76

FACTORS INFLUENCING DEHARDENING AND
REHARDENING OF FORSYTHIA x INTERMEDIA
STEMS

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DAVID F. HAMILTON

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FACTORS INFLUENCING DEHARDENING AND REHARDENING
OF FORSYTHIA X INTERMEDIA STEMS

TO: J. F. McLaughlin, Director May 16, 1972
Joint Highway Research Project Project: C-36-48C

FROM: H. L. Michael, Associate Director
Joint Highway Research Project File: 9-5-3

The attached Progress Report is submitted on the HPR, Part II, research project titled "Research in Roadside Development and Maintenance". This report is from Part II of this project "Selection, Establishment and Maintenance of Woody Ornamentals for Highway Plantings". The report has been authored by Mr. David F. Hamilton, Graduate Assistant in Research under the direction of Professor Philip L. Carpenter.

Resistance to dehardening and ability to reharden are essential to maintenance of cold hardiness in plants during periods of high temperature during the winter. The objective of this portion of the research was to study the relationship of dormancy to dehardening, environmental control of dehardening and rehardening, and modifications of dehardening with growth regulators. The results of the study are useful to highway department landscape groups in the area of proper storage and control of temperature of plantings prior to planting. Application of the findings to plantings made during cold weather should result in better survival and quicker establishment of the plantings.

The report is submitted for acceptance as partial fulfillment of the objectives of this research project. It will be submitted to the ISHC and the FHWA for review, comment and similar acceptance.

Respectfully submitted,

Harold L. Michael

Harold L. Michael
Associate Director

HLM:ms

cc: W. L. Dolch	M. E. Harr	M. B. Scott
R. L. Eskew	R. H. Harrell	J. A. Spooner
W. H. Goetz	M. L. Hayes	N. W. Steinkamp
W. L. Grecco	R. D. Miles	H. R. J. Walsh
M. J. Gutzwiller	J. W. Miller	K. B. Woods
G. K. Hallock	C. F. Scholer	E. J. Yoder

Progress Report

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OF FORSYTHIA X INTERMEDIA STEMS

by

David F. Hamilton
Graduate Assistant in Research

Joint Highway Research Project
and
Department of Horticulture
School of Agriculture

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The opinions, findings and conclusions expressed in this publication are those of the authors and not necessarily those of the Federal Highway Administration.

Purdue University
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ABSTRACT

Hardiness of stems of Forsythia x intermedia Zabel growing outdoors was determined from mid-November 1970 to early April 1971. At different times in winter, stem pieces from plants were subjected to different time-temperature combinations to study temperature required for dehardening and rehardening.

Once the cold requirement of dormancy had been fulfilled, the temperature and exposure required for significant dehardening decreased, reaching a minimum in late winter. The daily duration of low temperature required to prevent dehardening increased after dormancy was broken, but was constant throughout the remainder of winter. Stems failed to reharden beyond the level of hardiness found following dehardening, but before any exposure to low temperature. Attempts to modify dehardening with growth regulators applied in the fall to non-hardy plants were unsuccessful.

INTRODUCTION

The literature contains relatively little information on the relationship of dormancy to dehardening and rehardening. Resistance to dehardening and ability to reharden are essential to maintenance of cold hardiness in plants during periods of high temperature in winter.

The objectives of this research were:

1. to study the relationship of dormancy to dehardening in Forsythia x intermedia Zabel.
2. to study environmental control of dehardening and rehardening.
3. to modify dehardening with growth regulators.

REVIEW OF LITERATURE

Any attempt to review all the available literature on cold hardiness is unnecessary if not impossible. Studies of cold hardiness have been conducted for over a century. In 1935, Harvey compiled a bibliography of over 3400 articles on hardiness. The world literature in 1960 contained about 5,000 references on this subject, and at least 600 to 800 papers have been published since (Alden and Hermann, 1971). Thus this review is limited to an examination of research closely associated with environmental and growth regulator control of dehardening with special reference to dormancy.

Cold Hardening and Dormancy

Many workers (Levitt, 1941, Cooper, 1959, and Smith and Kefford, 1964) have agreed that development of cold hardiness and dormancy are intimately associated. In this research, the term dormancy will refer to the inability of a plant to produce normal growth under favorable conditions, while quiescence will refer to the nongrowing periods resulting from unfavorable external conditions.

According to Levitt (1941), dormancy is clearly prerequisite to cold hardening. Smith and Kefford (1964) in their three-phase explanation of development of dormancy also showed cold hardening to occur only after dormancy had been induced. These same authors said that while not all dormant buds become cold hardy, it is generally impossible in those that do develop cold hardiness to decide upon a strict demarcation between dormancy and development of cold hardiness.

Van Huystee (1964) showed that short photoperiods in the fall stimulated induction of cold hardiness in Cornus stolonifera Michaux. He attempted to explain this by saying that accumulation of growth inhibitors during fall induced dormancy and primed the hardening process. Davidson and Hamner (1957) found that in photoperiodic sensitive shrubs short-day induced dormancy resulted in greater winter hardiness than dormancy under long days. They further found that long days delayed dormancy which resulted in delayed maturity and a low degree of winter hardiness. Batchelor (1922) found that early frosts were more injurious to young than to mature walnut trees and explained that mature trees were less injured because they became dormant earlier.

