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Supercooling in Overwintering Azalea Flower Buds[1]

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ABSTRACT

Differential thermal analysis and nuclear magnetic resonance spectroscopy experiments on whole flower buds and excised floral primordia of azalea (*Rhododendron kosterianum*, Schneid.) proved that supercooling is the mode of freezing resistance (avoidance) of azalea flower primordia. Increase in the linewidth of nuclear magnetic resonance spectra for water upon thawing supports the view that injury to the primordia occurs at the moment of freezing. Nonliving primordia freeze at the same temperatures as living primordia, indicating that morphological features of primordial tissues are a key factor in freezing avoidance of dormant azalea flower primordia. Differential thermal analyses was used to study the relationship of cooling rate to the freezing points of floral primordia in whole flower buds. At a cooling rate of 8.5 C per hour, primordia in whole buds froze at about the same subfreezing temperatures as did excised primordia cooled at 37 C per hour. At more rapid cooling rates primordia in intact buds froze at higher temperatures.

Avoidance of freezing injury by supercooling has been implicated in the survival of many overwintering insects (2, 23–25). In contrast, as noted by Levitt (12), supercooling has rarely been demonstrated to be an avoidance mechanism in plants, although many early hardiness investigators believed that plants which survived winter did so by completely avoiding tissue freezing. Polyhydric alcohols and other antifreeze substances have been found in plant tissues, but not at concentrations likely to afford significant protection (22).

In 1906, Weigand (33) observed ice in living twigs during winter. He also showed, however, that certain living vegetative buds could be cooled to −26.5 C before ice crystals were observed. Recent work has verified that some plant tissues do in fact survive by avoiding freezing. Graham (8) demonstrated that dormant azalea flower primordia in winter do not freeze slightly below 0 C as do the stem tissues on which they are born. In mid-winter, excised primordia from buds of some hardy clones did not freeze above −40 C. Similar freezing avoidance has been observed in flower primordia of sweet cherry and may be a common phenomenon. In azalea, primordia invariably survived low temperatures if they did not freeze, but death occurred if a primordium froze (8). In contrast, hardy stems characteristically supercool only 2 to 6 C before freezing (15); however, they can tolerate the presence of this ice and survive.

Considerable research has been conducted on various horticultural crops to measure the seasonal patterns of flower bud hardiness (8, 18), the influence of environment on winter fluctuations in hardiness (19), and to identify genetic differences in bud hardiness (32). One question, however, has not been answered. If flower primordia do avoid freezing, how do they avoid freezing to such low temperatures? Specifically, do dormant buds contain antifreeze substances which lower their freezing point? Do they somehow supercool and resist nucleation via contiguous frozen stem tissue? Do scales, hairs, or other gross morphological features of the bud afford protection in some way? These experiments were designed to elucidate these questions and to provide a basis for hypothesizing how dormant flower primordia effectively avoid freezing.

The literature provides a clear but negative answer to the possibility that gross morphological features afford any significant protection. Weigand's work (33) in 1906 soundly disproved the then commonly held notion that bud scales, cottony hairs, or other bud parts provided thermal insulation from freezing winter temperatures. To quote Weigand, "To keep out the cold during an entire cold spell in winter would require, even in much thicker tissue, an almost absolute nonconductivity, and that is possessed by few if any substances in nature, much less by the bud scales. This erroneous impression has arisen probably through comparing the action of bud scales with that of clothing upon the human body, forgetting the fact that in the body there is a constant source of heat without which clothing could not keep it warm for more than a few minutes."

Remarkably, almost 70 years later, the idea that bud-scales and other bud parts provide protective thermal covering for meristematic tissues is still mentioned in some texts (6). Dorsey and Strausbaugh (4) noted that in dormant buds of plum, ice was found in the bud scales and axis, but not in the primordial region at −29.5 C. These studies did not implicate supercooling as the mechanism for the avoidance of freezing injury.

DTA[3] and NMR are used in the experiments reported here to observe the status of water in floral primordia. DTA and freezing curves in general have been used to monitor the formation of ice in plant tissues by the rise in tissue temperature associated with the heat of fusion of water (15, 21). NMR has frequently been employed to study water in animal tissues (31). Results show that the NMR line of cellular water is significantly broader than it is in pure water, which has been interpreted to mean that cellular water consists of several popula-

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[3] Abbreviations: DTA: differential thermal analysis; NMR: nuclear magnetic resonance.

