THURSDAY AFTERNOON SESSION

December 9, 1965

The afternoon session convened at 1:00 p.m. A question and answer session led by the mist symposium panel was held at the beginning of the afternoon session. The discussion is included in the Thursday Morning Session of the Proceedings. At the conclusion of the mist symposium discussion, Vice President Peter Vermeulen introduced Mr. David G. Leach who served as moderator for the balance of the afternoon session.

MODERATOR LEACH: We have three talks this afternoon with five minutes for questions after each talk. Our first speaker this afternoon is Dr. C. J. Weiser from the University of Minnesota. Dr. Weiser is a plant physiologist who specializes in problems of plant hardiness and as we all know he comes from about as tough a section of the country as anyone in this Society.

PRINCIPLES OF HARDINESS AND SURVIVAL AS THEY RELATE TO NEWLY PROPAGATED PLANTS[1,2]

CONRAD J. WEISER
Horticultural Science Department
University of Minnesota
St. Paul 1, Minnesota

Plant survival at low temperatures has been a vexing problem since man first gathered the fruits of the fields to provide sustenance for himself. Today a nurseryman in the Great Plains or an orange grower in Florida would both agree that low temperature injury is a most serious problem. In fact on much of the earth's surface low temperature is the single most limiting factor to plant growth and survival.

In the discussion to follow, we will attempt to provide a basis for the panel discussion to follow. I will emphasize research at the University of Minnesota, not because it is necessarily the best but because time is limiting and it is most familiar to me.

There are a number of factors which complicate the study of plant hardiness. Winter damage can be caused by several different environmental stresses. For example, desiccation, early fall or late spring frosts, rapid temperature changes, and extreme low temperatures in midwinter can cause damage either individually or in combination. It is obviously necessary to establish what type of stress is causing injury before you can intelligently cope with it. Unfortunately the cause of injury may be quite different in different years or in different parts of the same plant.

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[2]The author expresses his gratitude to Bailey Nurseries, Newport, Minnesota for cooperation on some of the studies reported here and to the Louis W and Maud Hill Family Foundation for support of the research

Newly propagated stock is frequently not well established, not in the best nutritional status, often has limited food reserves and probably has recently been subjected to an abrupt change of environment. It's easy to see why not many researchers have been willing to add these additional variables to a problem which already has too many intangibles.

To complicate matters even further, we still don't know the answer to the two basic questions: How does freezing kill plants? and, How do some plants acclimate to resist freezing injury? Attempts to find practical means of reducing injury or increase a plant's cold resistance are often frustrated for lack of the basic knowledge.

To contribute something positive to the discussion, let's consider what *is* known. Ice crystals in the plant are the factor which kills cells. Ice within a cell (intracellular) is invariably lethal while ice between cells (extracellular) may or may not cause death.

If a hardy plant is exposed to gradually lowering temperatures the first ice forms between the cells where water is purest. As the temperature continues to decline, water moves out of the cell to extracellular ice nuclei and the cell sap becomes increasingly more concentrated with a lower freezing point and less water available to freeze. Most hardy plants survive this type of freezing, probably by avoiding intracellular ice formation. It has also been observed that the permeability of the cell membrane to water increases as plants harden. This may be one facet of the cold acclimation process.

If a hardy plant is exposed to rapidly decreasing temperature over the freezing range, however, injury frequently occurs. In such a case it is thought that water cannot move out of the cell fast enough to keep pace with declining temperature. When this happens, the cell contents supercool and then suddenly freeze intracellularly, causing death. This was illustrated by a study on American arborvitae (1) which would resist -125° F. when freezing was slow (9° F per hour) but which were killed at 15° F. when freezing was fast (18° F. per minute). Rates of this magnitude were measured on arborvitae foliage outside in winter when the sun's radiation was suddenly interrupted by shading from a building or some other obstruction.

If small pieces of living tissue are cooled extremely fast by plunging them into liquid nitrogen at -320° F., water in the cells may be converted into a solid vitrified state with no injurious ice crystals. There are cases where plant tissues have survived such treatment when they were warmed rapidly enough to avoid ice crystallization during warming. This observation substantiates the statement that ice crystals and not low temperature are the cause of damage.

