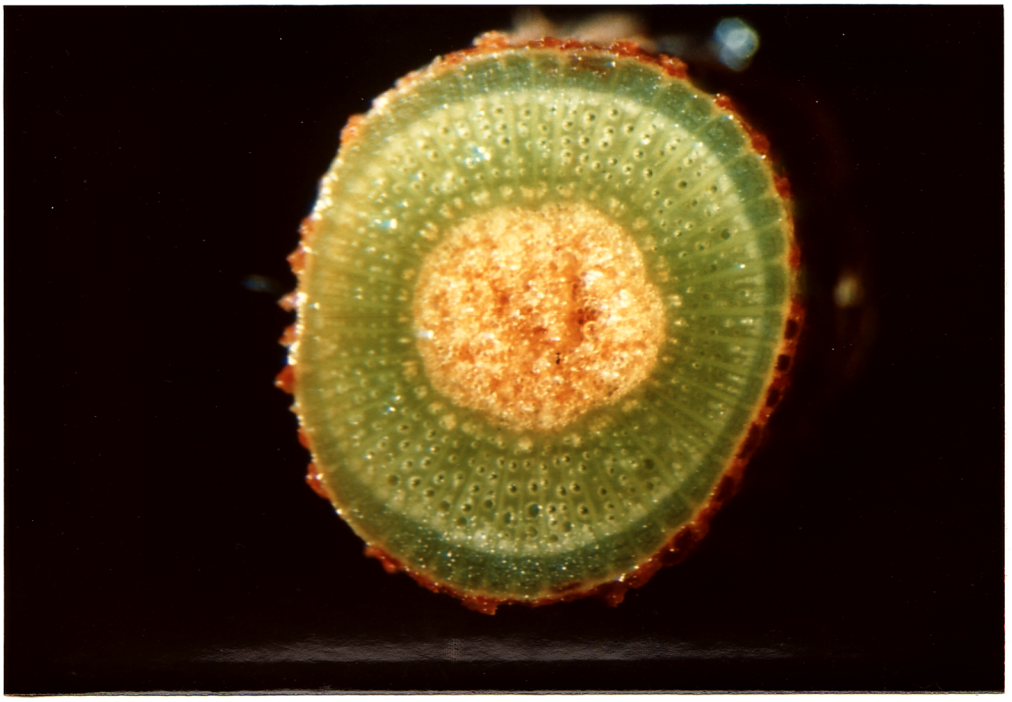
RESULTS AND DISCUSSION

Relationship between low temperature exotherm and viability of stems and buds: Figure 18 shows prints of V. riparia stem sections removed at points "a" through "e"(Fig. 17). It is evident that the first two exotherms are non-lethal and probably represent freezing of bulk water in extracellular spaces. The third exotherm is associated with injury to phloem parenchyma, the fourth(d) exotherm with injury to the phloem rays and the

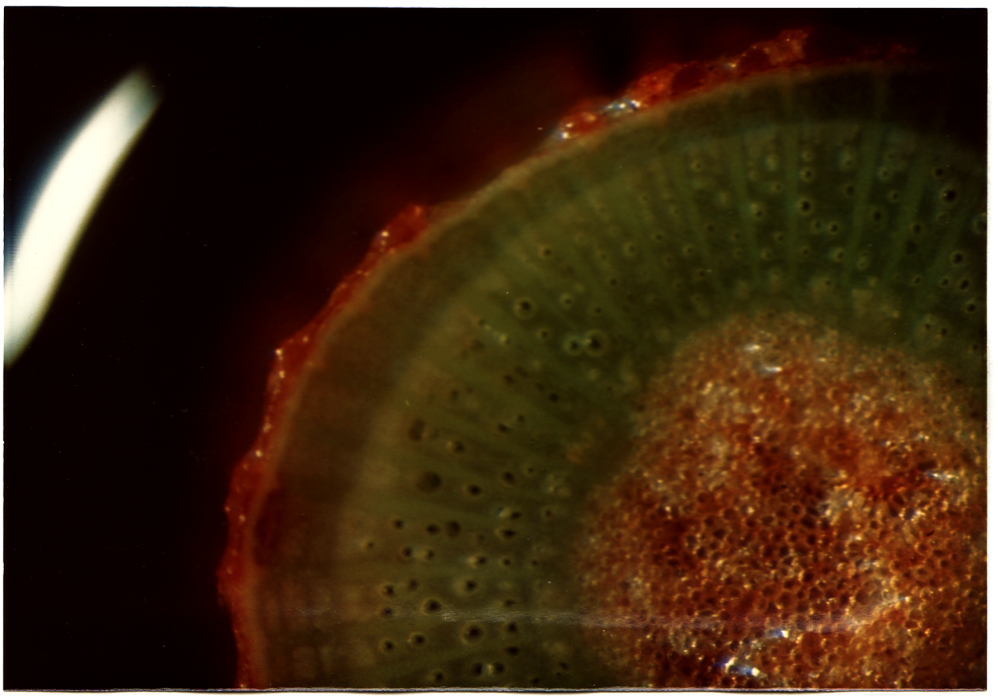
Figure 18. Cold injury in *Vitis riparia* stem sections. The letters a through e correspond to the sample removal points in Figure 17.