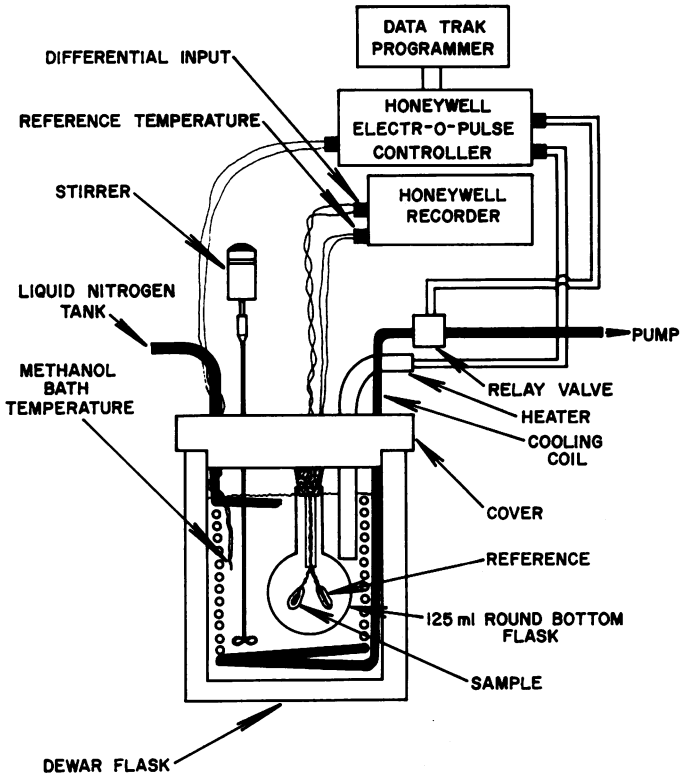

FIG. 1. Diagram of system used for differential thermal analysis.

tions of water molecules including a bound or ordered fraction in association with macromolecular constituents (9, 31). In addition to estimating the molecular associations of water molecules, the total amount of water which is not solid can also be measured. This is possible since the NMR line widths for solids (including ice) are usually 30,000 Hz or greater, while line widths of aqueous phases in tissues vary from a few Hz to 8000 Hz. Kuntz and coworkers (10) have used this property to study liquid water in frozen protein solutions. Toledo (30) and Sussman (28) have used it to measure quantitatively the liquid water in frozen wheat flour dough and fish muscle. Both DTA and NMR are convenient tools for investigation of water in living systems, since they are essentially nondestructive and yield useful dynamic and structural information concerning the status of cellular water.

MATERIALS AND METHODS

DTA studies were carried out on whole flower buds and excised primordia of deciduous azalea. NMR spectroscopy was performed on excised primordia. Tests were conducted on dormant materials during the period 12 February to 23 April 1973.

Plant Materials. Flower buds were collected as required from outdoor plantings of deciduous azalea (*Rhododendron kosterianum*, Schneid.) at the University of Minnesota Landscape Arboretum near Excelsior. Sufficient material for analysis was collected from an individual plant on a single date to complete one series of tests. Buds were stored at 5 C in stoppered flasks until tested. Storage did not exceed 7 days. Buds were picked by hand in a manner which separated the bud from the differentiated stem at the base of the bud.

Differential Thermal Analysis. Whole buds or excised primordia were placed in a small section of aluminum foil and attached to one side of a differential thermocouple so the thermocouple touched the bud (Fig. 1). A similar section of aluminum foil was placed around the reference thermocouple. The sample and reference were then enclosed in a 125-ml round bottom flask and submerged in a Dewar flask filled with methanol. A cooling coil and electric heater were used to vary the temperature of the methanol bath. Cooling (or thawing) was regulated automatically using a Honeywell Electr-O-Pulse (Model 80551-13) controller and Data Trak programmer (Model 5300). Temperature of the methanol bath was regulated to ± 0.25 C of the programmed value. Temperature difference between the sample and reference was recorded on a Honeywell Electronik 194 recorder.

Two series of tests using this procedure were performed: freezing-thawing analysis of whole buds and excised primordia and a cooling rate study on whole buds.

Freezing and Thawing Analysis of Whole Buds and Excised Primordia. Whole flower buds (one per test) were cooled at 8.5 C/hr until samples froze completely. Freezing was judged to be complete when dissipation of all the latent heat of fusion was achieved (as measured by DTA). The sample was then allowed to rewarm until all ice had melted as indicated by DTA measurement of heat absorption. For whole buds rewarming involved equilibration of the entire system to room temperature. Rates of warming did not exceed 11.0 C/hr.

Excised floral primordia (one per test) were cooled at 8.5 C/hr and rewarmed at 8.5 C/hr in the same manner as described above for the whole bud. The rewarming rate was controlled automatically in these tests.

Cooling Rate Study on Whole Buds. Whole buds obtained from an individual plant were cooled at 8.5 C/hr, 18.8 C/hr, and 37 C/hr. Excised primordia from a bud picked at the same time were cooled at 37.0 C/hr to determine differences between intact and excised primordia. Only one rate was used for excised primordia in view of Graham's finding (8) that excised primordia exhibit no significant difference in freezing temperatures when cooled at rates ranging from 3 C/hr to 180 C/hr. Although hardiness evaluation was not a primary objective of this investigation, injury was noted by visual inspection for browning of tissue after rewarming to room temperature.