Irving and Lanphear (1967) showed that development of cold hardiness in Acer negundo and Viburnum plicatum tomentosum was independent of induction of dormancy and that low temperatures independent of photoperiod would develop hardiness. They further said that it has been a mistake to assume that dormancy is a prerequisite for hardening simply because they occur in that order during the same season. In conclusion, they showed that development of cold hardiness was a photoperiodic phenomenon, but low temperatures effectively counteracted the influence of the long photoperiods.

Dehardening and Dormancy

Additional interest in the relationship of dormancy to cold hardiness arises from evidence that dormancy is also responsible for maintenance of cold hardiness during periods of high temperature. Lidforss (1907) found that evergreen branches brought indoors early in winter did not lose any cold resistance, whereas, in late winter a rapid decrease in hardiness resulted from exposure to indoor temperatures. Needles of Pinus rigida exposed by Meyer (1928) to laboratory temperatures for 3 weeks during early winter failed to lose their hardiness, but needles exposed to similar temperatures in late winter dehardened rapidly. Kessler (1935)

observed similar results. At the time of deepest dormancy (November) even 2 weeks indoors caused no dehardening.

Conifers of Siberia are characterized by a short period of winter dormancy and therefore were very sensitive to dehardening when temperature increased in January (Khlebnikova, Girs, and Kolovskii, 1963). In other conifers in which dormancy is completed in December, shoots became active in 7 to 8 days when exposed to high temperatures in early January, while only 3 days of high temperatures were required to activate the same species in March (Tumanov and Krasavtsev, 1955).

Irving and Lanphear (1967) found that dormancy retarded dehardening in Acer negundo and Viburnum plicatum tomentosum. One week's exposure to 21°C caused no dehardening in dormant Acer negundo plants, although considerable dehardening was found in non-dormant plants under the same conditions. In Viburnum plicatum tomentosum one week at 21°C in December, before dormancy was broken, caused dehardening of only 6°C, but similar temperatures in mid-February, after dormancy was broken, caused dehardening of 12°C.

Temperature and dehardening

Work on peaches by Edgerton (1954, 1960) indicated that even though dormancy retarded dehardening, it did not entirely prevent it. Exposure to 18.4°C for 4 days resulted in no dehardening, but after 7 days hardness was significantly reduced. In late winter, after dormancy had ended and the plants were merely quiescent, 4 mild days caused marked decreases in cold resistance.

Proebsting (1963) concluded that in dormant peach fruit buds there was a residual level of hardness that remained in spite of warm weather. He said that this value was constant until the end of dormancy, but decreased gradually as buds developed. In conclusion, he showed that dehardening can occur before

the end of dormancy provided hardiness greater than the minimum level has been attained previously.

Chaplin (1948), also working with peaches, found that the killing point of buds varied directly with temperature changes during winter months. Freezing tests showed that the killing temperature of fruit buds might rise as much as 11°C after a warm period, and fall as 5° to 6°C after a cold spell.

Brierly and Landon (1952) found that in Latham raspberry as short a period as 4 hours at 4°C resulted in some dehardening, and was followed by severe injury or killing at subsequent freezing temperatures. They suggested that daily exposure to temperatures lower than -3.5°C may be necessary for retention of a protective degree of cold resistance in raspberry.

Sakai (1966) hardened twigs of willow and poplar and then determined the amount of dehardening at different temperatures. At -3°C and lower, hardiness was maintained, while dehardening occurred at 0°C and above.

Zehnder and Lanphear (1966) found that dehardening of Taxus cuspidata leaves was related to duration of high temperature. Leaves dehardened 11°C in one week when given an 8-hour day at 24°C and a night temperature of 18.4°C. White and Weiser (1964) found that 5 to 7 year-old Thuja occidentalis trees which survived exposure to -69°C did not deharden after 5 days at 24°C in either January or February even though new growth was initiated in 10 to 14 days.

Photoperiod and Dehardening

According to Kramer (1936) development of cold hardiness probably is more dependent on photoperiod than is the low of hardiness. Temperatures high enough for growth rather than day length probably determine the time of resumption of growth in the spring.

Matzke (1936) found that lengthened days resulting from street lights caused a retention of leaves of Carolina poplar (Populus canadensis), London plane (Platanus acerifolia), sycamore (Platanus occidentalis), and crack willow (Salix fragilis) until extremely late in the growing season, thus delaying cold hardening. He further observed that the additional light did not cause leaves of the same trees to emerge earlier in the spring.

Cold Hardiness and Metabolism

According to Levitt (1956) and Alden and Hermann (1971) reduction of carbohydrate reserves in wintering plants reduces cold hardiness. Levitt listed more than 50 cases in which starch to sugar changes in bark tissues and evergreen leaves have been observed during dehardening.

Steponkus (1967) demonstrated that leaves and stems of *Hedera helix* 'Thorndale' hardened in the light dehardened more rapidly and to a greater extent at 21.1°C if they were exposed to light rather than kept in the dark. During dehardening, starch synthesis was much greater in lighted leaves than in leaves kept in the dark, while sugar content was correspondingly less. In stems, total sugars increased after 3 days of dehardening, and then declined after 7 days.

Dexter (1941) found that when deciduous shrubs are dehardened and then rehardened, they do not return to their original level of hardiness. He stated that full rehardening probably can not be accomplished without photosynthesis,

since each dehardening appears to permit rehardening to a lesser degree.

Several studies (Andrews, 1960, McGuire and Flint, 1962, and Pharis, Hellmers and Schuurmans, 1967) agreed with Dexter's hypothesis that increases in photosynthetic reserves contribute to greater hardiness. Pharis, Hellmers, and Schuurmans stated explicitly Dexter's implication that depletion of reserves during temperature fluctuations in winter months accounted for poor rehardening capabilities and subsequent cold injury.

