FACTORS INFLUENCING DEHARDENING AND REHARDENING OF *FORSYTHIA* x *INTERMEDIA* STEMS

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Technical Paper

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TO:	J. F. McLaughlin, Director Joint Highway Research Project	November 9, 1972
		Project: C-36-48C
FROM:	H. L. Michael, Associate Director Joint Highway Research Project	File: 9-5-3

The attached Technical Paper is from research on the HPR Part I Research Study titled "Research in Roadside Development and Maintenance". The Paper is titled "Factors Influencing Dehardening and Rehardening of *Forsythia* x *intermedia* Stems" and is authored by David F. Hamilton.

The paper is from research reported in an Interim Report of the same title as this paper.

The paper is proposed for publication in the Journal of the American Society for Horticulture Science. Approval of such publication is requested.

Respectfully submitted,

Harold L. Michael
Associate Director

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FACTORS INFLUENCING DEHARDENING AND REHARDENING OF *FORSYTHIA* x *INTERMEDIA* STEMS

by

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Abstract

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

Once dormancy had been broken, 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.

INTRODUCTION

Resistance to dehardening and ability to reharden are essential to maintenance of cold hardiness during intermittent periods of high temperature in winter. It is well known (2,3,5,9) that dehardening is retarded by dormancy. Irving and Lanphear (5) found that dormant *Acer negundo* plants failed to deharden measurably after one week at 21°C, but non-dormant plants under the same conditions dehardened considerably. According to Tumanov and Krasavtev (9) shoots of some conifers became active after 7 to 8 days of high temperature in early January, but after only 3 days in March. Edgerton (2,3) found that in early winter, peaches exposed to 18.4°C showed no significant dehardening until 7 days. In late winter, 4 mild days caused marked dehardening.

Brierly and Landon (1) found that Latham raspberry canes could reharden to some extent following dehardening in early winter. Edgerton (2) and Proebsting (6) concluded that as peach fruit bud development progressed their rehardening capability decreased. According to Howell and Weiser (4) dehardening of living bark of apple is only partially reversible. Once dehardening had begun, the bark did not reharden beyond the killing temperature on the day preceding the final day of dehardening. This killing temperature also increased with each successive day of dehardening.

The objective of this study was to determine the effect of different time-temperature combinations on dehardening and rehardening of *Forsythia x intermedia* Zabel at various times throughout the winter.

MATERIALS AND METHODS

A single clone of *Forsythia x intermedia* represented by a mature plant growing outdoors, was sampled periodically from November 1970 through April 1971 for dehardening and rehardening studies. Dehardening was carried out in a growth chamber at 21 ± 2°C, lighted 14 hrs daily at 2,000 to 2,500 ft.c. with cool-white fluorescent and incandescent lamps. Rehardening was carried out in another growth chamber at 4 ± 2°C lighted 8 hrs daily at 600-800 ft.c.

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 included all current season's growth between 2 and 20 cm from the shoot apex. Each piece was then cut into 2- to 5-cm sections, which were 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. Remaining boxes were placed in freezers and frozen at a rate not exceeding 3°C per hr to a series of test temperatures. Boxes were equilibrated at each test temperature for 2 hrs, and then left at 4°C to thaw slowly for 20-30 hrs.

Stem sections were then cut into 0.2- 0.5 cm segments, combined in 100 mg samples in test tubes. Segments within 0.1 cm of a node were discarded. Viability was determined by the refined triphenyl tetrazolium chloride (TTC) method (Steponkus and Lanphear, 8). Each experiment was subjected to analyses of variance and significance was determined by Duncan's multiple range test (Steel and Torrie 7).

Hardiness under natural conditions of detached stem pieces from the mother plant was measured at 10-day intervals or more often when abrupt changes in weather occurred throughout the testing period. The weekly minimum air temperature was also recorded throughout this period.

