better support where differences in carbon dioxide, oxygen and water saturation in pore spaces occurred.

LBC treated with IBA was established as the best system for ARF studies on developmental sequences in juvenile vs. mature *Ficus pumila*.

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EFFECTS OF FREEZING-THAWING (FAST AND SLOW) IN PLANTS

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A very informative paper by Dr. Robert Wright on the physiology of plant tops during winter appears in the 1977 IPPS Proceedings (Southern Region) (4). Following is a summary of certain concepts presented by Dr. Wright that are pertinent to a consideration of the effects of fast or slow freezing and thawing in plants.

Acclimation is the seasonal transition of plants from a tender growing condition to a hardy overwintering condition in species that go into a rest period. During rest internal factors prevent growth until certain biochemical and physiological requirements have been satisfied. After these changes have occurred, it is possible for growth to resume when there are good environmental conditions. Shortened days in fall and winter with decreased temperatures trigger various biochemical, biophysical and physiological changes in plants. Cells also undergo dehydration at this time. Plants can resist freezing either by avoidance or tolerance of low temperatures. Annuals live over by seeds that usually contain very little moisture. Herbaceous plants have crowns or roots with the ability to regenerate new buds and stems following a dormant period. Since the crowns and roots are below ground, they are protected from the lowest temperatures, and often snow and surface mulches prevent soils from freezing.

Levitt (1) lists four times when cells can be injured from freezing: (1) during freezing, (2) after freezing equilibrium is reached, (3) during thawing and (4) after thawing. Freezing can be either intracellular, within the cell; or, intercellular, outside the cell membranes between the walls of the individual cells. Scientists have believed that the expansion of water as it froze formed ice crystals that mechanically ruptured and tore cells apart. Much evidence exists to indicate that this is not the case in nature, but that the major cause of injury and death of cells is membrane destruction.

Asahina (1) reported that in *Tradescantia* staminal hairs ice crystals were first seen in the distal end. Ice crystals were seen in the cytoplasmic layers in the tonoplast and the vacuoles. When changes in temperature were rapid (at more than 20° F in 1 minute) ice crystals formed within the cells; however, such temperature changes rarely, if ever, happen in nature. Such temperatures disrupt the entire integrity of the cells with clotting of the protoplasmic layer and pulling away inwardly of the cell membrane from the cell walls. Membranes within the cell are destroyed and air spaces are squeezed out. Upon thawing, water fills in where these air spaces were, giving a translucent, water-soaked appearance to the frozen tissue.

Intracellular damage is irrepairable and results in certain death. It occurs in tender plants and those that remain unacclimated when temperature and moisture remain favorable for growth. These plants can and do acclimate under proper environmental conditions. In many tropical plants injury occurs at temperatures above freezing, but some are uninjured at −1 to −3°C. The freezing point could be depressed by cell inclusions and some supercooling. Usually cell membranes lose their semipermeability because the lipid structures in the proteinaceous membranes are altered and cell parts are destroyed.

Levitt (1,3) states that only circumstantial evidence exists that intracellular freezing occurs in nature. If it does, it would be fatal to plants. Asahina (1) reported that when temperature changes were very rapid, movement of water across the membrane barrier did not occur quickly enough to prevent internal freezing. Nucleators, of which there are few good ones within cells, are needed for formation of ice crystals. This is similar to seeding clouds to form raindrops. Silver iodide crystals are used, which start the vapor to condense into drops. In cells too, particular compounds and sites are needed to serve as nucleators for the formation of ice crystals. Some cells have no good nucleators within the membranes, but outside of them ice crystals form readily on the cell walls. As the fluid moves out of the cell membrane, the ice crystals grow between the cell walls. The cell membrane shrinks to the interior of the cell, and fluids within the cell become very viscous. Since it has become flabby and pulled inward, there is little danger that ice crystals will puncture the membrane. The fluids that move out across and membrane contain K^+ ions, as well as smaller amounts of some CA^{++}, sugars and other compounds.