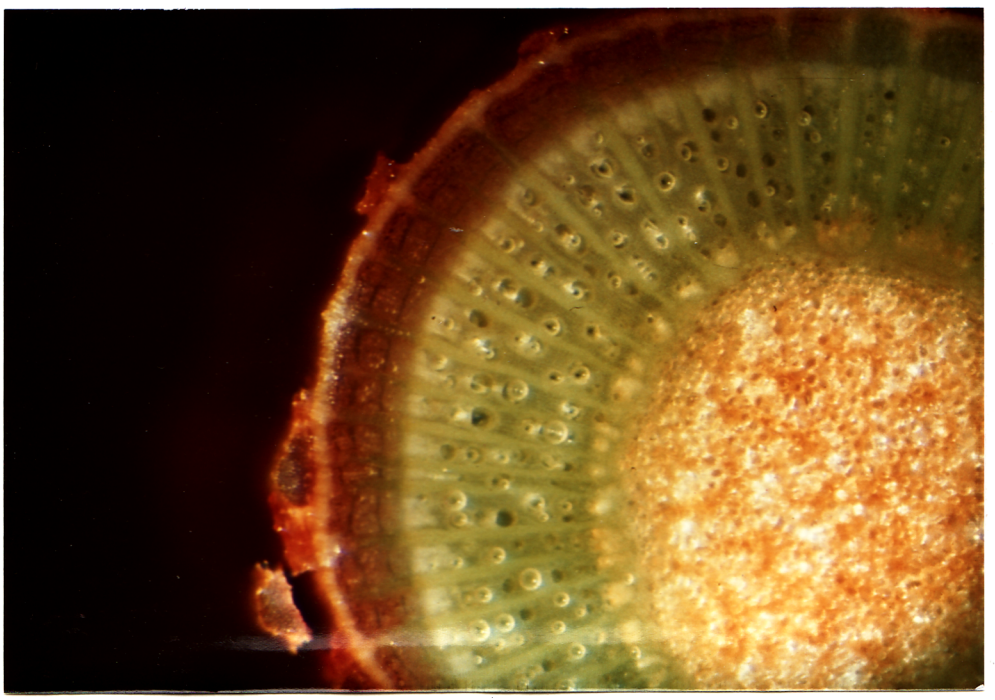
Control



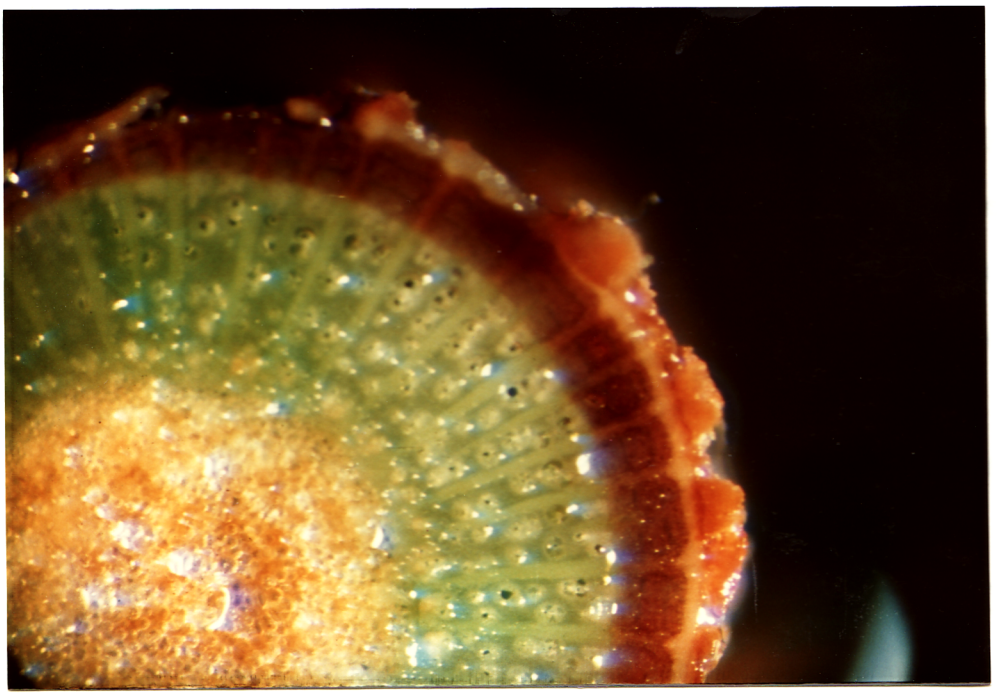
a



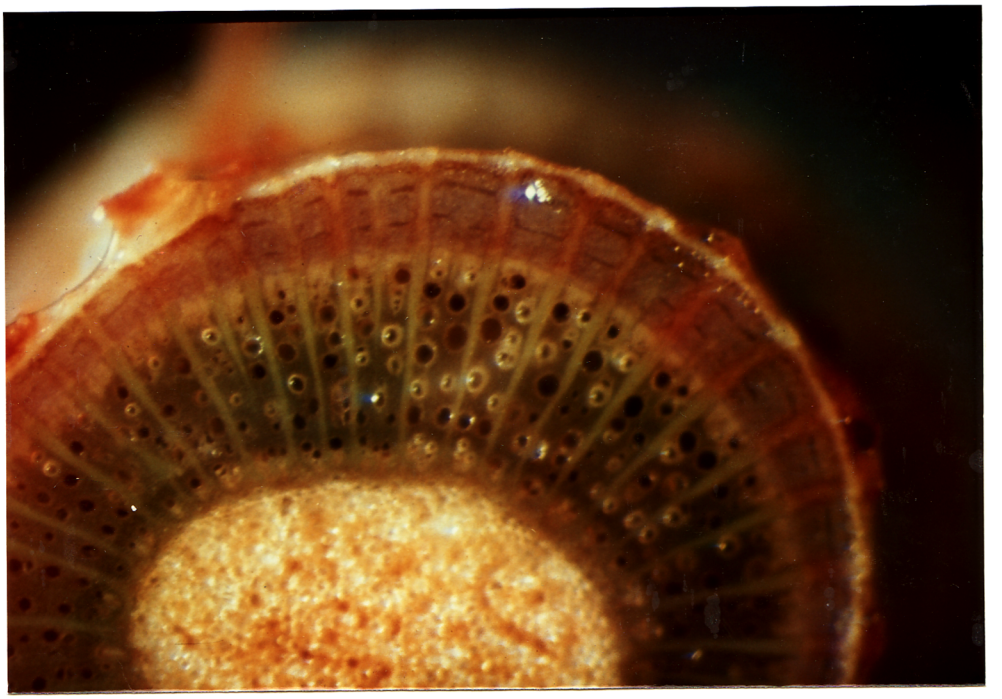
b



c



d



e

fifth(e) with injury to the xylem(Fig. 18).

Such a pattern is unique among woody species so far studied by DTA. For example, apple(130), blueberry(131) and azalea(57) stems exhibit three or more low temperature exotherms. In each case, however, only the last exotherm is associated with injury, and in each case the tissue injured is the xylem. This implies that in apple, blueberry and azalea, the various stem tissues are injured via different mechanisms; this does not appear to be true in V. riparia.

The order of stem tissue hardiness in V. riparia(Fig. 18) is not in agreement with the results of Pogosyan and Sakai(121). They found the hardiness of phloem rays and phloem parenchyma to be reversed from results in Fig. 16. However, they were working with different Vitis species, and may have been testing hardiness at a different time in the dormant season. Relative hardiness of tissues is known to change in some species over the course of a season(130).

The relationship between bud viability and low temperature exotherms is shown in Tables 3 and 4. In every case, if an exotherm was recorded, injury was noted. This implies that the low temperature exotherm in V. riparia buds is causally related to bud injury. This has also been shown to be true in a number of other woody species(17,51,54,57,58,127,129,165). The secondary and tertiary buds were, with only one exception, always hardier than the primary bud. Clark(36) and Stergios and Howell(157,158)

Table 3. Relationship between Vitis riparia bud viability and the low temperature exotherm. Buds removed immediately after exotherm.

