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INFLUENCE OF ROOTSTOCK ON THE RESPONSE OF SEYVAL GRAPEVINES TO ENVIRONMENTAL STRESS

By

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ABSTRACT

INFLUENCE OF ROOTSTOCK ON THE RESPONSE OF SEYVAL GRAPEVINES TO ENVIRONMENTAL STRESS

By

R. Keith Striegler

Experiments were conducted to determine if grapevine rootstocks can influence cold hardiness and flooding tolerance of scion tissues. Cold hardiness and water content of primary buds and canes were measured periodically during acclimation and deacclimation. Rootstocks had little effect on cold hardiness or water content during acclimation. However, significant rootstock effects were observed for the deacclimation period.

Canes from Seyval grafted on Cynthiana had greater cold hardiness and lower water content than the other graft combinations. Buds responded in a similar manner but to a lesser degree. Use of Cynthiana as a rootstock also reduced the percentage of shootless nodes.

The effects of rootstock and vine size on cold hardiness of Seyval grapevines were determined separately in a second study. Vine size effects were considered to be an indication of secondary rootstock effects since vine size modification is an important primary rootstock effect. Treatment effects on cold hardiness were determined by measurement of percent shootless nodes and the within vine distribution of canes with characteristics associated with increased cold hardiness.

Primary and secondary effects of rootstock on cold hardiness were observed. Percentage of shootless nodes was lowest when Seyval

was grafted on Couderc 3309. Vine size did not significantly influence the percentage of shootless nodes when canes of comparable quality were evaluated. Rootstock did not significantly affect the within-vine distribution of canes. Vine size effects on the within-vine distribution of canes were noted. Large vines had a greater number of poorly matured canes and canes with superior cold resistance. Large vines do not appear to be inferior to small vines if careful cane selection is practiced at pruning.

Flooding tolerance of own-rooted vines was determined under controlled conditions. St. George, Couderc 3309, and Riparia Gloire were tolerant of flooding while Kober 5BB, Seyval, and Cynthiana were intolerant of flooding. Flooding tolerance of Seyval was increased slightly by grafting onto Couderc 3309. Symptoms of flooding were desiccation of the shoot apex, flagging of leaves, necrotic areas on leaves, senescence of basal leaves, and regeneration of roots near the water surface.

This dissertation is dedicated to the memory
of my father, Curtis Striegler (1930-1984).

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TABLE OF CONTENTS

LIST OF TABLES

Page

SECTION II: INFLUENCE OF ROOTSTOCK ON THE RESPONSE OF SEYVAL GRAPEVINES TO FLOODING STRESS

EFFECT OF ROOTSTOCK ON THE GROWTH AND PHYSIOLOGY OF SEYVAL GRAPEVINES DURING FLOODING

APPENDICES

I. EFFECT OF FLOODING ON WATER RELATIONS, LEAF GAS EXCHANGE, AND GROWTH OF GRAPEVINES

II. RESPONSE OF SEYVAL GRAPEVINES TO FLOODING AT DIFFERENT STAGES OF DEVELOPMENT

LIST OF FIGURES

Page

II. RESPONSE OF SEYVAL GRAPEVINES TO FLOODING AT DIFFERENT STAGES OF DEVELOPMENT

INTRODUCTION

Stress has been defined as "any environmental factor potentially unfavorable to living organisms" (102). The importance of environmental stress has recently been summarized by Boyer (26). Most crops grown in the U.S. have a high genetic potential for yield which is not realized. Yield losses due to diseases, insects, and weeds account for 9.3 percent of yield potential. Soil problems and unfavorable climates result in yield losses which account for 69.1 percent of yield potential. While some of the reduction in yield caused by environmental stress (soil problems and unfavorable climates) could be alleviated with better management by growers, it appears that substantial reductions in yield are caused by environmental stress. Another consideration is reduced quality which may result from environmental stress (96). This is important for high value horticultural crops since quality is often as important as quantity of yield in determining profitability.

Exposure to freezing temperatures and flooding are major environmental stresses which limit viticulture in Michigan. Grape production in Michigan ranged from 29,090 to 54,432 metric tons during the 1983-87 period (57). Much of the variation in yield observed during this period was due to freezing injury. Freezing injury usually occurs during the dormant season or in the spring as buds begin to develop. Spring frost damage was not addressed in

this research because it appears that rootstocks do not influence the timing of budburst under Michigan conditions (109).

Flooding stress can be equally detrimental to vine performance but its impact is usually more localized due to the variable nature of soils in Michigan. Michigan soils are of glacial origin and it is not uncommon to find well-drained sandy soil in close association with imperfectly drained clay soil (186). Compacted zones can occur at various depths even in sandy soils which leads to restricted root growth and flooding during periods of heavy rainfall or excessive irrigation. Fruit trees or grapevines growing on poorly drained soil generally perform poorly and have increased mortality (94,113,115,118,173).

This dissertation was undertaken to gain an understanding of the involvement of the grapevine root system in scion responses to freezing and flooding stress. Various Vitis species and interspecific hybrids either grafted or on their own roots were used as probes to uncover root contributions to scion resistance. This approach should also yield useful information on root physiology and the mechanism of injury of freezing and flooding stress.

The experiments comprising this dissertation are arranged in chapters, each of which addresses an aspect of the research problem. Each chapter has its own introduction, materials and methods, results, discussion, and conclusions and it is intended that each will be submitted to the American Journal of Enology and Viticulture for publication.

A review of literature is included even though each chapter is designed to stand alone and has relevant literature reviewed in its introduction. The purpose of the literature review is to present a

detailed background on cold hardiness and flooding of woody plants. It is hoped that the review will serve as a reference for evaluation of the experiments in this dissertation. Emphasis will be placed on current literature since various aspects of cold hardiness (31,79, 80,183,184) and flooding (30,46,54,77,85,86,89,92,93,129,138, 167,170) have been recently reviewed.

LITERATURE REVIEW

I. COLD HARDINESS

The ability to withstand low temperatures is a requirement for the survival of woody plants in temperate regions of the world. Acclimation, deacclimation, mechanisms of freezing injury, and mechanisms of resistance to freezing are important aspects of this topic and will be covered in this portion of the literature review. Emphasis will be placed on literature pertaining to woody plants.

Woody plants have evolved elaborate strategies by which they resist freezing injury. The execution of these strategies is dependant on the ability of the plant to acclimate to freezing temperatures. New growth in the spring is tender and can only withstand temperatures a few degrees below 0°C. As fall approaches, woody plants somehow perceive certain environmental signals and begin the acclimation process. When acclimation is complete, some woody plants are capable of withstanding temperatures of -196°C depending upon the freezing event.

Deacclimation of woody plants occurs in the spring. During this process plants go from a state of maximum hardiness to being quite tender as growth begins. Much less is known about deacclimation than acclimation but it appears that deacclimation is not simply the reverse of acclimation.

The freezing of water in plant tissues does not always result in injury. Thus, interest in the mechanism of freezing injury has been great and debate on this subject has occurred for over 100 years. Recent evidence supports the idea that the plasma membrane is intimately involved in the injury resulting from freezing (168,177,205-207).

Resistance to freezing injury depends upon either avoidance or tolerance of freezing water in plant tissues. Many mechanisms have been identified which allow plants to resist freezing injury. This is also an area of active intervention by growers using such cultural practices as site and cultivar selection, addition of heat to orchards or vineyards, etc. A greater understanding of the mechanisms of freezing resistance is desirable since small increases in freezing resistance could result in a substantial reduction in crop losses and increased profitability for growers.

ACCLIMATION

The current hypothesis regarding cold acclimation of woody plants was proposed almost twenty years ago (183). With a few notable exceptions, the evidence accumulated since then supports the sequence of events presented in the hypothesis. The key element of the hypothesis is that acclimation in woody plants appears to involve three distinct phases.

The first stage of acclimation in most woody plants begins with the cessation of growth of vegetative shoots (183,184). Short days, low temperatures, and water deficits are environmental stimuli which have been associated with growth cessation and the initial stage of acclimation. Optimum conditions for acclimation of adapted species

appear to be exposure to short days and warm temperatures for a period of time followed by decreasing temperatures.

Decreasing day length in autumn has been shown to induce growth cessation, rest, and cold acclimation in several species of deciduous trees (183,184). Short day enhancement of growth cessation and acclimation was found to be mediated by phytochrome (184,190). The phytochrome system measures the length of the dark period and controls growth responses which are under photoperiodic control. Exposure of the phytochrome pigment in leaves to red light (660 nm) from normal sunlight or other sources induces the physiologically inactive form of the pigment to convert to the active form. When leaves containing phytochrome are in the dark or are exposed to far red light (730 nm), the active form of the pigment spontaneously reverts to the inactive form.

Water relations are also involved in the first stage of acclimation in woody plants (184). Reductions in tissue hydration occur after the plants stop growing in response to short days. Generally, the moisture content of plant tissues is inversely related to cold hardiness (102).

Cold acclimation of citrus (Citrus sinensis (L.) Osbeck) and red-osier dogwood (Cornus serica L.) was induced when trees were subjected to controlled water deficits (37-39,202). Exposure of citrus trees to a water deficit increased the sugar and proline levels in leaves and reduced freezing injury to leaves and stems (202). Decreased water potential and tissue moisture content were observed in red-osier dogwood trees during seven days of exposure to water deficit (38,39). Cold hardiness of stem tissues increased from -3 to -11^{o}C during this same period.

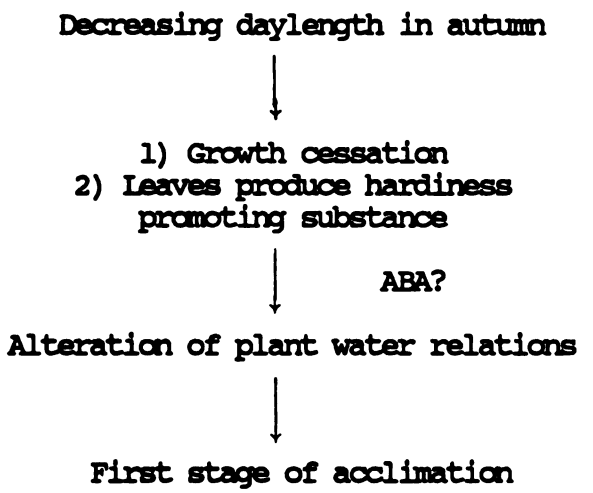
Plant roots appear to play an important role in the changes in water relations which occur during the initial stage of acclimation (108,119,188,201). The influence of root temperature on cold hardiness was examined in citrus (188,201). Exposure of roots to 5°C while shoots were exposed to a nonacclimating air temperature of 30°C resulted in decreased leaf xylem water potential and transpiration while leaf diffusive resistance and cold hardiness increased (188). Relative water content of leaves decreased in response to the 5°C root treatment but the differences were not statistically significant. Similar result were obtained when grafted citrus trees were exposed to a 5°C root temperature (201). Soil temperatures also influence acclimation of the root system (189).

Water relations, root conductivity, and cold hardiness of red-osier dogwood trees were measured under inductive and noninductive conditions for acclimation (108,119). Trees which were grown under inductive conditions (short days and low temperatures) displayed increased transpiration and cold hardiness. At the same time, stomatal resistance, root conductivity, and stem water content decreased. It appears that the reduction in tissue water content which occurs during acclimation in red-osier dogwood results from a decrease in stomatal resistance to water loss and an increase in resistance to water flow through the roots.

Short days and changes in water relations appear to elicit similar physiological responses during the induction of cold acclimation in red-osier dogwood (37). Do short days and alterations in water relations induce acclimation by independent mechanisms, or are these stimuli merely components of the same mechanism of induction? It is tempting to speculate that short days

and water relations function within the same mechanism. Studies with modified plants suggest a possible cooperative role for these environmental stimuli (183).

The environmental control of cold acclimation has been studied using plants divided by light or temperature barriers, partial or total defoliation at different times during acclimation, girdling to disrupt the translocation of substances in the phloem, and grafted plants composed of genotypes which differ in their ability to acclimate (183). These studies have shown that leaves exposed to short days produce a translocatable hardiness promoting substance (82,183,184). The translocatable hardiness promoter has not been identified, but evidence suggests that it functions by regulating plant water relations. The following proposed pathway of induction of Stage I acclimation is consistent with data collected from red-osier dogwood, apple (Malus pumila Mill.), and other hardy, woody plants:



As indicated above, it has been suggested that abscisic acid (ABA) is the hardiness promoter produced by short day leaves. Evidence from certain studies of acclimation of annual plants supports this

idea (116,144). However, a critical experiment to test this hypothesis has not been conducted in annual or perennial plants.

The second stage of acclimation is induced by low temperature (183,184). A significant increase in cold hardiness is noted around the time of the first killing frost in autumn. Although freezing temperatures trigger the induction of Stage II acclimation, considerable metabolic activity occurs during this period.

After the second stage of acclimation has been attained, prolonged, continuous exposure to freezing temperatures can induce further acclimation to the maximum levels of cold hardiness which can be achieved by woody species (183,184). The maximum level of cold hardiness attained is dependent on the mechanism by which the tissue in question resists freezing injury. Tissues which exhibit deep supercooling generally can survive to -40^{o}C while tissues which exhibit extracellular ice formation have survived to -196^{o}C. This type of hardiness is rapidly lost as tissues thaw. It appears that the third stage of acclimation results from structural changes in the protoplasm and does not involve active metabolic processes.

One of the more interesting facts which emerges from a review of the acclimation literature is the level of metabolic activity which occurs during this period when plants have quit growing and are entering dormancy. Weiser and his colleagues intensively studied metabolic changes in red-osier dogwood during acclimation (103,183). They found changes in total protein content, specific proteins, lipid unsaturation, starch, sugars, non-volatile organic acids, free and bound amino acids, organic and inorganic phosphorus, total RNA, DNA, tRNA, and rRNA. Similar changes have been observed in other species. Cold acclimation of Korean Boxwood (Buxus

microphylla var. Koreana Nakai) leaves was accompanied by increased rRNA activity and soluble and membrane-bound protein content (70). Increases in the content of proteins, nucleic acids, and phospholipids were observed during cold acclimation of the living bark of black locust (Robinia pseudoacacia L.) trees (160,161). Also, leaves of three citrus species displayed a substantial increase in polyamine and proline levels during acclimation (95).

Interpretation of results from these experiments is somewhat difficult because of the correlative nature of the data and the experimental techniques used. Are the observed metabolic changes involved in the initiation of cold acclimation or are they merely involved in the development of hardiness following the induction of acclimation? Also, many metabolic measurements in early studies were made on whole tissues rather than on specific tissues which are involved in the development of cold resistance. For instance, lipid alterations during acclimation were often studied using crude membrane preparations rather than highly purified plasma membrane fractions.

The development of improved separation procedures and molecular biology techniques has allowed greater precision in more recent studies of cold acclimation. Much of the recent research on metabolic changes during acclimation has been conducted using annual plants (71,72,106,111,112,145,152,177). One of the reasons for this is that it is difficult to extract and measure nucleic acids and proteins from woody plant tissues due to the presence of interfering substances (103).

Acclimation in several annual plant species has been shown to involve changes in the rate and pattern of protein synthesis and in

the population of translocatable mRNA (71,72,111,112,145, 152). In general, exposure to acclimating temperatures results in the de novo synthesis of proteins. These proteins have been characterized as cold acclimation proteins. However, caution must be exercised when interpreting these results due to the difficulty in determining cause and effect. For example, protein synthesis was analyzed in cold-tolerant winter wheat (Triticum aestivum L. cv. Fredrick and cv. Norstar) and cold-sensitive spring wheat (Triticum aestivum L. cv. Glenlea) during acclimation (152). One- and two-dimensional gel electrophoresis revealed that a high molecular weight protein (200 kDa) accumulated during acclimation. This protein was found in higher concentrations in the cold-tolerant cultivars than in the cold-sensitive cultivars suggesting a correlation between the degree of freezing resistance and accumulation of the protein. However, the fact that the protein accumulated in both cold-tolerant and cold-sensitive cultivars could indicate that its synthesis is part of the gross metabolic adjustment to low temperature rather than being specifically associated with the development of cold hardiness.

Cellular membranes appear to be intimately involved in cold acclimation and freezing injury. Thus, several studies have focused on changes in cellular membranes, particularly the plasma membrane, during cold acclimation (106,177,206). Uemura and Yoshida (177) reported slight changes in the composition of plasma membrane from rye (Secale cereale L.) seedlings during acclimation. In contrast, a more detailed study by Lynch and Steponkus found many changes in the plasma membrane composition of rye seedlings during this period (106). Acclimation alters virtually every lipid component in rye plasma membranes when the results are expressed on a molar % of the

total lipid content.

The influence of acclimation on the composition of the plasma membrane in woody, perennial plants has received less attention. An analysis of the lipid and protein composition of plasma membrane isolated from mulberry (Morus bombycis Koidz.) bark cells was accomplished by Yoshida (206). The level of phospholipids increased during cold acclimation. There were few qualitative changes in phospholipid composition during acclimation. Levels of phosphatidyl choline decreased slightly while levels of phosphatidyl ethanolamine increased slightly. Significant changes in the fatty acid composition of the plasma membrane phospholipids were observed. There was a substantial increase in the ratio of unsaturated to saturated fatty acids soon after growth cessation when a large increase in cold hardiness occurred. This change in the ratio of unsaturated to saturated fatty acids in plasma membrane phospholipids was primarily due to an increase in linoleate content.

The ratio of sterols to phospholipids in the plasma membrane decreased during acclimation principally due to the increase in phospholipids (206). Acclimation resulted in an increase in the fluidity and changes in protein content of plasma membrane. Most protein alterations occurred near the time of growth cessation. However, incorporation of certain high molecular weight proteins into the plasma membrane continued after growth cessation.

Ultrastructural examinations have also uncovered changes in the plasma membrane during cold acclimation (114,191). The plasma membrane was smooth and regular at times other than acclimation or deacclimation. During acclimation, irregularities of the plasma membrane were observed. These irregularities consisted of

invaginations and infoldings. Other activities were observed which were consistent with membrane component turnover and transformation. Collectively, these data indicate that the plasma membrane is in a dynamic state of change during acclimation.

Acclimation of grapevines (Vitis spp.) has not been studied extensively. However, recent studies have contributed significantly to our understanding of this process (58,59,154,195-198). Acclimation begins at the base of grapevine canes and proceeds in an acropetal manner (195-198). The progression of acclimation is closely associated with the vegetative maturity of canes and a reduction in tissue water content. Vegetative maturity of canes is signified by the development of periderm and the subsequent change in color of the cane from green to brown (130).

Wolpert and Howell (198) examined the effect of photoperiod on acclimation of Concord grapevines (Vitis labruscana Bailey). Vines were subjected to natural daylength (ND) or night interruption (NI) of ND with a white light source. NI delayed the cessation of shoot growth, but had no effect on the extent of shoot maturation, cold hardiness, or root conductance. Although light treatment had no effect on this physiological characteristic, root conductance decreased throughout the acclimation period. Somewhat different results were obtained by Fennell et al. (59). In this experiment, root hydraulic conductance decreased with decreasing daylength in Vitis riparia Michx. vines but not in Vitis labruscana Bailey vines.

These results indicate that growth cessation of grapevines is under photoperiodic control (198). However, growth cessation was not required for acclimation and acclimation was not under photoperiodic control. Cessation of growth of vines exposed to short days may be

related to cytokinin production by roots. Vines grown under a 15 hour photoperiod had twice the level of zeatin riboside in xylem exudate as vines grown under a 12 hour photoperiod (58).

White Riesling vines (Vitis vinifera L.) were subjected to temperature and photoperiodic treatments during acclimation (154). The only experimental treatment which substantially increased cold hardiness was natural acclimation (decreasing temperature and photoperiod). Short days or low temperature alone were not sufficient to cause acclimation. In addition, greenhouse control vines growing under noninductive conditions (warm temperatures and long days) increased in cold hardiness during autumn. This finding suggests that grapevines have an endogenous rhythm in cold hardiness as proposed by Howell and Weiser (183).

In summary, our present knowledge of grapevine acclimation is limited. Nevertheless, enough information is available to conclude that important differences exist between acclimation of grapevines and other woody, deciduous species. For example, grapevine acclimation is not under photoperiodic control as is acclimation in other woody species such as red-osier dogwood or apple (82,183,184,198). A better understanding of the acclimation process in grapevines would aid efforts to develop cultural practices which reduce the probability of freezing injury and new cultivars which resist freezing injury.

DEACCLIMATION

Deacclimation is the transition of plant hardiness from the hardy to the tender condition in the spring (184). Limited information is available on deacclimation of woody plants because

this process has been studied less than the acclimation process. The survival of woody plants in temperate regions is influenced by the deacclimation process. Woody plants having a deacclimation pattern which is not synchronous with environmental conditions are often susceptible to freezing injury. For instance, periods of fluctuating temperatures during the quiescent period following rest can result in significant freezing injury to woody species when tissues are exposed to relatively mild low temperatures. Thus, the timing and extent of deacclimation are important factors which can limit the adaptation of plants to a specific environment.

The relationship between root status and deacclimation has not been clearly defined (184). Resting plants have been reported to deacclimate to a lesser extent than quiescent plants when exposed to warm temperatures. Tanino et al. (174) treated red-osier dogwood plants with hydrogen cyanamide or 47°C water. These treatments had previously been shown to be effective in breaking the rest period of this species. Treated plants rapidly deacclimated when exposed to warm temperatures while control plants did not indicating that rest status can influence the extent of deacclimation. Contradictory results were obtained in other studies wherein tissues from resting plants deacclimated more rapidly than tissues from quiescent plants (184). It is possible that much of the conflicting data from these experiments can be attributed to species and cultivar differences.

The primary stimulus for deacclimation in deciduous woody plant species is temperature (25,41,49,81,184). Following the rest period, increasing temperatures in spring result in a loss of hardiness. The ability to reacclimate in response to sudden low temperatures is slowly lost as deacclimation proceeds (49,81).

Howell and Weiser suggest that irreversible protoplasmic changes are responsible for the observed hardiness losses which were only partially reversible (81). These changes seemed to prevent reacclimation beyond a certain basic level and the base level raised with each successive day of deacclimation.

Plant tissues undergo changes in water status, metabolism, and ultrastructure during deacclimation (25,70,91,114,150,191, 194,204,207). Increases in water content of grape (194) and blueberry (Vaccinium corymbosum L.) (25) buds were associated with decreased hardiness as deacclimation progressed. The freezing water in plant tissues can be detected by differential thermal analysis (DTA). Apple pith cells and xylem tissue exhibit a different exotherm pattern by DTA in the fall and spring (91). In the fall, pith and xylem exotherms could be separated. But in the spring, the two exotherms could not be distinguished which indicates that deacclimation is not simply the reverse of acclimation in apple tissues.

Cold hardiness of Korean Boxwood leaves was rapidly lost upon exposure to warm temperatures (70). During this period, a rapid synthesis of nucleic acids was observed. Deacclimation of mulberry trees resulted in significant decreases in the phospholipid content, the degree of unsaturation in phospholipid fatty acids, and membrane fluidity of plasma membranes (207). Also, the sterol to phospholipid ratio increased as cold hardiness decreased. Changes in protein and glycoprotein content of the plasma membrane were also noted. In a similar study, the protein and phospholipid content of bulk membranes of black locust trees declined during deacclimation (204). Arginine and proline pools are mobilized in stems of woody

plants during the latter stages of deacclimation just before active growth begins (150).

Changes in cell ultrastructure during deacclimation were observed in peach (Prunus persica [L.] Batsch) (191) and mulberry (114) stem tissue. The plasma membrane was smooth and regular except when plants were undergoing acclimation or deacclimation. During deacclimation, there was a gradual reappearance of large, centrally located vacuoles, invaginations of the plasma membrane, and the reappearance of complex membrane structures and vesicle aggregates along the plasma membrane. Most ultrastructural changes were related to the plasma membrane. This information, along with the previously discussed changes in plasma membrane composition, indicate that the plasma membrane is in a dynamic state of transformation during deacclimation.

MECHANISMS OF FREEZE INJURY

Freezing injury in woody plants is complex and involves a multiplicity of factors (31,183,184). A single or simple mechanism of freezing injury does not exist. In fact, multiple mechanisms are functional in woody plants and injury varies between species, tissues in a plant and stages of plant development. Our understanding of the mechanisms of freezing injury is limited. However, considerable experimental evidence suggests that the status of water in plant cells and the stability of the plasma membrane are critical elements in the development of injury during freezing.

The status and behavior of water in plant tissues plays an important role in freezing injury (31,169,183,184). During exposure to low temperatures, ice forms either inside (intracellular) or

outside (extracellular) of the cell. Intracellular freezing always results in death of the cell. Injury during intracellular freezing is due to the formation of numerous ice crystals in the protoplasm which disrupt cellular integrity. This type of freezing occurs in tender plants which are not capable of acclimation, in hardy plants before they acclimate or after they deacclimate, and in deeply supercooled tissues of hardy plants.

Extracellular freezing injury may or may not result in injury (31,183,184). Ice begins to form in the plant after a few degrees of supercooling and ice propagation progresses throughout the extracellular spaces. An extracellular vapor pressure deficit develops and water is drawn from the protoplasm to extracellular spaces where it freezes. The movement of cellular water to extracellular ice imposes a severe dehydration stress on the living protoplasm of cells. In addition, extracellular ice has been shown to be a direct cause of injury in certain plant species.

Dehydration stress resulting from extracellular freezing figures prominently in several hypotheses which have been proposed to explain freezing injury (31). These hypotheses include the sulfhydryl-disulfide hypothesis, the protein water shell hypothesis, the salting-out hypothesis, and the vital water hypothesis. A common theme in these hypotheses is that the dehydration stress imposed by extracellular freezing causes protein denaturation which disrupts cell structure and function. Although there is some evidence supporting one or more of the proposed hypotheses, further research is needed before the role of protoplasmic desiccation in freezing injury is clearly defined.

The stability of the plasma membrane is an important factor in freezing injury (31,168,169,183,184,205). Recent studies indicate that the primary site of freezing injury in plant cells is the plasma membrane (168,169,205). Three forms of lethal freezing injury have been identified in rye seedling plasma membranes (168,169). They are intracellular ice formation, dehydration-induced loss of osmotic responsiveness and expansion-induced lysis. These forms of injury have been studied intensively using isolated protoplasts, but they are not unique to protoplasts. Similar types of injury have been demonstrated in intact cells isolated from the same tissue as the protoplasts. The occurrence of any particular form of injury is based on probability and depends on the freeze-thaw protocol, the hardiness of the tissue from which the protoplasts were isolated, and the composition of the suspending medium.

Seeding by extracellular ice is thought to cause intracellular ice formation (168,169). The plasma membrane is an effective barrier to external ice crystals during freezing. Plasma membrane damage and loss of integrity are required before seeding by extracellular ice can occur. High resolution video recordings have shown that disruption of the plasma membrane occurs before intracellular ice formation. Thus, intracellular ice formation appears to be a consequence of membrane failure rather than a cause of membrane failure. Acclimated protoplasts have a lower incidence of intracellular freezing than nonacclimated protoplasts when subjected to similar cooling rates and temperatures. Also, water permeability of plasma membrane from acclimated and nonacclimated protoplasts was similar indicating that increased water permeability

of the plasma membrane was not responsible for increased resistance to intracellular freezing.

A second form of injury observed during freeze-thaw cycles of isolated rye protoplasts is dehydration-induced loss of osmotic responsiveness (168,169). This is the main type of injury in acclimated protoplasts which have been cooled to the LT_{50} at slow rates. Dehydration and contraction of the protoplasts during extracellular freezing apparently alters the semipermeable properties of the plasma membrane. The protoplasts exhibit characteristic osmotic behavior during cooling, but are osmotically inactive during warming and remain contracted. Possible causes of the loss of semipermeability are solute concentration, removal of water, electrical perturbations or thermotropic phase transitions.

Freezing and thawing of nonacclimated protoplasts can result in expansion-induced lysis (168,169). Formation of extracellular ice and the resulting dehydration and contraction of the protoplast cause a turnover of membrane material. A deletion of membrane components occurs during cooling. Vesicles are formed and endocytotic vesiculation of membrane material is observed. Membrane material is incorporated back into the plasma membrane after thawing. Sufficiently large surface area contractions are irreversible due to the limited amount of readily available material for incorporation and the rate at which it can be reincorporated. Therefore, the plasma membrane appears to be sensitive to mechanical stresses during osmotic contraction and expansion. In contrast, acclimated protoplasts undergo exocytotic extrusion of membrane material into surface polyps or tethered spheres during osmotically induced contractions from extracellular freezing. The formation of

exocytotic extrusions is fully reversible and contributes to the increased tolerance of acclimated protoplasts.

The use of protoplasts to study freezing injury must be evaluated carefully to insure that the relationship between freezing in isolated protoplasts and intact tissues is known. Intact cells and protoplasts from the same tissues of Vinca rosea L., Pyrus communis L., Distichlis spicata (L.) Greene and Spartina pectinata Link. were exposed to a freeze-thaw cycle (21). Protoplasts displayed greater freezing injury (measured by TTC reduction test) than intact cells in all species except Vinca. This indicates that the cell wall may have a protective role in certain species and cannot be ignored in the development of protocol for freezing studies.

In summary, freezing of plant tissues and subsequent development of injury are complex processes. The physical state of water and its location (intracellular vs. extracellular) change during freezing. These changes result in both direct and indirect injury to plant cells. The primary site of injury appears to be the plasma membrane. A greater understanding of freezing injury would be beneficial because this knowledge could be used to interpret the significance of correlative metabolic changes which occur during acclimation and deacclimation.

MECHANISMS OF RESISTANCE TO FREEZING

Woody plants survive low temperatures by avoiding or tolerating the freezing water in their tissues (31,184). Several important mechanisms have been identified which allow plants to resist freezing injury. Following acclimation, deciduous forest species

and fruit tree cultivars tolerate extracellular freezing in some tissues and avoid freezing in other tissues by deep supercooling. For instance, peach bark tissues exhibit extracellular ice formation and cellular dehydration while xylem ray parenchyma exhibit deep supercooling (18,33). Also, the use of cultural practices which maximize cold hardiness is an important means of resisting freezing injury (79,80).

Annual plants which have little cold resistance have evolved a very reliable mechanism for surviving low temperatures (31,184). Seeds are produced which avoid freezing due to their dehydrated state. In addition, regenerative tissues of herbaceous biennials and perennials develop only limited levels of cold resistance and survive primarily due to soil and snow cover. Woody plants in temperate regions develop significant levels of cold resistance. Important freezing avoidance mechanisms in woody plants are freezing point depression, supercooling, and deep supercooling.

Solutes in cells depress the freezing point and provide a few degrees of protection in plant tissues (31,184). Freezing point depression in plant tissues must be determined by measuring the temperature at which tissue water melts because some supercooling almost invariably occurs. Supercooling is observed when ice nucleating substances are lacking. Natural barriers which limit the propagation of ice between tissues within the plant might be involved in the development of supercooling. The extent of supercooling under field conditions is usually limited. Formation of ice on external plant surfaces results in seeding of internal tissues through lenticels, stomata, or wounds. Also, bacteria (Pseudomonas syringae van Hall and Erwinia herbicola (Lohnis) Dye)

have been shown to be effective external nucleators which reduce supercooling in annual plants (6,105). Ice nucleation in flower buds and vegetative tissues of woody perennial plants is promoted by intrinsic ice nucleating agents (5,11,16,17,68). Bacteria do not appear to be effective ice nucleators in tissues of woody plants.

Freezing point depression and supercooling probably are not significant avoidance mechanisms for hardy woody plants except in the fall before plants acclimate or in the spring as growth begins. However, in subtropical regions the few degrees of protection afforded by supercooling can be important. Orange (Citrus sinensis (L.) Osbeck) flowers displayed supercooling with freezing occurring between -3.8 and -6.1°C (203). Expression of the maximum capacity for supercooling in orange flowers would significantly reduce the hazard of freezing injury in central Florida groves.

Deep supercooling is an important mechanism of resistance to freezing injury in many deciduous woody plants (31,63,184). Pure water can supercool to -38°C when nucleating substances are not present. This temperature is the homogenous nucleating point for pure water or the temperature at which spontaneous ice nucleation occurs in the absence of nucleating substances. The presence of solutes and solvents in deeply supercooled water can further depress the temperature at which spontaneous nucleation occurs. Vitis riparia Michx. wood has been observed to deeply supercool to -55°C (69). Other species used in this experiment had no detectable low temperature exotherm in their wood.