There are several hypotheses about the destructive action of ice crystal formation. Some of the more frequently mentioned ideas include: A) A mechanical hypothesis considers that crystals puncture cell membranes or in some way disrupt cell continuity. B) A second hypothesis is that the removal of water from cells to ice crystals concentrates the salts in the cells to such an extent that proteins are irreversibly salted out and denatured. C) Levitt (2) has suggested that freezing injury is due to the formation of disulfide linkages between adjacent proteins when they come too close together due to water removal to ice crystals. D) Heber (3) has hypothesized that certain proteins need a water shell around them to maintain continuity. Death of cells results when freezing of water removes this water shell. At present, the first and fourth hypotheses seem the most reasonable.

How do plants develop resistance to freezing injury and what can we do culturally to protect them? These questions would be easier to answer if we knew which of the hypotheses of injury, if any, are correct.

Considering the first question, there are some ways in which cells could theoretically escape injury by avoiding freezing especially of the intracellular type. 1) High solute concentrations in the cell might give a freezing point depression just as a salt added to water lowers its freezing point. However, this usually can account for only a few degrees of protection. 2) Some insects are known to produce antifreeze substances such as glycerol and certain alcohols. But in plants, appreciable quantities have not been found. 3) Increases in water binding chemicals such as hydrophillic proteins may reduce the amount of free water available for freezing. Numerous workers have found that protein increases with cold resistance and we have recently verified this in red-osier dogwood (4). 4) Supercooling has been considered by some to be a means by which plants effectively avoid ice crystallization. Our studies usually indicate only a few degrees of supercooling however. Further, supercooling may be dangerous to a cell in that when crystallization finally occurs, it is very rapid and violent and is more likely to be intracellular. However, the cell membrane is a barrier to ice crystals and the possibility exists that the cell contents may supercool considerably even in the presence of extracellular ice. 5) Increases in membrane permeability to water have been observed during hardening and could enable plants to avoid intracellular ice by rapidly moving water out of the cell to extracell lular ice.

In this regard recent work by Kuiper (5) has indicated that decenylsuccinic acid, when taken up plant roots, increases the water permeability of the membranes six-fold. He also reports an increase in cold resistance of bean leaves and pear blossoms of as much as 10° - 12° F. after treatment. In nature an increased unsaturation of plant lipids during hardening has been observed in some plants including red-osier dogwood (6) which could account for increased water permeability of membranes.

In addition to escaping intracellular ice, there are some ideas about how plants may resist injury from extracellular ice. 1) It has been observed that hardened plants have a more elastic protoplasm than their non-hardened counterparts. This greater elasticity may allow the protoplasm to resist breaking and other mechanical disruption during frost plasmolysis or extreme dehydration. No one knows what makes the protoplasm more elastic. 2) Perhaps the most commonly observed chemical change in hardening plants is an increase in sugar and a decrease in starch. Heber (4), Sakai (7), Tumanov (8) and others have found that sugars can exert a considerable protective action against freezing. Heber thinks the sugars can substitute for water molecules as the protective shell around sensitive proteins by virtue of their -OH groups. Salts reversed the protective effects of sugars and other polyhydroxy compounds.

As introduction to the question of cultural means of increasing a plant's hardiness, we should first consider the phenomenon of natural acclimation. Figure 1 shows the seasonal cold resistance of the living bark of one year old red-osier dogwood twigs.

