Influence of Growth Retardants on Development and Loss of Hardiness of *Acer negundo*[1]

R. M. Irving[2]
Oklahoma State University, Stillwater

Abstract. Cold hardiness of *Acer negundo* was not increased by applications of CCC, B-Nine, and Amo if the plants were exposed to long day conditions and no subsequent hardening period was provided. Plants given short photoperiods during this time gained 8° in hardiness. Weekly applications of these compounds to plants given short days followed by a hardening period caused greater hardiness than those given short days alone.

Treating hardened, non-dormant plants with CCC, B-Nine, or Amo, either as a dip solution or soil drench, failed to retard the loss of hardiness after a dehardening treatment of 5 days at 70°F. Treatment with gibberellic acid by similar means distinctly accelerated the loss of hardiness under these conditions.

Introduction

The development of cold hardiness in *Acer negundo* has been shown to be a photoperiodic response (3, 4). Short photoperiods followed by low temperature exposure induced cold hardiness while long photoperiods were shown to inhibit it (3, 4). However, 2 growth retardants, B-Nine and Amo, caused an increase in hardiness of several degrees when applied to plants exposed to long days followed by a hardening period (6).

There are several reports which have indicated that growth retardants could bring about certain increases in cold hardiness of other plants. Modlibowski (9) sprayed CCC on pears in May of 1964 and obtained greater survival of flower after exposure to 26°F temperatures during the spring of 1965. Similarly, the per cent of tomato seedlings that survived a particular temperature was somewhat greater when they were grown in nutrient solutions in which CCC was added (8). Increased winter survival of cabbage was also reported by Marth (7) following fall spray applications of CCC and B-Nine. However, cold hardiness of peaches during the winter was not significantly affected by Alar sprays at 2000 ppm made in July (2).

Stewart and Leonard (13) indicated that winter hardiness of grapefruit and orange was increased when sprayed with maleic hydrazide. A slight increase in hardiness in lemon has also been reported when maleic hydrazide was applied (14). Modlibowski and Ruxton (10) found that raspberries treated with maleic hydrazide received less damage than control plants when exposed to 26.4° for 45 minutes during the "green bud" stage. These authors indicated that the effect of maleic hydrazide on frost resistance of buds was dependent on the degree of inhibition. When strong inhibition and chemical damage occurred, the tissues were more susceptible to frost. There was a point, however, where a lesser degree of inhibition was associated with an increase in frost resistance.

However, experiments of this type which show per cent survival at a single temperature provide information on the effectiveness of the compound only within a very narrow temperature range and do not yield data which would indicate any difference in survival at lower temperatures.

Recently, Irving and Lanphear (5) reported that dormant (resting) plants lost hardiness at a greatly reduced rate when compared to non-dormant plants. In addition, non-dormant plants which were severed from their root systems and placed in a solution of abscisic acid (dormin) lost much less hardiness during a given period of time than non-dormant plants placed in water.

The work reported herein was undertaken in order to measure the effect of growth retardants applied under both long and short photoperiods on cold hardiness of *Acer negundo* and to determine the ability of such compounds to reduce the amount of dehardening during warm temperature exposure.

Methods and Materials

Plant material and experimental conditions. The plants used in this study were seedlings of *Acer negundo* L., box-elder. Plants were grown for at least 3 months at approximately 75°F in a greenhouse under long photoperiods before being subjected to experimental conditions. Short day treatment was provided by covering daily with a blackcloth from 4:30 PM until 8:00 AM. Plants were given long photoperiods by means of incandescent supplemental lighting from 5:00 PM until 12:00 midnight.

Statistical analysis. A completely randomized experimental design was used and the analysis of variance was carried out. Duncan's new multiple range test was utilized for separation of treatment means.

Freezing test and viability determination. The standardized freezing procedure and the triphenyl tetrazolium chloride (TTC) technique for viability determinations was employed essentially as outlined by Irving and Lanphear (5). Briefly, the procedures are as follows: At least 5 tissue samples from each treatment were used in the standardized freezing test. A 6-inch section from each plant was cut into 6 equal pieces, individually wrapped in aluminum foil and 1 piece exposed to each temperature. One section at 40°F served as the control while the other samples were placed in styrofoam boxes which were placed in a freezer at 20°. The rate of temperature drop in the boxes was less than 6°/hr. When the temperature in the boxes reached 23° all the boxes except one were transferred to a freezer set at 10°. The temperature in the one remaining box was allowed to proceed to 20° and remained there for 2 hr. The process was repeated at +10, 0, −10, and −20°. After 2 hr. at the desired temperature, each box was removed and allowed to thaw at 40°. The samples were then placed in a plastic container under high humidity at room temperature for 36 hr.