Xylem ray parenchyma and flower bud tissues from a number of deciduous woody species exhibit deep supercooling. Deep supercooling has been observed in xylem ray parenchyma of apple

(91,133), grape (Vitis riparia Michx.) (126,127), Prunus species (133,135), Pyrus species (136), red cedar (Juniperus virginiana L.) (62), shagbark hickory (Carya ovata L.) (64) and several species of woody timberline species (23). Flower buds or primordia of azalea (Rhododendron kosterianum Schneid.) (65,66), blackberry (Rubus spp.) (181), blueberry (24), grape (12,132,193), peach (9,13,14,131,134), and sweet cherry (Prunus avium L.) (9,10) undergo deep supercooling to avoid freezing. Studies on freezing injury of hardy woody plants at the northern limits of the deciduous forest in North America and near the timberline in the Rocky Mountains of the western U.S.A. indicate that survival of freezing by deep supercooling is an important factor which limits plant distribution (63).

Injury to deeply supercooled tissues, such as xylem ray parenchyma and flower buds, is associated with a discrete and measurable freezing event. When the temperature falls below the homogeneous nucleation point, intracellular freezing is initiated causing death of deeply supercooled tissues. Differential thermal analysis (DTA) measures the release of heat when water freezes in plant tissues and is a convenient method of determining the occurrence of freezing events.

Flower buds display two types of freezing events or exotherms. The first exotherm generally occurs above -10°C and seems to be associated with ice formation in the bud scales and stem axis. This exotherm is followed by one or more low temperature exotherms (LTE's). LTE's are thought to result from the freezing of intracellular water in bud primordia. Injury to bud primordia is highly correlated with the LTE. The number of exotherms observed in buds is similar to the number of floral primordia when buds are

composed of a single primordium or are at an advanced stage of floral differentiation and composed of multiple primordia (181). Buds with multiple primordia exhibit a single LTE during the early stages of floral development.

The situation is considerably more complex in woody stems. Multiple exotherms are often observed and interpretation of results is difficult. The pattern of exotherms is influenced by species, acclimation status, and cooling rate among other factors. As an example, stems of some woody species do not display an LTE when fully acclimated (31,184).

The complexity of freezing events in woody tissues is illustrated by the work of Ketchie and Kammereck (91). Four exotherms were observed in woody tissues of apple by DTA. The first exotherm was measured at -6 to -9°C while the second and third exotherms varied with the time of year from -10 to -39°C. Freezing of extracellular water, pith cells, and xylem tissue were responsible for the first, second, and third exotherms, respectively. A LTE was recorded at -35 to -40°C consistently. The intensity of the LTE was reduced when the cooling rate was below 8°C/hour and was no longer detectable when the cooling rate was 4°C/hour. Use of sensors with high resolution (thermoelectric modules) was necessary for detection of the LTE which was a small exotherm. During acclimation, frequent sampling made it possible to observe the xylem exotherm move to lower temperatures and eventually become indistinguishable from the LTE. The combination of infrequent sampling and use of a single thermocouple for DTA measurement would give the appearance that the xylem exotherm was the LTE and moved to -40°C when the tissues was fully acclimated.

Tetrazolium reduction (2,3,5-triphenyltetrazolium chloride (TTC)) tests showed that the secondary xylem was the tissue with the greatest degree of cold resistance. Furthermore, TTC and tree stress tests indicated that the xylem exotherm and not the LTE was the critical temperature for survival of apple wood.

Extensive supercooling in xylem ray parenchyma and flower primordia while water in adjacent tissues is freezing indicates that barriers must exist which inhibit the seeding and spread of ice into supercooled tissues (31,184). Deep supercooling of xylem ray parenchyma depends on a structural feature of the stem. The ability to undergo supercooling was not related to seasonal changes in cell ultrastructure, but seemed to be due to some intrinsic structural factor present in tissues that supercool (191). It has been proposed that the structural barrier to ice seeding in xylem ray parenchyma may involve fine microcapillaries of the cell wall. The diameter of the microcapillaries would be of a sufficient diameter to prevent ice seeding through the cell wall.

Studies with polycarbonate membranes and controlled pore glass particles demonstrated that the presence of small diameter pores (microcapillaries) in cell walls would facilitate supercooling (15). This hypothesis has been tested using lanthanum nitrate to contrast the permeability of cell walls in stem tissues (192). The behavior of lanthanum in peach and flowering dogwood cell walls was compared with that of lanthanum in weeping willow cell walls. Peach and flowering dogwood (Cornus florida L.) exhibit deep supercooling while weeping willow (Salix babylonica L.) does not. The distribution of lanthanum crystals was similar in cell walls of all species. Primary and secondary walls of xylem cells exhibited low

permeability to lanthanum ions. However, the pit membranes were very permeable to lanthanum ions. This finding led the authors to speculate that the size of pores in the pit membrane region, rather than the entire cell wall, play the major role in defining the freezing behavior of a tissue.

A role for the size of pores in the plasma membrane in supercooling has also been suggested (20). Microcalorimetry was used to study water crystallization in the xylem of grapevine shoots treated with nistatin. "Nistatin pores" were formed in the plasma membrane and the extent of supercooling was reduced. It appeared that seeding of ice through plasma membrane pores was responsible for intracellular ice formation in supercooled xylem cells. Also, the temperature at which intracellular ice formation was detected decreased during acclimation and was due to decreasing pore sizes. The radius of channels in plasma membranes of xylem cells declined from 0.3 to 0.1 nm as shoots acclimated.

Deep supercooling of peach flower buds has been studied intensively by Ashworth (13,14). The barrier between supercooled water in the flower primordium and external ice crystals appears to be a composite of several features which enable the primordium to supercool. These include cellular features of the bud axis tissue, structural features which allow for redistribution of water and the isolation of water in the primordium, and morphological features which limit the development of vascular elements.

Deep supercooling of peach buds appears to require a redistribution of the water within the bud axis and scales (13). Ice formation begins in the bud axis and scales. The freezing is extracellular and water moves to regions in the bud scales. After

water in the bud axis and scales has moved to preferred sites and frozen, water in the primordium can supercool. Lack of viable bud axis cells or rapid cooling cause water in the bud to freeze as a unit and supercooling is prevented. It is thought that the redistribution of water cannot occur under these conditions. Consequently, ice crystals will seed the primordium thereby nucleating the tissue. Structural features of the bud are also important since loss of structural integrity prevents supercooling.

Water in the xylem vessels of peach and other woody plants freezes above -10°C (13). Thus, a continuous network of xylem vessels connecting the primordium to the rest of the plant would provide an avenue for ice seeding of the primordium. Dye uptake experiments and anatomical observations showed that xylem was not continuous into the primordium (13,14). Xylem vessel elements were not observed in the bud axis and primordium. Instead, discrete bundles containing procambial cells were seen. Vascular differentiation resumed and procambial cells developed into mature xylem vessel elements at the beginning of flower bud growth in the spring. There was a close correlation between the establishment of xylem continuity and the loss of the ability to deep supercool. It is not known if the features associated with deep supercooling of peach flower buds are present in flower buds of other species which exhibit this freezing avoidance mechanism.

Many plants survive exposure to low temperatures by tolerating extracellular freezing (31,184). This mechanism of resistance to freezing involves the redistribution of water in regard to location and biophysical state. Water moves from the protoplasm to ice crystals in extracellular spaces where it freezes. Loss of water in

this manner causes dehydration and contraction of the protoplasm. In general, hardy plants can survive a greater degree of dehydration stress than can less hardy plants.

How do woody plants survive extreme desiccation during extracellular freezing? Studies on anhydrobiotic organisms (organisms capable of surviving complete dehydration) suggest a protective role for cellular sugars (40,47,48). The ability of anhydrobiotic organisms to remain viable after the reversible loss of essentially all of their cellular water is proposed to occur because the lost water is replaced by a "complete" solvent, usually a sugar (40). This proposal is known as the water-replacement hypothesis.

Trehalose is a nonreducing disaccharide of glucose which is found in high concentrations in anhydrobiotic organisms (47). During dehydration, loss of water around polar head groups of phospholipids in membranes increases the packing density of these head groups. As a result, the opportunity for van der Waals interactions among hydrocarbon chains increases. Membranes in cells experiencing dehydration stress from extracellular freezing are therefore likely to undergo thermotropic phase transitions (liquid-crystalline state to gel state) at a higher temperature than are membranes in hydrated cells.

Dehydrated phosphatidyl choline has an elevated thermotropic phase transition over hydrated phosphatidyl choline (47). Also, dry palmitoyl phophatidyl choline (DPPC) in the presence of trehalose had a transition temperature similar to that of fully hydrated lipid, whereas DPPC dried without trehalose had a transition temperature about 30^{o}K higher. DPPC and trehalose appear to

interact by hydrogen bonding between the OH groups in the carbohydrate and polar head groups of DPPC. This hydrogen bonding alters the spacing of polar head groups and may decrease van der Waals interactions in the hydrocarbon chains of DPPC.

Sarcoplastic reticulum (SR) vesicle phase transitions were studied using NMR and thermal analysis (48). SR dried in the presence of trehalose did not undergo a transition from the lamellar phase to the hexagonal II phase. The addition of sorbitol to orchard grass plasma membrane during freezing resulted in a lowering of the temperature at which the thermotropic phase transition was detected (205). Freeze-induced fusion and leakage of small unilamellar vesicles composed of natural and synthetic phosphatidyl cholines was reduced when vesicles were frozen in the presence of sucrose (171). These results provide further support for the water replacement hypothesis.

Other cryoprotective mechanisms have recently been proposed (35,75). Hardy tissues of Populus balsamifera Moench. are thought to form intracellular glasses during slow cooling (75). Major components of the glass forming solutions are raffinose and stachyose. Formation of high temperature intracellular glasses would protect cells from intracellular ice formation at temperatures below -20^{o}C. Sugars, polyols, amino acids, methyl amines and lyotropic salts were tested for their ability to protect lactate dehydrogenase from damage during a freeze-thaw cycle (35). All compounds which have been shown to be preferentially excluded from contact with the surface of proteins in aqueous solution protected the enzyme to varying degrees. Also, solutes which bind to proteins (urea and guanidine HCl) were found to increase the inactivation of

lactate dehydrogenase during a freeze-thaw cycle. Based on these findings, it has been proposed that protein stability during freezing can be enhanced by solutes that are preferentially excluded from the protein surface in aqueous solution. This has been termed the preferential exclusion hypothesis.

The level of carbohydrates has been correlated with cold hardiness in many studies (4,83,88,128,175,180). However, the significance of these observations is not clear. Cold hardiness is a relatively weak sink for carbohydrates (79). This has been interpreted as an indication that carbohydrates are not directly involved in cold resistance unless the supply of carbohydrates is limiting. Other functions for carbohydrates during freezing are suggested by the water-replacement hypothesis and the preferential exclusion hypothesis. The mechanisms proposed in these hypotheses provide a means for carbohydrates to play a direct role in cold resistance of woody plant tissues.

II. FLOODING

The extent of flooding is an important site characteristic which can influence species composition in wetlands and crop selection and productivity in agricultural lands. Soils which support woody plants are frequently flooded. Many woody plants grow where they are only temporarily flooded, while others grow in swamps and on floodplains where they are flooded during much of the year. Poor soil aeration which accompanies flooding significantly influences the physiology, growth, and species composition of wetland forests. Although important differences among species in flooding tolerance exist, few woody plant species can survive and

grow on permanently flooded sites. The ecology of wetland forests is complex, and is influenced by flooding frequency and duration, a variety of soil factors including drainage, and flooding tolerance of seedlings.

Flooding and poor soil aeration are not limited to wetlands. Heavy rainfall or excessive irrigation can result in reduced aeration of agricultural soils. The problem is exacerbated when soils contain an impediment to drainage such as natural hardpan or compacted zone. A high water table can also result in periodic flooding of agricultural soils. Flooding of woody plants during the dormant season usually does not result in significant injury. However, woody plants which are exposed to flooding during the growing season are frequently injured. Flooding alters the anatomy, morphology, and physiology of most woody crop plants. Tree fruit species have been grouped according to their general tolerance of flooding by Rowe and Beardsell (148) as follows: quince (Cydonia oblonga Mill.) - extremely tolerant; pear (Pyrus communis L.) - very tolerant; apple (Malus pumila Mill.) - tolerant; citrus (Citrus spp.), plum (Prunus domestica L.), and cherry (Prunus avium L. and Prunus cerasus L.) - intermediate; apricot (Prunus armeniaca L.), peach (Prunus persica [L.] Batsch.), and almond (Prunus amygdalus Batsch.) - sensitive; and olive (Olea europaea L.) - very sensitive.

Important aspects of flooding which can be covered in this portion of the literature review are physical and chemical changes in the root environment, physiological responses of plants to flooding, mechanisms of flooding injury, and adaptations of plants to flooding. Emphasis will be placed on literature pertaining to woody plants.

Flooding rapidly alters the physical and chemical environment of the root-zone. Gaseous diffusion is effectively blocked by flooding and changes occur in the composition and concentration of gases in the soil atmosphere. Direct and indirect electrochemical changes in the soil are observed soon after flooding. Soil structure is also affected by inundation.

Woody plants which are flooded exhibit changes in growth, water relations, photosynthesis, production of plant growth substances, and uptake of inorganic ions from the soil solution. Physiological responses of plants to flooding vary according to species, plant organ, stage of development, and duration of flooding. Anoxia of roots has a dramatic effect on the growth and physiology of shoots. This indicates that communication between the root and shoot system plays a significant role in the physiology of flooded plants.

Flooding perturbs the metabolism of woody plants and results in numerous lesions which are potentially injurious. As soil O_2 is depleted during flooding, aerobic metabolism is increasingly replaced with anaerobic metabolism. This results in a net reduction of ATP available for cellular processes, increased concentration of potentially toxic anaerobic metabolites, and possibly, decreased cytoplasmic pH. In addition, the altered uptake of inorganic ions due to flooding may result in the accumulation of toxic concentrations of ions in shoots of affected plants. Susceptibility to soil fungal diseases also increases substantially during flooding.

Hydrophytes and mesophytes have evolved several adaptations to flooding whereby injury is avoided. Many of these adaptations appear to be functional in woody plants. A primary mechanism of

avoidance is the internal diffusion of O_2 from aerobic shoots via the stem or trunk to anaerobic roots. A certain level of aerobic metabolism is maintained and plant survival is enhanced. The development of adventitious roots on stems near the water surface provides at least partial replacement of the anaerobic root system and facilitates plant survival under flooded conditions.

PHYSICAL AND CHEMICAL CHANGES IN THE ROOT ENVIRONMENT

Flooding rapidly alters physical and chemical processes in the root environment (34,54,129). The physical effects of flooding include restriction of gas exchange between the air and soil, swelling of soil colloids, changes in the rheology of the soil, and destruction of soil structure (129). Changes in the chemistry of the root environment during flooding occur as a result of electrochemical and chemical transformations. Soil fertility and soil-forming processes are affected by the altered chemistry of the soil during flooding.

The most important physical effect of flooding is the restriction of gas exchange between the air and soil (54,129). In well-drained soil with adequate porosity, 10-60% of the soil volume is gas (129). The composition of gas in well-drained soil is fairly stable and similar to that of the atmosphere. Oxygen is an important component of soil gas because it serves as the terminal electron acceptor during respiration. The respiratory requirement for O_2 in soil is high, especially during the growing season (54). The flux of O_2 into the soil in response to respiration by roots and soil microorganisms has been reported to be between 3.5 and 17 L day^{-1} m^{-2} of land surface. Movement of O_2 into the soil occurs

primarily by gaseous diffusion (34,54,129). Fick's Law applies to gaseous diffusion in soils and can be briefly summarized as follows:

$$dg = -DA\ (dc/dx)_T\ dt$$

where D is the diffusion coefficient and dg the number of moles of the substance diffusing in time dt across a cross-sectional area A under a concentration gradient of dc/dx at temperature T (129).

The cross-sectional area or A decreases considerably when the water content of the soil increases. Gaseous diffusion is severely limited when less than 10% of soil pores are filled with gas. Flooding virtually eliminates gas-filled pores and exchange between the soil and air is possible only by molecular diffusion in soil water. Diffusion of O_2 in soil water is approximately 10^4 times slower than in air. As a result, O_2 movement in the soil is effectively halted. Microorganisms and roots consume the O_2 present in the water or trapped in the soil within a few hours of flooding. The oxygen diffusion rate (ODR) of flooded soils declines rapidly after flooding (8,22,43,115). ODR is measured using platinum microelectrodes and is a good indicator of O_2 supply in water saturated soils. Lemon and Erickson (101) characterized the use of the platinum microelectrode for this purpose and found that growth of tomato was well correlated with ODR. Another feature of restricted gas exchange in flooded soils is the accumulation of gases in the flooded soil which are by-products of soil processes. Nitrogen, carbon dioxide, methane, ethylene, and hydrogen are often found in high concentrations in flooded soils (34,129).

Other physical effects of flooding have less of an effect than the restriction of gas exchange and will only be of importance if the plant survives anoxia. Soil colloids absorb water and the soil

swells during flooding (129). Cohesion is reduced as dry soil is flooded. This is a result of increases in the thickness of water films between soil particles. Finally, flooding disrupts the aggregates in a soil and destroys soil structure. Partial restoration of soil structure is observed after excess water drains from the soil and reoxidation occurs.

Direct and indirect electrochemical changes occur when soil is flooded (34,129). The soil solution is diluted which increases pH, decreases electrical conductance, and alters the diffuse double layer of colloidal particles. Other more important changes are also occurring. As previously mentioned, free O_2 is exhausted soon after soil is waterlogged. Facultative and obligate anaerobic microorganisms use oxidized soil components and dissimilation products of organic matter as electron acceptors and reduce the soil. The sequence of the main reduction reactions after the disappearance of O_2 follows the thermodynamic sequence of:

$$2\ NO_3^- + 12\ H^+ + 10\ e^- \longrightarrow N_2 + 6\ H_2O$$

$$MnO_2 + 4\ H^+ + 2\ e^- \longrightarrow Mn^{2+} + 2\ H_2O$$

$$Fe(OH)_3 + 3\ H^+ + e^- \longrightarrow Fe^{2+} + 3\ H_2O$$

$$SO_4^{2-} + 10\ H^+ + 8\ e^- \longrightarrow H_2S + 4\ H_2O$$

Thus, flooded soils rapidly become very reduced and have a low redox potential (3,42,43,67,123-125). The redox potential is reduced sharply after flooding, then increases somewhat, and finally resumes its decline which occurs asymptotically with time (129).

The pH of an acid soil may increase while the pH of an alkaline soil may decrease during flooding (129). Most flooded mineral soils have a pH between 6.7 and 7.2 with the pH of the interstitial solutions being between 6.5 and 7.0. The increase in pH of acid

soils is due mainly to the reduction of Fe^{3+} to Fe^{2+}. Decreases in pH of alkaline soil during flooding are due to the accumulation of CO_2. If a soil has a low content of reducible ions, its pH may not rise above 5.0 even after months of submergence, and if the organic matter content of a high soil pH is low, the pH may not decrease below 8.0. In Michigan soils which are flooded, the predominant occurrence is increased CO_2 concentration and reduced solution pH (A.J.M. Smucker, personal communication, 1990).

The effects of flooding on pH may have secondary effects on plants through the action of pH on the concentration of ions (Fe, AlOH, Zn, Cu) in the soil solution (129). Flooding also results in changes in specific conductance and ionic strength, ion exchange, and sorption-desorption properties of soils.

It is important to note that flooding and the development of anoxic conditions in the field are usually non-uniform. When excessive water is applied to air-dry soil, an increasing proportion of micropores in aggregates and peds become water filled. Centers of aggregates may become anoxic but be surrounded by a shell of aerobic soil. Thus, plant roots may be subjected to various soil environments ranging from fully aerobic to hypoxic to anoxic. This adds a level of complexity to flooding experiments in the field which is seldom encountered in greenhouse work.

PHYSIOLOGICAL RESPONSES OF PLANTS TO FLOODING

Flooding of roots alters the physiology of roots, shoots, and reproductive organs of susceptible plants (1,86,93). Vegetative growth, reproductive growth, water relations, photosynthesis, production and metabolism of plant growth substances, and uptake of

ions from the soil solution are influenced by soil anoxia.

Vegetative and Reproductive Growth

Reduction of growth of roots, shoots and reproductive organs is a common response of plants to flooding (86). Roots are often affected first by flooding because they are exposed to the stress immediately. In fact, root development is often influenced by flooding before anoxia occurs in the soil. Early responses of roots to flooding are increased ethylene content and decreased internal concentration of O_2 (hypoxia). Root elongation can be inhibited in hypoxic roots because O_2 consumption and resistance to diffusion of O_2 into roots are large enough to create anoxic conditions within the root. Regions of the root with a high rate of metabolism, such as zones of cell division and elongation, are most susceptible to localized internal anoxia.

Anoxia severely inhibited the development of root length and number of growing root tips of five dry bean (Phaseolus vulgaris L.) cultivars (155). Dry weight of roots was reduced by flooding in red maple (Acer rubrum L.) (53), kiwifruit (Actinidia deliciosa Planch.) (153), apple (115), swamp tupelo (Nyssa silvatica var. biflora (Walt.) Sarg.) (78), loblolly pine (Pinus taeda L.) (78), blueberry (Vaccinium corymbosum L.) (3), bur oak (Quercus macrocarpa Michx.) (172), Pinus halepensis Mill. (200), and grape (Vitis vinifera L.) (61). Major factors which are involved in the decrease in dry weight of flooded root systems are the cessation of root growth and the degeneration of the existing root system (86). Regeneration of roots on the stem or trunk near the water surface can partially compensate for dry matter reductions in the existing root system

during flooding (53).

Dry matter allocation patterns during flooding can also be influenced by cultivar (61). Allocation of dry weight between leaves, shoots, and roots for seven Vitis vinifera L. cultivars was altered considerably by flooding. Tarrango, Muscat Gordo Blanco, and Shiraz had the highest reductions in dry weight and the reduction was uniform across plant part. Colombard had a low reduction in dry weight due to flooding and the reduction was uniform across plant part. Cabernet Sauvignon and Trebbiano showed a small reduction in dry weight from flooding with root dry weight being affected only slightly. Conversely, Semillon dry weight was reduced to a small extent by flooding with the dry weight of roots being reduced substantially while the dry weight of leaves and shoots was affected to a lesser degree.

Growth of above-ground organs of woody plants is also frequently reduced by flooding (86,92). Leaf growth appears to be sensitive to flooding in many species and shoot extension of susceptible species is almost always inhibited by flooding. Flooding reduced the leaf area of rabbiteye blueberry (Vaccinium ashei Reade) plants (43,44) and leaf size of highbush blueberry (3). On the other hand, leaf production (plastochron index) of grapevines was less sensitive to flooding than shoot extension rate (61).

The effect of flooding on shoot growth is dependant on species, timing of the flooding stress and duration of the flooding stress (3,9,43,61,78,115,172,185,200). In general, woody plants which are adapted to poorly-drained soils show little or no reduction in shoot growth when flooded while woody plants which are adapted to well-drained soils show significant reductions in shoot growth from

flooding. Flooding reduced the growth of loblolly pine seedlings (adapted to well-drained soils) but had little effect on growth of swamp tupelo seedlings (adapted to poorly-drained soils) (78). Shoot growth of Pyrus betulaefolia Bunge., a Pyrus species which survives flooding extremely well, was not significantly affected by one month of flooding in the spring (8). On the other hand, flooding for one month in the spring severely limited shoot growth of Pyrus communis L. (cultivar OH x F97), a Pyrus species which is more susceptible than P. betulaefolia Bunge. to flooding. Highbush blueberry (3), rabbiteye blueberry (43), apple (129), bur oak (172), and grapevine (61,185) displayed reduced growth during flooding. Flooding of Pinus halepensis Mill. seedlings for 43 days did not significantly affect plant height (200). However, this result may have been due to other factors which influenced the growth rate of control seedlings since both control and flooded seedlings stopped growing after approximately 22 days.

Timing and duration of the flooding episode are also important factors which influence the growth response of woody plants to flooding. Apple trees which were flooded during the summer exhibited a greater reduction in shoot growth than trees which were flooded in the spring or fall (115). Similarly, flooding of rabbiteye blueberries in the summer was more detrimental to stem length than flooding in the spring (43). Growth of Pyrus betulaefolia Bunge. seedlings was not significantly affected by one month of flooding while a year of continuous flooding resulted in flooded seedlings growing significantly less than control seedlings (8).

Reproductive growth of woody plants is altered by flooding (1,43,44,115). Apple trees which were flooded for 2 months in the

spring displayed lower fruit load (number of fruit per cm^2 of branch), lower yield, and higher return bloom the following year than control trees (115). Furthermore, cumulative effects of seasonal flooding on yield of apple trees were observed when trees were flooded during successive years. Flooding of highbush blueberry delayed bloom, decreased fruit set, increased fruit abscission, reduced the number of flower buds/shoot, decreased the number of flowers/bud, reduced berry weight and lowered % soluble solids of fruit (1). Similar, although less extensive, results were obtained when rabbiteye blueberry plants were flooded for up to 117 days (43,44). Flower bud formation, fruit set, and yield were significantly lower in flooded rabbiteye blueberry bushes.

Water Relations and Photosynthesis

Flooding also influences plant water relations and photosynthesis (30,93). Early observations that flooding causes cessation of growth, wilting, and basal leaf senescence suggested that water relations were involved in plant responses to flooding. Subsequent research has shown that stomatal aperture, transpiration, photosynthesis, and water absorption by roots are modified by flooding. Water potential of plants has also been shown to be affected by flooding but the effect is not clear-cut and is influenced by species, the manner in which flooding was imposed, and the duration of flooding. These results figured in the development of a hypothesis dealing with plant water relations during flooding. The sequence of events proposed to occur in flooded plants was: anaerobic stress reduces the water uptake; loss of water from the shoot exceeds the supply from the root, leading to a decrease in

leaf water potential (ψ_L) and wilting; stomata close in response to low ψ_L which restricts transpiration and allows the recovery of turgor (29). An important feature of this hypothesis is that a reduction in ψ_L precedes stomatal closure. This is often the observed sequence of events in leaves of plants subjected to water deficit.

Sojka and Stolzy (167) reviewed data on soil ODR, stomatal conductance and ψ_L. They found ODR to be highly correlated with stomatal conductance but not with ψ_L. This relationship was evident in several species and led the authors to conclude that "theories which point to increased root resistance resulting from low soil O_2 as the cause of stomatal closure do not fully explain some of the observed data". These findings do not support the hypothesis described above and indicate that regulation of stomatal aperture during flooding is complex and may be controlled by more than one mechanism. Further information on the relationship between ψ_L and stomatal conductance in flooded plants is presented later in this portion of the literature review.

Stonatal behavior is strongly influenced by plant species during flooding (93). Stomatal aperture is reduced soon after the imposition of flooding in susceptible species. Highbush blueberry (50,51), rabbiteye blueberry (42,44,51,52), bur oak (172), cherry (22), cherrybark oak (Quercus falcata var. pagodaefolia Ell.) (124), kiwifruit (153), pear (7), sweetgum (Liquidambar styraciflua L.) (123), and tomato (Lycopersicon esculentum Mill.) (27,29) display reductions in stomatal aperture soon after flooding begins. Stomatal behavior of species which are tolerant of flooding differs considerably from that of species which are sensitive to flooding.

Green ash (Fraxinus pennsylvanica Marsh.) seedlings exhibited stomatal closure rapidly after flooding was initiated (173). Stomata began to reopen when seedlings had been flooded for 6 days, however, stomatal conductance never increased to the level of control seedlings. The reopening of stomates was correlated with the production of adventitious roots. A similar result was observed for willow (Salix discolor Muhl.) which was subjected to one year of flooding (8). Transpiration is also reduced by flooding and the pattern observed generally parallels that of stomatal conductance (22,29,51,123,124,153,164).

Plants which are flooded often display a rapid decline in photosynthesis (22,29,51,123,124,153,164). Photosynthesis of flooded plants appears to be limited by stomatal and non-stomatal factors (93). Tupelo gum (Nyssa aquatica L.) seedlings were treated for one month as follows; (a) well-watered control; (b) well-watered with salinity; (c) flooded with saline water; and (d) flooded with tap water (122). Photosynthesis was reduced by both flooding and salinity treatments. Stomatal factors accounted for only 11 to 21 % of the observed reduction in photosynthesis. Non-stomatal factors limited photosynthesis to a greater degree than stomatal factors especially for seedlings subjected to salinity or salinity combined with flooding.

Davies and Flore (50-52) studied photosynthesis of flooded highbush and rabbiteye blueberry plants. Generally, flooded plants had significantly lower rates of photosynthesis than control plants after two days of flooding. Both stomatal and non-stomatal limitations to photosynthesis were observed during flooding and the relative importance of each type of limitation changed as flooding

duration increased. A decrease in stomatal conductance reduced carbon assimilation during short-term flooding (1-2 days) while longer flooding duration also decreased residual conductance (carboxylation efficiency) of the leaf (50). Stomatal limitations were most significant during short-term flooding while non-stomatal limitations became increasingly responsible for the observed decline in photosynthesis as flooding duration increased.

Photosynthesis of tomato leaves was measured before and after plants had been flooded for 24 hours (27). Carbon assimilation was reduced by flooding and the primary limitation to carbon assimilation appeared to come from non-stomatal factors since stomatal conductance limited the assimilation rate to approximately the same degree before and after flooding. Evaluation of the assimilation rate as a function of C_i (the intercellular CO_2 concentration) indicated that flooding primarily was affecting RuBP regeneration which includes photosynthetic electron transport, NADPH and ATP synthesis, and the reductive pentose phosphate cycle. It was proposed that RuBP regeneration is limited in flooded tomato plants due to low availability of P_i (orthophosphate). A reduction in sink activity of flooded roots might cause accumulation of sucrose in the leaves which could result in a build-up of triose phosphates and depletion of the cytoplasmic P_i pool. A consequence of this sequence of events would be the diversion of photosynthate into starch. Additionally, the cytoplasmic P_i level might be affected by the reduction in P uptake which occurs during flooding.

Soil flooding rapidly reduced photosynthesis of pecan (Carya illinoensis (Wangenh.) C. Koch) seedlings (164). Stomatal conductance to CO_2 was reduced by flooding while C_i was not

indicating that a reduction in the capacity of the mesophyll to assimilate CO_2 was the primary factor limiting assimilation.

Sour cherry (Prunus cerasus L.) trees responded to flooding by displaying a reduction in photosynthesis soon after flooding was initiated (22). Flooding affected carbon assimilation of sour cherry in several ways and the relative importance of each changed as flooding duration increased. Stomatal limitations to assimilation were small initially and the relative importance of this limitation appeared to decline as flooding continued. Mesophyll resistance seemed to be the most important limitation to assimilation. Initially, this effect was confined to RuBP regeneration capacity. As flooding continued, reductions in assimilation due to reduced RuBPC/O (ribulose bisphosphate carboxylase/oxygenase) amount or activity were observed.

The partitioning of photosynthate is also influenced by flooding (155). Labelled sucrose (^{14}C) was applied to leaves of dry bean plants which had been subjected to 72 hours of anoxia. There was a 50% reduction in translocation of ^{14}C label to anoxic roots as compared to control roots. Furthermore, most of the ^{14}C label translocated to anoxic roots was excluded from respiratory metabolism during a three hour pulse/chase period.

The flow of water across plant roots is described by the following equation:

$$J_v = L_p (\Delta P - \sigma \Delta \pi)$$

where J_v is the volume flow; L_p is the hydraulic conductivity; P is the hydrostatic component of the driving force; σ is the reflection coefficient and π is the gradient of osmotic potential between the xylem sap and the external solution (56). Anaerobic conditions in

the soil result in changes in the absorption of water by roots (7,29,42,50,56). Hydraulic conductivity of flooded plants of highbush blueberry (50), rabbiteye blueberry (42), and Pyrus species (7) fell below that of control plants soon after inundation. In tomato, L_p of flooded plants was lower than in control plants after 24 hours of flooding but as flooding continued for 48 hours the relationship changed (29). L_p of flooded plants was higher than for control plants and this result seemed to be related to the deterioration of the flooded root system (decreased fresh weight).