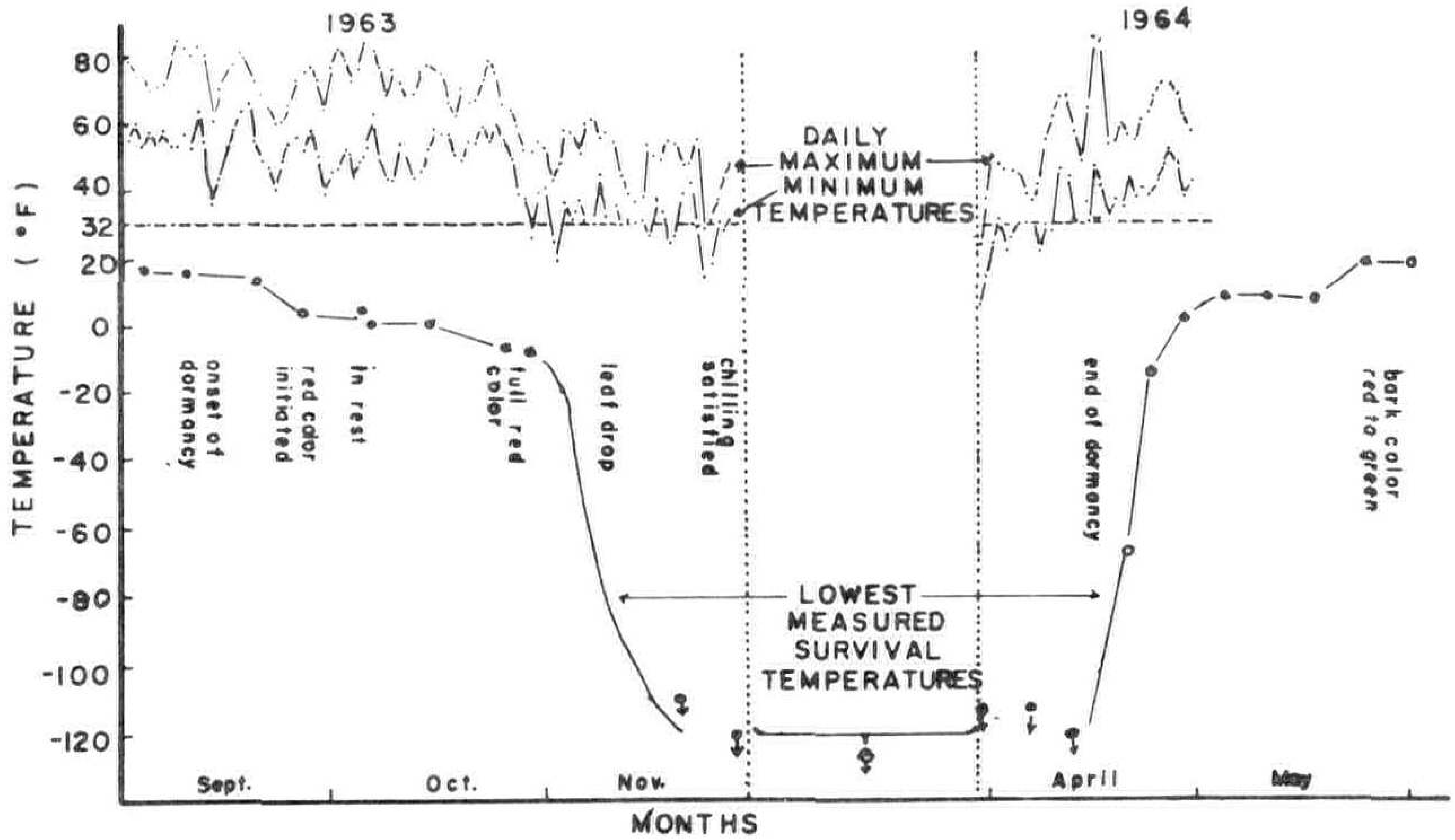

Figure 1. Seasonal changes in the cold hardiness of red-osier dogwood bark and other phenological changes ♀ indicates that the bark was not injured at the lowest test temperature.

The hardiness in this study was determined by exposing excised stem sections six inches long to a series of low temperatures in thermos bottles in a deep freeze (4). Thermocouples were used to measure the tissue temperatures which was gradually lowered at a rate not exceeding 9° F. per hour. After freezing, injury was determined by visual examination of bark correlated with regrowth ability and tetrazolium tests.

Cold acclimation occurred in two stages in the autumn. The first stage of hardening (to about 0° F.) occurred before any frosts in the fall. The second, and more dramatic stage occurred immediately after the first fall frosts and reached an undetermined level somewhere below-120° F. Hardiness was lost in the spring at the time growth started. This same pattern has been observed several years on American arborvitae as well as dogwood.

