AN ABSTRACT OF THE THESIS OF

Abbas M. Shirazi for the degree of Doctor of Philosophy in Horticulture presented on February 28, 1992

Title: Relationship of "Near-Lethal" Stress on Dormancy, Cold Hardiness and Recovery of Red-Osier Dogwood

Abstract Approved: Leslie H. Fuchigami

The effects of overcoming rest by application of "near-lethal" (NL) stress treatments on the subsequent development of cold hardiness of stem tissues of red-osier dogwood, *Cornus sericea* L., at different rest stages under three post-environments (0°, 23°, and natural condition) were studied. NL-heat stress (47°C for one hour) treatment during early (October) and late rest (December), overcame rest at 23°C post-environment. The treated plants broke bud within 22-35 days and 8-12 days, respectively. In contrast, the control treatments at early rest took more than 150 days to broke buds. Under natural post-environment conditions NL-heat stress treatment applied at the early and late stage of rest resulted in the same and 50-55 days earlier budbreak than control plants, respectively. Other type of NL-stresses e.g., NL-freeze stress (-7°C, for one hour), and NL-hydrogen cyanamide (0.5-1M), treatments at early rest and late rest also resulted in early (8-16 days) budbreak in a warm greenhouse.

To determine the effect of overcoming rest by NL-stresses on cold hardiness, NL-heat and NL-hydrogen cyanamide stress applications at early rest had a slight effect on cold acclimation at 23°C and no effect on cold hardiness under natural condition. In contrast NL-freeze stress applied at early rest caused the plants to rapidly increase in cold hardiness at 23°C post-environment. At late rest, all NL- stress treatments resulted in the rapid loss of hardiness at warm or natural post-environment conditions.

Recovery of plants from NL-stresses was dependent on the stage of development and temperature. Plants exposed to NL-heat stress recovered to a greater extent from October to December at 0°C post environment. All NL- heat-stressed plants recovered at 23° and natural post-environment conditions.

Conditions that favored recovery also favored glutathione production. At 23°C post-environment, reduced (GSH) and oxidized (GSSG) glutathione levels of NL-heat stressed plants increased, while at 0°C post-environment the levels of both decreased.

Studies on the heat tolerance of red-osier dogwood at different growth stages and its relationship to ethylene production, showed that heat tolerance of stem tissues was highest during the post-rest and early quiescent phases and lowest during the spring growth phase.

Heat stressed stem tissues did not produce ethylene during early rest, maximum rest, late-rest and quiescence. Highest levels of ethylene occurred during active growth. Application of 1-aminocyclopropane-1-carboxylic acid (ACC), methionine and indole acetic acid (IAA) during rest stages, enhanced ethylene production in non-heat and heat stressed stem tissues.

Relationship of "Near-Lethal" Stress on Dormancy, Cold Hardiness and Recovery of Red-Osier Dogwood

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed February 28, 1992

Commencement June 1992

APPROVED:

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Date thesis is presented February 28, 1992

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Dr. Leslie H. Fuchigami

Acknowledgements

I extend my thanks to all persons who helped me through my study. I express my warmest appreciation to Dr. Leslie H. Fuchigami, my major advisor for my development not only in research but also in life through his optimism, constant encouragement and understanding.

I thank the members of my committee, Drs. Tony Chen, Bill Gerwick, Bud Weiser, Paul Doecsher and Brayan Croft for their valuable advice and training.

Sincere thanks to Dr. Bud Weiser for his invaluable advise and encouragements which, I needed the most during my study.

My appreciation to Mrs. Vonda Peters, and Lisa Pace for their technical help.

I am also grateful to fellowship of dormancy and cold hardiness group for their support, assistance and friendships; Liping, John, Guak, Jorge, Peeradet, Ted, Dennis, Stefen, Gary, Pilar, Anawat, Balong, Renne, Alfonso, Zhao, Ron, Ursula, Paul, Karen and GiovanFranco.

I would like thank the Faculty, Staff and Graduate students at the Horticulture Department for their friendships, and moral support.

My sincere appreciation to Drs. P. Breen, J. Baggett, L. Daley, and R. Stebbins at the Department of Horticulture.

I also appreciate Mr. Jim Hay and greenhouse Crew and Staff for their valuable help during my study.

I also would like thank fellow graduate students at the Dr. Gerwick laboratory, at College of Pharmacy, and Dr. Powelson Laboratory in the Department of Botany and Plant Pathology.

My special thanks to visiting professor C. Jersa in the Forest Science Department for his Friendship and valuable advices.

My sincere appreciation to Drs, K. Bentson, G. Bradshow, B. Hawley, and N. Vance, at the USDA Forest Science Laboratory for their support and friendships.

I would like to thank following selection committees for their scholarships, Fellowships and awards. Dearborn Scholarship Committee for the Curtis Howard Dearborn Memorial Horticultural

Scholarship, Department of Horticulture, Oregon State University for the Kraus Graduate Fellowship, Oregon Nurserymen's Foundation for the Nurseryman's Memorial Award, and Office of International Education, Oregon State University for the Strickland Scholarships.

I appreciate Dr. Bruce Mackey and Mr. Thomas Bays for their help for my admission to Oregon State University. Special thank to my brother-in-law Mr. Richard. Bays for his help for coming to America again!

I am grateful to my kind mother Mrs. Kefayat Delavari, who devoted, and sacrifice her life for her children and to my late father Mr. RajabAli Shirazi. I also thank my brother Mr. Hooshang Shirazi, and my sisters, Shahla and Chehreh for their encouragement and support.

Finally, heartfelt gratitude is extended to my lovely wife Mahbubeh Keshtkaran. Her infinite patience, understanding, and compassion helped me through all these time " You are wonderful". And my thank to our sons, Eman and Ashkan for their patience and understanding "You are great".

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Relationship of "Near-Lethal" Stress on Dormancy, Cold Hardiness and Recovery of Red-Osier Dogwood

1. Introduction

The distance separating the planet earth from the sun has provided a unique physical environment that makes earth neither too cold nor too warm for higher forms of life (Gates 1980). Yet, of the total land area in the world only 11.3 % is arable (F.A.O. 1990). Weiser (1970) indicated that the remaining land not cultivated by humans (88.7%) is either too cold, too hot, too dry, too salty, too rocky, or too steep for farming. Of these conditions temperature emerges as the most important factor in the adaptation and cultivation of plants. The coldest reported air temperature is -89.2°C recorded in Vostok, Antarctica in1983, and the warmest air temperature recorded is 58°C in Al'Aziziya, Libya in 1922 (McFarlan 1992). Low temperature is responsible for the geographical distribution of many plants (Sakai and Larcher 1987). Levitt (1980) mentioned that the importance of temperature was known from the early era of plant biology, when two weeks of warm weather resulted in reducing freezing tolerance in plants (Goppert 1830, Levitt 1980), and exposure of the plants to low temperature increased their freezing tolerance (Haberlant 1875, Levitt 1980). Even though other environmental stresses other than temperature, (e.g., drought, salts, toxic ions, and athropogenic air pollutants) all influence crop productivity (Dilley *et al.* 1975). From 1948 to 1989 damage caused by low temperatures has resulted in the highest percentage rate of

crop losses in the United States followed by drought and excess heat (Agricultural Statistics 1990).

Temperate woody plants undergo ontogenic changes during their annual life cycle (Sarvas 1972, 1974, Fuchigami *et al.* 1982). Woody plants develop winter dormancy (rest) prior to the onset of sub-freezing temperatures (Levitt 1980, Weiser 1970). The termination of dormancy requires a period of chilling to overcome the rest period in many woody plant species native to temperate zones. Woody plants which satisfy their chilling requirement thereafter enter quiescence. Their release from the quiescence is dependent on warm temperature (Coville 1920, Doorenbos 1953, Vegis 1964, Fuchigami *et al.* 1982, Saure 1985).

Cold hardiness, "the ability of plants to survive subfreezing temperatures" (Alder and Herman 1971), or " the seasonal transition of plant hardiness from the tender to the hardy condition" (Weiser 1970), takes place during the annual cycle in response to distinct seasons (Sakai and Larcher 1987). Temperate woody plants first develop cold hardiness (cold acclimation) by responding to short days and warm temperature (Fuchigami 1971), second to low temperature (Tumanov and Krasavtsev 1959, Weiser 1970) and third to subfreezing temperature (Tumanov and Krasavtsev 1959). These three stages are believed necessary for complete cold hardiness development (Weiser 1970). In red-osier dogwood the rate of acclimation increases from the onset of rest to the acquisition of maximum rest. During this period plants do not deacclimate, even at relatively warm temperatures, e. g., 20°C. In contrast, following the late stage of rest to the time of bud-burst, the rate of acclimation

decreases and deacclimation increases in response to temperature (Kobayashi *et al.* 1982, 1983) .

The relationship of the rest period to cold hardiness is not well understood. While early researchers maintain that dormancy or "rest" is a prerequisite for cold hardiness (Chandler 1954), others later showed that the development of cold hardiness is independent of bud dormancy (Irving and Lanphear 1967). Greater cold hardiness has been attained after rest in red-osier dogwood (Van Hustee et al, 1967, Kobayashi and Fuchigami 1983) and in Douglas-fir seedlings (Hermann 1967). Low temperatures have been suggested to cause growth cessation and induce acclimation without dormancy development (Van Hustee 1967, Irving and Lanphear 1967, Howell and Weiser 1970).

Artificial breaking of the rest period has been practiced since the early 1900'S (Doorenbos 1953). Subfreezing temperature that results in premature breaking of rest of trees in cold regions of the world is a common phenomenon (Sakai and Larcher 1987). Research has shown that rest breaking treatments, require stress that is near the sublethal (near-lethal) point in order to be effective (Fuchigami and Nee 1987, Erez and Lavee 1974, Black 1952). Doorenbos (1953) proposed that near-lethal stress from different sources (e. g., chemicals, heat, freeze, light, drought, injury, anaesthetics, toxic substances, anoxia, etc.) are all capable of breaking the rest period in woody plants.

Recently, Fuchigami and Nee (1987) reported that the resting state of buds can be broken rapidly by exposing buds to near-lethal (NL) stresses. Nee (1986) showed that near-lethal stress treatments required to overcome the rest period caused electrolytes to leak from

cells, and stimulated ethylene and ethane production. Chemical and physical stresses applied at NL dosages overcame rest at any stage of rest.

There has been little research on the affects of breaking rest on the subsequent development of cold hardiness, and recovery of plants at different rest stages and under different environmental conditions. We hypothesize that NL- stress, which results in premature breaking of the rest, results in the loss of hardiness and recovery of the plants under warm and natural post-stress conditions. Therefore, the main objective of this study was to test the hypothesis of overcoming rest at different stages of rest with NL-stresses on the subsequent development of cold hardiness and recovery of plants under varying environmental conditions.

As we studied the relationship between rest and hardiness, we observed that plants exposed to NL-heat stress began to die when incubated at low temperature for several weeks. In contrast, the controls (without NL-stress) incubated at low temperature and NL-heat stressed plants incubated at warm temperature and natural condition all survived without injury. Thus, the second objective of this study was to determine the relationship of post-stress treatment on the recovery of plants exposed to NL-stresses.

Since glutathione, a tripepetide which is present in all higher plant cells has strong antioxidant activity, and its reduced form (GSH) has been found to be involved in the detoxification of many compounds associated with stress related effects (Alscher 1989, Rennenberg 1982), the third objective was to investigate the changes in

glutathione content of NL-heat stressed plants stored at 0° and 23°C post-environment conditions.

Ethylene production has been associated with many types of stresses in plant tissue (Lieberman 1979, Yang and Hoffman 1984). A high peak of stress ethylene usually results following stress treatments. This rise of ethylene has been proposed as being a good indicator of plants exposed to NL stresses (Field 1984, Harber and Fuchigami 1986). Kimmerer and Kozlowski (1982) showed that in paper birch water deficits, wounding, freezing and ozone exposure all enhanced ethylene production. In addition to its use as an indicator of stresses, ethylene levels has also associated with the growth status of temperate plants. Reduced ethylene production has been reported to be related to dormancy development in red-osier dogwood (Seibel and Fuchigami 1978) and coniferous species (Burr *et al.* 1985, Barbro *et al.* 1991).

The forth and fifth objectives of this study were to determine the heat tolerance and production of stress-ethylene in red-osier dogwood at different growth stages.

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2. Literature Review

2.1 Dormancy and Cold Hardiness

2.1.1 *Dormancy*

The dynamics of dormancy in woody plants has been of interest to biologists for many years (Knight 1801), and has been the subject of numerous reviews (Doorenbos 1953, Samish 1954, Romberger 1963, Wareing 1969, Perry 1971, Nooden and Weber 1978, Saunders 1978, Saure 1985). Dormancy has been described in several different ways. As defined by Doorenbos (1953), dormancy is a condition "When a tissue predisposed to elongate does not do so". Alternatively Samish (1954) has described dormancy as " the temporary suspension of visible growth" while Romberger (1963) has suggested that the term dormancy should be restricted only to meristematic tissues. In horticulture, dormancy has been recently defined as " The temporary suspension of visible growth of any plant structure containing a meristem" (Lang 1987). Lack of mitotic activity has been shown as indication of dormant stage in Douglas-fir (Owens and Moolder 1973), occurring from December through February. The term has also been defined ecologically as a survival mechanism (Saunders 1978), and a means of determining when stress resistance occur based on the absence of any mitotic activity (Lavender 1985).

The condition of dormancy consists of three basic phases. Romberger (1963) identifies the three phases of dormancy as 1) correlative inhibition 2) rest, and 3) quiescence. Correlative

inhibition occurs when buds are unable to grow because of inhibitory effects caused by apical dominance or lateral dominance (Samish 1954). This period has also been referred to as summer dormancy (Doorenbos 1953). The rest phase, also called winter dormancy (Doorenbos 1953) occurs when a bud will not grow because of the effects of some internal factor within the bud (Doorenbos 1953). Quiescence (or imposed dormancy (Doorenbos 1953)) refers to the condition when buds are inhibited from growing because of unfavorable environmental conditions (Samish 1954). Recently, the three phases of dormancy were referred to as ecodormancy (correlative inhibition), endodormancy (rest), and paradormancy (quiescence) (Lang 1987). In this review the terminology of Romberger(1963) and Lang (1987), last chapter, will be used.

Woody plants undergo seasonal changes during their annual life cycle (Sarvas 1972, 1974, Fuchigami *et al.* 1982). Fuchigami *et al.* (1982) developed a periodic growth model to quantify the annual cycle of temperate woody plants (Fig. 1.1). The model, with 360°GS (degree growth stages) consists of five physiological growth stages: 1) spring budbreak (0° GS). 2) maturity induction point (90 °GS), 3) vegetative maturity or onset of rest (180° GS), 4) maximum rest (270° GS), and 5) end of rest (315° GS). The °GS's coincide with the following growth stages: correlative inhibition (90° to 180°GS), rest (180° to 315°GS), and quiescence (315° to 360°GS) (Kobayashi 1983).

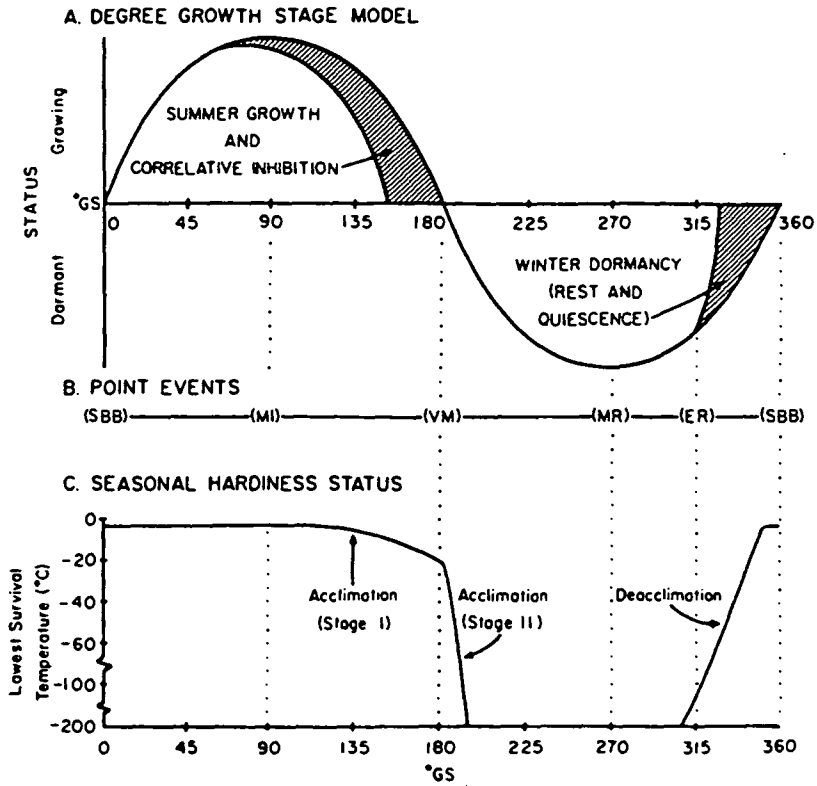

Figure 2.1 Degree growth stage model and rest breaking mechanisms in temperate woody plants (Fuchigami and Nee)

2.1.2 *Cold hardiness*

The cold hardiness of temperate woody plants has been studied by plant biologists for more than three centuries. In spite of extensive research on cold hardiness the physiological process of how plants acclimate are not known (Weiser 1970). Bobert (1683) describes the different types of damage on the various organs of the tree caused by freezing temperature (Vasilyv 1954). Senebier (1800) believed that " The tips of the branches are killed because they are not ripe" (Levitt 1941). [It is not clear whether the term ripe refers to dormancy and/or cold hardiness] .

The subject of cold hardiness has been reviewed extensively (Harvey 1918, Chandler 1954, Levitt 1956, Vasilyev 1956, Parker

1963, Weiser 1970, Alden and Hermann 1971). The term cold hardiness is defined by Alder and Herman (1971) as " the ability of plants to survive subfreezing temperatures". Cold acclimation by Weiser (1970) is defined as " the seasonal transition of plant hardiness from the tender to the hardy condition".

The development of cold hardiness in temperate woody plants, occurs in two or three stages (Fig. 1.1) (Tumanov and Krasavtsev 1959, Weiser 1970). Weiser (1970) maintains that growth cessation is a prerequisite for the first stage of acclimation. In red-osier dogwood short days and warm temperatures (Fuchigami *et al.* 1971a) triggered the development of the first stage of hardiness. The short day signal is received by the leaves which initiate production of the translocatable hardiness promoter (Fuchigami and Weiser 1971b). The short day response is mediated by phytochrome (Howell and Weiser 1970). These hardiness promotive compounds move from the leaves through the stems through phloem tissues (Fuchigami and Weiser 1971b). The second stage of acclimation is induced by low temperature (Tumanov and Krasavtsev 1959, Weiser 1970) and is independent of daylength (Fuchigami *et al.* 1982). During this stage low temperature promotes cold hardiness depending on the developmental condition of that plant (Sakai and Larcher 1987). Under natural conditions, however, one week of subzero temperature is sufficient to promote the second stage of cold hardiness (Sakai 1959, Van Hustee 1967, Tumanov *et al* 1973, Weiser 1970). The third stage of acclimation is induced by exposure to very low subfreezing temperatures. At this stage structural and physical changes occur rather than metabolic changes observed during the second stage of acclimation (Weiser 1970).

2.1.3 *Relationship between dormancy and cold hardiness*

The significance of the relationship between dormancy and cold hardiness has been described by Glerum (1976) as of " great physiological importance, but also of immense practical application" both in nursery practices and basic research.

Early work in this area has suggested a strong correlation between hardiness and dormancy. Hardy plants has been found to develop dormancy earlier than less hardy ones (Alsted 1889, Strausbough 1924, Levitt 1941, Knowlton and Dorsy 1927), The only reliable index of cold hardiness was a long rest period (Dorsy and Bushnell 1920). Some researchers reported that the correlation between dormancy and cold hardiness does not occur in all plants (Pojorkova 1924, Clements 1938, Walter 1949). Chandler (1954) reported that dormancy or "rest" is a prerequisite for cold hardiness. Others report that dormancy development is necessary in order to develop maximum cold hardiness in woody plants (Sakai 1955, Tumanov and Krasavtsev 1959, Tumanov *et al.* 1973). Van Hustee *et al* (1967), and Kobayashi and Fuchigami (1983) showed that greater cold hardiness was attained after rest in red-osier Dogwood plants. Hermann (1967) found the same relationships to be true for Douglas-fir seedlings . Maximum cold hardiness is apparently thought to depend on zero mitotic activity of bud cells (Lavender 1985). In contrast, Irving and Lanphear (1967) showed that development of cold hardiness was

independent of the bud dormancy in *Acer* and *Viburnum*. Weiser (1970) also suggested that growth cessation is also a prerequisite to cold hardiness development, rather than the occurrence of dormancy. Christersson (1978) showed that cold hardiness occurred under long days and 2°C , thus proving that the dormant stage is not necessary for the development of cold hardiness in Norway spruce.

Kobayashi *et al.* (1982, 1983) demonstrated that the rate of cold acclimation and deacclimation is dependent on the degree of rest of plants and temperature. From the onset of rest to the acquisition of maximum rest the rate of acclimation increases. During this period plants do not deacclimate, even at relatively warm temperatures, e. g., 20°C. In contrast, from late rest to spring bud-burst the rate of acclimation decreases and deacclimation increases in response to temperature. During this period the temperature range for either promoting or maintaining acclimation becomes narrower (e.g., lower temperatures are required for acclimation with later growth stages) and the temperature range for promoting deacclimation becomes wider (e. g., lower temperatures can cause deacclimation) with later growth stages.

Ritchie (1986) also found a physiological relationship between bud dormancy and cold hardiness in Douglas-fir seedling, independent of calender date. Ritchie (1986) speculated that a phenological verses a physiological relationship may exist since hardiness is controlled by short photoperiod which occurs at the same time yearly. In contrast, Cannel et al (1990) found no relationship between bud dormancy, and

cold hardiness in Sitka spruce and Douglas-fir seedlings. They observed cold hardiness development before bud dormancy, and greater cold hardiness in winter when dormancy decreased. This is regarded by the authors as " a phenological coincidence rather than a physiological relationship" between dormancy and cold hardiness.

A theoretical perspective of the relationship between dormancy and cold hardiness was described by Glerum (1976) based on work conducted by Romberger (1963). Romberger postulated that while dormancy was restricted to meristematic tissues, cold hardiness is an attribute common to all living tissues. He suggested a relationship does exists in the form of a signal, or translocatable factor, which translocates from the meristems to non meristematic regions.

Past research on the relationship between dormancy and cold hardiness indicate that the relationship of these processes may be species-specific. The specific linkage between dormancy and cold hardiness is neither clear nor well understood physiologically. Dormancy and cold hardiness may be coincidental outcomes regulated by similar environmental factors.

2.2 Breaking Rest Period in Temperate Woody Plants

2.2.1 *Natural release of rest*

The termination of the rest period in many woody plant requires a period of chilling, to overcome the rest period in plant species native to temperate zones (Doorenbos 1953, Nienstaedt 1967, Coville 1920). The duration of the chilling period and the temperature required to

overcome rest are genetically controlled (Samish 1954, Bennett 1950 Nooden and Weber 1978, Sherman *et al* 1977, Nakasu *et al* 1982).

The prerequisite of the chilling period for the termination of dormancy was first observed by Knight (1801), and subsequently studied by many researchers (Molish 1908, Coville 1920, Chandler *et al.* 1937). The effective chilling period ranges from 250 to 1000 hours (Samish 1954, Vegis 1964). Erez and Lavee (1971) indicated that 5°C is the optimum temperature to satisfy the chilling requirement in peach trees. Fluctuating temperature is thought to be more effective in satisfying the chilling requirements of deciduous fruit trees (Lyr *et al.* 1970, Samish and Lavee 1962, Erez *at al.* 1979). However, Lavender and Cleary (1974) found that fluctuating temperatures under natural condition are less effective for overcoming chilling requirements than constant temperature in coniferous species.

Chilling requirement may vary with age for a given plant species (Nienstaedt 1966). Variation within species also occurs (Sharik and Barnes 1976). Scalabrelli and Couvillon (1986) found that terminal buds require less chilling temperatures than lateral buds of 'Redhaven' peach.

Long photoperiods may partially compensate for the chilling requirement in many plant species (Olmested 1951, Nienstaedt 1966, Garber 1983). Erez *et al* (1966) found that light had a stimulating effect on budbreak only at the late stage of dormancy prior to budbreak. Erez *at al* (1966) postulated that the low temperature and photoperiod are not entirely independent, as long daylength could partially compensate for insufficient chilling. A continuous

photoperiod (24 hours) can cause the breaking of dormancy in many plant species (Wareing 1956, Kawase 1961, Downs and Borthwick 1956), while continuous dark photoperiod will cause budbreak of trees only after their chilling requirements has been satisfied (Wareing 1956).

An interuption of the chilling period by high temperature between 15° to 35°C will delay budbreak in many temperate woody plants. This process called the chilling negation (Farmer 1968, Thompson *et al.* 1975, Van Den Driessche 1975) in which high temperature can negate some of the chilling period.

2.2.2 *Breaking of rest artificially*

Artificial breaking of the rest period has been practiced since early 1900'S (Doorenbos 1953). Early attempts, of using dormancy breaking treatments, such as injury (Jost 1893), acethylene (Johanssen 1896), ether (Johanssen 1900), electric current (Bos 1907), and warm water bath (Molisch 1908, 1909) were used to force spring-flowering shrubs. Later, dormancy breaking treatments were used on temperate fruit trees grown in warm regions of the world which lack sufficient chilling temperature to overcome rest naturally (Black 1952). The amount and time of application of dormancy breaking agents, depends on the physiological growth stages, and genetic makeup of the plants (Fuchigami and Nee 1987, Erez 1987). Black (1952) indicated that most dormancy breaking treatments, should be applied at the sublethal (near-lethal) point in order to be effective. Erez and Lavee (1974) and Fuchigami and Nee

(1987) hypothesized that any treatment applied at near-lethal dosages will break rest. Near-lethal stresses from different sources (e. g., chemicals, heat, freeze, light, drought, injury, aneaesthetics, toxic substances, anoxia, etc) are all capable of inducing budbreak, but is it not known whether all stresses share the same mechanisms (Doorenbos 1953).

The commercial use of chemical breaking agents for stimulating early budbreak in subtropical regions is quite common. Oil sprays with DNOC (dinitro-o-cyclohexyl phenol) was first used by Chandler et al (1937) to overcome rest on deciduous fruit trees. Guthrie (1941) used oil spray with DNOC (dinitro-ortho-cresol) to break rest period in peaches. Samish (1945) used mineral oil with DNOC (dinitro-o-cresol) for early budbreak in apple trees. Erez et al (1971) showed that variety of chemical compounds such as DNOC, DNOC+mineral oil, thiourea, KNO3 (potassium nitrate), and growth regulators like gibberelic acid and cytokinins, are all effective in breaking rest period of peach trees. These authors state that the combination of DNOC-mineral oil, thiourea, and KNO3 was the best rest breaking agent for commercial use at that time.

Kuroi (1963) used calcium cyanamide as a rest breaking agents in grape plants. Since then, calcium cyanamide has been used widely for breaking rest in grapevine (Iwasaki 1980, Yang et al 1982, Shulman *et al.* 1983). Hydrogen cyanamide, a product of calcium cyanamide in the presence of CO2 (MacAdam *et al* 1965) has been shown to be effective in overcoming the rest period of many temperate woody plants, e.g., on grapes (Shulman *et al.* 1983, Lin *et al.* 1985, Nir *et al.* 1988), on apples, pears, and peaches (Morimoto and Kumashiro 1978,

Fernandes-Escobar and Martin 1987), on raspberry (Snir 1983), and red-osier dogwood (Nee 1986, Tanino *et al.* 1989).

Near-lethal freeze stress has also been shown to break the rest period in temperate woody plant species (Olmested 1951, Samish and Lavee 1962, Lyr *et al.* 1970, Sparks *et al.* 1976, Nee 1986: Sakai and Larcher 1987). Suare (1985) stated that breaking of rest by subfreezing temperature is not due to an acceleration of chilling requirements for rest release, but is the response of sublethal condition.

Near-lethal heat stress was first reported to be effective in overcoming rest by Molisch (1908, 1909). He placed the dormant shoots of various plants in a water bath at 40° to 50°C. Since then numerous researchers have reported that heat stress (43°-55°C) were effective in overcoming rest in several plant species (Bennett 1950, Huang and Powell 1981, Shulman 1982, Nee 1986, Tanino *at al.* 1989).