The TTC viability test was performed by weighing 50 mg samples of previously frozen plant material. The samples were cut into 2 mm sections and placed in test tubes and 3.0 ml of 0.6% TTC solution (buffered at pH7.4 in a 0.05 *M* phosphate-phosphate buffer, plus .01%

[1]Received for publication May 29, 1968. Journal Paper No. 1705, Oklahoma State University Agricultural Experiment Station.

[2]Present address—International Minerals & Chemical Corporation, Administrative Center, Skokie, Illinois 60076.

Ortho 77 wetting agent) was added. The samples were vacuum infiltrated at 2 inches of Hg for 4 min. The tubes were then stoppered and incubated for 15 hr. at 85°. The TTC solution was removed and the tissue rinsed with distilled water to remove any TTC not contained in the sample itself. The tubes were filled to approximately 9 ml with 95% ethanol and placed in a boiling water bath for 10 min to extract the reduced TTC. Tubes were cooled and filled to a 10 ml volume with additional ethanol. Absorbance at 530 mμ was recorded and the values divided by the absorbance of the 40° control to determine the per cent reduction. The temperature at which 50% of the tetrazolium reducing capacity was lost was taken as the killing point.

Results and Discussion

Effect of growth retardants under long days without a hardening period. As a means of determining whether growth retardants could increase hardiness under long days without a subsequent hardening period, groups of plants were exposed to either short days, long days, or long days plus weekly spray applications of Amo[3] at 1000 ppm and B-Nine at 3000 ppm (Table 1). The data indicate that weekly applications of these compounds did not increase the hardiness of *Acer negundo* if no hardening period followed the treatments. Short days, however, significantly lowered the killing point after 5 weeks of treatment. The effect of short days is consistent with results previously obtained by Irving and Lanphear (3).

Table 1. Effect of B-Nine and Amo on the hardiness of *Acer negundo* maintained under long or short days.

Treatment for 5 weeks[x]	Killing point (°F)[y]
Short days	8.4 a
Long days	16.5 b
Long days + Amo 1000 ppm (sprayed weekly)	16.1 b
Long days + B-Nine 3000 ppm (sprayed weekly)	15.8 b

[x]No hardening period was given.
[y]Killing points followed by different letters are considered significantly different at the 0.05 probability level.

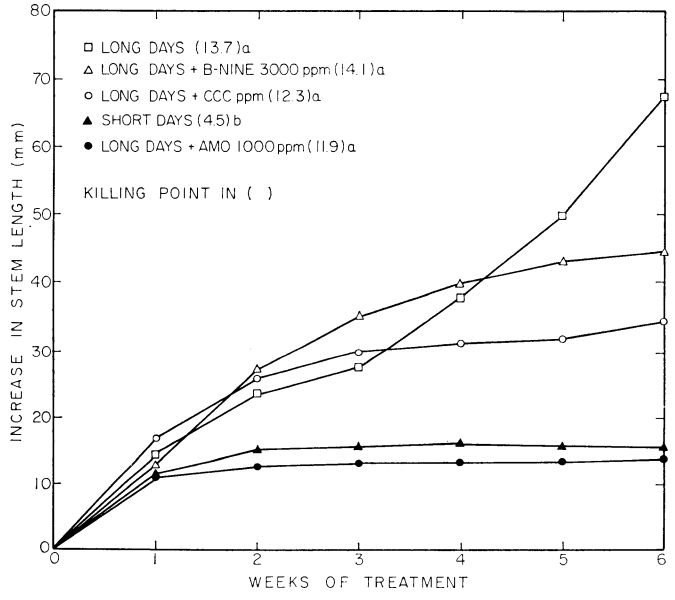

Fig. 1. Effect of growth retardants on growth and hardiness of *Acer negundo*. No hardening period was given. Retardants were applied weekly, B-Nine and Amo as sprays and CCC as a soil drench. Those killing points followed by different letters are considered significantly different at the 0.05 probability level.

A similar experiment was subsequently conducted wherein the amount of growth (increase in stem length) at weekly intervals and the killing points were determined at the end of 6 weeks of treatment (Fig. 1). Stem elongation of plants grown under long days continued in a linear fashion during the 6 weeks. Average growth during the period was 67 mm. Plants given either short days or long days plus Amo at 1000 ppm stopped elongating in about 2 weeks and grew only about 15 mm during this time. Intermediate growth rates were obtained under long days with B-Nine and CCC treatment. The rate of elongation of plants subjected to B-Nine and CCC was slowed somewhat, but not as sharply as with short days or Amo treatment.