In trials to artificially cold acclimate dogwood plants in controlled environment chambers, we found it necessary to *first* expose them to short days and then freezing temperatures. The short day treatment induced rest period and in some way preconditioned the plants so they were capable of acclimating rapidly when exposed to freezing. Frost was necessary for maximum acclimation. For example, 35° F. following short days did not induce any cold resistance. Also short days or freezing temperatures alone did not induce any acclimation, nor did a simultaneous short day and freezing treatment. It would be very useful to understand the nature of the short day induction response and the triggering mechanism of freezing temperature. A number of people are studying this and perhaps someday someone will be able to artificially induce cold acclimation.

The relationship of rest period, dormancy, and cold resistance is worth considering because of its practical cultural implications. Although the relationships are not fully understood, it is evident in the woody plants we have studied that: 1) Cold acclimation in the autumn begins at the time growth ceases due to short day rest induction. 2) Cold resistance is undiminished during the winter even after the chilling requirement for breaking rest has been satisfied. Arborvitae (1) and raspberry (9) maintained hardiness even when exposed to unseasonable dehardening conditions after rest was ended. 3) Cold resistance disappears with the beginning of spring growth. In summary, dormancy seems to be necessary for acclimation. Rest period may play a role in the induction of cold acclimation but does not appear to be absolutely essential for maintaining maximum cold resistance during the winter.

A great number of physiological changes occur during cold acclimation which may or may not be causally related to the process. In dogwood we have noted a decrease in starch and an increase in soluble carbohydrates (mainly raffinose) (10); a sharp increase in malic acid at the time of acclimation; an increase in proteins and polar amino acids in proteins (4); increased unsaturation of some lipid components (6); an increase in organic phosphorous and a decrease in inorganic phosphorous and an increase in ribonucleic acid. At this time it is difficult to say which if any of these changes bear a causal relationship to cold hardening. Perhaps the most likely relationship is between increase in sugars and hardening.

On the basis of this rather confusing background, let's go back to the beginning and see what possibilities there may be for increasing the survival of newly propagated plants.

The first step is to correctly diagnose the problem. In many respects, this is a most difficult step. We spent two years establishing that winter burn on arborvitae was commonly due to rapid temperature changes in Minnesota instead of desiccation. The third year we designed a shading experiment to eliminate rapid temperature change and most of the plants in the trial sustained cambium damage from what we believe to be an early fall frost instead.

Some species have certain organs or tissues which are especially subject to injury. The susceptibility of flower buds of forsythia and apricots is well known. Pellett (11) found that winter damage on some usually hardy junipers was due to root killing when they were grown in containers. In Minnesota, we have some strong reservations about the widespread use of Malling roots for apples because of their limited hardiness.

Recognizing the inherent limitations of the species, there are a number of factors we can influence to enhance prospects of survival:

Well established plants have a better chance of survival than newly rooted cuttings or seedlings which are undergoing transplanting shock and other stresses in addition to those imposed by winter. Proper timing of propagation, transplanting, and hardening off can overcome many of these difficulties.

Mineral nutrition has been shown to influence hardiness in some instances. In contianer grown arborvitae and juniper, Coultas (12) recently found that high levels of nitrogen increased injury while high levels of potassium generally reduced injury. This substantiates much earlier work. More unusual, however, was his observation that high phosphorous levels also predisposed plants to high injury. This is not generally recognized but may be a problem in intensive nursery culture where nutrients are maintained at a high level for maximum growth.

The reason that high K and P tend to decrease and increase winter damage respectively is not known. High N promotes vegetative development which does two things that may increase injury. Late autumn growth may delay hardening (13). This is especially true in plants which do not go into rest period early in response to shortening day length. Smithberg (14) found in red-osier dogwood that native clones collected at southern latitudes went into rest later than northern collections. All 35 clones in her study ultimately reached a high level of cold resistance (below -125° F.) by mid-winter but southern and maritime collections which grew late in the fall were already injured. Many of our choice ornamental and fruit plants are being grown north of their natural range and are subject to the same type of problem.

The second deleterious effect of nitrogen induced growth is the depletion of the carbohydrate reserves of the plant. If sugars have a direct protective action on cold sensitive proteins as suggested by Heber (3) this could reduce the plants capacity to harden.