2.3 Chemical Changes During the Breaking of Rest

The mechanisms involved in the breaking of rest by near-lethal stress in temperate woody plants have been associated with many factors. Early investigations by Fischer (1891) indicated that high level of organic substances, during rest will change during active growth (Doorenbos 1953). High sugar content resulting from the conversion of starch to sugar also has also been associated with the breaking of dormancy (Coville 1920). The high sugar content was

thought to increase the osmotic pressure of cells causing buds to overcome rest. Doorenbos (1953) suggested that high sugar content is the result and not the cause of dormancy release and is unrelated to breaking of rest. Muller-Thurgua (1885) (Doorenbos 1953) demonstrated that a rise in respiration occurs during budbreak. The increase in respiration at the time of budbreak has been reported by others (Buttler and Landsberg 1981, Wang *et al.* 1985, Hosaki 1984).

The effect of chilling temperature on overcoming rest has been related to membrane changes (Doorenbos 1953), and more specifically, to changes in membrane lipids (Raisin 1980). A change in the permeability of membrane during chilling was first reported by Coville (1921). Coville (1920) had suggested that the sudden increase of respiration was associated with necrotic tissue.

Low oxygen activity, has been postulated by Boresch (1928) as a mechanism involved in breaking of rest (Doorenbos 1953). Oil sprays, used to break dormancy caused the temporary restriction of oxygen (Samish 1954). Erez *at al.* (1980) also showed that low oxygen activity stimulated bud break in "Redheaven" peach trees.

The role played by enzymes in breaking dormancy by near-lethal stress is still not clear. Nir *et al.* (1984) reported that a reduction of catalase activity was responsible in breaking of rest by near-lethal stresses in grape plants. The reduction in catalase activity caused the stimulation of hydrogen peroxide which resulted in the acceleration of the pentose phosphate pathway (Nir *et al.* 1984).

Wang and Faust (1988) found that a change in the accumulation of fatty acids and a decrease in free sterol in apple buds during dormancy release occurs in the presence of thidiazuron (a plant

bioregulator which used to stimulate bud break). They also found nitroguanidines, a synthetic cytokinin, changed the sterol composition, increased both galactolipids and phospholipids, and the changed ratio of unsaturated fatty acids, in apple buds during the breaking of dormancy (Wang and Faust 1989a ,1989b).

The role of plant hormones in the breaking of dormancy is still obscure. Abscisic acid (ABA) is a hormone naturally occurring has been associated with the development of dormancy. Generally, ABA levels has been shown to decrease prior to budbreak (Wood 1983, Harrison and Saunder 1975, Seely and Powell 1981). Uyemura and Imanishi (1987) found ABA levels to decrease in freezia corm during the release of dormancy. In contrast, Phillips and Hoffman (1979) did not observe any changes in the level of ABA in terminal buds of *Acer*, even after bud break had occurred. Similarly, Wang *et al.* (1987) reported that the breaking of dormancy with thidiazuron did not change the ABA levels of treated buds.

The content of gibberellins (GA) has been reported increase during chilling (Walser *et al.* 1981). The increase in GA has been associated with budbreak following the satisfaction of the chilling requirement (Paiva and Robitaille 1978). Wood, however, (1983) found low levels of GA both prior to and following budbreak.

Cytokinins has also associated with the breaking of the dormancy and budbreak. Hewett and Wareing (1973) observed a peak in cytokinin levels two weeks prior to budbreak in poplar trees.. Borkorwska (1976) found a cytokinin high activity in March, at the time of bud swell in apple buds. However, Young (1989) showed a sharp decrease in cytokinin coincident with budbreak in apple buds.

Auxin levels during dormancy have been reported to be low during dormancy and has increased prior to budbreak in pecan (Wood 1983). Powell (1987) indicated that it is unlikely that auxin plays any role in bud dormancy.

Reduced ethylene production has been reported to be related to dormancy development in red-osier dogwood (Seibel and Fuchigami 1978) and coniferous species (Burr *et al.* 1985, Barbro *et al* 1991). Nee (1986) observed high ethylene production when resting buds of red-osier dogwood plants were broken by hydrogen cyanamide. Ethylene has also been used to break dormancy in gladiolus corms (Hoski 1984). In contrast, Paiva and Robitaille (1978) found no relationship between ethylene and budbreak in apple. Others have also suggested that ethylene actually plays an inhibitory role in dormancy release (Burg 1968, Liberman 1979). Saure (1985) indicated that dormancy breaking agents, act by removing the inhibition caused by ethylene.

The role of polyamines in the breaking of dormancy is not clear. Polyamines (spermine and spermidine) compete for the same substrate S-adenosyl methionine (SAM) that is used in ethylene biosynthesis (Slocum *et al* 1984). Ket and Romani (1988) showed that polyamines reduce ethylene levels. Polyamine levels where found to be low during dormant period and increased with budbreak (Wang 1985).

Glutathione, a tripepetide which is present in all higher plant cells (Rennenberg 1982, 1987), has strong antioxidant activity against free radicals (Kosower and Kosower 1978, Alscher 1989), and act as a substrate for dehydroascorbate reductase (Kappus 1985). The

reduced form of glutathione (GSH) reacts with free radicals to form the oxidized form (GSSG). Reduced glutathione has been found to be important for detoxification of many compounds produced as a result of stress, such as chilling (Wise and Naylor 1987), water stress (Burk *et al.* 1985), frost and salinity (De kok and Oosterhius 1983), herbicides (Martinge and Scalla 1988), ozone (Alscher and Amthor 1988).

The relationship of glutathione with dormancy and cold hardiness is still not well known. There has been an increase of glutathione content during winter in both *Picea abies* (Esterbauer 1978) and red-osier dogwood (Guy *et al* 1984, Siller 1991).

Fuchigami and Nee (1987) have postulated that glutathione, is involved in the breaking of the rest. Glutathione applied by stem injection was successful in breaking the dormancy in plants (Bennett and Skoog 1938, Guthrei 1940). Fuchigami and Nee (1987) proposed that glutathione acts as a strong reducing agent and protects the deactivation of proteins by reacting with oxidizing agents and or free radicals. They showed that hydrogen cyanamide, a rest breaking agent can bind with the glutathione thiol group non enzymatically.
Siller (1991) reported that the glutathione (GSH and GSSG) content decreased during onset of the rest, increased at maximum rest, and only GSH levels decreased at quiescent stage in 'Redhaven' peach.

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3. "Near-Lethal" Stress Effects on Dormancy and Cold Hardiness

3.1 Abstract

The effects of overcoming rest by application of "near-lethal" (NL) stress treatments on the subsequent development of cold hardiness of stem tissues at different rest stages under three post-environments were studied. Shoots of container grown red-osier dogwood, *Cornus sericea* L., plants were subjected to NL- heat stress, 47°C for one hour, during October, November and December (1988) and October and December (1989, 1990) and then placed into the following post-environment treatments (PET): constant 0° and 23° and natural conditions.

Regrowth tests to measure the degree of rest indicated that the NL-heat treatment overcame rest of the plants at early (October) and late (December) stages of rest. At 23° PET, NL-heat treatment during October caused all plants to break buds within 28-35 days(1988), 22-30 days (1989), and 27-35 days (1990), respectively. NL-heat stress applied during December caused all plants to break buds within 10-12 days(1988,1989), and 8-12 days (1990) after treatment. NL-heat treatment in November (1988), during deep rest, caused only 10% earlier budbreak of the plants. At 0°C PET, plants died after 9 weeks. Under natural conditions, bud break of NL-heat and control plants treated in October and Novomber (1988), and October (1989, 1990) broke buds at the same time. NL-heat treatment in December resulted in 50-55 days (1988, 1989, 1990), earlier budbreak than control plants under natural PET.

In 1988, the LT_{50}, the temperature at which 50% of the tissues were killed, of NL-heat stressed and non-heat stressed plants at early stage of rest was similar following incubation at both 23° and natural PET. At maximum rest NL-heat treatment caused faster deacclimation at 23° PET and had no effect on acclimation under natural PET. At late rest NL-heat treatment caused earlier deacclimation in both 23° and natural PET. In October, 1989 and 1990, the hardiness of NL-heat stressed plants was less than the non-heat stressed plants at 23° PET, but was similar to the non-heat stressed plants at natural PET. In December, 1989 and 1990, NL-heat treatment caused earlier deacclimation at both 23° and natural PET.

To compare the effect of other stresses on overcoming rest and cold acclimation, resting plants in October, 1988 and December, 1989, were exposed to either no stress (controls), NL-heat (47°C 1hr), freezing temperature (FT) (-7°C, 1hr), or Hydrogen cyanamide (HC), 1 M (October), 0.5 M (December). In October the LT_{50} of the NL-heat and NL-HC treatments after 3 weeks of 23°C PET was similar to the controls, whereas the LT_{50}, of the NL-FT treatment was enhanced after 3 weeks 23°C PET. After 12 weeks the LT_{50} of the of NL-heat stress and NL-HC was similar and occurred at a higher temperature than the control. In December the NL-heat stress was less hardy than the other treatments after 3 weeks. NL-stressed plants deacclimated faster after 12 weeks 23°C PET than the none stressed controls.

Incubation of NL-heat stressed plants at 0°, 5°, 10°, 15°, and 23°C PET in Oct. 20, 1989 caused progressively later budbreak

from 23°C PET (25-30 days), 15°C PET (34-42 days), to 10°C PET (53-58 days), respectively. At 0° and 5°C PET NL- heat stressed plants died after 8 weeks PET. All NL-stressed and non-stressed plants gained hardiness from 3 to 12 weeks at 10°, 15°, and 23°C PET, respectively.

These findings provide possible explanations for differences in acclimation and deacclimation of woody plants previously exposed to NL-stresses, and proves that the stem hardiness is independent of rest period. In addition, the breaking of rest by NL-stresses throughout the rest period may explain the reason for the failure of dormancy models, e.g. chill unit models, predicting the development and release of rest particularly in areas exposed to NL stress conditions.

3.2 Introduction

Woody plants undergo seasonal changes during their annual life cycle (Sarvas 1972, 1974, Fuchigami *et al* 1982). Dormancy and cold hardiness are protective mechanism in temperate woody plant species that allow them to survive stressful conditions. The significance of the relationship between dormancy and cold hardiness has been described by Glerum (1976) as of " great physiological importance, but also of immense practical application" both in commerce and research. However, an understanding of the physiological linkages between dormancy and cold hardiness is not well understood.

Early work on dormancy and cold hardiness relationship by Dorsy and Bushnell (1920) suggested that the only reliable index of cold hardiness was a long rest period. Others maintain that dormancy or "rest" is a prerequisite for cold hardiness (Chandler 1954), and that maximum cold hardiness development in woody plants is related to dormancy (Sakai 1956, Tumanov *et al* 1959, Tumanov *et al* 1973). Development of cold hardiness has been shown to be independent of bud dormancy in *Acer* and Viburnum species (Irving and Lanphear 1967). Greater cold hardiness in red-osier dogwood was obtained after rest (Van Hustee *et al.* 1967, Kobayashi and Fuchigami 1983), or just prior to the completion of the rest in peach buds (Hatch and Walker 1969). Low temperatures have been reported to cause growth cessation and induce acclimation without dormancy development (Van Hustee *et*

*al.*1967, Irving and Lanphear, 1967, Howell and Weiser 1970). Weiser (1970) suggested that growth cessation is a prerequisite to cold hardiness development, rather than the occurrence of dormancy.

Kobayashi *et al.* (1982,1983) demonstrated that the rate of cold acclimation and deacclimation is dependent on the degree of rest of plants and temperature. From the onset of rest to the acquisition of maximum rest the rate of acclimation increases and plants do not deacclimate, even at relatively warm temperatures, e. g., 20°C. In contrast, following the end of rest to the time of bud-burst the rate of acclimation decreases and deacclimation increases in response to temperature. During this period the temperature requirement for either promoting or maintaining acclimation becomes narrower (e.g.,. lower temperatures are required for acclimation with later growth stages) and the temperature range for promoting deacclimation becomes greater (e. g., lower temperatures can cause deacclimation) with later growth stages. These studies showed that the physiological status of the plant during winter was dynamic and influenced acclimation and deacclimation.

Fuchigami and Nee (1987) showed the resting state of buds can be broken rapidly by exposing the bud to near-lethal (sub-lethal) stresses. Erez and Lavee (1974) hypothesize that NL- stress treatment will break rest. Near-lethal stresses from different sources e. g. chemicals, heat, freeze, light, drought, injury, anaesthetics, toxic substances, anoxia, etc., are all capable of breaking rest (Doorenbos 1953). The NL-stress treatments

required to overcome rest causes increased electrolyte leakage from cells and stimulates ethylene production (Nee 1992). Chemical or physical stress, applied at NL dosages, can overcome rest at any stage, but there is not extensive research on the effects of breaking dormancy on the subsequent development of cold hardiness of plants. Tanino *et al.* (1989) showed that both hot water (47°C for one hour) and hydrogen cyanamide (2 M) treatments resulted in early budbreak and loss of hardiness at 250°GS in red-osier dogwood after three weeks of incubation in a warm greenhouse.

The objective of this study was to investigate the relationship of breaking rest at different stages of rest by NL-stresses on the subsequent development of cold hardiness under varying post-environmental conditions.

3.3 Materials and Methods

Plant Materials

A clone of red-osier dogwood, *Cornus sericea* L. from Wayland, MA, was used. These clone of dogwood was selected because previous work had established the relationship of stages of rest to cold hardiness in response to natural conditions at Corvallis, Oregon (Kobayashi *et al.* 1982). In addition, a companion study had shown that dogwood plants exposed to NL stress did not recover at low temperatures (Shirazi 1992). The specific growth stages were determined by °GS model (Fuchigami *et al.* 1982, Kobayashi 1983). The plants were propagated by single stem cutting in February. Rooted cuttings were transplanted into a mixture of sterilized bark, peat and soil (1:1:1) in 15 cm fibre pots, and grown in a lathhouse with 20 % shade. Plants were irrigated by overhead system twice daily (6 AM & 3 PM). Osmocote (Sierra Co.), a slow-release fertilizer (17-6-9) with micronutrients was applied at a rate of 4 grams per pot. Plants were trained to one stem in June & July. They were between 70 to 90 cm tall at the time of the experiments.

Establishment of Near-Lethal heat stress

Near-lethal heat stress treatment was determined by exposing internode (2nd, 3rd, and 4th) stem sections of dogwood, harvested on 18 Oct.to 40, 45, 47, 50, and 55°C for 0, 30, 60, and 120

minutes in a circulating water bath. The experiment was repeated twice (n=10). One hour after treatment the stem sections (2.5 cm) were devided into two groups. One group was placed in individual 10 cm petri dishes containing two sheets of moistened filter paper, incubated in the dark at 23°C, and evaluated visually for tissue death after 2 weeks. The second group of samples was evaluated for electrolyte leakage with a Markson Conductivity Meter. Each sample was placed in 15-ml of double-distilled water, shaken for 36 hours and the initial conductivity was taken. Samples were killed in a hot water bath at 80°C for 1.5 hour, and shaken for 24 hours, and final conductivity measurements were taken. Percent ion leakage was calculated by dividing the initial measurement by the final measurement and multiplying this by 100. The data is expressed as percent (%) electrical conductivity of each treatment (Fig. 3.1).

Near-lethal stress, was defined as any stress causing a slight increase in electrolyte leakage (~ 25%) compared to non-stressed samples (~ 13-14%) without apparent permanent damage to plant cell. Based on the above studies NL stress was obtained by exposing plants tissues to 47°C for one hour in a circulating water bath. Exposure of intact plants to NL-heat stress was done by completely enclosing the top portion of the plant in a 4 mil polyethylene bags, evacuating the air from the bag, and immersing the tops into the water. After immersion the plants were kept for approximately 24 hours at room temperature prior to post-treatment.

Determination of cold hardiness

Cold hardiness was determined using artificial freezing tests. Ten NL-heat stress and 10 control plants were randomly selected from each PET. Two centimeter stem segments were cut from the second, third, and fourth internodes and placed in 10- by 75-mm glass vials containing 0.5 ml double distilled deionized water. Samples were placed in a circulating refrigerated ethylene glycol bath (Model LT-SODD, Neslab Inc. Newington, N.H.). After 30 minutes at -1° the samples were nucleated with ice crystals and kept overnight at -1°C . The temperature was then lowered at 5°C per hour to the following test temperatures: -5°, -10°, -15°, -20°, -25°, -30° and -35°C The samples were held at each test temperature for 1 hour, removed, and thawed, at 4°C for 24 hours. Tissue viability was determined by electrical conductivity of each sample (n=5) as reported earlier. The LT_{50}, the temperature at which 50% of the tissues were killed, was calculated by first fitting a 3rd order polynomial through the data, and using the 50% midpoint to observe the LT_{50} point.

Post-temperature treatment effects on rest and cold hardiness

The effects of NL heat stress on rest and cold hardiness of plants at three PET (0°C and 23°C incubation in the dark, and natural conditions in the lathhouse at Corvallis, Oregon) were studied at different stages of rest (20 Oct., 9 Nov., and 5 Dec., 1988; 28 Oct., and 7 Dec.,1989 ; and 20 Oct., and 5 Dec., 1990).

Rest status of each plant was determined at 3 days intervals. Cold hardiness was determined after 3, 12, and 21 weeks of PET in 1988 and 3 and 12 weeks of PET in 1989 and 1990, respectively . Ten plants were used per treatment. The daily maximum and minimum temperatures in the lathhouse for 1988-1989, 1989-1990, and 1990-1991 are shown in Fig. 3.2.

Effect of different forms of NL-stresses on rest and cold hardiness

NL-freezing, hydrogen cyanamide, and heat stresses were used to determine the universal nature of NL-stress effects on rest and cold hardiness. On 20 Oct. and 5 Dec. 1990 dogwood plants were treated with either no stress (control), NL-heat (47°C for 1 hr), NL-freeze (-7°C for 1 hr), or NL-hydrogen cyanamide (1M in Oct, and 0.5M in Dec painted completely over the stem tissues). After treatment the plants were divided into two groups: one group of plants (n=30 for each treatment) was placed into either 0° or 23°C darkened PET to determine its effect on cold acclimation, and a second group of plants (n=30 for each treatment) was placed in a greenhouse (natural photoperiod and 24°/18°C (day/night) to determine the time of budbreak, a measure of the rest status of plants (Fuchigami, et al 1982). The treatments were arranged in a completely randomized design. The plants were observed every 3 days for bud break. Cold hardiness was determined as above, after 3, 12, and 21 weeks of PET.

Effect of different PET temperatures on rest and cold hardiness

To test the effect of NL stress on the development of rest and cold acclimation at different PET temperatures dogwood plants exposed to NL-heat stress (47°C , 1hr) and non-stressed (controls) were placed at 0°, 5°, 10°, 15°, and 23°C PET in the dark. Plants were observed for budbreak every 3 days. Cold Hardiness was determined as previously described after 3 and 12 weeks.

Statistical Analysis

Analysis of variance (ANOVA) for a completely randomized design was used for all experiments. Standard errors were used for all the studies illustrated with bar graphs. Statistical analysis was performed using SAS software (SAS Institute, 1988).

3.4 Results

Stress effects on electrical conductivity

Mean electrolyte leakage (EC) of the control, at zero exposure time, and at 40°C at all exposure times was approximately 13-14% (Fig. 3.1). The % EC of the 45-47°C and 50-55°C treatments exposed for 30-120 minutes averaged between 22-27%, and more than 48%, respectively. Visual browning of the stem tissues after two weeks incubation in a dark at 23°C +/- 3°C indicated that the stems exposed to 50 and 55°C were severely injured or dead, whereas the other treatments at the lower temperatures survived.

Post-temperature treatment effects on rest

NL-heat stressed plants stored at 0°C PET in October (1988, 1989,1990), died prior to 9 weeks of 0°C PET. None of the plants from the other treatments showed any signs of plant injury. In November and December,(1988, 1989, 1990) NL-heat stress treatments caused partial stem dieback at 0° PET after 12 and 15 weeks respectively. None of the plants from the other treatments were injured (Shirazi 1992).

Regrowth tests to measure the degree of rest indicated that the NL-heat treatment overcame rest during October and December. At 23° PET, NL-heat treatment during October caused all plants to break buds within 28-35 days (Table 3.1a 1988), 22-30 days (Table 3.2, 1989), and 27-35 days (Table 3.3,1990). NL-heat

stress applied during December caused all plants to break buds within 10-12 days (Table 3.1c, and Table 3.2 ; 1988,1989), and 8-12 days (Table 3.3,1990) after treatment. During November NL-heat stress caused only 10% earlier budbreak of the plants (Table 3.1b).

Under natural conditions, bud break of NL-heat and control plants treated in October and November (1988), and October (1989, 1990) broke buds at the same time. In contrast, NL-heat treatment in December resulted in 50-55 days (Table 3.1c, Table 3.2, and Table 3.3; 1988, 1989, 1990) earlier budbreak than control plants under natural PET.

NL-heat stress on hardiness development

LT_{50} of NL-heat stressed and non-heat stressed plants in October 1988 was similar following incubation at both 23° and natural PET (Fig 3.3). In November1988 NL-heat treatment caused faster deacclimation at 23° PET and had no effect on acclimation under natural PET(Fig 3.4). In December 1988 NL-heat treatment caused earlier deacclimation in both 23° and natural PET (Fig 3.5).

In October 1989, the NL-heat stressed plants were less hardy than the non-heat stressed plants at 23° PET (Fig. 3.6), but was similar under natural PET (Fig 3.6). In December1989, NL-heat stressed plants were less hardy than non heat stressed plants, after 3 weeks at 23° PET (Fig 3.7), and after 12 weeks at natural PET (Fig 3.7). In October 1989 and Dec. 1990 the effect of NL-heat

stress on acclimation were similar to the previous year (Fig. 3.8 and 3.9)

Other NL-stress effects on rest and cold hardiness

All NL-stress treatments (heat, freezing, and hydrogen cyanamide) caused faster budbreak, within 6-14 days (October) and 7-16 days (December) from the treatment date, than the control plants, which took 220-240 days (October) and 20-25 days (December) to budbreak from the treatment date (Table 3.4). Fig. 3.12 shows the the effects of different NL-stresses at early rest (20 Oct. 1990) on budbreak after 3 weeks incubation in a warm greenhouse (18°/24°C night/day).

In a companion paper we reported that NL heat, freezing and hydrogen cyanamide treatments all resulted in significant dieback when plants were incubated at 0°-dark post-treatment (Shirazi 1992). Dieback caused by freezing and hydrogen cyanamide was similar, but less than the injury caused by NL-heat stress. In October the hardiness of the NL-heat and NL-HC treatments after 3 weeks of 23°C PET were similar to the controls (Fig.3.10), whereas the hardiness of the NL-FT treatment was significantly greater than the other treatments after 3 weeks 23°C PET (Fig 3.10). After 12 weeks the hardiness of the of NL-heat, NL-FT and NL-HC were similar but less than the control (Fig. 3.10). After 21 weeks, the controls, NL-heat, NL-HC all deacclimated completely, while , NL-FT plants retained some hardiness (Fig. 3.10) In December the hardiness of NL-HC and NL-FT were similar to the controls, and greater than the hardiness of NL--heat stressed plants at 23°C PET.

By the 12 weeks of 23°C PET all treatments deacclimated completely (Fig. 3.10).

Effect of different PET temperatures on rest and cold hardiness

The post-environment temperature had a significant effect on time of budbreak of NL-heat stressed plants. The time required for budbreak decreased with increasing PET temperature, e. g., 25-30 days at 23° PET, 34-42 days at 15°C PET and 53-58 days at 10°C. Control plants did not have budbreak by 150 days. Plants incubated at 0° and 5°C PET did not have break buds and were dead after 7-8 weeks (Table. 5).

The effects of NL-heat stress on hardiness was dependent on the exposure time and PET temperature. After 3 weeks PET, the hardiness of the NL-heat treatments at 0° and 23°C PET was less than the controls, and at 5°, 10°, and 15°C were similar to the controls (Fig. 3.11). After 12 weeks PET, the only difference in hardiness between the NL-heat and controls occurred at 23°C where the NL-heat treatment was slightly less hardy than the controls.

3.5 Discussion

As reported previously NL-stresses are capable of overcoming rest in plants (Nee 1986, Fuchigami and Nee 1987). In this study NL-heat, -freeze and -hydrogen cyanamide treatments were all successful in overcoming rest in red-osier dogwood plants (Table 3.1, 3.2 3.3, 3.4, and 3.5). The time of the budbreak following the NL-stress treatment was a function of PET temperature, e.g. faster at warmer temperatures. Under natural conditions, when rest was broken in October and November by NL-stress, the time of bud break was the same as the untreated controls. In Dec., however, breaking rest with NL-heat stress caused the plants to broke buds about 50-55 days earlier than the controls. The reason for these differences is not known.

Although rest may be broken at any stage the resulting growth depends on the time of budbreak. Fuchigami and Nee (1987) described two stages of rest, bud release and stem elongation stages. The bud release stage occurs between 180° to 315°GS and the elongation stage occurring after 280°. The first stage appears to be localized within the bud while the second phase is not. NL-stress can overcome the bud release phase and not the elongation phase of rest. In our studies NL-stresses applied during October and November (200° and 270°GS) resulted in bud release and short (rosette-like) stem elongation, and application in December resulted in bud release and normal stem elongation , e.g. similar to elongation of buds during spring growth (data not shown). According to Fuchigami and Nee (1987) the requirement for stem elongation in

the same clone of dogwood was satisfied by December and not during October and November. Fuchigami and Nee (1987) attribute the shoot elongation at later stages of rest to the effect of cytokinin and/or other growth promoters. Whether the breaking of the elongation phase of rest had any bearing on the time of bud break under natural conditions is not known. Perhaps the release of the bud release phase of rest with NL stress in December activated the stem elongation phase thus causing growth to occur 50-55 days earlier under natural conditions. Kobayashi and Fuchigami (1983) also reported that the temperature range for overcoming quiescence increases with later growth stages after the chilling requirement is satisfied. Breaking of rest after the requirement for stem elongation was satisfied may have advanced the stage of development and thus increased the temperature range for growth.

The fact that NL-stresses in general are capable of breaking rest perhaps provide an explanation for the reasons why chilling models used to predict the end of the rest do not work satisfactorily under some environments. Our studies suggest that rest may be broken at any stage of rest by exposure to NL-stress. The fact that all three forms of NL-stress treatments were successful in overcoming rest implies that stresses in general may be universally involved in the breaking of rest. Siller (1991) also provide evidence that chilling temperatures may be a form of stress that causes the breaking of rest. He showed that chilling and NL-heat stresses causes increase in glutathione levels consistent with the breaking of rest in peaches. Chilling causes slow accumulation of glutathione while NL-stresses causes rapid increases in glutathione content. These studies

suggests that consideration of the plants exposure to stresses during rest development may be important in improving chilling models to predict the development of rest in temperate plants.

During maximum rest the NL-heat stress treatment was not completely successful in overcoming rest in all plants. Later studies indicated that plants at maximum rest required more stress to break rest (Shirazi 1992).

The relationship of dormancy and hardiness has been studied by numerous researchers (Irving and Lanphear 1967; Weiser 1970 ; Glerum 1976 ; Kobayashi 1983 ; Cannel 1990). In previous studies we have shown that the onset of hardiness under natural conditions in red-osier dogwood occurs at the beginning of the rest, 180 °GS, and the rate of the acclimation and deacclimation was a function of the stage of development (Kobayashi, 1983) . The rate of acclimation increases with later stages of rest reaching a peak near maximum rest, 270 °GS. Under natural conditions maximum hardiness is acquired after the rest period is complete, e,g, after 315° GS. Deacclimation begins during the late-rest phase, ~300 °GS, and the temperature range and rate increases with later stages of development (Kobayashi *et al.* 1983).

Our study suggests the development of cold acclimation and deacclimation may be altered by NL-stress treatments, however, the effect on hardiness was a function of the physiological status of the plant. When NL-heat stress is applied from early rest, e.g., 200 °GS, to maximum rest, 270 °GS, the development of stem hardiness was not affected under warm and natural PET in 1988, However, in 1989 and 1990 stem hardiness were slightly affected by NL-heat

stress at 23°PET only. If NL-heat stress is applied during the late-rest stage, e.g. 300 °GS, the loss of hardiness was significantly faster than the controls under both warm and natural conditions. Perhaps these studies suggest that the advancement of both bud and stem development is necessary for deacclimation of stem tissues and implies that the resting state of buds per se has no bearing on the overall hardiness of the plants. These results confirm the report by others that cold acclimation is independent of the resting bud (Cannel *et al.* 1990).