Exotherm Temp.	Removed at:	Viability* Primary	Secondary	Tertiary
-33°C	-34.5°C	-	+	none
-34.0	-34.5	-	+	+
-32.0	-33.8	-	+	+
-33.4	-33.8	-	-	+
-32.6	-33.7	-	+	+
-33.1	-33.7	-	+	none
-32.7	-33.6	-	+	none
-32.9	-33.6	-	+	none
$\overline{X}$ = -33.0				
"Control"(15 buds)		+(15)	+(15)	+(15)

*Viability determinations: "+" = live; "-" = dead.

Table 4. Relationship between Vitis riparia bud viability and the low temperature exotherm. Buds removed prior to occurance of exotherm.

Exotherm Temp(°C)*	Viability#		
	Primary	Secondary	Tertiary
None	+	+	+
-23.6	+	-	+
None	+	+	+
None	+	+	+
None	+	+	+
None	+	+	+
None	+	+	+
None	+	+	+
None	+	+	+
None	+	+	+
None	+	+	+

*All buds removed at -31.0.

#Viability determinations: "+" = live; "-" = dead.

found the same relationship in V. labrusca varieties. This arrangement may have adaptive value in Vitis species. Secondary and tertiary buds, though hardier, are less productive than primary buds(157,181). It is also known that large crops of fruit can stress a plant(21,34,42,71,148,157,177,181) since the fruits are the primary carbohydrate "sink." Thus after unusually cold winters, when many of the primary buds are killed, the secondaries and/or tertiaries will grow. Since they are less productive, the vine can grow and recover for a season without the stress of a heavy crop of fruit.

In many studies, it has been shown that different tissues in a woody plant exhibit different degrees of hardiness(17,21,28,36, 57,58,81,100,114,121,122,130,131,132,142,144,157,158,179). Therefore, as pointed out by Stushnoff(159,160), breeders must identify the tissue(s) that is(are) critical for survival of the species in question in their locality. Thus for V. riparia, the most critical tissues during midwinter appear to be the primary buds and phloem parenchyma.