Nuclear Magnetic Resonance Spectroscopy. A Varian A-60D high resolution NMR spectrometer was used to determine the spectra of water in excised primordia over a range +5 C to −50 C. The water spectra were monitored during freezing to −50 C and thawing to +5 C. At each intermediate temperature at which spectral measurements were made, the instrument was stabilized and allowed to equilibrate for approximately 20 min before the spectrum was recorded. Sample spinning was not necessary.

RESULTS

Differential Thermal Analysis. DTA recordings of cooling whole buds at 8.5 C/hr (Fig. 2) clearly show the major exotherms (release of heat of fusion) associated with ice formation. The large first exotherm results from freezing of water in the bud scales while succeeding exotherms result from freezing of the primordia (8). It was observed that the bud scale exotherm displayed no variation during the period when experiments were conducted. However, in early spring primordia exotherms occurred at warmer temperatures in agreement with Graham's work (8) (Fig. 2). An important result is that the number of exotherms produced in tests at 8.5 C/hr generally correspond to the total number of primordia even though a significant number may have been dead sometime before the tests (Table I). Primordia killed naturally were easily observed, since they were a deep brown color and more desiccated than primordia killed during the tests. Exotherms of dead primordia were in every way similar to those of the living primordia, but smaller

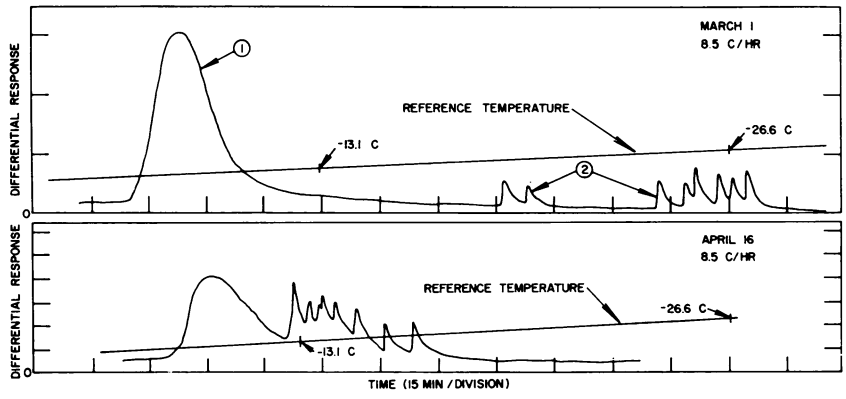

FIG. 2. DTA recordings of freezing (exotherm) profiles of whole azalea flower buds cooled at 8.5 C/hr. On 1 Mar, the bud is a typical mid-winter one. On 16 Apr the convergence of exotherms indicates loss of hardiness in early spring. Exotherm 1 corresponds to freezing of water in the bud scales and stem axis upon which the primorida are attached. Exotherms of type 2 indicate freezing of an individual primordium. The number of primordia exotherms generally equals the number of primordia in the bud at a cooling rate of 8.5 C/hr. Each division on the Differential Response scale equals approximately 1.4 C.

Table I. *Freezing Temperatures of Flower Primordia in Whole Azalea Buds Cooled at 8.5 C/hr and Excised Primordia Cooled at 37 C/hr*

Date	Tissue	Cooling Rate	Primordia Exotherm Temperatures[1]
		C/hr	*-C*
21 Mar	Whole bud	8.5	13.2, 15.8, 16.5, 16.9, 17.2, 18.9, 19.0, 19.3, 19.7, 20.9, 22.8
	Excised primordia[2]	37.0	17.2, 19.0, 21.3, 23.4, 25.0, 26.5
27 Mar	Whole bud	8.5	**18.1**, 19.9, **20.8**, 20.9, **21.5, 21.6, 22.7, 23.0, 23.1, 23.5**
	Excised primordia[3]	37.0	19.2, 20.9, 23.2, 24.7, 25.7, 26.2
16 Apr	Whole bud	8.5	12.8, 13.0(2), 13.2, 13.8, 13.9, 14.5, 15.3, 16.5, 17.6
	Excised primordia	37.0	12.3, 13.8, 14.5, 15.7, 16.7(2), 18.2, 20 1, 20.9, 22.6, 24.3
20 Apr[4]	Whole bud	8.5	**12.8, 13.1, 15.3, 18.4**, 19.1, **20.0, 20.9, 21.3, 21.5, 22.6, 22.8**
	Excised primordia	37.0	14.7, 17.2, 20.9

[1] **Bold type** temperatures correspond to exotherms of primordia that were dead before the test.

[2] Only living primordia were used in tests on excised primordia. Number of exotherm temperatures equal the number of excised primordia tested. For the test referenced, six living and four dead primordia were excised. Living primordia had water content 65.7% fresh wt and dead primordia had water content of 52.5% fresh wt.

[3] For the test referenced, six living primordia had water content 65% fresh wt and four dead primordia had water content 57.5% of fresh wt.

[4] Materials used here were taken from a plant removed from the Minnesota Landscape Arboretum 6 Mar and stored at +5 C.

in magnitude as a result of the decreased water content. Freezing of living primordia during cooling always produced browning of floral parts as noted by visual inspection after rewarming to room temperature. A third type exotherm was also observed (Fig. 3) and may be analogous to the D exotherm reported in apple stems by Quamme *et al.* (21). The third exotherm was not investigated further in this study.