The observation that the NL-freeze stress treatment on plant hardiness was different from those for NL-heat and NL-cyanamide stress was interesting. All three stress treatments overcame rest, however, the NL-freeze treatments in October caused the plants to rapidly increase in hardiness after 3 weeks of warm PET (23°). In contrast, the hardiness of the NL-heat and cyanamide treatments were similar to (23° PET) than the controls. By the 12th week of PET the hardiness level of all three NL-stress treatments were the same and slightly less than the controls. In December treatments, again we observed a faster rate of deacclimation for all the treatment , which could be an indication of the universal effect stresses on acclimation and deacclimation. These results suggests that the NL-freeze treatments had the same effect in overcoming rest in buds as other stresses, however, its effect on hardiness in the October treatment was different. Weiser (1970) reported that the second and third stages of acclimation were promoted by low temperatures and that frost at these stages enhanced its development. Our study suggests that the enhancement of the second and third stages is

unique to low temperatures and can not be triggered by all stresses. The cause of this difference is not known.

The theoretical perspective of the relationship between dormancy and cold hardiness has been described by Glerum (1976) based on work conducted by Romberger (1963) who postulated that while dormancy was restricted to meristematic tissues, cold hardiness is an attribute common to all living tissues. Glerum (1976) suggested that relationships may exist in the form of a signal, or translocatable factor, which translocates from meristems to non meristematic regions. Cannel *et al.* (1990) regard the relationship between dormancy and hardiness as " a phenological coincidence rather than a physiological relationship". We do not know the relationship between bud and cambium dormancy. The fact that the time of application with regard to stage of development influenced the growth and hardiness of the plants suggests that the physiological development of the bud and stem tissues are important.

The interaction of NL-stresses and post-temperature effects on plant dormancy and cold hardiness may be of significant importance to plant survival. It is likely that the unexplained dieback of plants observed at high elevations in several countries may be due predisposition to the NL-stresses and the post-temperature interactions reported in this paper and an earlier paper (Shirazi 1992). This study shows that bud dormancy (rest) and cold hardiness of stem tissues are independent processes, and the effects of overcoming rest on hardiness development was a function of the physiological state of the plant. A significant implication of these findings to agriculturalists, ecologists and others are the possible

strategies needed to protect plants predisposed to NL-stresses, particularly in cold regions.

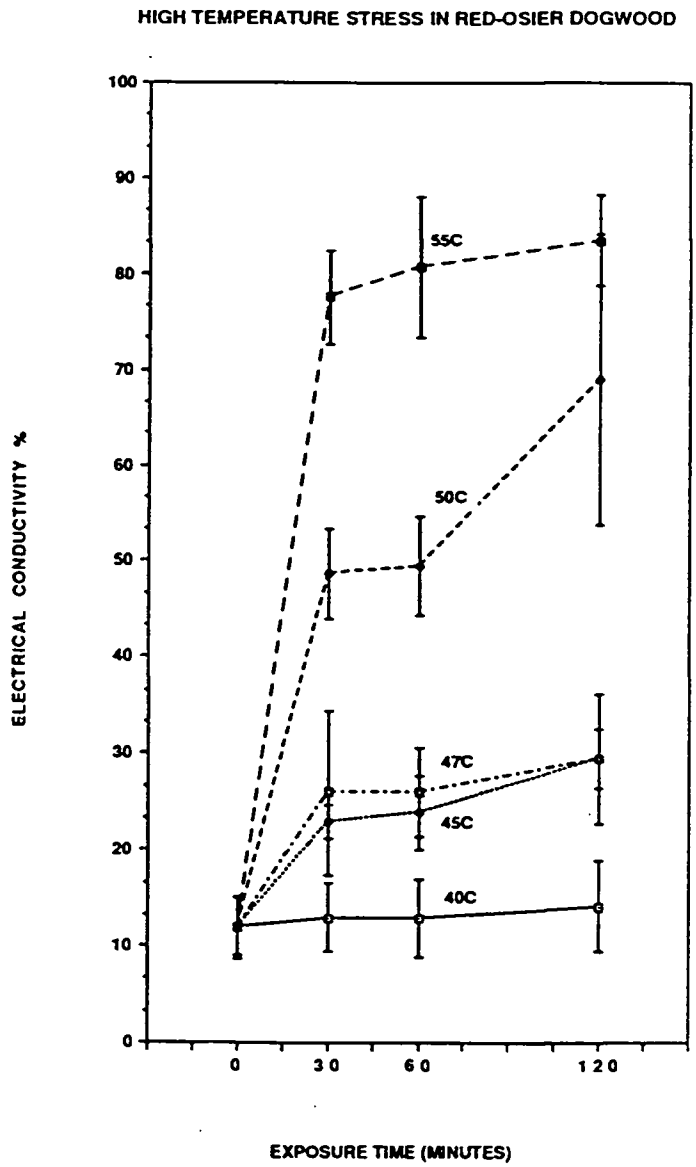

Figure 3.1 Determination of NL heat stress (40°, 45°, 47°, 50°, and 55°C) of internode stem segments of red-osier dogwood following 0, 30, 60, and 120 minutes exposure time.

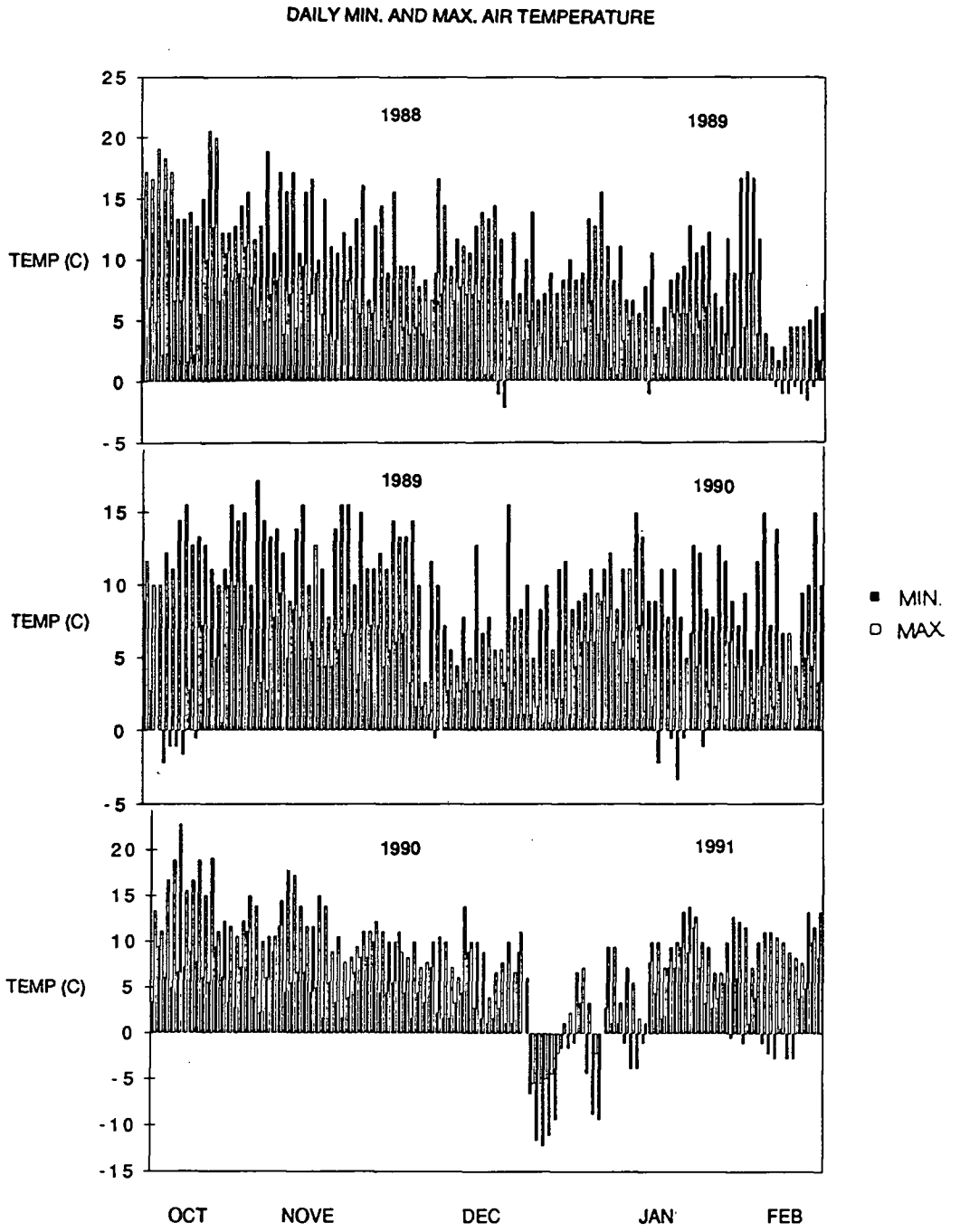

Figure 3.2 Daily minimum and maximum air temperature in the lathhouse from late October to early February for 1988-89, 1989-90 and 1990-91 in Corvallis, Or (44°, 37', 59" N and 123°, 11', 32" W).

Table 3.1 (a, b, c) Effects of NL-heat stress (47°C for one hour) on of red-osier dogwood (*Cornus sericea* L.) plants at three growth stages (20 Oct. 1988, 220°GS; 9 Nov. 1988, 270°GS; and 5 Dec.1988, 300°GS.) and three post-environment treatment (PET) (0°C, 23°C and natural condition at Corvallis, Or).

Dormancy Stages	Treatment	Storage	Days to Bud Break
October 20, 1988 (EARLY)	Control (Unstressed)	23°C	>150 days
		0°C	No Bud Break
		Natural Condition	130-135 days
	Heat Stressed	23°C	28-35 days
		0°C	No Bud Break
		Natural Condition	130-135 days

TABLE a

Dormancy Stages	Treatment	Storage	Days to Bud Break
November 9, 1988 (DEEP)	Control (Unstressed)	23°C	>150 days
		0°C	No Bud Break
		Natural Condition	110-115 days
	Heat Stressed	23°C	* 10-12 days
		0°C	No Bud Break
		Natural Condition	110-115 days

TABLE b

* Only 10% of plants had bud break.

Dormancy Stages	Treatment	Storage	Days to Bud Break
December 5, 1988 (LATE)	Control (Unstressed)	23°C	110-125 days
		0°C	No Bud Break
		Natural Condition	85-90 days
	Heat Stressed	23°C	12-15 days
		0°C	No Bud Break
		Natural Condition	30-35 days

TABLE c

Dormancy Stages	Treatment	Storage	Days to Bud Break
October 28, 1989 (EARLY)	Control (Unstressed)	23°C	>150 days
		0°C	No Bud Break
		Natural Condition	125-133 days
	Heat Stressed	23°C	22-30 days
		0°C	No Bud Break
		Natural Condition	125-133 days
December 7, 1989 (LATE)	Control (Unstressed)	23°C	30-35 days
		0°C	No Bud Break
		Natural Condition	90-95 days
	Heat Stressed	23°C	10-12 days
		0°C	No Bud Break
		Natural Condition	30-35 days

Table 3.2 Effects of NL-heat stress (47°C for one hour) on budbreak of red-osier dogwood (*Cornus sericea* L.) plants at two growth stages (28 Oct., 1989, 220°GS and 7 Dec.,1989, 300°GS) and three post-environment treatment (PET) (0°C, 23°C and natural condition at Corvallis, Or).

Dormancy Stages	Treatment	Storage	Days to Bud Break
October 20, 1990 (EARLY)	Control (Unstressed)	23°C	>150 days
		0°C	No Bud Break
		Natural Condition	115-120 days
	Heat Stressed	23°C	27-35 days
		0°C	No Bud Break
		Natural Condition	115-120 days
December 5, 1990 (LATE)	Control (Unstressed)	23°C	35-45 days
		0°C	No Bud Break
		Natural Condition	70-75 days
	Heat Stressed	23°C	8-12 days
		0°C	No Bud Break
		Natural Condition	18-25 days

Table 3.3 Effects of NL-heat stress (47°C for one hour) on budbreak of red-osier dogwood (*Cornus sericea* L.) plants at two growth stages (20 Oct., 1990, 220°GS and 5 Dec., 1990, 300°GS.) and three post-environment treatment (PET) (0°C, 23°C and natural condition at Corvallis, Or).

TREATMENT	Days to BUDBREAK	
	OCTOBER	DECEMBER
Control	220-240 days	20-25 days
Heat (47°C)	6-10 days	7-10 days
Freeze (-7°C)	8-12 days	12-14 days
Hydrogen Cyanamide (1M October, .5M December)	8-14 days	10-16 days

Table 3.4 Effects of different NL-heat (47°C for one hour), freeze (-7°C for one hour), and hydrogen cyanamide (1M October1988., and 0.5 December1989) stress on budbreak of red-osier dogwood (*Cornus sericea* L.) plants at greenhouse (24°/18°C ; day/.night) post- environment treatment (PET).

POST-ENVIRONMENT TREATMENT	TREATMENT	Days to BUDBREAK
OC	CONTROL	NO BUDBREAK
	HEAT	NO BUDBREAK
5C	CONTROL	NO BUDBREAK
	HEAT	NO BUDBREAK
10C	CONTROL	>150 Days
	HEAT	53-58 DAYS
15C	CONTROL	>150 Days
	HEAT	34-42 DAYS
23C	CONTROL	>150 Days
	HEAT	25-30 DAYS

Table 3.5. Effects of NL-heat stress (47°C for one hour) on budbreak of red-osier dogwood (*Cornus sericea* L.) plants in 20 Oct., 1989 (220°GS) at 0°, 5°,10°, 15°, and 23°C dark post-environment treatment (PET).

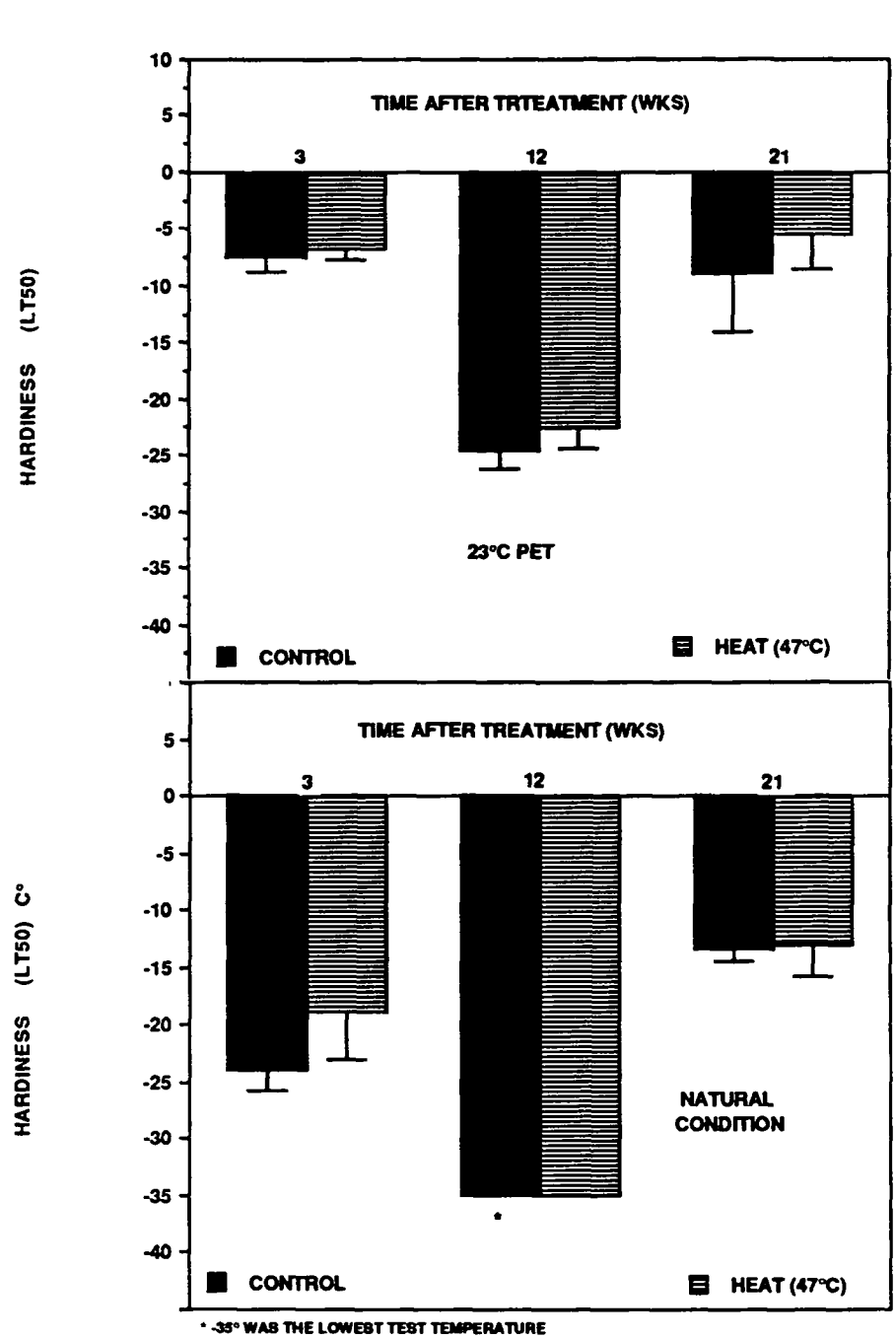

Figure 3.3 Effects of NL-heat stress (47°C for one hour) on cold hardiness of red-osier dogwood (*Cornus sericea* L.) plants at early rest stage (20 Oct., 1988, 220°GS) and two post-treatment environments (23°C and natural condition at Corvallis, Or).

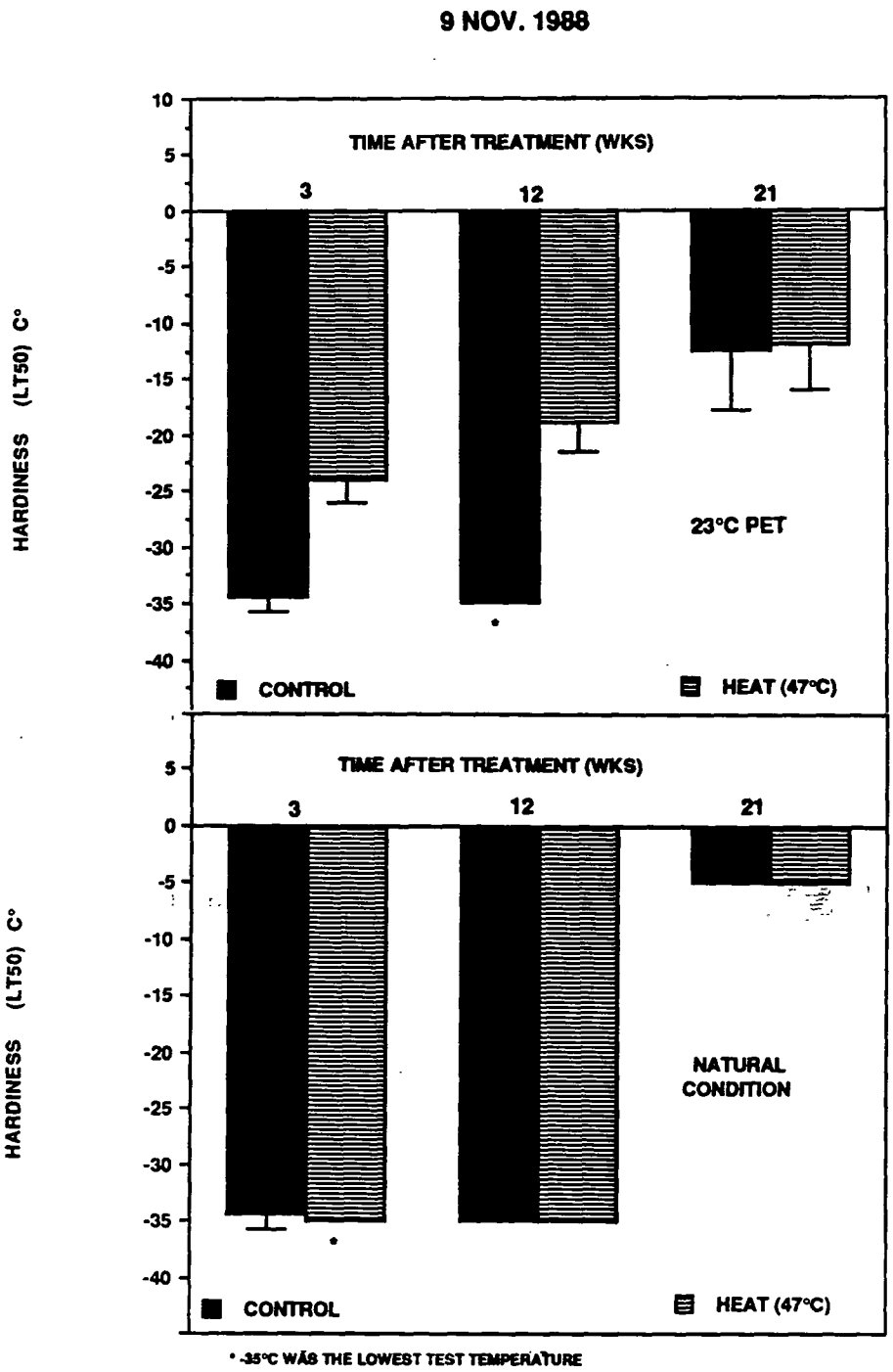

Figure 3.4 Effects of NL-heat stress (47°C for one hour) on cold hardiness of red-osier dogwood (*Cornus sericea* L.) plants at deep stage of rest growth stages (9 Nov.,1988, 270°GS) and two post-treatment environments (PET) (23°C and natural condition Corvallis, Or).

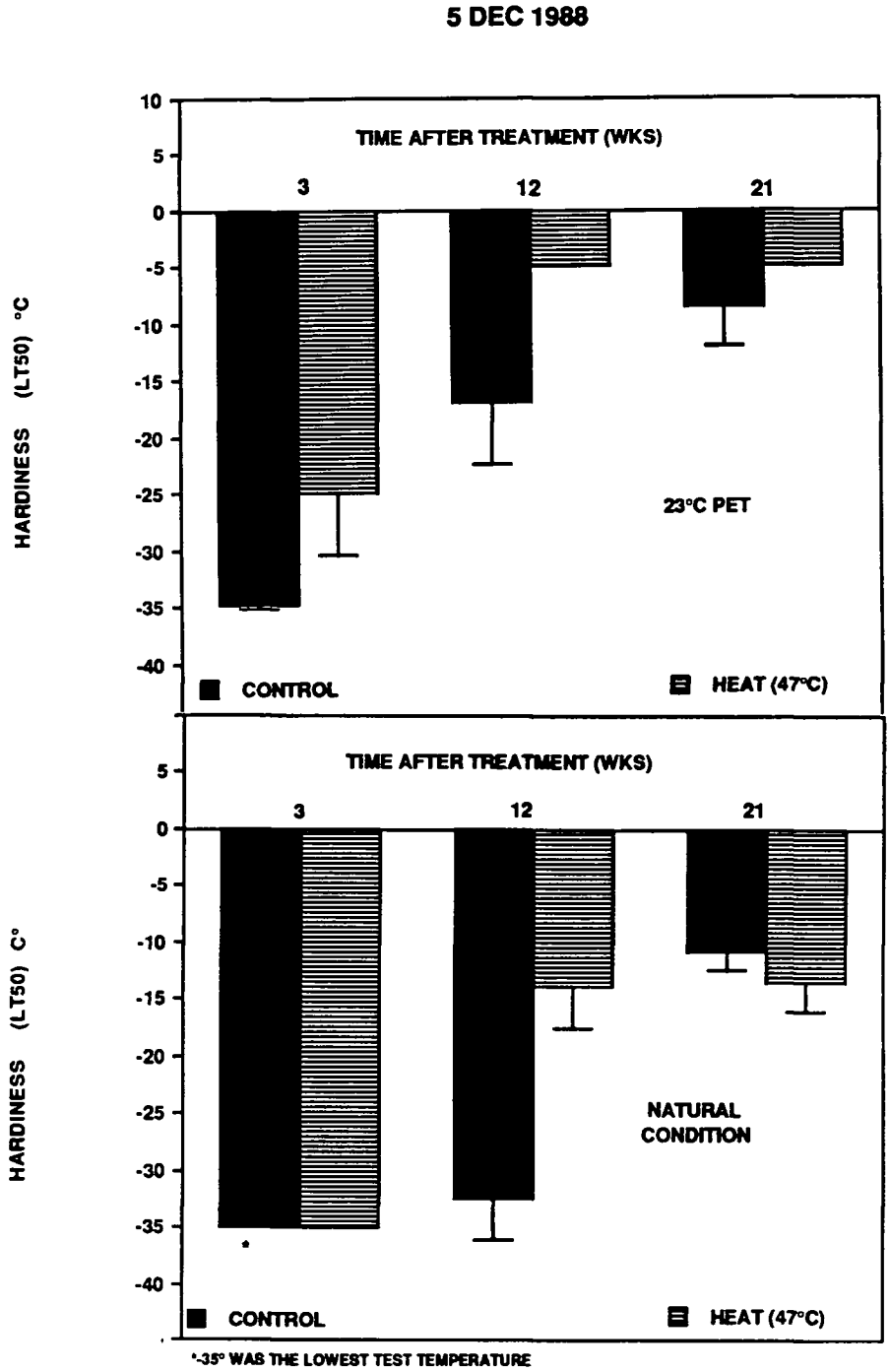

Figure 3.5 Effects of NL-heat stress (47°C for one hour) on cold hardiness of red-osier dogwood (*Cornus sericea* L.) plants at rest stages (20 Oct.,1988, 220°GS) and two post-treatment environments (PET) (23°C and natural condition at Corvallis, Or).

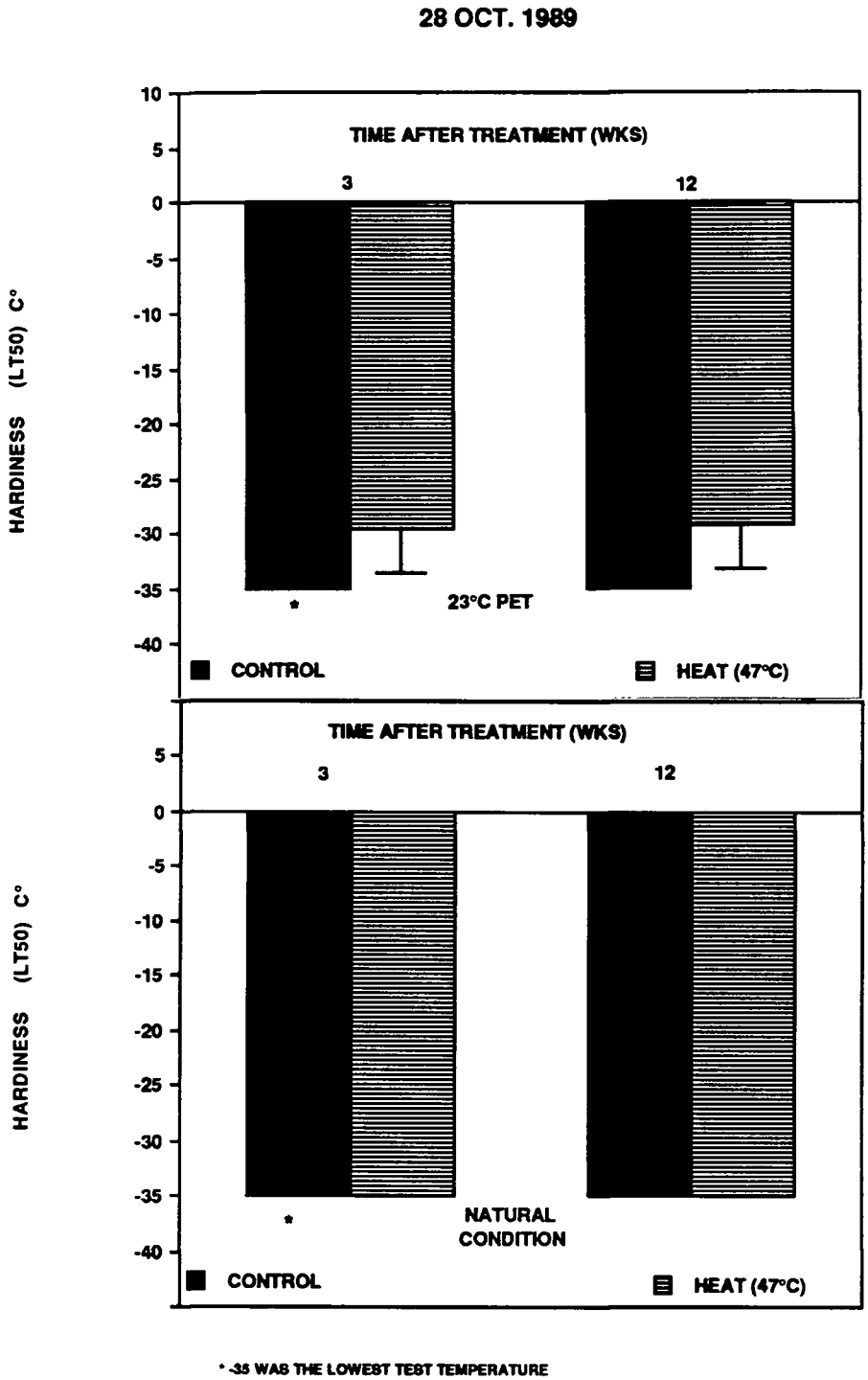

Figure 3.6 Effects of NL-heat stress (47°Cfor one hour) on cold hardiness of red-osier dogwood (*Cornus sericea* L.) plants at early rest stage (28 Oct., 1989, 220° GS) and two post-environment treatment (PET) (23°C and natural condition at Corvallis, Or).

