He was immediately hired by the University of Massachusetts where he served as assistant professor from 1953 to 1957, then associate professor from 1957 to 1959. That year he returned to Oregon State University as associate professor, advancing to full professor in 1967.

Our recipient has been a member of the IPPS since 1955 and was a charter member in the founding of the Western Region in 1960. He was Western Region president in 1967-68. He has worked on Membership, Long Range Planning, and Convention Planning Committees.

He has also been very active in the American Rhododendron Society, serving on the National Board of Directors, and as the Society's Secretary-Treasurer, and as President.

Our recipient has received many award and honors, among then being:

Gold Medal, American Rhododendron Society

Honorary Membership, Oregon Holly Growers Assoc.

Research Achievement Award, Oregon Association of Nurserymen

Horticultural Achievement Award, Oregon Federation of Garden Clubs

Horticultural Achievement Award, National Council of State Garden Clubs

Jackson Dawson Gold Medal, Massachusetts Horticultural Society.

It is a great honor to announce our 1985 Award of Merit recipient as Dr. Robert L. Ticknor, Professor of Horticulture, Oregon State University, North Willamette Experiment Station, Aurora, Oregon.

PRINCIPLES OF PLANT FREEZING RESISTANCE AND INJURY

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Water Properties: The unique properties of water are of central importance in plant freezing processes. Pure water freezes at 0°C or 32°F. Impurities depress the freezing point of plant tissue water, by 1° to 2°C in most plants. Additionally the water in some plant tissues supercools substantially below its actual freezing temperature.

Small volumes of water supercool to a greater extent than large volumes. Very fine droplets or thin sheets of pure water can supercool down to, but not below, -40°C which is the Spontaneous Nucleation Temperature of water. In plant tissues which deep supercool water can avoid freezing a few degrees below -40°C because of soluble impurities in cellular water (1).

Liquid water becomes denser as it cools, but at the moment water crystallizes into ice it increases 4 percent in volume — and releases a large amount of heat (540 calories per gram of water). The heat released when liquid water crystallizes into solid ice is called the Heat of Fusion. The practice of protecting plants from freezing injury by continuously sprinkling to maintain an ice-water interface on the plant surface is based on this energy release from water at the moment it freezes.

Plant Freezing Processes: Tender plant species like tomatoes, beans and melons are killed at the moment they freeze — and some "chilling sensitive" tropical species like bananas, rice and poinsettias are actually killed by cool temperatures well above freezing (5° to 15°C). In contrast many hardy woody plants can survive prolonged freezing at extremely low temperatures in winter — including submersion in liquid nitrogen at -196°C or -320°F. Many cultivated trees and shrubs fall between these hardiness extremes. Even the hardiest plant species are killed at temperatures just slightly below freezing (eg at -3°C or 27°F) during active spring and summer growth. With rare exceptions growth cessation (dormancy) is a prerequisite to cold acclimation in plant species capable of hardening (3).

When ice forms at one or more locations in a plant it is capable of spreading (often rapidly) throughout the plant. Rapid cooling/freezing gives rise to rapid ice spread and formation of many small ice crystals. Slow cooling rates give rise to formation of fewer large ice crystals or lenses, and slower spread of ice throughout the plant. Some plants have tissue zones which ice does not propagate through readily. For example, barriers to the spread of ice from frozen stems into dormant overwintering flower buds have been found at the base of buds in several woody genera including *Prunus* (4).

At the microscopic level rapidly cooled cells are observed to "flash" or freeze intracellularly as thousands of tiny ice crystals form within the living cytoplasm of the cell. Such intracellular freezing is invariably lethal. In plant tissues cooled slowly on the microscope stage water is observed to freeze outside of the living cell (extracellular freezing) in spaces between cells. Some plants have "preferred sites" of ice

formation where large ice masses often form without causing serious damage to the plant.