L_p of Pyrus communis L. (susceptible to flooding) and Pyrus betulaefolia Bunge. (tolerant of flooding) was measured periodically during flooding for 30 days (7). Significant reductions in L_p were noted for Pyrus communis L. and Pyrus betulaefolia Bunge. after 4 and 20 days of flooding, respectively. Furthermore, reductions in stomatal aperture occurred concomitantly with changes in L_p. Collectively, these data suggest that the root system of Pyrus betulaefolia Bunge. contributes to the tolerance of this species to flooding. Further studies were conducted with Pyrus communis L. to determine the site of increased root resistance during flooding. L_p was measured for intact root systems, root systems with feeder roots detached, and stems with all roots removed. Removal of the feeder roots only partially restored rates of L_p indicating that feeder roots were not the only site of increased resistance. Resistance to flow in the xylem vessels progressed further up the tree with increasing duration of flooding. Thus, resistance appeared to be primarily from longitudinal movement of the water through xylem vessels and not from radial movement of water (entry of water into the root). The authors felt that the results were indicative of an

occlusion of the xylem vessels (xylem plugging).

In general, changes in L_p have been equated with changes in J_v (56). The potential contribution of changes in the osmotic component of the driving force to reductions in J_v under anaerobic conditions has received little attention. Everard and Drew measured changes in J_v across detopped, 7-day-old maize (Zea mays L.) roots during the initial 24 hours of anoxia (56). J_v through anoxic roots fell below that of aerobic control roots one hour after the equilibrium oxygen partial pressure in the bathing medium dropped below 2.0 kPa (air = 20.6 kPa). A nullification of the diurnal rhythm in L_p was primarily responsible for the reduction in J_v (the L_p of flooded plants did not increase during the light period of the light cycle). However, about 25% of the reduction in J_v could be accounted for by a smaller osmotic component of the driving force (σ) on water movement. Thus, measurement of L_p appears to provide a reliable, but not precise, estimate of J_v in stressed roots.

The effect of flooding on water potential remains equivocal. Water potential of flooded bur oak (172), cherrybark oak (124), green ash (173), pecan (164), and sweet gum (123) was unchanged or higher than that of control seedlings. Pyrus rootstocks exhibited a significant decline in leaf water potential during 30 days of flooding (7). However, stomatal closure either preceded or occurred simultaneously with the observed changes in water potential. Flooded kiwifruit plants had higher water potential than control plants for up to 5 days of flooding (153). After 5 days of flooding, water potential of flooded plants decreased and became more negative than control plants. Stem water potential of cherry trees was significantly reduced after 24 hours of flooding (22).

Significant reductions in stomatal conductance and carbon assimilation were also observed after 24 hours of flooding.

Collectively, these findings strongly suggest that the previously described hypothesis concerning the mechanism involved in the flooding effect on plant water relations is not valid for most woody plants. Stomatal closure precedes or occurs at the same time as reductions in root hydraulic conductivity and leaf water potential. The effect of flooding on transpiration is similar to that observed for stomatal conductance. Photosynthesis is generally affected somewhat later during the flooding episode. In summary, stomatal aperture of flooded plants appears to be regulated by a different mechanism than in plants subjected to water deficit since stomatal closure seems to prevent a decrease in ψ_L rather than being the result of a leaf water deficit. Also, the root system is a likely source of positive or negative messages which may regulate stomatal aperture during flooding.

Bradford and Hsiao (29) conducted a detailed analysis of water relations of tomato plants during short-term flooding. This work provided useful information on the mechanism of regulation of stomatal aperture during flooding. Stomata of flooded tomato plants exhibited a diurnal cycle but maximal opening was only approximately 60% of control plants. Also, stomatal closure occurred earlier on each successive day of flooding. Plants flooded in the morning showed no detectable response on the first day of flooding but on the second morning of flooding stomata on flooded plants did not open as widely as those on control plants. Water potential measurements indicated that this was not due to a cycle of stomatal opening, transient wilting, and recovery. This sequence of events

was induced by flooding plants at the beginning of the dark period. In this case (flooded during the dark period), plants wilted within 30 minutes of illumination on the following morning and then recovered within the next 30 minutes. Subsequent days produced stomatal behavior similar to that of plants flooded in the morning.

The evidence suggests that timing of flooding may be important in determining whether stomatal adaptation prevents or follows an episode of low leaf water potential. When plants were flooded in the morning, transpiration was occurring as the anaerobic conditions developed. The authors proposed that this might allow the transport of a "signal" from the roots which would limit the extent of stomatal opening on the next day. Imposition of flooding during the night, when transpiration is negligible, would reduce root conductance of water without the opportunity to communicate this event to the shoot. As a result, when leaves are illuminated the following morning, stomata would open, transpiration would exceed water uptake by the roots, and wilting would occur until stomata closed sufficiently to allow equilibrium between transpiration and water uptake. It is speculated that the "signal" coming from the flooded root might be ABA (positive message) or the lack of some factor such as cytokinins or gibberellins (negative message). In summary, regulation of stomatal behavior in tomato was observed to occur by two mechanisms. The extent to which either mechanism regulated stomatal behavior was dependant on the time of day when flooding was imposed.

Plant Growth Substances

Soil flooding alters the pattern of synthesis and metabolism of plant growth substances (30,32,85,138-140,159). In general, the content of auxin, ethylene, and ABA increases during flooding, while those of gibberellins and cytokinins decreases during flooding. Plant growth substance interactions during flooding are complex and our understanding of them is limited. Unfortunately, there has been much speculation and little critical experimentation on the role of plant growth substances in plant responses to flooding. As a result, emphasis will be given to plant growth substance effects during flooding which have been well documented in the literature.

The involvement of ethylene in the response of plants to flooding has been studied to a greater degree than has the involvement of other plant growth substances. Ethylene plays a role in the epinastic curvature of leaves of waterlogged tomato plants, formation of aerenchyma, and in the development of hypertrophy in submerged stems (85).

Soil flooding rapidly induces the downward growth of tomato petioles known as epinasty (30,138). Epinasty occurs when cells on the upper adaxial side of the petiole expand more rapidly than cells on the lower abaxial side. Tomato plants display epinasty when exposed to ethylene even at very low concentrations. The appearance of flooded tomato plants is similar to plants which have been exposed to ethylene. Levels of ethylene in flooded plants are greater than those in control plants. Furthermore, the development of epinasty in flooded plants is prevented by inhibitors of ethylene action such as Ag^{+} or benzothiadiazole derivatives. Collectively,

this evidence confirms that ethylene is involved in the epinastic response of tomato plants to flooding.

The ethylene which accumulates in shoots of flooded tomato plants has its origin in the root system (30,138). Anaerobiosis of roots stimulates the production of 1-aminocyclopropane-1-carboxylic acid (ACC) which is translocated to the shoots via the transpiration stream. ACC, which is the immediate precursor of ethylene in the ethylene biosynthetic pathway, is converted to ethylene in the aerobic shoot system. Also, a portion of the ACC produced in anaerobic roots is metabolized to N-malonyl-ACC (conjugated form) either in the root or shoot.

The time course of epinastic curvature provides evidence which confirms the involvement of ACC from anaerobic roots in flooding-induced epinasty. ACC export from the roots increased to a maximum after 48 hours of flooding and then declined. Control plants did not have detectable levels of ACC in their xylem sap. Flooding increased ethylene production in petioles which lagged behind the appearance of ACC by 12 to 24 hours. Furthermore, ethylene synthesis in shoots was stimulated when ACC was supplied to excised shoots at concentrations equal to those measured in flooded plants.

Other physiological changes which occur in flooded tomato plants are rapid stomatal closure and reduced photosynthesis. Bradford (28) exposed tomato plants to elevated levels of ethylene and found no effect on stomatal conductance or photosynthetic capacity. Thus, ethylene does not appear to be responsible for the characteristic changes in stomatal behavior and carbon assimilation which are observed in flooded tomato plants.

Formation of aerenchyma is a common response of maize and other herbaceous species to soil flooding (85,138). Aerenchyma are internal, longitudinally connected gas-filled intercellular spaces caused by cell separation or by breakdown of cells in the cortex or pericycle. The development of aerenchyma facilitates gas diffusion between the root and the aerial environment which allows survival of the roots during anoxia. In maize, formation of aerenchyma is promoted by hypoxia and increased ethylene levels. Roots exposed to 3-12 kPa O_2 show increased ethylene production and aerenchyma formation. Also, application of inhibitors of ethylene biosynthesis or action prevent aerenchyma formation under hypoxic conditions.

The relationship between ethylene production, ACC concentration, and aerenchyma formation was studied in nodal roots of maize by Atwell et al. (19). Production of ethylene and ACC accumulation were closely correlated in different regions of hypoxic roots. The root apex had the highest ethylene production and ACC concentration on a fresh weight basis. Aerenchyma formation was observed in more mature regions of the root approximately 20 mm behind the apex. Exposure of intact root tips to anoxia inhibited aerenchyma formation in the mature root axis. The same effect was observed when root apices were excised or subjected to high osmotic pressures. These findings indicate that an intact, functional apex is required, in addition to low O_2 and increased ethylene, for high rates of aerenchyma formation in adjacent root tissue.

Aerenchyma and lenticel formation were induced by exogenous ethylene or hypoxia in pond pine seedlings (Pinus serotina Michx.) (175). The aerenchyma formed were primarily schizogenous rather than lysigenous as in many herbaceous species. Redox dye

experiments showed that the aerenchyma formed by exogenous ethylene allowed longitudinal diffusion of atmospheric O_2 to submerged roots. The relevance of these results to flooding of pond pine seedlings under field conditions is not explicit because the concentration of ethylene in root tissues was not measured, the concentration of exogenous ethylene used may not have been in the physiological range which would be expected in flooded soils and seedlings exposed to hypoxia and exogenous ethylene produced a somewhat different response than seedlings exposed to hypoxia or exogenous ethylene. It is not clear whether ethylene is involved in the development of aerenchyma in woody plants or whether the results obtained were artifacts of the experimental procedures which were employed. Further investigation is needed so that the role of ethylene in the development of aerenchyma in woody plants can be more clearly defined.

Another morphological change often observed in flooded woody plants is the development of hypertrophy in stems or trunks (92). Increases in ethylene concentration of stems have been correlated with the development of hypertrophy in flooded stems (85,172,173, 199,200). Hypertrophy increases the internal porosity of stems and enhances internal aeration.

In summary, ethylene appears to be involved in a wide range of adaptive responses of plants to flooding. A better understanding of ethylene physiology in flooded plants would likely allow the development of genetic and cultural strategies to reduce crop losses from flooding.

The ABA content of leaves increases during flooding (60,87,159,208). The increase in ABA is not caused by a decrease in

leaf turgor as is the case when plants are subjected to water deficit. Stomatal closure and elevated ABA concentrations are often observed in leaves of flooded plants in the absence of a significant reduction in plant water potential.

Zhang and Davies (208) studied the ABA accumulation in roots and leaves of flooded pea (Pisum sativum L.) plants. Within a few hours after flooding began, the content of free-ABA in roots of flooded plants increased as compared to roots of control plants. The increase was not statistically significant until the beginning of the second day of flooding. Significant increases in free-ABA content of flooded leaves were detected after approximately 36 hours of flooding. Stomatal conductance of flooded plants was reduced by approximately 50% after 12 hours of flooding.

The free-ABA content of leaves and roots of plants that had been flooded for several days exhibited substantial diurnal variation. The maximum content of free-ABA was recorded 3 hours or more after the beginning of the light period. When illumination ceased, there was a rapid decline in free-ABA content which was likely due to the free form being metabolized to the conjugated form. The authors propose that flooded pea roots produce increased levels of ABA which are then transported to the leaves where it accumulates and causes stomatal closure prior to a reduction in leaf water potential. This hypothesis does not appear to fit the experimental data because stomatal closure occurred before significant increases in free-ABA were recorded in flooded leaves. The authors suggest that the discrepancy between the timing of the reduction in stomatal conductance and the increase in free-ABA in leaves can be explained if soon after increases in ABA are

detectable in roots, ABA is translocated via the transpiration stream to the external surface of the plasmalemma of guard cells which is the site of action for closing of stomata by ABA.

In another study, water relations and ABA content of pea plants were followed during a three day flooding treatment (87). Flooding of pea plants resulted in a significant decrease in leaf conductance approximately 24 hours after the stress was imposed. During three days of flooding, the plants did not wilt and leaf water potential of flooded plants increased rather than decreased. Concomitant with these changes, there was a 10-fold increase in endogenous ABA of flooded leaves. No evidence was found that increases in ABA were preceded or accompanied by loss of leaf hydration. Leaves detached from control plants and maintained in vials of water for up to three days behaved in a similar manner as leaves on flooded plants (stomatal closure in the absence of a water deficit but in association with increased ABA content). In another experiment, ABA was supplied to freshly detached leaflets via the transpiration stream. Stomata closed partially within 15 minutes and the extractable concentration of ABA associated with this closure was similar to that found in flooded plants. Flooding of ABA-deficient mutant pea and tomato plants provided further evidence supporting a role for ABA in flooding-induced stomatal closure. When the ABA-deficient 'wilty' mutant of pea was flooded, stomates of mutant plants closed to a lesser degree than stomates of normal plants. In addition, the associated increase in foliar ABA was not as great in mutant plants as in normal plants. Similarly, flooding resulted in stomates of tomato plants closing within 24 hours while closure of stomates was not observed in ABA-deficient 'flacca' mutant plants.

These data provide strong evidence that the ABA which accumulates in leaves of flooded plants promotes stomatal closure. The authors propose that the accumulation of ABA is due to reduced transport from leaves to the roots. Flooded roots represent a reduced sink for ABA synthesized in leaves and this change would favor accumulation of ABA in leaves.

The conflicting hypotheses concerning the source of ABA which accumulates in leaves of flooded plants were tested by Flore et al. (60). Plants of normal ('Alisa Craig') and ABA-deficient mutant ('flacca') tomato cultivars were either flooded or maintained as unflooded controls. Leaf photosynthesis, stomatal conductance, ABA content of leaf and xylem sap, and cytokinin content of leaf and xylem sap were measured at 24 hour intervals. After 48 hours of flooding, photosynthesis and stomatal conductance were inhibited in both normal and mutant plants, however, the decrease in stomatal conductance was not as great in the ABA-deficient mutant plants. The ABA content in leaves of both normal and mutant plants increased after 48 hours of flooding, but there were no significant changes in the cytokinin content of leaves. Flooding did not affect the xylem sap concentration of ABA or cytokinin, but, the flow rate decreased by 50% or more after 48 hours of flooding. Thus, these data are consistent with the proposal that the increase in ABA content of the leaf during flooding is a result of ABA not being translocated out of the leaf and are not consistent with the proposal that ABA is synthesized in flooded roots and subsequently translocated to leaves where it accumulates.

Auxins also appear to be involved in the response of plants to flooding but the evidence for this is somewhat limited (138).

Flooding of sunflower (Helianthus annuus L.) plants is associated with an increase in the auxin content of shoots and the development of stem hypertrophy. Unlike most physiological responses of plants to flooding, these changes were not related to the development of anoxia in flooded soil. Sunflower plants which were flooded with aerated water displayed a similar response as plants which were flooded with non-aerated water. This result has been referred to as the "water effect". Possible explanations for the observed results are that flood-induced changes in auxin physiology may be caused by slight reductions in the O_2 supply to tissues (aerated water is intermediate in O_2 concentration between the aerobic control and the flooded, anaerobic treatment) or the presence of water itself may somehow interfere with root metabolism, perhaps by leaching substances from the roots or by interfering with normal gas exchange between roots and the soil. Further research is required so that the role of auxins in plant responses to flooding can be more clearly defined.

Conclusive evidence linking cytokinins or gibberellins with a specific plant response to flooding is lacking (138). However, there is much indirect evidence which suggests that these plant growth substances are involved in plant responses to flooding.

Cytokinins and gibberellins are produced in the root system (141). During flooding, the content of cytokinins and gibberellins diminishes in the shoots (32,139,140). Chlorosis of leaves, leaf senescence, and reduced shoot elongation are symptoms of flooding stress. It is interesting to note that the application of exogenous cytokinins promotes chlorophyll retention and delays senescence of detached leaves. Also, shoot elongation is promoted by

gibberellins. The reopening of stomates on leaves of flooded Fraxinus pennsylvanica Marsh. seedlings was correlated with the development of adventitious roots indicating that a factor from the root system might be involved in stomatal regulation during flooding (173).

It has been proposed that nonstomatal inhibition of photosynthesis during flooding might be due to a reduced supply of cytokinins available to the shoots. Flore et al. (60) found that the cytokinin concentration in the xylem sap of tomato plants was unchanged during 48 hours of flooding. However, the total amount of cytokinins translocated to shoots would have been reduced considerably due to a 50% reduction in root hydraulic conductivity which occurred after 24 hours of flooding. Application of exogenous cytokinins to flooded tomato plants prevented the decrease in stomatal conductance and photosynthesis which usually occurs during flooding (28). Based on these studies, it appears that cytokinins may be involved in the loss of photosynthetic capacity during flooding. Additional research is needed so that our understanding of cytokinin synthesis and metabolism in flooded plants is based on good data rather than speculation.

Ion Uptake

Flooding has a significant impact on the uptake of inorganic ions from the soil solution (34,54,93). The effects of flooding on plant mineral nutrition are complex. The nutrient absorption mechanisms and physiology of the species being studied, initial soil conditions, and the extent to which flooding alters soil properties are involved in flooding effects on plant mineral nutrition (93).

Nitrogen concentrations in plant tissues generally decline when plants are flooded and in nearly every case total nitrogen content of plant tissues is reduced (93). The initial soil nitrogen status, denitrification, and the uptake responses of plants to flooding interact to determine the amount and distribution of nitrogen in tissues of flooded plants. Uptake of the ammonium ion by prune scions grafted on Myrobalan 29C rootstocks was inhibited by anaerobiosis (146). Dry bean root systems subjected to anoxia for three days absorbed only 60% as much nitrate as did aerated control root systems (157). The concentration of nitrogen in swamp tupelo (78), citrus rootstock (73), and pecan (182) leaves was lower when trees were flooded than when trees were well-aerated. Also, flooding reduced the level of nitrogen in loblolly pine needles, but the effect was observed only when phosphorus was applied (78).

Potassium uptake is influenced in a similar manner as nitrogen uptake by flooding (93). A reduction in potassium uptake is often observed during flooding and this response may limit plant growth. Anoxic dry bean roots absorbed only 2% as much potassium as control roots (157). Roots of the cultivar Pinto III which were subjected to localized anoxia (a split-root system with half of the root system subjected to anoxia and the other half subjected to aerobic conditions) displayed a greater uptake of potassium per unit root weight in the aerated half of the root system than in roots from aerobic control plants. Uptake of potassium was also inhibited by anaerobiosis in grafted prune trees (146). Leaf content of potassium was reduced by flooding in loblolly pine (78) and pecan (182). In contrast, swamp tupelo exhibited an increase in leaf potassium content during flooding (78).

Flooding generally lowers the tissue concentration and total content of phosphorus when phosphorus in the soil is adequate (93). In soils moderately or severely deficient in phosphorus due to the phosphorus being tied up in iron or aluminum phosphates or tightly held on anion-exchange sites of clays and hydrous oxides of ferric iron and aluminum, flooding produces a different result. Flooding under these conditions often leads to a greater availability of phosphorus for uptake by the roots. Thus, plant phosphorus content can be increased by flooding if phosphorus uptake is not severely limited by anaerobiosis and levels of available phosphorus in the soil before flooding were not inordinately high.

Uptake of phosphorus ions by dry bean roots was reduced by anoxia (157). The phosphorus content of citrus rootstock (73), loblolly pine (78), and pecan leaves (182) decreased during flooding. Swamp tupelo did not display a reduction in phosphorus content of leaves during flooding (78).

Tissue concentrations of calcium and magnesium often respond to flooding in a similar manner (93). Calcium and magnesium concentrations are usually not altered as much by flooding as are concentrations of nitrogen, potassium and phosphorus; however, concentrations may decrease slightly and the total content of these ions declines appreciably due to reduced plant growth. There does not appear to be close coupling between active uptake mechanisms and calcium and magnesium accumulation by plants which may explain the lesser effect of flooding on tissue concentrations of these elements.

Dry bean root systems showed reduced uptake of calcium and magnesium ions during anoxic treatment (157). Concentrations of

calcium and magnesium in pecan leaves on flooded trees were lower than those on control trees after 31 days of flooding (182). Loblolly pine and swamp tupelo seedlings display distinctly different responses to flooding for calcium and magnesium leaf concentration (78). Leaf concentrations of loblolly pine were decreased by flooding whereas the calcium and magnesium concentration in swamp tupelo leaves was unaffected by flooding.

The sodium and chloride content of plants generally increases during flooding (93). This is possibly due to a reduction in the efficiency of salt exclusion mechanisms which are likely to be active processes requiring energy. Toxicity may result when sensitive species are flooded. Flooding increased the total uptake of sodium and chloride of grapevines and increased the amount of these ions transported into shoots (185). Leaf damage was visible within five days of waterlogging.

The uptake of micronutrient ions from the soil solution during flooding is strongly influenced by soil factors (93). Tissue concentrations of these ions increase, decrease, or remain the same during flooding depending on soil conditions. High levels of iron and manganese in flooded plants may result in phytotoxicity in certain species. Loblolly pine seedlings had decreased levels of zinc and manganese and dramatically increased levels of iron when flooded (78). Flooding of pecan seedlings reduced tissue concentrations of zinc, iron, and manganese (182). The manganese content of swamp tupelo seedlings was reduced by flooding, but the content of zinc and iron showed no effect due to flooding (78). More information is needed on the effects of flooding on micronutrient uptake and translocation in woody plants.

MECHANISMS OF FLOODING INJURY

Symptoms of flooding injury include a reduction in root growth, partial stomatal closure, inhibition of photosynthesis, a reduction in leaf area and shoot elongation, chlorosis and senescence of leaves, and wilting of leaves (86,92). In addition, flooding injury is often involved, either directly or indirectly, in the mortality of affected plants. Several mechanisms have been proposed to account for the changes in growth, physiology, morphology, and anatomy of flooded plants. Little is known about the relative importance of each of the proposed mechanisms or whether they act singularly, simultaneously, or synergistically. Toxicity of anaerobic metabolites, cytoplasmic acidosis, the reduced energy charge of anaerobic cells, cytokinin and gibberellin "starvation", accumulation of toxic levels of ions from the soil solution, accumulation of damaging levels of ethylene from anaerobic soils, and the increased susceptibility of flooded plants to soil diseases appear to be involved in the injury of plants during flooding.

Accumulation of Anaerobic Metabolites

As soil O_2 is depleted during flooding, metabolism in roots shifts increasingly from respiration to glycolysis. Consequently, anaerobic metabolites accumulate in roots of many species of plants during flooding (45,46,86,110,163). However, certain species of plants which are tolerant of flooding did not display an increase in ethanol production or an induction of alcohol dehydrogenase (ADH) activity (45). This finding led to the proposal that flooding tolerance was related to the capacity to avoid synthesis of potentially toxic ethanol from pyruvate at the end of glycolysis.

Flooding-susceptible species were thought to produce ethanol in larger amounts than flooding-tolerant species during anaerobiosis due to increased ADH activity and/or a Pasteur effect. Reduced ethanol production in flooding-tolerant species was thought to occur because these plants were able to produce malate rather than ethanol from pyruvate. Flooding-tolerant plants were purported to lack malic enzyme (ME) activity. This proposal became known as the metabolic theory of flooding tolerance.

Jackson and Drew (86) recently examined the experimental evidence relating to this theory. Little information is available which supports the notion that flooding-tolerance derives from a specialized metabolism which results in the avoidance of ethanol accumulation in tissues. In fact, there is a significant body of data which conflicts with the proposed mechanism of injury. A summary of arguments against the metabolic theory of flood tolerance are listed below:

1. Ethanol production.

   Ethanol is produced by plant cells under anaerobic conditions, but it is doubtful whether this response in roots is related to the susceptibility to flooding. Plant species, such as rice, winter wheat, and rye, which are fairly flooding-tolerant produce considerable amounts of ethanol when waterlogged.

2. Ethanol toxicity.

   Ethanol does not appear to accumulate to toxic levels in tissues of flooded plants.

3. ADH activity.

   ADH activity increases during anaerobiosis, but it is unclear if this is related to flooding tolerance. Also, the synthesis of ethanol may be limited more by the activity of pyruvate decarboxylase (PDC) than by ADH.

4. Malate and ethanol.

An examination of three flooding-tolerant species showed that malate failed to accumulate under anaerobic conditions and appreciable activity of the ME was found.

5. ATP production.

The synthesis of malate as proposed would result in no net ATP production. It is unlikely that plant survival would be enhanced by a respiratory pathway which gives no net yield of ATP.

6. Substrates for respiration.

The metabolic theory proposes that glycolysis should be stimulated under anaerobic conditions in flooding-intolerant species, causing ethanol to accumulate more rapidly than in flooding-tolerant species. However, there is much evidence which is in conflict with this proposal. Survival of rice seedlings under anoxia is closely associated with rapid production of ethanol. Furthermore, the survival of roots of wetland and non-wetland species is prolonged rather than decreased when the supply of respirable substrate is increased by addition of glucose to anaerobic media.

In summary, the metabolic theory of flood tolerance does not appear to be supported by the experimental record.

Cyanogenic glucosides were identified in the roots of apricot, peach, and plum seedlings (147). When seedlings were subjected to anaerobiosis, cyanogenic glucosides were hydrolyzed which released hydrogen cyanide. A close association was observed between flooding tolerance, glycoside hydrolysis, and cyanogenesis under anaerobic conditions. Seedlings of apricot and peach were more sensitive to flooding than plum seedlings. Also, the cyanogenic glucoside content and the proportion of it that was hydrolyzed during flooding was greater in peach than in plum roots. It is not clear whether hydrogen cyanide is the primary cause of mortality in flooding-intolerant Prunus species or a secondary, contributing factor.

Cytoplasmic Acidosis

Roberts et al. (142,143) have proposed that cytoplasmic acidosis is responsible for meristematic death in hypoxic root tips of maize and pea. In this hypothesis, regulation of cytoplasmic pH is believed to be an important factor in the survival of plants under hypoxia. The following sequence of events is thought to occur in the cytoplasm of root meristematic cells of higher plants which have been subjected to hypoxia. Lactate dehydrogenase (LDH), the enzyme which catalyzes the production of lactate from pyruvate, has an alkaline pH optimum and lactic acid production begins soon after oxidative phosphorylation ceases. Lactic acid accumulation results in a slight acidification (pH falls from 7.4 to 6.8 approximately) of the cytoplasm which inhibits further lactic acid production and stimulates ethanol production. Ethanol production is stimulated by slight acidification of the cytoplasm because PDC, the enzyme which catalyzes the first reaction in the pathway from pyruvate to ethanol, has an acid pH optimum. Cytoplasmic pH stabilizes and ATP production can proceed without cytoplasmic acidosis for several hours. Factors which prevent the regulation of cytoplasmic pH allow acidification of the cytoplasm and death of root meristem tissues. Also, longer exposure to hypoxia will eventually result in cytoplasmic acidosis and death of root meristem tissues due to leakage of acid from acidic vacuoles. The differential sensitivity of maize and pea roots to flooding was explained by the observation that leakage of acid from vacuoles occurred earlier during hypoxia in pea root tips than in maize root tips.

On the other hand, LDH activity in barley (Hordeum vulgare L.), wheat (Triticum aestivum L.), rye (Secale cereale L.), and maize

roots increased up to 20-fold during several days of severe hypoxia (76). Furthermore, [^{14}C] glucose was supplied to induced barley roots under hypoxic conditions resulting in lactate acquiring label but to a lesser degree than ethanol or alanine. Most of the [^{14}C] lactate was secreted into the medium whereas most other labelled products were retained in the roots. These findings do not agree with the cytoplasmic acidosis hypothesis. Hypoxic induction of LDH and lactate secretion by induced roots would not be expected if lactate glycolysis ends soon after the beginning of hypoxia due to acidosis brought about by lactate accumulation in the cytoplasm as is proposed in the cytoplasmic acidosis hypothesis. The authors speculated that the induced LDH may have an unrecognized function beyond that of glycolysis during anoxia.

Reduced ATP Supply

Lack of energy for cellular processes may be another mechanism by which plants are injured during flooding (86). The oxidation of one mol of glucose to CO_2 and H_2O yields 38 mol of ATP under aerobic conditions. A net yield of two mol of ATP is generated from one mol of glucose by glycolysis under anaerobic conditions. This represents a 95% reduction in ATP production and the ATP available may not be sufficient to support cell maintenance and growth.

Maintenance of glycolysis during anaerobiosis may be limited by assimilate availability. Reduced photosynthesis (22,29,51,123,124,153,164) and assimilate translocation (155) would likely provide inadequate substrate for glycolysis at some point during anaerobic stress. In addition, the efficiency of use of photoassimilates is also decreased by flooding (165). Most of the

ethanol and other metabolites produced in roots under anaerobic conditions appear to be secreted to the surrounding medium thereby preventing their accumulation to toxic levels in tissue. Losses of carbon by this mechanism may account for up to 33% of the soluble photoassimilated carbon in Phaseolus vulgaris L. (165).

Reduced Supply of Plant Growth Substances

The concentration of cytokinins and gibberellins in xylem exudate is reduced by flooding (32,60,138,139). Reduced export from roots to shoots might be a partial cause of the lowered level of cytokinins and gibberellins in shoots of flooded plants. Nonstomatal inhibition of photosynthesis, chlorosis and premature senescence of leaves, formation of adventitious roots, and reduced shoot elongation may be influenced by cytokinin and gibberellin "starvation" (28,138).

Accumulation of Toxic Levels of Ions

Factors which injure plants may also originate in the soil (54,86). Ion pumps which function to exclude certain ions from the cytoplasm are disrupted during flooding. Anaerobic metabolism may provide insufficient energy to maintain the activity of the ion pumps or plasma membrane damage may cause dissipation of protein-concentration gradients which would end energy-dependant ion transport. Passive uptake of ions from the soil solution without selectivity occurs with accumulation of certain ions to toxic levels.

Ethylene Accumulation in Flooded Soils

High levels of ethylene in vineyard soil were found to influence the growth and physiology of grapevines under field and green house conditions (84,121). Soil compaction-induced iron chlorosis appeared in vineyards around the time of bloom and was correlated with soil water content (121). The severity of symptoms was greatest when soil moisture content was high and shoot growth was proceeding rapidly. The O_2 and CO_2 content of soil did not appear to be involved in compaction-induced iron chlorosis. Measurement of O_2 and CO_2 levels in different soil layers of adjacent healthy and chlorotic vineyards showed no significant differences. However, concentrations of ethylene in soil were found to be higher in chlorotic than in healthy vineyards. Since iron is assimilated mainly by young, unsuberized roots and ethylene in concentrations above 1 ppm completely inhibits growth of grapevine roots, the root surface able to assimilate iron would be reduced. Under conditions of rapid growth, uptake of iron by the reduced root system would not be able to meet the demands of the shoot system resulting in iron chlorosis.

Production of ethylene in vineyard soil was also influenced by the addition of organic matter. In general, organic matter that produced the most ethylene in laboratory tests also produced the greatest chlorosis in the vineyard. Oil radish has been grown in vineyards to reduce soil water content at bloom and thereby reduce compaction-induced chlorosis. This approach has been somewhat successful in Swiss vineyards.

Various organic materials were applied to soil and the evolution of ethylene measured after flooding (84). Application of

organic materials stimulated ethylene production in waterlogged soils. Dead and fresh grapevine leaves, dead citrus leaves, fresh citrus roots, dead pear leaves, dead peach leaves, dead persimmon leaves, and rice straw greatly increased ethylene evolution. Levels of ethylene of up to 4000 nl 10 g $soil^{-1}day^{-1}$ were recorded. Evolution of ethylene from organic materials in the soil during flooding seemed to require microbial activity and was markedly affected by soil temperature, moisture content, and aeration. The application of dead grape leaves, which caused the greatest ethylene evolution, inhibited shoot growth, total fresh weight, root fresh weight, main root length, and succinate dehydrogenase activity of roots of grapevine cuttings grown for three weeks in the greenhouse.

Disease Interactions

Plants are often predisposed to disease by flooding (170). This effect may result from anoxia-induced changes in the root or the release of exudates by stressed roots.