After 6 weeks of treatment, the killing point of the long day treated plants was 13.7°. This compared to a 4.5° killing point for plants exposed to short days. Treatment with the 3 growth retardants under long day conditions failed to effectively alter the level of hardiness. The killing points of groups treated with B-Nine, CCC and Amo were 14.1, 12.3, and 11.9° respectively. Thus, it would appear that although 2 of these compounds have been shown to increase hardiness under long days when treatment was followed by a hardening period, they were unable to stimulate hardiness without the benefit of low temperature exposure. In addition, the data indicate that cessation of growth does not automatically confer greater plant hardiness as maintained by Rosa (12). Growth rates of plants given short days or long days plus Amo were almost identical, yet the hardiness levels after 6 weeks were distinctly different (Fig. 1). These results are in general agreement with those of Tumanov and Trunova (14) who indicated that growth retardation does not always reflect favorably on plant resistance to cold.

Effect of growth retardants under short days followed by a hardening period. Although treatment of plants with Amo and B-Nine under long days followed by a hardening period has been shown to increase hardiness (6), the influence of these compounds during short days had not been tested. In order to determine this effect, groups of plants were given short days or short days plus weekly applications of either B-Nine at 3000 ppm, Amo at 1000 ppm, or CCC at 1000 ppm for 5 weeks. These treatments were followed by a hardening period of 3 weeks in darkness at 40°. Weekly applications of the growth retarding compounds under short days significantly increased the hardiness of *Acer negundo* (Table 2). The gain in hardiness from B-Nine, Amo and CCC was 6.2, 7.3 and 4.5° respectively in Experiment 1. In the second experiment, all 3 treatments promoted greater hardiness than did the control, however, the difference in the case of CCC was not statistically significant. These results indicate that application of growth retarding chemicals in conjunction with short days may increase the hardiness gained within a given time period. Whether the ultimate hardiness level of the plant is correspondingly increased is not conclusively known at this time. However, these data do suggest that the hardiness potential may be increased with multiple applications of these compounds when done so in conjunction with short photoperiods and subsequent hardening temperatures.

[3]Chemicals used in this paper include Amo (Amo 1618) which is 4-hydroxy-5-isopropyl-2-methylphenyl trimethyl-ammonium chloride, 1-piperidine carboxylate; B-Nine (Alar) which is succinic acid 2,2-dimethyl hydrazide, supplied courtesy UniRoyal Chemical Company, Bethany, Connecticut; CCC (Cycocel), which is (2-chloroethyl) trimethylammonium chlorida; GA, the 10% K salt of gibberellic acid.

Table 2. Effect of B-Nine, Amo, and CCC on hardiness of *Acer negundo* given short days followed by a hardening period.

Treatment for 5 weeks	Hardening at 40°	Killing point (°F)[y] Expt. 1	Expt. II
Short days	3 weeks	−16.7 a	−14.1 a
Short days + B-Nine 3000 ppm (weekly)[z]	3 weeks	−22.9 b	−19.9 b
Short days + Amo 1000 ppm (weekly)	3 weeks	−24.0 b	−20.1 b
Short days + CCC 1000 ppm (weekly)	3 weeks	−21.2 b	−16.9 a

[y]Killing points followed by different letters are considered significantly different at the 0.05 probability level.
[z]B-Nine and Amo were applied as sprays and CCC as a soil drench.

Effect of growth regulating compounds on the loss of hardiness. Since abscisic acid (dormin) has been shown to reduce the rate of dehardening of nondormant plants, the use of Amo, CCC, and GA on hardiness loss in this same species was also tested. Amo and CCC, like abscisic acid have, in certain tests, been shown to interfere with gibberellin biosynthesis (1, 11). Test plants were hardened by exposure to long days and 40° nights for 5 weeks in order to provide plants with a measure of hardiness without being dormant. This treatment was followed by a 3 week hardening period in darkness at 40°. Four groups of 5 plants each were then dehardened at 70° for 5 days. One group was irrigated with water during the 5 day period while the other groups received either Amo, CCC, or gibberellic acid at 1000 ppm as a soil drench. A fifth group was timed so as to receive the same pretreatment with no dehardening. Table 3 indicates that neither Amo nor CCC were able to retard the loss of

Table 3. Effect of Amo, CCC, and GA applied as a soil drench on the loss of hardiness of *Acer negundo*.

