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Red-osier dogwood plants were grown outdoors in a lathhouse and under two temperature regimes in growth chambers to determine the time of vegetative maturity, the transition between summer and winter dormancy. Xylem water potential (XWP), tissue moisture content, ethylene and ethane production, and electrical impedance were used to try to find a quantitative measure of vegetative maturity that could be used by researchers and nurserymen to predict maturity. XWP showed a significant correlation with tip dieback, but the variability within samples precludes its use as an index of maturity. The change in moisture content was too gradual to be of value as a predictive index. Ethylene production of stem sections correlated with tip dieback, and deserves future testing. Frozen stem sections produced ethane and ethylene, both of which declined as maturity progressed. Electrical impedance values showed promise as the most accurate measure of maturity in red-osier dogwood.

A second objective was to relate vegetative maturity to the first stage of cold acclimation in red-osier dogwood. Previous research has suggested that the onset of cold acclimation occurred before rest and about the time that the plants matured. Confirmation that vegetative maturity and the onset of cold acclimation coincide is presented in these studies.

Hardiness-Maturity Relations in Red-osier Dogwood (*Cornus stolonifera* Michx.)

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HARDINESS-MATURITY RELATIONS IN RED-OSIER DOGWOOD (CORNUS STOLONIFERA MICHX.)

INTRODUCTION

The production of deciduous nursery stock is a multimillion dollar industry in the Pacific Northwest. The mild climate, with a long growing season and ample moisture, can easily produce 6 feet of new growth in a single season in some species (48). Defoliated trees are harvested mechanically and either heeled bare-root outdoors in sawdust or stored in refrigerated warehouses before shipping to markets, mainly in the East and Midwest.

Since many of the nursery-grown trees are not native to the Pacific Northwest, the mild climate encourages continued growth late into the autumn when most of the native species are already dormant. This delay in dormancy development delays natural leaf abscission which causes serious delays in harvesting of the crop. Harvesting done with leaves intact causes increases in weight and bulk during handling, while continued respiration of intact leaves during storage can cause serious depletion of food reserves. Decaying leaves in storage and handling areas can increase the danger of disease or insect infestation and create problems with sanitation.

Any process which can advance the time of defoliation and harvest so that more digging could take place before the arrival of winter rains in November would benefit the grower. Heavy rains

make the use of mechanical harvesters extremely difficult in the saturated soils. Efficiency of field digging operations is as much as 15 times greater before the rains set in (48).

Several methods of circumventing the problems of delayed defoliation and harvest have been used. In Washington state, hand defoliation is used by some growers, but it is expensive and time-consuming (70). Undercutting to promote defoliation in advance of harvest has been used by evergreen nurseries (20) and rose growers (102), but it has not been used widely by growers of deciduous trees. Many defoliants have been tried (18, 57, 58, 67, 71), but the amount of defoliation and injury vary with the time of application, variety, location, and concentration. Because these procedures have not worked well, most growers wait until natural defoliation takes place before harvesting.

As reported previously (32, 33, 48), one of the major problems confronting the successful defoliation of deciduous trees is not knowing when plants can be defoliated safely. Deciduous plants defoliated too early, before vegetative maturity, develop stem dieback during winter storage decreasing their salability.

The primary objective of this research was to find a quantitative method for predicting the earliest possible time for defoliating deciduous nursery stock without causing tip injury. Such an index of vegetative maturity would provide a safe, reliable method for predicting the

effectiveness of defoliants with regard to the reduction in phytotoxicity at the time of application (48).

STAGES OF DEVELOPMENT IN DECIDUOUS PLANTS

Dormancy and Vegetative Maturity

Summer dormancy (23), or correlative inhibition (132), describes the stage of development at which the lateral buds are inhibited by leaves and the terminal bud. Removing the leaves or the terminal bud while a plant is still in summer dormancy removes the source of inhibitors, and the lateral buds will grow. The end of summer dormancy occurs when defoliation fails to stimulate growth of the lateral buds (23, 132).

As summer dormancy ends, the development of winter dormancy begins. Winter dormancy is the period when growth is inhibited by endogenous factor(s) (23). Putting the plant under favorable growing conditions will not stimulate bud break once the development of winter dormancy is completed. Exposure to a period of winter chilling is required by many woody plants to overcome the effects of winter dormancy (132). Others call this stage "rest" (131), "innate dormancy" (132), or "true dormancy" (62).

Quiescence is the stage of dormancy when external conditions, such as low temperatures or moisture stress, prevent growth (131). Exposure to an environment conducive to growth will result in its rapid resumption.

Vegetative maturity, according to Fuchigami (33), is the stage

in a plant's seasonal development at which it is able to survive the winter without injury. Defoliation before a plant is vegetatively mature will result in tip dieback and possibly death (33, 48). Seibel (106) has recently demonstrated that timing of the onset of winter dormancy coincides closely with the onset of vegetative maturity. She hypothesizes that the two phenomena may be one and the same.

The following reviews discuss dormancy in detail (24, 45, 62, 73, 97, 132, 151).

Cold Acclimation

Many deciduous trees, including red-osier dogwood, acclimate to sub-freezing temperatures in a typical two-phase pattern (34, 49, 54, 124, 129). The first stage of acclimation is induced by short days (SD) and the production of a translocatable hardiness promoter (34, 49, 114) that is not present, or is inhibited in, plants under long day (LD) conditions (49, 54). McKenzie et al. (83) reported that the translocatable hardiness factor could be abscisic acid. The first phase of acclimation can occur under SD in the absence of cold temperatures (34, 53). In fact, low temperatures can actually inhibit the first stage of acclimation (34).

The first stage of acclimation takes place after the end of summer dormancy, but before the completion of winter dormancy (33, 53). Van Huystee et al. (129) reported that the first stage of

acclimation occurred in mid-September; during that time, hardiness increased from -7° to -18°C. The first stage of acclimation is not indicative of the maximum levels of hardiness that can be attained.

The second stage of acclimation is apparently stimulated by frost (123, 129), after which hardiness levels can reach -85° C. and lower. The low temperature-induced hardiness stimulus is not translocated (49). A plant exposed to SD without freezing temperatures will not attain this level of hardiness.

A third stage of acclimation induced by prolonged low temperatures (-30° to -50° C.) has been demonstrated by Tumanov and Krasavtsev (123). Hardiness can be increased below -196° after exposure to the low temperatures, but the effects are quickly lost when the temperature increases.

Many studies have been carried out relating the changes in plant metabolites to acclimation. The increase in sugars in stems and leaves has been related to hardiness (76, 103). There is evidence that *in vitro* applications of certain sugars stimulate a slight increase in hardiness (103). Starch levels decrease during acclimation, possibly because of their conversion to sugars (76).

Evidence for an increase in free amino acids was presented by Li *et al.* (76), with the exceptions of alanine, glutamate, and aspartate. These simple amino acids may be used more readily in protein synthesis than other amino acids. Free proline was reported to be abundant in dormant white spruce buds (117), possibly for storage of

CO_2 or carbohydrates (118). High concentrations of sugar and water stress tended to increase the amount of free proline (118).

During the first stage of acclimation, the amount of protein in the plant increases (44, 74, 108, 113). This increase lasts for just a short time; protein levels then decline as acclimation proceeds. Protein synthesis in apple leaves remained at normal levels until after the first frost when it began to decrease (113). Proteolytic enzymes, RNase, proteases, and polyphenol oxidases all increase their activity after the first frost (113). RNA levels increase until the first stage of acclimation begins; then it slowly declines (74, 108). The amount of DNA remains about constant or declines slightly (74, 108, 113).

Plant water status during acclimation plays an important role in determining resistance of living organisms to freezing injury. In red-osier dogwood, water stress can induce cold-acclimation (83), and artificial dehydration of stem sections can cause a rapid increase in hardiness of 5° to 10°C. (75). Decreases in xylem water potential (48) and stem water content (83) coincide with the development of vegetative maturity and cold acclimation, respectively. Nuclear magnetic resonance studies (17) show a broadening of spectral lines at the onset of acclimation, indicating that a decline in the amount of free water occurs at that time. These changes in plant water status signify the possible realignment of the internal structure of the cells

to a more stress-resistant form capable of surviving sub-freezing temperatures (35).