Rehardening

In spite of the fact that the rehardening capabilities of plants during temperature fluctuations in winter determine the amount of cold injury incurred, the literature contains relatively little information on the phenomenon of rehardening. Much of the work in this area is intimately related to vernalization. Vernalization is the requirement of many perennial cereals and grasses for a period of low temperature (-0°C to -10°C) to stimulate flowering (Milthorpe and Ivins, 1966). Rudorf (1938) subjected winter cereals that had been vernalized to varying degrees to a temperature of 10°C at different photoperiods, and determined the rate of dehardening and the degree to which the plants could then be rehardened. He concluded that dehardening and ability to rehardened was directly related to activity of the plant.

Dexter (1941) stated that rehardening was possible in vernalized wheat plants, but more likely in unvernallized plants. He also found rehardening of alfalfa roots to occur following dehardening due to warm spells in winter. Rehardening was likely to be incomplete, however, particularly if any growth had occurred.

Brierly and Landon (1952) in Minnesota found Latham raspberry canes could rehardened to some extent following dehardening in early winter, but not enough to escape injury at temperatures below -17.8°C . They further found that some

rehardening may occur naturally under alternating temperatures below that at which growth begins (6.1°C). If growth does occur it is likely that injury will follow any subsequent exposure to temperatures lower than -6.7°C .

In work with peaches, both Edgerton (1954) and Proebsting (1963) found that as bud development progressed the rehardening capability of fruit buds was retained but occurred more slowly.

In recent work with living bark of apples, Howell and Weiser (1970) reported that short-term changes in cold resistance were closely related to air temperatures of the preceding day. In controlled studies, hardy plants dehardened as much as 15°C in one day in a warm greenhouse, and rehardened 15°C in 3 days when held at -15°C . Dehardening was only partially reversible. Once dehardening had begun, the bark did not reharden beyond a certain base level. The base level increased with each successive day of dehardening and usually corresponded to the killing temperature on the day preceding the final day of dehardening. Dexter (1941) had previously found similar results in rehardening studies with deciduous shrubs.

Effects of Growth Regulators on Cold Hardiness

Cold hardiness has been altered significantly in plants following treatment with growth regulators. Irving and Lanphear (1968) found that gibberellin, a growth promoter, prevented the induction of cold hardiness, while N-dimethylamino succinamic acid, a growth retardant, enhanced hardiness. Irving (1969) has also shown 2-chloroethyl trimethylammonium chloride (CCC) and Amo 1618 to increase cold hardiness of Acer negundo. Treated plants given short photoperiods for 5 weeks gained 4.5°C in hardiness over untreated plants.

Stewart and Leonard (1960) showed that winter hardiness of grapefruit and orange trees was increased when they were sprayed with maleic hydrazide. Maleic hydrazide has also been used to increase hardiness of lemon trees (Tumanov and Trunova, 1958).

Growth regulators may also be as important in controlling dehardening as in development of hardiness. Irving and Lanphear (1968) applied dormin, a naturally-occurring gibberellin antagonist, to non-dormant Acer negundo plants and found that the rate of dehardening was retarded. Applications of gibberellin to dormant plants broke dormancy but did not accelerate dehardening at 21.1°C. Irving (1969) has also observed that treating hardened, non-dormant plants with CCC, Amo 1618, and succinic acid 2, 2 dimethylhydrazide failed to retard dehardening after 5 days at 21.1°C. However, treatment with gibberellin distinctly accelerated dehardening under these conditions.

The effects of growth regulators on hardening of non-dormant plants and on dehardening of dormant plants have been thoroughly studied. Little work has been done to study residual effects of growth regulators upon dehardening, when they were applied to non-hardy plants the previous year.

Proebsting and Mills (1964) attempted to delay flowering in peaches with applications of gibberellic acid (GA), to reduce chances of late spring frost injury. They applied GA at 80-100 ppm to mature Elberta peach trees in late August, September, and November. Applications in August and September delayed flowering up to 7 days in one year, but had no effect the next year. In similar studies, Edgerton (1966) found no residual effects of either GA or 2-chloroethyl trimethylammonium.

Modlibowski (1965) sprayed CCC on pear trees in May and obtained greater survival of flowers after exposure to -3°C during the following spring. Modlibowski and Ruxton (1954) found that raspberries treated with maleic hydrazide in the fall were damaged less than control plants when exposed to -3.5°C for 45 minutes during the "green bud" stage in the following spring.

Weaver (1959) reported delayed bud break of Vitis vinifera and Prunus avium following application of gibberellin the previous year. The higher the concentrations of gibberellin used, the longer dormancy was prolonged.

Brian, Petty, and Richmond (1959) made weekly applications of GA to several species of deciduous trees between mid-August and late November. Development of dormancy in autumn and bud break in spring were delayed by one to 3 weeks. They concluded that prolongation of dormancy was not necessarily a consequence of delayed onset of dormancy in autumn. However, they did feel that treatment with GA in the autumn could be used to delay flowering the following spring in areas of frost danger.

GENERAL MATERIALS AND METHODS

Plant Material

All plants of Forsythia x intermedia Zabel used in the experiments were of a single clone, represented by a mature parent plant growing outdoors. Samples of the previous year's stem growth were taken from the parent plant for periodic observations of hardiness under natural conditions. Rooted cuttings from the same plant were grown for 8 to 12 weeks in a greenhouse at $21^{\circ} \pm 4^{\circ}\text{C}$ and under a 14 hour photoperiod, before being moved outdoors and treated with growth regulators.