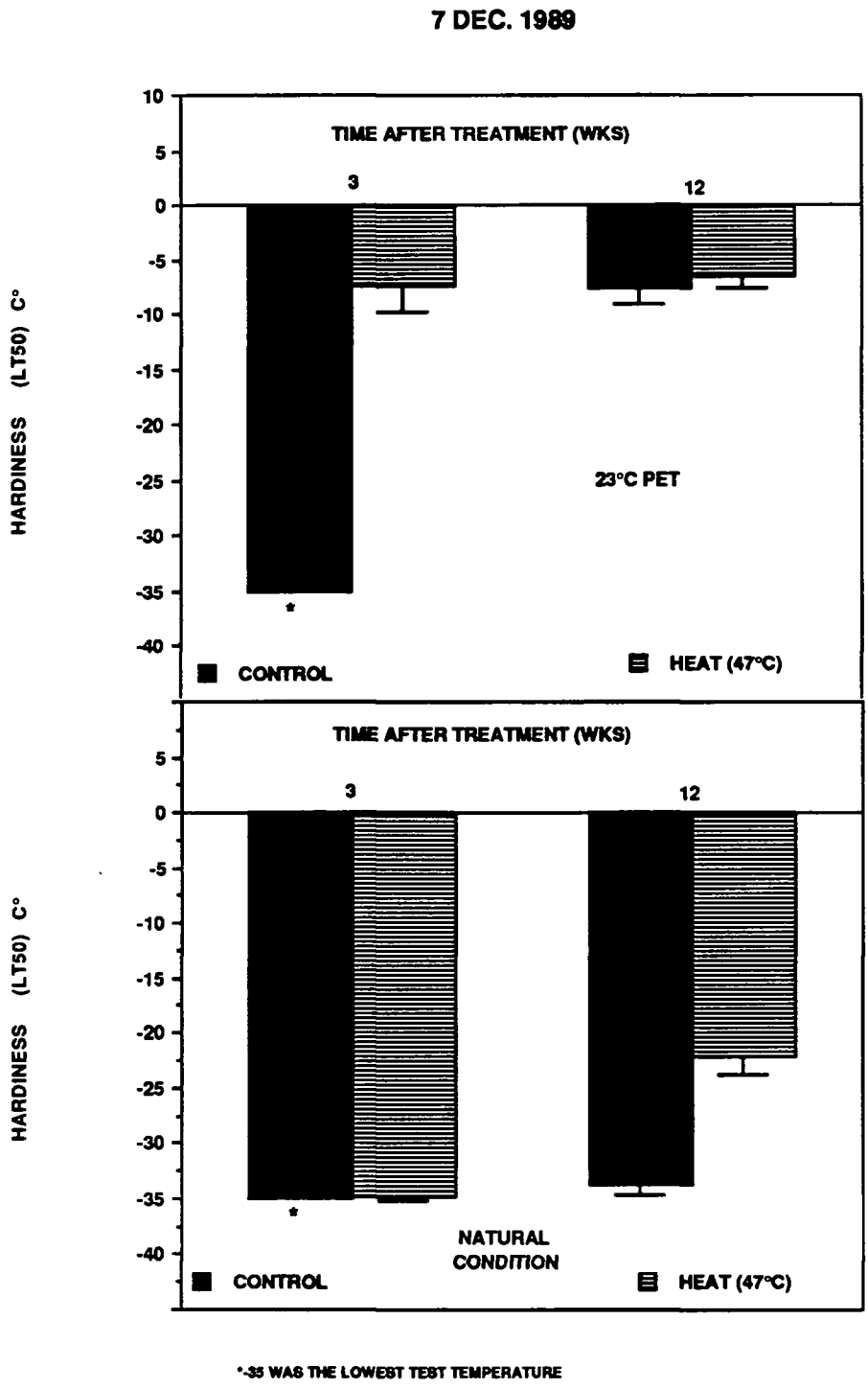

Figure 3.7 Effects of NL-heat stress (47°C for one hour) on cold hardiness of red-osier dogwood (*Cornus sericea* L.) plants at late rest stages (7 Dec.,1989, 300°GS.) and two post-environment treatment (PET) (23°C and natural condition at Corvallis, Or).

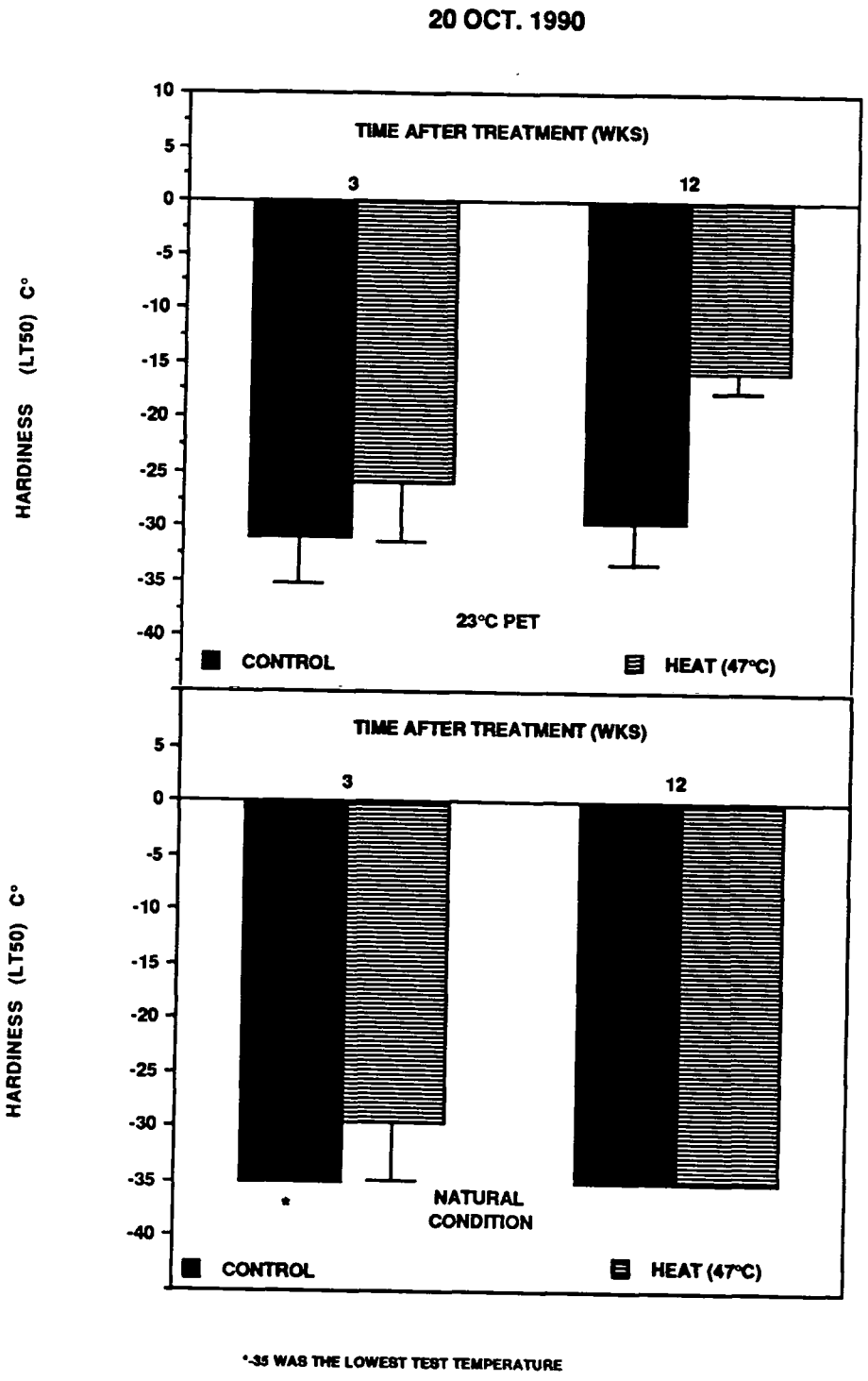

Figure 3.8 Effects of NL-heat stress (47°C for one hour) on cold hardiness of red-osier dogwood (*Cornus sericea* L.) plants at early rest stage (20 Oct., 1990, 220°GS) and two post-environment treatment (PET) (23°C and natural condition at Corvallis, Or).

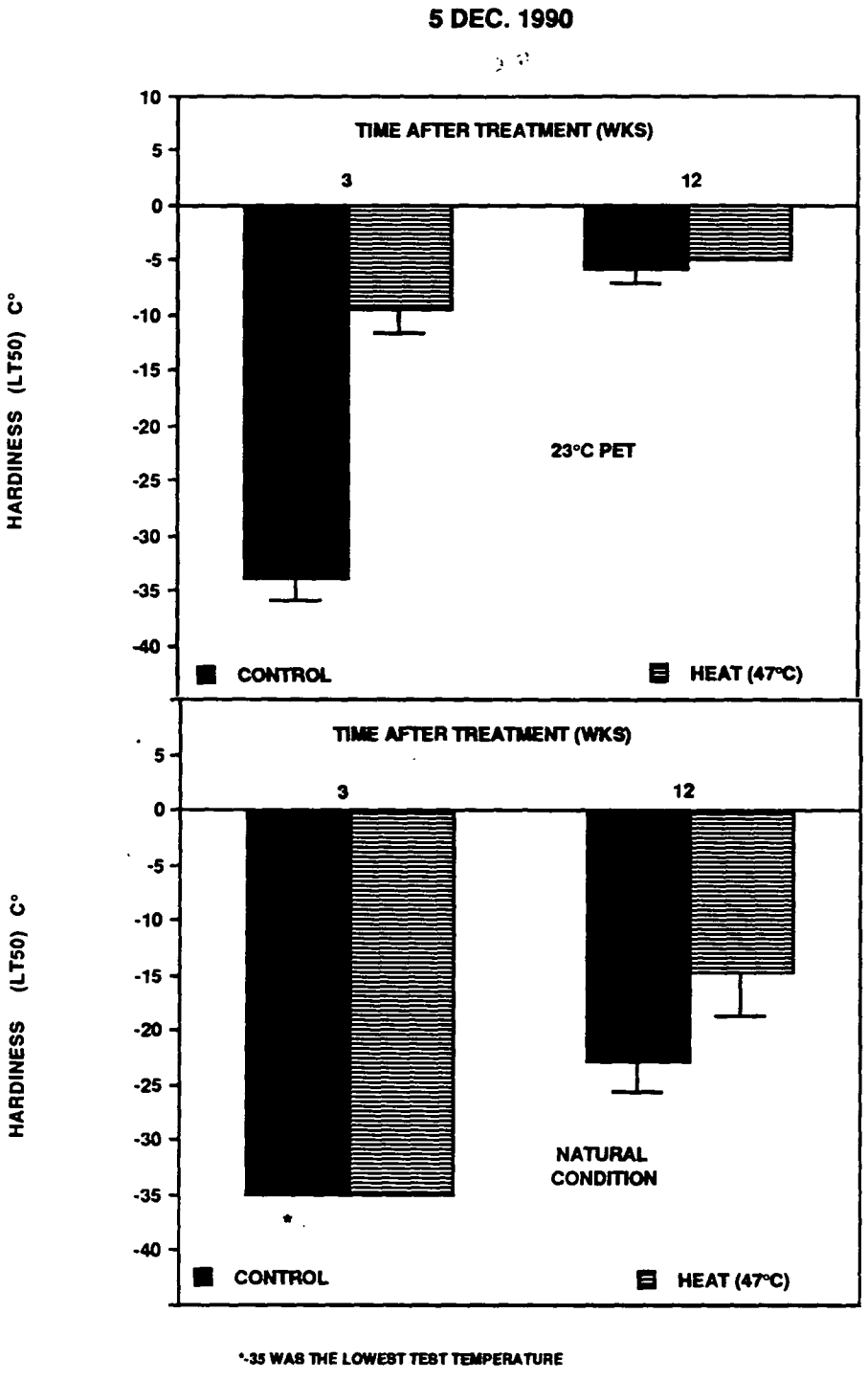

Figure 3.9 Effects of NL-heat stress (47°C for one hour) on cold hardiness of red-osier dogwood (*Cornus sericea* L.) plants at late rest stages (5 Dec., 1990 300°GS.) and two post-environment treatment (PET) (23°C and natural condition at Corvallis, Or).

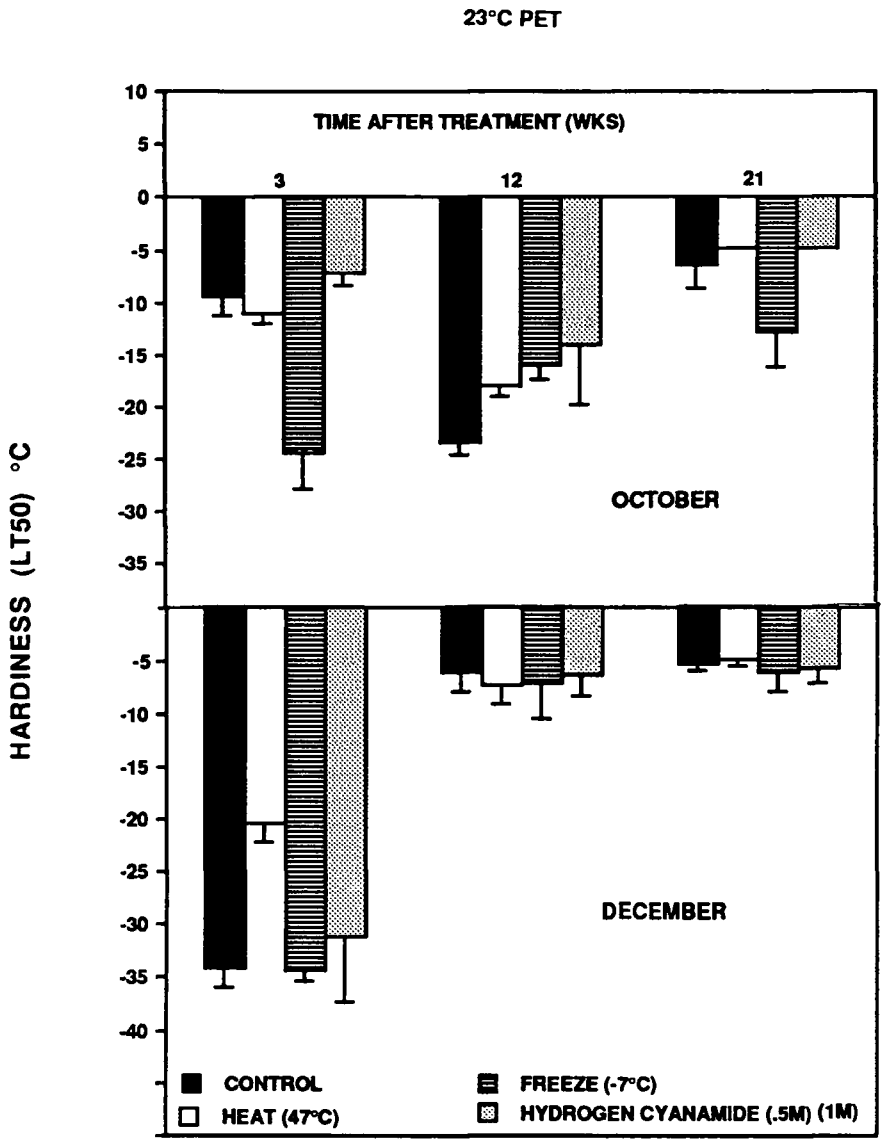

Figure 3.10 Effects of different NL-heat (47°C for one hour), freeze (-7°C for one hour), and hydrogen cyanamide (1M October 1988 and 0.5 M. December 1989) on cold hardiness of red-osier dogwood (*Cornus sericea* L.) plants at 23°C PET.

Figure 3.11 Effects of NL-heat stress (47°C for one hour) on cold hardiness of red-osier dogwood (*Cornus sericea* L.) plants in 20 Oct 1989, 220°GS at 0°, 5°, 10°, 15° and 23°C dark post-environment treatment (PET).

OCT 20 1989

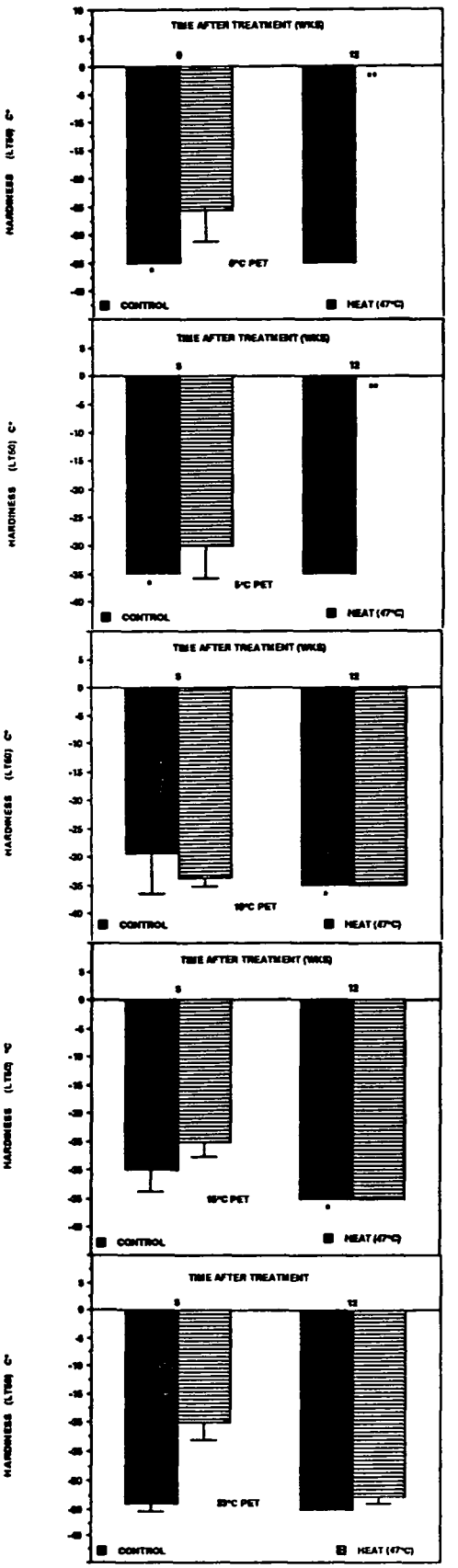
TIME AFTER TREATMENT (WKS)
HARDINESS (LT99) C°
5°C PET
CONTROL
HEAT (47°C)
HARDINESS (LT50) C°
10°C PET
HARDINESS (LT90) °C
15°C PET
HARDINESS (LT99) C°
20°C PET

Figure 3.12 Effects of different NL-(47°C for one hour), freeze (-7°C for one hour), and hydrogen cyanamide (1M) stress, in 20 Oct. 1991 on budbreak of red-osier dogwood (*Cornus sericea* L.) plants after 3 weeks in a warm greenhouse (24°/18° day/night).

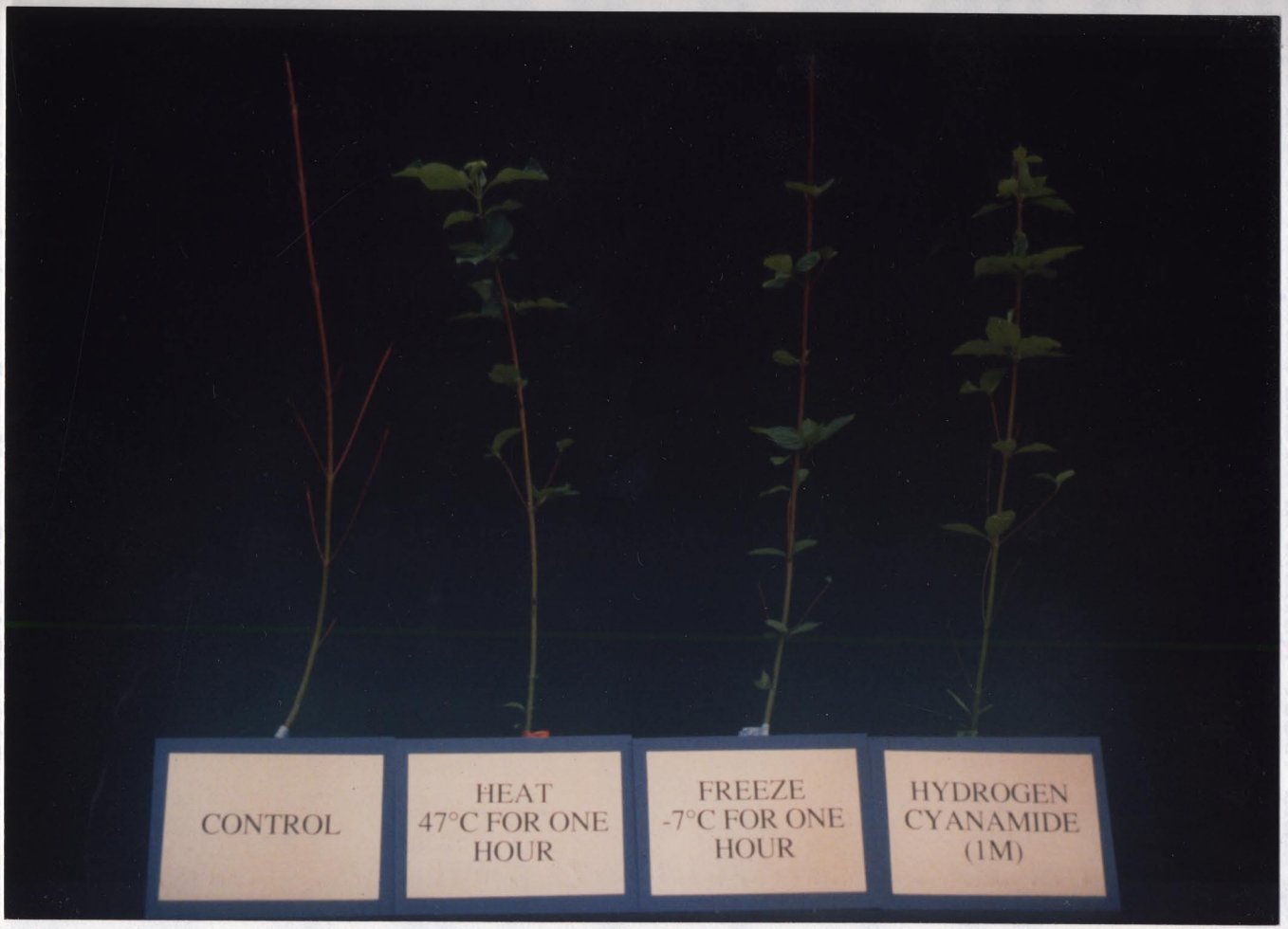
CONTROL
HEAT
47°C FOR ONE
HOUR
FREEZE
-7°C FOR ONE
HOUR
HYDROGEN
CYANAMIDE
(1M)

Figure 3.12

3.6 References

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4. Recovery of Plants from "Near-Lethal" Stress

4.1 Abstract

The effects of "near-lethal" (NL) stress as a means of overcoming dormancy on the subsequent development of hardiness and recovery from stress was measured under three post-stress environmental conditions. This study reports on the dieback and recovery after varying lengths of post-treatment. Shoots of red-osier dogwood, *Cornus sericea* L., plants were subjected to 47°C for one hour during October, November and December, and then placed into either constant 0°C or 23°C (dark condition) or kept in natural conditions at Corvallis, Oregon. In October, plants exposed to NL-heat stress died prior to 9 weeks of 0°C post-treatment. None of the plants from the other treatments showed any signs of plant injury. For plants exposed to NL-heat stress during November and December, partial stem dieback occurred during the next 12 and 15 weeks respectively. None of the plants from the other treatments were injured.

Post-stress temperatures of 0° or 5°C following NL-heat in October were lethal while temperatures above 10°C allowed recovery. The effects of post-stress exposure to 0°C injured excised stem within 48 hours, whereas the irreversible damage to whole plants occurred by two weeks. Dormant plants in October exposed to other stresses, e. g., freezing temperature and hydrogen cyanamide, at NL dosages showed that these stresses also caused plant dieback at 0°C and little or no dieback at 23°C post-treatment environment.

4.2 Introduction

Temperate woody plants develop winter dormancy (rest) prior to the onset of sub-freezing temperatures. Researchers have associated this as a necessary prerequisite for achieving complete cold acclimation (Levitt 1980, Weiser 1970, Saunnders 1978). The rate of cold acclimation and deacclimation is dependent on the degree of rest of the plant and temperature (Kobayashi *et al.* 1982). From the onset of rest to the acquisition of maximum rest the rate of acclimation increases and plants do not deacclimate, even at relatively warm temperatures, e. g., 20°C. In contrast, following the end of rest to the time of bud-burst the temperature requirement for either promoting or maintaining acclimation becomes narrower (e.g.,. lower temperatures required for acclimation with later growth stages) and the temperature range for promoting deacclimation becomes greater (e. g., lower temperatures can cause deacclimation) with later growth stages. Although dormancy may not be directly related to cold hardiness development, studies showed that the physiological status of plants in general during winter was dynamic and influenced acclimation and deacclimation (Sarvas 1972, 1974 ; Weiser 1970, Tumanov *et al* 1973; Kobayashi *et al.* 1982,1983, Sakai and Larcher 1987, others).

We recently showed that the resting state of buds can end rapidly and growth stimulated by exposing the bud to near-lethal (sub-lethal) (NL) stresses (Fuchigami and Nee 1987). The stress treatments required to overcome rest causes electrolytes to leak

from cells and stimulates ethylene production (Nee 1986). Chemical or physical stress, applied at the NL dose, can overcome rest at any stage (Doorenbos 1953, Erez and Lavee 1974).

Subfreezing temperature that results in premature breaking of rest of trees in cold regions of the world e.g., Sapporo, Japan is quite common phenomenon (Sakai and Larcher 1987). Suare (1985) indicated that breaking rest by subfreezing temperature is not the the result of accelerating of chilling requirements for rest release, but is the response of sublethal condition. Doorenbos (1953) proposed that NL-stress from different sources (e. g., chemicals, heat, freeze, light, drought, injury, anaesthetics, toxic substances, anoxia, etc) are all capable of breaking rest period in woody plants, however it is not known that all stresses share the same mechanisms by overcoming rest.

In a companion study , we found that the effect of overcoming the resting state of buds on hardiness was dependent on the stage of plant development. In contrast loss of rest at the later stages of rest stimulated plant growth and deacclimation at a wider range of temperatures, thus causing rapid loss of hardiness under natural conditions. overcoming rest with NL-stress at the earlier stages of rest development stimulated bud break at high temperatures and without affecting the hardiness of the stem tissues under the controlled environment and natural condition (Shirazi 1992).

While investigating the relationship between rest and hardiness , we observed that plants exposed to NL-heat stress began to die when incubated at low temperature for several weeks. In contrast, the controls (without NL-stress) incubated at low temperature and

NL-heat stress plants incubated at warm temperature and natural condition (Corvallis, Or) all survived without injury . Investigation of previous literature to explain this was unsuccessful, Therefore the following experiments were done to verify the above observation, and if verified, to determine the effects post-environment treatment on dieback and recovery, to determine whether the physiological status of plants influenced the observed effects, and to determine whether other NL-stresses had similar effects on plant recovery during post-treatment.