Extracellular ice crystals grow larger as the temperature is lowered because water in the living cell cyptoplasm migrates through the cell membrane and cell wall to the external ice nuclei, in response to the vapor pressure deficit outside of the cell. When this occurs the living cytoplasm of cells dehydrates and shrinks — sometimes to ¼ of its original volume without injury (3). Hardy plant cells can survive extracellular freezing and thawing to varying degrees. Tender plant cells cannot.

Plant and animal cells can be artificially cooled extremely rapidly in the laboratory, by plunging into liquid nitrogen or helium. Under such conditions cell water can become a solid-liquid, like window glass, with no ice crystal lattice formation. Such "vitrified" cells can survive this extreme cooling — illustrating that ice formation, not low-temperature, is the cause of injury. The vitrification of water in living cells, by cooling and rewarming so rapidly that ice crystallization does not occur, is used in cryopreservation of plant and animal cells — such as sperm and red blood cells.

Intracellular freezing is a cataclysmic event at the cellular level. Cell membrances and enzyme compartmentalization are destroyed and death occurs rapidly and dramatically. Plant tissues become flaccid, water soaked and undergo rapid oxidative browning as soon as they thaw.

Rapid cooling rates (eg. about 10°C per minute) are used in the laboratory to produce intracellular freezing in plant cells. In nature the air temperature never drops that rapidly, but lethal intracellular plant freezing probably does occur in nature when deep supercooled tissues suddenly crystallize, and under some other conditions. For example arborvitae foliage on the southwest side of plants exposed to bright winter sun on a cold winter day can thaw and warm as much as 20°F above the air temperature. When such plants were suddenly shaded the foliage temperature dropped 15°F in one minute. The sun-warmed arborvitae foliage was killed above 0°F when it cooled rapidly and refroze (intracellularly) — even though it was hardy enough to survive slow extracellular freezing to −25°F (5).

Scientists do not know how or why extracellular freezing kills hardened plant cells that are capable of surviving tissue ice formation down to a certain critical temperature. It is known that plant water content decreases markedly as plants harden; that hardy plants can tolerate greater water stress. That extracellular freezing imposes increasingly severe dehydration stress on cells as the temperature decreases; and that

hardy plants survive when more of their water is frozen than less hardy plants (3,4). It is likely that death from extracellular freezing occurs when the living protoplasm becomes too dehydrated.

Important Points

* Tender plants can only avoid freezing injury by not freezing. Ice nucleating bacteria, such as *Pseudomonas syringae*, are widely distributed and prevent plants from supercooling below about −2°C. If no ice nucleating bacteria are present some tender plants can supercool and avoid freezing and injury down to temperatures of about -8°C or 17°F. Eliminating ice nucleating bacteria from woody plants usually does not lower their freezing point (increase their supercooling). This suggests that many woody species may have naturally occurring internal (endogenous) ice nucleators in their tissues. It also suggests that eliminating ice nucleating bacteria may prove to be an effective technique for fostering freeze avoidance in tender herbaceous plants — but not in woody plants.

* Hardy plants can avoid freezing injury by tolerating extracellular ice in their tissues — or by not freezing.

* Some hardy plants (eg. Eastern deciduous forest species) have both avoidance and tolerance mechanims for surviving (1). Many cultivated trees and shrubs, for example, tolerate extracellular freezing in their vegetative buds, phloem, xylem vessels and trachieds, cortex and cambium tissues — and avoid freezing (down to as low as -40°C or F) in other adjacent stem tissues which deep supercool such as pith and xylem ray parenchyma cells (in pears, apples and Eastern hardwoods), and in dormant flower buds (in cherries, apricots, peaches, blueberries, deciduous azaleas and conifers).

* Rapid and lethal intracellular freezing likely occurs in nature when the water in deep supercooled tissues suddenly crystallizes, and when sun-warmed (thawed) plant tissues of small mass are suddenly shaded (cooled) on cold bright winter days (5).

