Research Reports:

Leaf and Stem Cold Hardiness of Four Selections of *Raphiolepis umbellata* Makino[1]

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Abstract

Leaves and stems of four selections of *Raphiolepis umbellata* Makino were evaluated for cold hardiness at various times throughout the winter over a two year period and compared to the hardiness of their cold hardy parent plant (PI 277653). All four selections were equally or more cold tolerant than the parent plant on all test dates over both years tested. The leaves of these plants were hardier than the stem tips, but were similar to the cold hardiness of two year old stems. During midwinter the leaf and two year old stem cold hardiness ranged from − 15 to − 18°C (5 to 0°F). These data indicate that the new selections have good cold hardiness potential and may increase the range of adaptability of raphiolepis.

Index words: freeze tolerance, acclimation, adaptability range

Significance to the Nursery Industry

Due to its attractive evergreen foliage, white or light pink flowers, and bluish-black berries, raphiolepis has always been a popular landscape plant. However, low temperatures currently limit its northern range of adaptability. Breeding efforts are being made to improve their cold hardiness, but test winters do not occur regularly so their actual cold hardiness, throughout the season, is not known.

Methods of screening for cold hardiness under laboratory conditions can significantly shorten the normal evaluation procedure which is largely weather dependent. Multiple date laboratory cold hardiness testing holds great promise to provide an index of a particular plant's low temperature tolerance. It can provide useful information about the low temperature adaptability of a particular taxon.

Introduction

Yeddo raphiolepis or Indian Hawthorn (*Raphiolepis umbellata* Makino.) is a popular plant throughout USDA hardiness zones 8 through 10 (3). Its lustrous dark green leaves,

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fragrant white or light pink flowers, and 10–12 cm (0.38–0.5 in) purple-black to bluish-black berries that persist through the winter, make raphiolepis an attractive and desirable landscape plant (3). Increasing its cold tolerance may increase its geographic range of adaptability. Twenty species and cultivars of raphiolepis were evaluated by Corley (1, 2) at the Georgia Experiment Station in Griffin, Georgia. He found *R. umbellata*, 'Ovata' and an unnamed Plant Introduction (PI 277653) to be the most hardy after exposure to −19°C (−3°F) in 1983. The PI had the least cold injury, while 'Ovata', even though seriously injured, regrew the fastest. In 1985, these plants were exposed to −22°C (−8°F) at the same location. All species and cultivars were killed to the ground, and the PI was the only selection that regrew the following spring.

In an attempt to find other cold hardy raphiolepis plants, crosses were made between the PI and 'Ovata'. Four of approximately 2000 seedlings have been selected for desirable horticultural characteristics, cold hardiness, and leaf spot resistance. However, the degree of cold hardiness of these selections throughout the winter season is not known since extremely cold winters do not occur regularly in middle Georgia. Therefore, the objective of this paper is to evaluate the cold hardiness of leaves and stems of the cold hardy raphiolepis parent and crosses ('Ovata' × PI) at various times throughout the fall, winter, and spring over two winter seasons.

Material and Methods

Seedlings of the cold hardy PI, originating from *R. umbellata* seed collected by USDA plant explorer John Creech in Japan during 1962, were planted into field plots at the Georgia Experiment Station in 1963. Cross between the PI and *R. umbellata* 'Ovata' was made in 1973, and selected plants were planted into the field in 1975, and maintained by using common horticultural practices. Four selections from the above mentioned cross were used in this study. The plants were sampled six times for cold hardiness estimates in 1989–90 on October 30, November 20, December 18, January 22, February 20, and March 26 and three times in 1991 on January 29, February 27, and March 27. To estimate cold hardiness on each test date, thirty-six (36), uniform, 10 cm (4 in) long stem segments were removed from each plant. In the first year, only stem tips were used since they appeared to be the most easily damaged by freezing. In the second year, however, 20 cm (7.9 in) of stem tissue was removed to allow testing of both stem tips, from the past season's growth and stem segments from the second year growth. In either case, the leaves were removed from the stems. Samples, wrapped in moistened paper towels, were put in plastic bags then placed on ice in an insulated container for transport to the lab. Within 2 hours of the collection the stems and leaves were prepared for freezing.