Dehardening experiments were conducted to determine whether dehardening varies with temperature within the dehardening range on to the length of exposure to dehardening temperature. Preliminary experiments had shown the time required for dehardening at 21°C to be approximately 4-6 days. Naturally-hardened detached stems were placed at a series of temperatures from 4°C to 27°C, and hardiness was determined after selected periods.

A second 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°C and 21°C under a 9-hr photoperiod, with the low temperature given during the dark period. Tests were conducted monthly, and hardiness was determined after 2, 4, and 6 days' exposure to alternating temperatures.

Beginning February 4, 1971, detached stems that had been hardened naturally and then dehardened at 21°C for different lengths of time were placed at 4°C to promote rehardening. Hardiness was determined after different lengths of exposure to 4°C.

RESULTS AND DISCUSSION

Hardiness of stems under natural conditions increased as weekly minimum air temperature decreased (Figure 1). Fluctuations in air temperature resulted in lesser fluctuations in stem hardiness. Dehardening occurred rapidly in late February and early March as weekly minimum air temperature increased.

In dehardening experiments in December (Table 1), significant dehardening was found only after 6 days exposure to 27°C. In January (Table 2), significant dehardening was found after 6 days exposure to 16°C. Further significant dehardening occurred at 21°C. In March (Table 3), dehardening occurred more rapidly than in December and January. Significant dehardening was found after only 4 days at 16°C. No further significant dehardening was found at this time at higher temperatures, but further dehardening did occur after 6 days at 21°C and higher.

When stems were subjected to diurnally alternating temperatures in mid-December (Table 4), no dehardening was found until after 6 days of 21°C. At this time as little as 4 hrs exposure to 4°C per day was sufficient to prevent significant dehardening. However, in January (Table 5), the same exposure was only partially effective. When the same treatments were applied beginning March 7 (Table 6), 6 hrs of low temperature daily were required to prevent dehardening. It is evident that the daily amount of low temperature required to prevent dehardening during temperature fluctuations increases as dehardening increases.

In rehardening experiments in early February (Table 7), significant dehardening had occurred after 5 days at 21°C. An additional 6 days at 4°C gave no rehardening; the low temperature only prevented further dehardening. In stems dehardened for 4 and 6 days beginning March 7 (Table 8), 2 days exposure to 4°C were required to stop dehardening. After 4 days at 4°C, dehardening was reversed and after 6 days at 4°C significant rehardening had occurred. It is possible that further exposure to 4°C would cause additional. 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 reserves.

As in some other woody species (2,3,5,8), dormancy retards dehardening in *Forsythia*, but does not prevent it entirely. Exposure of dormant stems to dehardening temperatures for more than 6 days would presumably cause continued, and eventually complete dehardening. Even though dehardening occurs more rapidly as winter progresses, 5 to 6 days of warm weather apparently are required before it reaches a maximum. The rate of dehardening appears to remain constant at temperature greater than 21°C.

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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.