In summary, the production of maximum growth by high levels of N and P fertility are sometimes not compatible with maximum cold hardening. High levels of K are generally beneficial. In tender species which have a tendency to grow late in the autumn, it would be advisable to withhold N and possibly P late in the growing season to slow down growth and promote the accumulation of sugars. Although our discussion has centered around high fertility levels, it is generally recognized that unthrifty plants suffering from low fertility are also subject to winter damage. Cultural practices such as heavy late shearing or pruning which induce late fall growth in some plants have the same undesirable effects as high nitrogen fertilization.

Light is important in hardening plants for two reasons. As we have already mentioned, short days are the triggering mechanism which induces rest period and preconditions plants to respond to the hardening influence of low temperatures. It is important that plants to be overwintered out-doors, are exposed to short days in the autumn. This is especially a problem if artificial illumination is used to lengthen the day during or immediately after rooting of cuttings or seed germination.

While hardening may be inhibited by long days we want the plants to accumulate sugars through photosynthesis. This means that bright light during the short day is desirable. The common practice of shading newly rooted cuttings to harden them off after mist propagation limits their already depleted carbohydrate reserves and probably reduces their capacity to acclimate to cold. Shading during autumn should be kept to an absolute minimum.

The undesirable effects of shading on winter survival were graphically illustrated to us in a study where the effects of late and early fall shading, fall pruning, and high and low nutrient levels on winter survival of one year old globe arborvitae were evaluated. The plants shaded early in October with a 50 percent lath shade covered with burlap was severely injured while the control and those shaded later, in early December, were injured much less. In this study the pruning and nutrient status had little if any effect.

While intense sunlight favoring maximum photosynthesis is desirable during the autumn hardening period, it is often not desirable later in the winter (1) because it contributes to rapid temperature changes in foliage or bark and to desiccation. Some type of shading put on as late as possible probably has merit for protecting many evergreens. In Minnesota rhododendrons (*R. catawbiense* and *R. maximum*) have survived -35° F. when they are protected by heavy shade. We are presently testing reflective flocking agents which are sprayed on plants with a water soluble adhesive as a protection from this type of injury on rhododendrons, strawberries, *Pachistima canbyi* and American arborvitae. A large number of plants can be treated in a short time in massed plantings such as a container stock area in much the same way as Christmas trees are flocked. A major problem is to find an adhesive which will wash off readily in the spring and yet withstand fall rains.

Temperature is also critical to the process of cold acclimation. As pointed out earlier, freezing temperature is necessary for the development of maximum hardiness in dogwood following the photoperiodically induced first stage of hardening. In the case of late propagated stock, it is important not to make the transition from the protected environment of the propagating area to an outdoor overwintering area too abrupt. At the same time, plants need low temperatures to harden providing it's not too low too soon. Good timing and semi-protected hardening areas such as cold frames offer a possible solution to the problem.

Little is known about root hardening, but in dogwood, low temperature exposure contributes to root hardening which can reach -4° F. (6). In apple rootstocks, preliminary field data indicate that the hardiness of roots is essentially the same under mulch as under bare soil or snow cover. The gradual cooling of the soil mass in nature probably gives ample time for root hardening in most cases but in containers, root injury is more of a problem as in the case of juniper (11) and needs further study.

Water is often discussed in relation to hardiness. Obviously if there is little free water in plant cells, there will be less available to form ice crystals. The danger in this reasoning is that withholding water may retard the cold acclimating process and also contribute to desiccation injury. The moisture level in plant tissues is lower in winter than summer (11), but this is largely internally controlled and heavy watering does not increase tissue moisture appreciably unless the plants are quite desiccated.

It is doubtful that withholding water is beneficial in hardening most plants and may actually interfere with natural hardening. Probable exceptions to this are plants which do not go into rest in the fall and need unfavorable conditions to stop their growth. Some herbaceous plants such as spring bedding stock develop a few degrees of hardiness from exposure to low water, high light, and cool temperatures but the contribution of low water to this type of hardening is uncertain. Caution is called for in withholding water except in special cases.

Special techniques of a mechanical and chemical nature are being used to aid in overwintering plants. The most widely used mechanical type of protection is the temporary plastic shelter erected over nursery beds and blocks of container stock. These shelters are kept reasonably air-tight to prevent drying out of stock over winter. They are apparently quite successful. If an appreciable degree of shading is involved, plants should be covered as late in the fall as feasible. Many evergreens maintain good color under such shelters. A complete discussion of this and other mechanical means of protection such as mulches, mulch in cold frames, and refrigerated storage would require more time than we have here.