Enviromental Conditions

Growth chambers were used for all experiments unless otherwise stated. For cold acclimation the chamber temperature was held at $4^{\circ} \pm 2^{\circ}\text{C}$. Light was provided for 8 hours daily at an intensity of 600 to 800 ft-c supplied by cool white fluorescent and incandescent lamps. The artificial cold acclimation period lasted 6 to 8 weeks.

Dehardening was carried out in a chamber held at $21.2 \pm 2^{\circ}\text{C}$. Light was supplied by cool white fluorescent and incandescent lamps at 2000 to 2500 ft-c at a photoperiod of 14 hours, except as otherwise noted.

Method of Sampling and Freezing

Experiments were arranged in a completely randomized design. Each replicate was composed of stem pieces from a single plant, and 3 to 5 replications were used. Stem pieces were selected to include all current season's growth within 8 inches of the plant apex. To minimize variation the terminal one-inch of stem was removed and discarded. The remainder of the stem was then cut into 1- to 2-inch sections to insure that a section from each stem was exposed to the entire range of test temperatures. Sections were then randomly assigned to test temperatures, wrapped individually in aluminum foil, and placed in insulated boxes. One box was left at 4°C as a control, while remaining boxes were placed in a freezer at -1°C. When the temperature within the boxes dropped below 0°C, all except the -1°C treatment box were transferred to the next freezer set at a lower temperature. This process was repeated in a similar manner for successively lower temperatures until the lowest temperature was reached. The temperature of the box remaining in each freezer after others were transferred was allowed to equilibrate with the temperature of the freezer and remained at this temperature for 2 hours. Then the box was moved to a 4°C growth chamber and left to thaw slowly for 20 to 30 hours. After thawing stem sections were cut into segments from .2-.5 cm in length, combined in 100 mg samples and placed in test tubes. To avoid possible differences between nodal and internodal tissues, no segments within 0.1 cm of a node were used.

This method of freezing produced a rate of temperature drop not exceeding 3°C per hour. Viability after freezing and thawing was determined by the refined triphenyl tetrazolium chloride (TTC) method (Steponkus and Lanphear, 1967).

Sample Preparation for the TTC Test

Stem sections were cut into segments from 0.25 to 0.5 cm in length to facilitate penetration of the triphenyl tetrazolium chloride solution. To avoid possible differences between nodal and internodal tissues, no segments within 0.1 cm of a node were used. The remaining portion of each stem section was placed in vermiculite under intermittent mist. Visual observation of viability was made after 10 and 20 days as a comparison with the TTC test.

The Triphenyl Tetrazolium Chloride Method

Segments prepared for use in the TTC method were combined in 100 mg samples and placed in test tubes. Then 3 ml of 0.6% TTC solution (buffered at pH 7.4 in a 0.5M phosphate - phosphate, with .01% Ortho X-77 added as a wetting agent) was added to each test tube. Samples were vacuum-infiltrated at 28 psi for a minimum of 2 min, or until the samples had lost enough air to sink in the TTC solution. Test tubes were then stoppered and incubated at 30°C for 15 hours. After 15 hours the TTC solution was removed and the tissue was rinsed with distilled water to remove any TTC not contained within the sample. After rinsing was complete, 7 ml of 95% ethanol was added to each test tube. Tubes were then placed in a boiling water bath for 10 min for extraction of the TTC. After removal from the bath and cooling, additional ethanol was added to each test tube to bring the total volume to 10 ml. Within one hour after cooling each tube was thoroughly shaken, and absorbance at 530 mμ was measured immediately in a Bausch and Lomb Spectronic 20 colorimeter or a Beckman Model B spectrophotometer.

Extrapolation and Analysis of the Killing Temperature

Even though freezing damage may be sustained over a range of temperatures, it is desirable to use a single "killing temperature". The amount of TTC remaining in samples was determined as a percentage of that at the 4°C controls, and was plotted as a function of freezing temperature. For purposes of comparing tests, killing temperature was defined as that required to give 50% decrease in absorption of TTC, according to Steponkus and Lanphear (1967), and Mityga (1969).

Analyses of variance (Steel and Torrie, 1960) were performed on the individual experiments to determine significant variables. Significance of means was determined by Duncan's multiple range test.

SPECIFIC METHODS AND RESULTS

Experiment 1: Cold hardiness of stems under natural conditions

The purpose of this experiment was to measure levels of cold hardiness of Forsythia x intermedia under natural conditions throughout late fall, winter, and early spring. Hardiness of detached stems was measured at approximately 10-day intervals from November 20, 1970, until April 10, 1971, or when any abrupt changes in weather occurred during this period (Figure 1). Minimum air temperatures were also recorded throughout this period (Figure 1). Hardiness of stems increased as the average minimum air temperature decreased. Maximum hardiness and the lowest air temperatures were found from early to mid-February. Temperature fluctuations throughout this period caused no extensive fluctuations in hardiness. After February 20, the average minimum air temperature increased, while dehardening occurred rapidly.

Figure 1. Killing Temperature of Naturally - Hardened Stems of Forsythia x intermedia over Late Fall, Winter, and Early Spring 1970-1971, with Weekly Minimum Air Temperature.

Figure 1.