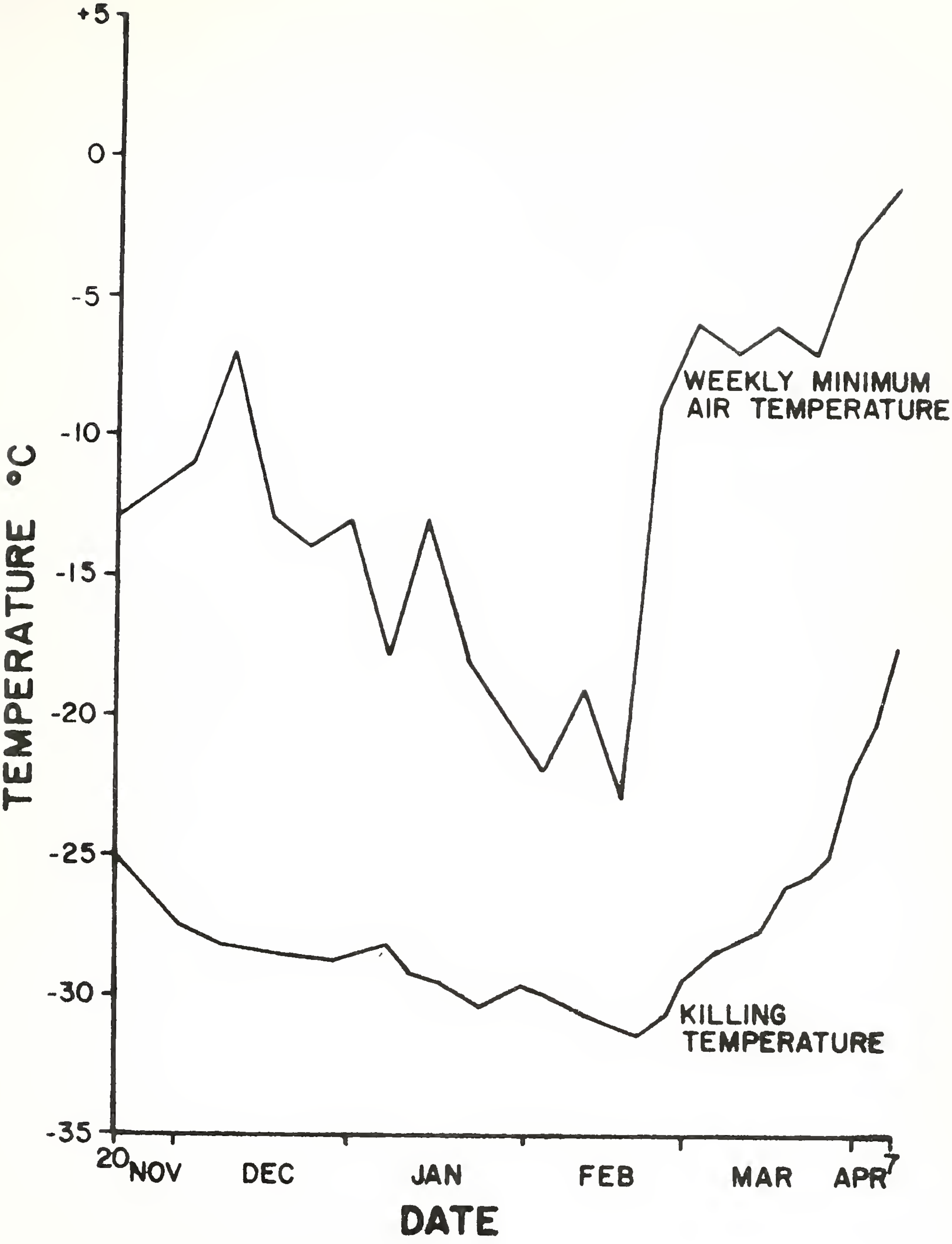
+5
0
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Table 1. Effect of temperature on dehardening of naturally-hardened stems of *Forsythia* x *intermedia* beginning December 16, 1970.

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.

Table 2. 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)	
4.4	-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.

Table 3. Effect of temperature on dehardening of naturally-hardened stems of *Forsythia x intermedia* beginning March 7, 1971.

Temperature for dehardening (^{o}C)	Killing Temperature (^{o}C)*	
	Exposure (days)	
	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.

Table 4. 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.

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

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

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

Table 6. 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	Killing Temperature (°C)* Length of treatment (days) 4	6
24	0	-27.9 a	-28.4 a
6	18	-27.6 a	-27.4 a
4	20	-22.0 b	-22.4 b
2	22	-21.8 b	-18.4 c
0	24	-20.8 b	-16.9 c

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

Table 7. 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	6
0	-29.9 a	-24.4 b	-19.0 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.

Table 8. 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	4	6
0	-28.6 a	-20.8 b	-16.7 c
1		-16.1 c	-14.2 cd
2		-16.1 c	-11.9 d
4		-18.0 bc	-16.5 c
6		-20.5 b	-15.8 c

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