Root exudates in the rhizosphere have a significant impact on microbial activity (170). Components of root exudates directly affect propagule germination, mycelial growth, and reproduction of pathogens. Zoospores of *Pythium* spp. and *Phytophthora* spp. are attracted to roots and exudates from roots. Amino acids and ethanol have been shown to attract zoospores (positive chemotaxis). Positive chemotaxis has also been demonstrated for several aldehydes, alcohols, and fatty acids. The region of cell elongation in roots appears to produce more exudates and attract more zoospores than other root tissues.

Anaerobiosis reduced plant growth and increased the exudation of ethanol, amino acids, and sugars by pea roots (166). A concomitant increase in the severity of Fusarium spp. root rot of anaerobic roots was also observed. In addition, flooding increased the infection rate and mortality of Fraser fir (Abies fraseri (Pursh.) Poir.) seedlings (90), walnut (Juglans spp.) rootstock seedlings (107), and almond seedlings (187) from root and crown rot caused by Phytophthora spp.

In summary, injury to plants during flooding occurs by a number of mechanisms. Due to the wide range of factors which are injurious to plants during flooding, efforts by plant breeders to develop cultivars resistant to flooding appear to have a low probability of success. Selection of characteristics which promote survival of plants during transient, short term flooding appears to have a greater chance of success.

ADAPTATIONS OF PLANTS TO FLOODING

The mechanisms by which plants adapt to flooding are complex and have been studied very little except for morphological changes which allow plants to avoid anoxia (34,77,92). Survival of plants usually does not result from a single adaptation but rather from a combination of adaptations.

Metabolic Adaptations

Plants do not adapt to flooding by becoming resistant to anoxia. Instead, survival is enhanced by adaptations which allow the plant to tolerate or avoid anoxia (77). Commonly, crop plants are only exposed to anoxia for short periods of time. Little information is available on metabolic adaptations which allow plants

to survive and recover from this type of flooding episode.

The metabolic theory of flooding tolerance as proposed by Crawford (45,46) has been discussed in the previous section. In contrast to this theory, it appears that increased glycolytic activity and secretion of metabolites from root cells may be associated with survival of roots during short term flooding (76,77,142,143,149,151). Furthermore, the induction of ADH (149,179) and LDH (76) under anaerobic conditions suggests that a specialized glycolytic metabolism must contribute in some way to survival. The contribution of ethanol glycolysis to tissue survival is likely to involve the sustained production of a certain level of ATP for cellular maintenance.

The importance of ethanol glycolysis for survival of anoxia was highlighted by the work of Saglio et al. (151). Young, intact maize plants were exposed to hypoxia (2-4 kPa partial pressure O_2) for 18 hours before root tips were excised and placed under anoxic conditions. Hypoxic preconditioning resulted in larger amounts of ATP, larger ATP/ADP and aldehyde energy charge ratios, and higher rates of ethanol production when excised root tips were subsequently made anoxic, compared with root tips transferred directly from aerobic to anoxic media. The improved energy metabolism achieved by hypoxic preconditioning was associated with increased ADH activity and the induction of ADH-2 isozymes. Consequently, hypoxically pre-conditioned root tips were able to survive 22 hours of anoxia while root tips which were not preconditioned were only able to survive 8 to 9 hours of anoxia. These results indicate that maize root tips were able to metabolically acclimate to hypoxic conditions which resulted in improved energy status and tolerance of anoxia.

Metabolic acclimation appeared to be closely linked with induction of an effective ethanolic fermentation pathway.

In another study, apricot seedlings were flooded for approximately one month (74). Flooding reduced root respiration and seedling survival. Maintenance of root respiration was correlated with the ability to survive flooding. Also, wetland species which are tolerant of flooding were found to produce high levels of ethanol (163). Survival during long-term flooding was apparently linked with ethanol glycolysis in these species.

Another metabolic adaptation that can result in greater flooding-tolerance is rapid stomatal closure (30,93). ABA accumulates in leaves of flooded plants and is the likely cause of stomatal closure soon after flooding begins (60,87). Rapid stomatal closure prevents the loss of turgor in leaves and subsequent wilting (29,123).

Anatomical Adaptations

Adaptations which allow plants to avoid anoxia have been studied to a greater extent than have metabolic adaptations which allow plants to tolerate anoxia. The most common anoxia-avoidance adaptations are the development of aerenchyma, hypertrophy and hypertrophied lenticels, and adventitious roots (34,77,89,92).

Aerenchyma are internal, longitudinally connected gas-filled intercellular spaces caused by cell separation (schizogenous) or dissolution of cells in the cortex or pericycle (lysigenous) (85,89). The internal porosity of roots and submerged stems or trunks is increased by aerenchyma formation. Aerenchyma facilitate the exchange of O_2 between aerobic shoots and anoxic roots during

flooding. Oxidation of the rhizosphere is also associated with aerenchyma formation. Internal transport of oxygen occurred over a distance of at least 210 mm in maize roots with aerenchyma (55). The transport of oxygen in roots with aerenchyma resulted in higher values for ATP content, adenylate energy charge, and ATP/ADP ratios than in roots without aerenchyma. A number of species including Vaccinium corymbosum L. (2), Gmelina arborea Roxb. (118), Pinus serotina Michx. (175), Tectona grandis L. (118), and Zea mays L. (19) develop aerenchyma in response to flooding.

Morphological Adaptations

Hypertrophy and hypertrophied lenticels are adaptations which are associated with the avoidance of injury during anoxia (77,92). The development of hypertrophy and hypertrophied lenticels may facilitate the movement of O_2 from aerobic shoots to anaerobic roots. Also, hypertrophied lenticels may provide a means for dissolved gases and anaerobic metabolites such as ethylene, ethanol, lactic acid, and acetaldehyde to exit the stem. Formation of hypertrophied lenticels has been observed in many species of woody plants including Quercus macrocarpa Michx. (172), Fraxinus pennsylvanica Marsh. (67,173), and Actinidia deliciosa Planch. (153). A comparison between green ash (flooding-tolerant) and water oak (Quercus nigra L.) (less flooding-tolerant than green ash) seedlings during long term flooding revealed the adaptive significance of hypertrophy and hypertrophied lenticel formation (67). After approximately two weeks of flooding, hypertrophied lenticels and basal swelling were observed in green ash seedlings whereas water oak seedlings exhibited only slight enlargement of

basal lenticels and no discernable swelling. Green ash seedlings maintained higher O_2 and lower CO_2 and ethylene concentrations in roots during flooding than water oak seedlings. O_2 was apparently able to diffuse from the stem to roots in green ash seedlings and this resulted in a greater degree of rhizosphere oxidation than in water oak seedlings.

Adventitious rooting is a common adaptation of woody plants to flooding (34,54,77,92). Roots are very sensitive to O_2 availability with root metabolism being disrupted soon after flooding is initiated. The root:shoot ratio decreases during flooding reflecting a greater reduction in root growth than in shoot growth. Also, decay of roots often results in a loss of dry matter during anaerobic stress. The development of adventitious roots along submerged stems at least partially compensates for the reduced growth and metabolism of the existing root system.

Fraxinus pennsylvanica Marsh. seedlings formed adventitious roots on submerged stems during flooding (173). The development of adventitious roots was correlated with stomatal reopening. Leaf water potential was higher in flooded than in control seedlings during the course of the experiment. Adventitious roots increased water uptake in flooded seedlings which contributed to the maintenance of leaf turgor. Other woody species which exhibit adventitious rooting during flooding are Acer rubrum L. (53), Vitis vinifera L. (61), Actinidia deliciosa Planch. (153), Gmelina arborea Roxb. (118) and Tectona grandis L. (118).

Compensatory root growth can play an important role in plant survival due to the heterogenous nature of anaerobiosis in flooded soils. Phaseolus vulgaris L. seedlings were grown using a split-

root system (156). Treatments consisted of an aerated control, a non-aerated control in which both halves of the root system were subjected to anoxia using N_2, and localized anoxia in which one-half of the root system was aerated and the remaining half subjected to anoxia using N_2. Shoot and root growth were reduced by the non-aerated control treatment but not by the localized anoxia treatment. Root growth was greatest in the aerated portion of the localized anoxia treatment. Plants responded to anoxia by increasing root growth in aerobic regions. Compensatory root growth allowed plants subjected to anoxia in part of their root volume to maintain growth.

Rootstock Selection

Grafting is a cultural modification which can be used to increase the flooding tolerance of woody crop species (99,100,110). The growth and physiology of eight apple rootstocks was examined during flooding (99). Malus prunifolia (Willd.) Borkh. trees displayed the greatest tolerance to flooding while M26 and M9 trees displayed the least tolerance to flooding. Further testing showed that scions grafted on Malus prunifolia (Willd.) Borkh. were more tolerant of flooding than scions grafted on M26 and M9 (100).

A similar result was observed for Prunus rootstocks (110). Prunus japonica Thunb. (extremely tolerant of flooding), Prunus persica L. (intolerant of flooding), Prunus mume Sieb. et Zucc. (intolerant of flooding), and Prunus salicina Lindl. (intolerant of flooding) were used as rootstocks in a flooding experiment. After eight days of flooding, scions grafted on Prunus japonica Thunb. had much lower injury ratings than scions grafted on the other rootstocks.

Further exploitation of this method of increasing the flooding tolerance of woody crop species appears to have merit. Significant seedling variability in flooding tolerance has been observed for Juglans nigra L. (36), Juglans hindsii Jeps. (36), and Populus trichocarpa Torr. & Gray (162). These findings suggest that careful screening and selection of seedling material may produce rootstocks with increased levels of flooding tolerance.

In summary, plant survival during flooding is not associated invariably with a single adaptation. Metabolic, anatomical, and morphological features of roots and shoots combine to allow various degrees of anoxia tolerance or anoxia avoidance.

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SECTION I

INFLUENCE OF ROOTSTOCK ON THE RESPONSE OF SEYVAL GRAPEVINES TO FREEZING STRESS

I. EFFECT OF ROOTSTOCK ON THE COLD HARDINESS OF SEYVAL PRIMARY BUDS AND CANES DURING ACCLIMATION AND DEACCLIMATION

ABSTRACT

Experiments were conducted to determine if grapevine rootstocks can directly influence cold hardiness of scion tissues during acclimation and deacclimation. Vines in an established vineyard were used in the 1985-1986 and 1986-1987 dormant seasons. Treatments included own-rooted Seyval (Sey/own), and Seyval grafted to Seyval (Sey/Sey), Harmony (Sey/Harm), Kober 5BB (Sey/5BB), and Couderc 3309 (Sey/3309). A second experiment was conducted during the 1987-1988 dormant season using potted vines. In this experiment, rootstocks were selected which have been reported to differ in length of vegetative cycle and time of bud burst. Treatments were own-rooted Seyval (Sey/own), and Seyval grafted to Seyval (Sey/Sey), Cynthiana (Sey/Cyn), Riparia Gloire (Sey/RGl), and St. George (Sey/StG). Cold hardiness and water content of primary buds and canes were measured periodically during acclimation and deacclimation. Rootstock had little effect on cold hardiness or water content during acclimation. However, significant rootstock effects were observed for the deacclimation period. Sey/Cyn canes had greater cold hardiness and lower water content than the other treatments during the 1988 deacclimation period. Buds on Sey/Cyn vines responded in a similar manner but to a lesser degree. Percentage shootless nodes was also reduced by use of Cynthiana as a rootstock in 1988. Primary buds and canes increased in cold hardiness and decreased in water content during acclimation. An inverse relationship was observed during deacclimation with cold

hardiness decreasing and water content increasing as deacclimation proceeded. Changes in the relationship between cold hardiness and water content of canes after the first killing frost in the fall suggest that grapevine cold acclimation can be viewed as a two stage process.

INTRODUCTION

Insufficient cold hardiness is a major factor which limits viticulture in the eastern United States and Canada. The potential for cold injury and resultant economic loss exists each dormant season. Cultural manipulation of vine cold hardiness has previously been reviewed by various authors (7,11,21,24,25). Practices used to minimize cold injury in vineyards can be categorized into those which are done prior to establishment and those which are done after the vineyard is established (7). The most critical of these are pre-establishment decisions such as site and cultivar selection. When a Vitis vinifera L. or less vigorous interspecific hybrid cultivar is chosen, cultivar selection will also involve the selection of an appropriate rootstock.

Rootstocks have recently received attention as a possible means of increasing grapevine cold hardiness (15,17,18,19,23, 29,35). Miller et al. (18) compared the cold hardiness of Kober 5BB (5BB), Couderc 3309 (3309), and Selection Oppenheim No. 4 (SO4) rootstocks for 3 seasons. Differences in bud hardiness were variable and seldom statistically significant. However, consistent and often statistically significant differences between rootstocks in cane cold hardiness were observed. Canes from 3309 vines were more cold resistant than canes from 5BB vines. SO4 canes were generally

intermediate in cold hardiness.

In a subsequent experiment, cold hardiness of White Riesling growing on its own roots and grafted to 5BB, 3309, and SO4 was examined (19). Rootstock influenced scion cold hardiness to a limited extent. Grafted vines had significantly hardier canes in one of three years studied and scions grafted to 3309 had significantly fewer shootless nodes in one of the four years studied. Other reports have indicated more substantial increases in scion cold hardiness by rootstock (15,21,23). Rootstock selection has also been suggested as a means of reducing cold injury to roots in areas of China where soil temperatures reach extremely low levels during the winter (35). Differences in root cold hardiness were measured and V. amurensis Rupt. x V. riparia Michx. hybrids were recommended over the less cold hardy and commonly used Beta rootstock.

In contrast, Shaulis et al. (25) found that vine cold hardiness was not affected by various rootstocks during a long term study at Geneva, NY. Similar results were obtained when primary bud cold hardiness of Chardonnay grafted to Elvira and 3309 was measured (29). Rootstock did not have a consistent effect on primary bud cold hardiness.

Lack of agreement concerning the influence of rootstock on scion cold hardiness is not surprising considering the complexity of the problem. The design and conduct of rootstock experiments is difficult due to the confounded nature of stock-scion relationships (10). The ability to separate primary effects from secondary effects is crucial for accurate interpretation of results.

Timing and frequency of sampling are also important in cold hardiness experiments. Cold injury occurs primarily due to intracellular freezing or desiccation stress resulting from extracellular freezing (5). The type and amount of injury observed is often related to when the freezing episode occurs during the dormant season. For example, -15°C would cause little injury in the winter when vines are at their maximum hardiness, but could cause considerable injury in the fall before vines become fully hardy or in the spring as vines are losing hardiness. Thus, it is instructive to subdivide the dormant season into three periods which are acclimation, mid-winter, and post-rest/deacclimation (11).

Sampling for cold hardiness in most of the previously reported studies was concentrated in the mid-winter period. The effect of rootstock on cold hardiness of grapevine scion tissues has not been adequately investigated during acclimation and deacclimation. Reports of reduced root hydraulic conductance with declining daylength during acclimation (8,34) and the close association between tissue water content and cold hardiness during acclimation and deacclimation (4,30-34) provide a possible mechanism for primary rootstock effects.

Thus, the objectives of this study were twofold. First, to determine if rootstock has a direct (primary) effect on cold hardiness of primary buds and canes of Seyval during acclimation and deacclimation. Secondly, to define the relationship between water content and cold hardiness of Seyval primary buds and canes during acclimation and deacclimation.

MATERIALS AND METHODS

Two experiments were conducted over a three year period. Experiment I was conducted during the 1985-86 and 1986-87 dormant seasons, while Experiment II was conducted during the 1987-88 dormant season.

Experiment I

Vines used in this study were located at the Clarksville Horticulture Experiment Station, Clarksville, MI. Treatments included own-rooted Seyval (Sey/own) and Seyval grafted to Seyval (Sey/Sey), Harmony (Dog Ridge x Couderc 1613) (Sey/Harm), Kober 5BB (Vitis berlandieri Planchon x Vitis riparia Michx.) and Couderc 3309 (Vitis riparia Michx. x Vitis rupestris Scheele) (Sey/3309). These rootstocks were selected because of their range in cold hardiness (10,18,Striegler and Howell,unpublished data,1981-82).

Vines were planted in 1983 in a uniform Kalamazoo sandy loam soil. Vineyard spacing was 2.4 m x 3.0 m (within row x between row) and row orientation was north to south. The training system employed was Hudson River Umbrella with fruiting wood retained as five-node canes. During the first season of this study cordons were established at pruning and all fruit was subsequently removed. In the second season, vines were pruned to a 10 + 10 pruning severity (10 nodes retained/0.45 kg of cane prunings) and flower clusters were thinned to one per developing shoot. An upper limit of 50 nodes retained/vine was set to avoid overcropping. All other cultural practices were conducted according to Michigan Agricultural Experiment Station recommendations (14,16,22).

Experiment II

This experiment was conducted at the Horticulture Research Center, East Lansing, MI. Treatments included own-rooted Seyval (Sey/own), and Seyval grafted to Seyval (Sey/Sey), Cynthiana (Vitis aestivalis Michx.) (Sey/Cyn), Riparia Gloire (Vitis riparia Michx.) (Sey/RGl), and St. George (Vitis rupestris Scheele) (Sey/StG). These rootstocks were selected based on reported differences in length of vegetative cycle and timing of bud burst (20). Riparia Gloire has a short vegetative cycle and early bud burst; St. George has a long vegetative cycle and early bud burst; and Cynthiana has a long vegetative cycle and late bud burst. Sey/RGl vines were only used during the 1987 acclimation period due to a shortage of plant material.

Vines were obtained from a commercial nursery and planted into 18.9 L pots using a sterile medium of 50% sandy loam soil, 30% spagnum peat, and 20% sand (by volume) in early June. Potted vines were placed on a flat gravel-covered area and arranged in blocks. Vine spacing was 0.9 m x 0.9 m. A single wire trellis (1.7 m height) was constructed and shoots were trained upward along jute twine which was tied to the wire. Two shoots were allowed to develop per vine. The vines were defruited and lateral shoots removed on a regular basis. Vines were watered as needed (generally twice per week). Soluble 20-20-20 fertilizer was mixed with water and applied to vines on 23 June, 8 July, 23 July, and 30 July. Each vine received 480 mg of N, P, and K, respectively, per application. All vines appeared healthy and there were no visible symptoms of nutrient deficiency. Applications of mancozeb, triadimefon, and carbaryl were applied at seven to ten day intervals to control

fungal diseases and insects. Weeds around pots were controlled with applications of paraquat and fluazifop-butyl.

In both experiments, canes were sampled periodically during the acclimation and deacclimation portion of the dormant season. Selection of canes was based on exposure status, cane diameter, and internode length (13). Persistent lateral status was not considered in Experiment I due to the high percentage of nodes having persistent laterals. In Experiment II, lateral shoots were not present because they were removed as part of the training procedure. Canes were selected which had been well-exposed during the previous growing season and were of medium diameter (7-10 mm) and internode length (5-9 cm).

The number of nodes and the number of mature nodes were counted on vines during the 1987 acclimation period (Experiment II) to determine the extent of cane maturation. Tissue maturation was assessed visually according to browning of periderm. Mature nodes were expressed as a percentage of total nodes.

At the time of sampling, entire canes were removed from vines, cut to sixteen nodes, and randomly divided into two groups for separate determination of cold hardiness and water content. Canes were returned to the laboratory within two hours of sampling and stored at 1°C until they were prepared. Samples were stored for no longer than 12 hours in this manner. In both experiments, samples consisted of node-internode pieces which were 3 to 6 cm long. Node-internode pieces were prepared and segregated according to their position on a cane. The following categories were used: nodes one to four, nodes five to eight, nodes nine to twelve, and nodes thirteen to sixteen. Nodes were counted from the base to the apex

of the cane. Each category was equally represented in a replicate so that cane positional effects on cold hardiness and water content were avoided (31-34).

Controlled freezing tests were used to measure cold hardiness. The freezing technique used was similar to that of Wolpert and Howell (31). Four samples (one from each of the cane position categories) per treatment were inserted into each of several vacuum flasks and placed into a chest freezer (Revco Ultra Low^{TM}). Samples were in contact with aluminum foil to facilitate heat removal and moist cheesecloth to inoculate samples which prevented supercooling. Freezer temperature was manually lowered to provide a sample cooling rate of 5^{o}C/h or less. Tissue temperature was monitored by a thermocouple (26 gauge copper-constantan) which was inserted into the pith of a representative sample in each flask. Flasks were removed at selected temperatures and allowed to warm slowly overnight at 1^{o}C. A temperature range was chosen such that the warmest temperature provided no injury and the coldest temperature was lethal to all tissues. Test temperatures were replicated four times in the freezer. Thawed samples were placed in humid chambers for 10 to 14 days, after which, tissues were sectioned and rated as alive or dead by the method of tissue browning (27). Dead buds had primordia which were brown and water soaked, while dead canes had phloem and cambium layers which were brown. The modified Spearman-Karber equation was used to calculate T_{50} values (temperature at which 50% of tissues would be killed) from tissue viability data (3). Cold hardiness was expressed as T_{50} values.

Water content was determined on primary buds (primordium + bud scales) and 2 cm cane segments by placing four to eight tissues (one

or two from each of the cane position categories) per treatment into each of four glass weighing vials fitted with ground-glass stoppers. Tissues were weighed, oven-dried for 36 hours at 70°C (vials open), and reweighed. Water content was expressed as grams water/gram tissue dry weight.

Shootless nodes were counted in the spring of each season following bud burst. Shoots were allowed to grow approximately 15 cm before data were collected. Shootless nodes were expressed as a percentage of total nodes.

Data within sample dates were subjected to AOV and mean separation was by Tukey's HSD test or Duncan's Multiple Range Test (26). Arcsin transformation was performed on percentage data before AOV (26).

RESULTS

Experiment I

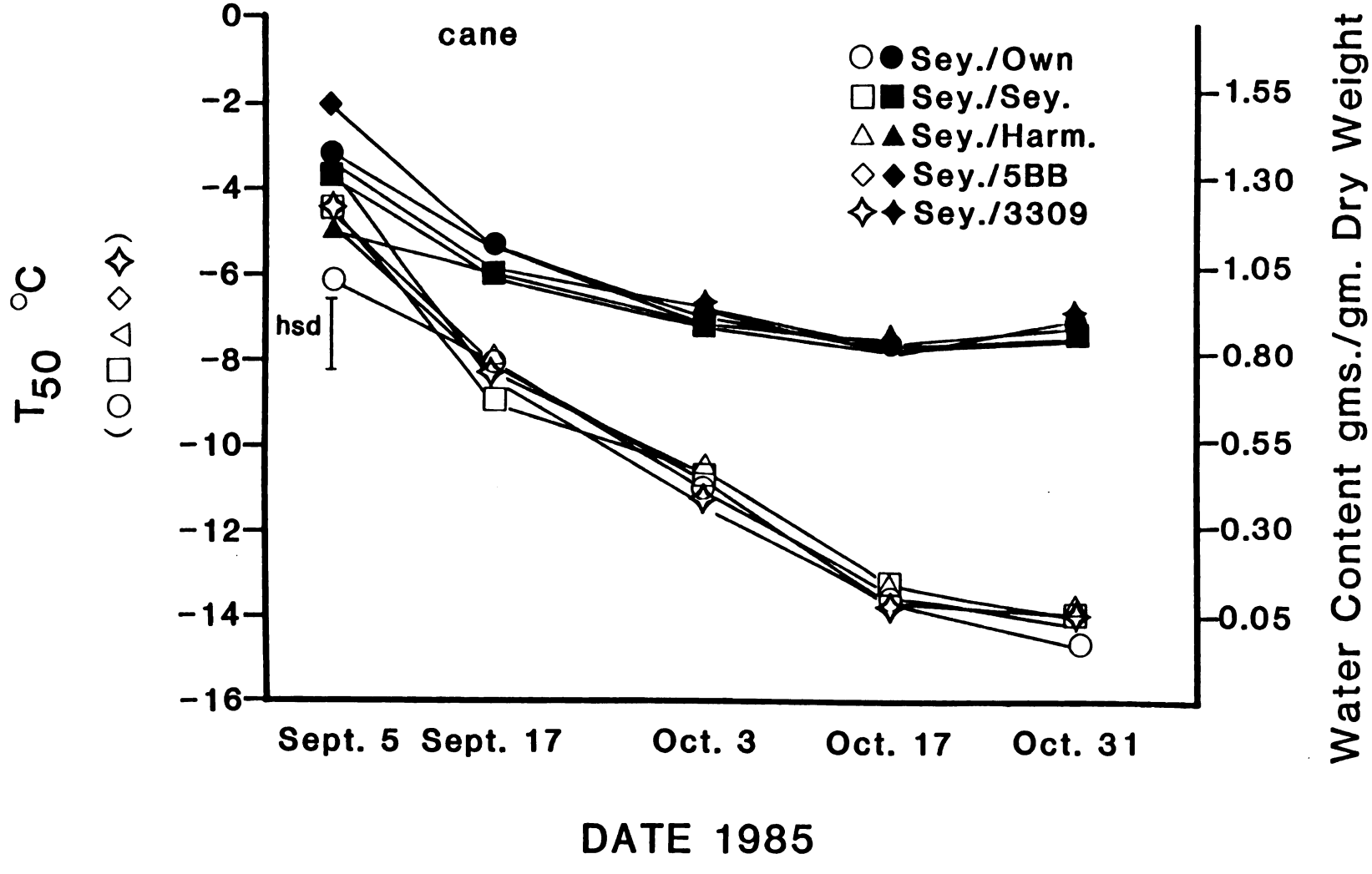
Cold hardiness and water content of primary buds was not significantly affected by rootstock during acclimation in 1985 (Figure 1). Primary bud cold hardiness increased and water content decreased during acclimation.

Canes from own-rooted vines were significantly more cold resistant than canes from grafted vines on 5 September (Figure 2). No other significant differences were observed for canes during acclimation in 1985. Cold hardiness of canes increased continually throughout the acclimation period. Cane water content declined until mid-October where a plateau of 0.80 to 0.89 g/g tissue dry wt was reached. Increases in cold hardiness after mid-October did not appear to be related to water content. A killing frost was recorded

Figure 1. Effect of rootstock on the cold hardiness (T_{50}) and water content of primary buds of Seyval grapevines during acclimation. 1985. Clarksville, MI.

Water Content gms./gm. Dry Weight
(● ■ ▼ ◆ ✦)
1.55
1.30
1.05
0.80
0.55
0.30
0.05
bud
○● Sey./Own
□■ Sey./Sey.
△▲ Sey./Harm.
◇◆ Sey./5BB
✧✦ Sey./3309
T50 °C
(○ □ △ ◇ ✧)
0
-2
-4
-6
-8
-10
-12
-16
Sept. 5
Sept. 17
Oct. 3
Oct. 17
Oct. 31
DATE 1985

Figure 2. Effect of rootstock on the cold hardiness (T_{50}) and water content of canes of Seyval grapevines during acclimation. 1985. Clarksville, MI.


cane
○● Sey./Own
□■ Sey./Sey.
△▲ Sey./Harm.
◇◆ Sey./5BB
✧✦ Sey./3309
hsd
0
-2
-4
-6
-8
-10
-12
-14
-16
1.55
1.30
1.05
0.80
0.55
0.30
0.05
Sept. 5
Sept. 17
Oct. 3
Oct. 17
Oct. 31
DATE 1985
T50 °C
(○□△◇✧)
Water Content gms./gm. Dry Weight
(●■▲◆✦)

on 17 October (Figure 3). Its occurrence seems to be closely associated with the end of the phase of grapevine cold acclimation in which increasing cold hardiness is correlated with decreasing water content. Vines were totally defoliated by 31 October.

Rootstock had little effect on cold hardiness or water content of primary buds during deacclimation in 1986 (Figure 4). Buds on Sey/3309 vines had a greater water content than buds on Sey/own or Sey/Harm vines on 26 March. Water content of buds increased and cold hardiness decreased as deacclimation proceeded. Primary buds were at scale crack on 23 April (1).

Sey/3309 canes were more cold resistant than canes from the other treatments on 26 March (Figure 5). Rootstock effects on cold hardiness or water content of canes were not evident at other sample dates during deacclimation in 1986. In general, water content of canes increased and cold hardiness decreased during deacclimation. Considerable fluctuation in air temperature occurred during the 1986 deacclimation period (Figure 6). It is interesting to note that canes and buds did not seem to be very responsive to rehardening temperatures.

Rootstock did not significantly affect primary bud cold hardiness or water content during acclimation in 1986 (Figure 7). Primary bud water content decreased and cold hardiness increased throughout the acclimation period. Cane cold hardiness and water content were only slightly influenced by rootstock during acclimation in 1986 (Figure 8). Sey/5BB canes were higher in water content than canes from other treatments on 4 September. Canes from Sey/3309 vines were significantly more cold resistant than canes from Sey/5BB on 30 October. Unlike the 1985 data, cane cold

Figure 3. Maximum and minimum air temperatures (^{o}C) during acclimation. 1985. Clarksville, MI.

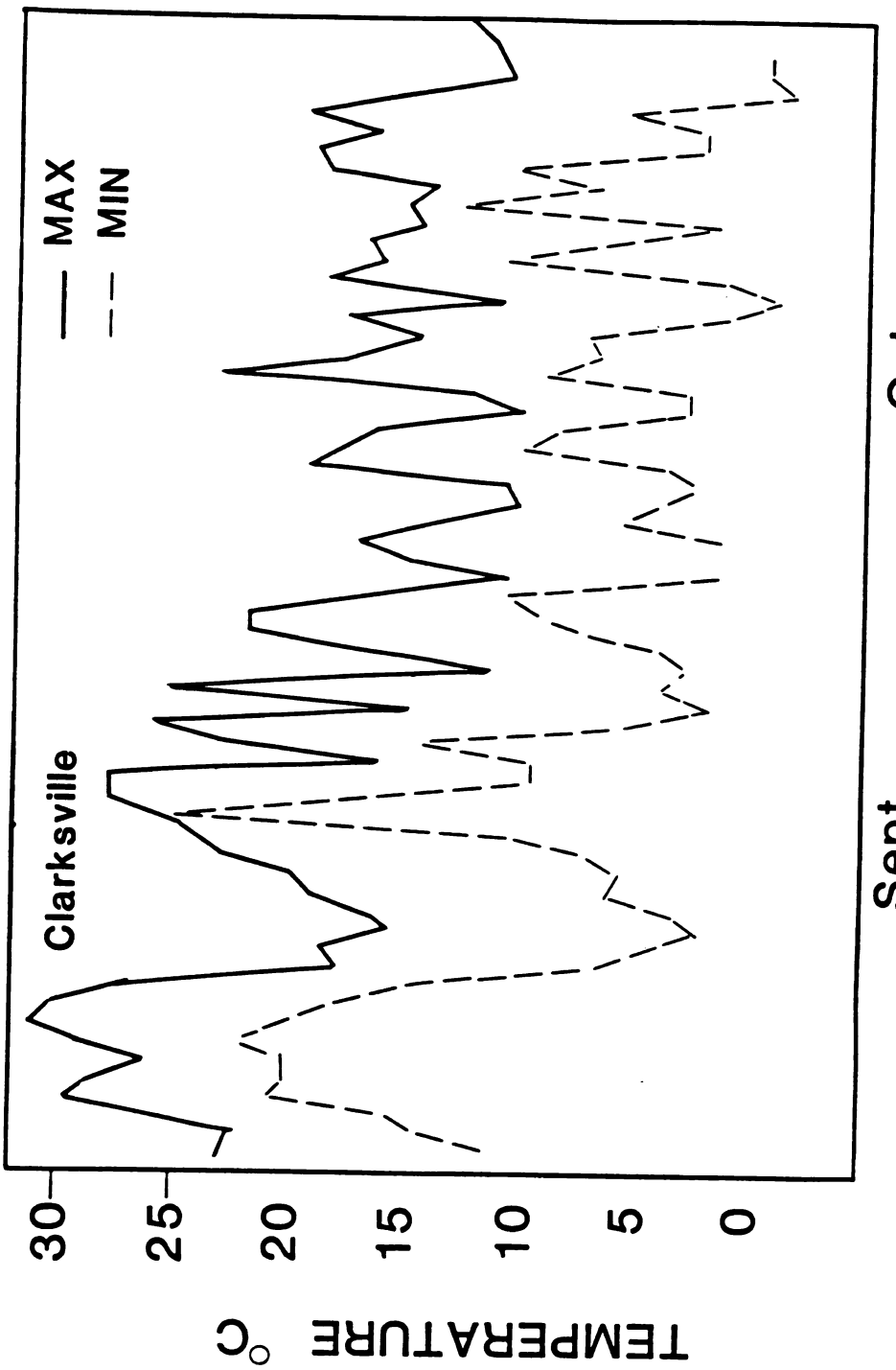

Clarksville
MAX
MIN
30
25
20
15
10
5
0
TEMPERATURE °C
Sept.
Oct.
DATE : 1985

Figure 4. Effect of rootstock on the cold hardiness (T_{50}) and water content of primary buds of Seyval grapevines during deacclimation. 1986. Clarksville, MI.