Defoliation

At vegetative maturity, a plant can be maintained in a defoliated state without suffering visible injury. Causes of premature defoliation include temperature extremes, water stress, insect damage, disease, or the use of chemical defoliants by man. Early leaf-fall can inhibit vegetative or reproductive growth, prevent shoot elongation, cause bud mortality and tip dieback, and increase susceptibility to insects and disease (64). Other effects of early defoliation include changes in food reserves and hormonal balance, alterations in water status, and over-all metabolic 'shock' (48). In most cases, the effects of defoliation are visible the same year (37, 66, 81, 141). If leaves are removed later in the season, at the time of vegetative maturity, the effects may not be visible until the following year. For example, spring bud break may be delayed (32, 33).

Carbohydrate levels in the roots and root exudates of defoliated trees have been studied to determine the changes that take place when the leaves are removed. The amount of starch in the roots of defoliated trees declines to low levels (96, 104, 136). Partially defoliated trees maintain near-normal starch levels after defoliation (104, 136), and the resurgence of growth typical of completely defoliated

trees does not occur. Parker and Houston (96) observed an increase in the levels of fructose and glucose following defoliation, but Wargo et al. (136) found no significant changes in sugars. Smith (112) tested root exudates for carbohydrates, organic acids, and amino acids, and found that quantities of the various metabolites varied from year to year.

Evert et al. (29) followed the decline in cambial growth in sugar maple after blocking the phloem by girdling the stem. Starch levels below the cut area declined, but remained at levels high enough to indicate that a simple absence of starch was not responsible for the lack of cambial growth. They hypothesized that movement of starch could take place through the ray and axial parenchyma, but that normal hormonal movement could not. Leaves were apparently the perception sites controlling hormonal production and movement through the phloem.

To be effective as a defoliant, a chemical must cause senescence and abscission without directly killing the leaf and leaving it attached to the plant (92). It must not injure the growing point or cause severe alterations in growth patterns the following year. Between 50% (67) and 80% (58) of the leaves should abscise within a 2-3 week period after spraying. In any case, leaves not directly removed by spraying should be sufficiently loose so that they abscise during harvest and handling.

The use of artificial defoliants began after 2,4-D was discovered during World War II (92). Milbraith (87) successfully defoliated roses with ethylene gas as early as 1940. Early researchers used a variety of chemicals, and came up with a variety of results, depending on the time of application, type of chemical, species or cultivar, concentration, or location (18, 59, 99, 102). Roberts (102) expressed the need for a reliable measure of rose maturity before defoliants could be safely used.

Recent workers have faced the same problems of variability with their experiments. Jones et al. (57, 58) have had some success with chemical defoliants such as potassium iodide and bromodine, especially when using repeated applications. Larsen (67, 68, 69, 71) has attained successful defoliation with KI and bromodine, but he has also tested ethylene-producing compounds such as ethephon with favorable results. Jones et al. (58) stated that "ethephon has little value as a defoliant in deciduous nurseries." However, Sterrett et al. (115, 116) successfully used combinations of ethephon and endothall to defoliate seedlings, and other workers (21, 25, 99) indicate that ethylene-producing chemicals may be very useful in defoliation-inducing compounds.

Since a plant that is vegetatively mature is indistinguishable from one that is not, the timing of defoliant applications must be based on the use of some physiological index of maturity. Application

of defoliants at or after the time of vegetative maturity would increase their effectiveness and reduce injury (33). Others have also reported that late applications of defoliants are more effective and cause less injury (57, 70, 115, 116).

METHODS OF MEASURING PHASES OF DEVELOPMENT

Xylem Water Potential

The basic measure of plant water status is its water potential (ψ_w). Water potential is the specific free energy of water in a plant system, which includes the cellular contents, cells, tissues, and the soil rhizoplane (111).

The water potential is the sum of three component values: $\psi_w = \psi_s + \psi_p + \psi_m$ where ψ_s is the osmotic potential, ψ_p is the pressure potential, and ψ_m is the matric potential. Osmotic potential is the decrease in ψ_w due to solutes and their colligative properties. The effects of hydrostatic pressure, or tension, are known as pressure potential. The matric potential is determined by the water status of the liquid-solid interfaces in the system: cell membranes, macromolecules, and micelles, for example. In a turgid plant, the matric potential is close to 0, so for general use, $\psi_w = \psi_s + \psi_p$ (111).

When a turgid plant is water stressed, the ψ_w decreases (becomes more negative). The initial decline in ψ_w is due mainly to changes in ψ_s. If the stress is not alleviated and ψ_w continues to decrease, the change in ψ_s subsides and the declines in ψ_p and ψ_m become more important in determining total XWP.

One device for measuring ψ_w is the pressure bomb (105, 134).

Proper use of the values obtained from the pressure chamber has been emphasized by Boyer (14, 15). Osmotic potentials and matric potentials may not be fully accounted for, especially at low ψ_w. However, the speed and simplicity for field use, and the shortcomings of methods such as thermocouple psychrometry, make the pressure bomb a valuable tool (125).

Some physiological changes that take place in response to decreasing water potential include a decline in the quantum yield of photosynthates by chloroplasts (90), a reduction in cytokinins (55), an increase in abscisic acid (13), and an increase in the permeability of cell membranes to water (41, 84). Other processes that are affected include a decline in cell division and cell wall synthesis, a reduction in protein and carbohydrate metabolism, a hastening of leaf senescence, and a possible change in the apical meristem from vegetative growth to reproductive growth (48). In red-osier dogwood, an increase in plant water stress can also bring about an increase in plant hardiness (19, 75). Several reviews of plant response to water stress have been published recently (35, 50, 51, 59, 60, 61, 110).

An excellent review on the effects of water stress on plant hormones has been written by Livne and Vaadia (78). Recent discoveries and their implications with regard to dormancy development will be discussed in the following paragraphs.

The role of abscisic acid (ABA) has been reviewed by Milborrow

(86). ABA was shown to increase in wilted wheat leaves (145, 146). It increased de novo, that is, not from a water-soluble form already present, in wilting spinach leaves (150). Exposure to ABA caused stomatal closure in excised leaves, inhibiting transpiration (88). Excised grape leaves showed a rapid increase in the amount of ABA-like inhibitors while ψ_w was declining and the stomates were closing (79). Zabadal (149) determined a threshold level for increased production of ABA in excised ragweed leaves of -10 to -12 bars. Raschke et al. (100) cold treated leaves of Xanthium strumarium, and found that chilling increased ABA levels. Chilling, in conjunction with decreasing moisture levels, increased ABA levels much higher than would have been expected from either variable alone. Thus, it would seem that the declining ψ_w during dormancy development (48) and the cool nights of autumn are closely associated with the increased amounts of ABA present during senescence and abscission.

Concurrent with the increase in ABA during the decline in water potential, is the decline in cytokinins (8, 55) and the cytokinin/ABA ratio (89). A decrease in levels of cytokinins during dormancy development also occurs (8).

Water stress can increase ethylene production in cotton, promoting abscission of the bolls (43, 85). Guinn (43), however states that the increase in ethylene may be due to nutrient deficiencies caused by water stress, rather than by the water stress itself.

Hormonal activity has been shown to be secondary to the effects of water content with regard to enzymes such as RNase (9, 10). The major effects of hormones may be determined by the presence and availability of water in the plant.

Ethylene and Ethane

The role of ethylene in plant physiology has been the subject of recent reviews (2, 3, 98, 147). While the exact role and function of ethylene is unclear, the action appears to be via an indirect pathway. Ethylene may control a secondary process, such as protein synthesis, ABA levels, or auxin transport (3), which in turn direct abscission or senescence. Ethylene in this regard takes on the role of an intermediary hormone, much as cyclic AMP does in animals (3). However, it may still be responsible for initiation of certain processes such as leaf senescence (56).

The methionine pathway is the major source of ethylene in higher plants (3, 101). The formation of ethylene requires ATP (107), but specific inhibitors and stimulators of ethylene synthesis appear to act on the enzymes involved with ethylene production, rather than on the ATP cycle (101). Evidence also indicates that a single enzyme system is responsible for all ethylene production, whether it be under normal conditions or due to stress or hormonal activity (4).

