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FACTORS INFLUENCING DEHARDENING AND  
REHARDENING OF FORSYTHIA × INTERMEDIA  
STEMS

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BY  
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Technical Paper

FACTORS INFLUENCING DEHARDENING AND REHARDENING  
OF FORSYTHIA x INTERMEDIA STEMS

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

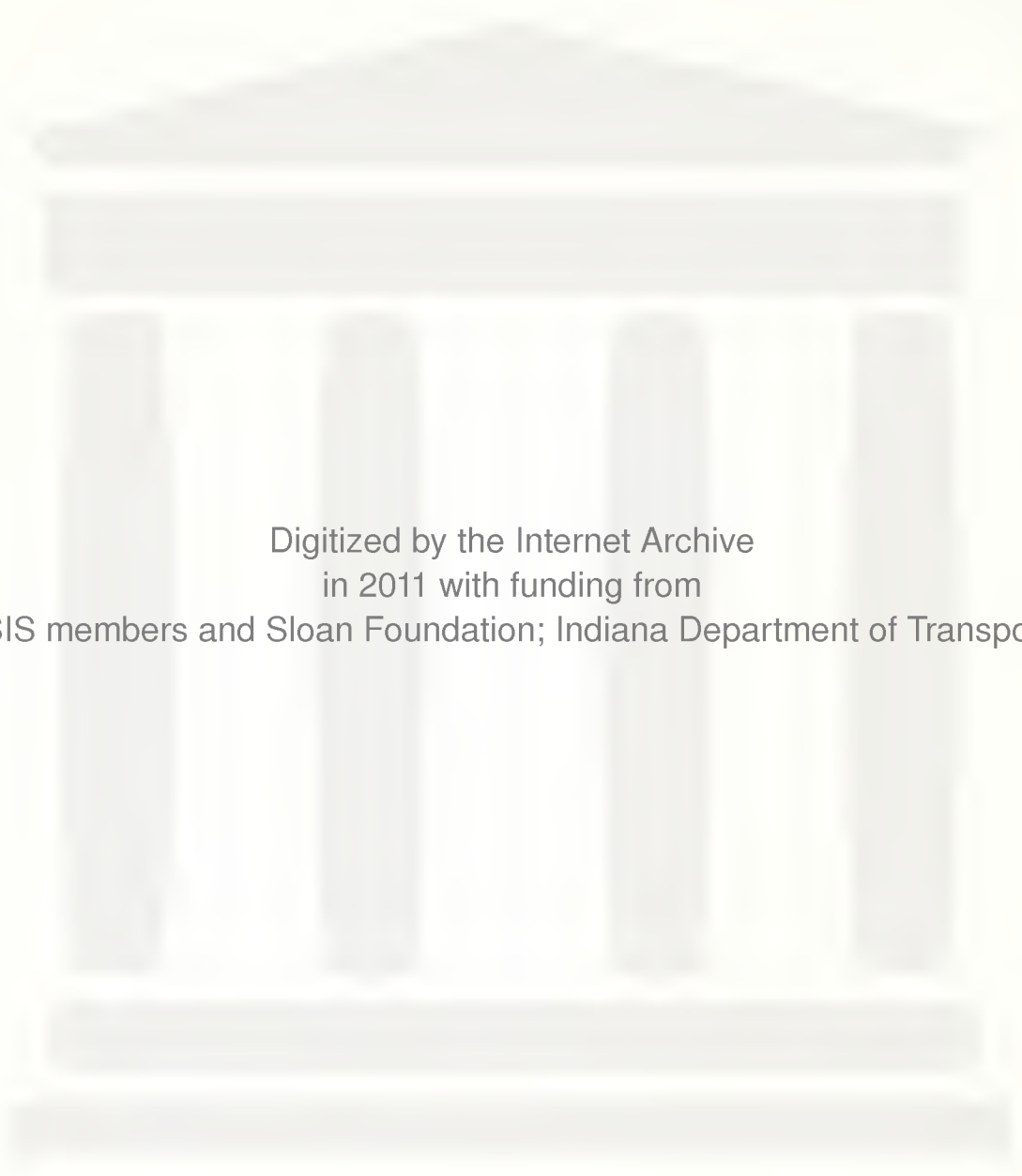
Respectfully submitted,



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Technical Paper

FACTORS INFLUENCING DEHARDENING AND REHARDENING  
OF FORSYTHIA x INTERMEDIA STEMS

by

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Joint Highway Research Project  
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and the

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Purdue University  
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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

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

15 Brierly and Landon (1) found that Latham raspberry canes could  
16 reharden to some extent following dehardening in early winter.  
17 Edgerton (2) and Proebsting (6) concluded that as peach fruit bud  
18 development progressed their rehardening capability decreased.  
19 According to Howell and Weiser (4) dehardening of living bark of apple  
20 is only partially reversible. Once dehardening had begun, the bark  
21 did not reharden beyond the killing temperature on the day preceding  
22 the final day of dehardening. This killing temperature also increased  
23 with each successive day of dehardening.  
24

1  
2 The objective of this study was to determine the effect of  
3 different time-temperature combinations on dehardening and rehardening  
4 of Forsythia x intermedia Zabel at various times throughout the winter.