Freezing and thawing analysis of whole buds and individual primordia by DTA gave exotherm and endotherm profiles shown in Figure 3. Thawing was essentially complete at a temperature of −2 ± 1 C. Area under the exotherm profiles was always within ± 20% of the area under the endotherm profiles. Comparison of the areas is somewhat difficult because the length of the strip chart recordings was such that small changes in drawing of the base line produced 10 to 15% variation in area determination.

Temperatures and the profile of primordia exotherms vary considerably with an increase in the cooling rate from 8.5 C/hr to 18.8 C/hr, whereas little variation is exhibited between rates of 18.8 C/hr and 37 C/hr (Fig. 4). Cooling at 18.8 C/hr and 37 C/hr elevated the temperature of primordia nucleation and showed that many primordia freeze at or near the same temperature. This result was common through all tests until early spring. In early spring, cooling at 37 C/hr caused primordia to freeze over the same range as the other bud parts and no distinct exotherms were observed. Exotherm temperatures of primordia in whole buds cooled at 8.5 C/hr approached those of excised primordia cooled at 37 C/hr (Table I).

Nuclear Magnetic Resonance Spectroscopy. In cooling to −50 C, the linewidth of the H_2O spectra remained at 210 ± 20 Hz at all temperatures until no NMR signal (liquid water) could be detected (Fig. 5). No change in the amplitude of the spectra (after correction for the Boltzmann effect) was distinguished up to the time of freezing at −26 C. Amplitude remained constant at the new level to −34 C when the band disappeared indicating all detectable water had crystallized. The freezing at −26 and −34 C was completed in less than 2 min.

In other tests performed, but not shown here, freezing occurred at only one temperature and all observable NMR signal (liquid water) was lost at the freezing point. These variations are assumed to result from the separation of floral parts when the primordia were excised from the bud, allowing individual parts to freeze at somewhat different temperatures. On rewarming from −50 C, the first detectable liquid water was observed at a temperature slightly greater than −26 C, but extensive thawing did not occur until temperature approached 0 C. Spectra were recorded during warming at −26 C, −20 C, and +5 C. At each point the temperature was stabilized for approximately 20 min to observe if continued thawing would occur. Spectra were always found to reach a constant amplitude within 5 min of a change in temperature and no variation could be detected in the remaining 15 min. Linewidth for all spectra recorded on rewarming was 460 ± 20 Hz.

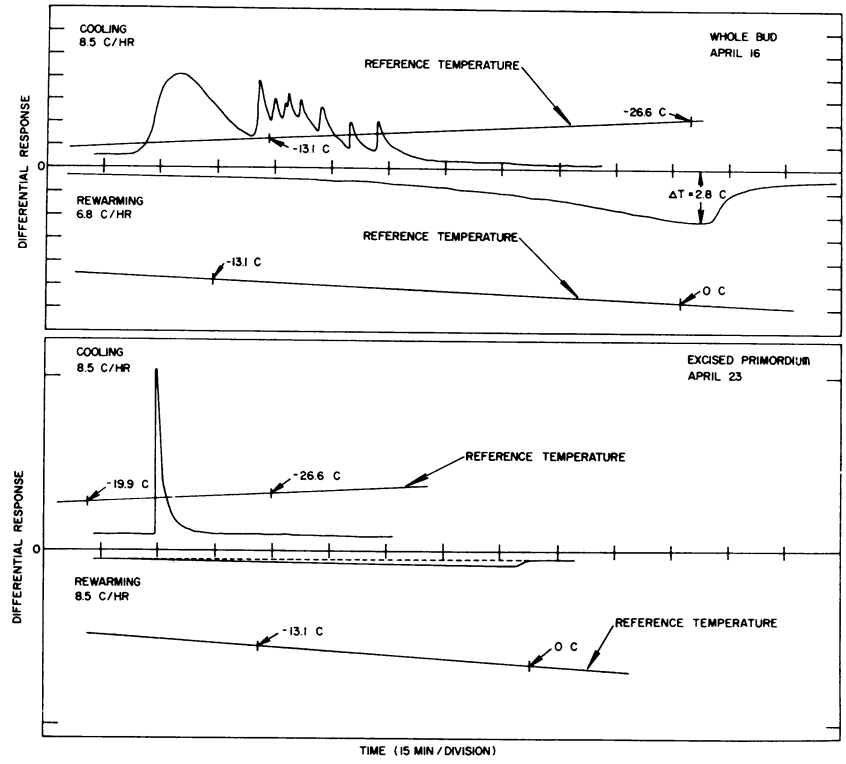

FIG. 3. Freezing (exotherm) and thawing (endotherm) profiles of a whole azalea flower bud and an excised floral primordium recorded by DTA. Each division on the Differential Response scale equals approximately 1.4 C.