Two possible modes of ethylene action during leaf senescence

have been described by Abeles (3). In its passive role, the sensitivity occurs when other factors fall below certain levels, allowing the ethylene to function. As juvenility factors (auxins, cytokinins) decline during senescence, the effect of the ethylene would increase. As an active substance, ethylene production increases in senescing leaves, and it becomes the prime factor there. Both roles have been shown to be present at certain times in some plants.

The effects of hormones and ethylene on leaf abscission have been reviewed by several authors (3, 7, 64, 86). As long as juvenility factors are present in the abscission layer, the cells will be insensitive to ethylene action (1). Externally applied IAA stimulated ethylene production in peas (14), while other cytokinins and gibberellins may be involved in the modulation or control of the auxin-ethylene system and the sensitivity of the abscission zone to ethylene action (93). Gibberellic acid also promoted an increase in both the rate and amount of leaf-drop in cotton plants exposed to ethylene (91). Since endogenous levels of both ethylene and auxins are high in young leaves, a reduction in auxin levels in the abscission zone must occur before the leaves will abscise (7, 12). Both ethylene and synthetic inhibitors of auxin transport reduce the movement of auxins to the abscission zone promoting more rapid leaf abscission (11). Evidence also indicated that ethylene controlled the production of cellulase, the enzyme responsible for abscission (5, 47). Ethylene

was also responsible for a well-documented increase in membrane permeability during dormancy development (6).

The activities of ethylene during dormancy development at sites other than the abscission zone have not been clearly established. Production of ethylene by white pine buds decreased from measurable levels in summer to almost undetectable amounts by October. The production of ethylene increased again the following spring.[1] Seibel (106) traced the decline in ethylene production of excised nodes, leaves, and internodes of red-osier dogwood during maturity development. All three tissues showed similar declines in ethylene production from high levels in the summer to low levels as maturity approached.

Originally, ABA was believed to be the naturally-occurring abscission accelerator, but there were too many incongruities in its activity for this to be true (93). ABA will enhance abscission rapidly in older tissue (7), but repeated applications are required to promote abscission in young leaves (26). When explants are used, however, ABA is very effective at inducing abscission (7). ABA appears to be more involved with regulating the production of ethylene, rather than acting as a direct inducer of ethylene or abscission (36).

The evidence today points to another hormone that may be

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[1] A. C. Leopold and K. M. Brown, unpublished results.

responsible for the process of leaf senescence and abscission. Called the senescence factor (SF), it accelerates the process of abscission and stimulates the production of ethylene (94). This is in contrast to auxin (IAA) which can stimulate ethylene but not abscission, and ABA which stimulates abscission but can inhibit the production of ethylene. ABA is found in both young and old leaves; SF is found only in senescent or near-senescent leaves (94).

Ethylene production increased in stressed or injured tissues (27, 46, 140, 148). These increases are dependent upon the presence of physiologically disturbed, but living, cells (27, 52, 72). Severe damage to cells caused a decline in ethylene production (148) accompanied by an increase in the amount of ethane produced (27). Ethane is normally present in quantities that are minute in comparison to the amount of ethylene (22). Homogenization of apple tissue promoted an increase in ethane production with a concurrent, though much greater, decline in that of ethylene (77). Point freezing of sugar beet leaf discs increased ethane production as the area of frozen tissue increased to 100% (27). Ethylene production reached a maximum when 50% of the tissue was killed by freezing. It then declined to negligible levels as the area of frozen tissue increased (27). Ethylene production requires the presence of living cells with ATP, while ethane is formed as a by-product of cellular breakdown without use of energy (27).

Electrical Impedance

Electrical impedance is a measure of the apparent internal resistance to an alternating current. Unlike resistance, impedance measurements refer only to alternating currents and take into account the capacitance, the property that determines the amount of charge (ions) that can be stored across tissue or membrane boundaries (120). Impedance reduces the variability that is inherent when using resistance alone (119).

The preliminary work on electrical impedance with regard to tissue injury was written by Osterhout (95) in 1921. Luyet (80), another early researcher, demonstrated that impedance measured at a frequency of one kilohertz (kHz) decreased with increasing injury reaching a minimum at death. At one megahertz (mHz), the impedance remained almost constant whether the tissue was living or dead. In general, higher frequencies yield lower impedance values (119).

Reasons for differences between the two frequencies have become more clear as our understanding of ion flow has become more complete (40). Low frequency measurements tend to record the impedance of the cell walls and membranes, so if those membranes are disrupted the impedance will decline. High frequency measurements record cytoplasmic impedance. Changes in the cytoplasm during freezing are less perceptible than the changes in the

membranes, so megahertz readings do not vary as much as kilohertz values (40).

Electrical measurements have been used to detect differences due to freeze-damage and to predict hardiness in various species. Increasing amounts of injury decreased electrical resistance (38, 121, 141, 143), conductance (28, 128), and impedance (30, 39, 40). Wilner (142) tested apples for hardiness by measuring resistance, and found that the resistance was highest in the hardiest varieties, but that the differences were significant for only a brief period in early October. Svejda (119) measured electrical impedance in roses and observed that impedance tended to be highest in the hardiest varieties. Weaver et al. (137) attempted to predict hardiness in peaches using impedance. There was a positive correlation between increasing impedance and hardiness, but there was too much variability due to scion diameter. Van den Driessche (126, 127), showed that electrical impedance increased in Douglas fir seedlings during the autumn, but that the values obtained from prefreezing tests for impedance were not a reliable indicator of hardiness.

Fejer (30) observed that electrical impedance could be used to effectively detect tissue injury, but that it could not be used to predict hardiness in advance. Van den Driessche (127) used electrical measurements taken after freezing tests to predict hardiness, but Evert and Weiser (28), using conductance ratios, predicted hardiness

within 2°C. in red-osier dogwood without freezing the plants.

Fensom (31) recommended the use of pulsed resistance, or short bursts of current, for measuring electrical resistance. Skutt et al. (109) measured the decrease in pulsed resistance in decaying wood and were able to distinguish it from healthy wood. Wargo and Skutt (135) were able to detect damage caused by gypsy moth defoliation by measuring the decrease in pulsed resistance.

The use of an impedance ratio (Z kHz/Z mHz, where Z is the impedance and kHz and mHz are the frequencies at which it is measured) as a means of determining injury was first discussed by Greenham and Daday (42) for use on alfalfa and clover. Glerum (39) used impedance ratios to determine vitality in several species, and found that impedance ratios decreased after injury due to freezing or boiling. Van den Driessche (127) measured impedance ratios before and after freezing attempting to predict frost-hardiness. Pre-freezing impedance ratios did not predict hardiness as well as measurements taken after freezing. Impedance ratios decline during the development of dormancy (40).

The decrease in impedance in injured tissue may be due to increased passage of electrolytes through the disrupted membranes into the intercellular fluid, changing the electrical properties (119, 120). This high concentration of mobile ions caused a decrease in electrical resistance and impedance. Higher concentrations of

mobile ions caused a decrease in electrical resistance and impedance. Higher concentrations of mobile ions, such as K^+ and Ca^+, were reported in injured tissue while the resistance was declining (122).

Changes in electrical measurements during dormancy may be dependent on the availability of free water (119). Both xylem water potential and stem moisture content declined during dormancy development (48, 83). The composition of cell membranes in Cornus spp. changed from molecules shorter than C^{18} to longer molecules, up to C^{24}, as hardiness increased (139). Changes in membrane structure could lead to increased permeability of the cell membrane to water (28), 130). Realignment of the internal structure of the protoplasm to a more stress-resistant form due to changes in the structure and arrangement of macromolecules and the formation of reversible hydrophobic bonds (35, 82) may force water out of the cells. The dehydrated protoplasm may then be capable of surviving sub-freezing temperatures after this reorganization.

Research Approach

The purpose of this research was to identify a reliable index of plant maturity, and to evaluate the relationship of maturity to the onset of cold acclimation. A maturity index could be used to determine when a plant is physiologically able to withstand the effects of defoliation without causing tip dieback.

The results of a hand defoliation test for maturity (33) were compared with the results of a series of laboratory tests in order to determine the predictive value of such procedures. Analysis of variance and linear correlations were used to compare the data.

This thesis is written in scientific manuscript format. The following papers will be submitted to HortScience for publication.