Chemical protectants for the most part have the property of either reducing desiccation or retarding growth. The beneficial effects of such treatments have generally not been too exciting. A polyvinyl chloride (Wilt Pruf) had no detectable influence on foliage moisture levels in overwintering arborvitae foliage (1) but Smith and Chadwick (15) have reported that an acrylic copolymer (Foli-Gard) was more effective than Wilt-Pruf in reducing water loss from transplants.

Growth retardants such as B-Nine, Cycocel (16), Maleic Hydrazide and others have increased hardiness to a limited extent in some plants. The major potential of these chemicals may lie in their capacity to stop fall growth on species which don't go into rest.

The report that the water permeability increasing substance decenyl succinic acid (5) induced a substantial increase in hardiness was very encouraging, but the lack of substantiating evidence from other sources suggests the need for further critical work.

LITERATURE CITED

1 White, W C and C J Weiser 1964 The relation of tissue desiccation, extreme cold, and rapid temperature fluctuations to winter injury of American arborvitae Proc Amer Soc Hort Sci 85:554-563.

2 Levitt, J 1962 A sulfhydryl-disulfide hypothesis of frost injury and resistance in plants Jour Theoret Biol. 3 355-391

3 Heber, U W. and K. A. Santarius. 1964 Loss of adenosine triphosphate synthesis caused by freezing and its relationship to frost hardiness problems Pl Phys 39·712-719.

4 Huystee, R B van 1964 Cold acclaimation and accompanying metabolic changes in red-osier dogwood with emphasis on proteins Ph D Thesis Univ of Minnesota. 106 p.

5 Kuiper P. J C. 1964 Inducing resistance to freezing and desiccation in plants by decenylsuccinic acid. Science 146.544-546.

6 Van der Schans, C 1965. Cold acclimation of bark and roots of *Cornus stolonifera* and accompanying changes in the saturation of fatty acids Ph D thesis. Univ. of Minnesota. 34 pp.

7 Sakai, A 1958 The frost-hardening process of woody plants II Relation of increase of carbohydrate and water soluble protein to frost resistance. Low Temp Science B 16:24-34.

8 Tumanov, I I and Trunova, T 1957. Hardening tissues of winter plants with sugar absorbed from the external solution. Fisiol. Rast. 4·663-673

9 Miles, N W 1965 A study of rest period in September and Latham red raspberry and its association with winter hardiness Ph D thesis Univ of Minnesota. 94 pp

10. Li, P. H, C. J. Weiser, and R van Huystee 1965. Changes in metabolites of red-osier dogwood during cold acclimation Proc Amer. Soc Hort Sci. 86·723-730.

11 Pellett, N. 965. Cold acclimation of container grown Hetzi juniper. Ph.D. thesis Univ of Minnesota. 113 pp

12 Coultas, L 1965 The Influence of Fertilizers on the Nutrition and Performance of Certain Container Grown Evergreens. Ph.D. thesis. Univ of Minnesota.

13. van Huystee, R, C. J Weiser, and P. Li 1965. Some postulated relationships among autumn phenomena in woody plants. Nature 208·89-90.

14 Smithberg, M. 1964 Patterns of variation among some climatic races of red-osier dogwood. M.S thesis. Univ of Minnesota. 137 pp.

15 Smith, E M. and L C Chadwick. 1964 The use of anti-desiccants in establishing liners. Proc. Internat. Pl. Prop. Soc. 14:88-94.

16. Marth, P C. 1965. Increased frost resistance by application of plant growth-retardant chemicals Agr. and Food Chem. 13 (4) :331-333.

JACK HILL: I would like Dr. Weiser to go through step by step the process which leads to cracking of stems in *Arbor vitae.* Would you repeat that in the ABC form?

DR. WEISER: The cracking of the stem showed up in the spring as a kind of peeling back of the bark from the wood. We think that was due to a fall frost that injured the cambium. It didn't show up until the following spring. But we don't have any definitive information on this. I wish we had good data to support it but we think this is what caused the injury. Do you have this type of problem?