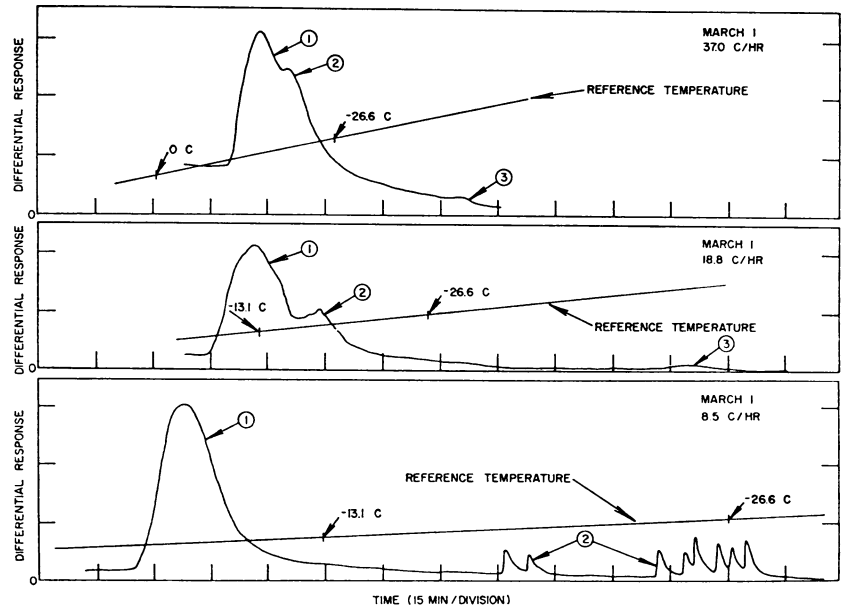

FIG. 4. Freezing (exotherm) profiles of whole azalea flower buds, collected on 1 Mar, cooled at 37.0 C/hr, 18.8 C/hr, and 8.5 C/hr, recorded by DTA. Exotherm 1 corresponds to freezing of water in the bud scales and stem axis upon which the primorida are attached. Exotherms of type 2 refer to feezing of the primordia. A third exotherm is not shown for the 8.5 C/hr cooling rate. Each division on the Differential Response scale equals approximately 1.4 C.

DISCUSSION

Most plant tissue when cooled and then rewarmed slowly will follow the general rule that freezing and completion of thawing will occur at the same temperature. This was clearly not the case for primordia of azalea flower buds in this study. Results of DTA and NMR unquestionably demonstrate the presence of a "metastable equilibrium" of liquid water (*i.e.* supercooling) in primordia at subzero temperatures.

The other possible explanation of the freezing avoidance capabilities of floral primordia would be that some substantial freezing point depression or eutectic freezing phenomenon exists. While solutes in the living cell undoubtedly depress the freezing point somewhat and progressively lower the freezing

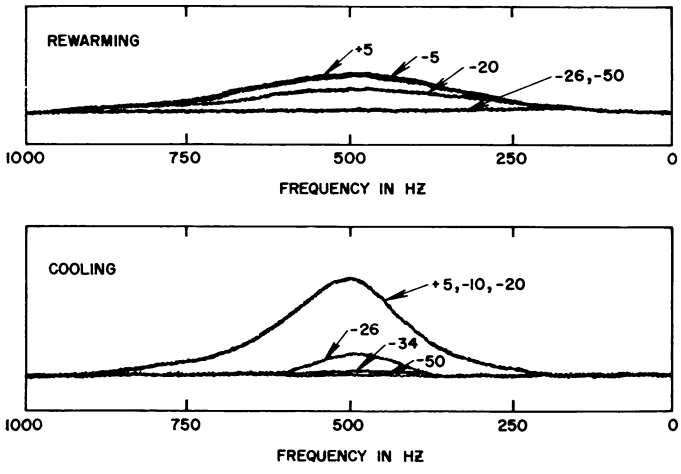

FIG. 5. NMR spectra of unfrozen water at selected temperatures (C) in an excised azalea floral primordium cooled from +5 to −50 C and rewarmed to +5 C. While cooling, spectra remained constant at temperatures between those where recordings were made. While rewarming, spectra changed in a continuous fashion between −26 C and complete thawing.

point as the cell sap becomes more concentrated during freezing, the contribution of this type of "protection" to the freezing avoidance observed seems minor. Freezing point depression and eutectic freezing phenomenon are well defined solid-liquid equilibrium processes that exhibit the same freezing and thawing points if examined under equilibrium conditions (7, 16). As stated by Glasstone (7), "the term metastable is used to describe a definite equilibrium, which is nevertheless not the most stable equilibrium at the given temperature; a metastable system undergoes a spontaneous change on the addition of the stable phase." A metastable system will not undergo solid to liquid phase change at the same temperature as the liquid to solid transition. This is precisely what was observed in the experiments reported here (Figs. 4 and 5). Freezing of primordia always occurred at a much lower temperature than the temperature at which thawing was completed. Complete thawing of water as observed by DTA and NMR was over a range slightly greater than the freezing point to −2 ± 1 C. During thawing equilibration of the test systems (DTA or NMR) at any temperature in this range stopped the solid to liquid transition and it did not continue until warming was resumed.