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THE RELATIONSHIP BETWEEN VEGETATIVE MATURITY AND THE FIRST STAGE OF COLD ACCLIMATION[1,2]

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Abstract. Red-osier dogwood (Cornus stolonifera Michx.) plants were grown outdoors in a lathhouse to study the relationship between vegetative maturity and the first stage of cold acclimation. Both microscopic observations and electrical impedance ratios used to measure damage of frozen stem sections verified the close association of the onset of the first stage of cold acclimation and vegetative maturity. The relationship of these processes to dormancy development is discussed.

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Vegetative maturity of deciduous plants was recently defined as that stage of development when a plant is no longer dependent on leaves for survival during prolonged winter storage (1, 5). Plants defoliated before this stage either die completely or suffer some level of tip dieback. Unfortunately, the relationship of this stage to other known processes of dormancy development is not well understood. Fuchigami et al. (1) showed that maturity occurs several weeks prior to rest. Seibel (7) recently observed that maturity is closely related, and may be identical, to the the transition period between summer and winter dormancy. This stage is also referred to as the end of correlative inhibition, when leaves are no longer necessary to prevent the lateral buds from growing (11).

Fuchigami et al. (2) reported that leaves are necessary for cold acclimation and related defoliation to the first stage of cold acclimation. From this data, a close relationship between the first stage of acclimation and vegetative maturity can be inferred. The first stage of acclimation, which is induced by short days, depends on the presence of a translocatable hardiness promoter produced in the leaves (8, 9). Defoliation eliminates the receptor sites for the short-day stimulus and/or the source of the hardiness promoter (2, 6). Previous studies to relate the onset of cold acclimation to a specific stage of development have suggested that the onset of hardiness occurs during winter dormancy or rest (10). More recent evidence

demonstrates that cold acclimation occurs prior to rest (2, 6).

Red-osier dogwood plants were propagated from single node cuttings in the summer of 1975 and transplanted into 5 in. pots in a mixture of sand, soil, and peat (1:1:1). After over-wintering in a lathhouse, they were pruned to a single leader and selected for uniformity on the basis of height and growth habit. The plants were from from 70 to 130 cm. tall when experiments began in early July of 1976.

On each sampling date, 10 plants were defoliated and left in the lathhouse under natural temperature and daylength. Temperature and humidity in the lathhouse were monitored with a Weathermeasure hygrothermograph. Plants were observed daily, and any new growth was removed. In March, tip dieback of these plants was measured and recorded as $\frac{\text{dead stem length}}{\text{total stem length}} \times 100$.

For hardiness determinations, another 10 plants were selected and the stems cut into 2-cm. sections. Four sections from each plant were wrapped in aluminum foil and frozen in a freezing chamber to -10° C. at 3°/hr. Nonhardy red-osier dogwood plants survive to -6° (1). Therefore, survival below this temperature provides an indicator of the first stage of cold acclimation.

From microscopic observations of frozen stem sections, an injury scale of 0 to 3 was used to rate progressive stages of injury due to freezing. A value of 0 indicated no injury; 1 corresponded to pith damage; 2 displayed cambial injury; and 3 indicated injury to

bark cortex and phloem as well as pith and cambial tissues. After freezing, stem sections were incubated for 48 hours at room temperature (23° C.). Microscopic evaluations were then made and the data from all 40 sections from each sample were averaged to plot the graph shown in Fig. 1.

Impedance ratios (3, 4) were measured with a meter on loan from Weyerhaeuser, Inc. Stem sections were frozen, thawed, and warmed to room temperature as previously discussed, before measuring impedance. The scale of the meter was not designed for the changes in electrical properties that occurred during dormancy development, so an arbitrary scale was designed to record the data. Stem sections that registered on the meter were designated 'on scale'. Those that did not were called 'off scale'. The curve plotted in Fig. 1 is drawn from $\frac{\text{no. of 'on scale' readings}}{40} \times 100$, where 40 is the total number of samples that were measured at each sampling period. This scale was accurate and adequate for showing the changes that occurred during maturation.

Fig. 1 illustrates the close association between the development of vegetative maturity and the onset of cold acclimation. Complete vegetative maturity occurred on September 21, approximately the same time that a pronounced change in hardiness, as determined by microscopic observation and electrical impedance, was observed. The change in impedance prior to September 21 may indicate

differences between individual plants or an increase in hardiness not detectable by microscopic examination. Electrical impedance has been used by researchers and industry to determine the extent of freezing injury, and is thought to be more sensitive than other tests (3).

Fuchigami et al. (1) reported that vegetative maturity occurred several weeks, approximately 6, before the development of rest in red-osier dogwood. Therefore, on the basis of their findings and results presented here, the onset of cold acclimation occurs before rest and at the vegetative maturity stage of development (Fig. 2). Recently, Seibel (7) showed that vegetative maturity and the transition between summer and winter dormancy are probably the same stage of development. Therefore, relating dormancy to cold acclimation, the first stage of acclimation occurs at the transition stage between summer and winter dormancy, the onset of winter dormancy (Fig. 2).

These studies are supportive of previous reports that hardiness occurs before rest (2, 6). This suggests that other deciduous plants, and possibly all deciduous species, may begin hardening at the onset of winter dormancy. Experiments similar to those used here, relating these processes, would be of interest and necessary to determine how widespread these relationships actually are.

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Fig. 1. Electrical impedance values, microscopic hardiness ratings of tissue frozen to -10° C. and tip dieback in lathhouse plants. Electrical impedance (◉—◉); hardiness (●———●); tip dieback (●-----●). Vegetative maturity was complete on 9/21.