Plants that survive low freezing temperatures are those with extracellular freezing. Asahina (1) said extracellular freezing is governed by both external and internal factors involving the grade of supercooling, cooling rate, hardiness of the cells and their amount of freezable liquid. These cells continuously lose moisture outside the membrane, and ice masses show remarkable growth in spaces between the walls of the cells. Their protoplasm must resist the stresses caused by dehydration and contraction as the cell volume is reduced. Solutes become very concentrated within the cell membrane, and pH changes occur as many ions move outside the membrane. Asahina (1) states that damage is physiochemical rather than mechanical. If the temperature drop is gradual enough, the freezable liquid moves out of the cell walls even in tender plants.

Very hardy plants which are fully acclimated can withstand very low temperatures without damage. Winter wheat tolerates temperatures down to −25°C; and in a dogwood species, temperatures down to −196°C did not cause injury. However, temperatures must drop slowly if plants are to survive. Cells of these plants tolerate dehydration and contraction of their protoplasm. Most of these plants lack nucleating substances within the cells or have barriers to prevent internal freezing. Often supercooling occurs. As long as there is no damage to the membranes, cells will reabsorb liquids upon slow thawing and then appear normal again.

Pure water can be supercooled to −38°C without freezing. Once crystals form freezing is very rapid. Certain plant tissues supercool to −15°, or −59°F, but not all to the same extent under field conditions. If soils are not cold, moisture can move up from the roots to prevent dessication. In dessicated tissues ice crystals do not spread uniformly, and injury and death can result. This is the case in winter when it is very windy and the frozen soil makes absorption and translocation of moisture impossible.

In winter, fully acclimated apple tissue can go down to -40° C without injury. If temperatures fall below the homogeneous nucleation temperature, freezing occurs. Both supercooling and extracellular freezing are involved. Hoar frosts can enter lenticels, stomates and wounds to start ice formation. Nucleation points are constant in plants. Water migrates from cambial and phloem tissue to the outer cortex where ice forms on the surface of the cell walls without injury, as described by Wiegand (3).

Slow extracellular freezing occurs in large ice masses at specific sites. Sakai (1) in 1965 reported stems could survive −60°C in midwinter if freezing occurred slowly due to a gradual dropping of the temperature. However, water does not have a chance to migrate to these sites. Levitt (3) reported that killing occured at higher temperatures when rate of freezing was rapid. This was reported in apple twigs by Hildreth in 1926, raspberry buds by Schwartze and trees by Day and Peace in 1937. Rapidly frozen apple twigs were killed at −19°F while those frozen slowly were killed at −25° to −40°F, according to Beach and Allen. This was the case in apple trunks, pine shoots and cabbage tissue. Weiser (1) reported that evergreen foliage was injured at −80°C during slow freezing when acclimated; however, tissue was killed at −19°C when temperatures were dropped 8 to 10°C per minute. Lethal intracellular freezing occurs in hardy plants when temperatures drop fast, as reported by Olien (1). However, Sakai and Suka (1) observed that if ice crystals were small no injury would result from rapid thawing. Some plants were reported to be so frost hardy that no injury occurred regardless of rate of thawing (3).

Repeated freezing and thawing has an amplifying effect on injury in some cells, according to Olien (1). The first time cells were exposed to sublethal temperatures, injury was only slight. Fully acclimated winter wheat could be exposed to −19°C without injury the first time. However, cells were killed after a second freezing and thawing. Gusta (1) (unpublished) stated that when large ice masses thaw, resulting pools of bulk water lead to injury at higher temperatures.