The small freezing point depression observed in DTA experiments (−2 ± 1 C) is typical of woody plants in general (15). Primordia avoid freezing and associated injury by supercooling and avoiding freezing at low temperatures. It should also be noted here that the broadening of the NMR spectra for water upon warming indicates that a change had occurred in the ordered state of water (31). Graham's freezing curve studies of overwintering azalea flower buds showed that flower primordia were killed either when they froze or during the thawing process (8). When whole buds subjected to freezing stress were rewarmed after a specified number of primordia had frozen (as noted by the associated exotherm), the number of dead primordia always corresponded to the number of exotherms. Remaining unfrozen primordia were green and apparently not injured. Change in the NMR line width circumstantially supports the view that injury occurs as a result of water crystallization and not as a result of thawing or rewarming. While attempts have not been made to observe ice formation microscopically in flower primordia during cooling it seems highly likely that this sudden freezing of substantially supercooled tissue involved intracellular freezing. This supposition is consonant with the invariably lethal affects of freezing on the primordia in which it occurs and with the rapidity with which freezing takes place. This study does not provide the basis for any explicit description of how the primordia supercool to such low temperatures. It is possible, however, to visualize some hypothetical factors which could account for the supercooling behavior of flower primordia.

Many investigators have shown that very pure water droplets can be supercooled to low temperatures, but never below approximately −40 C (3, 5, 11, 29). A relationship between the homogeneous nucleation temperature and droplet diameter has been developed by Fletcher (5) based on phase transition (or nucleation) theory. From this it can be estimated that in a volume of pure water 1 cm^3, one ice nucleus of critical radius will form/sec at about −30 C. If the volume is reduced to 10^{-5} cm^3, the rate of formation will be 10^{-5} nuclei/sec. In a system consisting of 10^6 such small (10^{-5} cm^3) volumes about 10^1 nuclei would form per day at −30 C. The dimension and number of volumes used as examples here have been based on electron microscopy studies of cellular size in *Rhododendron* flower buds by Schneider (26) and measurement of the actual dimensions of azalea floral primordia.

It should be noted that the numerical value for the flux of nuclei formation is by no means absolute owing to the inherent difficulties in applying nucleation theory to solid-liquid phase transitions (1, 5), especially in assuming a value for the surface tension between the solid and liquid phases. In addition, this discussion is based on the assumption that the water droplets are very pure. Foreign surfaces and suspended insoluble particles promote heterogeneous nucleation at higher temperatures. The degree of freezing point elevation depends on the size and surface properties of the impurities (5). Solutes, however, generally increase the supercooling necessary for crystallization in small droplets (20, 29). Recent work by Rasmussen and Mackensie (29) using emulsified aqueous solutions in heptane and silicon oil have shown that solutes including glucose, urea, PVP, NaCl, glycerol, ethylene glycol, and NH_4F not only increase the supercooling required for nucleation of ice, but depress the nucleation temperature (as compared to distilled water) more than they depress the melting point.

Although one would reason that the living cell provides many sites for heterogeneous nucleation, MacKenzie *et al.* (13) indicate that ice-like structures necessary for heterogeneous nucleation of ice do not generally exist in the cell and that the cytoplasm behaves much like a dilute aqueous solution. Their proposal is based on experiments in which individual yeast cells apparently survived supercooling to −37 C before freezing by homogeneous nucleation.

Considering again the system of small volumes outlined above, suppose these small volumes are isolated from each other by some form of nucleation barrier (Fig. 6). If this barrier consists of small capillary connections the freezing point of the whole system may be depressed significantly depending on the diameter of the capillaries. Mazur (14) has considered this problem in relation to ice penetration of the plasma membrane. His derivations determined that to depress the freezing point −30 C the diameter of the capillaries must be approximately 10^{-7} cm. Capillaries of this size or smaller could prevent the propagation of ice throughout the whole system at temperatures above −30 C. Above −30 C the spontaneous formation of an ice crystal of the critical radius in any small volume would only nucleate that one volume, while below −30 C the first nucleation will propagate ice through the entire 10^6 small volumes.