In Table 5 are shown the results of cooling naturally "dehydrated" buds to -46.5°C. None of the buds exhibited low temperature exotherms, which implies that they had lost all freezable water. In such a state they should be hardy to very low temperatures. However, the primary buds were all killed. They most likely were not dead prior to cooling, since all "control" buds were alive(Table 5). The fact that the primary buds were all

Table 5. Viability* of naturally "dehydrated" Vitis riparia buds cooled to -46.5°C.

Primary	Secondary	Tertiary
-	+	+
-	+	+
-	-	+
-	+	+
-	+	+
-	+	+
-	+	+
-	+	+
-	+	+
-	+	+
Control(12) +(12)	+(12)	+(12)

*Viability determinations: "+" = live; "-" = dead.

killed and the secondaries and tertiaries survived may mean that primary buds are injured by dehydration while the latter are not. Freeze dehydration has been shown to cause injury in some plants (31,63,89,90,146). If this is shown to be the case with the primary buds but not the secondaries or tertiaries, it would mean that there is a qualitative as well as a quantitative difference in the cold resistance mechanisms of these buds.

However, a more likely explanation seems to be that the secondary and tertiary buds had become completely dehydrated, while the primaries still contained a small amount of freezable water, an amount too small to be detected with the available equipment. Dehydrating conditions(intense cold, low relative humidity) of a longer duration would probably have caused the primary buds to dehydrate completely also.

The ability of a genotype to dehydrate and thereby avoid freezing is a mechanism of importance to breeders. In blueberries, Biermann, et al(17) found that this mechanism was unique to a cold tolerant hybrid and was not present in the less hardy commercial cultivar Rancocas. They felt that DTA could be used in a breeding program to rapidly identify the most hardy genotypes, those whose buds can dehydrate in midwinter. The same technique might also be an effective means of identifying in a breeding program the most hardy Vitis genotypes.

DTA as a viability test: Table 6 and Figures 19 and 20 show the results of this study. In all cases, low temperature exo-

therms were recorded in tissues previously killed by freezing to -50°C. In most cases, the exotherms of dead tissue occurred at slightly warmer temperatures than those of live tissue(Table 5). It has been speculated that the ability of plant tissues to maintain water in a supercooled state is largely dependent on structural integrity of that tissue(54,130,132). Apparently the initial intracellular freezing injures the tissue so that in subsequent cooling cycles, water cannot be supercooled to as low a temperature as before.

The important point, however, is that dead tissue as well as live tissue exhibits low temperature exotherms. Therefore, DTA cannot be used as a viability test in V. riparia. Quamme, et al(132) obtained the same results with apple stem tissue, and George, et al(54) found similar results with azalea florets.

Stergios and Howell(156), however, felt that DTA was an accurate viability test for 'Concord' grape. They noted that live stem tissue exhibited two low temperature exotherms and dead tissue only one. In reviewing their work, however, it is difficult to ascertain the significance of the exotherms they recorded. They did not specify how long the samples had been dead before being re-frozen; similar results may have been obtained with the V. riparia samples had the incubation period been longer than 48 hours. Conversely, the applicability of DTA as a test for viability may be species-specific in the genus Vitis.

Figures 19 and 20 reveal two interesting results. With the

Table 6. Low temperature exotherms of live and dead *Vitis riparia* buds.

Treatment#	EXOTHERM POSITION(°C): Primary	Secondary	Height ratio*
Initial	-35.5, -37.6	-38.6, -39.6	
Immediately refrozen	-33.7, -35.1	-35.9, -36.8	1.94, 1.56
Initial	-35.8, -40.0	-38.2, -41.5	
Refrozen 24 hr. later	-35.2, -37.6	-37.6, -39.3	1.6, 1.3
Initial	-36.6, -36.6	-38.3, -39.0	
Refrozen 48 hr. later	-36.5, -42.8	-41.8, -40.1	0.75, 0.77

#Two buds per treatment

*Height ratio = exotherm magnitude of refrozen bud/initial exotherm magnitude.

Figure 19. Differential thermal analysis profiles of Vitis riparia stem sections which were frozen, thawed and then refrozen at the specified intervals.