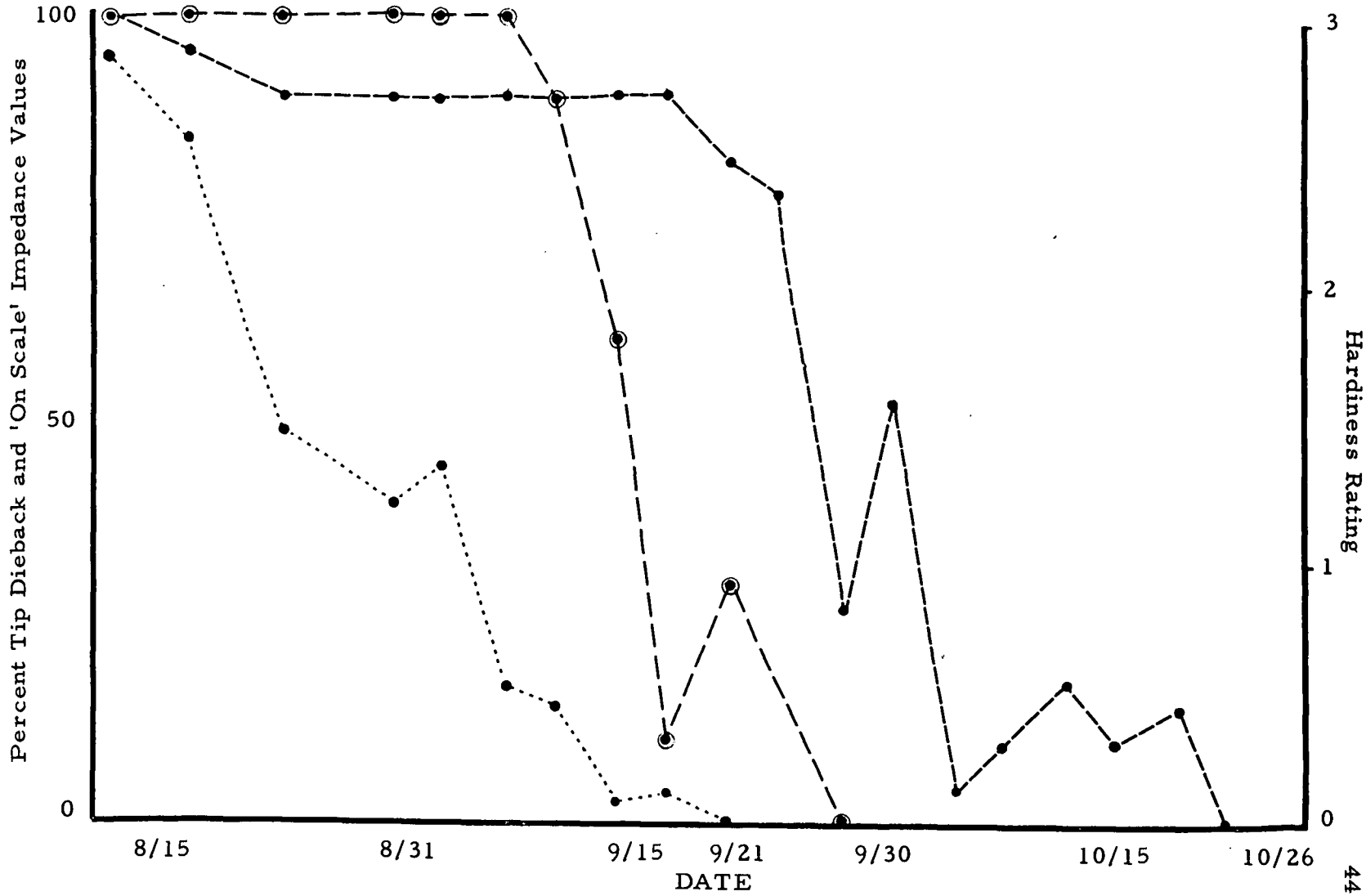
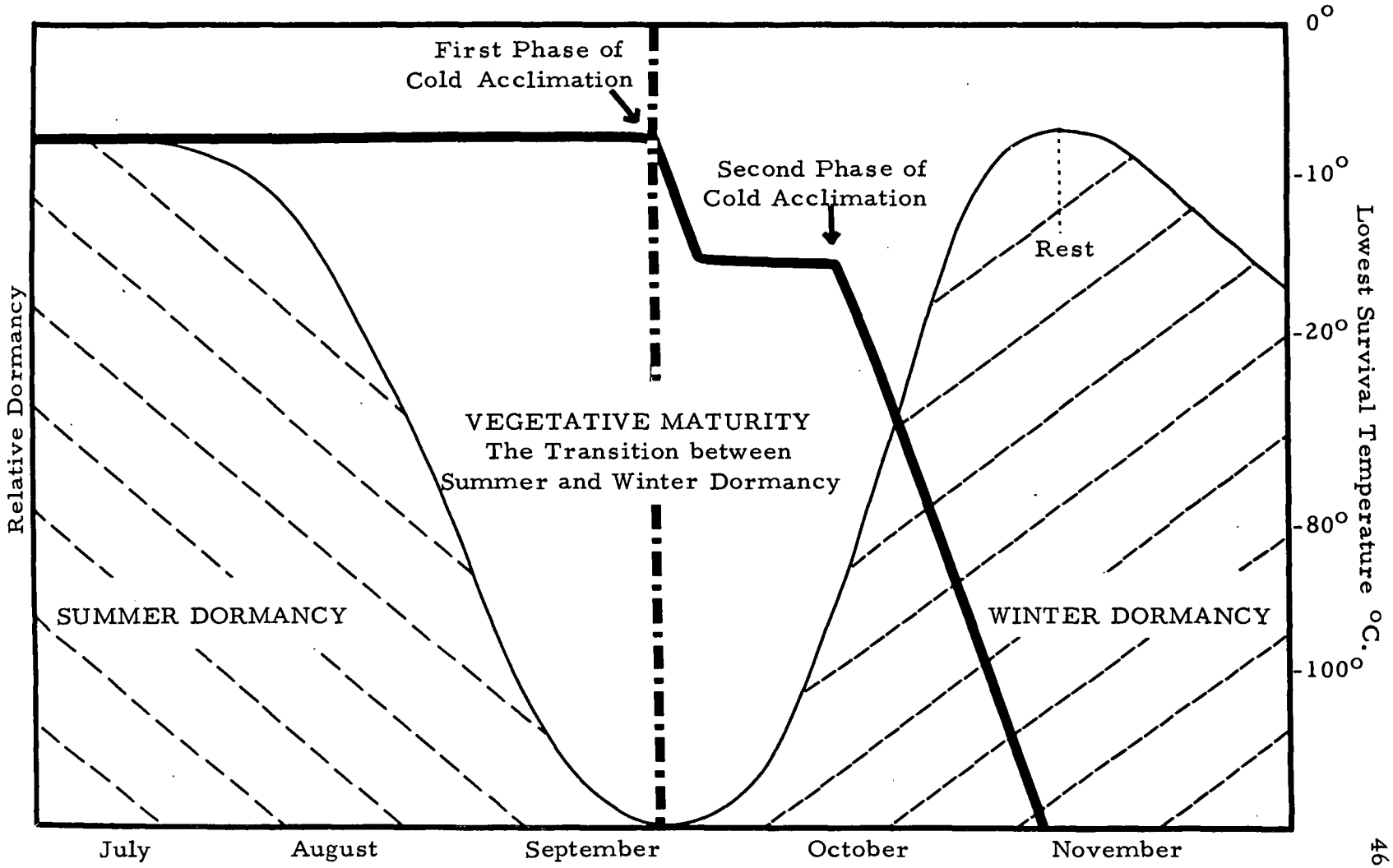

100
50
0
Percent Tip Dieback and 'On Scale' Impedance Values
3
2
1
0
Hardiness Rating
8/15
8/31
9/15
9/21
9/30
10/15
10/26
DATE

Fig. 2. Relationship between hardiness and dormancy development in red-osier dogwood.


First Phase of
Cold Acclimation
Second Phase of
Cold Acclimation
Rest
VEGETATIVE MATURITY
The Transition between
Summer and Winter Dormancy
SUMMER DORMANCY
WINTER DORMANCY
Relative Dormancy
Lowest Survival Temperature °C.
0°
-10°
-20°
-80°
-100°
July
August
September
October
November

XYLEM WATER POTENTIAL AND ELECTRICAL IMPEDANCE RATIOS AS MEASURES OF VEGETATIVE MATURITY IN RED-OSIER DOGWOOD (CORNUS STOLONIFERA MICHX.)[1]

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Additional index words. Dormancy, deciduous, defoliation

Abstract. Xylem water potential and electrical impedance ratios were used to predict the time of vegetative maturity in red-osier dogwoods grown under two temperature regimes in growth chambers and in a lathhouse under natural conditions. The decline in XWP correlated with the development of vegetative maturity as measured by tip dieback after defoliation. In two growth chamber conditions, average XWP values reached a minimum at the time of vegetative maturity. In all cases, however, variability within samples was so large as to preclude the use of XWP as an accurate, reliable index for determining plant maturity. A change in electrical impedance ratios at and after maturity caused the impedance meter to go 'off scale'. In comparison to the XWP readings, the change in electrical appearance ratios was more consistent and shows promise as a predictor of vegetative maturity.

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A decline in plant moisture occurs during the development of dormancy (4, 6). Some of the changes in a plant's physiological and biochemical processes that are correlated with the decline in moisture content include an increase in the permeability of cell membranes to water (6), an increase in the amount of abscisic acid (7), declines in cell division, protein and carbohydrate synthesis and metabolism (4), and lower yields from photosynthesis (8). Leaf senescence is accelerated and plant hardiness can be increased (5) when the available moisture declines.

This decline in plant moisture coincides with the decline in many plant processes, so a measure of plant water status to determine the stage of seasonal development of the plant seems possible (4). Vegetative maturity, the transition between summer and winter dormancy, is the term used to identify the phase of development when deciduous plants are able to survive the effects of fall defoliation (2, 4). A plant that is mature does not visibly differ from one that is not mature. Since the onset of vegetative maturity can occur at natural leaffall or up to a month or more in advance (4), another method not dependent on visual characteristics must be used to determine the time of maturity.

Recently, vegetative maturity was related to the onset of the first stage of cold acclimation (9). Because of this association and the relationship of declining water content with hardiness, it seemed

logical that XWP could be used to measure vegetative maturity.

In addition to XWP, electrical impedance ratios of stem sections were measured. Electrical resistance has been used to detect injury due to freezing (1, 3) or to predict hardiness (11, 12). A decline in impedance ratios has been associated with the development of dormancy (3), so a useful relationship between impedance ratios and vegetative maturity seemed plausible.