#### 6 MATERIALS AND METHODS

7 A single clone of Forsythia x intermedia represented by a mature  
8 plant growing outdoors, was sampled periodically from November 1970  
9 through April 1971 for dehardening and rehardening studies.

10 Dehardening was carried out in a growth chamber at  $21 \pm 2^{\circ}\text{C}$ , lighted  
11 14 hrs daily at 2,000 to 2,500 ft.c. with cool-white fluorescent and  
12 incandescent lamps. Rehardening was carried out in another growth  
13 chamber at  $4 \pm 2^{\circ}\text{C}$  lighted 8 hrs daily at 600-800 ft.c.

14 Experiments were arranged in a completely randomized design. Each  
15 replicate was composed of stem pieces from a single plant, and 3 to 5  
16 replications were used. Stem pieces included all current season's  
17 growth between 2 and 20 cm from the shoot apex. Each piece was then  
18 cut into 2- to 5-cm sections, which were randomly assigned to test  
19 temperatures, wrapped individually in aluminum foil and placed in  
20 insulated boxes. One box was left at  $4^{\circ}\text{C}$  as a control. Remaining  
21 boxes were placed in freezers and frozen at a rate not exceeding  $3^{\circ}\text{C}$   
22 per hr to a series of test temperatures. Boxes were equilibrated at  
23 each test temperature for 2 hrs, and then left at  $4^{\circ}\text{C}$  to thaw slowly  
24 for 20-30 hrs.



1

2 Stem sections were then cut into 0.2- 0.5 cm segments, combined in  
3 100 mg samples in test tubes. Segments within 0.1 cm of a node were  
4 discarded. Viability was determined by the refined triphenyl  
5 tetrazolium chloride (TTC) method (Steponkus and Lanphear, 8). Each  
6 experiment was subjected to analyses of variance and significance was  
7 determined by Duncan's multiple range test (Steel and Torrie 7).

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

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

19 A second experiment was designed to find out the extent to which  
20 short periods of low temperature during mild weather in winter prevent  
21 excessive dehardening. Detached stems were subjected to alternating  
22 temperatures of 4<sup>o</sup>C and 21<sup>o</sup>C under a 9-hr photoperiod, with the low  
23 temperature given during the dark period. Tests were conducted  
24 monthly, and hardiness was determined after 2, 4, and 6 days' exposure

1  
2 to alternating temperatures.

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

7  
8 RESULTS AND DISCUSSION

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

14 In dehardening experiments in December (Table 1), significant  
15 dehardening was found only after 6 days exposure to 27°C. In January  
16 (Table 2), significant dehardening was found after 6 days exposure to  
17 16°C. Further significant dehardening occurred at 21°C. In March  
18 (Table 3), dehardening occurred more rapidly than in December and  
19 January. Significant dehardening was found after only 4 days at 16°C.  
20 No further significant dehardening was found at this time at higher  
21 temperatures, but further dehardening did occur after 6 days at 21°C  
22 and higher.

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

11 In rehardening experiments in early February (Table 7), significant  
12 dehardening had occurred after 5 days at 21°C. An additional 6 days  
13 at 4°C gave no rehardening; the low temperature only prevented further  
14 dehardening. In stems dehardened for 4 and 6 days beginning March 7  
15 (Table 8), 2 days exposure to 4°C were required to stop dehardening.  
16 After 4 days at 4°C, dehardening was reversed and after 6 days at 4°C  
17 significant rehardening had occurred. It is possible that further  
18 exposure to 4°C would cause additional. Without photosynthesis, it is  
19 doubtful that rehardening beyond the level preceding any dehardening  
20 can be accomplished, since each period of warm temperature causes  
21 further depletion of reserves.  
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2 As in some other woody species (2,3,5,8), dormancy retards  
3 dehardening in Forsythia, but does not prevent it entirely. Exposure  
4 of dormant stems to dehardening temperatures for more than 6 days  
5 would presumably cause continued, and eventually complete dehardening.  
6 Even though dehardening occurs more rapidly as winter progresses,  
7 5 to 6 days of warm weather apparently are required before it reaches  
8 a maximum. The rate of dehardening appears to remain constant at  
9 temperature greater than 21<sup>o</sup>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.