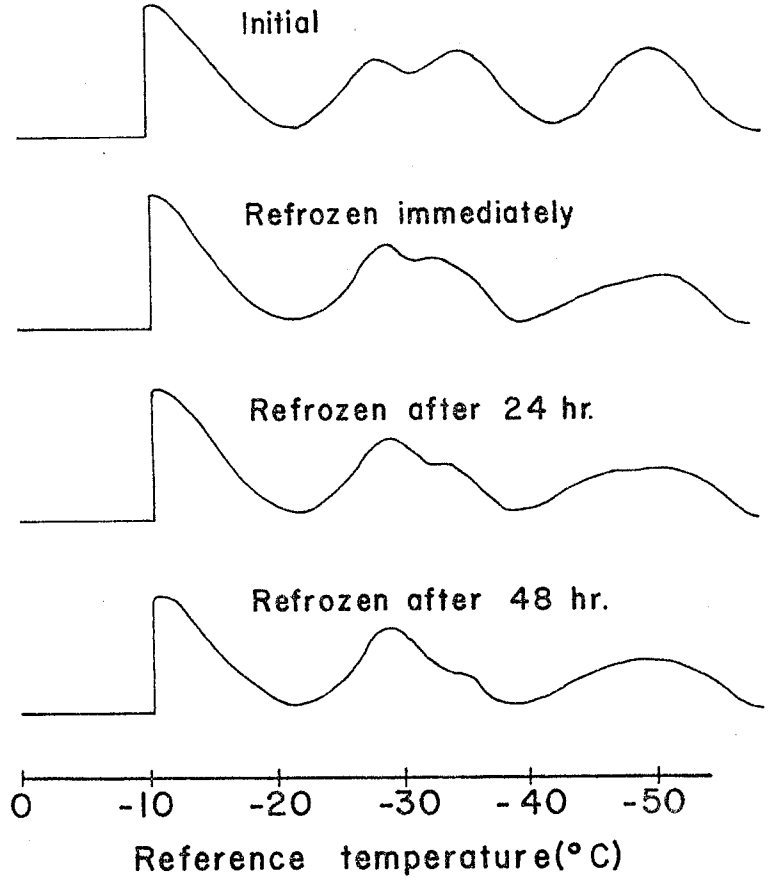
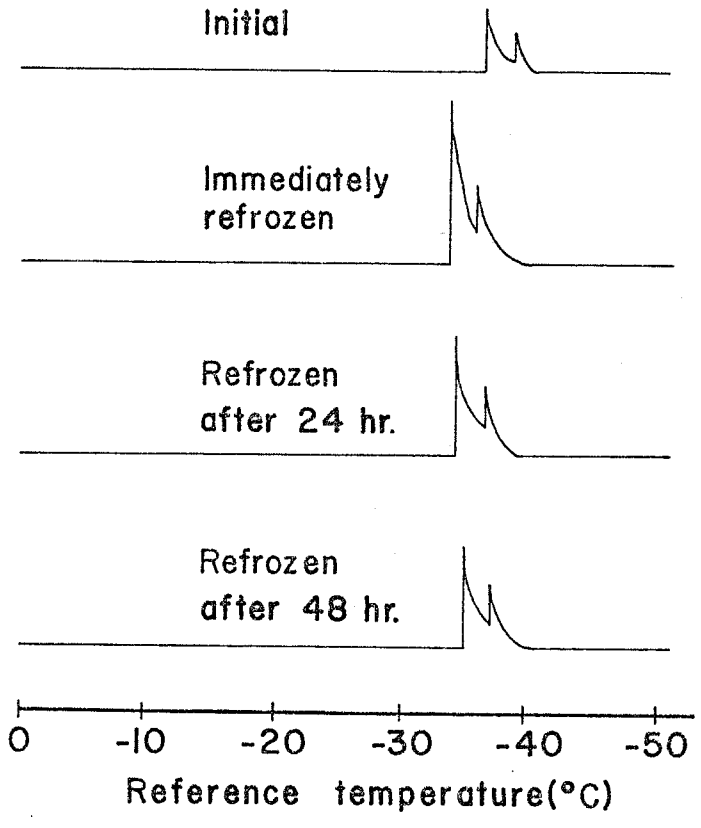

Initial
Refrozen immediately
Refrozen after 24 hr.
Refrozen after 48 hr.
0
-10
-20
-30
-40
-50
Reference temperature(°C)

Figure 20. Differential thermal analysis profiles of Vitis riparia buds which were frozen, thawed and then refrozen at the specified intervals.


Initial
Immediately refrozen
Refrozen after 24 hr.
Refrozen after 48 hr.
0
-10
-20
-30
-40
-50
Reference temperature(°C)

stems, exotherms corresponding to "d" and "e" in Figure 17 declined in magnitude after being initially frozen to -50°C. This might have been due to injury to the membrane when initial intracellular freezing took place. The cells may lose water due to this injury. Loss of water would be expressed as an exotherm of reduced size. Membrane injury has been implicated as an expression of freeze injury(88,89,90,108,177).

In contrast to the results with stem exotherms, the bud exotherms were dramatically larger upon re-freezing than during the initial freezing(Fig. 20). An explanation for this phenomenon is lacking; all that can be done is to relate the results at this time.

Periodic DTA of buds and stems: Figures 21 and 22 illustrate the results of this study. In all cases, a "free water" exotherm was recorded in the stem tissue(Fig. 21). As shown previously, this is a non-lethal exotherm. On one date(Feb. 12), two free water exotherms were recorded. The exotherms associated with tissue injury occurred at temperatures from -20° to -47°C. The exotherm corresponding to "e" in Fig. 17 was very stable after November 9, indicating that the xylem had reached its maximum hardiness at this point. The locations of the other exotherms fluctuated greatly.

One may speculate as to whether the location of the exotherms in fact represents the hardiness of the tissue on that date, since it is known that the hardiness of some species is

Figure 21. Differential thermal analysis profiles of *Vitis riparia* stem sections during the winter of 1976-77. Average of four to six samples.