This study attempted to relate plant water status, using XWP as measured with the pressure bomb (10, 13) and percentage moisture, with vegetative maturity as measured by tip dieback after defoliation (2). In addition, electrical impedance ratios were compared with vegetative maturity of the lathhouse plants.

Red-osier dogwood plants were propagated from single node cuttings and transplanted into 5 in. pots containing a mixture of sand, peat, and soil (1:1:1) in the spring of 1976. The plants were between 30 and 40 cm. high when the growth chamber experiments began in early July. They were trimmed to 2 shoots and uniform plants were selected into groups of 3 and arranged in 5 blocks of 30 plants each in the growth chambers. The growth chambers were set at $13^{\circ} \pm 1^{\circ}$ C. and $29^{\circ} \pm 1^{\circ}$, with a light intensity of 213.67 lux and a photoperiod of 10 hours in both chambers.

Weekly, a pair of plants was randomly chosen from each of the 5 sections. One plant from each pair was defoliated and placed in a

warm (21^{+o} C. days, 18^{o} nights) greenhouse with natural daylength. Defoliated plants were observed daily and any new leaves removed. Tip dieback was measured in March, 3 months after the plants were moved into a plastic-covered lathhouse. Percentage tip dieback was recorded as $\frac{\text{dead stem length}}{\text{total stem length}} \times 100$.

Pressure bomb measurements of XWP were taken from the remaining plants using the top 5 to 8 cm. of stem. XWP was recorded in bars, and taken before the lights were turned on in order to reduce variations due to transpiration. To measure percentage moisture, 1 cm. sections of internodes were cut, weighed, and dried for 48 hours in a 60^{o} C. drying oven before the final weighing.

The lathhouse plants, ranging from 70 to 130 cm. tall, were selected for uniformity based on height and growth habit. Ten plants were defoliated twice each week, and left in the lathhouse. Tip dieback was recorded as described previously. At the same time, XWP and moisture content were measured on a similar group of plants.

The percent moisture declined gradually as the plants matured in all 3 growing conditions (Fig. 1 and 2). These results support the findings of others (4) showing that deciduous plants lose water during dormancy development. The decline in percent moisture is too gradual and not definitive enough to warrant its use as an index of maturity.

A significant correlation (r=.404) between tip dieback and the

decline in XWP in the lathhouse plants was in agreement with the results reported by Hotze (4). However, the span of values for XWP ranged from -3.5 to -14 bars. Of these values, 99% of the plants with XWP of -7.5 or lower were mature, but almost 40% of the plants with XWP of greater than -7.5 were also mature. Under growth chamber conditions, similar trends were observed, with the lowest average XWP coinciding with the time of vegetative maturity. Unfortunately, variability within all the samples decreased the value of XWP as a tool for predicting maturity. This data supports the conclusions drawn by Hotze (4): "It is not likely that the XWP measurements will become a nursery practice for determining defoliation timing."

Electrical impedance ratios were measured on 2-cm. internode sections of the lathhouse plants using methods described in the previous paper (9). Impedance values showed a rapid decline beginning a few weeks before maturity development (Fig. 1). No plants recorded 'on scale' values after the time of vegetative maturity. Changes in moisture content and the internal arrangement of the cells in response to this decline in moisture may be responsible for the changes in impedance values (3).

The rapid change in impedance values was quite consistent in all tissues measured and suggests that this technique may be used to predict vegetative maturity. Further testing to determine the critical

changes in impedance values is necessary before their use as an index of vegetative maturity can be recommended.

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Fig. 1. Average XWP, moisture content, and tip dieback in plants grown at 13° C. XWP (●———●); % moisture content (●– –●); tip dieback (●------●). XWP values listed with ± one standard deviation. Vegetative maturity occurred during week 7.

Percent Tip Dieback and Moisture
0
50
100
2
4
6
8
10
Time in Weeks
-10
-8
-6
-4
Xylem Water Potential; Bars

Fig. 2. Average XWP, moisture content, electrical impedance values, and tip dieback in plants grown under natural conditions. XWP (●——●); % moisture content (●– –●); Electrical impedance values (●– – –●); and tip dieback (●-----●). Maturity was complete on 9/21.

Percentage
Tip Dieback, Moisture, and 'On Scale' Impedance Values
0
50
100
8/15
8/31
9/15
9/21
9/31
10/15
10/26
DATE
-10
-9
-8
-7
-6
-5
-4
-3
-2
-1
0
Xylem Water Potential; Bars

CHANGES IN ETHYLENE AND ETHANE PRODUCTION OF FROZEN AND UNFROZEN STEM SECTIONS OF RED-OSIER DOGWOOD DURING DEVELOPMENT OF VEGETATIVE MATURITY[1]

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Additional index words. Dormancy, deciduous, Cornus stolonifera Michx.

Abstract. Ethylene and ethane production of frozen and unfrozen stem sections of red-osier dogwood was compared with the development of vegetative maturity and cold acclimation. In unfrozen stem sections, ethylene production decreased and no ethane production occurred as vegetative maturity development progressed. After freezing, the production of ethylene decreased and ethane increased with increasing injury to stem tissues. The possibility of using ethylene and ethane to quantitatively measure freezing injury is discussed.

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The importance of vegetative maturity, a stage in the seasonal development of deciduous plants, and its relationship to the transition between summer and winter dormancy has recently been described (14). Besides being important for the defoliation of deciduous nursery stock and prolonging winter storage, it is also related to the delay of spring bud-break (6) and to the onset of cold acclimation (12).

Recently, Seibel (14) reported that ethylene production of excised leaves, internodes, and nodes decreases significantly with increasing levels of maturity development in Cornus stolonifera Michx. Lowest levels of ethylene were reached at and after full maturity development. Leopold and Brown[2] reported that the measurable amounts of ethylene produced in white pine stems during the summer decreased to undetectable levels by October.

Applications of ethylene, via ethephon treatments, during the development of vegetative maturity in C. stolonifera caused a significant delay in maturity development at all levels tested (6). Maintaining high ethylene levels during maturity development, when natural levels are declining, may inhibit maturity development directly or through an influence on hormonal activity.

Ethylene has also been associated with the hardiness of plants. Dollwet and Kumamoto (4) reported an increase in cold hardiness in

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[2]Unpublished results.

some species after applications of ethylpropylphosphonate, a compound which releases ethylene. Fuchigami et al. (7), however, observed that direct applications of ethylene had no effect on the induction of cold hardiness in red-osier dogwood. The different results of the 2 groups may be explained by differences in timing of application with respect to maturity, differences in plant response, or differences between the chemicals used.

When compared to endogenous ethylene production, the amount of ethane present in plants under normal conditions is minute (3). Wounding or stressing tissues increases ethylene production (5, 10), and damage severe enough to cause membrane breakdown, as during freezing injury, may stimulate the formation of ethane (5). The 2 compounds, though closely related in structure, are apparently formed via 2 very different pathways (5). Ethylene requires the presence of intact cells and a source of ATP (5, 13). Ethane is produced as a by-product of cellular disintegration, and, therefore, does not require energy in its formation (5).

The objectives of this study were three-fold: 1) to follow the trend of decreasing ethylene production during maturity development; 2) to observe the changes in ethylene production by frozen stems as maturity progressed; and 3) to document the presence of ethane as a by-product of cellular damage caused by freezing in woody stems.

Red-osier dogwood plants were propagated from single node

stem cuttings and grown in 5 in. pots in a lathhouse in Corvallis, OR. Plants were pruned to a single leader, and selected for uniformity on the basis of height and growth habit. The plants ranged in height from 100 to 150 cm. when the ethylene measurements were begun in mid-August.

A total of 20 plants were sampled twice each week beginning on August 13 and ending on October 26. Half of these plants were defoliated on each date and left in the lathhouse under natural daylength and temperatures. They were observed daily and any new growth was removed. In March, the amount of tip dieback was recorded.

To determine the amount of ethylene produced in frozen and unfrozen samples, 2-cm. internode stem sections were used. One section from each plant was wrapped in aluminum foil and frozen to -10° C. at 3°/hr. in a freezing chamber. After reaching -10°, stem sections were put into thermos jars and slowly thawed in a 1° refrigerator. At the same time, another stem section was wrapped in foil and refrigerated at 1°. Both samples were refrigerated for 24 hours so that both frozen and unfrozen stem ethylene measurements could begin simultaneously. Preliminary data indicated that short-term refrigeration did not significantly decrease total ethylene production.