* Freezing injury is caused by ice formations in the plant — not by low temperature — except in chilling sensitive plants of tropical origin.

* Stage of plant development is critically important to freezing tolerance. Even the hardiest plant species, capable of surviving submersion in liquid nitrogen or helium during the winter, are killed at temperatures just slightly below freezing (-3°C or 27°F) when they are in the spring flush of growth.

* Growth cessation (dormacy) is a necessary prerequisite to cold hardening in hardy/woody plants — with rare exceptions (2).

Freezing Resistance/Cold Acclimation: Hardy plant species are extremely adaptable organisms. They are capable of increasing in hardiness from about −3°C during the spring growth flush to −30° or −50° or even below −196°C in midwinter. Seasonal patterns of cold hardiness can be established by sampling plants in the field at frequent intervals throughout the year, subjecting them to controlled freezing tests, and plotting their lowest survival temperatures during the year (3).

Growth chamber experiments, exposing different branches on a single plant to different environments, and studies of grafted, girdled, and defoliated plants have been conducted to establish how environmental signals regulate the processes of hardening and dehardening (3). Metabolic studies, microscopic studies, and measurements of the heat of fusion released as plants freeze identify changes occurring in plants at the cellular level during hardening and dehardening — and the lethal and non-lethal freezing events occurring in plants (4).

Important Points:

Studies of cold acclimation (hardening) in hardy and semi-hardy species which are capable of acclimating indicate the following:

* The developmental stage of a plant has a strong influence on whether, how rapidly, and to what extent the plant can acclimate or deacclimate (2).

* Actively growing plants during spring and summer are not hardy.

* Many hardy woody plants appear to acclimate in three distinct stages under optimum conditions.

* The optimum sequence of developmental stages and environmental stimuli for achieving maximum hardiness development in hardy species is:

a) Exposure to short days and warm temperatures (Stage I).
(Plant vegetative maturity and dormancy.)

b) Exposure to cool temperatures and frost (Stage II).
(Initiation of physiological rest.)

c) Prolonged exposure to sub-freezing temperature (Stage III)

* Stage I of acclimation is induced best by short days and warm temperatures. Many woody species achieve a hardiness of about 0°F or -18°C during this initial stage of acclimation. Short days concurrently induce dormancy in adapted species. The short-day induction of hardiness involves a "biological clock" (a photo reversible enzyme-pigment system called phytochrome) which is located in the leaves; and a translocatable

hardiness promoting factor that carries the "clock" message from the photoreceptive leaves to overwintering plant tissues. The translocatable factor is probably a hormone. It moves through the living bark (phloem) (3).

* *Stage II* of acclimation is induced by low temperatures/ frosts. In contrast to short-day induction the hardiness induced by low temperature is a localized (not a translocated) response. Roots, (or branches) which are not exposed to low temperatures do not become as hardy as exposed stems (or roots). Hardy plant species which are exposed to frost after reaching Stage I increase in hardiness very rapidly — as much as 10° to 20°F per day during Stage II of acclimation (4).

* *State III* Russian researchers have demonstrated with hardy birch species that the maximum hardiness achieved in Stage II can be gradually increased even further by prolonged plant exposure (several weeks) to continuous subfreezing conditions. The additional hardiness may amount to 10° or 20°C. This additional increment of hardiness *(Stage III)* is lost as soon as plants thaw (3).

* The optimum sequence of conditions which have been described lead to the maximum and most rapid development of hardiness — but it is possible to induce, and eventually achieve, maximum hardiness in hardy species without exposure to short days. i.e. low temperature exposure alone can eventually induce maximum hardiness in dormant plants (3).

* Hardiness studies of different climatic races of a plant species that have evolved in climatically divergent parts of the natural range of the species illustrate the important role of the plant's biological clock. A Minnesota field study of climatic races of red-osier dogwood races native to Minnesota, North Dakota and Seattle, Washington illustrates this point (3). The ND clone acclimated (Stage I) earliest in the fall in Minnesota and the MN clone next. Both survived without injury. The Seattle, WA clone was killed back from the branch tips almost to the ground by the first severe fall frost in Minnesota because it failed to stop growing and acclimate to Stage I.