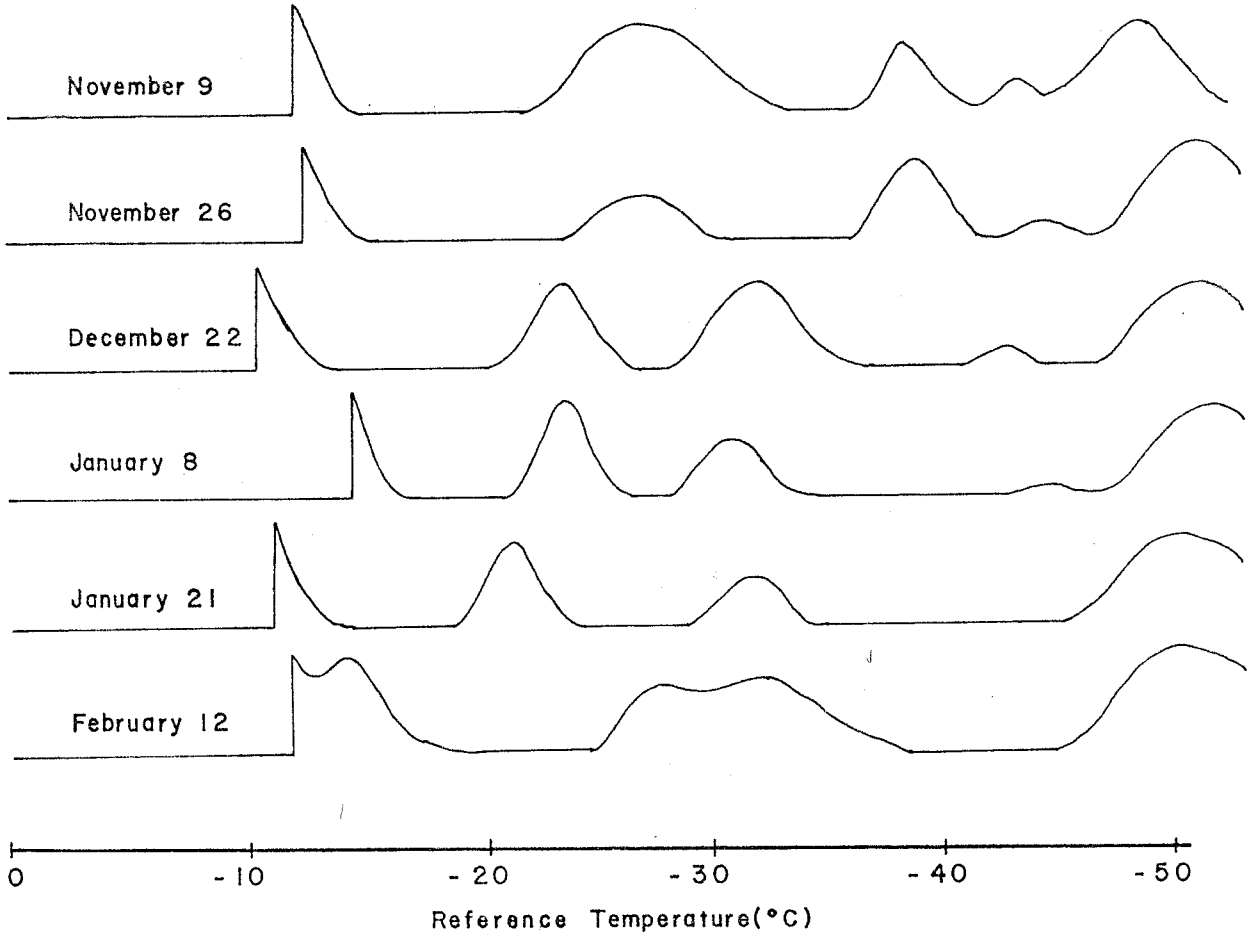
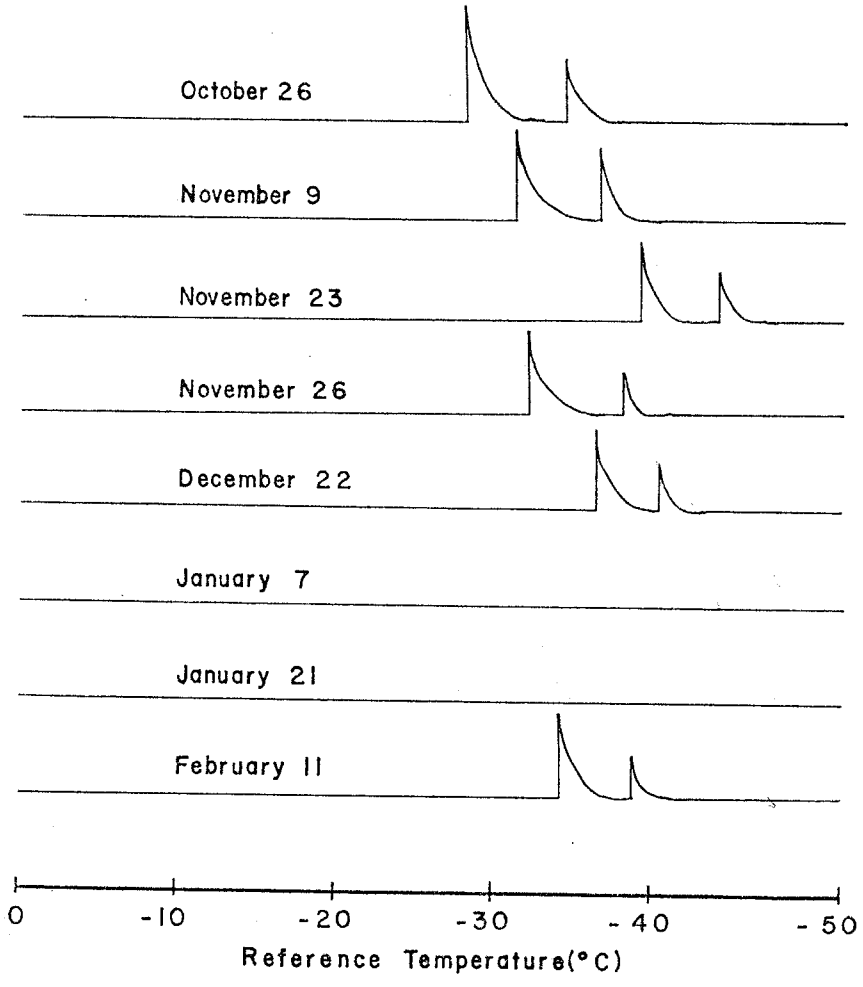