Experiment 2: Time required for dehardening at constant temperature

The initial step in the dehardening studies was to determine the time necessary for dehardening in naturally-hardened stems of Forsythia. Dehardening tests were conducted periodically from December 1970 through March 1971. At each sampling, detached stems in test tubes containing 5 ml of distilled water were placed at 21.2°C. Hardiness was determined by controlled freezing, followed by the TTC Test, after 8 hours and again after 1, 2, 4, and 6 days exposure to 21.2°C.

By December 10, stems had become hardy to -28.1°C (Table 1). After 6 days at 21.2°C stems were not found to be significantly less hardy than those at 4°C. Visual observations indicated killing temperatures comparable to those obtained by the TTC test.

Table 1. Effect of length of exposure to 21.2°C on the killing temperature of stems of naturally-hardened Forsythia x intermedia beginning December 10, 1970.

Length of Exposure to 21.2°C	Killing Temperature (°C)*	
	TTC	Visual
none	-28.1 a	-23 to -30
8 hours	-28.0 a	-23 to -30
1 day	-27-9 a	-23 to -30
2 days	-28.1 a	-23 to -30
4 days	-27.2 a	-23 to -30
6 days	-25.5 a	-23 to -30

* Killing temperatures not followed by the same letter are significantly different at the 5% level.

On January 12 (Table 2), stems were found to be hardy to -29.2°C . Exposure to 21.2°C for 4 days or less gave no significant change in hardiness, while 6 days exposure resulted in a significant decrease in hardiness.

Table 2: Effect of length of exposure to 21.2°C on the killing temperature of stems of naturally-hardened Forsythia x intermedia beginning January 12, 1971.

Length of exposure to 21.2°C	Killing Temperature ($^{\circ}\text{C}$)*	
	TTC	Visual
none	-29.2 a	-23 to -30
8 hours	-28.9 a	-23 to -30
1 day	-28.6 a	-23 to -30
2 days	-28.6 a	-23 to -30
4 days	-28.5 a	-23 to -30
6 days	-19.6 b	-23 to -30

*Killing temperatures not followed by the same letter are significantly different at the 5% level.

On March 1 (Table 3), stems had hardened to -29.6°C . Significant dehardening was first observed after 4 days of exposure to 21.2°C . Additional dehardening occurred after 4 days, with a loss of hardiness of 12.9°C after 6 days.

Table 3. Effect of length of exposure to 21.2°C on the killing temperature of stems of naturally-hardened Forsythia x intermedia beginning March 1, 1971.

Length of exposure to 21.2°C	Killing Temperature (°C)*	
	TTC	Visual
none	-29.6 a	-23 to -30
8 hours	-29.2 a	-23 to -30
1 day	-28.8 a	-23 to -30
2 days	-26.9 a	-23 to -30
4 days	-20.8 b	-18 to -23
6 days	-16.7 b	-12 to -18

*Killing Temperatures not followed by the same letter are significantly different at the 5% level.

Experiment 3: Comparison of dehardening at various temperatures

This experiment was conducted to determine whether dehardening is a function of temperature within the dehardening range as well as to the length of exposure to dehardening temperature, as shown in Experiment 1. Immediately following each test in Experiment 1, additional naturally-hardened stems were placed at a series of temperatures from 4.4°C to 26.8°C. Hardiness was determined after 4 and 6 days at each temperature.

In December (Table 4), no significant dehardening was found at any temperature following exposure for 4 days. After 6 days, significant dehardening was found only at the highest temperature used (26.8°C).

Table 4. Effect of temperature on dehardening of naturally-hardened stems of Forsythia x intermedia beginning December 16, 1970.

Temperature for dehardening (°C)	Killing Temperature (°C)*	
	Exposure (days) 4	6
4.4	-28.2 a	-28.5 a
10.0	-27.7 a	-28.1 a
15.6	-28.1 a	-26.1 a
21.2	-27.1 a	-25.5 a
26.8	-25.7 a	-23.7 b

*Killing temperatures not followed by the same letter are significantly different at the 5% level.

In January (Table 5), no significant change in hardness was found after 4 days at any temperature, but significant differences were found after 6 days. At this time, significant dehardening had occurred at 15.6°C and a further significant decrease in hardness had occurred at 21.2°C, but no further significant change was found at 26.8°C.

Table 5. Effect of temperature on dehardening of naturally-hardened stems of Forsythia x intermedia beginning January 18, 1971.

Temperature for dehardening (°C)	Killing Temperature (°C)* Exposure (days)	
	4	6
4.4	-29.6 a	-28.9 a
10.0	-28.8 a	-26.3 a
15.6	-29.1 a	-23.9 b
21.2	-28.3 a	-19.6 c
26.8	-27.6 a	-18.6 c

*Killing temperatures not followed by the same letter are significantly different at the 5% level.

Dehardening was found to occur more rapidly in March (Table 6), than in December and January. Significant dehardening was found after only 4 days at 15.6°C. At this time no further significant dehardening was found at higher temperatures. However, dehardening measured after 6 days at 21.2°C and 26.8°C was significantly more than dehardening at 15.6°C during the same period.

Table 6. Effect of temperature on dehardening of naturally-hardened stems of Forsythia x intermedia beginning March 7, 1971.

Temperature for dehardening (°C)	<u>Killing Temperature (°C)*</u>	
	<u>Exposure (days)</u>	
	4	6
4.4	-28.6 a	-28.2 a
10.0	-27.8 a	-26.9 a
15.6	-24.4 b	-23.8 b
21.2	-21.9 b	-15.3 c
26.8	-21.6 b	-15.2 c

*Killing temperatures not followed by the same letter are significantly different at the 5% level.