Palta and Li (1) reported tissue frozen for 12 days at −4°C had 0 to 50% intercellular spaces infiltrated with water and all cells alive 7 to 12 days after thawing. In −11°C cells there were 80 to 100% intercellular spaces infiltrated with water and all cells were dead. These cells showed the highest conductivity readings. Semipermeability properties of the membranes were apparently uninjured since cells regained turgor. However, severe damage to the active ion and sugar transport mechanisms was the cause of death. Therefore, since plants vary greatly in their response to low temperature, it is not possible to generalize as to the extent of supercooling, of intracellular or extracellular freezing. Plants that can be and are fully acclimated withstand very low freezing temperatures if their membranes tolerate stress from contraction and dehydration during freezing. The most serious damage occurs to the ion transport mechanisms in cell membranes. This damage is lethal to frozen cells. Fast freezing and thawing usually cause much greater damage to cells than do slow changes; however, in very hardy plants, rates have no effect on injury.

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QUESTIONS FOLLOWING NURSERY BUSINESS FORUM

RICHARD VAN LANDINGHAM: Question for all speakers. Have any of you done a cost study on the use of different herbicides in a container operation? We recently used Ronstar several times and have used other herbicides in the past. I would like to know which one any of you has found is the cheapest and the most effective to use.

BRAD MAY: We were using Lasso, but it was hurting our quality. It is a little cheaper to put out, but plant quality was going down, so we went to Ronstar. Our coverage was not any better but the quality of our plants returned to what we would like to have. We use Ronstar at the recommended rate every 10 to 12 weeks, depending on the weather.

HARRY HOPPERTON: Question to Henry Chase. Henry, you said you waited for a rain to activate the Treflan. What if it didn't rain for two weeks?

HENRY CHASE: As long as you aren't getting any rain, you are not going to be getting any germination. The Treflan will remain in place. Many times during the summer a heavy dew will solve that problem.

SIDNEY MEADOWS: I'm going to take just one minute here to inject something that I believe in that has worked for us extremely well. We plant in our cans in April, assign a certain number of cans to two people, and tell them to keep these cans clean until September 15 for a certain amount of money. In recent years, we have used a half million cans for two people for all summer, and they have done it. In fact, they have come out ahead by not having to work full time on it. By working in extra credit somewhere else, they can receive a nice bonus check. Surprisingly, this costs less than a cent a can. The worst job on the place is made a prestige job because they work hard to eliminate any source of weed seed. It works best to start the contract program with newly canned plants, otherwise there will be a build-up of weed seed in the container.

PETER VAN DER GIESSEN: Question for Sidney Meadows. Does the 1 cent that you are paying involve the salary that these people are going to make?

SIDNEY MEADOWS: That is their pay. The contract amount is based on a rate of $2.65 an hour for an 8½ hour day, 42½ hours a week for that period of about 15 weeks. It figures out to be a little less than a penny a can. These people usually earn about $300 bonus on the contract system.

KERMIT MORRIS: Is the weeding in addition to the use of herbicides?

SIDNEY MEADOWS: We don't use any herbicides in this program. If we keep the weeds out of the walk and the perimeter, the contract workers will keep them out of the cans.

BOB LOGNER: Question for Sidney Meadows. What is the difference in cost and the ease of using a plastic pot in comparison with the ease of using a peat pot?

SIDNEY MEADOWS: I've never analyzed it but will give you my opinion. I just tell myself that the ease of using a peat pot is worthwhile because of the amount of trouble in removing a plastic pot from the root ball, storing it and reusing it. I would much prefer to use a peat pot because it is put out there and gone. It is certainly easy just to pick it up and pot it. We do use the 3-inch square plastic in a flat, as Brad discussed, but I believe using the peat is cheaper.

The final choice depends on the character of the root system and the length of time required for rooting. If we are going to stick something in and plant it quickly, we use the peat pot. If the cuttings are going to be in the pot for a long time, Burford holly, for example, we put them in plastic pots. Azalea goes in a peat pot. If the plant remains in a peat pot very long, the pot disintegrates. With many broad leaves we stick them in the peat pots and then transfer them in about 60 days. A peat pot is first choice, and a plastic pot is second choice. As far as rooting quality is concerned, I don't think there is any difference.