Such a mechanism of supercooling seems plausible in flower

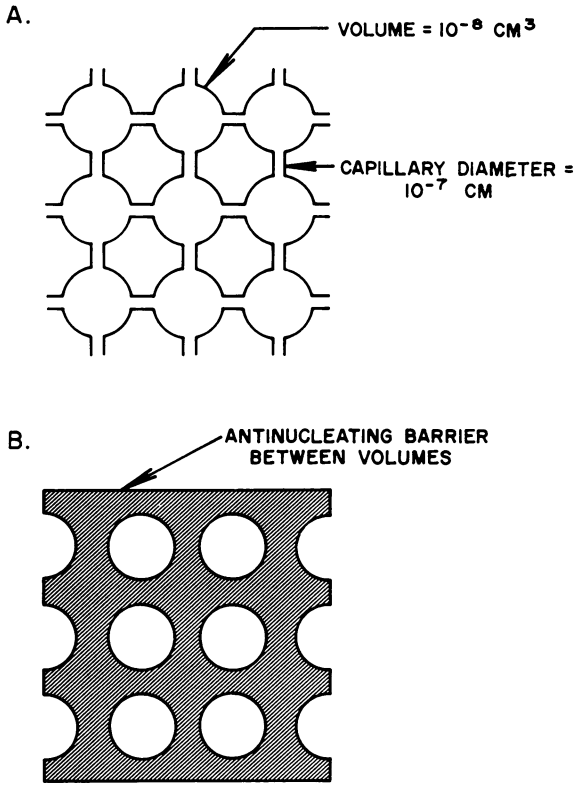

FIG. 6. Schematic representation of a hypothetical system capable of supercooling to approximately −30 C. A: Small volumes separated by capillaries; B: small volumes separated by an antinucleating barrier. Supercooling appreciably below −40 C cannot be explained by this arrangement (5).

primordia in which cells are small and tightly packed, where there are no large intercellular spaces, and where the cell walls are thin (26). Graham (8) observed in his studies that the water content of primordia was closely correlated with the freezing (killing) temperature. One can visualize the redistribution of water away from larger volumes as a result of surface properties thereby reducing the likelihood of ice nucleation and propagation. Equally attractive as a capillary barrier is the possibility that the nucleation barrier between small volumes may contain chemical antinucleating agents which could prevent ice propagation. A final possibility would be that as the probability of forming a nucleus of critical radius reaches its threshold value (5), all small volumes nucleate over a small temperature range. Threshold nucleation of all cells in a primordium at approximately the same temperature appears unlikely since separated primordium parts nucleate at different temperatures, while intact primordia freeze completely at one temperature.

Further investigation may well reveal that some biochemical mechanisms play a major role in the resistance of primordia to freezing. However, the finding that primordia which have been dead for some time still freeze in the same manner as living primordia leads one to the conclusion that the structural nature of the tissue is the key factor. Investigation of the physical and chemical properties of the cell wall would seem to be a logical point for future work to further elucidate the supercooling properties of dormant buds. The structural role of the cuticle in the supercooling of insects has been discussed in some detail by Salt (25).

Regardless of the exact nature by which the primordia are able to supercool, DTA can be useful for quickly estimating hardiness of azalea flower buds provided the test cooling rates cause primordia to freeze at the same temperatures they would freeze at under natural conditions. Freezing tests conducted on whole buds (Fig. 4) revealed that the freezing temperatures of primordia approached limiting values at a cooling rate of 8.5 C/hr. These limiting values are approximately the same as the freezing temperatures of excised primordia cooled at 37 C/hr. Excised primordia freezing temperatures are independent of cooling rate (8). This evidence seems to support the reasoning that attachment of the primordia in the bud via the vascular network may play an important role in determining the nucleation point at different cooling rates. Freezing and associated injury to the floral primordia in winter cereals (27) seem to depend on the ability of the vascular tissue to stop the advance of the freezing boundary. Nonequilibrium freezing as discussed by Olien (17) may produce mechanical injury in tissues of rigid structure. Although no definite conclusions on this point may be drawn from the findings in this report, it is possible that nonequilibrium freezing and associated mechanical stress near the region of attachment of the primordium to the stem axis in the flower bud may be involved in the observed effects of cooling rate on primordium freezing. In early spring when growth processes begin, cell division and development of large intercellular spaces in the primordial tissues (26) could contribute to the ease with which ice nucleates and propagates into the floral primordia at higher cooling rates. This may explain why in early spring primordia are no more resistant to nucleation than other bud parts at a cooling rate of 37 C/hr. Similar studies (unpublished) in this laboratory on sweet cherry flower buds which were just breaking dormancy have shown that while excised floral primordia did not nucleate above −20 C, primordia in whole buds froze along with other bud parts at about −10 C; an indication that nucleation proceeds via the bud stem axis to the floral primordia at this stage of development.

DTA and NMR spectroscopy experiments have shown that supercooling is the mechanism of resistance to freezing and associated injury in the floral primordia. Influence of cooling rate on the freezing point of floral primordia in azalea flower buds has been demonstrated.