T50 °C
(○ □ △ ◇ ✧)
9
5
1
-3
-7
-11
-15
-19
-23
○● Sey./Own
□■ Sey./Sey.
△▲ Sey./Harm.
◇◆ Sey./5BB
✧✦ Sey./3309
bud
hsd I
Mar. 12
Mar. 26
Apr. 9
Apr. 23
DATE 1986
3.00
2.50
2.00
1.50
1.00
0.50
0.00
-0.50
Water Content gms./gm. Dry Weight
(● ■ ▲ ◆ ✦)

Figure 5. Effect of rootstock on the cold hardiness (T_{50}) and water content of canes of Seyval grapevines during deacclimation. 1986. Clarksville, MI.

Water Content gms./gm Dry Weight
(● ■ ▲ ◆ ✦)
1.20
1.00
0.80
0.60
0.40
cane
○● Sey./Own
□■ Sey./Sey.
△▲ Sey./Harm.
◇◆ Sey./5BB
✧✦ Sey./3309
hsd
Mar. 12
Mar. 26
Apr. 9
Apr. 23
DATE 1986
T50 °C
(○ □ △ ▽ ◇ ✧)
-5
-9
-13
-17
-21
-25

Figure 6. Maximum and minimum air temperatures (^{o}C) during deacclimation. 1986. Clarksville, MI.

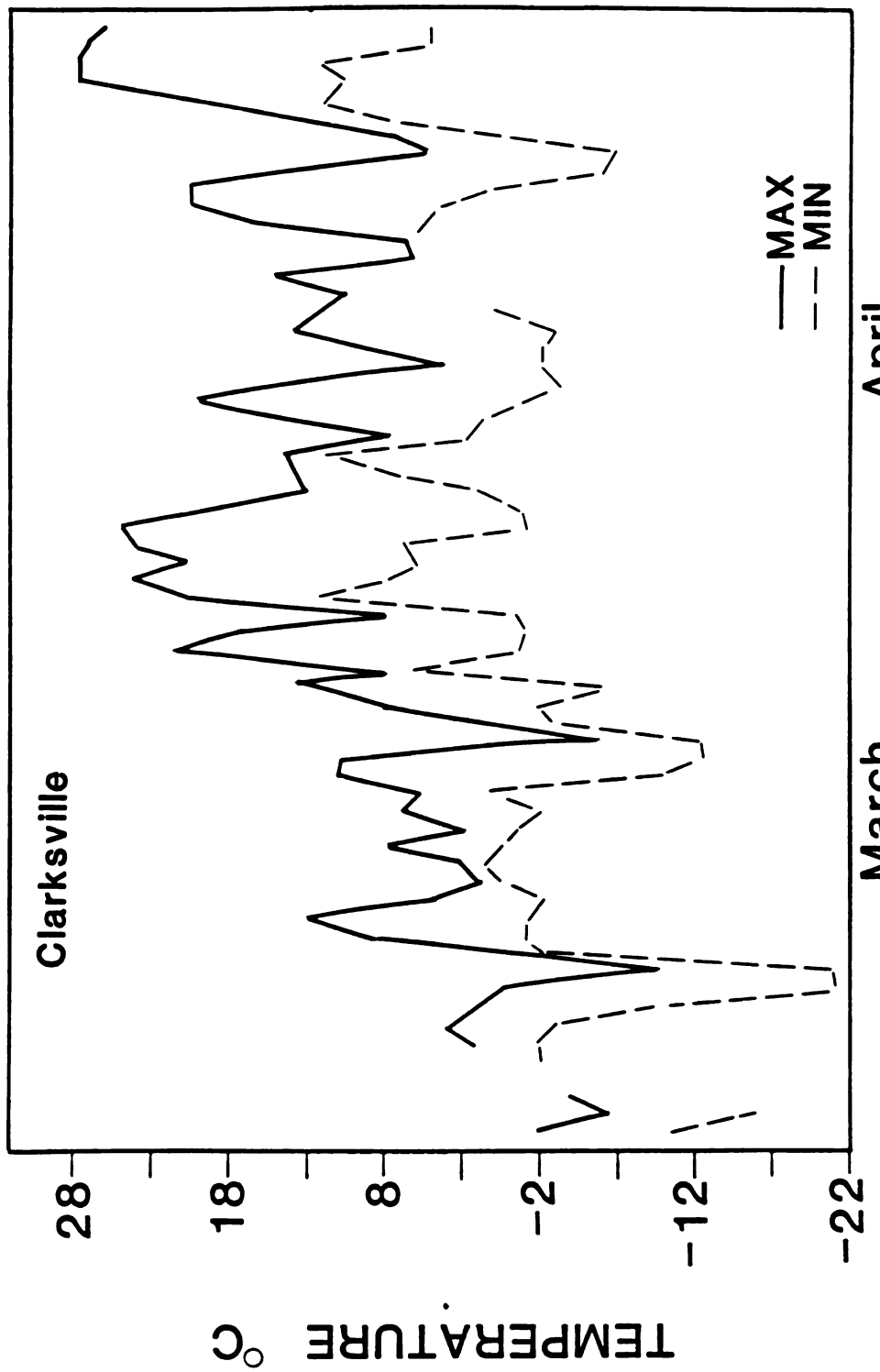

Clarksville
MAX
MIN
March
April
28
18
8
-2
-12
-22
TEMPERATURE °C
DATE : 1986

Figure 7. Effect of rootstock on the cold hardiness (T_{50}) and water content of primary buds of Seyval grapevines during acclimation. 1986. Clarksville, MI.

T50 °C
(○ □ △ ◇ ✧)
2
0
-2
-4
-6
-8
-10
-12
-14
bud
○● Sey./Own
□■ Sey./Sey.
△▲ Sey./Harm.
◇◆ Sey./5BB
✧✦ Sey./3309
Sept. 4
Sept. 18
Oct. 2
Oct. 16
Oct. 30
DATE 1986
2.75
2.25
1.75
1.25
0.75
0.25
Water Content gms./gm. Dry Weight
(● ■ ▲ ◆ ✦)

Figure 8. Effect of rootstock on the cold hardiness (T_{50}) and water content of canes of Seyval grapevines during acclimation. 1986. Clarksville, MI.

T50 °C
(○□△◇✧)
3
-1
-5
-9
-13
hsd
cane
○● Sey./Own
□■ Sey./Sey.
△▲ Sey./Harm.
◇◆ Sey./5BB
✧✦ Sey./3309
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Water Content gms./gm. Dry Weight
(●■▲◆✦)
Sept. 4
Sept. 18
Oct. 2
Oct. 16
Oct. 30
DATE 1986

hardiness increased and water content decreased continuously throughout the acclimation period. This discrepancy may be due to the lack of a killing frost during the sampling period in 1986 (Figure 9). Defoliation of vines was only approximately 30% complete on the final sample date (30 October).

Significant rootstock effects were observed on 11 March and 28 March during deacclimation in 1987 (Figure 10). Primary buds of Sey/Harm were hardier than buds of Sey/own on 11 March. On 28 March Sey/own and Sey/Sey buds had a lower water content than buds from the other treatments. Water content increased and hardiness decreased during deacclimation except for the final sampling date where water content and cold hardiness increased. This result appears to be anomalous based on the 1986 and 1988 deacclimation data for primary buds.

Differences among treatments were evident for canes during the 1987 deacclimation period (Figure 11). Sey/Sey canes had a lower water content than canes from the other treatments on 11 March. This trend continued on 28 March as Sey/own and Sey/Sey canes exhibited the lowest water content. On 9 April Sey/3309 canes were the most cold hardy and Sey/own canes were the least cold hardy. Canes of Sey/own and Sey/Sey were less cold hardy than canes from the other treatments on 17 April. From 28 March through the remainder of the deacclimation period, Sey/3309 and Sey/5BB canes tended to be more cold resistant than Sey/own and Sey/Sey canes. In general, water content increased and cold hardiness decreased as deacclimation of canes progressed. Air temperature data for the 1987 deacclimation period were fragmentary due to equipment malfunction (Figure 12). By the final sample date (17 April), bud growth had

Figure 9. Maximum and minimum air temperatures (^{o}C) during acclimation. 1986. Clarksville, MI.

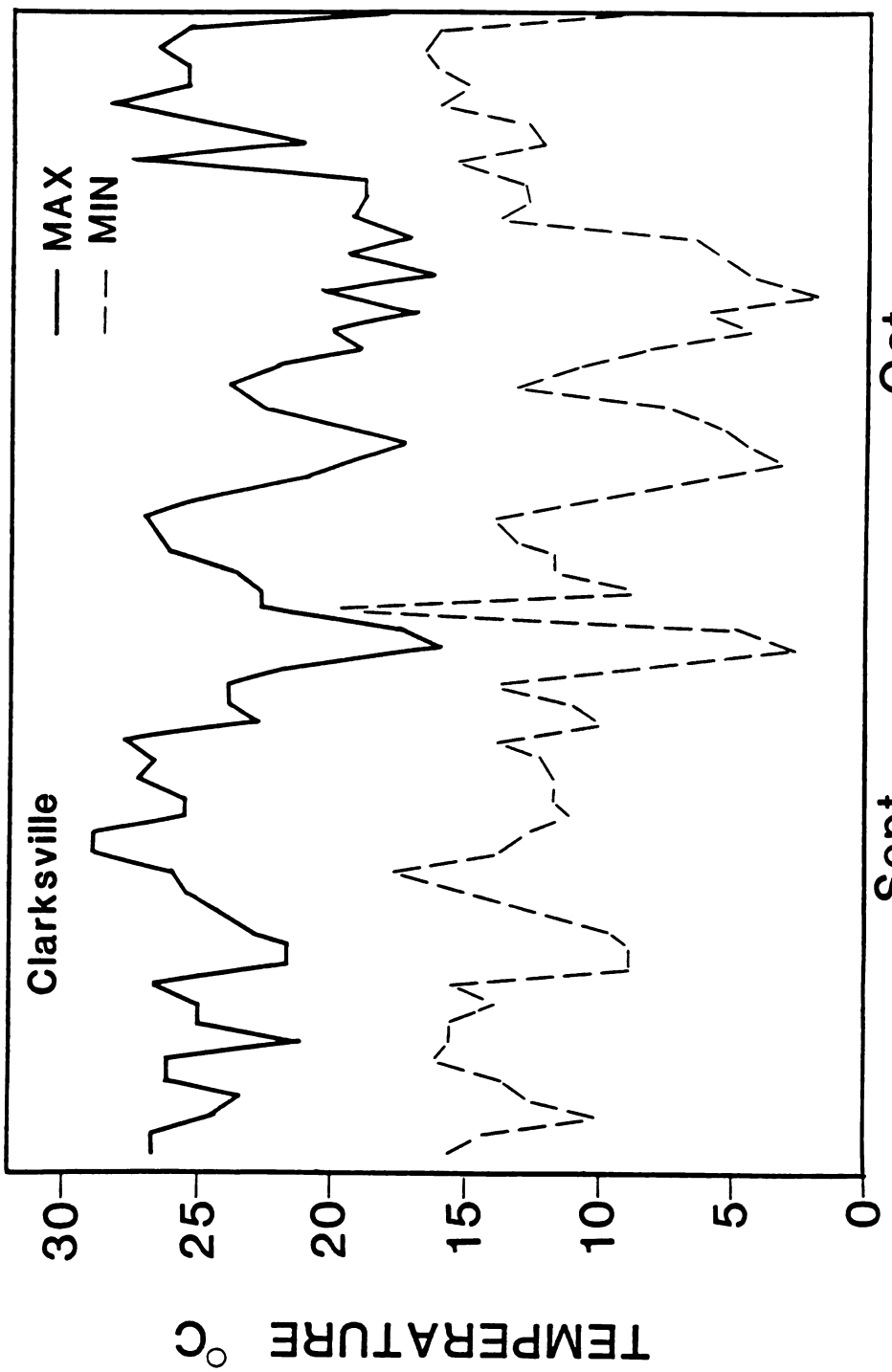

Clarksville
MAX
MIN
30
25
20
15
10
5
0
TEMPERATURE °C
Sept.
Oct.
DATE : 1986

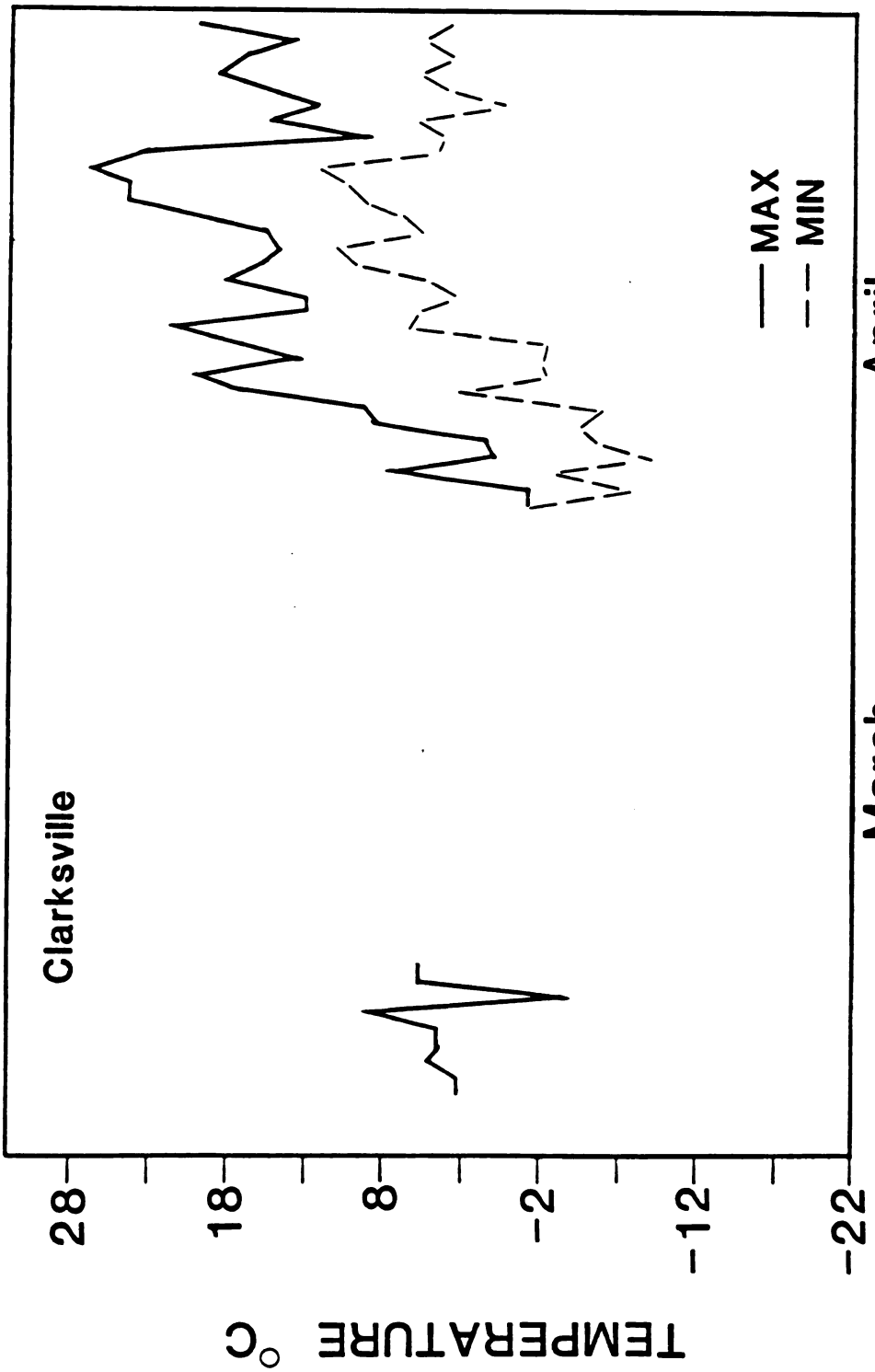
Figure 10. Effect of rootstock on the cold hardiness (T_{50}) and water content of primary buds of Seyval grapevines during deacclimation. 1987. Clarksville, MI.

T50 °C
(○□▽◇✧)
7
3
-1
-5
-9
-13
-17
-21
bud
○ ● Sey./Own
□ ■ Sey./Sey.
△ ▲ Sey./Harm.
◇ ◆ Sey./5BB
✧ ✦ Sey./3309
hsd
Mar. 11
Mar. 28
Apr. 9
Apr. 17
DATE 1987
Water Content gms./gm. Dry Weight
(●■▼◆✦)
2.25
1.75
1.25
0.75
0.25
-0.25
-0.75

Figure 11. Effect of rootstock on the cold hardiness (T_{50}) and water content of canes of Seyval grapevines during deacclimation. 1987. Clarksville, MI.

T50 °C
(○□△◇▽□○)
2
-3
-7
-11
-15
-19
-23
Mar. 11
Mar. 28
Apr. 9
Apr. 17
DATE 1987
cane
○● Sey./Own
□■ Sey./Sey.
△▲ Sey./Harm.
◇◆ Sey./5BB
✧✦ Sey./3309
hsd I
Water Content gms./gm. Dry Weight
1.20
1.00
0.80
0.60
0.40
0.20
(●■▼◆✦)

Figure 12. Maximum and minimum air temperatures (^{o}C) during deacclimation. 1987. Clarksville, MI.


Clarksville
MAX
MIN
March
April
28
18
8
-2
-12
-22
TEMPERATURE °C
DATE : 1987

begun and buds were considered to be at the scale crack stage of development (1).

Rootstock had little effect on percentage of shootless nodes (Table 1). No effect was observed in 1986 and grafted vines were superior to own-rooted vines in 1987.

Experiment II

Sey/Sey canes had a higher percentage of mature nodes than canes from the other treatments early in the 1987 acclimation period (Table 2). By 1 October cane maturation was essentially complete and only minor differences existed between the treatments. The reduction in percentage of mature nodes observed for most treatments on 29 October is probably an artifact caused by the difficulty encountered in counting total nodes. Counting of total nodes on 29 October was problematic because green shoot tips of canes had been killed by frost and were brown, dehydrated, and shrunken. Although the differences were not always statistically significant, tissue maturation appeared to be closely related to cold hardiness during the early stages of acclimation.

Sey/Sey primary buds were more cold hardy than buds from the other treatments on 3 September (Figure 13). Rootstock effects were also observed on 15 October during acclimation in 1987. On that date, Sey/own and Sey/Sey buds had a lower water content than buds from the other treatments. Also, cold hardiness differences were observed on 15 October when primary buds of Sey/own were hardier than buds of Sey/RGl or Sey/StG. Primary bud water content decreased and cold hardiness increased as acclimation proceeded.

Table 1. Effect of rootstock on the percentage of shootless nodes of Seyval grapevines. Clarksville Horticulture Experiment Station.

Rootstock	Shootless nodes (%) 1986	1987
Own	11.0	36.9a[z]
Seyval	19.1	9.1b
Harmony	10.2	14.8b
Kober 5BB	14.5	17.7ab
Couderc 3309	13.5	7.8b
	n.s.	

[z] Mean separation by Duncan's Multiple Range Test, $\alpha = 0.05$.

Table 2. Effect of rootstock on the number and percentage of mature[y] nodes during acclimation. 1987. Horticulture Research Center, East Lansing, MI.

	Sample date									
	3 Sept.		17 Sept.		1 Oct.		15 Oct.		29 Oct.	
Rootstock	Number of mature nodes	% of mature nodes	Number of mature nodes	% of mature nodes	Number of mature nodes	% of mature nodes	Number of mature nodes	% of mature nodes	Number of mature nodes	% of mature nodes
Own	0.2b[z]	0.7b	9.1ab	31.8ab	16.7	61.1b	19.3b	67.0a	18.7	60.4bc
Seyval	4.8a	15.4a	11.1a	39.3a	19.1	64.5a	21.8a	65.5ab	20.5	62.5ab
Cynthiana	0.0b	0.1b	6.5b	23.5b	15.4	60.4b	17.5b	66.9a	19.6	63.8ab
Riparia Gloire	0.3b	0.3b	7.1b	27.6b	16.5	61.3b	14.7c	59.2c	18.6	65.6a
St. George	0.4b	0.3b	8.1ab	25.9b	17.3	64.6a	14.9c	61.8bc	20.3	56.6c

[y] Cane maturation was determined visually according to browning of periderm.

[z] Mean separation by Duncan's Multiple Range Test, $\alpha = 0.05$.

Figure 13. Effect of rootstock on the cold hardiness (T_{50}) and water content of primary buds of Seyval grapevines during acclimation. 1987. East Lansing, MI.

T50 (°C)
(○ □ △ ◇ ✧)
-0.0
-5.0
-10.0
-15.0
-20.0
bud
Sey./Own ● ○
Sey./Sey. ■ □
Sey./Cynth. ▲ △
Sey./Rip. Gloire ◆ ◇
Sey./St. George ✦ ✧
hsd
hsd
hsd
Sept. 3
Sept. 17
Oct. 1
Oct. 15
Oct. 29
DATE 1987
Water Content (g/g dry wt.)
(● ■ ▲ ◆ ✦)
2.80
2.40
2.00
1.60
1.20
0.80

Rootstock had little effect on cold hardiness and water content of canes during acclimation in 1987 (Figure 14). Sey/Sey canes had a greater degree of cold resistance than canes from the other treatments on 3 September. Canes from Sey/StG vines had the highest water content on 15 October and 29 October. Water content of canes decreased until mid-October where a plateau of 0.75 to 0.85 g/g tissue dry wt was reached. Cane cold hardiness increased throughout the acclimation period. Increases in cold hardiness after mid-October were not related to water content. A killing frost was recorded on 12 October (Figure 17) and its occurrence seems to be closely associated with the point in the acclimation process that increasing cold hardiness is no longer related to decreasing water content. This was also observed with field-grown vines in Experiment I during the 1985 acclimation period and in a previous experiment using Concord (32).

Primary bud cold hardiness and water content were influenced by rootstock primarily late in the 1988 deacclimation period (Figure 15). On 31 March, Sey/own buds had a lower water content than buds from the other treatments and Sey/Cyn buds were hardier than Sey/StG buds. Water content of Sey/own and Sey/Sey buds was significantly lower than that of Sey/StG on 14 April. Primary buds exhibited increased water content and decreased cold hardiness during the deacclimation period. Large increases in water content of primary buds were seen between 31 March and 14 April.

Significant rootstock effects on cane water content and cold hardiness were perceived during deacclimation in 1988 (Figure 16). Sey/StG canes deacclimated earlier than canes from other treatments. Although the differences were not always statistically significant,

Figure 14. Effect of rootstock on the cold hardiness (T_{50}) and water content of canes of Seyval grapevines during acclimation. 1987. East Lansing, MI.

cane
Sey./Own ● ○
Sey./Sey. ■ □
Sey./Cynth. ▲ △
Sey./Rip. Glore ◆ ◇
Sey./St. George ✦ ✧
hsd
hsd
hsd
T50 (°C)
(○ □ △ ◇ ✧)
-0.0
-5.0
-10.0
-15.0
-20.0
Water Content (g/g dry wt.)
(● ■ ▲ ◆ ✦)
2.20
1.80
1.40
1.00
0.60
Sept. 3
Sept. 17
Oct. 1
Oct. 15
Oct. 29
DATE 1987

Figure 15. Effect of rootstock on the cold hardiness (T_{50}) and water content on primary buds of Seyval grapevines during deacclimation. 1988. East Lansing, MI.

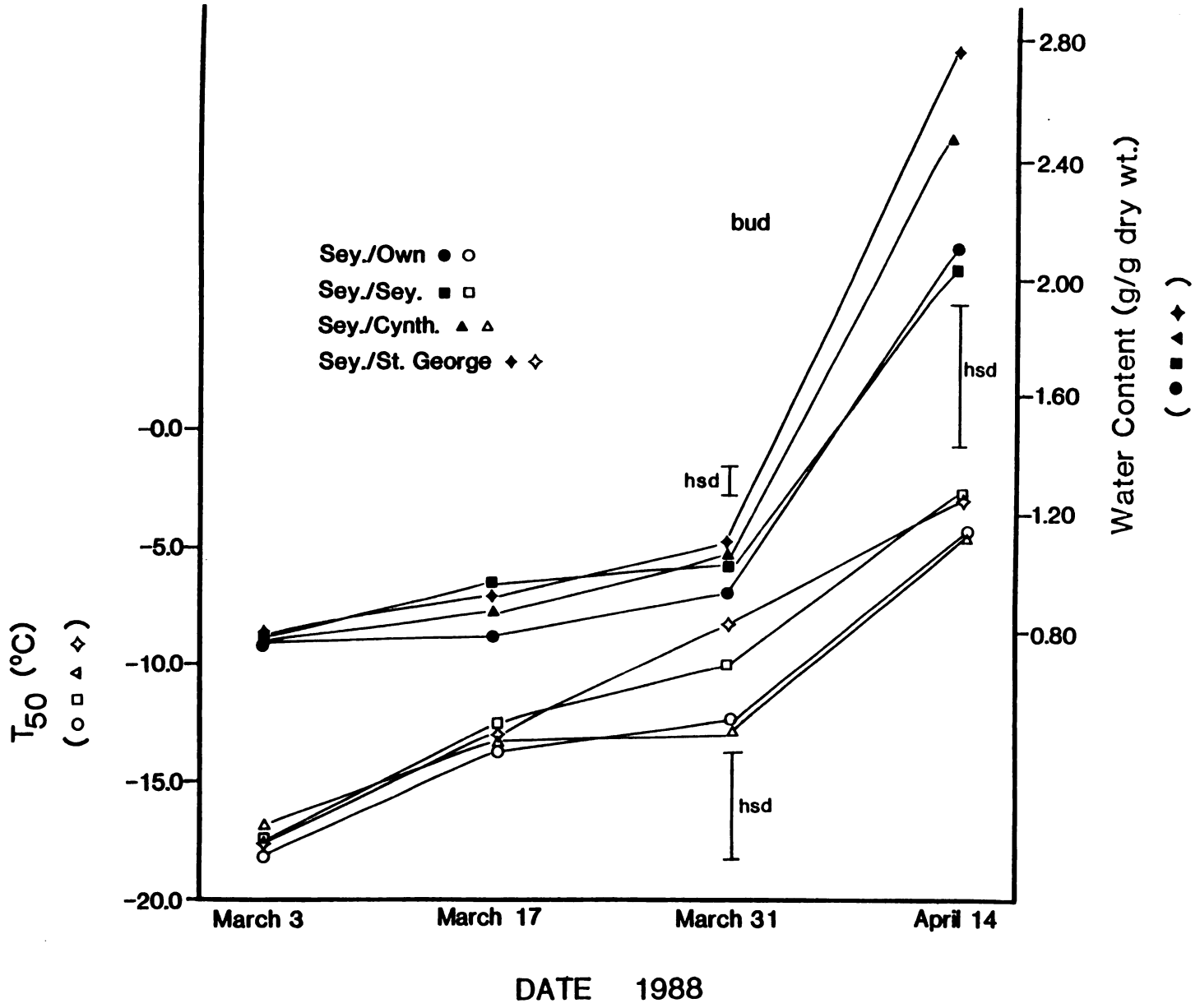

bud
Sey./Own ● ○
Sey./Sey. ■ □
Sey./Cynth. ▲ △
Sey./St. George ◆ ✧
hsd
hsd
hsd
-0.0
-5.0
-10.0
-15.0
-20.0
2.80
2.40
2.00
1.60
1.20
0.80
March 3
March 17
March 31
April 14
T50 (°C)
(○ □ ▲ ✧)
Water Content (g/g dry wt.)
(● ■ ▲ ◆)
DATE 1988

Figure 16. Effect of rootstock on the cold hardiness (T_{50}) and water content of canes of Seyval grapevines during deacclimation. 1988. East Lansing, MI.

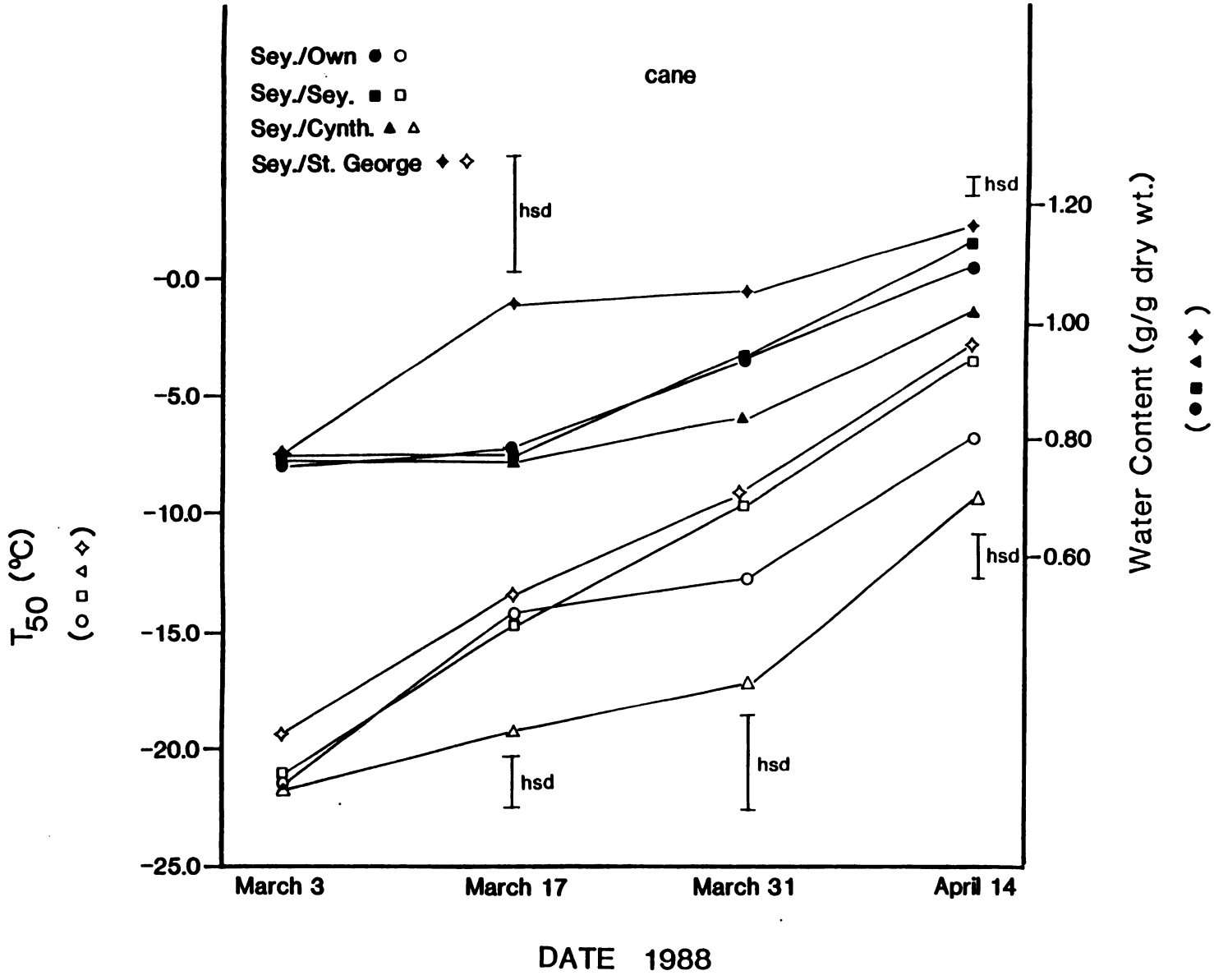
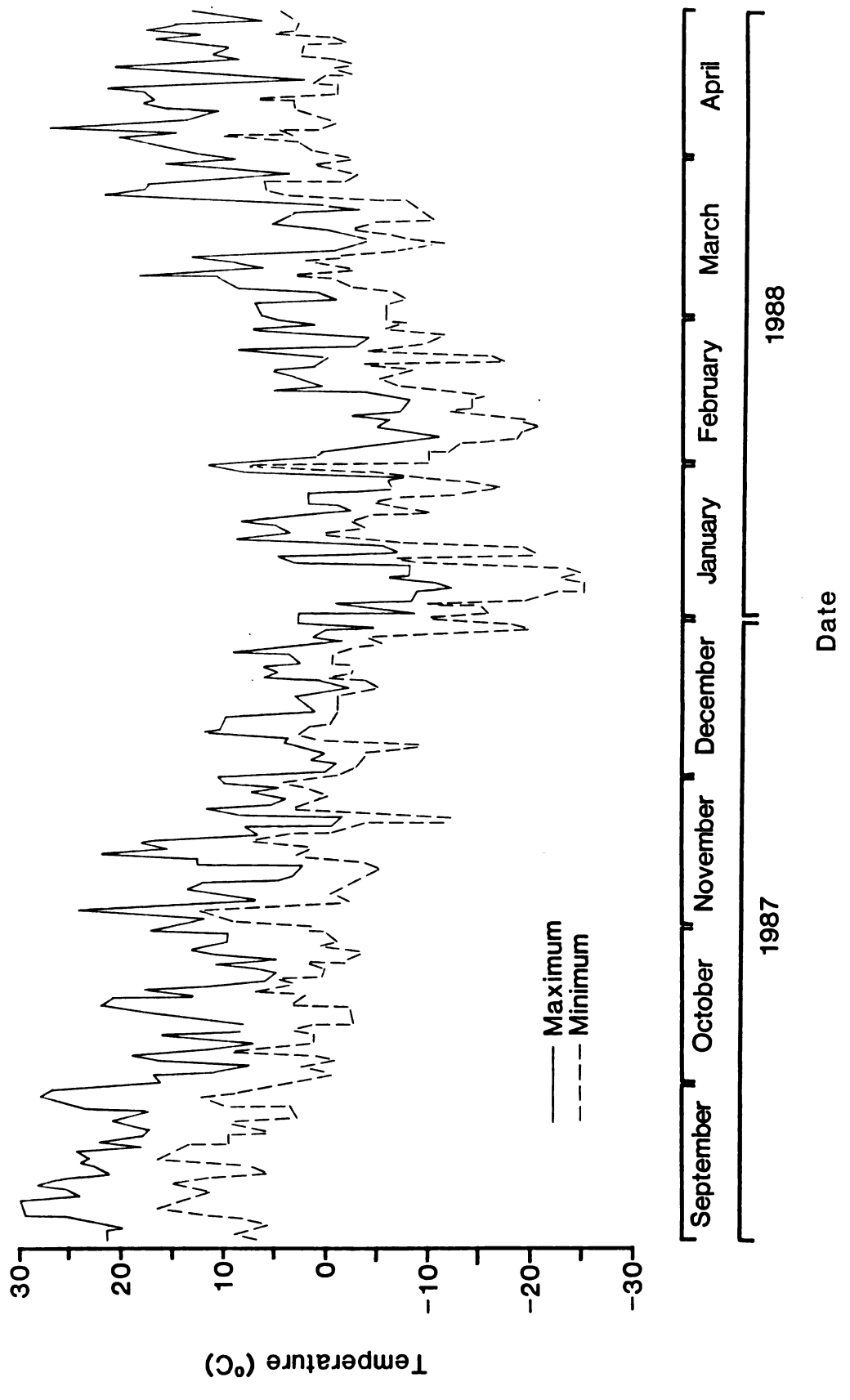

Sey./Own ● ○
Sey./Sey. ■ □
Sey./Cynth. ▲ △
Sey./St. George ◆ ◇
cane
hsd
hsd
hsd
hsd
hsd
-0.0
-5.0
-10.0
-15.0
-20.0
-25.0
1.20
1.00
0.80
0.60
March 3
March 17
March 31
April 14
T_{50} (°C)
(○ □ ▲ ◇)
Water Content (g/g dry wt.)
(● ■ ▲ ◆)
DATE 1988

Figure 17. Maximum and minimum air temperatures (^{o}C) during acclimation, mid-winter, and deacclimation. 1987-88. East Lansing, MI.