Experiment 4: Dehardening under alternating temperatures

This experiment was designed to find out the extent to which short periods of low temperature during mild weather in winter prevent excessive dehardening. Detached stems were subjected to alternating temperatures of 4.4°C and 21.2°C under a 9-hour photoperiod. They were exposed to the low temperature during the dark period. The experiment was conducted monthly throughout the winter. Killing temperatures were determined followed controlled freezing and the TTC test after 2, 4, and 6 days of exposure to alternating temperatures.

In mid-December (Table 7), no differences in hardness were found between treatments after 4 days of alternating temperatures. However, as no dehardening had been found after 4 days at 21.2°C a few days earlier (Table 1), no differences were anticipated at this time.

Following 6 days of treatment, significant dehardening occurred only in stems exposed to 21.2°C for at least 22 hours daily. As little as 4 hours exposure to 4.4°C per day was sufficient to prevent significant dehardening.

Table 7. Effect of alternating temperatures on dehardening of stems of naturally-hardened Forsythia x intermedia beginning December 16, 1970.

Hours daily at 4.4°C	Hours daily at 21.2°C	Killing Temperature (°C)*	
		Length of treatment (days)	
		4	6
24	0	-28.3 a	-28.8 a
6	18	-28.1 a	-27.9 a
4	20	-27.6 a	-26.7 a
2	22	-26.9 a	-24.6 b
0	24	-27.2 a	-24.4 b

*Killing temperatures not followed by the same letter are significantly different at the 5% level.

In treatments beginning January 18 (Table), as in mid-December, no differences in hardness were found after 2 and 4 days of alternating temperatures. Although 4 hours daily at 4.4°C for 6 days retarded dehardening in December, it did not in January. Significant dehardening was found after 6 days exposure to 4 hours of low temperature daily, and further significant dehardening was found in stems receiving only 2 hours of low temperature daily.

Table 8. Effect of alternating temperatures on dehardening of stem of naturally-hardened Forsythia x intermedia beginning January 18, 1971.

House daily at 4.4°C	Hours daily at 21.2°C	<u>Killing Temperature (°C)*</u>		
		<u>Length of treatment (days)</u>		
		2	4	6
24	0	-29.6 a	-28.8 a	-27.9 a
6	18	-28.9 a	-29.0 a	-26.1 a
4	20	-29.0 a	-28.4 a	-24.5 b
2	22	-28.3 a	-27.9 a	-20.8 c
0	24	-28.4 a	-27.8 a	-19.5 c

*Killing temperatures not followed by the same letter are significantly different at the 5% level.

When the same treatments were applied beginning March 7 (Table 9), no significant differences in hardness were found after 2 days. After 4 days, dehardening had occurred in treatments where none had been found in January. Six hours daily at 4.4°C was effective in preventing dehardening for 6 days. Four house daily at 4.4°C was ineffective in retarding dehardening after 4 days, but was partially effective after 6 days.

Table 9. Effect of alternating temperatures on dehardening of stems of naturally-hardened Forsythia x intermedia beginning March 7, 1971.

Hours daily at 4.4°C	Hours daily at 21.2°C	<u>Killing Temperature (°C)*</u>		
		<u>Length of treatment (days)</u>		
		2	4	6
24	0	-29.2 a	-27.9 a	-28.4 a
6	18	-28.2 a	-27.6 a	-27.4 a
4	20	-27.3 a	-22.0 b	-22.4 b
2	22	-26.9 a	-21.8 b	-18.4 c
0	24	-26.6 a	-20.8 b	-16.9 c

*Killing temperatures not followed by the same letter are significantly different at the 5% level.

Experiment 5: Dehardening and Photoperiod

As stated by Kramer (1936) and YOUNG (1961), photoperiod probably does not effect dehardening. However, evidence eliminating photoperiod as a limiting factor in dehardening is somewhat vague. To study the relationship of photoperiod to dehardening, detached naturally-hardened stems were subjected to a constant temperature of 21.2°C, known to permit dehardening (Experiment 1). Different lots of stems were placed under a 9-hour day and under a 14-hour day. Killing temperatures were determined by controlled freezing and the TTC test after 4 and 6 days of treatment in January and again in March.

Significant dehardening was found in stems under long and short photoperiods after 6 days of high temperature beginning January 18 (Table 10), but differences between photoperiod treatments were insignificant.

Table 10. Comparison of killing temperatures of short and long photoperiods following dehardening of naturally-hardened stems of Forsythia x intermedia beginning January 18, 1971

Photoperiod (hours)	Killing Temperature (°C)*		
	Length of treatment (Days)		
	0	4	6
9	-31.1 a	-29.6 a	-21.3 b
14	-31.1 a	-38.8 a	-20.1 b

* Killing temperatures not followed by the same letter are significantly different at the 5% level.

Beginning March 7 (Table 11), 4 days exposure to 21.2°C resulted in significant dehardening under both photoperiods. As in January, no significant differences were found due to photoperiod.

In late January, dehardening had been found to occur after 6 days at 21.2°C but not after 4 days (Table 2). In this experiment, to obtain adequate dehardening and to further pinpoint the time of dehardening, 5 and 6 days exposure to high temperature were given before attempts at rehardening. Five days exposure to 21.2°C gave significant dehardening but an additional 6 days at 4.4°C gave no rehardening. Further significant dehardening occurred with 6 days exposure to 21.2°C, but again no rehardening was found after an additional 6 days at 4.4°C.