4.3 Materials and Methods

Plant material

A clone of red-osier dogwood, *Cornus sericea* L. from Wayland, MA, was used. *Cornus sericea* L. was selected because previous work had established the relationship of stages of dormancy to cold hardiness in response to natural conditions at Corvallis, Oregon (Kobayashi *et al.* 1982) and the role of near-lethal stresses on overcoming dormancy (Nee 1986). The plants were propagated in February by stem cuttage. Rooted cuttings were transplanted in a mixture of sterilized bark, peat and soil (1:1:1 v/v) in 15 cm paper pots, and grown in a lathhouse with 20 % shade. Plants were irrigated by overhead system twice daily (6 AM & 3 PM). Osmocote (Sierra Co.) slow release fertilizer (17-6-9) with micronutrients was placed and mixed at rate of (4 grams) per pot. Plants were trained to one stem in June-July. They were 70-90 cm respectively at the time of the experiments. The stage of development of the plants at the time of treatment was determined as reported by Kobayashi *et al.* (1982).

Establishment of "Near-Lethal" heat stress

Near-lethal heat stress treatment was determined by exposing internode (2nd, 3rd, and 4th) stem sections of dogwood, harvested on 18 October to 40, 45, 47, 50, and 55°C for 0, 30, 60, and 120

minutes in a circulating water bath. The experiment was repeated twice (n=10). One hour after treatment the stem sections (2.5 cm) were devided into two groups. One group was placed in individual 10 cm petri dishes containing two sheets of moistened filter paper, incubated in the dark at 23°C, and evaluated visually for tissue death after 2 weeks. The second group of samples was evaluated for electrolyte leakage with a Markson Conductivity Meter. Each sample was placed in 15-ml of double-distilled water, shaken for 36 hours and the initial conductivity was taken. Samples were killed in a hot water bath at 80°C for 1.5 hour, and shaken for 24 hours, after which final measurements were taken. Percent ion leakage (expressed as percent (%) electrical conductivity) was calculated by dividing the first measurement by the final measurement and multiplying this by 100 (Fig. 3.1).

Near-lethal stress, was defined as any stress causing increased electrolyte leakage (as compared to the non-stressed samples) without damage to plant cell. For majority of these studies, NL stress was obtained by exposing plants tissues to 47°C for one hour in a circulating water bath. The average % electrolyte leakage was about 25%. Exposure was achieved by enclosing the plants in 4 mil polyethylene bags with the air removed to enhance good plant exposure with the water temperature. After immersion, the plants were kept for approximately 24 hour at 23°±3°C prior to post-environment treatment.

Post-treatment temperature effects on recovery

The effects of NL heat stress on recovery of plants at three post-treatment environments (0°C and 23°C both in the dark, and natural conditions in the lath house) were studied at three dates (October 20, November 9, and December 5) when the plants were at early rest (220°GS), maximum rest (27°GS), and late rest (300°GS), respectively (Fuchigami et al 1982). The plants (n=5), were observed for stem dieback at three week intervals. The maximum and minimum temperatures in the lath house for 1988-1989 are shown in (Fig 4.1). The experiment was repeated three times (In 1988, 1989, and 1990). Since the data were similar in all three years only the 1988 data were presented.

To test the effects of post-treatment temperature on plant recovery, NL-heat stressed and control plants in October (220°GS) were incubated in the dark at 0°, 5°, 10°, 15° and 23°. Percent surviving plants was determined after 0, 3, 6, 9 and 12 weeks following the NL-heat stress treatment.

To determine the amount of post-treatment time at 0°C following NL-heat stress required to cause injury, plant internode stem segments 2.5 cm long were excised from natural condition on February 4, 1991. These segments were exposed to 23 and 51°C (higher temperature was used for NL stress) for one hour and then placed into incubators without light at the 23 and 0°C post-treatment environments. The experiment was repeated twice (n=5). Electrical conductivity of the stems was measured after 0, 1,

2, 4, 8, 16, 24, 48, 96 and 192 hours of exposure to 0°and 23°C post-treatments

To further test the effects of exposure time on plant recovery, dogwood plants treated with NL-heat stress were placed into alternating 0° and 23°C post-treatment temperatures. Treatment time was a total of six weeks. Temperature treatments were either 0, 1, 2, 3, 4, 5 or 6 weeks at one temperature followed by either 6, 5, 4, 3, 2, 1 or 0 of the other temperature, and vice a versa. The treatments were replicated 10 times in a completely randomized design. Percent of stem dieback was observed after 6 weeks.

Post-treatment light effects on recovery

To determine if the light may have played a role in recovery of plants from NL-heat stress under natural conditions, plants subjected to 47°C heat stress for 1 hour were divided into two groups. One group was placed in the dark at 0°C and the other group was placed in 12 hours of light at 0°C. Incandescent light (58 umol· s^{-1}·m^{-2} PAR) was provided at the plant height. Ten plants per treatment were completely randomized. Plants were observed for stem dieback at 3 weeks intervals for 21 weeks.

Other NL-stresses effects on recovery

Two other NL-stresses, freezing and hydrogen cyanamide, were compared with NL-heat stress to determine the universal nature of NL-stresses effects on plant recovery. In October dogwood plants

were treated with either no stress (control), NL-heat stress for 47°C for 1 hr., NL-freeze stress at -7°C (Nee 1986)) for 1 hr., or NL-hydrogen cyanamide (1.0M) (Nee 1986) painted completely over the stem tissues. Hydrogen cyanamide is a strong oxidizing agent reported to overcome rest by NL-dosages (Fuchigami and Nee 1987). The plants were placed into either 0° or 23° darkened post-treatment chambers, respectively. Ten plants per treatment were arranged in a completely randomized design. The plants were observed for stem and bud dieback (complete browning of the treated part of the plants) at 3 weeks intervals for 21 weeks.

Statistical Analysis

Analysis of variance (ANOVA) for a completely randomized design was used for all experiments. Means were separated by Duncan's Multiple Range Test ($P = 0.05$) for all the studies illustrated with bar graphs. Standard Errors were used for the line graphs. Statistical analysis was performed using the SAS software (SAS Institute, 1988).

4.4 Results

Near-lethal heat treatment on electrolyte leakage

Mean leakage of the control, at zero exposure time, and at 40°C in all exposure times was approximately 13-14%. The % leakage of the 45° and 47°C treatments exposed for 30-120 minutes averaged between 22-27%, and the 50° and 55°C treatments averaged more than 48% leakage. Browning of the tissues of the stems after two weeks incubation in a dark high humidity chamber at 23°C ±3°C indicated that the stems exposed to 50° and 55°C died, whereas those at ≤47°C survived (Fig. 3.1)

Post-treatment temperature effects on recovery

The growth stage, when NL-heat stress treatment was administered, and the post-treatment environment, significantly affected the recovery of plants (fig. 4.2). Plants exposed to NL-heat stress during October, November and December suffered decreasing levels of stem dieback (% recovery) respectively when exposed to 0°C in the dark . None of the plants exposed to 23°C in the dark and natural post-treatment conditions, and none of the controls without NL-heat stress treatment suffered any dieback.

Cross sections of internode and the treated and untreated part of the internode of red-osier dogwood treated at early rest (20 Oct. 1990; 220°GS) stage are shown in Fig. 4.6.. and Fig 4.7.

after 9 weeks storage at 0°C post-environment

The post-treatment temperature greater than 10°C-dark condition caused recovery of plants from the NL-heat stress treatment in October (Table 4.1). Plants exposed to 10°, 15°, and 23°C recovered completely, without any injury. In contrast, the plants exposed to 0 and 5°C-dark post-treatment caused complete stem dieback after 9 weeks of incubation.

Internodal stem sections of dogwood exposed to NL-heat stress and 0° and 23° -dark post-treatments indicated that the cells lost approximately 10-15% more electrolytes than the controls immediately following the NL-heat treatment (fig. 4.3). At 23° post-treatment the leakage from the NL-heat stressed segments did not change significantly throughout the duration of the study. The controls at 23° post-treatment showed a slight decrease in electrolyte loss after 48 hours of incubation. At 0° post-treatment a large increase in electrolyte leakage occurred after 48 hours incubation in the NL-heat stressed stem sections. The leakage increased from approximately 25% to approximately 75%. The controls w/o NL heat stress at 0°C post-treatment also showed an increase in electrolyte leakage after 48 hours incubation.

Alternating the post-treatment temperature from 0° to 23° and 23° to 0° after NL-heat stress had a significant influence on the dieback and recovery of plants after only 6 weeks incubation (Table 4.2 and 4.3). Plants exposed to 0° for either 2, 3, 4 or 5 weeks followed by either 4, 3, 2 or 1 weeks at 23° suffered 25, 56, 14 and 10% stem dieback, respectively (Table 4.2). Plants exposed to 1 week 0° followed by 5 weeks 23°, 6 weeks 0° and the controls without NL-stress treatment showed no signs of plant dieback after

6 weeks total incubation time (Table 4.2). By reversing the order of the post-treatment temperature, e.g.,. 23° first then 0°, less stem dieback was observed (Table 4.3). The only treatments that showed some stem dieback (8-10%, involving only 1 out of 10 plants) were the 3--3 (3 weeks 23° followed by 3 weeks 0° post-treatment), 4--2 and 6--0 treatments (Table 4.3).

Post-treatment light effects on recovery

Exposure of plants to light during post-treatment had slight, but significant effect on recovery at 0°. After 9 weeks of post-treatment at 0°-dark the plants were completely killed (Fig. 4.4). In contrast, plants exposed to 0°-light post-treatment had no visible signs of dieback at 9 wks, 50% dieback at 12 weeks and complete dieback at 15 weeks. None of the control plants was affected by the post-treatments.

Other NL-stress effects on recovery

Near-lethal heat, freezing and hydrogen cyanamide treatments all resulted in significant dieback when plants were incubated at 0°-dark post-treatment (Fig.4.5) The extent of dieback caused by freezing and hydrogen cyanamide was similar, but less than the injury caused by NL-heat stress. None of the plants treated with either freezing or hydrogen cyanamide suffered complete dieback. After 21 weeks of incubation at 0°-dark post-treatment approximately 40-50% dieback occurred. The NL-heat treatment were completely killed by 9 weeks

of 0°-dark post-treatment. The controls at 0°-dark and 23°-dark post-treatment were not injured. The NL heat and freezing stress treatments incubated at 23° dark post-treatment all survived. Slight injury, 10 and 20%, occurred in the hydrogen cyanamide treatment after 18 and 21 weeks incubation at 23°C-dark post-treatment.

4.5 Discussion

In previous studies of dormancy release by NL-stress treatments exposure to warm post-treatment temperature has been the standard procedure. This obviously caused us to overlook the problem of plant dieback at low temperature treatments following exposure to NL-stresses. The significance of this "accidental" finding of plant dieback at low temperature following exposure to NL-stresses is not known. This may explain some of the causes of plant dieback in cold regions when plants are exposed to severe environmental stress. Horticulturist have often observed unexplained dieback of plants months after the plants were exposed to stresses. Although speculative, is it possible that the dieback of forest trees and plants observed in many temperate regions throughout the world may be due in part to NL-stress effects followed by cold post-stress environments?

The reasons for the decreased plant dieback (increased % recovery) observed from October to December could be related to higher thermostability of the plants due to the greater cold hardiness development. Alexandrov (1977) describes that the greater thermostability of many different plants will achieve by cold hardening treatment, under natural and laboratory conditions, and this includes even for the summer plants. Levitt (1980) describes the relationship of thermotolerance and freezing tolerance as two opposite relations: one that summer heat increase thermostability, and second that increase of freezing tolerance during fall and winter result in higher thermostability. In other studies we have

shown red-osier dogwood to have greater heat tolerance from October through December (Shirazi 1992), which corresponds with the increasing level of freezing tolerance (Van Hustee *et al* 1967, Kobayashi and Fuchigami 1983).

Although still preliminary, results from the electrolyte leakage study of excised internodal stem sections suggests that the cellular damage at 0°C post-treatment occur within 48 hours . At this time more than 50% of the electrolytes leaked from the cell membranes. Tissues leaching more than 50% electrolyte leakage are considered dead. Visual observations of the internodal stem sections indicated that the tissues of the stems were severely injured/killed following the 48 hours and longer 0°C post-incubation treatment.

Alternating the post-treatment temperatures between 0° and 23° (Tables 4.2 and 4.3) indicated that the dieback of intact plants occurred between the first 1 and 2 weeks of 0° post-treatment. The reason for this slower development of injury in whole plant systems is not known. Perhaps the greater plant mass in the whole plant system and the untreated root system of the NL-heat stress treatments may have afforded either some protection or better recovery of the NL-heat stressed tissues. In addition, plants may have partially recovered in the first 24 hours at room temperature prior to incubation at 0°. The greatest damage occurred after a brief exposure (e. g., 2 weeks) to 0° post-treatment followed by a long exposure (e. g., 4 weeks) to 23° post-treatment. This may be due to injury caused at 0° and the faster expression of the injury at 23° (Table 4.2 and 4.3). Similarly, the less amount of plant dieback

with longer 0°C followed by 23° post-treatments may be due to the time required for expression of the symptoms.

Light may aid in the recovery of the NL-stressed plants. The data suggest that light had a slight beneficial influence on recovery. Perhaps the complete survival of the plants exposed to post-treatment natural conditions may be due in part to the effects of light. In a separate study, when plants were covered completely with aluminium foil to exclude light under natural condition, we did not observe any dieback in heat-stressed or control plants (Shirazi 1992). These studies suggests that although light may have some effects on recovery, temperature appears to have a dominating influence on either plant recovery or dieback.

The effects of NL-freezing and NL-hydrogen cyanamide treatments on post-treatment plant recovery were similar to the effects of NL-heat treatment. This suggests that the effects of the low temperature post-treatment dieback observed were not unique to the NL-heat stress and may be universal to all NL-stresses. Although the extent of dieback caused by NL-freezing and NL-hydrogen cyanamide was less than the NL-heat stress (Fig. 4.5). It is likely that the degree of stress imposed by those treatments was not as severe as the NL-heat treatment.

The reason for the plant dieback and recovery is not known. Levitt (1980) suggests that stresses may cause plant injury by inducing a secondary stress which requires an extended time period to develop and results in injury. Gate (1980) reported that the ability of an organism to survive extreme temperatures is dependent on their repair mechanisms.

The extend of balance between cellular damage caused by temperature stresses determines the efficiency of the repair mechanism (Gates 1980)

The implications of the interaction of NL-stresses and post-temperature effects on plant recovery and dieback may be of significant importance to plant survival. It is likely that the unexplained dieback of plants exposed to plant stresses (e.g., forest decline) may be due to the NL-stress X post-temperature interaction reported in this paper. This study suggests that any NL stress applied at a particular stage of plant development may cause plant dieback when exposed to unfavorable cold (nonfreezing) post-stress environments. A significant and important implication of this finding to agriculturalist and others is the possible strategies that are needed to recover plants predisposed to NL-stresses particularly in cold regions.

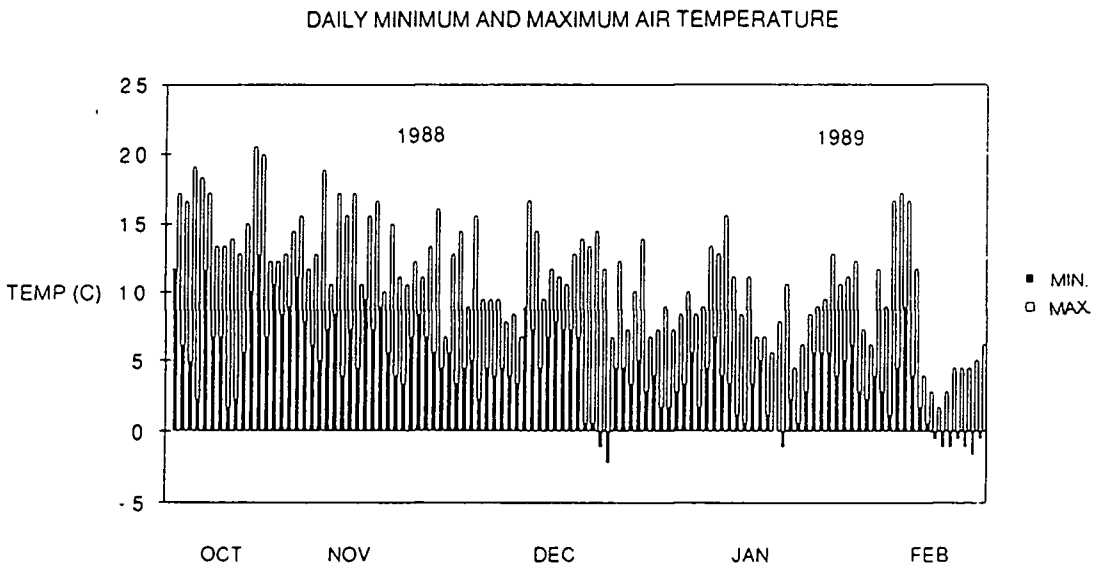

Figure 4.1 Daily minimum and maximum air temperature in the lathhouse from late Oct. to early Feb. for 1988-89, in Corvallis, Or (44°, 37', 59" N and 123°, 11', 32" W).

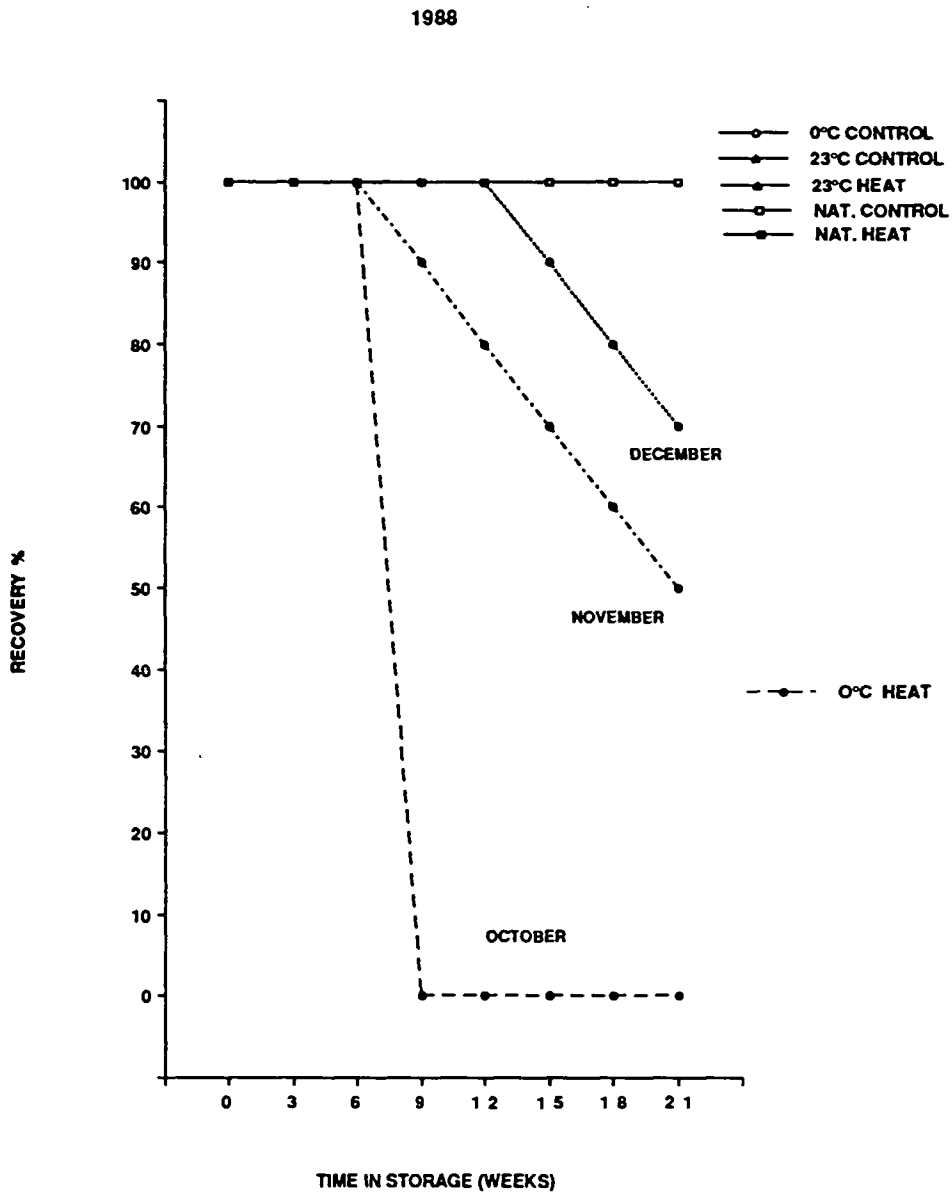

Figure 4.2 Effects of NL-heat stress (47°C for one hour) on the % recovery of red-osier dogwood (*Cornus sericea* L.) plants at three sampling dates (20 Oct. ; 9 Nov. ; 5 Dec.) and three post-environment treatments (PET).

POST-TREAT. DURATION (WEEKS)	HEAT TREATMENTS	POST-TREATMENT CONDITIONS				
		0°C	5°C	10°C	15°C	23°C
		% SURVIVAL				
0	CONTROL	100	100	100	100	100
	HEAT	100	100	100	100	100
3	CONTROL	100	100	100	100	100
	HEAT	100	100	100	100	100
6	CONTROL	100	100	100	100	100
	HEAT	100	100	100	100	100
9	CONTROL	100	100	100	100	100
	HEAT	(0.00)*	(0.00)	100	100	100
12	CONTROL	100	100	100	100	100
	HEAT	(0.00)	(0.00)	100	100	100

(00.0)* = HEAT TREATED PART OF PLANTS WERE DEAD.

Table 4.1 Effect of NL heat stress (47°C for one hour) in Oct. on the recovery of red-osier dogwood (*Cornus sericea* L.) plants following 0 to 12 wks of incubation in the dark at five post-environment treatment (PET)(0°, 5°, 10°, 15°, 23°C).

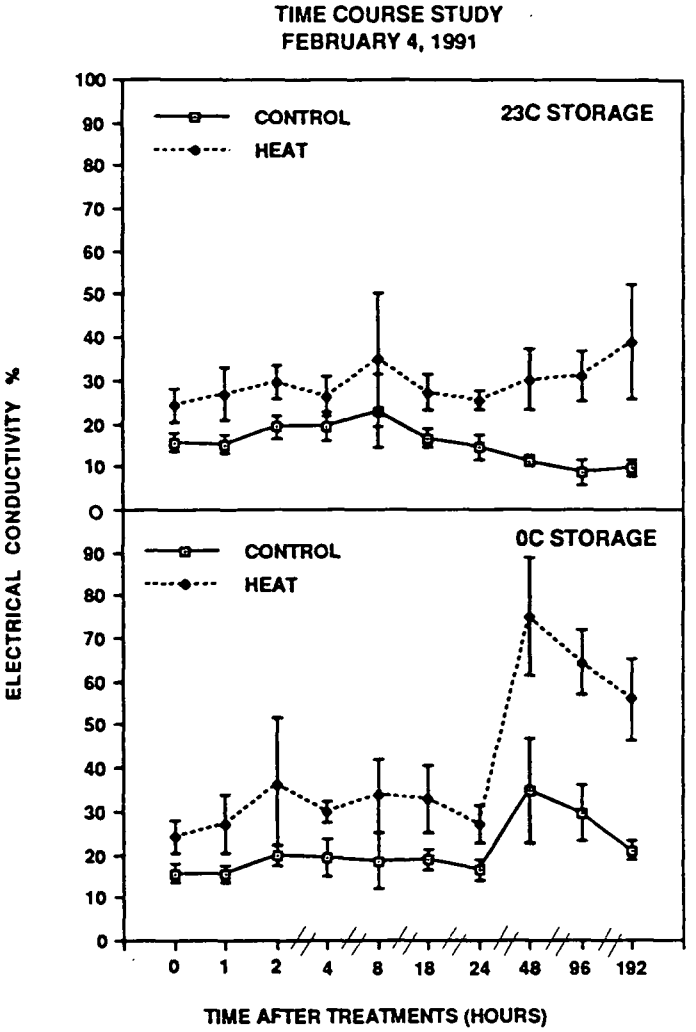

Figure 4.3 Time course of the effect of NL heat stress (51°C for one hour) on the electrical conductivity of stem segments (2.5 CM) of red-osier dogwood (*Cornus sericea* L.) on 4 Feb. 1991 following 0, 1, 2, 4 ,8, 16, 24, 48, 96., and 192 hours of either 0° or 23°C post environments.

POST-TREATMENT CONDITIONS & DURATION (WEEKS) 0°C	23°C	HEAT TREATMENT	NUMBER OF PLANTS WITH DIEBACK*	% AVERAGE STEM DIEBACK
1	5	CONTROL	0	0.00
1	5	HEAT	0	0.00
2	4	CONTROL	0	0.00
2	4	HEAT	4	25 ± 19.2
3	3	CONTROL	0	0.00
3	3	HEAT	3	56 ± 20.6
4	2	CONTROL	0	0.00
4	2	HEAT	3	14 ± 5..2
5	1	CONTROL	0	0.00
5	1	HEAT	2	10 ± 7.07
6	0	CONTROL	0	0.00
6	0	HEAT	0	0.00

*TOTAL OF 10 PLANTS PER TREATMENT.

Table 4.2 Effects of NL heat stress (47°C for one hour) in 6 Nov., 1989 on the % dieback of red-osier dogwood (*Cornus sericea* L.) plants at alternating 0° followed by 23° post-environment temperatures 1--5 (1 wk 0° followed by 5wk 23°), 2--4, 3--3, 2--4, 1--5, and 0--6. Data on dieback was taken after 6 wks post-environment treatment.

POST-TREATMENT CONDITIONS & DURATION (WEEKS) 23°C	0°C	HEAT TREATMENT	NUMBER OF PLANTS WITH DIEBACK*	% AVERAGE STEM DIEBACK
1	5	CONTROL	0	0.00
1	5	HEAT	0	0.00
2	4	CONTROL	0	0.00
2	4	HEAT	0	0.00
3	3	CONTROL	0	0.00
3	3	HEAT	1	10 ± 0
4	2	CONTROL	0	0.00
4	2	HEAT	1	8 ± 0
5	1	CONTROL	0	0.00
5	1	HEAT	0	0.00
6	0	CONTROL	0	0.00
6	0	HEAT	1	10 ± 0

*TOTAL OF 10 PLANTS PER TREATMENT.

Table 4.3 Effects of NL heat stress (47° for one hour) in 6 Nov. on the % dieback of red-osier dogwood(*Cornus sericea* L.) plants at alternating 23° followed by 0° post-treatment temperatures (1--5 =1 wk 0° followed by 5wk 23° , 2--4, 3--3, 4--2, 5--1, and 0--6). Data on dieback was taken after 6 wks post-environment treatment.

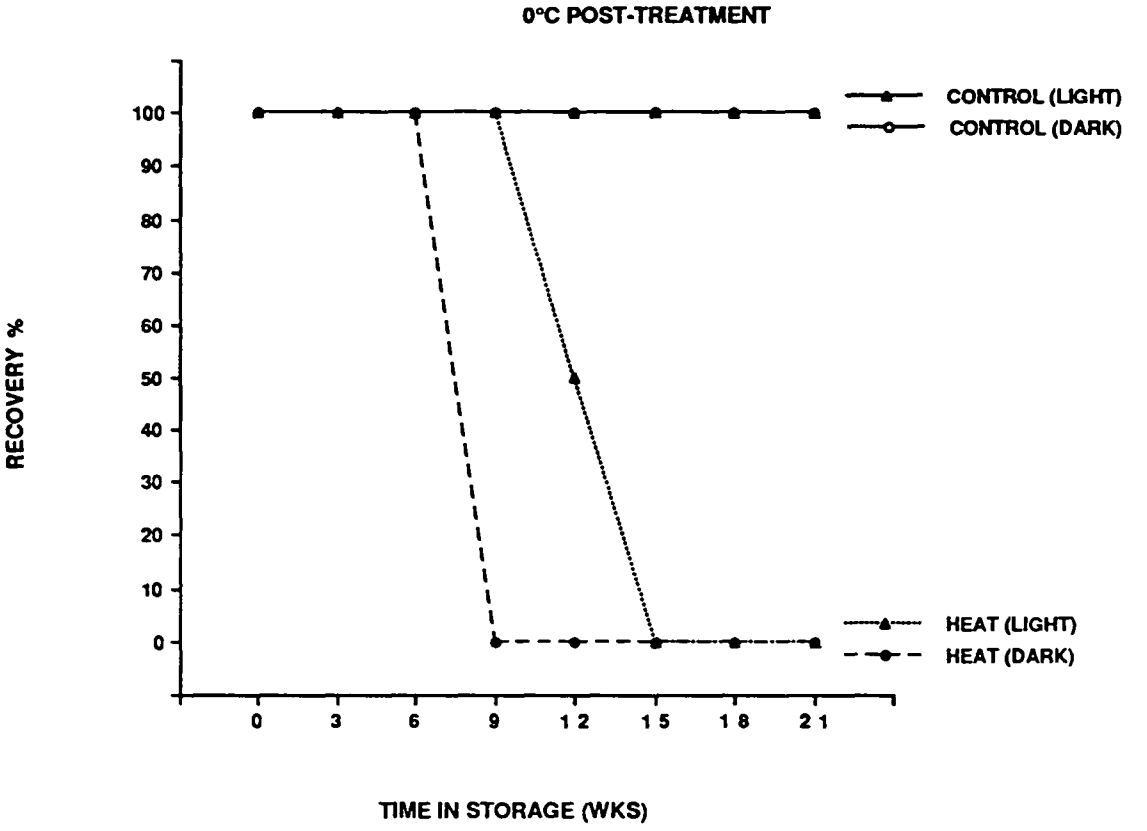

Figure 4.4 Effects of NL heat stress (47°C for one hour) in October 1989 on % recovery of red-osier dogwood (*Cornus sericea* L.) plants following 0 to 21 wks storage either light or dark condition at 0°C post-environment.