JACK HILL: Yes we do. I think there are very few people in the room who are not familiar with this problem. When you say a fall frost — we always have a fall frost — what is the difference, if any, in the frost which causes the damage.

DR. WEISER: We had plants in rather high nutrition that had been sheared rather heavily and they were let's say pushing in the fall. Our first frosts in the fall were rather severe — we didn't have any 30° F. or 29° F. frost — we had one that went down to about 19 or 20° F., the first frost of the fall. And at this time this is one place where the curve of frost resistance and the environmental tension do come close together. In the fall in *Arbor vitae,* it is a critical stage. Most of our data were recorded in the two previous years when we had not had any of that type of injury. We had only peripheral winter burn.

CARL GULLO: Dr. Weiser, where do you get the decenyl-succinic acid and at what concentration do you use it?

DR. WEISER: It is available from a chemical supply house. I don't remember the name right now. It's used at 10^{-3} molar concentration. But in Wageningen, Holland, and in East Malling and in Germany research workers have used the material without success. I am sure some of the other people in the room tried this.

DR. LANPHEAR: We tried this on the roots of *Taxus* to see if we could increase hardiness and we were not successful.

DR. WEISER: This has been the experience of most people. Maybe some one here from Connecticut is familiar with Dr. Kuiper. He has published in Science, has shown pictures, and has some very striking results — showing a very marked increase in the hardiness of bean plants and also the flowers of apple and pear. So far no one else has been able to repeat the work.

DR. FLINT: Have you tried bringing this material in direct contact with tissues, without having to go through external surfaces?

DR. WEISER: We have in progress some root up take studies which is how Dr. Kuiper did it. So far we haven't tried any any spraying, its been mainly trying to reproduce what he reported.

DR. FLINT: Have you tried any excised tissue?

DR. WEISER: No.

DR. FLINT: I gather from the literature that perhaps part of the problem, anyway, may be getting the material into the plant to begin with. It might be interesting to see what the effect would be on excised tissues.

DR. WEISER: It's a paraffin like material, its rather hard to dissolve without dissolving it in hot alcohol first and then in water.

MODERATOR LEACH: Unfortunately Mr. Wagner ran into some mechanical difficulties at home and is not able to be with us. However, Bill Curtis, president elect of the International has kindly consented to give Mr. Wagner's paper. This reminds me of the fellow who gave up smoking because he feared cancer — he took to chewing toothpicks and died of Dutch Elm Disease.

BILL CURTIS: Before I begin I should point out that the C and R nursery is located in Wenatchee, Washington and they have entirely different growing conditions, I think, than many of you people do here, at least entirely different from what we have in Western Washington and Western Oregon.

PRE-EMERGENCE WEED CONTROL IN NURSERY STOCK

RUDY WAGNER
C & O Nursery
Wenatchee, Washington

It is a real pleasure to be here with you to-day to discuss a common problem which we all seem to have, WEEDS. As it is the nurserymen's most costly problem, we are all searching for ways to bring down the high cost of weed control. In the spring of 1961 we at C & O Nursery decided to do something about it, and tried using Chemical weed control in our ornamental stock. I am here to-day to tell you of some of the experiences we have had in the last four years.

The term pre-emergence when in reference to weed control means an application of chemicals after planting but before emergence of weeds. The selection of the chemical to be used for pre-emergnce weed control will depend on whether it is being used or applied as a direct or an over all spray on lining out stock. Certain sprays cannot be used on liners even if it is directed at the base of the plants, without taking some chance of producing some injury. But the same chemical may be used quite safely as a granual applied in dry form when the foliage is dry, with good results. We have selected Simazine as the chemical in our operation and we are using it exclusively for the time being as it has performed very well in tests made at one of the state experiment stations. Pre-emergence treatments seemed the best method to use in our case. Most weeds are much easier to kill about the time they germinate and it is very important to kill them before they get established.

In pre-emergence applications of Herbicides a thin film of chemical is applied on the surface of the soil which will prevent growth of young weed seedlings. They are killed before they even become visible. We apply the herbicide as an over all spray and find this the easiest and most accurate way of application as it is very simple and can be accomplished with little difficulty. This brings us to the equipment to apply the herbicide. The