For the freezing test a 7 cm (2.9 in) section of each stem was selected, wrapped in moist cheesecloth and placed into a test tube (25 × 200 mm) (1 × 7.9 in). Whole leaves randomly selected from those removed from the stem segment described above were prepared in a similar manner. The tubes were then submerged in ethylene glycol-water solution (1:1) in a temperature bath precooled to −2 ± 0.5°C (28 ± 1°F). Stem and leaf temperatures were measured by thermocouples placed next to the samples and recorded by a Campbell Scientific CR7-X datalogger. Crushed ice crystals were applied to the wet cheesecloth to insure that samples did not undercool. The temperature of the samples was held constant at −2 ± 0.5°C (28 ± 1°F) for approximately 14 hours. Samples were then cooled at a rate of ≤ 4°C (7°F) per hour. Four leaves and/or stems of each taxon were removed from the bath at progressively lower 3°C (5°F) temperature intervals. Controls were prepared and kept at 4°C (36°F) for the duration of the freezing test.

After thawing at 4°C (36°F) for overnight, the samples were removed from the tubes and placed in disposable, round, 100 × 15 mm petri dishes containing filter paper saturated with water to maintain a 100% relative humidity. The petri dishes were placed on their sides at room temperature (22 ± 2°C) (72 ± 4°F) for 10–14 d. At this time the samples were visually evaluated for injury as described by van Huystee et al. (10); Smithberg and Weiser (8); Fuchigami et al. (4); Stergios and Howell (9); and Hummel et al. (5). Stem segments showing brown discoloration and breakdown of cells in the cambium and phloem were rated as dead. Similarly injured leaves were identified by tissue browning and water soaking. The nonfrozen controls and samples not injured by the freezing treatments remained green and showed no discoloration in the cambium, phloem or leaves. The number of stems or leaves killed at each temperature was recorded and the lowest survival temperature (LST) was determined from the data. The LST was the lowest temperature at which little or no injury was observed, as described by Sakai et al. (7). In most cases there was no variability between replicates when determining the LST for a specific cold hardiness determination. The standard deviation was reported where variability was present. The lack of variation between replicates can be explained since individual, uniform taxa were used and cold hardiness was determined only within a 3°C (5°F) range.

Results and Discussion

The four selections of raphiolepis were, in general, as cold hardy or slightly hardier in both the stems and leaves as the cold hardy parent (PI 277653) on all dates tested over two winter seasons (Table 1 and 2). During the first year of testing, when only leaves and stem tips were used, the four selections acclimated at a similar rate as the PI (Table 1). The stem tips of all the selections were generally less cold hardy than the leaves at all sampling periods and were as cold hardy as the PI. On January 22, 1990 the leaf cold hardiness remained relatively unchanged from the previous sampling date, however, none of the stem tips survived, including the unfrozen controls. The outdoor temperatures dipped to −13°C (9°F) and −15°C (5°F) on December 23 and 24, respectively, killing the stem tips. On January 22 when samples were taken, the injury had not yet manifested itself, but when samples were placed in the laboratory, the injury became apparent. Therefore there is no estimate of cold hardiness of the stem tips on this or later sampling dates during the first year of testing. It is reasonable for the stem tips to be killed by the freezes indicated above since the stem tip cold hardiness on December 18, 1989 ranged from −6 to −9°C (21 to 16°F), (Table 1), which was well above the temperatures experienced on December 23 and 24, 1989. Stem tip injury was evident on all plants in the field later in the winter. Due to the injury of the stem tips, stem sections from the second year growth were used on February 20 and March 26 sampling dates. The two year

Table 1. The lowest survival temperatures (°C ± SD) of 4 selections of *Raphiolepis umbellata* compared to their cold hardy parent (PI 277653) from October 30, 1989 through March 26, 1990.