November 9
November 26
December 22
January 8
January 21
February 12
0
-10
-20
-30
-40
-50
Reference Temperature(°C)

Figure 22. Differential thermal analysis profiles of *Vitis riparia* buds during the winter of 1976-77. Average of four to six samples.


October 26
November 9
November 23
November 26
December 22
January 7
January 21
February 11
0
-10
-20
-30
-40
-50
Reference Temperature(°C)

freezing rate dependent(the V. riparia stems were frozen at an unnaturally fast rate, 110-120°C/hr.). It seems likely that the location of the "e" exotherm does in fact reflect xylem hardiness on that date. Hardiness of xylem has been shown to be independent of freezing rate in all species studied by DTA(57,130,132). Also, one would expect the "e" exotherm to occur at temperatures higher than -45 to -47°C if it were freezing rate dependent.

The phloem parenchyma and rays, however, may have been more hardy than Figure 21 indicates. In hardy apples, phloem injury is freezing rate dependent, whereas xylem injury is not(130). More work is needed to uncover the relationship between freezing rate and tissue injury in V. riparia.

It is evident that, unlike bud tissue(Fig. 22), stem tissues do not naturally dehydrate during mid-winter. Therefore, they can always be injured by temperatures of -47°C or above, since this is the lower limit of predicted homogeneous nucleation point for plant tissue water(53). Thus V. riparia can be added to the list of those species that are limited in their northern distribution by the characteristic of deep supercooling of intracellular water.

Figure 22 shows representative DTA profiles of V. riparia buds throughout winter. It is apparent that buds enter the winter in a less hardy and more hydrated state, and can become dehydrated in midwinter. The position of the bud low temperature exotherm at fast freezing rates most likely accurately reflects

bud hardiness on that date. Although the freezing point of azalea and blueberry flower buds is freezing rate dependent (17,54), both exhibit "free water" exotherms which represent water freezing in extracellular spaces. At very fast freezing rates, this free water may freeze with such energy that it can permeate the cell membranes and "seed" the bud tissue with ice, causing them to be killed at higher temperatures(54). V. riparia buds exhibit no such free water exotherm (Fig. 22). Therefore, the freezing points in Fig. 22 most likely represent the actual hardiness of the buds on the date. When Bittenbender and Howell froze excised blueberry florets(19), they found no relationship between hardiness and freezing rate. This can be explained because excised florets are no longer associated with a mass of bulk water which, upon freezing at very fast rates, can seed the floret tissues with ice.

Very rapid freezing is an obvious advantage because larger numbers of samples can be screened for hardiness. The relationship between V. riparia bud hardiness and freezing rate should be further investigated to demonstrate conclusively the effects of freezing rate. It seems safe to say, however, that fast freezing rates have less effect on V. riparia bud hardiness than on bud hardiness of other plants.