Pretreatment[x]	Dehardening treatment	Killing point (°F)[y]
5 weeks LD +40° nights	No dehardening	−7.5 a
5 weeks LD +40° nights	5 days at 70° + H_2O (as soil drench)	+4.7 b
5 weeks LD +40° nights	5 days at 70° + Amo 1000 ppm (as soil drench)	+3.2 b
5 weeks LD +40° nights	5 days at 70° + CCC 1000 ppm (as soil drench)	+6.5 b
5 weeks LD +40° nights	5 days at 70° + GA 1000 ppm (as soil drench)	+11.5 c

[x]Followed by a hardening period of 3 weeks in darkness at 40°.
[y]Killing points followed by different letters are considered significantly different at the 0.05 probability level.

hardiness when compared to plants treated with water. Losses in hardiness ranged from 10.7 to 14.0° during the 5 day period. However, the application of gibberellin increased the rate of dehardening causing a loss of 19.0° during this time.

This result may be explained by the difference in growth made during the dehardening period. Although all treatments—including those treated with the growth retardants—did produce some growth, gibberellin treated plants elongated as much as 40 mm during the 5 days. The 2 effects appear to be consistent, since one would not expect a growth retardant to reduce loss of hardiness if it were also unable to inhibit growth of the plants.

A second experiment along these lines was conducted by applying growth regulating compounds as a dip treatment. Plants were hardened by the same procedure as the previous experiment. The stems were then immersed for 1 minute in a tube containing either (1) water, (2) Amo at 1000 ppm, (3) CCC at 1000 ppm, (4) B-Nine at 1000 ppm, or (5) GA at 1000 ppm (Table 4). Dehardening for 5 days at 70° followed. In this test Amo reduced the killing point slightly when compared to water, while CCC and B-Nine had no apparent effect on the killing point. Once again, treatment with GA markedly stimu-

Table 4. Effect of Amo, CCC, B-Nine, and GA applied as a dip solution on the loss of hardiness of *Acer negundo*.

Pretreatment[x]	Dehardening treatment	Killing point (°F)[y]
5 weeks LD +40° nights	No dehardening	−8.1 a
5 weeks LD +40° nights	5 days at 70° + H_2O	+3.4 b
5 weeks LD +40° nights	5 days at 70° + Amo 1000 ppm (as a dip)[z]	+1.9 b
5 weeks LD +40° nights	5 days at 70° + CCC 1000 ppm (as a dip)	+3.9 b
5 weeks LD +40° nights	5 days at 70° + B-Nine 1000 ppm (as a dip)	+4.0 b
5 weeks LD +40° nights	5 days at 70° + GA 1000 ppm (as a dip)	+10.7 c

[x]Followed by a hardening period of 3 weeks in darkness at 40°.
[y]Killing points followed by different letters are considered significantly different at the 0.05 probability level.
[z]The dip treatment was provided by immersing the stems for 1 minute in a tube containing the chemical.

lated the loss of hardiness. The killing point shifted from −8.1 to + 10.7° when treated with GA, compared to a rise from −8.1 to + 3.4° for the control. The ability of GA to stimulate the loss of hardiness of non-dormant plants is in sharp contrast to its effect on dormant plants. Application of GA by the dip method failed to stimulate the loss of hardiness of dormant *Acer negundo* plants, even though gibberellin applications by this method at the same concentration were able to break bud dormancy (5).

These data indicate that single applications of growth retarding compounds, whether as a soil drench or as a dip, are not able to lessen significantly the degree of hardiness lost during a period of warm temperatures. Although visible growth occurred in both experiments, plants treated by the dip method produced much less growth during the same period of time.

Low temperature damage in spring often occurs on ornamentals which have initiated some growth during a warm period prior to a return to cold weather. It is not yet known if these retardants could sufficiently retard the growth of ornamentals under natural conditions where soil temperatures would be much lower, since in these tests soil temperatures, as well as air temperatures, were maintained at 70°. In view of the stimulating effects of GA on growth and loss of hardiness, it would appear that growth retardants could be expected to retard the rate of dehardening only with a concomitant inhibition of growth.

These data do not support the idea that growth retardants applied to ornamentals under natural conditions immediately prior to a warm period would be effective in delaying production of new growth and insuring increased survival. Multiple applications of these compounds at an earlier date may be the only possible means to provide sufficient retardation in development to allow plants to evade or escape damage from impending low temperatures.