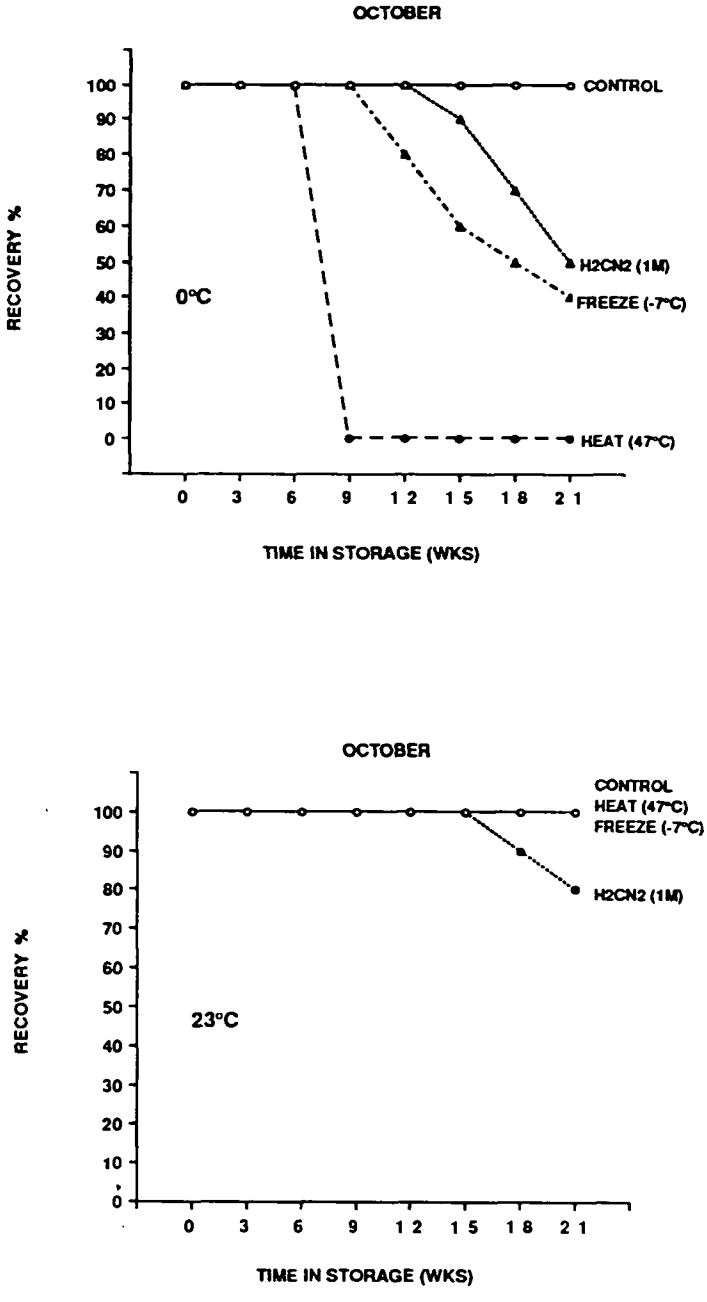

Figure 4.5 Effects of NL -heat (47°C for one hour), freeze (-7°C for one hour), and hydrogen cyanamide (1M) in October on the % recovery of red osier (*Cornus sericea* L.) plants at two post-environments (0°C, and 23°C).

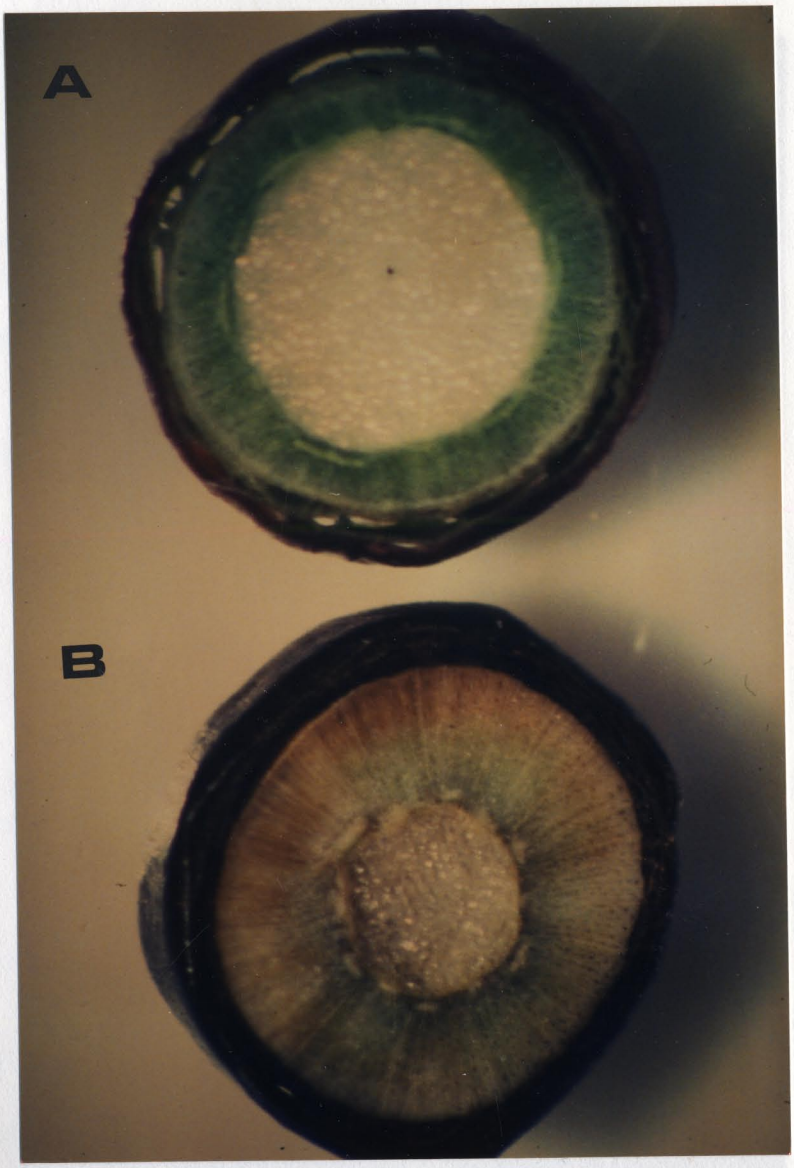

Figure 4.6 Cross sections of internode of red-osier dogwood (*Cornus sericea L.*) plants at 0°C post-environment: A, Control (nonstressed) ; B, NL-heat stress (47°C for one hour).

Figure 4.7 Internode of red-osier dogwood (*Cornus sericea L.*) at 0°C post-environment: Top, NL-heat stress (47°C for one hour) and Bottom, Nontreated part.

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5. Role of Glutathione on Recovery of Red-Osier Dogwood Plants from "Near-Lethal" Heat Stress

5.1 Abstract

This study reports on the relationship of glutathione on the recovery and death of red-osier dogwood plants from NL-heat stress. Shoots of dormant red-osier dogwood, *Cornus sericea* L., plants were exposed to 51° for one hour during early February, and then incubated in the dark at either 0° or 23°C PET. Reduced and oxidized forms of glutathione (GSH & GSSG, respectively) were measured with HPLC at 365 nm absorbance after 0, 24 hrs, and 2, 4 weeks PET, respectively. Incubation of the NL-heat stress treatments for 24 hours at 23°±3°C prior to post treatments resulted in a significant decrease of GSSG and slight decrease of GSH. In contrast, the sudden exposure of NL-stressed plants to 0°C for 24 hours resulted in a significant reduction of both GSH and GSSG levels throughout the PET test period. At 23° PET, GSH and GSSG levels of NL-heat stressed plants increased, while at 0° post treatment environment the levels of both decreased.

5.2 Introduction

Glutathione, a tripepetide which is present in all higher plant cells (Rennenberg 1982, 1987), has strong antioxidant activity (Alscher 1989). The reduced form of glutathione has been found to be involved in the detoxification of many compounds associated with stress related effects, such as chilling (Wise and Naylor 1987, Siller-Cepeda *et al* 1991), water stress (Burke *et al* 1985), drought (Dhindsa, 1987) frost and salinity (De kok and Oosterhius 1983), heavy metals (Grill *et al* 1985), herbicides (Martinge and Scalla 1988), ozone (Guri 1983, Mehlhorn et al 1982), sulfur dioxide (Grill and Esterbauer 1978 , Grill 1979 ; Shu-Wen *et al* 1982, Chiment *et al* 1986), heat stress (Nieto-Sotelo 1986), and low temperature stress (Esterbauer and Grill 1979, Guy and Carter 1984, Kuroda *et al* 1990a, 1990b). In addition, an increase in water soluble thiol compounds have been observed in spruces (Grill *et al* 1988) and lichens (Guttenberger *et al* 1991) growing at high altitudes.

Recently, we reported that NL-stresses from varied sources e.g., heat, freeze, and hydrogen cyanamide, resulted in complete , or partial dieback of dormant red-osier dogwood plants, when stored at low temperatures (0°-5°C) PET, whereas the plants exposed to ≥10°C and natural PET (Corvallis, Or ; 44°, 37', 59" N and 123°, 11', 32" W) recovered. We proposed that the recovery of plants exposed to NL stresses is a function of the environment following exposure to stress. Because glutathione is a strong antioxidant and because we have found glutathione to be involved in detoxifying various compounds (Nee, 1986), the objective of this study was to investigate the changes of

glutathione content in NL-heat stressed plants stored at 0° and 23°C PET.

5.3 Materials and Methods

A clone of red-osier dogwood, *Cornus sericea* L. from Wayland, MA, was used, because previous work had established the relationship of NL-stresses on overcoming rest (Nee 1986) and causing plant injury at low temperature (Shirazi 1992). The plants were propagated and grown as previously described (Kobayashi *et al* 1982, Shirazi 1992). NL-heat stress was achieved by exposing defoliated stems to 51°C for one hour in a circulating water bath in 6 Feb. 1991. The tops of the plants were enclosed in 4 mil clear polyethylene bags with the air removed to enhance plant contact with the water temperature. After immersion, the plants were kept for approximately 24 hour at 23°±3°C prior to post-environment treatment (PET) (0° and 23°C dark condition for 2 and 4 weeks). In another study , after NL-heat treatment, the plants were placed immediately into 0°C PET and gluthatione levels determined 24 hours later.

Reduced and oxidized form of glutathione were measured as described by (Siller-Cepeda *et al* 1991). Bark tissues (n=3) were homogenized with 5 ml of 10% perchloric acid with 1mM BPDS (bathophenanthroline disulfonic acid). The homogenates were centrifuged at 10,000 g for 20 minutes at 4°. The supernatant was collected, and the tissues rehomogenized and centrifuged two additional times. The supernatant from all three extractions were pooled and 0.5 ml of the aliquot was added to 50 ul of 0.5 mM *Y*-glutamyl-glutamate (internal standard). The sample was carboxy-methylated with iodoacetic acid, and derivatized with 2,4-dinitro-

1-flourobenzene as reported previously (Siller-Cepeda *et al* 1991). A Beckmam Gold HPLC with a 3-aminopropyle-sepherisorb column (4.6 X 200mm, 5u) (Custom L. C. Inc Houston TX.) was used to analyze for glutathione at room temperature with flow rate of 1.5 ml/minute. Absorbance was monitored at 365 nm.

5.4 Results

The level of GSH was not affected while GSSG level increased significantly by the NL-heat stress treatment immediately after exposure to heat stress (Fig. 5.1). Twenty four hours after treatment, the NL-heat stressed plants incubated at 23° PET showed a slight but significant decrease in GSH levels and a significantly large decrease (e.g., more than 2x) in GSSG levels. The GSSG and GSH levels of the non-stressed control plants were not significantly affected between 0 and 24 hrs of 23° PET. In contrast, after 24 hrs of 0° PET, the levels of GSH and GSSG decreased by more than 2.5x in the NL-heat stressed treatments. The levels of both compounds in the non-stressed controls at 0° PET were similar to the levels at 23° PET after 24 hrs.

At 23°C PET, GSH levels of NL-heat and non-stressed plants were similar after 0, 2 and 4 weeks (Fig. 5.2). A significantly small increase in GSSG occurred between the NL-heat and non-stressed treatment immediately following The NL-heat treatment (0 wks of PET). After 2 weeks PET, GSSG levels of NL-heat and control treatments were similar.

In contrast, at 0° PET, the levels of GSH and GSSG in the NL-heat stressed plants decreased significantly from 0 and 2-4 wks of PET treatment (Fig. 5.3). In the controls, GSH remained unchanged throughout the 0° PET treatment, and GSSG levels remained the same between 0 and 2 weeks and increased significantly between the 2nd and 4th week of 0° PET. The amount of GSH and GSSG in the controls of 0° and 23°C PET from 0 to 4 weeks were similar (Fig. 5.2 and Fig. 5.3).

5.5 Discussion

As stated previously, we reported that dormant plants exposed to NL-stresses were either severely injured or killed during low PET temperatures, and recovered without injury at warm PET temperatures. The cause of this dieback at low PET temperatures and recovery at warm PET temperatures is not known. Siller (1991) found that the levels of GSH in dormant peach plants exposed to natural "chilling units" to overcome rest decreased during early rest and increased to a maximum level when rest was broken, He also showed that hydrogen cyanamide, a strong oxidizing agent used to overcome rest, also caused an initial decrease followed by an increase in GSH levels. Based on this work we hypothesized that the recovery of plants exposed to NL-stresses was dependent on the metabolism of antioxidants such as GSH at warm temperatures.

The results of this study supports the hypothesis. Within 24 hours after exposing NL-heat stressed plants to 0° PET the level of GSH and GSSG decreased dramatically. Significant decreases in both compounds were also observed after 24 hrs. of 23° PET. After 2 and 4 weeks of 0° PET the levels of GSH and GSSG from the NL-heat stressed treatments remained low while the levels in the controls remained high, with the GSSG showing a significant increase after 4 weeks of PET. In contrast, at the warm (23°) PET temperature treatment, the levels of GSH and GSSG from the NL-heat treatments increased to the same extent as the non-stressed control plants. Therefore, these studies suggests that plants exposed to NL-stresses results in a rapid (within 24 hours after exposure) depletion of GSH

and GSSG . "Recovery" of GSH and GSSG levels thereafter is dependent on warm temperatures.

Although we cannot conclude from this study that the recovery and death of plants from NL-stresses is a function of GSH and GSSG levels, we do provide evidence that show a strong relationship between the PET conditions that results in recovery or death and the levels of glutathione. Glutathione has also been associated more strongly than other antioxidants (e.g. α-tocopherol and ascorbic acid) with oxidative stresses caused by air pollutants (Mehlhorn *et al* 1986). In this and other reports glutathione has been found to protect cells from the deleterious effect of free radicals resulting from oxidative stresses. For example, Barclay (1988) indicated that " Relatively high concentration of GSH play a key role in protecting sensitive biological membrane from free radical damage by trapping oxygen radicals in the aqueous phase".

Alcher and Amthor (1988) discussed the processes of maintenance and/or repair in cells exposed to oxidative processes resulting in free radical production. For general maintenance of the cell, an enzymatic induction and biosynthesis of macromolecules is required; while for repair processes the resynthesis of new macromolecules (which are lossed and/or damaged during oxidative stresses, e.g. membrane lipid bodies), reestablishment of the ionic balances across membranes, and re-reduction of the SH enzymes are necessary (Alcher and Amthor 1988).

It is possible that the NL-stress treatment resulted in the production of free radicals that could present a problem to the cells if not reduced. Failure to produce glutathione by stressed plants at the

low temperature PET may have resulted in an accumulation of free radicals which eventually lead to the death of the cells. In contrast, at the high temperature PET the production of glutathione enabled the cells to reduce the levels of the free radicals thus enabling the cells to recover from the NL-stress treatment.

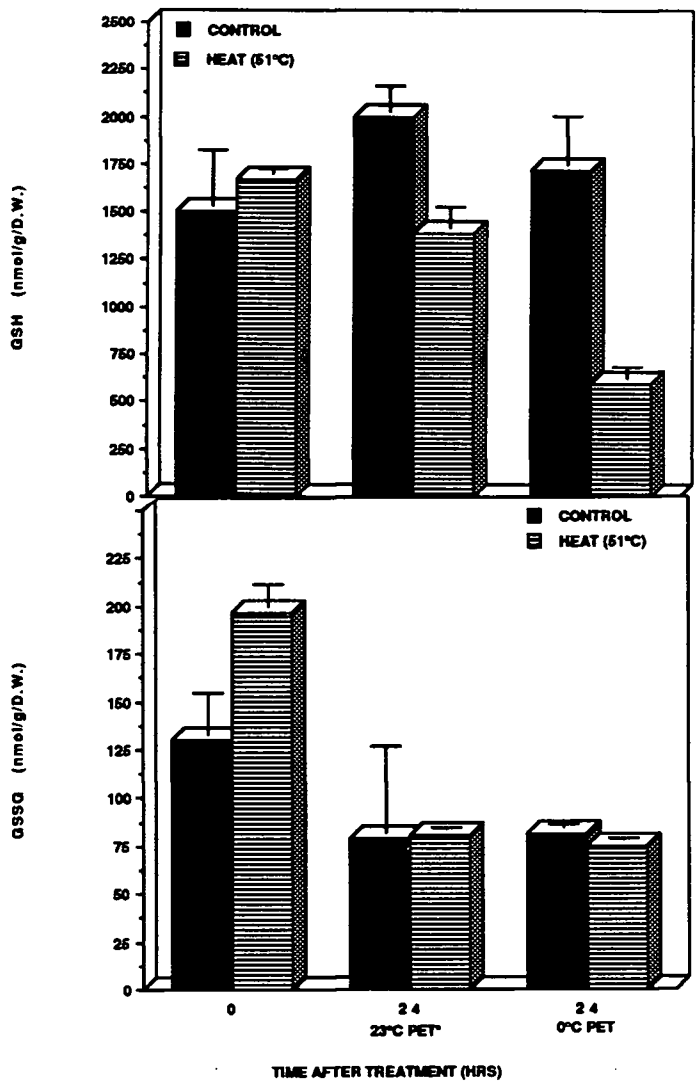

Figure 5.1 Levels of reduced (GSH) and oxidized (GSSG) glutathione of control (nonstressed) and NL-heat stressed red-osier dogwood (*Cornus sericea* L.) plants stored at 23°C and 0°C post-environment treatment (PET) for 0 and 24 hours.

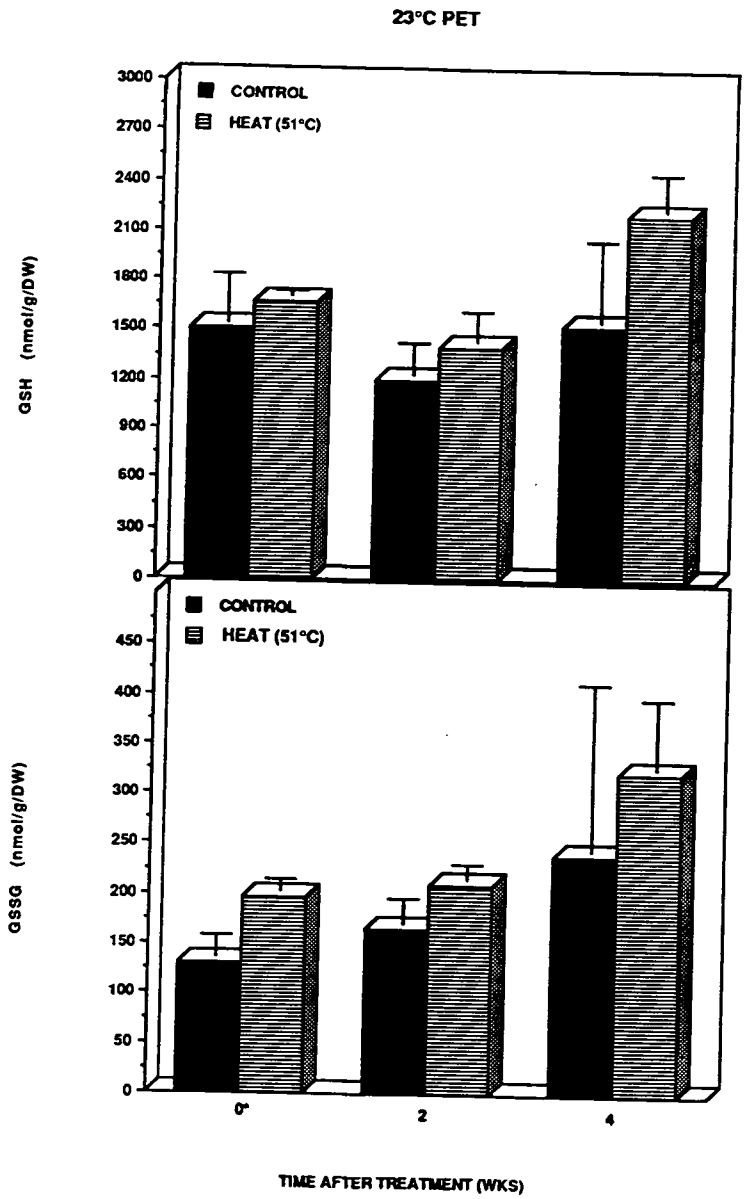

Figure 5.2 Levels of reduced (GSH) and oxidized (GSSG) glutathione of control (nonstressed) and NL-heat stressed red- osier dogwood (*Cornus sericea* L.) plants stored at 23°C post environment treatment (PET) for 0, 2, and 4 weeks. *0 weeks: Plants were held at 25°±3°C for approximately 24 hrs prior exposure to 23°C (PET)..

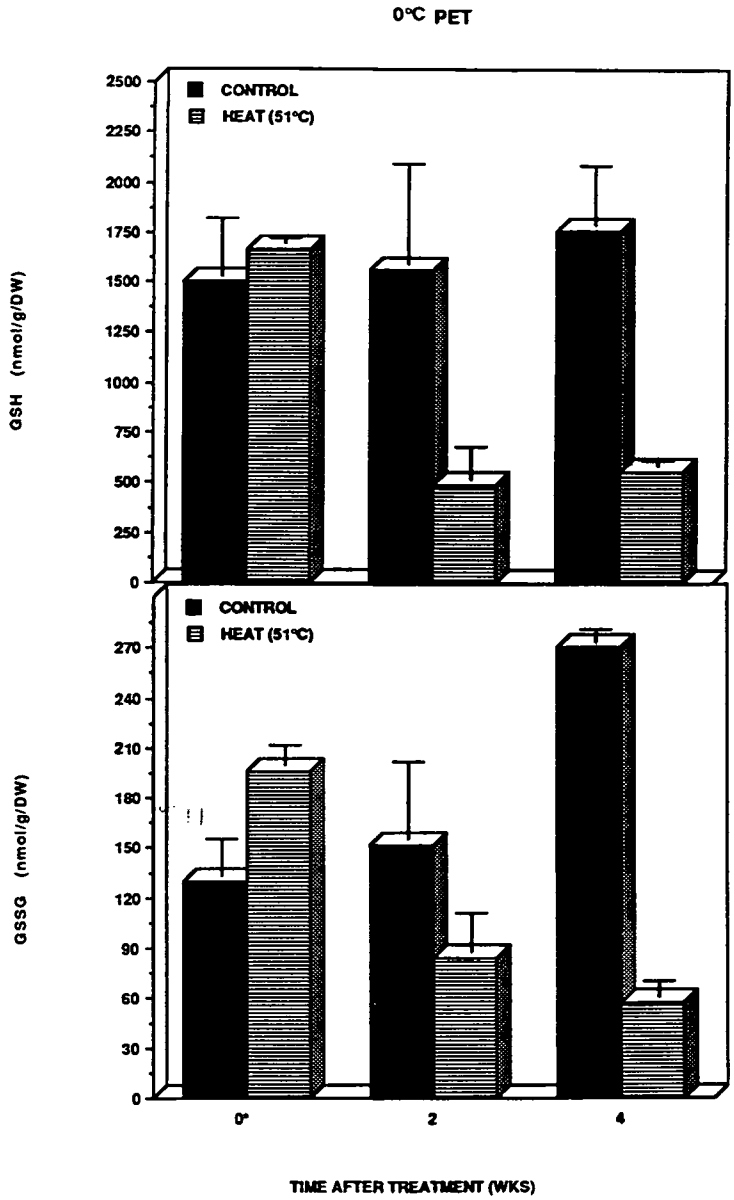

Figure 5.3 Levels of reduced (GSH) and oxidized (GSSG) glutathione of control (nonstressed) and NL-heat stressed red-osier dogwood (*Cornus sericea* L.) plants stored at 0°C post environment treatment (PET) for 0, 2, and 4 weeks.
*0 wks: Plants were held at 25°±3°C for approximately 24 hrs prior exposure to 0° PET.

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6. Heat Tolerance and Ethylene Production of Red- Osier Dogwood Stem Tissues at Different Growth Stages

6.1 Abstract

Heat tolerance of red-osier dogwood, *Cornus sericea* L., was determined at different growth stages and tissue viability was measured by electrical conductivity and ethylene production. Heat tolerance of tissues were highest during the later stages of endodormancy and early ecodormancy phases and lowest during the spring growth phase. During active growth the previous seasons stem tissues increased in heat tolerance and were similar to the tolerance of tissues during early endodormancy.

Ethylene production of heat treated stem tissue was dependent on the stage of development and a function of the degree of stress. During early endodormancy and during active growth heat stress of stem tissues stimulated ethylene production, reaching a peak at 40°C, followed by a steady decrease at higher temperatures. Highest levels of ethylene from stressed tissues occurred in May, July, September and March from the early stages of endodormancy to ecodormancy heat stressed stem tissues did not produce ethylene (late October to January. Application of 1-aminocyclopropane-1-carboxylic acid (ACC) to stem segments at late endodormancy (December), methionine and indole acetic acid (IAA) at maximum endodormancy (November) enhanced ethylene production of non-heat and heat stressed stem tissues.

6.2 Introduction

Heat tolerance of temperate woody plants species has been reported by many researchers (Levitt 1980, Seidel 1986, Helgerson 1990) . Heat tolerance variability has been related to seasonal changes, plant size, age, and genetic make-up of plants (Parker 1971, Levitt 1980, Seidel 1986, Helgerson 1990) . Heat tolerance of temperate woody plants has also been related to bud developmental stages (Koppenal and Colombo1988).

Heat stress has been used to successfully overcome bud dormancy of woody plants (Molisch 1908,1909, Bennett 1950, Nee 1987, Tanino *et al.* 1989) and flowering bulbs (Hosoki 1984) . The critical level and duration of heat stress to overcome dormancy is near the lethal temperature (Nee 1986, Tanino *et al.* 1989, Shirazi 1992).