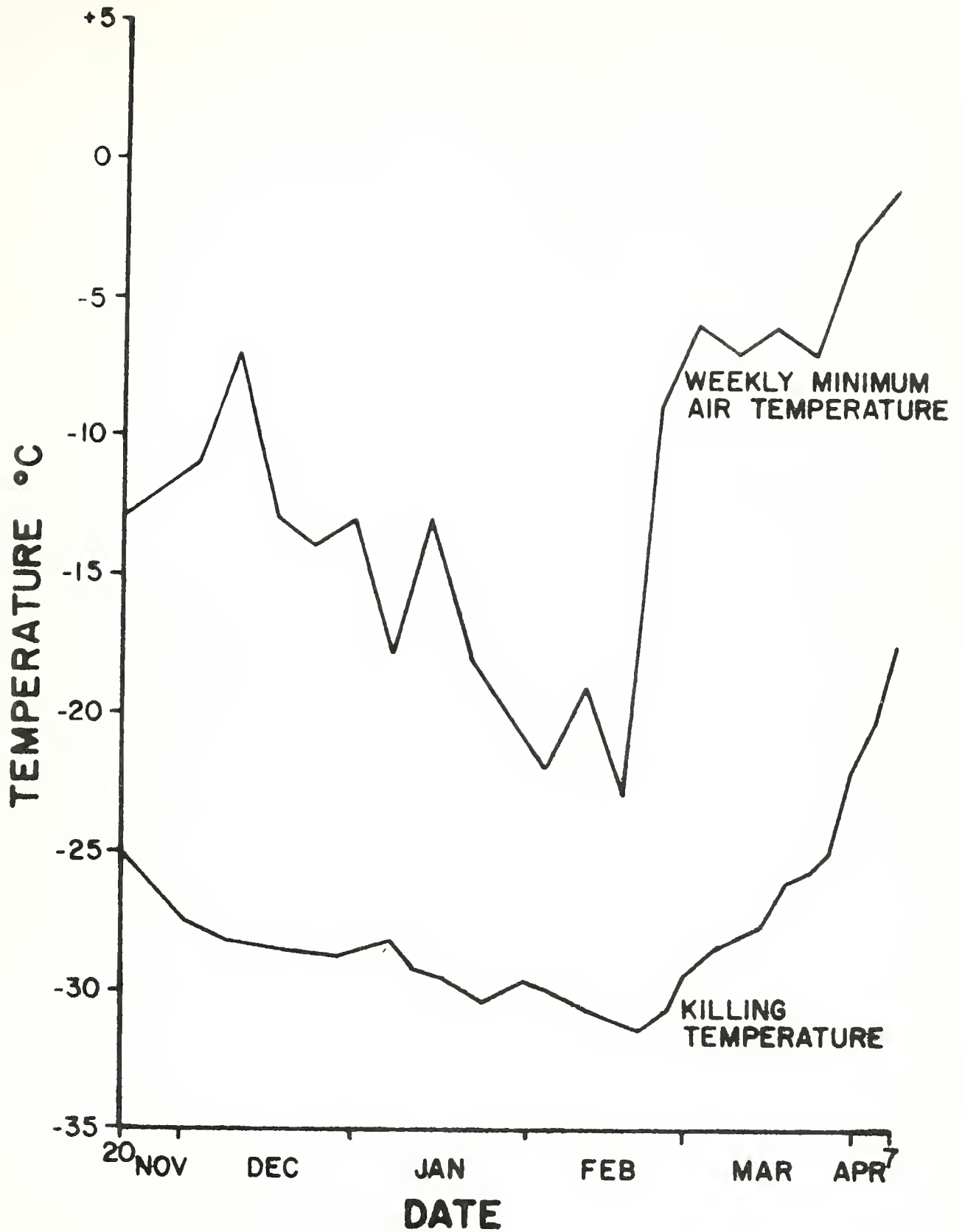




Table 1. Effect of temperature on dehardening of naturally-hardened stems of Forsythia x intermedia beginning December 16, 1970.

Temperature for dehardening ( $^{\circ}\text{C}$ )	Killing Temperature ( $^{\circ}\text{C}$ ) <sup>*</sup>	
	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 ( $^{\circ}\text{C}$ )	Killing Temperature ( $^{\circ}\text{C}$ )*	
	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 ( $^{\circ}\text{C}$ )	Killing Temperature ( $^{\circ}\text{C}$ ) <sup>*</sup>	
	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.

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7 Table 4. Effect of alternating temperatures on dehardening of stems  
8 of naturally-hardened Forsythia x intermedia beginning  
9 December 16, 1970.

Hours daily at 4.4°C	Hours daily at 21.2°C	<u>Killing Temperature (°C)*</u>	
		<u>Length of treatment (days)</u>	
		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

19 \*Killing temperatures not followed by the same letter are significantly  
20 different at the 5% level.  
21  
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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)	<u>Killing Temperature (°C)*</u>		
	<u>Length of pretreatment at 21.2°C (days)</u>		
	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)	<u>Killing Temperature (°C)*</u>		
	<u>Length of pretreatment at 21.2°C (days)</u>		
	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.