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LITERATURE CITED

1. ADAMSON, A. W. 1966. Physical Chemistry of Surfaces. Interscience Publishers, New York.
2. ASAHINA, E., K. AOKI, AND J. SHINOZAKI. 1954. The freezing process of frost hardy caterpillars. Bull. Entomol. Res. 45: 329-339.
3. BIGG, E. K. 1953. The supercooling of water. Proc. Phys. Soc. B. 66: 688-694.
4. DORSEY, M. J. AND P. D. STRAUSBAUGH. 1923. Winter injury to plum during dormancy. Bot. Gaz. 76: 113-142.
5. FLETCHER, N. H. 1970. The Chemical Physics of Ice. Cambridge University Press, Cambridge.
6. GALSTON, A. W. AND P. J. DAVIES. 1970. Control Mechanisms in Plant Development. Prentice-Hall, Inc., Englewood Cliffs, N. J. pp. 135-136.
7. GLASSTONE, S. 1946. Textbook of Physical Chemistry, Ed. 2. D. Van Nostrand Company, Inc., New York.
8. GRAHAM, R. P. 1971. Cold injury and its determination in selected *Rhododendron* species. Master's thesis. University of Minnesota, St. Paul.
9. HAZELWOOD, C. F., B. L. NICHOLS, AND N. F. CHAMBERLAIN. 1969. Evidence for the existence of two phases of ordered water in skeletal muscle. Nature 222: 747-750.
10. KUNTZ, I. D., T. S. BRASSFIELD, G. D. LAW, AND G. V. PURCELL. 1969. Hydration of macromolecules. Science 163: 1329-1330.
11. LANGHAM, E. J. AND B. J. MASON. 1958. The heterogeneous and homogeneous nucleation of supercooled water. Proc. Roy. Soc. London A 247: 493-504.
12. LEVITT, J. 1972. Responses of Plants to Environmental Stresses. Academic Press, New York.
13. MACKENZIE, A. P., D. H. RASMUSSEN, AND M. N. MACAULAY. 1973. Deep supercooling of *Saccharomyces cerevisiae*. Society for Cryobiology, 10th Ann. Meeting, Key Biscayne, Fla.

14. MAZUR, P. 1966. Basis of freezing injury. *In:* H. T. Meryman, ed., Cryobiology. Academic Press, New York. pp. 213–315.
15. MCLEESTER, R. C., C. J. WEISER, AND T. C. HALL. 1968. Seasonal variations in freezing curves of stem sections of *Cornus stolonifera* Michx. Plant Cell Physiol. 9: 807–817.
16. MERYMAN, H. T. 1966. Review of biological freezing. *In:* H. T. Meryman, ed., Cryobiology. Academic Press, New York. pp. 3–114.
17. OLIEN, C. R. 1967. Freezing stresses and survival. Annu. Rev. Plant Physiol. 18: 387–408.
18. PROEBSTING, E. L. 1972. A comparison of hardiness responses in fruit buds of "Bing" cherry and "Elberta" peach. Amer. Soc. Hort. Sci. 97: 802–806.
19. PROEBSTING, E. L. 1970. Relation of fall and winter temperatures to flower bud behavior and wood hardiness of deciduous fruit trees. Hort. Sci. 5(5): 22–24.
20. PRUPPACHER, H. R. 1963. Some relations between supercooling and the structure of aqueous solutions. J. Chem. Phys. 39: 1586–1594.
21. QUAMME, H., C. STUSHNOFF, AND C. J. WEISER. 1972. The relationship of exotherm to cold injury in apple stem tissues. Amer. Soc. Hort. Sci. 97: 608–613.
22. SAKAI, A. 1961. Effect of polyhydric alcohols to frost hardiness in plants. Nature 189: 608–613.
23. SALT, R. W. 1950. Time as a factor in the freezing of undercooled insects. Can. J. Res. D, 28: 285–291.
24. SALT, R. W. 1961. Principles of insect cold-hardiness. Annu. Rev. Entomol. 6: 55–74.
25. SALT, R. W. 1963. Delayed inoculative freezing of insects. Can. Entomol. 95: 1190–1202.
26. SCHNEIDER, E. F. 1972. The rest period of *Rhododendron* flower buds. J. Exp. Bot. 77: 1021–1038.
27. SINGLE, W. V. 1964. Studies on frost injury to wheat. II. Ice formation within the plant. Aust. J. Agr. Res. 15: 869–875.
28. SUSSMAN, M. V. AND L. CHIN, 1966. Liquid water in frozen tissue: Study in nuclear magnetic resonance. Science 151: 324–325.
29. RASMUSSEN, D. H. AND A. P. MACKENZIE. 1972. Effect of solute on ice-solution interfacial free energy; calculation from measured homogeneous nucleation temperatures. *In:* H. H. G. Jellinek, ed., Water Structure at the Water-Polymer Interface. Plenum Publishing Corporation, New York. pp. 126–145.
30. TOLEDO, R., M. P. STEINBERG, AND I. A. NELSON. 1968. Quantitative determination of bound water by NMR. J. Food Sci. 33: 315–317.
31. WALTER, J. A. AND A. B. HOPE. 1972. Nuclear magnetic resonances and the state of water in cells. Prog. Biophys. 23: 3–20.
32. WEAVER, G. M., H. O. JACKSON, AND F. D. STROUD. 1968. Assessment of winter hardiness in peach cultivars by electric impedance, scion diameter and artificial freezing studies. Can. J. Plant Sci. 48: 37–47.
33. WEIGAND, K. M. 1906. Some studies regarding the biology of buds and twigs in winter. Bot. Gaz. 103: 373–424.