Ethylene production has been associated with many types of stresses in plant tissue (Abeles 1973, Lieberman 1979, Yang and Hoffman 1984). The relationship of heat stress and ethylene production has not been investigated in woody plants. Hosoki (1984) reported that hot water treatment (46°C for one hour) had no significant effect on ethylene production in dormant gladiolus corms and Easter lily bulbs. Ethylene levels decreased during dormancy development in deciduous (Seibel and Fuchigami 1978) and coniferous trees (Burr *et al.* 1985, Barbro *et al.* 1991). Generally, the production of ethylene increases in response to increasing stress up to a certain point and thereafter decreases (Leopold *et al.* 1972, Kobayashi *et al.* 1981, Harber and Fuchigami1986, Field 1981, 1985). Kimmerer and Kozlowski (1982) showed that in paper birch, water

deficit, wounding, freezing and ozone exposure all enhanced ethylene production.

The suggested pathway of stress ethylene biosynthesis is from methionine. Ethylene has been produced from methionine (Lieberman *et al.* 1965, Yang *et al.* 1966). Methionine is metabolized (Yang and Hoffman 1984) to S-adenosylmethionine (SAM), to 1-aminocyclopropanecarboxilic acid (ACC), to ethylene (Adams and Yang 1979, Yang and Hoffman1984, Liberman 1979). ACC synthase which converts SAM to ACC is a key enzyme in ethylene biosynthesis, and EFE (ethylene-forming enzyme) which converts ACC to ethylene is thought to be membrane-bound (Kende 1989). Its activity is thought to be related to the membrane integrity of the plant cell (Imaseki and Watenabe 1978, Kende 1989).

Indole acetic acid also has stimulatory effects on ethylene production in plants (Yu *et al.* 1979 ; Yoshi and Imaseki 1982). Auxin-induced ethylene production is due to the enhancement of the ACC-synthase activity (Jones and Kende 1979, Yu and Yang 1979, Yoshi and Imaseki 1981).

The objective of this study was to investigate the heat tolerance of red-osier dogwood at different growth stages and its relationship to ethylene production.

6.3 Materials and Methods

A clone of red-osier dogwood, *Cornus sericea* L. from Wayland, MA was used for these studies. The growth stages of the plants were determined by the °GS model (Fuchigami *et al.* 1982, Kobayashi 1983) . The plants were propagated by single node stem cuttings in Feb., 1988. Rooted cuttings were transplanted into a mixture of sterilized bark, peat and soil (1:1:1 v/v) in 15 cm fibre pots, and grown in a lathhouse with 20 % shade. Plants were irrigated by overhead sprinklers twice daily (6 AM & 3 PM). Osmocote (Sierra Co.), a slow-release fertilizer (17-6-9) with micronutrients, was applied at a rate of 4 grams per pot at planting. plants were trained to one stem in Jun & Jul. plants were 70 to 90 cm tall at the time of the experiments.

Five detached 50 cm terminal stem tissues were placed in a 4 mil polyethylene bag, the air evacuated and the bag sealed to prevent water entry. The bags were then placed in a water bath and exposed to either 30°, 35°, 40°, 45°, 50°, 55°, or 60°C for one hour at each test temperature. The controls were held at room temperature (23°±3°C). After heat treatment, 2.5 cm internodal stem segments (n=5) were cut from the 2nd, 3rd, and 4th internodes.

For conductivity determination, each internodal sample (5 replications) was placed in a 25 ml glass vial with 15 ml of double-distilled water Samples were evaluated for electrolyte leakage with a Markson Conductivity Meter held at room temperature. Samples were shaken for 36 hrs in an orbital shaker at 100 rmp at room

temperature before the initial conductivity was taken. Samples were then killed in a hot water bath at 80°C for 1.5 h, shaken for 24 hrs, and the final conductivity measurements determined. Total ion leakage was calculated by dividing the initial by the final measurement multiplied by 100. The LT_{50}, the temperature at which 50% electrolyte leakage occurred, was calculated by first fitting a 3rd order polynomial through the data, and using the 50% midpoint as the LT_{50} point.

For ethylene measurements 5 internode samples were randomly selected, placed in a scintillation vial, closed with a serum stopper, incubated for 8 hrs in the dark and 1 ml samples were drawn from the vials and injected into a Carle 211 gas chromatograph equipped with a 1.22 m x 3.8 mm 80/100 mesh activated alumina column with a flame ionization detector. The flow rate was 20 ml/min, and the column and detector temperature was 80°..

To determine whether ACC was limiting in dormant tissues internode samples (heat treated and nontreated) in 4 Dec. 1988 were treated with either .25 ml of 10 mM ACC (Sigma Co.) or double distilled water and shaken on a orbital shaker at 100 rpm for one hour in the dark at 25°C. To determine if the methionine and IAA can stimulate ethylene production in dormant tissues internode samples (heat treated and nontreated) obtained on 8 Nov. 1989 were treated with .25 ml of 100 mM methionine or 10mM IAA (Sigma Co.) or double distilled water and shaken on a orbital shaker at 100 rpm for18 hrs in the dark at 25°C. The water on the surface of the samples were removed with Kimwipes, placed individually into scintillation vials as described above. Samples

were incubated for 8 and 24 hrs and ethylene measurement s taken as described previously.

Analysis of variance (ANOVA) for a completely randomized design was used for all experiments. Standard Errors were used for the line graphs. Statistical analysis was performed using the SAS software (SAS Institute, 1988).

6.4 Results

Heat tolerance

The LT_{50} of heat stressed red-osier dogwood stem tissues varied throughout the sampling period from Septemberl 988 to July 1989 (Fig. 6.1). At the onset of endodormancy, September (180°GS), the LT_{50} was 55°. As plants developed endodormancy the LT_{50} first decreased to 51° in October and then increased to 54° at maximum endodormancy in November. Highest tolerance to heat stress occurred during the late stage of endodormancy and early ecodormancy phase (December and January, respectively). At spring bud break (March, 360°GS) the LT_{50} decreased to 48° and thereafter the LT_{50} increased to about 54° in July (90°GS) (Fig. 6.1)

Electrolyte leakage of control (no heat stress) internodal stem tissues of red-osier dogwood obtained from plants growing under natural conditions in the lathhouse varied between Sepember 1988 to July 1989 (Fig 6.2.). Leakage of electrolytes increased during the development of endodormancy (September to November, 180° to 270°GS, respectively), decreased during late endodormancy (November to December, 270° to 315°GS, respectively), and remained low during the ecodormancy and active growth period (December to July, 315° to 90°GS, respectively).

Ethylene production

Ethylene production of non-stressed (controls) and heat-stressed internodal stem tissues varied with sampling date (Fig. 6.3). The controls produced higher ethylene levels during the active growth period (March to July) than during the dormant period (September to January). During early endodormancy (September and early October) ethylene levels increased with increasing heat stress, peaking at 40°, and decreasing sharply beyond 45°. At later stages of endodormancy through the ecodormancy period (October to January), heat stress had no effect on ethylene production. In March, when spring bud break occurred, increasing heat stress caused ethylene levels to decrease. After this period, May and July, heat stress promoted stress ethylene production, increasing to a peak at about 40° and decreasing sharply at 45° and thereafter (Fig. 6.3).

ACC application to internodal stem sections in December 1988 promoted ethylene production in the non-stressed and heat-stressed (30° to 45° and very low at 50°) tissues. In contrast, non-stressed treated tissues produced low levels of ethylene (approx. 1 nl/g/hr) in all treatments (Fig. 6.4) . Methionine stimulated ethylene production after 8 hrs for the 30°-45° heat treated samples and after 24 hrs for the 30°-50° heat treated samples (Fig. 6.5) . Significant difference between 8 and 24 hrs incubation was observed for the 45° and 50° heat treated samples only (Fig. 6.5). The control sample treated with methionine also produced substantial amounts of ethylene. In contrast, nontreated tissues produced low levels of ethylene (approx. 0.5 nl/g/hr) in all treatments (Fig. 6.5).

IAA stimulated ethylene production after 8 and 24 hrs for the 30°-45° heat treated samples respectively (Fig. 6.6) . Significant difference between 8 and 24 hrs of incubation was observed for the 45° heat treated samples only (Fig. 6.6). The control sample treated with the IAA also produced substantial amounts of ethylene. In contrast, tissues without IAA produced very low levels of ethylene (approx. 0.5 nl/g/hr) in all treatments (Fig. 6.6).

6.5 Discussion

Early studies established that resting tissues can tolerate higher temperature than active tissues (Just 1877, Levitt 1980). Our results also showed that heat tolerance depends on the physiological growth stage of plants. Highest levels of heat tolerance, during the dormant period, was obtained during December and January. Previous studies had shown that the cold hardiness of this clone of dogwood stem tissues are hardiest during December and January when the plants are in the quiescent (ecodormant state) (Kobayashi *et al.* 1983, Shirazi 1992). Others have also reported that the cold hardiness of plants are greatest following the rest period (Sarvas 1972, Van Hystee *et al.* 1967). Comparison of the heat tolerance results with previous cold hardiness reports (Kobayashi *et al.* 1983), suggests a close relationship between heat tolerance and cold hardiness.

During spring growth the heat tolerance of stem tissues decreased sharply to its lowest level and then increased thereafter from May to July. The reason for this change in heat tolerance during this period is not known. Englert (1992) reported that desiccation tolerance of Washington hawthorn were lowest at spring growth and increased thereafter.

The increase in ion leakage for control plants starting at the early endodormancy period could be an indication of membrane permeability changes due to the chilling temperature. Increased membrane permeability during chilling temperatures in woody plants has been previously reported by Coville (1920) and Doorenbos (1953).

Changes in ethylene production during plant development are well known. Seibel and Fuchigami (1978) reported differences in ethylene levels in red-osier dogwood during active growth and the development of endodormancy. Higher ethylene levels were found in non-dormant stem tissues than dormant tissues. Hosoki (1984) also has shown that heat treatment did not enhance ethylene production in dormant gladiolus corms and Easter lily bulbs. Our studies confirm these results.

The production of stress ethylene has also been shown to be dependent on the growth status of plants (Seibel and Fuchigami 1978, Burr *et al.* 1985, Barbro *et al.* 1991). Typically, with most tissues, increasing stress causes plant tissues to increase production of ethylene, reaching a peak, and then decreasing until the tissue is killed (Harber and Fuchigami 1986 , Fields 1985). Because of the changes in ethylene levels with changes in stress treatment the production of ethylene has been used as a measure of stress and injury to plant tissues (Leopold *et al.* 1972, Tingey *et al.* 1976, Harber and Fuchigami 1986, Kacperska and Kubacka-Zebalska 1989, Mehlhorn *et al.* 1991). Increasing levels of ethylene is thought to relate to the degree of non-lethal stress that the tissues are exposed to. Following the peak in ethylene, further stress cause ethylene levels to decrease and this decrease correlates inversely with increases in membrane permeability and ethane production (Peiser and Yang 1979, Kobayashi *et al.* 1981, Field 1981, Kimmerer and Kozlowski 1982, Harber and Fuchigami 1986).

In our studies, we found that the typical bell-shaped curve of ethylene production with increasing levels of heat stress during the

active growth period (March to July) and during early endodormancy (September and early October). During the later stages of endodormancy and the ecodormancy period (late October to January) heat stress of stem tissues had no effect on ethylene production. Barbro et al (1991) suggested that the low ethylene levels in *Pinus sylvestris L.* during the winter is due to low temperature and low metabolic activity. Application of ACC in December and methionine and IAA in November to the tissues following the heat stress treatments all promoted ethylene production. The level of ethylene produced was not due to heat stress as the control tissues without heat stress produced similar levels of ethylene as the heat stressed tissues. The bell-shaped curve, which typifies stress ethylene production was not observed with increasing heat stress. Thus, these studies suggests that the low levels of ethylene in dormant tissues is due to the low levels of substrate rather than the lack of enzyme activity required for ethylene production. Methionine has been shown to be very low in poplar twigs (Sagisaka 1972), and red-osier dogwood stem tissues (Li *et al.* 1966) during the dormancy period.

These studies suggests that ethylene measurement would not be useful in determining the degree of stress of dormant plant tissues . However, the absence of ethylene peak following heat treatment could be used as a quick measure of endodormancy and ecodormancy in temperate woody plants.

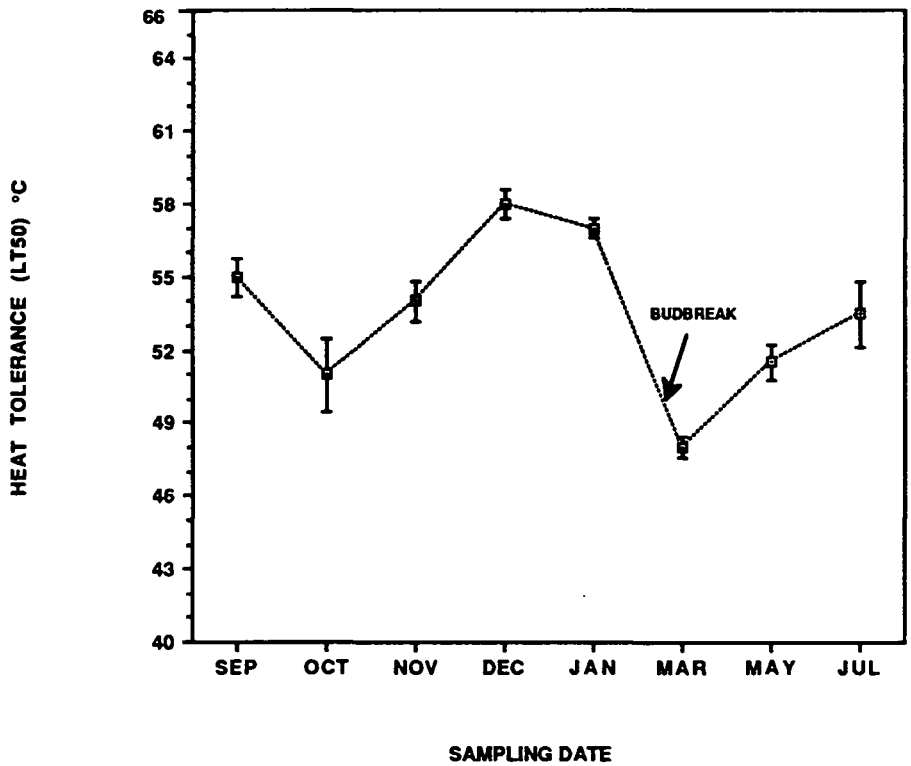

Figure 6.1 Heat tolerance of red-osier dogwood (*Cornus sericea* L.) stem tissues at different sampling dates.

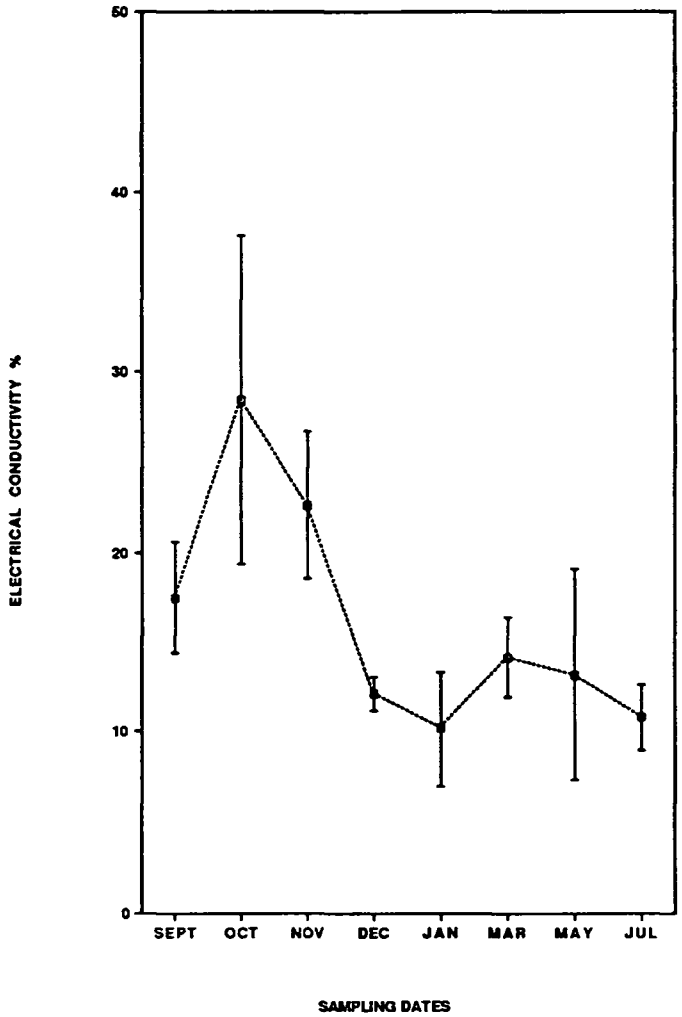

Figure 6.2 Ion leakage (% of the control plants) at different sampling dates in red-osier dogwood (*Cornus sericea* L.).

Figure 6.3 Ethylene production in red-osier dogwood (*Cornus sericea* L.) stem tissues at different sampling dates.

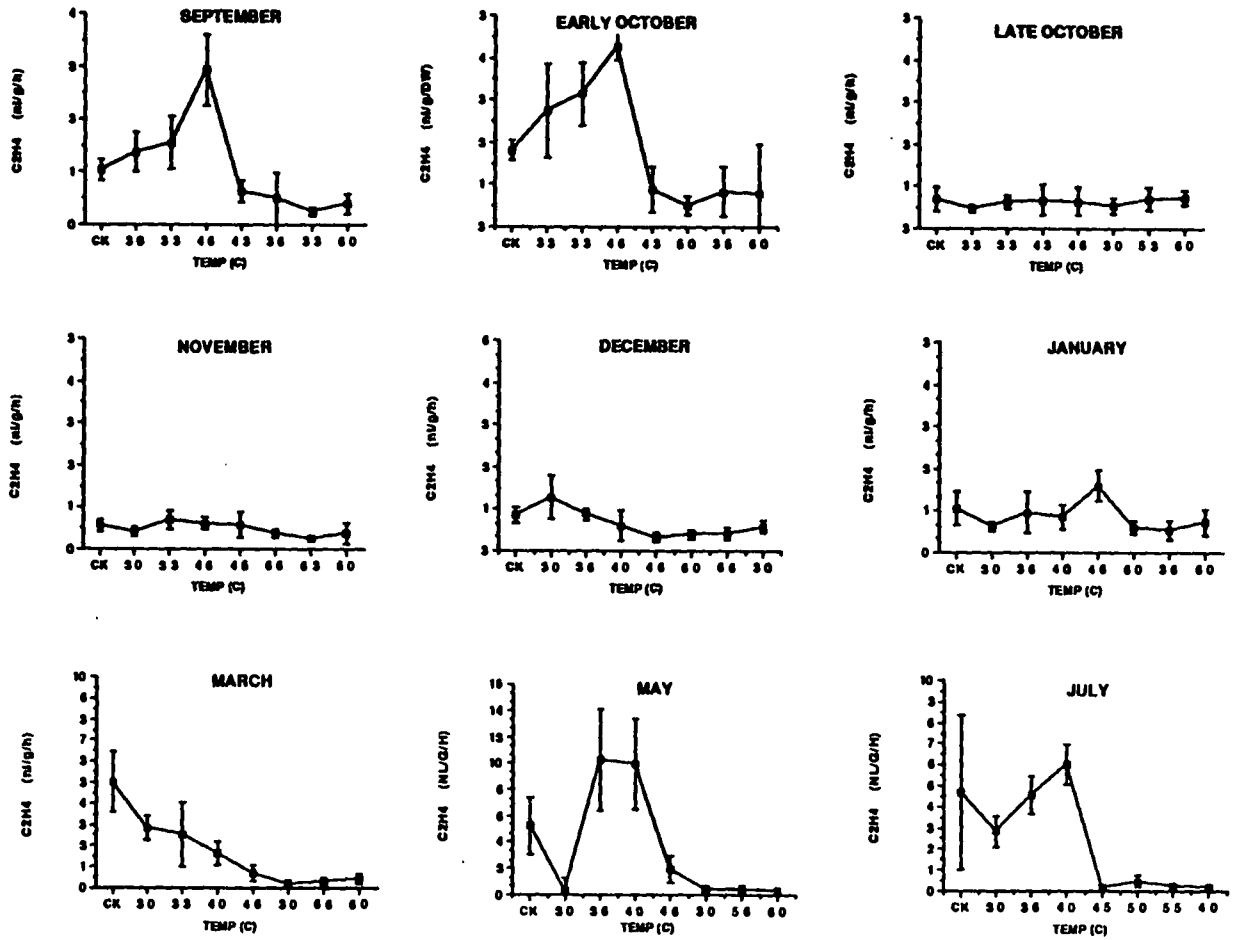
SEPTEMBER
EARLY OCTOBER
LATE OCTOBER
NOVEMBER
DECEMBER
JANUARY
MARCH
MAY
JULY
TEMP (C)

Figure 6.3

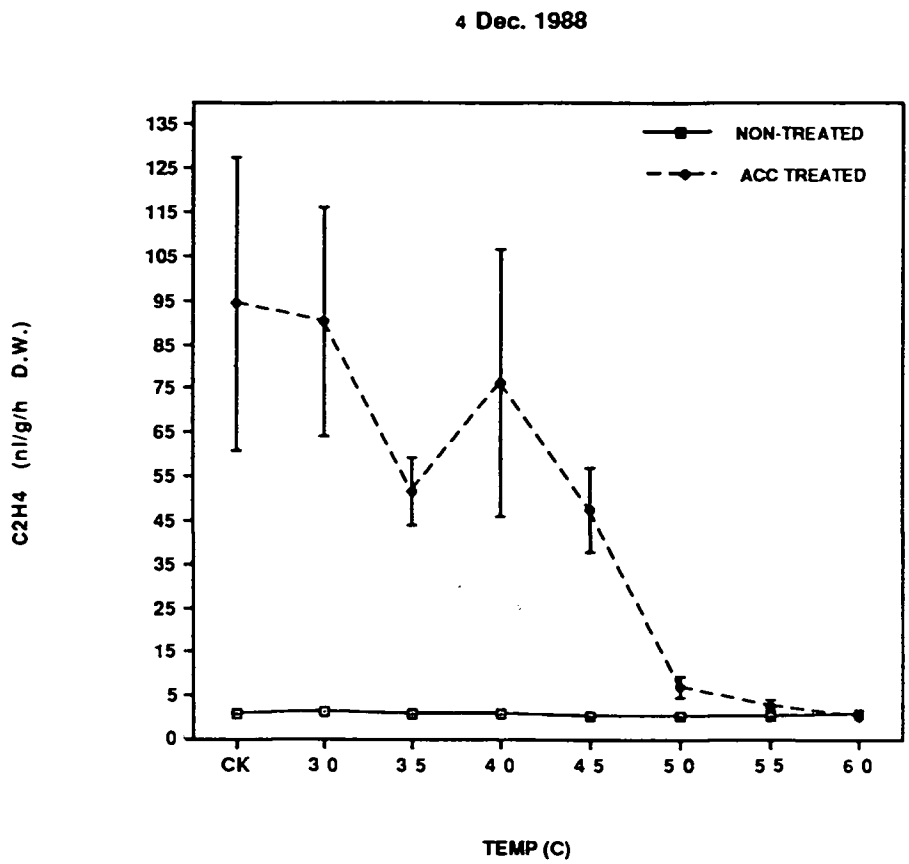

Figure 6.4 Effect of 10mM ACC on ethylene production of heat treated and non treated red-osier dogwood(*Cornus sericea* L.) stem tissues after 8 h in 4 Dec. 1988.

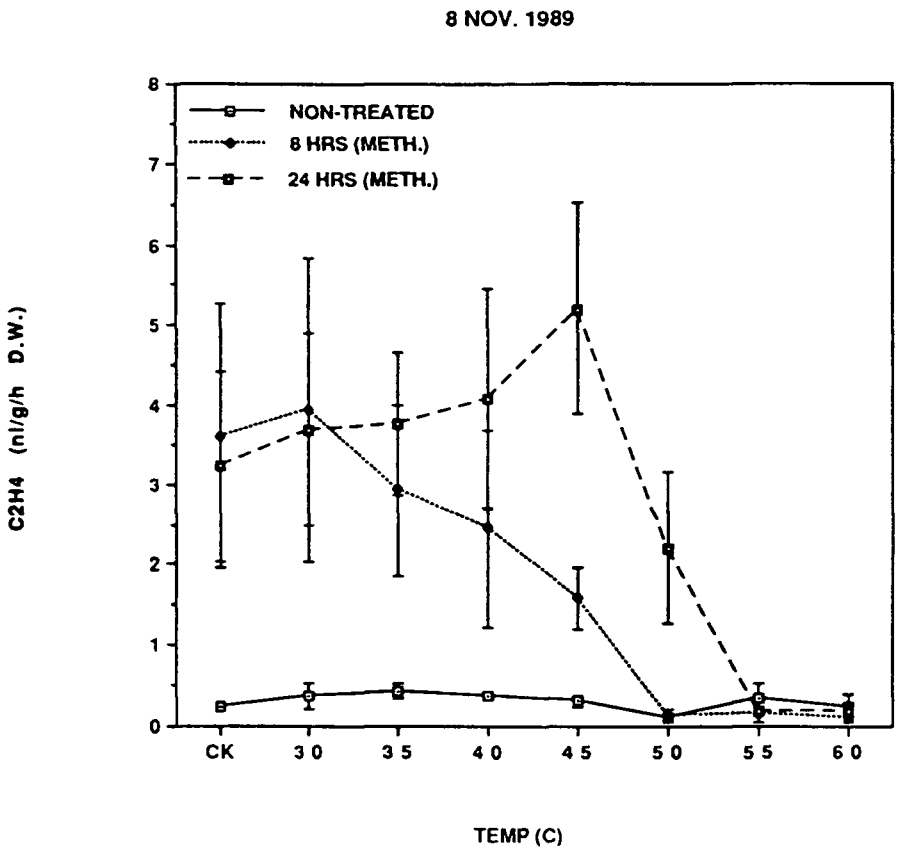

Figure 6.5 Effect of 100mM methionine on ethylene production of heat treated and non treated red-osier dogwood (*Cornus sericea* L.) stem tissues after 8 and 24 h in 8 Nov.,1989.

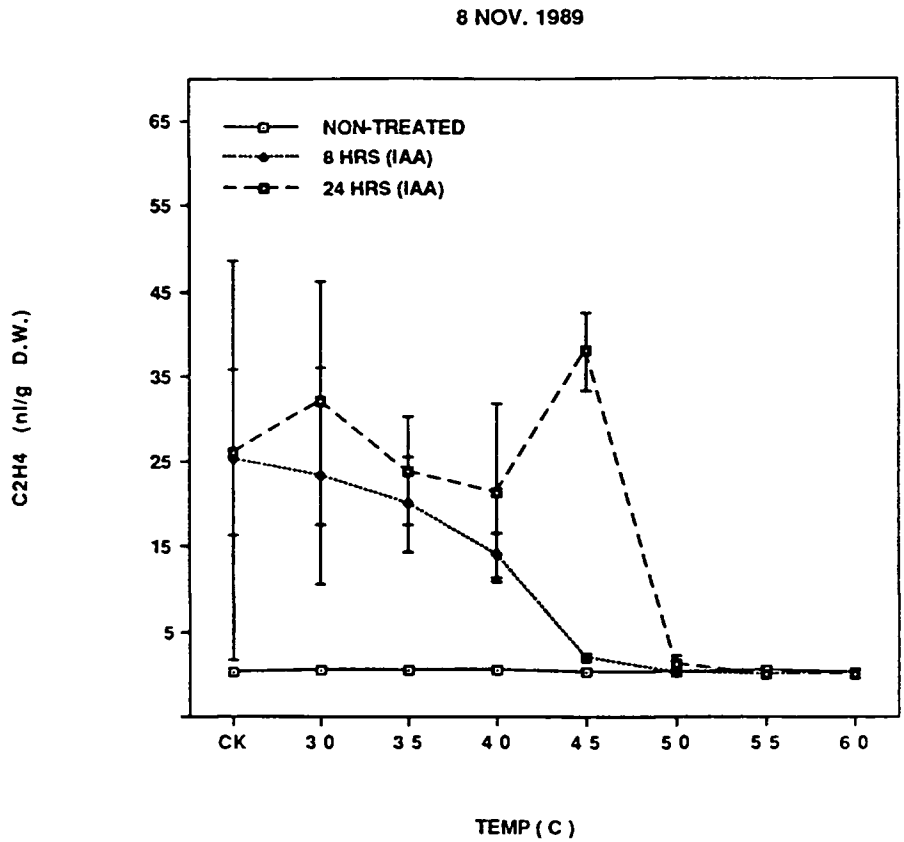

Figure 6.6 Effect of 10mM IAA on ethylene production of heat treated and non treated red-osier dogwood (*Cornus sericea* L.) stem tissues after 8 and 24 h in 8 Nov., 1989.