After refrigeration, stem sections were placed into 2-dram vials sealed with serum stoppers and incubated in a 23° C.

temperature-controlled dark room for 6 hours. One ml. samples of air from the vials of both frozen and unfrozen stems were injected into a Carle 210 analytical gas chromatograph with flame ionization detector and a 4' by 1/8" 80/100 mesh activated Alumina column that was maintained at 100° with a flow rate of 20 ml./min. (14).

The amount of ethylene produced by unfrozen stem sections decreased as plants matured. There was a strong correlation (r=.727) between ethylene production in unfrozen stem sections and tip dieback, indicating that it may be possible to predict vegetative maturity by measuring the decline in ethylene production during dormancy development. Seibel (14) pointed out the absence of a threshold value for using ethylene as a predictor of plant maturity. No threshold value is seen with the results presented here (Fig. 1), and the variability within samples is also shown.

Results of ethylene production by frozen stem sections during vegetative maturity development are reported in Fig. 2. Initial production was low in comparison to the levels reported in unfrozen stem sections. During the development of vegetative maturity a slight increase occurred, and at the time of vegetative maturity, high levels of ethylene and extreme variations among samples was evident. After vegetative maturity, ethylene concentration declined sharply and reached levels similar to those of unfrozen stem sections (Fig. 1 and 2).

Microscopic observations of frozen stem sections (Fig. 2) suggest that the level of ethylene production by frozen stem sections is related to the amount of tissue damage. According to microscopic observation, the stem tissues did not appear to survive freezing until after maturity was reached. After this stage, the tissue appeared to be increasingly less damaged until 3 or 4 weeks after maturity, when no tissue appeared damaged by exposure to -10° C. treatments.

The low levels of ethylene production in frozen stem sections before maturity was apparently due to severe internal disorder. ATP and enzymes necessary for ethylene synthesis (13) may not be available to the damaged tissue (5).

The amount of ethane production in frozen stem sections is reported in Fig. 3. None of the unfrozen stem sections, throughout the sampling period, produced measurable amounts of ethane. This is consistent with previous reports (3, 5, 11). The production of ethane from stem sections subjected to the freezing test is directly related to the amount of tissue damage and, therefore, to maturity and hardiness development (Fig. 3). Similar findings relating tissue damage and ethane levels have been reported (5).

As plants matured and hardiness increased (12), more cells were able to survive the low temperatures, thus resulting in greater ethylene production from stressed tissues and cells. These living cells, though possibly injured, produced large quantities of wound

ethylene in response to the damaged cells around them and their own injury. Variations in ethylene production between sampling dates are most likely due to the extreme sensitivity of the method, in combination with the relatively uncontrollable effects on cellular damage and/or stress. Any sampling variation could cause changes in the amount of injured tissue, and since the tissues themselves are changing, some variation can be expected.

In conclusion, these studies suggest a definite relationship between ethylene levels and the development of vegetative maturity and the onset of cold acclimation. The production of ethylene decreases with increasing vegetative maturity and dormancy development. Ethane is not produced by live tissue in measurable quantities, but is related to freezing injury. Comparison of ethylene-ethane levels with freezing damage suggests that stressed and injured tissues produce high levels of ethylene, and if damage is severe enough, ethane.

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Fig. 1. Ethylene production of unfrozen stem sections and tip dieback in lathhouse-grown plants. Maturity occurred 9/21. Ethylene (o——o); tip dieback (o------o).

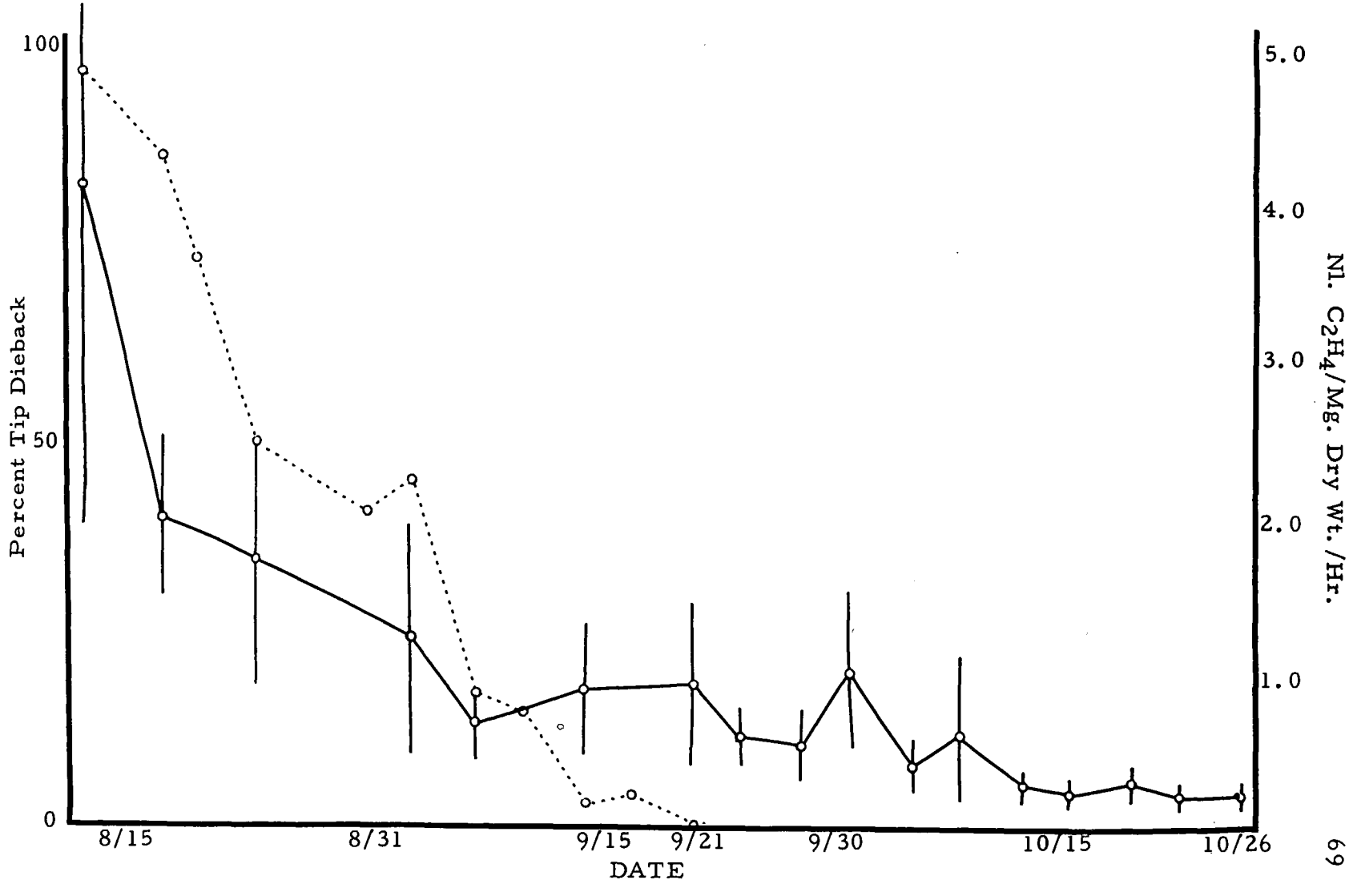

100
50
0
Percent Tip Dieback
8/15
8/31
9/15
9/21
9/30
10/15
10/26
DATE
5.0
4.0
3.0
2.0
1.0
Nl. C_2H_4/Mg. Dry Wt./Hr.

Fig. 2. Ethylene production of frozen stem sections, microscopic hardiness ratings, and tip dieback in lathhouse-grown plants. Plants were mature on 9/21. Ethylene (o——o); hardiness ratings (●– – –●); tip dieback (o------o). Numbers on hardiness curve refer to injury rating system.

For additional information on the hardiness ratings, see Fig. 1, page 45.