Temperature (°C)
30
20
10
0
-10
-20
-30
Maximum
Minimum
September
October
November
December
January
February
March
April
1987
1988
Date

Sey/StG canes had the highest water content and lowest cold resistance throughout the deacclimation period. In addition, Sey/Cyn canes were delayed in their deaccliamtion response when compared to Sey/StG canes. Canes from Sey/Cyn vines had the lowest water content and highest cold resistance during deacclimation. Sey/Cyn canes were 8.5°C hardier than Sey/StG canes on 31 March. The general trend observed was that canes increased in water content and decreased in cold hardiness as deacclimation proceeded.

Sey/Cyn vines had significantly fewer shootless nodes than vines from the other treatments in 1988 (Table 3). Air temperatures for the 1987-88 dormant season are given in Figure 17.

DISCUSSION

Rootstock had limited impact on cold hardiness or water content during acclimation. Couderc 3309, which has been shown to increase scion cold hardiness of certain *V. vinifera* L. cultivars during mid-winter period (19,21), did not consistently improve Seyval cold resistance during acclimation. This may indicate that rootstock factors which are important for scion cold hardiness in mid-winter are not as important during acclimation. Alternatively, the lack of effect by Couderc 3309 may be related to scion characteristics. Seyval is more cold hardy than White Riesling. The mechanism of rootstock-induced cold hardiness increase in mid-winter may have been operating during acclimation but was not observed due to a masking effect of the innate hardiness level of Seyval. The relationship between treatment cold hardiness, exposure to freezing temperatures, and expression of cold injury has been discussed by Howell (12). In this instance, temperatures during the acclimation

Table 3. Effect of rootstock on the percentage of shootless nodes of Seyval grapevines. Horticulture Research Center. 1988.

Rootstock	Shootless nodes (%)
Own	55.9ab[z]
Seyval	46.3b
Cynthiana	22.2c
St. George	66.8a

[z] Mean separation by Duncan's Multiple Range Test, $\alpha = 0.05$.

period may not have been severe enough to allow expression of rootstock effects on cold hardiness.

Rootstock had a small effect on cane maturation during acclimation in 1987. Sey/Sey canes had a greater percentage of mature nodes early in the acclimation period. Although a rootstock effect was noted, this result does not support the idea that rootstocks with a short vegetative cycle (early acclimation) positively influence scion cane maturation and cold hardiness since Sey/RGl canes did not exhibit accelerated cane maturation or increased cold hardiness. Riparia Gloire vines are reported to have the shortest vegetative growth cycle among the rootstocks used in this study (20). Similar results were obtained by Basler using well-exposed shoots on grafted vines in the field (2). He found no differences in cane maturation due to rootstock even though several of the rootstocks he used were reported to impart early or late cane maturation. These findings suggest that the observed differences in cane maturation among various Vitis species (and consequently rootstocks) may result from factors originating in the shoot system rather than in the root system. Thus, the observation that endogenous cytokinin levels in V. riparia Michx. root exudate decline with decreasing photoperiod is significant for acclimation of own-rooted vines but may not be important for acclimation of vines grafted to V. riparia Michx. (9).

Generally, water content decreased and cold hardiness increased during acclimation. There was one notable exception to this pattern. Increases in cane cold hardiness were not accompanied by further decreases in water content of canes following a killing frost in 1985 and 1987. Apparently, there is a change in the cold

acclimation process of grapevines which is closely associated with the first killing frost. These data and those of Wolpert and Howell (32) suggest that grapevine cold acclimation is characterized by two distinct stages. During the first stage of grapevine cold acclimation, cold hardiness increases are closely associated with advancing tissue maturity and declining tissue water content. The second stage of acclimation begins after the first killing frost and increases in cold hardiness seem to be related to decreasing air temperature. A two stage model of woody plant acclimation has previously been proposed by Weiser (28).

Rootstock effects were evident during the deacclimation period, especially in 1988. Sey/3309 and Sey/5BB canes were generally more cold resistant than canes from other treatments during 1987. The differences were not always statistically significant, their magnitude was small, and they were inconsistent between seasons. The present data are insufficient to judge whether the observed increase in cold hardiness by Couderc 3309 and Kober 5BB was real or an artifact. Further study is needed to determine if these rootstocks can consistently increase scion cold hardiness during acclimation of Seyval grapevines.

Consistent rootstock effects on cold hardiness and water content were observed during deacclimation when rootstocks which differ widely in relative timing of bud burst were evaluated. Primary bud cold hardiness was affected less by rootstock than was cane cold hardiness. However, Sey/Cyn buds were hardier than Sey/StG buds on the last two sample dates. Examination of data for canes in 1988 revealed that Sey/Cyn canes were lower in water content and higher in cold hardiness than Sey/StG canes during the

entire deacclimation period. Cumulative injury was also reduced by rootstock as Sey/Cyn vines had significantly lower percentage of shootless nodes than Sey/StG vines.

The slower rate of deacclimation observed for Sey/Cyn canes may be due to the ability of this treatment to resist deacclimation during exposure to warm temperatures or to reharden upon exposure to low temperatures once deacclimation has begun. Both of these mechanisms have been observed for grapevine buds (6). Further research on the involvement of roots in the deacclimation process is necessary before the observed delay in deacclimation by Cynthiana rootstock can be fully explained.

In general, water content increased and cold hardiness decreased during deacclimation. Large increases in water content of primary buds were often observed late in the deacclimation period. Buds were at the scale crack stage of development by the final sample dates in 1986 and 1987. Water content at scale crack reached levels of 1.80 to 3.17 g/g tissue dry wt.

CONCLUSIONS

Choice of rootstock had little effect on cold hardiness or water content during acclimation. A different situation existed during deacclimation. Sey/Cyn canes had a lower water content and higher level of cold hardiness than the other treatments throughout the deaccliamtion period. Sey/Cyn primary buds responded in a similar manner but to a lesser degree. The following proposals are consistent with the data collected in this study: (1) rootstocks with early bud burst deacclimate sooner than rootstocks with late bud burst; (2) the root system appears to be equally important as

above-ground tissues in determining the rate of deacclimation; and (3) under certain circumstances, rootstocks are able to transmit differences in the rate of deacclimation to scion tissues.

Significant rootstock effects were observed for cumulative cold injury as indicated by percent shootless nodes data. Grafted vines had lower percentage of shootless nodes than own-rooted vines in 1986. Percentage shootless nodes was significantly lower for Sey/Cyn vines when compared with Sey/own, Sey/Sey, or Sey/StG vines in 1988.

Primary buds and canes increased in cold hardiness and decreased in water content during acclimation. One significant exception was noted. Increases in cold hardiness after the occurrence of a killing frost were not accompanied by further decreases in water content. This suggests that grapevine cold acclimation occurs in two stages: (1) cold hardiness increases in the first stage were closely associated with advancing tissue maturity and declining water content of tissues; and (2) the second stage of acclimation began after the first killing frost and increases in cold hardiness were no longer related to tissue maturation and water content.

Cold hardiness decreased and water content increased in primary buds and canes during deacclimation. Primary buds showed large increases in water content during deacclimation with levels reaching 1.80 to 3.17 g/g tissue dry wt at the scale crack stage of bud development. More research on grapevine cold acclimation and deacclimation is needed before we can significantly increase our understanding of the complex interactions occurring between rootstock and scion during these periods.

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II. PRIMARY AND SECONDARY EFFECTS OF ROOTSTOCK ON COLD HARDINESS OF SEYVAL GRAPEVINES

ABSTRACT

The effect of rootstock and vine size on cold hardiness of Seyval grapevines were determined separately. Vine size effects were considered to be an indication of potential secondary rootstock effects since vine size modification is an important primary rootstock effect. Own-rooted Seyval (Sey/own) and Seyval grafted to Seyval (Sey/Sey), Kober 5BB (Sey/5BB), and Couderc 3309 (Sey/3309) were the rootstock treatments used in this study. Large, medium, and small vine size classes were established within each rootstock treatment.

Rootstock effects on cold hardiness were determined by measurement of cumulative injury to buds as percentage of shootless nodes and the within-vine distribution of canes with characteristics associated with increased cold hardiness (medium diameter and well-exposed to sunlight during the growing season). Both primary and secondary effects of rootstock on cold hardiness were observed. Sey/3309 vines had the lowest percentage of shootless nodes among the rootstock treatments. Vine size did not significantly influence the percentage shootless nodes when canes of comparable quality were evaluated. Rootstock did not significantly affect the within-vine distribution of canes. Large vines had a greater number of poorly matured canes and canes with superior cold resistance. Large vines do not appear to be inferior to small vines in cold hardiness if careful cane selection is practiced at pruning.

INTRODUCTION

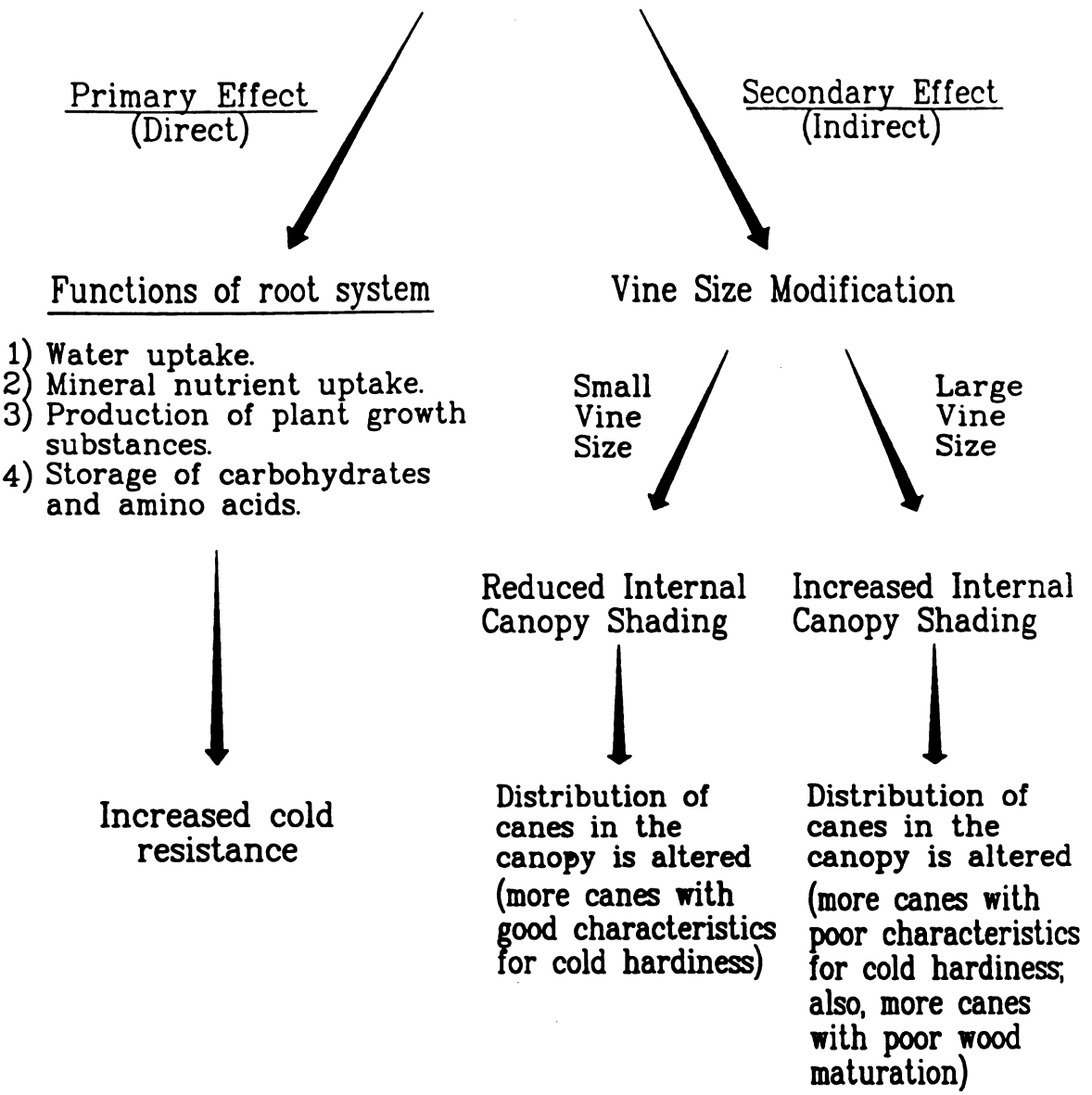
Rootstock-scion relationships in the grapevine are complex. The performance of a rootstock-scion combination represents the sum of an additive rootstock contribution, an additive scion contribution, and a nonadditive contribution of the rootstock x scion interaction (3,5,11). This situation creates considerable difficulty for the viticulture researcher who endeavors to measure rootstock contributions to scion characteristics. Primary rootstock effects must be separated from secondary rootstock effects so that data can be accurately interpreted.

The major functions of the grapevine root system are vine water relations, uptake and translocation of nutrients, synthesis and metabolism of plant growth substances, and storage of carbohydrates (10). Primary rootstock effects are likely mediated through one or a combination of these functions. Grapevine rootstocks have a primary effect on vine size (kg cane prunings/vine) (2,3,7,8). Increases in vine size when canopy length is fixed result in crowding of shoots and internal canopy shading (17). The negative consequences of internal canopy shading on yield, fruit quality, and wine quality are well-documented (12-15,17,18). Most secondary effects of rootstock are mediated through rootstock influences on vine size and internal canopy shading. Possible mechanisms of rootstock involvement in cold hardiness of grapevine primary buds and canes are outlined in Figure 1.

Within-vine variation in cold hardiness is considerable (4,16,20,21). The importance of this factor for vine cold resistance was first recognized by Shaulis (16). Most of the within-vine variation in cold hardiness which is observed is due to

Figure 1. Potential mechanisms of rootstock involvement in cold hardiness of grapevine primary buds and canes.

Rootstock


Primary Effect
(Direct)
Secondary Effect
(Indirect)
Functions of root system
1) Water uptake.
2) Mineral nutrient uptake.
3) Production of plant growth substances.
4) Storage of carbohydrates and amino acids.
Increased cold resistance
Vine Size Modification
Small Vine Size
Large Vine Size
Reduced Internal Canopy Shading
Increased Internal Canopy Shading
Distribution of canes in the canopy is altered (more canes with good characteristics for cold hardiness)
Distribution of canes in the canopy is altered (more canes with poor characteristics for cold hardiness; also, more canes with poor wood maturation)

internal canopy shading. Differences in cold hardiness of primary buds and canes varied by up to 12°C depending on the presence of periderm, periderm color, cane diameter, persistent-lateral status, and leaf exposure to sunlight during the growing season (4). Cold hardiness was increased by exposure to sunlight during the growing season, dark-colored periderm, medium cane diameter, and lack of persistent lateral canes. The importance of exposure status and tissue maturation, as indicated by periderm status and color, for maximum cold resistance of tissues has been confirmed in recent studies (20,21).

This information allows for the development of rational sampling procedures for grapevine cold hardiness studies. Current knowledge dictates that canes which are sampled should have similar diameter, sunlight exposure status, persistent lateral status, and cropping stress. Howell (3) used these criteria to sample comparable canes from 1-year-old potted vines, 2-year-old nonbearing vines, 15-year-old mature bearing vines and 25-year-old abandoned vines of the same cultivar at the same location on the same date and found no hardiness difference. This provides strong evidence that non-treatment variation can be reduced substantially by using a critical sampling procedure. The detection of primary rootstock effects on cold hardiness of scion tissues depends on the use of critical sampling.

Grapevine rootstocks have been shown to have a primary effect on scion cold hardiness (6,Striegler and Howell,unpublished data, 1988,see Chapter I). Cold hardiness of canes was increased by rootstock during mid-winter and deacclimation. Effects on bud cold hardiness of canes were also noted but usually only when cumulative

injury (percentage of shootless nodes) was examined.

Exposure to sunlight during the growing season and cane maturation, two factors which are associated with increased primary bud and cane cold hardiness, are not uniformly distributed in most grapevine canopies. Exterior canes had 9 to 14 mature nodes while canes from the canopy interior had 0 to 2 mature nodes (16). Thus, treatments which influence internal canopy shading can alter the distribution of canes which possess characteristics of maximum cold resistance and thereby affect vine cold hardiness. Vine size increases while canopy space is fixed provide a mechanism for secondary rootstock effects through alteration of the within-vine distribution of canes with maximum potential for cold hardiness. Chardonnay vines grafted on Couderc 3309 (large vine size) had a greater number of canes with 0 to 3 and more than 10 mature nodes than Chardonnay grafted on Elvira (small vine size) (20). Apparently, factors other than periderm status which are associated with increased cold hardiness were not considered when within-vine cane distribution was measured. Also, this is not conclusive evidence of a secondary rootstock effect since rootstock and vine size effects were confounded. Determination of separate rootstock and vine size effects would allow us to increase our understanding of primary and secondary rootstock effects on cold hardiness. As pointed out by Howell (3), this matter is of considerable practical importance. If primary effects of rootstock are noted, genetic improvement can be undertaken to modify the characteristic of interest. On the other hand, if secondary effects are noted, the question becomes one of cultural management and not rootstock.

Therefore, the purpose of this experiment was to separately determine rootstock primary and secondary effects on vine cold hardiness. Canopy development, productivity, and fruit quality were also determined due to their interrelationship with cold hardiness.

MATERIALS AND METHODS

This experiment was conducted in a grafted Seyval vineyard at the Clarksville Horticulture Experiment Station, Clarksville, MI. Rootstock treatments included own-rooted Seyval (Sey/own) and Seyval grafted to Seyval (Sey/Sey), Kober 5BB (Vitis berlandieri Planchon x Vitis riparia Michaux) (Sey/5BB), and Couderc 3309 (Vitis riparia Michaux x Vitis rupestris Scheele) (Sey/3309).

Vines were planted in 1983 in a uniform Kalamazoo sandy loam soil. Vineyard spacing was 2.4 m x 3.0 m (within row x between row) and row orientation was north to south. The training system employed was Hudson River Umbrella with fruiting wood retained as five-node canes. Vines were pruned to a 10 + 10 pruning severity (10 nodes retained/0.45 kg of cane prunings) on 18-19 April 1986. An upper limit of 50 nodes retained/vine was set to avoid overcropping.

Vines of small (0.45-0.91 kg cane prunings/vine), medium (1.14-1.59 kg cane prunings/vine), and large (1.82-2.27 kg cane prunings/vine) vine size were identified within each rootstock treatment after pruning. Six single vine replicates were randomly selected within each vine size class.

All vines were flower-cluster-thinned to one cluster per shoot with the basal cluster on each shoot being retained. Developing shoots were counted on 13 June 1986 when vines were at full bloom.

Length of canopy per vine was measured after canopy development was complete on 13 September 1986. These data were used to calculate the percentage of occupation by canopy of trellis space. This information was deemed important because failure to fill the allotted trellis space has been a problem for own-rooted Seyval vines in Michigan.

Individual vine yield and the number of clusters per vine were determined on 17 September 1986. Prior to harvest, samples of five apical berries from 20 randomly selected clusters were taken to give a 100 berry sample for each replicate. Berry samples were transported to the Viticulture and Enology Laboratory in the Department of Horticulture where they were weighed and then stored at 1°C for later analysis. Sample analysis was completed within two days of sampling.

At the time of analysis, berries were crushed in a mortar and pestle and the juice strained through two layers of cheesecloth. Soluble solids were measured using a Bausch and Lomb Abbe 3-L refractometer. A 5 ml aliquot of juice was diluted to 100 ml with deionized H_2O and then titrated to pH 8.2 with 0.1N NaOH to determine acidity (1). Acidity was expressed as grams of tartaric acid per 100 ml of juice. The pH of juice was measured using a Fisher Accumet (model 620) pH meter.

In late November, canes on the vines were rated according to the extent of maturation, diameter, and exposure to sunlight during the growing season. These characteristics were chosen because they have been associated with increased cold resistance (4). Our primary interest was to determine the influence of rootstock and vine size on the within-vine distribution of canes with superior

cold resistance. Nodes one through five (base ---> apex) were rated since this was the bearing unit retained at pruning. All ratings were visual and subjective. The categories used were: (1) having 5 mature nodes, medium diameter (7-10 mm), and well-exposed to sunlight during the growing season; (2) all other canes having five mature nodes; and (3) canes with fewer than five mature nodes. Cane diameter was measured between nodes four and five. Persistent lateral status was not considered due to the high percentage of canes having persistent laterals at nodes one through five. Persons collecting data were provided with a well-exposed cane piece of medium diameter as a reference. The data are presented on a per vine and percentage basis. This was done so that methods of presenting this type of data could be compared.

Shootless nodes were counted after budburst. Shoots were allowed to grow approximately 15 cm before measurement. Data were analyzed as a 4 x 3 factorial with rootstock and vine size class serving as factors. Data were subjected to analysis of variance and mean separation was done by Duncan's new multiple range test. The arcsin transformation was performed on percentage data prior to analysis of variance (19).

RESULTS

Rootstock had little effect on growth and canopy development (Table 1). Sey/3309 vines were able to occupy more of their allotted canopy space than Sey/own, Sey/Sey, or Sey/5BB vines. Vine size had a greater effect on growth and canopy development than rootstock. Nodes retained/vine, shoots/vine, shoot density, and percentage of occupation of trellis space were directly related to

Table 1. Effect of rootstock and vine size on growth and canopy development of Seyval grapevines. 1986. Clarksville, MI.

Treatment	Vine size (kg/vine)	Nodes retained/ vine	Shoots/ vine	Shoot density (shoots m^{-1} of canopy)	Occupation of trellis space (%)
Rootstock					
Own	1.35	30	40	24	68b[z]
Seyval	1.32	28	38	25	65b
Kober 5BB	1.41	31	44	27	67b
Couderc 3309	1.43	31	43	22	79a
	n.s.	n.s.	n.s.	n.s.	
Vine Size (kg/vine)					
0.45-0.91	0.77c	17c	29c	20c	60b
1.14-1.59	1.39b	30b	40b	23b	74a
1.82-2.27	1.98a	43a	55a	30a	76a

[z]Mean separation by Duncan's Multiple Range Test, $\alpha = 0.05$.

vine size.

Productivity was only slightly affected by rootstock (Table 2). Sey/5BB vines had the highest yield and the lowest berry weight. Clusters from Sey/own vines were larger than clusters from vines of the other rootstocks. Vine size had a somewhat greater impact on productivity than rootstock. Yield and the number of cluster per vine increased with increasing vine size. Small vines had slightly larger berries than vines in the medium or large vine size classes. Fruitfulness was not significantly reduced by large vine size.

Rootstock and vine size effects on fruit quality were limited to soluble solids (Table 3). The differences observed were inversely related to yield. Rootstock did not affect the distribution of canes within the vine in relation to cold resistance (Table 4). The effect of vine size on the within vine distribution of canes in relation to cold resistance varied according to the manner in which the data were reported. Increases in vine size resulted in a greater number of total canes, canes with superior cold resistance, and cane with less than five mature nodes when reported on a per vine basis. In contrast, presentation of the data as a percentage of total canes indicated that increasing vine size decreased the percentage of canes with superior cold resistance and canes with inferior cold resistance. The percentage of canes with less than five mature nodes increased with increasing vine size.

Rootstock effects were present in the percentage of shootless nodes data (Table 5). Sey/own vines had the highest and Sey/3309 vines the lowest percentage of shootless nodes. Vine size did not significantly affect the percentage of shootless nodes when comparable canes were evaluated.

Table 2. Effect of rootstock and vine size on productivity of Seyval grapevines. 1986. Clarksville, MI.

Treatment	Yield (MT/ha)	Clusters/ vine	Berries/ cluster	Berry weight (g)	Cluster weight (g)	Fruitfulness (kg fruit/ retained node)
Rootstock						
Own	17.8ab[z]	37	199	1.89	376.1a	0.46
Seyval	15.0b	35	178	1.86	329.9b	0.40
Kober 5BB	19.0a	46	195	1.68	325.1b	0.47
Couderc 3309	15.8b	39	179	1.80	320.9b	0.41
		n.s.	n.s.	n.s.		n.s.
Vine Size (kg/vine)						
0.45 - 0.91	10.4c	23c	189	1.87a	350.8	0.46
1.14 - 1.59	17.0b	41b	186	1.77b	328.0	0.43
1.82 - 2.27	23.3a	54a	189	1.78b	335.2	0.41
			n.s.		n.s.	n.s.

[z] Mean separation by Duncan's Multiple Range Test, = 0.05.

Table 3. Effect of rootstock and vine size on fruit quality of Seyval grapevines. 1986. Clarksville, MI.

Treatment	Soluble Solids (%)	Titratable Acidity (g/100 ml)	pH
Rootstock			
Own	19.2a[z]	1.06	3.15
Seyval	19.0ab	1.05	3.14
Kober 5BB	18.2b	1.08	3.16
Couderc 3309	19.4a	1.07	3.19
		n.s.	n.s.
Vine size (kg/vine)			
0.45 - 0.91	20.2a	1.09	3.16
1.14 - 1.59	19.0b	1.05	3.17
1.82 - 2.27	17.6c	1.06	3.16
		n.s.	n.s.

[z] Mean separation by Duncan's Multiple Range Test, = 0.05.

Table 4. Effect of rootstock and vine size on the within vine distribution of canes in relation to cold resistance. 1986. Clarksville, MI.

Treatment	Superior cold resistance[z] (canes/ vine)	(%)	Inferior cold resistance[y] (canes/ vine)	(%)	Less than 5 mature nodes (canes/ vine)	(%)	Total canes/ vine
Rootstock							
Own	10	29	8	23	17	49	35
Seyval	10	32	8	26	13	42	31
Kober 5BB	9	24	8	22	20	54	37
Couderc 3309	9	25	9	25	18	50	36
	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Vine Size (kg/vine)							
0.45 - 0.91	8b[x]	36a	7	32a	7c	32c	22c
1.14 - 1.59	9ab	27b	9	27b	15b	45b	33b
1.82 - 2.27	11a	23c	8	17c	29a	60a	48a
			n.s.				

[z] Cane characteristics = 7-10 mm in diameter and well-exposed to sunlight during the growing season.

[y] Cane characteristics = canes having 5 or more mature nodes which were not 7-10 mm in diameter and/or well-exposed to sunlight during the growing season.

[x] Mean separation by Duncan's Multiple Range Test, = 0.05.

Table 5. Effect of rootstock and vine size on the percentage of shootless nodes. 1987. Clarksville, MI.[z]

Treatment	Shootless Nodes[y] (%)
Rootstock	
Own	58a[x]
Seyval	31b
Kober 5BB	15bc
Couderc 3309	9c
Vine Size (kg/vine)	
0.45 - 0.91	28
1.14 - 1.59	25
1.82 - 2.27	28
	n.s.

[z] Measurements were made on canes which were retained at pruning. Canes were 7-10 mm in diameter and had been well-exposed to sunlight during the growing season.

[y] Arcsin transformation was performed before AOV. Means represent detransformed data.

[x] Mean separation by Duncan's Multiple Range Test, = 0.05.

DISCUSSION

The number of shoots per vine increased with increasing vine size. This, coupled with a fixed canopy space allotted to each vine, resulted in an increase in shoot density as vine size increased. Increases in shoot density result in greater leaf area per unit row length and shade within the canopy (12,17). Seyval vines with 6 shoots per 30 cm of row had greater internal canopy shading than vines with 2 or 4 shoots per 30 cm of row (9). Although occupation of trellis space increased with increasing vine size, it is doubtful that this is a practical method of solving the problem due to the greater internal canopy shading that would occur with increased vine size at a fixed canopy space. Better training of cordons and medium vine size would likely yield a canopy with the desired characteristics.

The relationship between vine size and yield is not surprising in that node number per vine is based on vine size. A greater number of nodes per vine results in increased numbers of shoots and clusters. Cluster number and yield are directly related for thinned vines such as in this study. Sey/5BB vines had the highest yield among rootstock treatments. The data are insufficient to declare this as a primary rootstock effect since Sey/own vines also displayed increased yield. However, synthesis and metabolism of cytokinins by grapevine roots and the involvement of cytokinins in the floral development and fruit set provide a possible avenue for primary rootstock effects on scion yield (10). Rootstock and vine size differences in soluble solids were related to yield. Competition between "sinks" for photosynthate and the resulting

reduction in fruit or vegetative maturity are well-documented in the grapevine (22).

The lack of effect of rootstock and the considerable effect of vine size on the within-vine distribution of canes with superior cold resistance indicates that rootstock influences on vine cold hardiness through this mechanism were of a secondary nature. The problem then is not one of rootstock but of cultural management. Management of vine size to increase vine cold hardiness is a subject of considerable interest to growers.