Table 11. Comparison of killing temperatures of short and long photoperiods following dehardening of naturally-hardened stems of Forsythia x intermedia beginning March 7, 1971.

Photoperiod (hours)	Killing Temperatures (°C)*		
	Length of exposure (days)		
	0	4	6
	-28.6 a	-23.9 b	-15.3 c
14	-28.6 a	-21.9 b	-15.2 c

*Killing temperatures not followed by the same letter are significantly different at the 5% level.

Experiment 6: Rehardening following dehardening

Rehardening capabilities of plants during temperature fluctuations in winter determine the amount of cold injury incurred. To study rehardening following dehardening, detached naturally-hardened stems were subjected to different lengths of time at 21.2°C for dehardening and then placed at 4.4°C to promote rehardening. Killing temperatures were determined by controlled freezing and the TTC test for 1, 2, 4, and 6 days at 4.4°C.

In late January, dehardening had been found to occur after 6 days at 21.2°C but not after 4 days (Table 2). In this experiment, conducted in early February (Table 12), 5 and 6 days exposure to high temperature were given before attempts at rehardening to obtain significant dehardening and to further pinpoint the time of dehardening. Five days exposure to 21.2°C gave significant dehardening, but an additional 6 days at 4.4°C gave no rehardening. Further significant dehardening occurred with 6 days exposure to

21.2°C, but again no rehardening was found after an additional 6 days at 4.4°C.

Table 12. Rehardening at 4.4°C in stems of Forsythia x intermedia following dehardening beginning February 4, 1971.

Length of exposure to 4.4°C (days)	Killing Temperature (°C)*		
	Length of pretreatment at 21.2°C (days)		
	0	5	
0	-29.9 a	-24.4 b	-19.9 c
1		-24.3 b	-19.6 c
2		-24.2 b	-18.8 c
4		-25.0 b	-19.8 c
6		-24.8 b	-19.9 c

*Killing temperatures not followed by the same letter are significantly different at the 5% level.

In stems exposed to 4 and 6 days of 21.2°C beginning March 7 (Table 13), dehardening continued after exposure to 4.4°C. Hardiness following 2 days at 4.4°C was significantly less than hardiness prior to any cold exposure. After 4 days at 4.4°C, dehardening was reversed. Following 6 days at 4.4°C the killing temperature of stems did not significantly differ from that of stems before exposure to 4.4°C.

Table 13. Rehardening at 4.4°C in stems of Forsythia x intermedia following dehardening beginning March 7, 1971.

Length of exposure to 4.4°C (days)	Killing Temperature (°C)*			
	Length of pretreatment at 21.2°C (days)			
	0	2	4	6
0	-28.6 a	-26.9 a	-20.8 b	-16.7 c
1		-25.7 a	-16.1 c	-14.2 cd
2		-26.9 a	-16.1 c	-11.9 d
4		-28.8 a	-18.0 bc	-16.5 c
6		-27.2 a	-20.5 b	-15.8 c

*Killing temperatures not followed by the same letter are significantly different at the 5% level.

Experiment 7: Differences in Hardiness of Nodes and Internodes

In previous experiments, after 3 weeks under intermittent mist, nodal tissue often appeared alive, while internodal tissue appeared dead. To study differences, detached stems were exposed to 21.2°C for 2, 4, and 6 days beginning March 1, and killing temperatures determined following controlled freezing and the TTC test. For TTC tests, nodal samples included axillary buds and all tissue within 0.1 cm of them, while internodal samples included all remaining tissue.

No differences in hardiness were found between nodes and internodes using the TTC method (Table 14). Differences appeared in visual observations, but they are probably insignificant as the maximum and minimum killing temperatures overlap.

Table 14. Comparison of hardness of nodes and internodes of naturally-hardened stems of Forsythia x intermedia following exposure to 21.2°C beginning March 1, 1971.

Length of exposure to 21.2°C (days)	Type Tissue	Killing Temperature (°)*	
		TTC	Visual
0	node	-29.6 a	-30 to -35
	internode	-28.5 a	-23 to -30
2	node	-26.9 a	-23 to -30
	internode	-27.3 a	-23 to -30
4	node	-20.8 b	-23 to -30
	internode	-21.0 b	-18 to -23
6	node	-16.7 c	-18 to -23
	internode	-14.9 c	-12 to -18

*Killing temperatures not followed by the same letter are significantly different at the 5% level.

Experiment 8: Residual effects of growth regulators on dehardening

Hardening and dehardening have been significantly altered in many plants with growth regulators. Relatively little work has been done to study the residual effects of growth regulators on dehardening when applied to non-hardy plants in the fall. To study residual effects of N-dimethylamino succinamic acid (Alar), a growth retardant, and gibberellic acid (GA), a growth promoter, on dehardening, treatments were applied to non-hardy Forsythia in the fall. Treatments consisted of GA applied at 100 and 500 ppm and Alar at 1000 and 3000 ppm. One group of plants was treated September 15 and another group October. To insure uniform coverage, the aerial portion of the plant was dipped in a

solution of the growth regulator for 10 to 20 sec. All plants were removed from the greenhouse maintained at 21.2°C to 26.8°C one week before the first treatment and placed under outdoors until early December, when they were moved to a growth chamber at 4.4°C to avoid injury due to low temperatures outdoors.