Plant selection	Date collected											
	10-30-89		11-20-89		12-18-89		01-22-90		02-20-90		03-26-90	
	Leaf	Stem[y]	Leaf	Stem[y]	Leaf	Stem[y]	Leaf	Stem[y]	Leaf	Stem[x]	Leaf	Stem[x]
1	−9	−6	−12	−7 ± 4	−15	−9 ± 2	−15	C[z]	−11 ± 2	−15	−9	−12
2	−9	−6	−9	−6	−15	−9	−15	C[z]	−14 ± 2	−15	−9	−14 ± 2
3	−9	−6	−12	−6	−15	−9	−15	C[z]	−12	−12	−9	−12
4	−9	−6	−12	−9	−18	−9	−15	C[z]	−12	−15	−9	−11 ± 2
PI	−9	−6	−9	−6	−15	−9	−15	C[z]	−12	−12	−6	−9

[z]Non-frozen control dead.
[y]One year old stem tips.
[x]Two year old stem segments.

old stems had the same or 3°C less cold hardiness than leaves on these dates indicating that in turn they are more cold hardy than the stem tips. This is substantiated by the fact that they survived the exposure to the minimum temperatures experienced on December 23 and 24. It is also well established that stem tips are less cold hardy than older wood (6). On the March 26, 1990 sampling date, both the leaves and the stems of all the plant selections were hardier than the PI (Table 1).

On the second year of testing, stem tips, stem sections from second year's growth, and leaves were evaluated for cold hardiness on three different dates. On all dates the one year old stem tips were less cold hardy than the two year old stem sections or leaves (Table 2) and there was little difference in the cold hardiness of the stem tips of the plant selections and the PI. Leaf and two year old stem cold hardiness closely paralleled each other on January 29 and February 27 sampling dates, but, on March 27 the leaves had deacclimated and were less cold hardy than the stems. On March 27, 1991, as in the first year of the experiment both the leaves and two year old stems of the plant selections were more cold hardy than those of the PI (Table 2). No difference in hardiness was found in the stem tips of the selection and the PI.

The four raphiolepis selections in this study, considering the data collected over two years, are as cold hardy as the parent PI. In fact, they appear hardier in the spring (March) than does the PI. This indicates that the selections may be more able to survive late spring frosts than the PI and that the selections could be grown in areas where the PI is injured or killed.

The two year old stems are hardier and retain their hardiness longer than the stem tips. Therefore it is important to choose the appropriate tissue sample to estimate the cold hardiness. In an ornamental plant such as raphiolepis it is not desirable to have tender tissue (such as stem tips) killed regularly during the winter. On the other hand the older stem segments give a more accurate reflection of the survivability of the plant. Cold hardiness estimates of both the stem tips and older tissue is useful to the grower, but caution should be taken to sample the appropriate tissue for the problem being studied.

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Table 2. The lowest survival temperatures (°C ± SD) of old and new stem segments and leaves of 4 selections of *Raphiolepis umbellata* to their cold hardy parent (PI277653) from January 29 through March 27, 1991.

Plant selection	Date collected								
	1-29-91			2-27-91			3-27-91		
	Leaf	Stem (new)[z]	Stem (old)[y]	Leaf	Stem (new)[z]	Stem (old)[y]	Leaf	Stem (new)[z]	Stem (old)[y]
1	−18	−12	−18	−18	−12	−18	−11 ± 2	−6	−18
2	−18	−11 ± 3	−18	−18	−15	−18	−12	−8 ± 2	−18
3	−18	−12	−21	−18	−12	−14 ± 3	−11 ± 2	−6	−18
24	−18	−14 ± 4	−18	−18	−12	−18	−12	−6	−18
PI	−15	−12	−18	−15	−12	−15	−9	−6	−12

[z]One year old stem tips.
[y]Two year old stem segments.