It is commonly accepted that small vines are superior in cold hardiness to large vines due to their reduced internal canopy shading. Our data do not support this view. Large vines had a greater number of poorly matured canes but also had more canes with superior cold resistance. More importantly, large vines had a sufficient number of canes with superior cold resistance to meet the requirements of the training system and pruning severity used in this study. This suggests that with careful cane selection during pruning, large vines would not be inferior to small vines in cold hardiness.

Presentation of cane distribution data on a per vine basis was more appropriate than on a percentage basis since percentage data did not always denote viticulturally significant differences. As an example, the fact that small vines had a higher percentage of canes with superior cold resistance than large vines is not viticulturally important as long as large vines had sufficient canes with superior cold resistance to meet the requirements of the training system and pruning severity. It appears that reporting within vine cane distribution data on a percentage basis can result in inaccurate

interpretation of the data.

The shootless node data provide further evidence against the concept of small vines having a greater degree of cold resistance than large vines. Vine size did not affect the percentage of shootless nodes when comparable canes (medium diameter, well-exposed canes retained at pruning) were evaluated. Primary rootstock effects were observed among the rootstock treatments. Sey/3309 vines had significantly lower percentage of shootless nodes then Sey/own or Sey/Sey vines.

CONCLUSIONS

Vine size generally had a greater impact on the parameters measured than did rootstock. Vine size was directly related to the number of shoots/vine, shoot density, percentage of the trellis space that was occupied, number of clusters/vine and yield/vine and was inversely related to berry weight and % soluble solids. The higher shoot density of large vines as compared with small vines would result in a greater degree of internal canopy shading in large vines (12,17).

Evidence of primary and potential secondary rootstock effects on vine cold hardiness were observed. The within-vine distribution of canes with superior cold resistance was altered by vine size but not rootstock. This indicates that rootstock effects on cold hardiness by this mechanism (vine size modification) are possible and would be of a secondary nature. Our data do not support the commonly held view that small vines are superior in cold hardiness to large vines. Large vines had a greater number of canes with superior cold resistance than did small vines. The number of canes

with superior cold hardiness on large vines was sufficient to meet the requirements of the pruning severity used in this study. When canes with superior cold hardiness were retained at pruning, vine size had no effect on percent shootless nodes.

Vines grafted to Couderc 3309 had the lowest percentage of shootless nodes indicating a primary effect on scion cold hardiness. Further study is needed to determine the seasonal variation in the within-vine distribution of canes. Assessment of the within-vine distribution of canes with superior cold hardiness appears to be a useful method of determining secondary treatment effects on vine cold hardiness. This aspect of vine cold hardiness should receive greater attention by viticulture researchers.

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SECTION II

INFLUENCE OF ROOTSTOCK ON THE RESPONSE OF SEYVAL GRAPEVINES TO FLOODING STRESS

EFFECT OF ROOTSTOCK ON THE GROWTH AND PHYSIOLOGY OF SEYVAL GRAPEVINES DURING FLOODING

ABSTRACT

Own-rooted St. George, Couderc 3309, Riparia Gloire, Kober 5BB, Seyval, and Cynthiana vines were subjected to soil flooding under greenhouse conditions. The rate of shoot elongation (RSE), net photosynthesis (P_n), stomatal conductance (g_s), transpiration (Tr), and water use efficiency (WUE) were measured at one to four day intervals as an estimate of sensitivity to flooded conditions. In general, RSE was the most sensitive and WUE the least sensitive parameter to flooding. St. George, Couderc 3309, and Riparia Gloire were the most tolerant cultivars, while Kober 5BB, Seyval, and Cynthiana were the most susceptible cultivars to flooding.

Symptoms of flooding were desiccation of the shoot apex, flagging of leaves, necrotic areas on leaves, senescence of basal leaves and regeneration of roots near the water surface. Oxygen diffusion rate (ODR) values which have been shown to be damaging to woody plants (ODR value of less than 20 g O_2 x 10^{-8} cm^{-2} min^{-1}) were attained within three hours of flooding.

The effect of rootstock on flooding tolerance of a susceptible scion was measured during an 8 day flooding period. Treatments were own-rooted Seyval and Seyval grafted on Seyval, Couderc 3309, and St. George. Flooding tolerance of Seyval was increased slightly by grafting onto Couderc 3309.

INTRODUCTION

Soil drainage is an important aspect of site selection for fruit crops (8,22,37,39). Soils with poor internal drainage or a

high ground water level are generally unsuitable for fruit production because they are periodically flooded (11,17,20-22, 33,34). Vineyard soils in the Great Lakes region of the eastern USA are commonly of glacial origin and can display considerable heterogeneity (34,38). Well-drained sandy loam soils are often found in close association with poorly drained clay loam soils.

The performance of Seyval grapevines growing on two distinct soil types was monitored during a five year irrigation study (6,Douglas Welsch,personal communication,1988). Soil type had a greater influence on vine performance than irrigation treatment. Vines grown on Kalamazoo loam soil (somewhat poorly-drained) had lower vine size, yield, and survival than vines grown on Oshtemo sandy loam soil (well-drained). These observations suggested that flooding might be a significant problem in poorly-drained vineyard soils in Michigan.

Flooding has significant effects on the anatomy, morphology, and physiology of roots and shoots of woody plants (4,9,10,25, 28,32). Anatomical and morphological responses to flooding include the development of adventitious roots, stem hypertrophy, hypertrophied lenticels on stems, and aerenchyma tissue (9). Flooding influences the physiology of woody plants in a number of ways. Root and shoot growth, stomatal conductance, transpiration, photosynthesis, and root hydraulic conductance are generally reduced by flooding (4,9,10). In addition, flooding alters the uptake of ions from the soil solution (9), production of plant growth substances (23), and susceptibility to disease caused by soil fungi (31).

Vegetative growth, yield, and survival of woody fruit crops are reduced by flooding (2,3,4,12,13,18,28). Flooding injury is influenced by species, time of the year in which flooding occurs, and the duration of flooding (9). There is considerable variation in flooding tolerance both between and within fruit tree species (28). Quince (Cydonia oblonga Mill.), pear (Pyrus communis L.), and apple (Malus pumila Mill.) are tolerant of flooding while apricot (Prunus armeniaca L.), peach (Prunus persica [L.] Batsch.), almond (Prunus amygdalus Batsch.), and olive (Olea europaea L.) are sensitive to flooding. Species which are intermediate in tolerance to flooding are citrus (Citrus spp.), plum (Prunus domestica L.), and cherry (Prunus avium and Prunus cerasus L.). In general, flooding of woody plants during active growth results in a greater degree of injury than flooding during dormancy or other periods when growth is minimal (4,9,18,28). Injury commonly becomes more severe as the duration of flooding increases (2,3,4,9,28). For example, the composition of woody species on floodplain soils is often directly related to the duration of flooding during the growing season (9).

There are few reports in the scientific literature concerning grapevine responses to flooding. Most information that is available comes from field observations and is subjective. Viala and Ravaz (35) summarized the findings of European viticultural scientists of the 19th century on flooding tolerance of grapevine species and certain cultivars. Vitis cinerea Engelmann, Vitis candicans Engelmann, Vitis vinifera L. x Vitis cinerea Engelmann hybrids, Rupestris du Lot, Solonis, Vitis vinifera L. x Vitis rupestris Scheele hybrids, Vitis vinifera L. x Vitis riparia Michx. hybrids,

Vitis riparia Michx. and Vitis rupestris Scheele were regarded as having tolerance to flooding. More recently, the flooding tolerance of rootstock cultivars was compiled by Pongracz (23). Rootstocks tolerant of flooding included Riparia Gloire, Richter 110, Paulson 1103, SO-4, Kober 5BB, Malegue 44-53, Couderc 3309, and Millardet 101-14. Rupestris du Lot, Richter 99, Millardet 41B, and EM 333 were reported to be sensitive to flooding. It is apparent that the subjective evaluation of flooding tolerance under field conditions is problematic when one considers the contrasting rating given Rupestris du Lot. This rootstock is rated as being tolerant of flooding by Viala and Ravaz (35) and intolerant of flooding by Pongracz (23).

Limited data are available on morphological and physiological responses of grapevines to flooding. Shoot growth of six Vitis vinifera L. cultivars was reduced by flooding (36). Furthermore, flooding of vines exposed to salinity increased the uptake of Na and Cl, increased the amount of Na and Cl transported to shoots, and resulted in damage to leaves within five days of flooding.

Freeman subjected seven Vitis vinifera L. cultivars to 40 days of flooding (5). A reduction in shoot growth was not observed until vines had been flooded for 12 days. All shoot growth ceased after 34 days of flooding. The rate of shoot extension was more sensitive to flooding than the plastochron index. Flooding reduced vine dry weight by 30 to 69%. The pattern of dry matter allocation between leaves, shoots, and roots during flooding differed among cultivars. Symptoms of flooding were basal leaf senescence and the development of adventitious roots near the water surface.

The rhizosphere of potted Delaware and Muscat of Alexandria vines was supplied with 0, 5, 10, or 20% O_2 (7). Anoxia resulted in decreased growth of roots and shoots, photosynthesis, and root respiration. In addition, concentrations of N, P, and K in leaves and N, P, K, and Mg in roots were reduced by low soil O_2 content. Roots exposed to anoxia displayed swelling of the root tip, a brownish coloration of the roots, an increase in the number of roots with broken lenticels, and a decrease in the number of lateral roots.

The problems associated with flooding of grapevines on poorly-drained soils can be surmounted by soil improvement or plant improvement (28). Installation of drainage tiles, deep cultivation of soil to shatter compacted zones, and culture of vines on raised beds can be used to improve soil conditions. The usefulness of these practices is often limited because they are difficult to implement and expensive. Furthermore, the effectiveness of soil improvement methods is reduced when soil heterogeneity exists.

Plant improvement can also be used to reduce the impact of flooding in poorly-drained vineyard soils (28). Cultivars can be selected which are tolerant of flooding or flooding tolerance can be increased by grafting susceptible cultivars on more tolerant rootstocks. Grafting has been used to successfully increase the flooding tolerance of fruit trees (2,3,12,13,15).

The possible contributions of grapevine rootstocks to scion performance during flooding have not been examined. Identification of rootstocks which could be used where soil drainage is inadequate would be beneficial. Reduction of flooding injury in poorly-drained areas of vineyards would improve productivity and increase grower

profitability. Also, it is likely that use of flooding tolerant rootstocks would provide some benefit even on well-drained soils following excessive rainfall or irrigation.

Thus, the purposes of this study are to 1) evaluate the flooding tolerance of selected grapevine cultivars under controlled conditions and 2) determine if the flooding tolerance of a susceptible scion cultivar can be influenced by grafting.

MATERIALS AND METHODS

A series of experiments was conducted during a three year period. All experiments were located in the Plant Science Greenhouses, Michigan State University, East Lansing, MI. Experiment I was conducted in May-June 1986, Experiment II was conducted in July-August 1987, and Experiment III was conducted in March-April 1988.

Experiment I

St. George (*Vitis rupestris* Scheele; synonymous with Rupestris du Lot), Couderc 3309 (*Vitis riparia* Michx. x *Vitis rupestris* Scheele), Riparia Gloire (*Vitis riparia* Michx.), Kober 5BB (*Vitis berlandieri* Planchon x *Vitis riparia* Michx.), Seyval (complex interspecific hybrid), and Cynthiana (*Vitis aestivalis* Michx.) were obtained as one-year-old rootings from a commercial nursery. These cultivars were selected based on differences in flooding tolerance as reported by Pongracz (23). St. George has a low tolerance of flooding while Couderc 3309, Riparia Gloire, and Kober 5BB have a high tolerance of flooding. The flooding tolerance of Seyval and Cynthiana was unknown. However, previous field observations suggested that Seyval is intolerant of flooding (6).

Vines were planted in 11.3 L plastic pots using a sterile medium of 50% sandy loam soil, 30% spagnum peat, and 20% sand (by volume) in late May. Potted vines were moved into a greenhouse where they grew for 27 days before treatments were imposed. During this period, vines were fertilized once with soluble 20-20-20 fertilizer. Each vine received 480 mg of N, P, and K, respectively. Water was applied based on tensiometer readings from representative pots. Two shoots were allowed to develop per vine. Bamboo stakes were placed in pots and developing shoots trained upward along a stake. Flower clusters and lateral shoots were removed as they developed.

Vines were blocked according to uniformity of growth on 18 June and treatments were randomly assigned within blocks. Healthy leaves of similar age and size were selected on each shoot and tagged. Gas exchange measurements were made on the same leaves during the course of the experiment.

Initial measurements were taken on 19 June. Measurements included the rate of shoot elongation (RSE), net photosynthesis (P_n), stomatal conductance (g_s), transpiration (Tr), and water use efficiency (WUE). Gas exchange data were collected using an ADC LCA-2 portable photosynthesis system with the broadleaf Parkinson Leaf Chamber (Analytical Development Co., Hoddesdon, United Kingdom). All gas exchange measurements were conducted between 0800 and 1200 h under conditions of light saturation. Gas exchange parameters were calculated as described by Moon and Flore (16).

Treated vines were flooded at 1600 h. The method of flooding was as follows: 18.9 L plastic pots were lined with two polyethylene bags (one inside the other); then, vines in 11.3 L pots were placed

inside the polyethylene bags which were immediately filled with water. The water level was monitored daily and maintained approximately 5 cm above the soil surface. Control vines were also placed inside 18.9 L plastic pots and exterior pots were covered with aluminum foil to maintain similar soil temperatures among treatments.

Vine responses to flooding were evaluated over a 13 day period. Shoot elongation measurements were made at 1-2 day intervals and subsequent gas exchange measurements were conducted at 2-4 day intervals. Mean maximum/minimum air temperatures during the period of flooding were 34/18^{o}C.

A randomized complete block experimental design was used. Data were analyzed by analysis of variance (30).

Experiment II

Seyval grapevines were obtained as one-year-old rootings from a commercial nursery. Vines were planted in mid July and grown for 29 days without treatment. Planting, training, and maintenance of vines were accomplished as described in Exp. I.

Vines were blocked according to uniformity of growth on 13 August and treatments were randomly assigned within blocks. Treated vines were flooded at 1830 h. Flooding was imposed in the same manner as in Exp. I. Vines were flooded for 15 days and soil oxygen diffusion rates (ODR) were measured periodically using an oxygen diffusion rate meter equipped with an $Ag^{+}/AgCl$ reference electrode. Five 25-gauge platinum electrodes were inserted approximately 10 cm into the soil midway between the rim and the center of each pot. Oxygen diffusion rate (ODR) measurements were taken after a three

minute equilibration period using an applied voltage of 0.65 V (14,31). Air temperatures were not monitored. A randomized complete block experimental design was used. Data were analyzed by analysis of variance (30).

Experiment III

Rootstock treatments used in this experiment were own-rooted Seyval and Seyval grafted to Seyval, Couderc 3309 (Vitis riparia Michx. x Vitis rupestris Scheele), and St. George (Vitis rupestris Scheele). These rootstocks were selected based on their range of flooding tolerance as determined in Exp. I.

Vines were obtained from a commercial nursery and planted in mid March. Potted vines were moved into a greenhouse where they grew for 28 days before treatments were imposed. Planting, training, and maintenance of vines were accomplished as described in Exp. I.

Vines were blocked according to uniformity of growth on 15 April and treatments were randomly assigned within blocks. Healthy leaves of similar age and size were selected on each shoot and tagged. Gas exchange measurements were made on the same leaves during the course of the experiment.

Collection of data began on 16 April. Measurements included RSE, P_n, g_s and Tr. Gas exchange measurements were conducted using an ADC LCA-2 portable photosynthesis system as described in Exp. I.

Flooding was imposed at 1300 h after initial measurements had been taken. The manner in which flooding was imposed has been illustrated in Exp. I. Vines were flooded for eight days. Data were collected at two day intervals during the period of flooding. Mean

maximum/minimum air temperatures during this time were 25/18°C.

A randomized complete block experimental design was used. Data were analyzed by analysis of variance (30).

RESULTS

Experiment I

Evaluation of control vines on 21 June 1986 indicated that Seyval and Cynthiana had lower RSE, P_n, g_s, and Tr than the other cultivars (Table 1). WUE was not significantly affected by cultivar.

St. George, Couderc 3309, and Riparia Gloire vines displayed considerable tolerance of flooding (Tables 2,3,4). RSE of St. George vines was significantly reduced after five days of flooding, but growth continued during the 13 day flooding period (Table 2). P_n, g_s, Tr, and WUE were not significantly lowered by flooding.

Couderc 3309 vines did not exhibit a significant reduction in RSE, P_n, g_s, or Tr until 13 days of flooding (Table 3). WUE was not affected by flooding. The response of Riparia Gloire vines to flooding was intermediate between the responses of St. George and Couderc 3309 (Table 4). Riparia Gloire vines demonstrated a significant reduction in RSE after five days of flooding. However, shoots on flooded vines continued to elongate during the remaining eight days of flooding. P_n, g_s, and Tr were not significantly reduced until vines had been flooded for 13 days. WUE was not affected by flooding.

Kober 5BB, Seyval, and Cynthiana vines were less tolerant of flooding than St. George, Couderc 3309, and Riparia Gloire vines (Tables 5,6,7). RSE, P_n, g_s, and Tr of Kober 5BB vines were

Table 1. Growth and physiology of selected grapevine cultivars[x]. 21 June 1988.

Cultivar	Rate of shoot elongation ($mm\ day^{-1}$)	Net photosynthesis ($mgCO_2 dm^{-2} hr^{-1}$)	Stomatal conductance ($cm\ sec^{-1}$)	Transpiration[y] ($mgH_2O cm^{-2} sec^{-1}$)	Water use[y] efficiency (moles CO_2/ moles H_2O)
St. George	28.4b[z]	25.1a	0.67a	10.8a	2.9
Couderc 3309	28.8b	21.0ab	0.57a	9.2a	2.6
Riparia Gloire	40.6a	18.9b	0.50a	8.2a	2.8
Kober 5BB	36.2ab	20.2ab	0.57a	9.6a	2.7
Seyval	11.8c	12.5c	0.24b	4.6b	2.9
Cynthiana	9.4c	7.1c	0.16b	3.5b	2.4
					n.s.

[x] Data are from non-flooded vines.

[y] Values have been multiplied by 10^3.

[z] Mean separation by Duncan's Multiple Range Test, $\alpha = 0.05$.

Table 2. Effect of flooding on the growth and physiology of St. George grapevines.

Days of flooding[x]	Treatment	Rate of shoot elongation (mm day^{-1})	Net photosynthesis ($mgCO_2 dm^{-2} hr^{-1}$)	Stomatal conductance (cm sec^{-1})	Transpiration[y] ($mgH_2O cm^{-2} sec^{-1}$)	Water use[y] efficiency (moles CO_2/ moles H_2O)
0	Control	34.2	14.3	0.49	7.3	2.2
	Flooded	34.0	15.2	0.40	6.1	3.0
		n.s.	n.s.	n.s.	n.s.	n.s.
2	Control	28.4	25.1	0.67	10.8	2.0
	Flooded	27.0	23.8	0.67	12.0	2.5
		n.s.	n.s.	n.s.	n.s.	n.s.
5	Control	52.6	24.8	0.54	8.7	3.3
	Flooded	30.8	20.6	0.51	8.6	2.8
		*[z]	n.s.	n.s.	n.s.	n.s.
9	Control	60.0	27.3	0.62	12.7	2.6
	Flooded	30.6	19.5	0.50	10.4	2.0
		**	n.s.	n.s.	n.s.	n.s.
13	Control	48.0	21.3	0.69	12.6	2.0
	Flooded	16.2	13.7	0.46	9.4	1.8
		**	n.s.	n.s.	n.s.	n.s.

[x] Vines were flooded on 19 June 1986 after initial measurements were taken.

[y] Values have been multiplied by 10^3.

[z] * and ** indicate statistical significance at $\alpha = 0.05$ and 0.01, respectively.

Table 3. Effect of flooding on the growth and physiology of Couderc 3309 grapevines.

Days of flooding[x]	Treatment	Rate of shoot elongation (mm day^{-1})	Net photosynthesis ($mgCO_2 dm^{-2} hr^{-1}$)	Stomatal conductance (cm sec^{-1})	Transpiration[y] ($mgH_2O cm^{-2} sec^{-1}$)	Water use[y] efficiency (moles CO_2/ moles H_2O)
0	Control	48.0	16.0	0.51	6.9	2.8
	Flooded	43.2	16.3	0.62	8.3	2.4
		n.s.	n.s.	n.s.	n.s.	n.s.
2	Control	28.8	21.0	0.57	9.3	2.6
	Flooded	31.8	22.3	0.68	11.9	2.3
		n.s.	n.s.	n.s.	n.s.	n.s.
5	Control	45.8	20.3	0.55	8.6	2.8
	Flooded	28.6	18.1	0.46	8.1	2.7
		n.s.	n.s.	n.s.	n.s.	n.s.
9	Control	48.0	20.0	0.42	9.4	2.7
	Flooded	36.4	14.2	0.28	6.9	2.4
		n.s.	n.s.	n.s.	n.s.	n.s.
13	Control	36.2	18.1	0.58	11.4	1.8
	Flooded	14.8	8.0	0.19	4.5	2.2
		*[z]	**	**	**	n.s.

[x] Vines were flooded on 19 June 1986 after initial measurements were taken.

[y] Values have been multiplied by 10^3.

[z] * and ** indicate statistical significance at $\alpha = 0.05$ and 0.01, respectively.

Table 4. Effect of flooding on the growth and physiology of Riparia Gloire grapevines.

Days of flooding[x]	Treatment	Rate of shoot elongation (mm day^{-1})	Net photosynthesis ($mgCO_2 dm^{-2} hr^{-1}$)	Stomatal conductance (cm sec^{-1})	Transpiration[y] ($mgH_2O cm^{-2} sec^{-1}$)	Water use[y] efficiency (moles CO_2/ moles H_2O)
0	Control	64.2	13.8	0.42	6.5	2.5
	Flooded	64.4	13.3	0.40	6.1	2.5
		n.s.	n.s.	n.s.	n.s.	n.s.
2	Control	40.6	18.9	0.50	8.2	2.8
	Flooded	49.8	20.5	0.58	10.7	2.3
		n.s.	n.s.	n.s.	n.s.	n.s.
5	Control	82.0	21.3	0.45	7.0	3.6
	Flooded	46.6	14.0	0.31	5.2	2.9
		*[z]	n.s.	n.s.	n.s.	n.s.
9	Control	84.8	23.5	0.52	11.1	2.7
	Flooded	45.0	13.9	0.29	6.2	2.1
		*	n.s.	n.s.	n.s.	n.s.
13	Control	58.6	19.7	0.62	11.7	1.9
	Flooded	24.0	7.8	0.23	5.3	1.5
		*	**	**	**	n.s.

[x] Vines were flooded on 19 June 1986 after initial measurements were taken.

[y] Values have been multiplied by 10^3.

[z] * and ** indicate statistical significance at $\alpha = 0.05$ and 0.01, respectively.

Table 5. Effect of flooding on the growth and physiology of Kober 5BB grapevines.

Days of flooding[x]	Treatment	Rate of shoot elongation (mm day^{-1})	Net photosynthesis ($mgCO_2 dm^{-2} hr^{-1}$)	Stomatal conductance (cm sec^{-1})	Transpiration[y] ($mgH_2O cm^{-2} sec^{-1}$)	Water use[y] efficiency (moles CO_2/ moles H_2O)
0	Control	42.6	19.0	0.43	6.7	3.0
	Flooded	45.4	19.4	0.48	7.4	3.0
		n.s.	n.s.	n.s.	n.s.	n.s.
2	Control	36.2	20.2	0.57	9.6	2.7
	Flooded	30.2	18.1	0.52	10.6	2.1
		n.s.	n.s.	n.s.	n.s.	n.s.
5	Control	53.2	20.5	0.56	9.0	2.7
	Flooded	26.2	7.6	0.19	3.6	2.3
		*[z]	**	**	**	n.s.
9	Control	55.4	20.7	0.47	10.5	2.4
	Flooded	8.2	3.1	0.08	2.4	1.0
		**	**	**	**	*
13	Control	46.2	16.1	0.53	10.2	1.7
	Flooded	5.0	5.9	0.11	3.0	1.5
		**	**	**	**	n.s.

[x] Vines were flooded on 19 June 1986 after initial measurements were taken.

[y] Values have been multiplied by 10^3.

[z] * and ** indicate statistical significance at $\alpha = 0.05$ and 0.01, respectively.

Table 6. Effect of flooding on the growth and physiology of Seyval grapevines.

Days of flooding[x]	Treatment	Rate of shoot elongation (mm day^{-1})	Net photosynthesis ($mgCO_2dm^{-2}hr^{-1}$)	Stomatal conductance (cm sec^{-1})	Transpiration[y] ($mgH_2Ocm^{-2}sec^{-1}$)	Water use[y] efficiency (moles CO_2/ moles H_2O)
0	Control	13.4	7.7	0.18	3.4	2.6
	Flooded	16.6	5.8	0.15	2.9	2.3
		n.s.	n.s.	n.s.	n.s.	n.s.
2	Control	11.8	12.5	0.24	4.6	2.9
	Flooded	6.0	6.4	0.13	3.0	2.2
		n.s.	n.s.	n.s.	n.s.	n.s.
5	Control	13.8	16.6	0.35	6.6	2.9
	Flooded	3.0	5.3	0.12	2.4	2.0
		*[z]	**	**	**	n.s.
9	Control	18.0	17.5	0.39	9.3	2.3
	Flooded	3.2	3.3	0.09	2.2	1.1
		**	**	**	**	*
13	Control	19.2	16.0	0.50	10.3	1.8
	Flooded	1.8	1.1	0.03	0.9	0.9
		**	**	**	**	n.s.

[x] Vines were flooded on 19 June 1986 after initial measurements were taken.

[y] Values have been multiplied by 10^3.

[z] * and ** indicate statistical significance at $\alpha = 0.05$ and 0.01, respectively.

Table 7. Effect of flooding on the growth and physiology of Cynthiana grapevines.

Days of flooding[x]	Treatment	Rate of shoot elongation (mm day^{-1})	Net photosynthesis ($mgCO_2 dm^{-2} hr^{-1}$)	Stomatal conductance (cm sec^{-1})	Transpiration[y] ($mgH_2O cm^{-2} sec^{-1}$)	Water use[y] efficiency (moles CO_2/ moles H_2O)
0	Control	10.6	9.2	0.25	4.4	2.5
	Flooded	14.0	8.2	0.27	5.0	1.9
		n.s.	n.s.	n.s.	n.s.	n.s.
2	Control	9.4	7.1	0.16	3.5	2.4
	Flooded	8.2	3.9	0.13	2.5	1.0
		n.s.	n.s.	n.s.	n.s.	n.s.
5	Control	10.6	9.7	0.25	4.6	2.8
	Flooded	1.0	1.7	0.08	1.6	1.0
		**[z]	**	*	*	*
9	Control	15.0	11.1	0.22	5.4	2.4
	Flooded	1.6	1.6	0.05	1.2	0.7
		*	**	*	**	*
13	Control	10.6	9.0	0.21	5.3	2.0
	Flooded	1.4	0.4	0.02	0.5	0.7
		*	**	**	**	*

[x] Vines were flooded on 19 June 1986 after initial measurements were taken.

[y] Values have been multiplied by 10^3.

[z] * and ** indicate statistical significance at $\alpha = 0.05$ and 0.01, respectively.

significantly reduced following flooding for five days (Table 5). WUE was not consistently affected by flooding.

Flooding had a fairly rapid impact on the growth and physiology of Seyval vines (Table 6). Although not statistically significant, flooded vines displayed a reduction in RSE, P_n, and g_s of approximately 50% after two days of flooding. Five days of flooding produced statistically significant reductions in all parameters except WUE. The growth of shoots on flooded vines was negligible by the end of the experiment.

Cynthiana vines exhibited a significant decrease in all parameters when the flooding duration reached five days (Table 7). Shoot elongation and gas exchange were almost non-existent after vines were flooded for 13 days.

Symptoms observed on flooded grapevines were desiccation of the shoot apex, flagging of leaves, necrotic areas on leaves, senescence of basal leaves and regeneration of roots near the water surface. Root regeneration was observed in all cultivars except Cynthiana.

Experiment II

Flooding produced a rapid reduction in soil ODR (Figure 1). Within three hours, ODR fell below 20 g O_2 x $10^{-8}cm^{-2}min^{-1}$ in flooded soil. Soil ODR gradually declined during 15 days of flooding.

Experiment III

Flooding tolerance of Seyval was increased by grafting onto Couderc 3309 (Table 8). The effect was limited to RSE and P_n and its magnitude was small. A significant reduction in RSE and P_n was not observed for this graft combination until vines had been flooded

Figure 1. Effect of flooding on oxygen diffusion rate of potted Seyval grapevines measured at 10 cm depth.

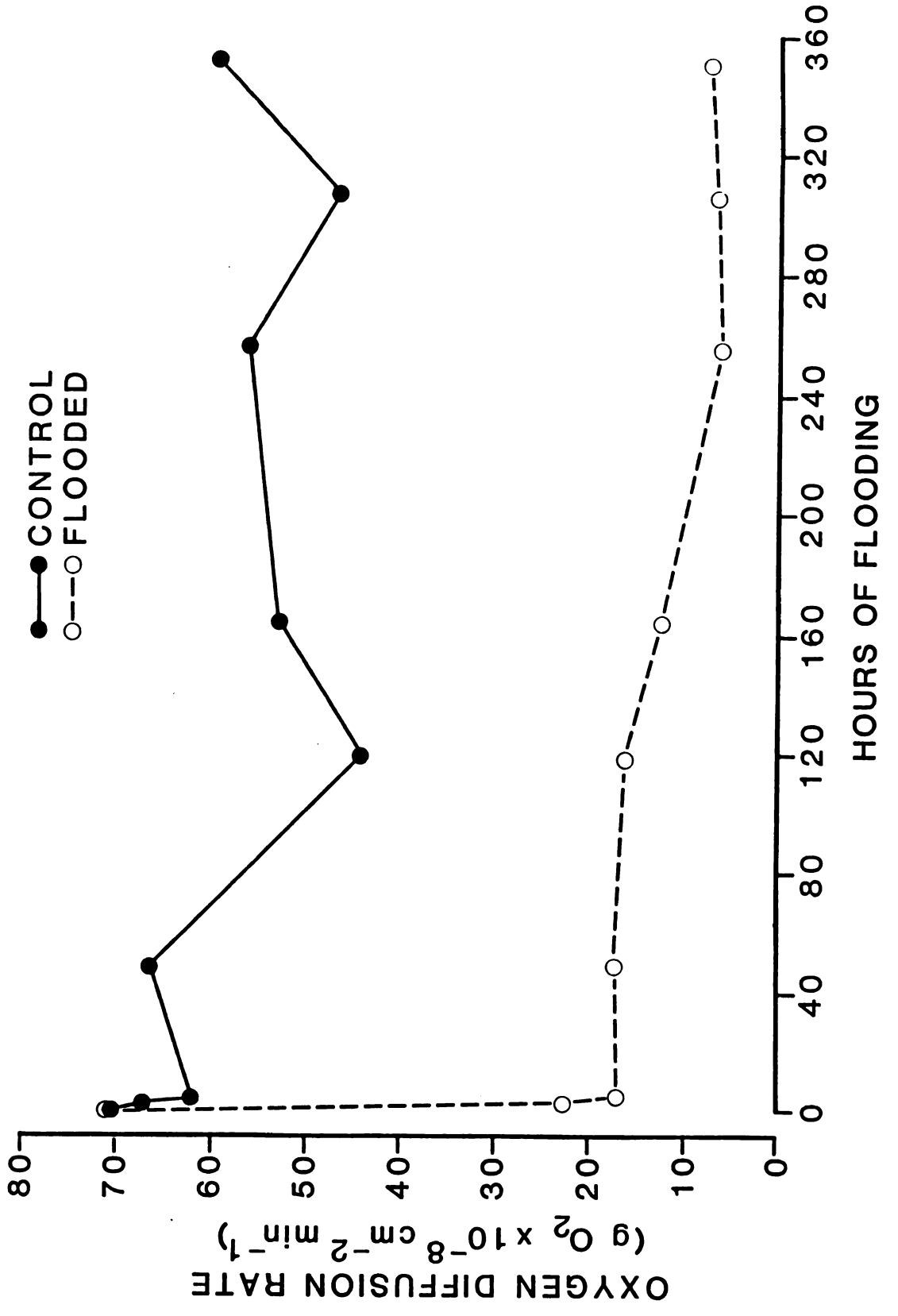
CONTROL
FLOODED
OXYGEN DIFFUSION RATE
(g O_2 x 10^{-8} cm^{-2} min^{-1})
0
10
20
30
40
50
60
70
80
0
40
80
120
160
200
240
280
320
360
HOURS OF FLOODING

Table 8. Number of days of flooding required for flooded vines to show a significant[y] reduction in growth and physiology.