By midwinter however the surviving branch bases of the WA clone were just as hardy (below -196°C) as the ND and MN clones. This illustrates that all three climatic races had the inherent capacity to acclimate to below -196°C — but the WA clone lacked the proper biological clock to stop growing and begin acclimating early enough in the fall to avoid severe injury in Minnesota.

* Most cultivars of landscape plant are grown far from their original sites of origin, and many have been hybridized — to further confuse their biological clocks. The plant biologi-

cal clock is genetically controlled — and cannot be "reset" by progresively growing late acclimating plants in more severe climates.

* Plant cultivars which stop growing and set terminal buds in the field at a given location in late summer are likely to acclimate before severe fall frosts — to achieve maximum hardiness rapidly (2). If their inherent mid-winter hardiness exceeds the mid-winter minimum temperatures at the location they will prove to be well adapted, and survive without injury. Such species can be given optimum growing conditions (water, fertilizer, etc.) throughout the summer and fall without reducing their hardiness — in fact healthy plants often acclimate faster and to a greater extent than less vigorous plants of the same cultivar.

* Plant cultivars which lack the biological clock settings to respond to short days and to stop growing and set terminal buds in late summer at a given location are likely to cold acclimate late, and to sustain freezing injury during the fall and early winter (2). The hardiness and survival of such cultivars can be enhanced by management practices which induce/impose dormancy and initiate Stage I hardening before fall frosts. Withholding late season irrigation and fertilizer, avoiding late summer fertilizer, avoiding late summer pruning or defoliation, interplanting a competitive cover crop, or applying chemical growth inhibitors are practices that have been used to induce dormancy, and initiate cold acclimation in such "unadapted" cultivars. Observations of when and whether cultivars set terminal buds in the field is an easy and effective way to estimate whether or not a cultivar is adapted. Such visual observations can provide a basis for designing appropriate irrigation, fertilization, pruning, defoliation and digging schedules for each cultivar. Plants dug before they are vegetatively mature often sustain die-back in storage and transit.

* Different tissues within the same plant can differ markedly in hardiness. Tissues which avoid freezing by deep supercooling often acclimate earlier in the fall and deacclimate later in the spring than tissues of the same plant that survive by tolerating extracellular freezing — but the deep supercooling tissues seldom become as hardy by midwinter. In apple, for example the pith and xylem ray parenchyma cells in the wood are killed in midwinter at 20°C higher temperatures than the living bark tissues (cambium, phloem and cortex). The lower limit of hardiness for deep supercooling flower buds and wood tissues is about -40°C. Fortunately most plants survive even when these tissues are killed (4).

* Temperature, and stage of plant development, are the primary factors that regulate day-to-day changes in plant de-

hardening and rehardening in late winter and spring. Unseasonably warm days in late winter, when bud chilling requirements (rest period) have been satisfied can cause rapid losses of hardiness (10°F per day). Rehardening does occur, but slowly, in response to subsequent exposure to cool temperatures. The temperature of the preceeding day correlates well with day-to-day change in hardiness at this time of the year (4).

* Hardiness is fully and irreversibly lost when plants enter the spring flush of growth. The only effective management practices to delay dehardening and spring growth are those which reduce/delay plant exposure to warm temperatures. Shading, and misting to promote evaporative cooling are effective up to a point — but temperatures as low as 5°C can promote growth and dehardening in the spring when plant development has progressed through physiological rest (2).

There are still many unanswered questions about plant freezing resistance and injury, but an undertstanding of the principles involved can provide the basis for intelligent nursery managment strategies to enhance hardiness and reduce freezing injury. Selection of the proper plant materials for each locale is still the most effective and important management decision we make — and observation and experience the best teachers.

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