CONCLUSIONS

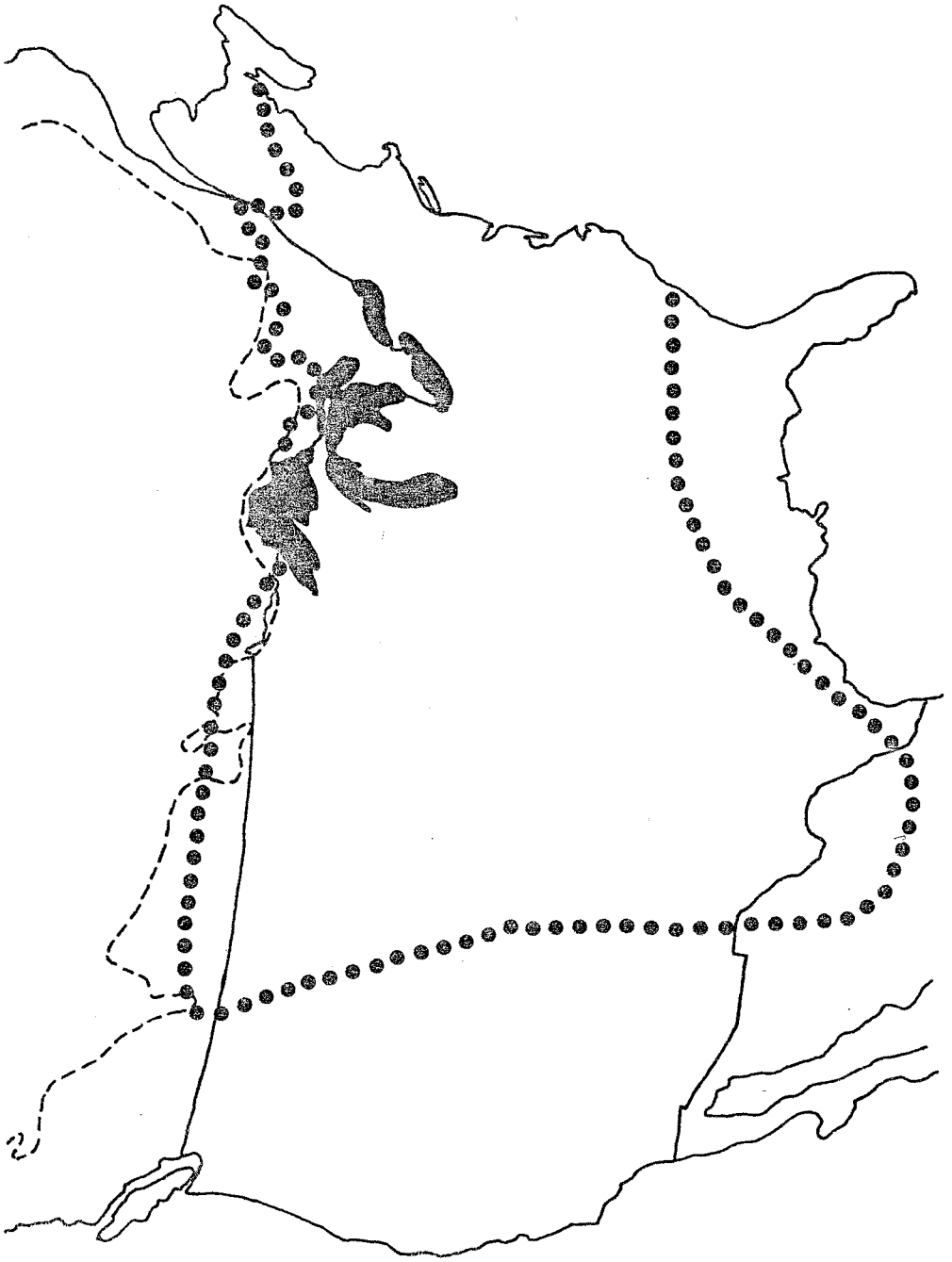
1. Supercooling of intracellular water appears to be the method of freezing avoidance in Vitis riparia. Because of this, the species is limited in its northern distribution to its present boundary, which coincides closely with the -40°C average winter minimum temperature isotherm(Fig. 23). This mechanism places a limit on the hardiness which can be achieved through breeding, unless genotypes can be found which do not possess supercooling as a means of freezing avoidance.

2. V. riparia is unique among those woody plants investigated by DTA, in that all its tissues(buds, phloem and xylem) apparently have a common means of cold injury avoidance, i.e. deep supercooling of intracellular water.

3. During intense cold periods, buds can dehydrate and thereby withstand very low temperatures(to at least -46.5°C). If, as in the case of blueberries(17), less hardy species do not exhibit the phenomenon of bud dehydration, DTA could be used in a breeding program as a rapid means of identifying genotypes possessing the capacity of bud dehydration.

4. DTA at fast rates can be used as a rapid method of determining bud and xylem hardiness in V. riparia. Further studies are necessary to determine if other Vitis species utilize supercooling as a means of freezing avoidance, and if in V. riparia DTA at very fast rates can accurately determine hardiness

Figure 23. Natural range of Vitis riparia(dotted line) and -40°C average winter minimum isotherm(dashed line).



of tissues other than buds and xylem.

5. DTA cannot be used as a viability test in V. riparia, because dead tissues also exhibit low temperature exotherms. Some other viability test, such as tissue browning, must therefore be used. Further work is necessary to determine if the same relationship holds true in other Vitis species.

6. In V. riparia stem tissues may be ranked according to decreasing order of hardiness as xylem > phloem rays > phloem parenchyma, and in buds, secondary, tertiary > primary. This means that the phloem parenchyma and primary buds, being the most critical tissues, should be the focal point of hardiness studies in V. riparia.

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