Data from studies on bud dormancy and winter hardiness indicate that there are certain periods or stages during the winter when the plant does not produce significant amounts of gibberellin. Therefore, timing of growth retardant applications is of critical importance since the treatments must be made early enough to suppress development but yet late enough to be present when production of gibberellin is about to occur.

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The Interaction of Resistant Rootstock to the Nitrogen, Weed Control, Pruning and Thinning Effects on the Productivity of Concord Grapevines[1]

Nelson Shaulis[2]
New York State Agricultural Experiment Station, Geneva, and
Robert G. D. Steel[3]
North Carolina State University, Raleigh

Abstract. This reports a 1960–1964 study of the responses of 'Concord' grapevines, own-rooted as well as grafted on the phylloxera resistant rootstock 'Couderc 3309', to pruning severity, thinning of fruit, N fertilization and weed control in a factorial experiment at Fredonia, New York.

Although phylloxera were present, moderate size of vine and large yield were attained with own-rooted vines. Here, '3309' rootstock did not have a unique effect on 'Concord'.

As an initial response, in 1960, when the range in vine size was 1.8 to 3.0 lb. of cane prunings per vine, increases in vine size were associated with increased yield because fruitfulness was not then seriously depressed by the vine size increasing treatments of '3009' rootstock, cultivation, and N fertilization.

As an equilibrium response in 1963–1964, in the range in vine size of 1.9–4.9 lb. of cane prunings per vine, when vine size was above 3.5 lb. of cane prunings, the decline in fruitfulness prevented a gain in yield, and there was either no increase or a decrease in fruit maturity.

A 1966 sequel, affording 8′ and 16′ of canopy length for each 8′ spaced vine, showed fruitfulness to be closely associated with node number per unit length of canopy. Where the canopy length is fixed, crowding was likely the basis for the declines in fruitfulness which accompanied increases in vine size and node number.

The effect of the resistant rootstock '3309' was similar to that of the other vine-size increasing treatments in that it decreased fruitfulness and fruit maturity and had a small effect on yield and on soluble solids/vine.

Introduction

The unique effect of resistant rootstocks in affording adequate growth and yield of grapevines is evident at sites with either phylloxera as shown by Lider (8), or with root-knot nematodes as shown by Harmon and Snyder (5) and Lider (9), or where there is vine replanting as discussed by Winkler (24) and Lider and Shaulis (10). That unique effect is more frequently encountered with commercial varieties of *Vitis vinifera* than with some of the hybrids with *Vitis labrusca,* particularly 'Concord'.

Research reported by Gladwin (4), Oberle (12), Shepard (21), Magoon (11), Vaile (18), and Reynolds and Vaile (14) has shown that the vine size increase due to the use of resistant rootstocks was less for 'Concord' than for other American hybrids as 'Campbell Early', 'Moore Early', or 'Delaware'. Although there has been a decade of work on the significance of phylloxera and plant parasitic nematodes to 'Concord' grapes the answer is not clear according to Taschenberg (22).

For 'Concord', the major grape variety in New York, resistant rootstocks are very seldom used even though vine size is below the optimum as defined by Kimball and Shaulis (6). To maintain or increase the vine size of 'Concord' in New York, it is generally necessary to afford weed control, N fertilization and crop reduction by a more severe pruning than is used for 'Concord' in Washington State (1). The major question is: how does the use of a phylloxera resistant rootstock affect the responses of 'Concord' to the growth increasing treatments of weed control and nitrogen fertilization, and to the crop reduction treatments of either pruning or flower cluster thinning?

Materials and Methods

The planting was made at Fredonia, New York in 1956. The soil was Howard gravelly loam, which is acid, very well drained, and more than 10 feet deep. The site, at the Vineyard Laboratory of the New York State Agricultural Experiment Station, had not had a vineyard or grape nursery for at least 15 years prior to the 1956 planting.

Vine spacing was 8 ft in rows spaced at 9 ft. In each of 3 replicates there were 8 rows each of six 6-vine plots. The following treatments were selected to afford a wide range of vine size and of yield:

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[1]Received for publication September 17, 1968. Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 1666.
[2]Department of Pomology.
[3]Department of Experimental Statistics.
The consistent support by the New York State Concord Grape Production Research Fund, Inc. and the splendid work by Mr. Donald Crowe and Mrs. Sheldon Hubbard is gratefully acknowledged.