Exploratory studies showed that 6 days at 21.2°C was sufficient for dehardening in untreated plants in January. Beginning January 22, (Table 15), plants in all treatments were exposed to 21.2°C for 6 days. Plants treated with GGA, with the exception of plants treated at 100 ppm in October, had dehardened significantly more than untreated plants. Treatment with Alar had no significant effect on dehardening in January.

Table 15. Residual effects of growth regulators on dehardening of Forsythia x intermedia beginning January 22, 1971.

Treatment	Killing Temperature (°C)*
none	-23.2 a
GA at 100 ppm, September 15	-20.5 b
GA at 500 ppm, September 15	-18.5 b
GA at 100 ppm, October 15	-21.3 a
GA at 500 ppm, October 15	-20.0 b
Alar at 1000 ppm, September 15	-23.0 a
Alar at 3000 ppm, September 15	-24.0 a
Alar at 1000 ppm, October 15	-22.8 a
Alar at 3000 ppm, October 15	-22.8 a

*Killing temperatures not followed by the same letter are significantly different at the 5% level.

Killing temperatures determined in February (T 1e 16), showed results similar to those found in January. Plants treated the previous fall with GA were significantly less hardy than untreated plants, while plants treated with GA in September in had dehardened significantly more than those treated with GA in October. Treatment with Alar in the fall had no significant effect on dehardening in February.

Table 16. Residual effects of growth regulators on dehardening of Forsythia x intermedia beginning February 22, 1971.

Treatment	Killing Temperature (°C)*
none	-21.1 a
Ga at 100 ppm, September 15	-11.0 b
GA at 500 ppm, September 15	-11.8 b
GA at 100 ppm, October 15	-16.0 c
GA at 500 ppm, October 15	-15.8 c
Alar at 1000 ppm, September 15	-20.0 a
Alar at 3000 ppm, September 15	-20.8 a
Alar at 100 ppm, October 15	-21.5 a
Alar at 3000 ppm, October 15	-19.9 a

* Killing temperatures not followed by the same letter are significantly different at the 5% level.

DISCUSSION AND CONCLUSIONS

Dehardening

Dormancy was found to retard dehardening in Forsythia, but not to prevent it entirely. In December, before dormancy was completed, 6 days exposure to 21.2°C did not give significant dehardening, but the same treatment did give significant dehardening in mid-January. It is likely that exposure of dormant stems to dehardening temperatures for more than 6 days would cause continued dehardening and possibly total loss of hardness. In similar studies with Acer negundo and Viburnum plicatum tomentosum, Irving (1967) found that dormancy and dehardening were independently controlled, but that dormancy significantly restricted dehardening. He found that the eventual degree of dehardening during prolonged periods of high temperature was not significantly different in dormant and non-dormant plants. From these results, it is apparent that maintenance of cold hardness is more dependent upon continued low temperature than upon dormancy.

Once the cold requirement of dormancy had been fulfilled, the temperature and exposure required for significant dehardening decreased, reaching a minimum in late winter. Even though dehardening occurred more readily throughout the winter, it appears that 5 to 6 days of warm weather are required before dehardening reaches a maximum. The rate of dehardening may remain constant at temperatures greater than 21.2°C, as in these experiments, higher temperature did not further stimulate dehardening. Additional studies are needed to determine the effects on dehardening of prolonged exposure to higher temperatures than were used here.

A relationship was also found between dormancy and the daily duration of low temperature required to prevent dehardening. In mid-December, 4 hours exposure to low temperature (4.4°C) daily prevented dehardening for 6 days, while 6 hours daily were required in January and March. However, as both dormancy and low temperature retard dehardening, it is possible that the daily duration of low temperature required to prevent dehardening would be less during dormancy.

Rehardening

Extensive rehardening of Forsythia stems following dehardening appears unlikely. Stems dehardened in late January and then re-exposed to low temperature did not reharden, but no further dehardening occurred. In late winter, stems apparently required a short adjustment period (2 days) to low temperature, before dehardening stopped. After the 2-day adjustment period, dehardening stopped and rehardening began, and at the end of the rehardening period, the stems had returned to the level of hardness found before exposure to low temperature. It is possible that longer exposure than 6 days to 4.4°C would cause further rehardening. However, without photosynthesis, it is doubtful that rehardening beyond the level preceding any dehardening can be accomplished, since each period of warm temperature causes further depletion of reserve foods.

Practical Applications

These results indicate that plant material used in highway landscapes should be stored under controlled temperatures, at the nursery and also by the highway department prior to planting. Premature warming of storage

facilities will increase respiration rates and rapidly deplete food reserves. This means that by the time plant materials reach the planting site they are in a weakened state and stand little chance of survival in the adverse conditions usually found at these sites. If plant materials receive the proper low temperatures to prevent dehardening and depletion of stored foods, better survival and quicker establishment are likely under the stress of highway planting sites.

Low temperatures need not be provided constantly to maintain plant material in a hardened state or state of low metabolic activity. As shown in this research 6 hours of low temperature (4°C) daily are adequate to maintain hardiness. Without a daily exposure to low temperature, plant materials lose hardiness in as little as 2 to 4 days of high temperature (21°C). Although this study utilized Forsythia intermedia, other woody ornamentals may be expected to behave similarly. Further implications are that plant materials may be transported only 15-16 hours without cold temperatures to prevent dehardening.

Rehardening studies show that once plant materials are dehardened they are unlikely to rehard. Of course without foliage and the proper temperature no metabolites are manufactured.

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