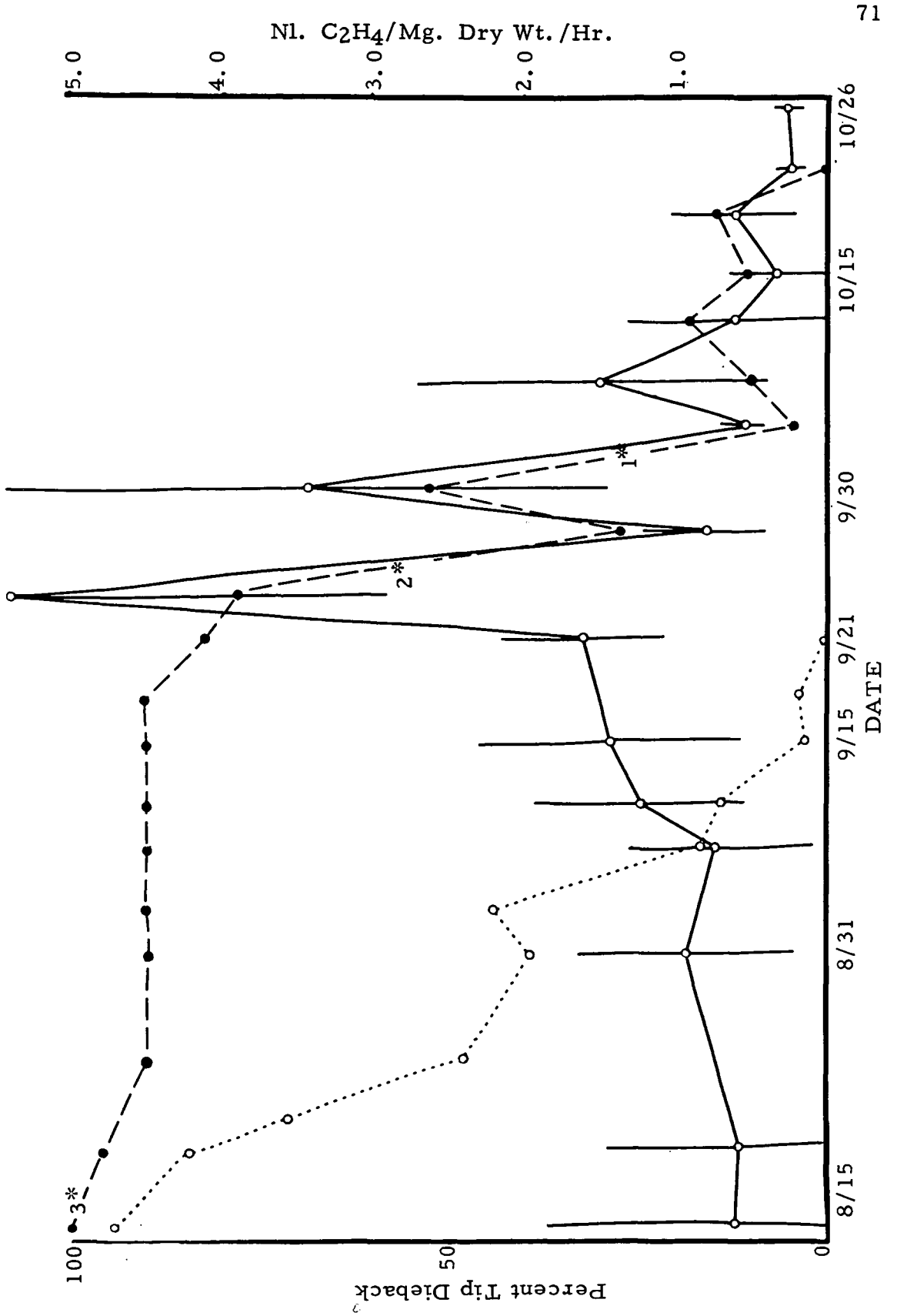
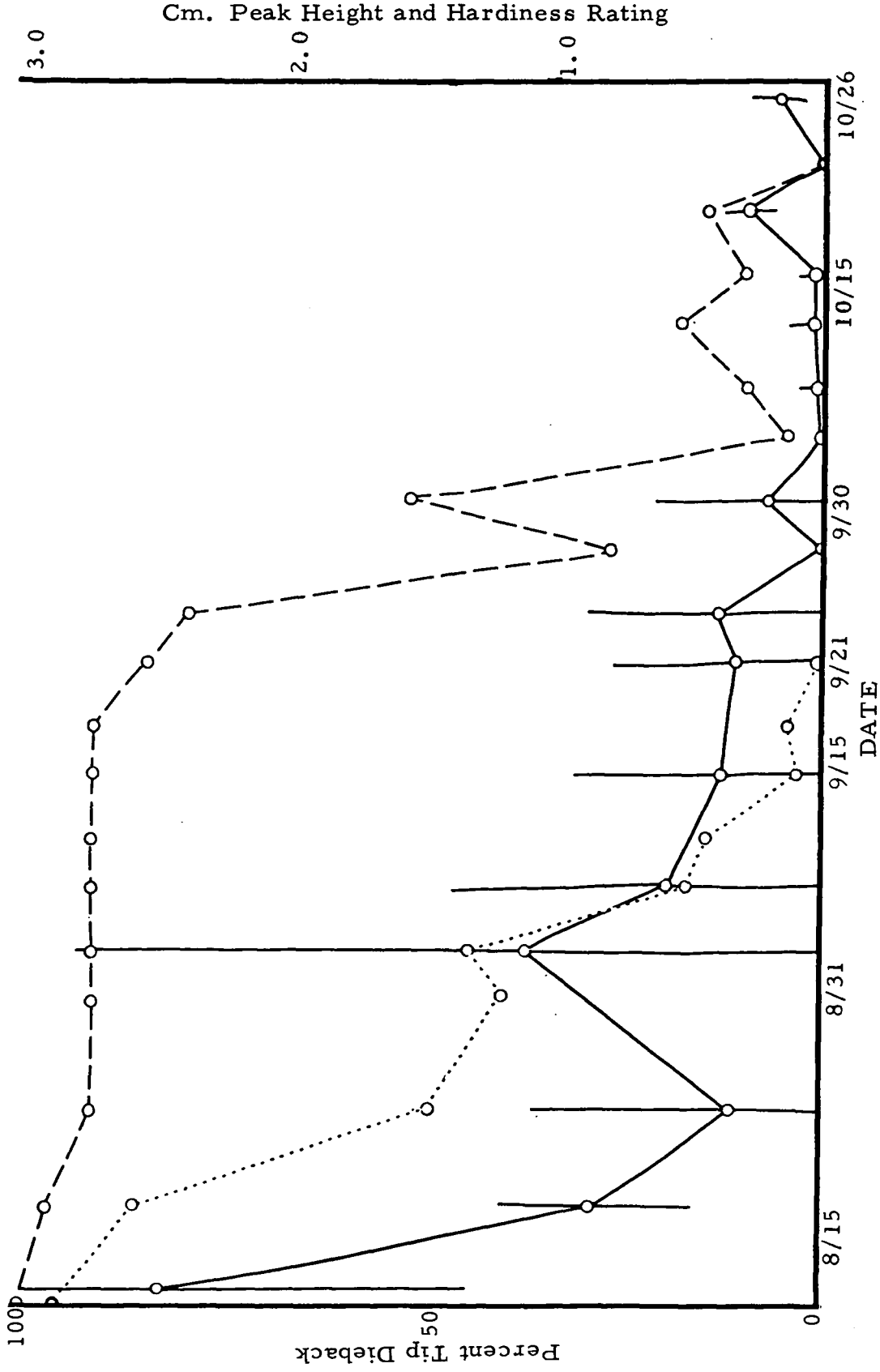

Nl. C_2H_4/Mg. Dry Wt./Hr.
5.0
4.0
3.0
2.0
1.0
10/26
10/15
9/30
9/21
9/15
8/31
8/15
DATE
1*
2*
3*
100
50
0
Percent Tip Dieback

Fig. 3. Ethane production, microscopic hardiness ratings and tip dieback in lathhouse-grown plants. Plants were mature on 9/21. Ethane (o———o), measured in cm. peak height; hardiness ratings (o — —o); tip dieback (o------o).

For additional information on the hardiness ratings, see Fig. 1, page 45.


Cm. Peak Height and Hardiness Rating
3.0
2.0
1.0
10/26
10/15
9/30
9/21
9/15
8/31
8/15
DATE
100
50
0
Percent Tip Dieback