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Appendices

Appendix 1a.

Dormancy Stages	Treatment	% Recovery *		
		Post-Environment Treatment		
		0°C	23°C	Natural Condition
28 Oct. 1989 (Early)	Control	100	100	100
	Heat	0.00**	100	100
7 Dec. 1989 (Late)	Control	100	100	100
	Heat	80**	100	100

* % Recovery after 21 weeks of Post-Environments
** Dieback of the heat treated part of the plants occurred in 10-11 weeks for October, and 18-19 weeks for December treatments at 0°C, respectively.

Table A1. Effects of NL-heat stress (47°C for one hour) on the % recovery of red-osier dogwood (*Cornus sericea* L.) plants at two sampling dates (28 Oct. 1989 and 7 Dec. 1989), and three post-environment treatments (PET) (0°C, 23°C and natural condition at Corvallis, Or).

Appendix 1b.

Dormancy Stages	Treatment	% Recovery *		
		Post-Environment Treatment		
		0°C	23°C	Natural Condition
20 Oct. 1990 (Early)	Control	100	100	100
	Heat	0.00**	100	100
5 Dec. 1990 (Late)	Control	100	100	100
	Heat	60**	100	100

* % Recovery after 21 weeks of Post-Environments
** Dieback of the heat treated part of the plants occurred in 7-8 weeks for October, and 14-15 weeks for December treatments at 0°C, respectively.

Table A2. Effects of NL-heat stress (47°C for one hour) on the % recovery of red-osier dogwood (*Cornus sericea* L.) plants at two sampling dates (20 Oct. 1990 and 5 Dec. 1990), and three post-environment treatments (PET) (0°C, 23°C and natural condition at Corvallis, Or).

Appendix 2.

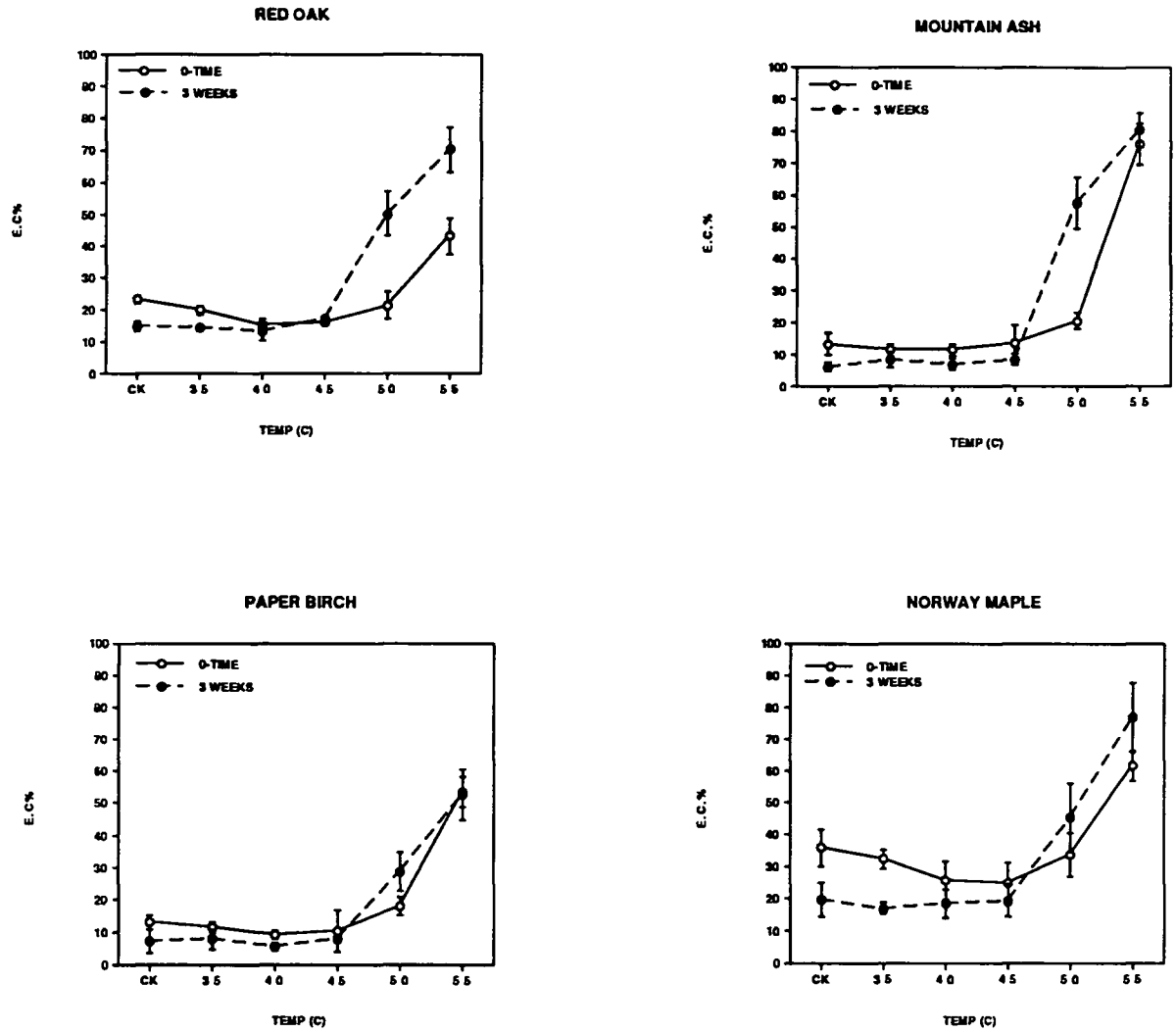

Figure A.2 Effects of heat treatments: Control (23°±3°C), and Heat(35°. 40°, 45°, 50°, and 55°C for one hour) on % electrical conductivity of two years old seedlings of dormant red oak (*Quercus rubra* L.), paper birch (*Betula papyrifera* Marsh.), European mountain ash (*Sorbus* aucuparia L.), and Norway maple (*Acer platanoides* L.) stem tissues (2 cm) after 0 and 3 weeks of 0°C post-environment on 4 May, 1991.

Appendix 3a.

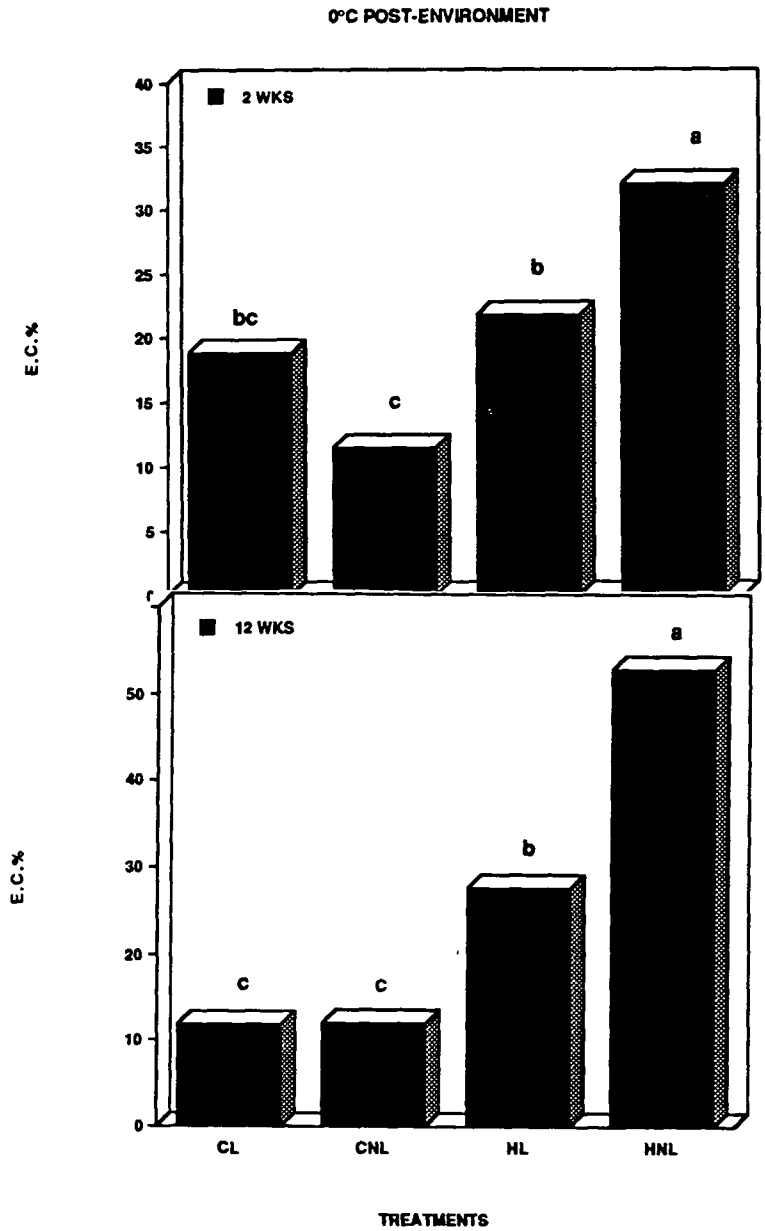

Figure A2. Effects of leaching on % electrical conductivity of heat stressed and non stressed stem tissues (2.5 cm) of red-osier dogwood (*Cornus sericea* L) on an orbital shaker (100 rpm) for 48 hours in15 ml DD-H2O prior to incubation at 0°C post-environment for 2 and 12 weeks on 22 Jan. 1991.
Abbreviations: CL: control leached ; HL: heat (49°C for one hour) leached ; CNL: control nonleached ; HNL: heat (49°C for one hour) nonleached

Appendix 3b.

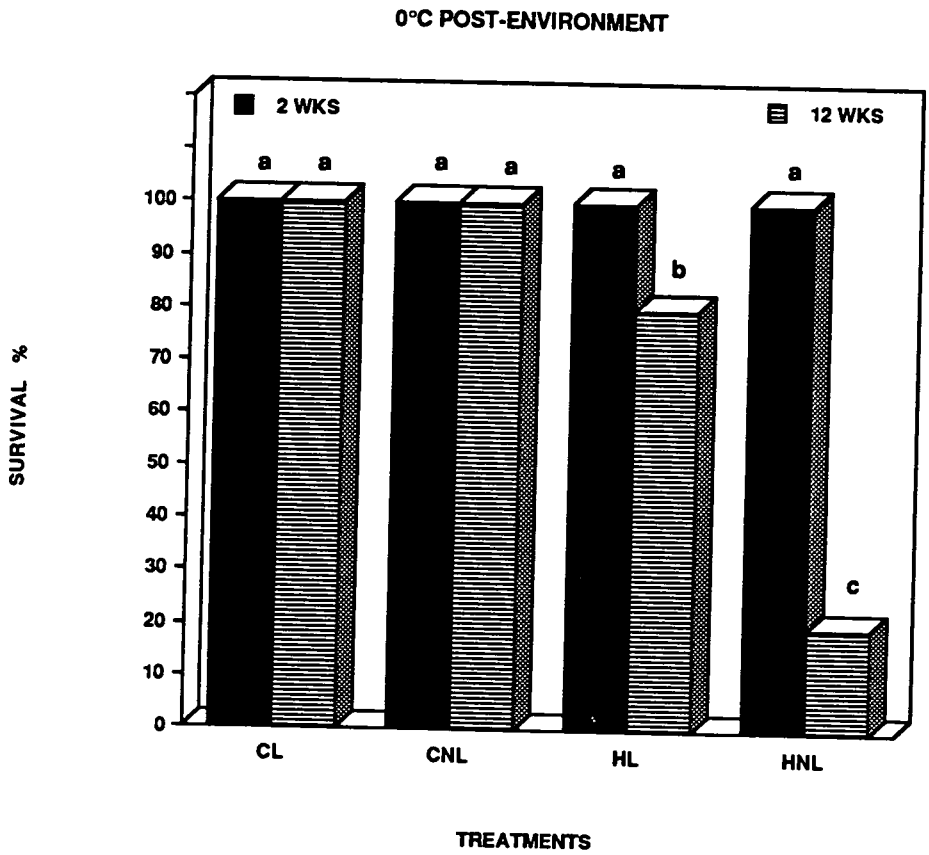

Figure A3. Effects of leaching on % survival of heat stressed and non stressed stem tissues (2.5 Cm) of red-osier dogwood (*Cornus sericea* L.) on an orbital shaker (100 rpm) for 48 hours in15 ml DD-H2O prior to incubation at 0°C post-environment for 2 and 12 weeks on 22 Jan. 1991. Abbreviations: CL: control leached ; HL: heat (49°C for one hour) leached ; CNL: control nonleached ; HNL: heat (49°C for one hour) nonleached.

Appendix 4.

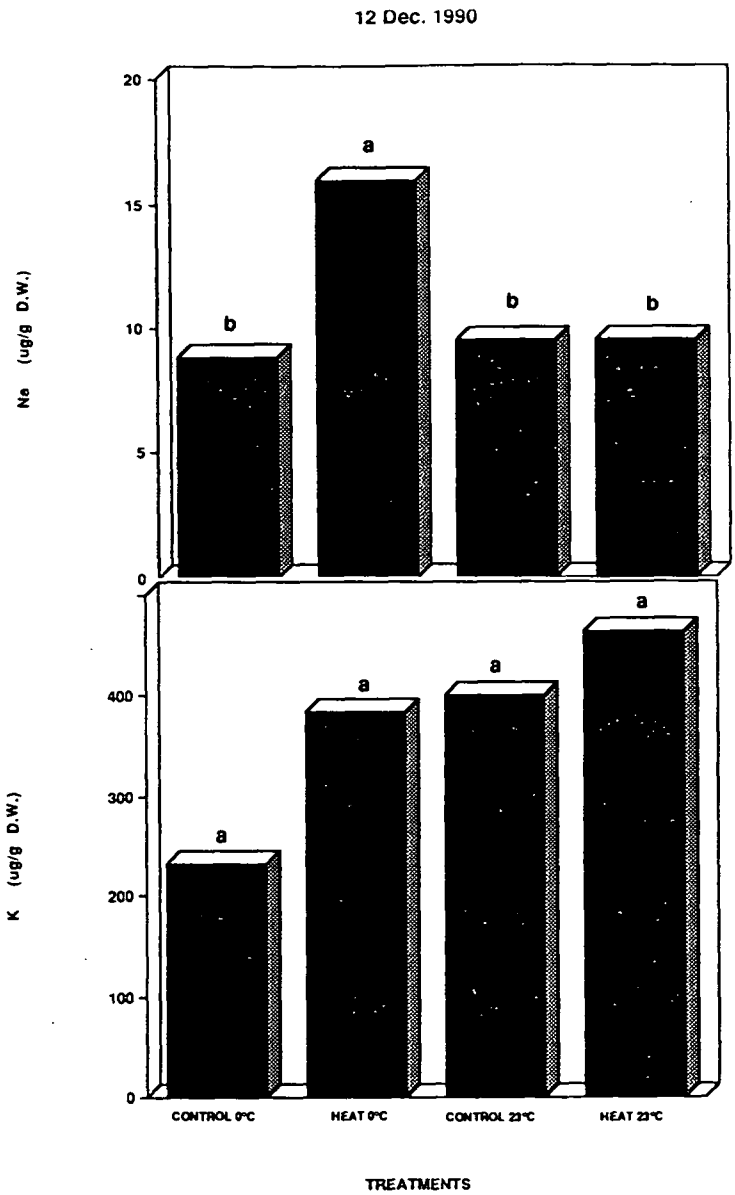

Figure A4. Effect of heat stress (50°C for one hour) on specific ion leakage (sodium, Na and potassium, K) of stem tissues (2.5 cm) of red-osier dogwood (*Cornus sericea* L.) after 36 hours in an orbital shaker (100 rpm) with 15 ml DD-H20 in 10 Dec. 1990.

Appendix 5.

Daily minimum and maximum air temperature and humidity in the lathhouse, Oregon State University, Corvallis, Oregon.

(October 1988 to February 1989)

Date	Temperature (°C)		Humidity (%)	
Oct. 1988	Min.	Max.	Min.	Max.
20	11.6	17.2	43	80
21	6.1	16.6	48	82
22	4.9	16.0	23	82
23	2.2	18.3	24	83
24	11.6	17.2	47	79
25	6.6	13.8	62	85
26	6.6	13.3	41	83
27	1.6	13.8	20	83
28	2.2	12.7	34	79
29	5.5	14.9	45	82
30	9.9	20.5	38	77
31	12.7	19.9	47	75
Nov. 1988				
1	6.6	12.2	80	85
2	10.5	12.2	73	83
3	8.3	12.7	69	82
4	8.8	14.4	61	83
5	11.1	15.5	56	82
6	7.7	11.6	52	83
7	6.1	12.7	62	87
8	4.9	18.8	70	85
9	7.2	10.5	82	85
10	8.3	17.2	72	84
11	3.8	15.5	76	85
12	7.2	17.2	70	83
13	4.4	10.5	77	83
14	9.4	15.5	79	80
15	7.2	16.6	73	84
16	8.8	9.9	82	83
17	5.5	14.9	70	83
18	3.8	11.1	77	84
19	3.3	10.5	80	84
20	6.6	12.2	79	84
21	8.3	11.1	83	84

Appendix 5 continued

22	6.6	13.3	81	84
23	5.5	16.0	75	83
24	4.4	6.6	83	84
25	5.5	12.7	79	84
26	3.3	14.4	77	83
27	4.4	8.8	83	84
28	---	---	---	---
29	4.9	15.5	73	83
30	2.2	9.4	79	84
Dec. 1988				
1	4.4	9.4	76	83
2	3.8	9.4	81	83
3	4.4	7.7	82	83
4	3.8	8.3	82	83
5	3.3	6.6	83	84
6	8.8	16.6	73	82
7	7.2	14.4	73	83
8	4.4	9.4	81	83
9	6.6	11.6	81	83
10	7.7	11.1	80	82
11	7.2	10.5	81	82
12	7.2	12.7	82	83
13	---	---	---	---
14	1.6	13.8	70	83
15	.55	13.3	73	83
16	.55	14.4	72	82
17	-1.1	11.6	72	81
18	-2.2	6.6	77	82
19	4.4	12.2	75	85
20	4.4	7.2	84	86
21	3.3	9.9	75	85
22	4.9	13.8	71	84
23	2.7	6.6	83	85
24	3.8	7.2	82	84
25	1.6	8.8	70	84
26	1.6	7.2	76	85
27	2.7	8.3	71	85
28	3.3	9.9	74	84
29	3.3	8.3	84	85
30	5.5	8.8	83	84
31	1.6	13.2	77	84
Jan. 1989				
1	4.4	12.6	74	84
2	6.6	15.5	73	82
3	3.8	8.3	81	83
4	3.3	11.1	74	83
5	1.1	8.3	77	84

Appendix 5 continued

6	55	11.1	72	83
7	3.3	6.6	83	84
8	---	---	--	---
9	4.9	6.6	80	80
10	1.1	5.5	73	80
11	0.0	7.7	77	80
12	-1.1	10.5	71	80
13	2.2	4.4	78	81
14	.55	6.1	73	80
15	2.7	8.3	73	81
16	5.5	8.8	81	82
17	5.5	9.4	78	82
18	5.5	12.7	79	82
19	3.8	10.5	78	83
20	4.9	11.1	77	83
21	6.1	12.2	77	83
22	2.7	7.2	83	84
23	2.2	6.1	82	87
24	---	---	---	---
25	---	---	---	---
26	3.8	11.6	78	87
27	2.7	8.8	82	86
28	1.1	16.6	75	85
29	4.4	17.2	74	84
30	8.8	16.6	75	79
31	3.8	11.6	65	81
Feb. 1989				
1	1.6	3.8	70	83
2	.55	2.7	80	86
3	-.55	1.6	84	86
4	-1.1	2.7	81	86
5	-1.1	4.4	79	86
6	-.55	4.4	78	86
7	-1.1	4.4	77	86
8	-1.6	4.9	77	86
9	-.55	6.1	77	86
10	1.6	5.5	79	86
11	1.6	7.7	81	86
12	2.2	13.3	79	87
13	7.2	11.1	80	86
14	3.3	16.0	72	86
15	-1.1	14.9	69	85
16	4.9	9.4	82	86
17	6.6	10.5	83	85
18	7.2	11.6	81	85
19	4.4	16.6	76	85

Appendix 5 continued

Daily minimum and maximum air temperature and humidity in the lathhouse, Oregon State University, Corvallis, Oregon

(October 1989 to February 1990)

Date	Temperature (°C)		Humidity (%)	
Oct. 1989	Min.	Max.	Min.	Max.
26	8.8	11.6	44	85
27	2.7	9.9	50	80
28	0.0	9.9	30	80
29	-2.2	12.2	26	82
30	-1.1	11.1	35	80
31	-1.1	14.4	20	80
Nov. 1989				
1	-1.6	15.5	15	72
2	2.7	12.7	30	85
3	-0.5	13.3	30	82
4	7.2	12.7	40	80
5	2.2	11.1	44	79
6	4.9	9.9	53	80
7	0.5	11.1	56	82
8	9.9	15.5	60	80
9	9.9	14.4	55	82
10	7.2	14.9	57	85
11	4.4	9.9	60	80
12	---	---	---	---
13	3.3	17.2	55	84
14	3.3	14.4	75	82
15	2.7	13.3	78	84
16	7.7	13.8	80	84
17	9.4	12.2	80	83
18	8.8	14.9	66	83
19	8.3	13.8	58	78
20	7.7	15.5	55	80
21	4.9	9.9	64	78
22	6.1	12.7	65	80
23	4.9	11.1	56	82
24	4.4	7.7	75	80
25	4.4	13.8	70	80
26	5.5	15.5	72	84
27	6.6	15.5	66	82

Appendix 5 continued

28	6.6	9.9	75	80
29	3.8	14.9	73	82
30	3.3	11.1	80	85
Dec. 1989				
1	---	---	---	---
2	7.2	11.1	84	86
3	7.2	12.2	80	80
4	4.4	11.1	80	83
5	5.5	14.4	72	80
6	6.1	13.3	71	81
7	6.6	13.3	65	84
8	3.3	14.4	70	82
9	1.6	9.9	60	81
10	1.6	3.3	78	80
11	1.1	11.6	66	76
12	-0.5	9.9	64	80
13	0.0	7.2	73	82
14	2.7	5.5	80	83
15	2.2	4.4	80	82
16	2.7	7.7	74	83
17	3.3	4.9	80	83
18	2.7	12.7	75	83
19	2.7	6.6	82	83
20	1.6	7.7	78	83
21	2.2	5.5	81	83
22	2.2	5.5	81	82
23	3.3	15.5	74	82
24	2.7	7.7	78	82
25	---	---	---	---
26	1.1	8.3	77	82
27	1.1	9.9	76	82
28	1.1	4.9	79	82
29	1.6	8.3	78	82
30	0.5	9.9	74	82
31	0.5	5.5	77	82
Jan. 1990				
1	2.2	11.1	79	82
2	2.2	11.6	74	78
3	1.1	8.3	72	76
4	4.4	8.8	80	81
5	6.1	9.4	80	80
6	6.1	11.1	78	80
7	6.1	9.4	69	80
8	8.8	11.1	82	84
9	7.7	12.2	80	82
10	6.1	8.3	80	81
11	5.5	11.1	78	84

Appendix 5 continued

12	3.3	11.1	76	82
13	4.9	14.9	75	82
14	7.2	13.3	75	81
15	3.8	8.8	78	74
16	1.6	8.8	73	79
17	-2.2	11.1	70	78
18	0.0	7.7	70	78
19	-0.5	11.1	68	80
20	-3.3	7.7	71	79
21	-0.5	4.9	74	78
22	6.6	12.7	56	81
23	4.4	12.2	68	81
24	-1.1	8.3	75	81
25	2.7	7.7	78	83
26	1.6	12.7	68	82
27	0.0	11.6	66	84
28	6.1	8.8	77	86
29	4.4	7.2	80	83
30	2.7	9.4	78	83
31	1.1	5.5	80	83
Feb. 1990				
1	2.2	11.6	76	84
2	4.4	14.9	75	83
3	1.1	7.2	83	84
4	1.6	13.8	76	83
5	3.3	6.6	70	80
6	0.5	6.6	77	80
7	0.0	4.4	74	78
8	2.2	9.4	77	82
9	4.9	9.9	79	83
10	4.4	14.9	59	81
11	3.3	9.9	75	83

Appendix 5 continued

Daily minimum and maximum air temperature and humidity in the lathhouse, Oregon State University, Corvallis, Oregon.

(October 1990 to February 1991)

Date	Temperature (°C)		Humidity (%)	
Oct. 1990	Min.	Max.	Min.	Max.
20	3.3	13.3	46	84
21	9.4	11.1	75	87
22	6.1	16.6	39	88
23	4.9	18.8	44	85
24	6.6	22.7	35	88
25	7.2	15.5	62	87
26	8.8	16.6	58	87
27	7.7	18.8	37	87
28	6.1	14.9	47	85
29	5.5	16.0	39	87
30	9.4	11.1	85	86
31	6.1	12.2	68	85
Nov. 1990				
1	3.3	11.6	46	88
2	2.7	10.5	53	87
3	7.2	12.2	75	86
4	11.1	14.9	56	86
5	3.8	13.8	52	86
6	2.2	9.9	54	86
7	6.1	10.5	80	86
8	8.8	10.5	68	85
9	11.6	14.4	52	84
10	4.4	17.7	42	83
11	5.5	17.2	44	87
12	6.6	13.8	54	88
13	6.6	11.6	64	87
14	4.4	11.6	55	87
15	4.9	14.9	35	84
16	1.6	13.8	50	86
17	5.5	8.8	79	86
18	3.3	10.5	43	84

Appendix 5 continued

19	1.6	7.7	66	88
20	3.8	8.3	72	88
21	6.6	9.4	71	87
22	8.3	11.1	80	85
23	8.3	11.1	63	83
24	9.9	12.2	70	83
25	4.4	11.1	66	84
26	4.4	9.9	64	84
27	5.5	9.9	64	87
28	1.1	8.8	65	87
29	4.4	8.3	58	85
30	1.6	9.9	64	86
Dec. 1990				
1	4.4	7.2	62	85
2	3.3	7.7	72	86
3	7.2	9.9	53	78
4	2.2	10.5	55	86
5	1.6	9.9	55	86
6	1.6	7.2	68	85
7	3.3	6.1	67	85
8	4.4	13.8	45	83
9	8.8	9.9	84	86
10	2.7	9.9	63	77
11	1.6	8.8	65	85
12	1.1	3.8	78	86
13	1.6	6.6	76	88
14	2.7	7.7	50	86
15	1.1	9.9	43	84
16	1.1	6.6	70	84
17	8.8	11.1	70	87
18	0.0	6.1	69	85
19	-6.6	-5.5	48	85
20	-11.6	-5.5	28	50
21	-12.2	-4.9	22	31
22	-11.1	-4.4	40	58
23	-1.6	-2.2	32	62
24	-1.6	1.1	43	61
25	-9.4	2.2	61	77
26	-1.1	6.6	69	78
27	3.3	7.2	62	87
28	-4.4	3.3	40	85
29	-8.8	-2.2	29	60
30	-9.4	0.0	55	69
31	2.7	9.4	42	80
Jan. 1991				
1	1.1	9.4	50	82
2	1.1	3.3	77	87

Appendix 5 continued

3	-1.1	7.2	33	85
4	-3.8	5.5	38	78
5	-3.8	1.6	65	89
6	-1.1	1.1	67	82
7	7.7	9.9	72	88
8	4.4	9.9	58	86
9	1.6	7.2	68	87
10	7.2	9.4	84	87
11	7.2	9.9	82	85
12	9.4	13.3	66	83
13	8.8	13.8	54	84
14	11.6	12.7	70	85
15	7.2	9.9	64	85
16	3.3	9.4	63	87
17	2.7	6.6	79	88
18	3.8	6.6	82	86
19	5.5	9.9	45	85
20	-0.5	12.7	18	75
21	6.1	12.2	26	78
22	-1.1	11.6	38	85
23	1.1	7.2	58	80
24	3.8	9.9	36	82
25	-1.1	11.1	25	84
26	-2.2	11.1	30	76
27	-2.7	10.5	38	84
28	0.5	9.9	49	85
29	-2.7	8.8	39	85
30	-2.7	8.3	48	85
31	3.8	7.7	80	92
Feb. 1991				
1	4.9	13.3	48	48
2	9.9	11.6	80	85
3	8.3	12.3	62	85