Rootstock	Rate of shoot elongation ($mm\ day^{-1}$)	Net photosynthesis ($mgCO_2 dm^{-2} hr^{-1}$)	Stomatal conductance ($cm\ sec^{-1}$)	Transpiration[z] ($mgH_2O cm^{-2} sec^{-1}$)
Own	2	2	2	2
Seyval	2	2	2	2
Couderc 3309	4	4	2	2
St. George	2	2	2	2

[y] Level of $\alpha = 0.05$.

[z] Values have been multiplied by 10^3.

for four days. Own-rooted Seyval and Seyval grafted on Seyval and St. George displayed significant reductions in all parameters after two days of flooding.

Symptoms observed on flooded vines were similar to those in Exp. I except for root regeneration. Few adventitious roots were observed near the water surface after eight days of flooding.

DISCUSSION

Anaerobic conditions occurred in soil of potted vines shortly after flooding was imposed. ODR of flooded soil fell below 20 g O_2 x $10^{-8}cm^{-2}min^{-1}$ within three hours of flooding. Critical ODR values have not been determined for grapevines. However, decreased fruit tree performance and survival have been associated with ODR values of 15 to 30 g O_2 x $10^{-8}cm^{-2}min^{-1}$ (2,3,18).

Growth and physiological responses of grapevines to flooding were similar to those observed in other woody fruit crops (1-4,13,18,19,28,29). In general, flooding significantly reduced RSE, P_n, g_s, and Tr. Flooding had little effect on WUE. Significant reductions in RSE occurred either simultaneously with or preceding a significant reduction in P_n, g_s, Tr, or WUE by flooding. This indicates that RSE was more sensitive to flooding than the gas exchange parameters measured during the study. RSE was previously shown to be more sensitive to flooding than the plastochron index (5).

Considerable variation in flooding tolerance was observed between grapevine cultivars. St. George, Couderc 3309, and Riparia Gloire were more tolerant of flooding than Kober 5BB, Seyval, and Cynthiana. A clear relationship between cultivar differences in RSE

and the gas exchange parameters of non-flooded vines and flooding tolerance was not observed. For example, cultivars with statistically higher values of RSE, P_n, g_s, and Tr are found in both the flooding-tolerant group of cultivars and the flooding-intolerant group of cultivars.

The ranking of rootstock flooding tolerance obtained in this study deviates from that of Pongracz (23). St. George is considered intolerant and Kober 5BB tolerant of flooding by Pongracz. Data collected in this experiment are in agreement with Viala and Ravaz (35) concerning the flooding tolerance of St. George. Differences in the ranking of rootstock flooding tolerance likely result from the manner in which flooding tolerance was evaluated.

St. George has been reported to have low resistance to the soil pathogen Phytophthora cinnamomi Rands (40). The effects of P. cinnamomi can be confounded with flooding effects during field evaluation of rootstock flooding tolerance. Also, avoidance mechanisms such as adventitious rooting, development of aerenchyma tissue, and formation of hypertrophied lenticels are more likely to contribute to plant survival under field conditions than during short term flooding under controlled conditions in the greenhouse.

Adventitious roots developed near the water surface after 13 days of flooding. However, adventitious rooting did not appear to improve vine performance since both tolerant and intolerant cultivars produced adventitious roots. The development of anaerobiosis in flooded soils is commonly not uniform. Regeneration of roots into aerobic areas of the soil would likely improve vine performance and survival during flooding in the field. The formation of adventitious roots is an important flooding avoidance

mechanism in many tree species (2,9,28).

The grapevine rootstocks used in this study were less effective than apple (12), peach (15), or pear (2) rootstocks in improving the performance of a susceptible scion during flooding. Flooding tolerance of grafted vines was not conveyed by a simple mechanism. Responses of grafted vines to flooding suggest a complex interrelationship between rootstock and scion.

Increased flooding tolerance was obtained when a sensitive scion was grafted on a more tolerant rootstock. This data provides evidence of a rootstock contribution to scion performance. Grapevine roots are involved in vine water relations, uptake and translocation of nutrients, synthesis of plant growth substances, and storage of carbohydrates and amino acids (26). Rootstock effects on flooding tolerance are likely mediated through one or more of these root functions.

However, the effect of rootstock on flooding tolerance was not consistent among flooding tolerant rootstocks, i.e., Couderc 3309 increased flooding tolerance while St. George did not. This suggests that factors other than rootstock were involved in determining flooding tolerance of a specific graft combination. Rives (27) has proposed that the performance of a graft combination is the sum of an additive scion contribution, an additive rootstock contribution, and a non-additive, interactive contribution specific to the graft combination. Flooding tolerance of grafted grapevines may result from rootstock, scion, and possibly graft union factors (affinity).

CONCLUSIONS

Soil ODR values rapidly declined following flooding. Levels of ODR which have been shown to be damaging to other woody species were reached within three hours of flooding. ODR of flooded soil remained low during a 15 day flooding period.

Flooding altered the growth and physiology of grapevine cultivars used in this study. Species and duration of flooding were important factors in determining the severity of flooding injury. St. George, Riparia Gloire, and Couderc 3309 displayed a greater degree of flooding tolerance than Kober 5BB, Seyval, and Cynthiana. Significant reductions in RSE were obtained after five days of flooding for all cultivars except Couderc 3309. This cultivar was able to maintain growth of flooded vines at the same level as control vines for more than 9 days of flooding.

Gas exchange measurements of the more tolerant cultivars were not significantly reduced until vines had been flooded for 13 days. Conversely, the flooding intolerant cultivars showed a decline in gas exchange after five days of flooding. WUE was not consistently affected by flooding. In general, RSE was the most sensitive and WUE the least sensitive parameter to flooding.

Symptoms observed on flooded vines were desiccation of the shoot apex, flagging of leaves, necrotic areas on leaves, senescence of basal leaves, and regeneration of adventitious roots near the water surface. Root regeneration could be an important avoidance mechanism when grapevines are flooded in the field. This response was observed in all cultivars except Cynthiana.

Flooding tolerance of a susceptible scion was increased by grafting. Use of Couderc 3309 as a rootstock increased the flooding tolerance of Seyval. The effect was limited to RSE and P_n and its magnitude was small. However, the importance of a 1-2 day increase in flooding tolerance should not be underestimated. Flooding in agricultural soils is usually transitory. The ability to maintain shoot growth and photosynthesis during short-term flooding would likely result in greater productivity. Further research is needed to determine if Couderc 3309 can influence flooding tolerance under field conditions.

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APPENDICES

I. EFFECT OF FLOODING ON WATER RELATIONS, LEAF GAS EXCHANGE, AND GROWTH OF GRAPEVINES

Table 1. Effect of flooding on root hydraulic conductivity, leaf gas exchange, and growth of Seyval grapevines.

Days of flooding[v]	Treatment	Root hydraulic[w] conductivity (ml sec^{-1})	Stomatal conductance (cm sec^{-1})	Transpiration[x] ($mgH_2Ocm^{-2}sec^{-1}$)	Net photosynthesis ($mgCO_2dm^{-2}hr^{-1}$)	Rate of shoot elongation (mm day^{-1})
0	Control	---[y]	0.22	7.2	21.57	24.9
	Flooded	---	0.23	7.4	21.89	24.5
			n.s.	n.s.	n.s.	n.s.
4	Control	3571	0.26	10.5	19.47	26.8
	Flooded	316	0.15	6.3	11.07	9.1
		**[z]	*	*	*	***
11	Control	5437	0.23	7.5	22.47	33.5
	Flooded	306	0.05	1.9	5.73	4.3
		***	***	***	***	***

[v] Vines were flooded on 8 March 1988 after initial measurements were taken.

[w] Values have been multiplied by 10^6.

[x] Values have been multiplied by 10^3.

[y] Data not available.

[z] *, **, and *** indicate statistical significance at α = 0.05, 0.01, and 0.001 respectively.

Table 2. Effect of flooding on leaf water potential, leaf gas exchange, and growth of Seyval grapevines.

Days of flooding[x]	Treatment	Leaf water potential (bars)	Stomatal conductance (cm sec^{-1})	Transpiration[y] ($mgH_2Ocm^{-2}sec^{-1}$)	Net photosynthesis ($mgCO_2dm^{-2}hr^{-1}$)	Rate of shoot elongation (mm day^{-1})
0	Control	-4.2	0.39	11.1	24.34	26.3
	Flooded	-4.3	0.35	10.5	24.73	24.0
		n.s.	n.s.	n.s.	n.s.	n.s.
2	Control	-11.6	0.24	8.1	21.83	19.2
	Flooded	-12.9	0.15	5.4	15.23	12.0
		*[z]	*	*	*	*
4	Control	-9.3	0.23	10.0	23.26	24.1
	Flooded	-11.3	0.07	3.5	8.61	8.7
		*	***	***	***	***
6	Control	-7.9	0.28	7.9	27.01	24.3
	Flooded	-12.1	0.08	2.8	10.86	2.3
		**	***	***	***	***
8	Control	-9.1	0.29	8.7	27.35	22.9
	Flooded	-12.9	0.05	1.9	7.09	1.5
		**	***	***	***	***

[x] Vines were flooded on 28 April 1988 after initial measurements were taken.

[y] Values have been multiplied by 10^3.

[z] *, **, and *** indicate statistical significance at α = 0.05, 0.01, and 0.001 respectively.

Table 3. Effect of flooding on leaf water potential, leaf gas exchange, and growth of Couderc 3309 grapevines.

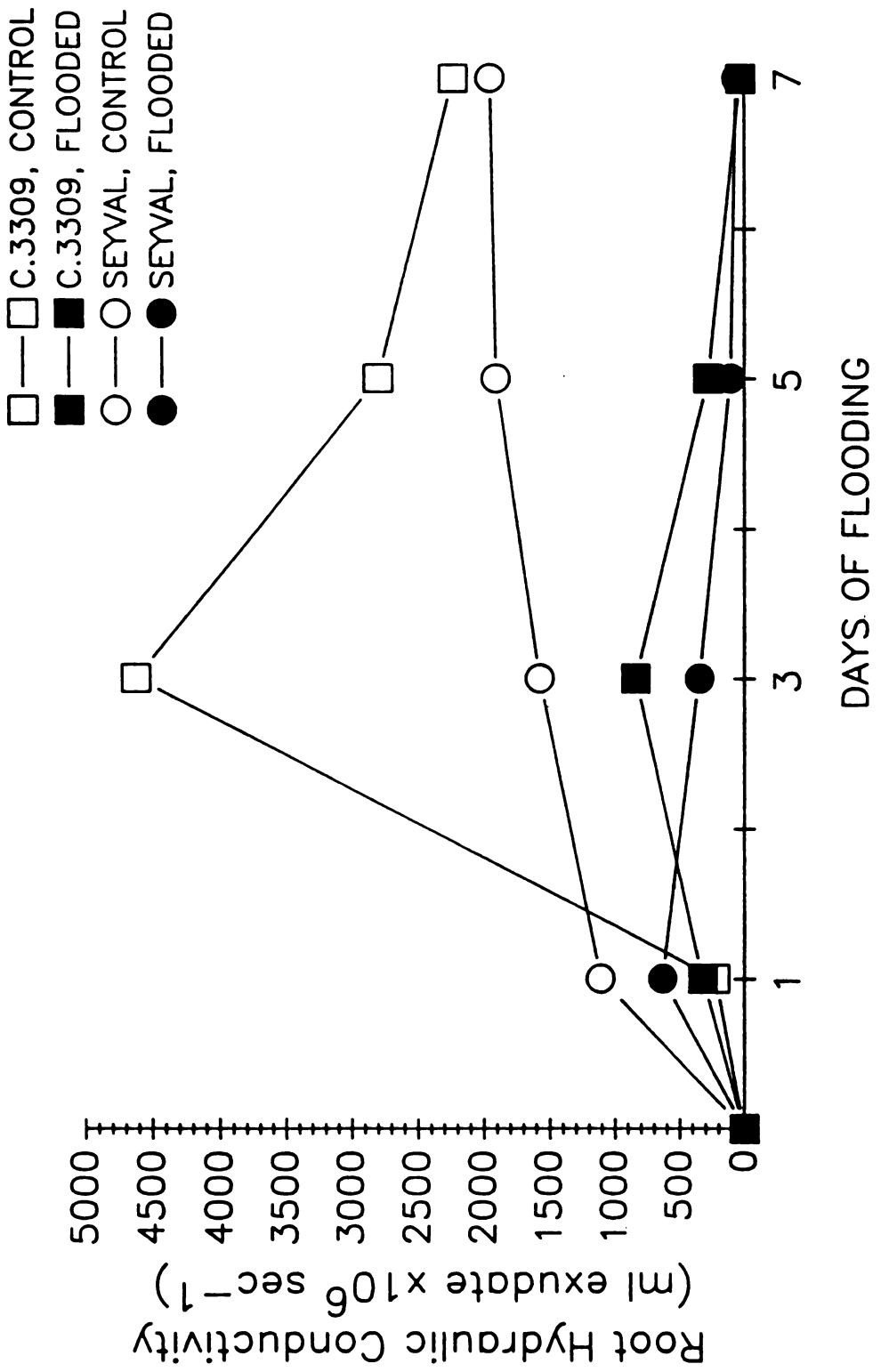
Days of flooding[x]	Treatment	Leaf water potential (bars)	Stomatal conductance (cm sec^{-1})	Transpiration[y] ($mgH_2Ocm^{-2}sec^{-1}$)	Net photosynthesis ($mgCO_2dm^{-2}hr^{-1}$)	Rate of shoot elongation (mm day^{-1})
0	Control	-9.1	0.27	10.8	24.33	30.8
	Flooded	-8.6	0.31	12.0	25.92	29.1
		n.s.	n.s.	n.s.	n.s.	n.s.
2	Control	-13.4	0.22	7.8	20.70	18.9
	Flooded	-16.2	0.22	7.9	19.51	13.4
		***[z]	n.s.	n.s.	n.s.	n.s.
4	Control	-10.9	0.18	8.4	20.32	21.1
	Flooded	-14.7	0.12	5.8	13.72	11.1
		**	n.s.	n.s.	*	**
6	Control	-11.0	0.26	7.1	24.02	21.3
	Flooded	-14.3	0.13	4.1	15.75	6.8
		**	**	**	**	**
8	Control	-10.2	0.26	7.8	23.57	18.9
	Flooded	-14.0	0.08	3.0	11.53	4.6
		***	***	***	***	***

[x] Vines were flooded on 28 April 1988 after initial measurements were taken.

[y] Values have been multiplied by 10^3.

[z] *, **, and *** indicate statistical significance at $\alpha = 0.05$, 0.01, and 0.001 respectively.

Figure 1. Effect of flooding on root hydraulic conductivity of Couderc 3309 and Seyval grapevines.


C.3309, CONTROL
C.3309, FLOODED
SEYVAL, CONTROL
SEYVAL, FLOODED
Root Hydraulic Conductivity
(ml exudate x10⁶ sec⁻¹)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
1
3
5
7
DAYS OF FLOODING

II. RESPONSE OF SEYVAL GRAPEVINES TO FLOODING AT DIFFERENT STAGES OF DEVELOPMENT

Table 1. Effect of time of flooding[w] on cumulative growth and cane maturation[x] of Seyval grapevines.

Sample date	Treatment	Nodes/ Vine	Mature Nodes/ Vine	Mature Nodes[y] /Vine (%)
1987				
10 Sept.	Control	28a[z]	4	14.3
	Flooded in July	24b	3	12.5
	Flooded in Sept.	28a	4	14.3
			n.s.	n.s.
25 Sept.	Control	28a	13	46.4
	Flooded in July	24b	12	50.0
	Flooded in Sept.	29a	13	44.8
			n.s.	n.s.
7 Oct.	Control	28a	17	60.7
	Flooded in July	24b	15	62.5
	Flooded in Sept.	29a	17	58.6
			n.s.	n.s.

[w] Vines flooded for 1 week (11-18 July 1987 and 10-17 September 1987).

[x] Cane maturation determined visually according to browning of periderm.

[y] Arcsin transformation was performed before AOV. Means represent detransformed data.

[z] Mean separation by Duncan's Multiple Range Test, $\alpha = 0.05$.

Table 2. Effect of time of flooding[x] on cold hardiness of Seyval grapevines.

Treatment	Sample Date		
	7 Oct. 1987	3 Dec. 1987	7 Jan. 1988
		Primary Buds	
Control	-11.4	-18.6	-18.8
Flooded in July	-11.2	-18.6	-18.2
Flooded in Sept.	-10.6	-18.8	-17.4
	n.s.	n.s.	n.s.
		Canes	
Control	-10.4	-16.8	-19.2a[z]
Flooded in July	-10.2	-17.0	-17.6b
Flooded in Sept.	-10.4	-17.4	-18.6a
	n.s.	n.s.	

[x] Vines flooded for 1 week (11-18 July 1987 and 10-17 September 1987).

[y] T_{50} in °C.

[z] Mean separation by Duncan's Multiple Range Test, $\alpha = 0.05$.

Table 3. Effect of time of flooding[x] on the percentage of shootless nodes of Seyval grapevines.

Treatment	Shootless Nodes[y] (%)
Control	18.8b[z]
Flooded in July	46.5a
Flooded in Sept.	35.1ab

[x] Vines flooded for 1 week (11-18 July 1987 and 10-17 September 1987).

[y] Arcsin transformation was performed before AOV. Means represent detransformed data.

[z] Mean separation by Duncan's Multiple Range Test, $\alpha = 0.05$.

Figure 1. Effect of time of flooding on net photosynthesis of Seyval grapevines.

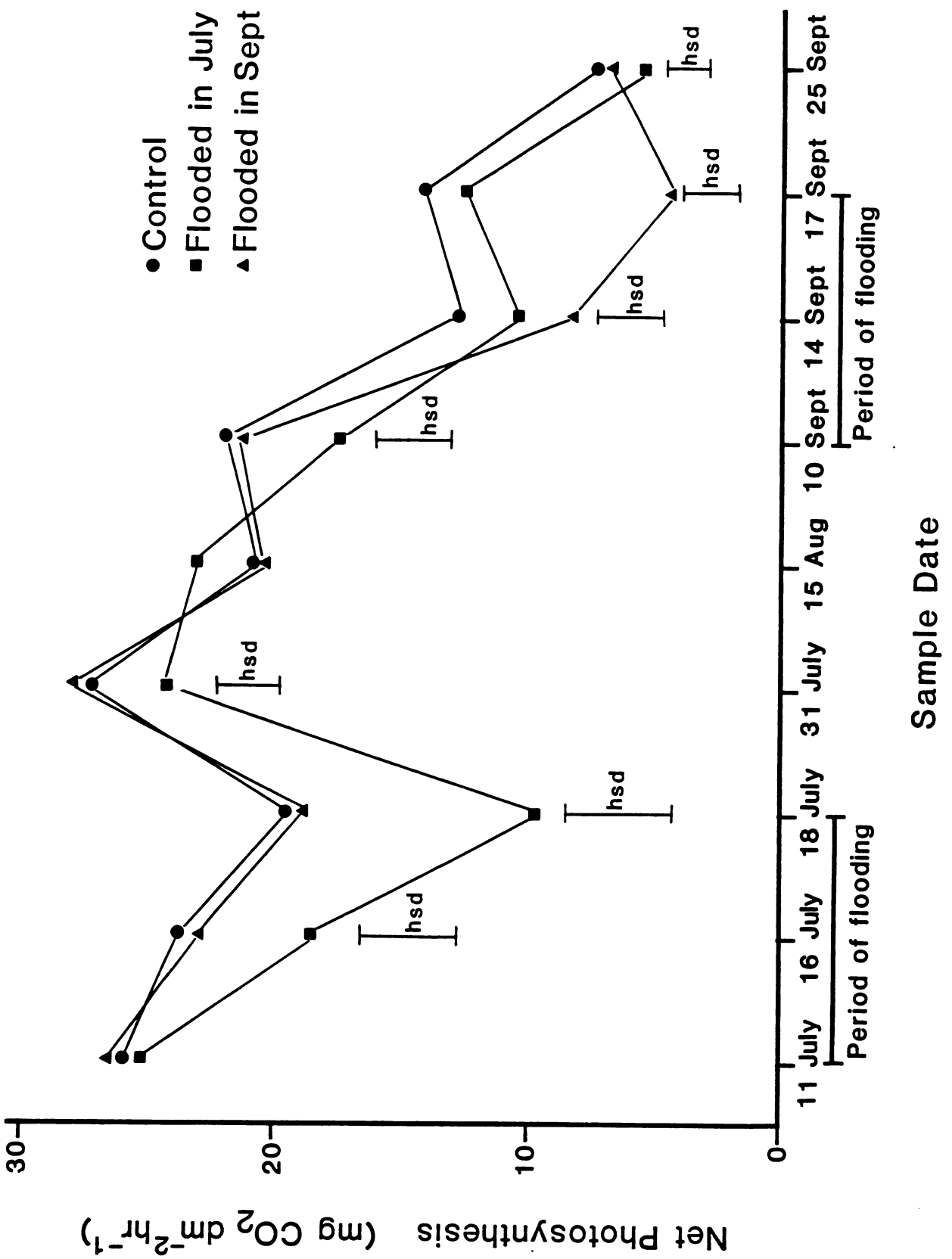

Control
Flooded in July
Flooded in Sept
hsd
hsd
hsd
hsd
hsd
30
20
10
0
11 July
16 July
18 July
31 July
15 Aug
10 Sept
14 Sept
17 Sept
25 Sept
Period of flooding
Period of flooding
Sample Date
Net Photosynthesis (mg CO_2 $dm^{-2}hr^{-1}$)

Figure 2. Effect of time of flooding on stomatal conductance of Seyval grapevines.

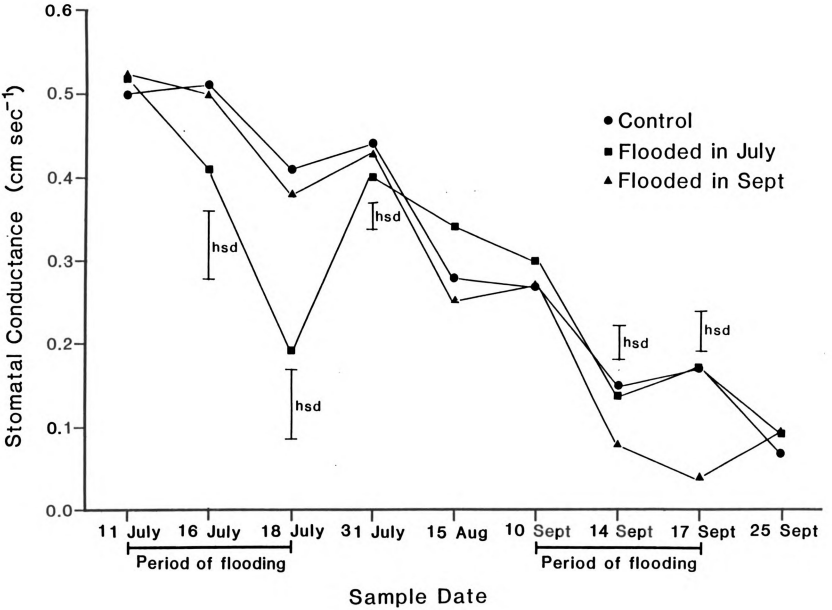

0.6
0.5
0.4
0.3
0.2
0.1
0.0
Stomatal Conductance (cm sec⁻¹)
Control
Flooded in July
Flooded in Sept
hsd
hsd
hsd
hsd
hsd
11 July
16 July
18 July
31 July
15 Aug
10 Sept
14 Sept
17 Sept
25 Sept
Period of flooding
Period of flooding
Sample Date

Figure 3. Effect of time of flooding on transpiration of Seyval grapevines.

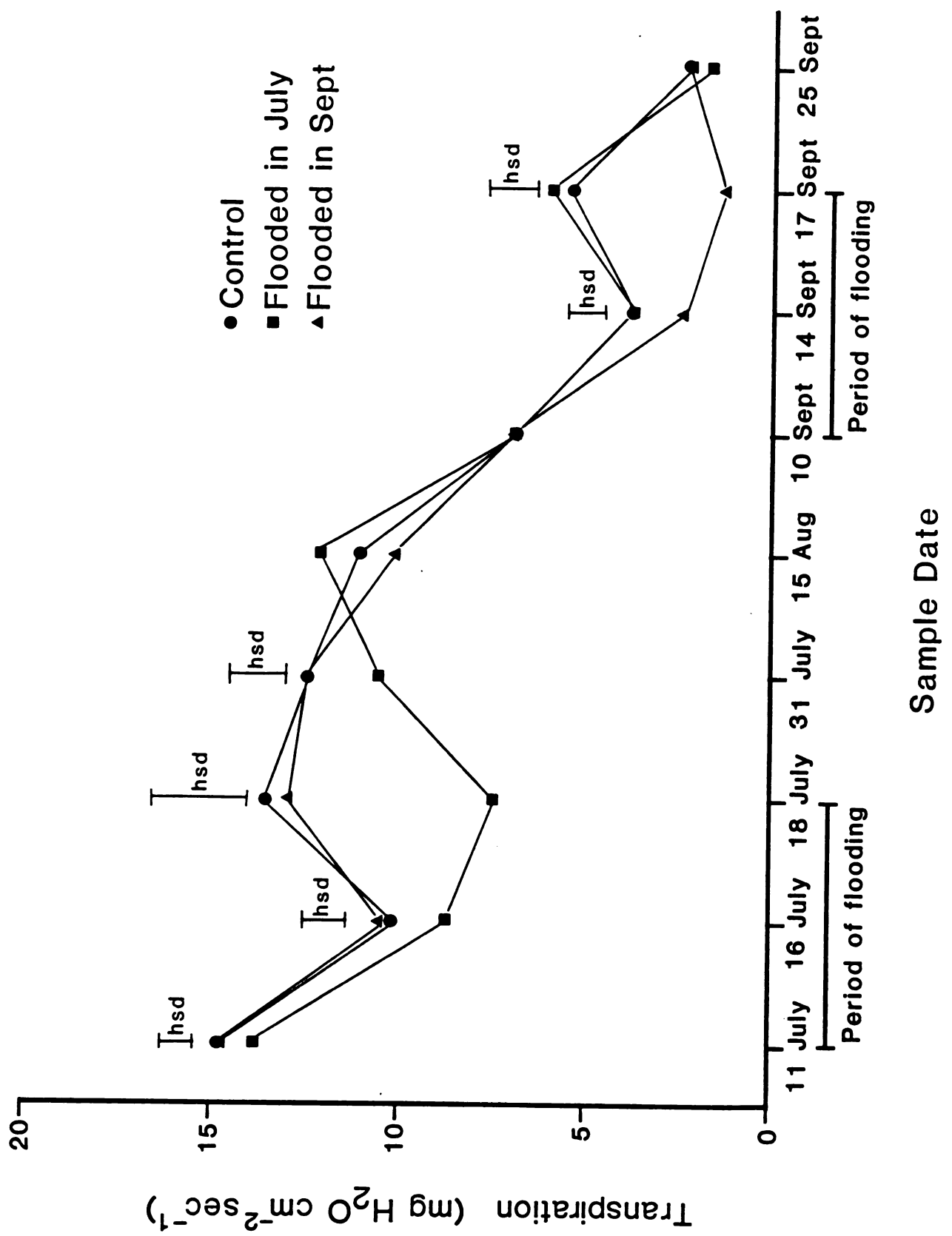
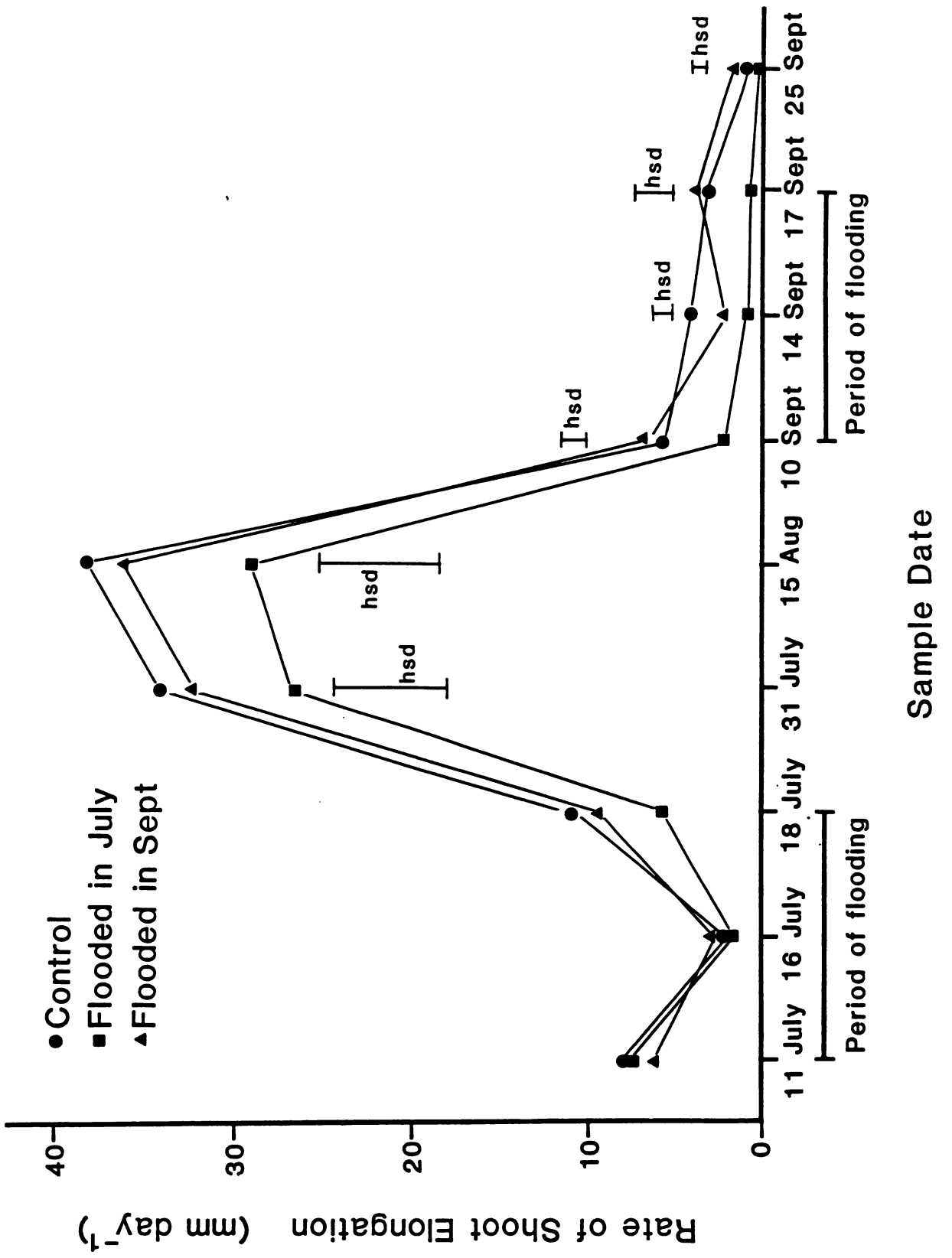

Transpiration (mg H_2O $cm^{-2}sec^{-1}$)
20
15
10
5
0
● Control
■ Flooded in July
▲ Flooded in Sept
hsd
hsd
hsd
hsd
11 July
16 July
18 July
31 July
15 Aug
10 Sept
14 Sept
17 Sept
25 Sept
Period of flooding
Period of flooding
Sample Date

Figure 4. Effect of time of flooding on the rate of shoot elongation of Seyval grapevines.


Rate of Shoot Elongation (mm day⁻¹)
40
30
20
10
0
●Control
■Flooded in July
▲Flooded in Sept
hsd
hsd
hsd
hsd
hsd
11 July
16 July
18 July
31 July
15 Aug
10 Sept
14 Sept
17 Sept
25 Sept
Period of flooding
Period of flooding
Sample Date