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Validity of Screening for Foliage Cold Hardiness in the Laboratory[1]

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Abstract

Thirty-five broadleaf evergreens which are evergreen in USDA cold hardiness Zone 7 were screened using leaf discs in the laboratory for Zone 6 (–20C) temperatures. Field data were collected from the plants in the field after two consecutive nights with lows of –20C. Correlations between lab and field scores were high, demonstrating the usefulness of leaf discs in initially screening broadleaf evergreens for evergreeness. The method allows for a more thorough sampling of a taxon and may therefore give better results than using a limited number of larger leaf samples, such as whole leaves. A number of the tested taxa should prove evergreen in Zone 6, including: *Camellia oleifera* Abel 'Lu Shan Snow', *Viburnum* L. Arrowwood 'Conoy', *Viburnum rhytidophyllum* Hemsl. 'Cree', and certain selections of *Pyracantha* M.J. Roem, *Illicium anisatum* L., *Quercus acuta* Thunb., *Quercus myrsinifolia* Blume and *Daphniphyllum macropodum* var. *humile* Miq.

Index words: cold hardiness, broadleaf evergreens, laboratory-field correlations.

Species used in this study: *Camellia japonica* L., *Camellia oliefera* Abel 'Lu Shan Snow', *Daphniphyllum macropodum* Miq., *Gardenia* Ellis. 'Klines Hardy', *Gardenia* 'Daisy', *Illicium anisatum* L., *Illicium mexicana* A.C. Sm., *Itea oldhami* Schneid., *Lithocarpus henryi* Rehd. & E. H. Wils., *Pyracantha* M.J. Roem., *Quercus acuta* Thunb., *Quercus glauca* Thunb., *Quercus laurifolia* Michx., *Quercus myrsinifolia* Blume, *Quercus salicina* Blume, *Raphiolepis indica* (L.) Lindl., *Raphiolepis umbellata* (Thunb.) Mak., *Sycopsis sinensis* D. Oliver., *Viburnum* L. Arrowwood 'Conoy', *Viburnum rhytidophyllum* Hemsl. 'Cree', *Viburnum* 'Eskimo'.

Significance to the Nursery Industry

A broadleaf evergreen cultivar may survive in a certain cold hardiness zone but may not be evergreen. As new broadleaf evergreens become available it is important that the grower know in which zones they can expect the plant to remain evergreen. The simple procedure described has proven to be a reliable tool for predicting whether a plant will remain evergreen in a particular zone. An examination of 35 taxa using this procedure found a number of plants which should be suitable for Zone 6 conditions. These include: *Viburnum* 'Conoy', *Viburnum rhytidophyllum* 'Cree', two pyracantha selections, *Camellia* 'Lu Shan Snow', an *Illicium anisatum* selection, selections of *Quercus acuta* and *Q. myrsinifolia* and a *Daphniphyllum macropodum* var. *humile* selection.

Introduction

Broadleaf evergreens are important landscape plants, especially in the southern cold hardy zones (Zone 7b and warmer (11)). The number of broadleaf evergreens suitable for use in Zone 7 is limited, and the choices become more limited in the colder hardiness zones (Zone 6 and below). Users in the colder climates would welcome additional broadleaf evergreen choices for their gardens. As new broadleaf evergreens come onto the market through breeding and exploration efforts, foliage cold hardiness needs to be quickly and accurately determined so growers will know whether a given plant will be evergreen in a given hardiness zone. Laboratory tests have been relatively successful in determining relative hardiness of stems and leaves and may provide a quick and easy method for initial evaluation of plants (2, 4, 5, 7).

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Sampling of whole leaves can be a problem when either the number of leaves on a plant are limited or when the number of leaves (replicates) needed to characterize a plant exceeds the capacity of the laboratory equipment. A simple solution to using whole leaves is to use leaf discs; however, it is first necessary to demonstrate that leaf discs accurately predict cold hardiness in the field. Leaf disc sampling has been shown effective with cabbage (*Brassica oleracea* var. *capitata* L.) (1), but not with southern magnolia (*Magnolia grandiflora* L.) (3).

Another problem with an assessment of 'evergreeness' is in the definition of evergreen. A number of factors must be considered; these include the intensity of yellowing or bronzing and the percent of foliage which is damaged at cold temperatures. In the end, the answer will be seen in the retail yard as consumers decide whether to buy a specific broadleaf evergreen or not. This concept differs significantly from that of Steponkus and Lanphear (10) for tissue viability, in that the method used for assessment should eliminate bias associated with visual observations. In this paper we define the loss of evergreen character (ie. nonevergreen) as the irreversible yellowing or browning of foliage.

The objectives of this study were to: (1) compare laboratory cold hardiness results to field data and determine if leaf discs provide a reliable means of estimating foliage cold hardiness, and (2) screen 35 broadleaf evergreen taxa in the laboratory for foliage cold hardiness to identify potential broadleaf evergreens for zone 6.

Materials and Methods

Two separate experiments were conducted during the winters of 1992/93 and 1993/94. During the winter of 1992/93 (Experiment 1), twelve unnamed hybrid pyracantha taxa and the cultivars 'Mohave', 'Navaho', and 'Apache' were selected in the fall of 1992 from the breeding program at the U.S. National Arboretum on the basis of superior ornamental traits. All 15 pyracantha taxa had been evergreen in Washington DC (Zone 7) for the previous four years . The plants were exposed to low temperatures of –14 (7), –10 (14), –10 (14), and –11C (13F) for 1989, 1990, 1991, and 1992 respectively. *Viburnum* 'Conoy', *Viburnum* 'Eskimo', and *Viburnum rhytidophyllum* 'Cree' were also examined. Both 'Conoy' and 'Cree' are reliably evergreen in Washington DC, while 'Eskimo' is semi-evergreen.

Leaf samples for Experiment 1 were collected on December 18, 1992, and January 21, 1993. Conditions were unusually mild throughout the test period: the minimum temperature previous to the first collection was –4C (25F) and previous to the second collection minimum temperatures reached only –7C (19F) on the day of collection and the previous day. Thus plants were not exposed in the field to temperatures below –10C (14F) and may not have reached the ordinary level of hardiness attained by these dates in typical Zone 7 conditions. The January sample only included a subset of the December sample and represented the more cold hardy selections from the December screening. For the pyracantha taxa (except #12) six to eight shoots between 8 and 10 cm (3 and 4 in) long were collected from each plant and stored in plastic bags at 4C (25F) in moist conditions until laboratory equipment became available for freezing, on December 21, 1992, and February 2, 1992 . Random leaves were sampled from the viburnum taxa and pyracantha #12.

In Experiment 2, 15 and 18 broadleaf evergreen taxa as listed in Table 3 were sampled December 22, 1993, and January 14, 1994, respectively. Six to eight branch tips approximately 12 cm (4.5 in) long were sampled from each taxon. On the December 22 sampling date, leaves were kept refrigerated until leaf discs were sampled from the leaves and the freezing cycle begun that same day. On the January 14 sampling date, the branches were placed in plastic bags with moist paper towels and stored at 4C (25F) until the equipment became available for freezing on February 4.

In Experiment 1, two to three leaf discs (7 mm in diameter) were sampled from 10 to 20 leaves per plant using a standard one-hole paper punch. For a few pyracantha selections, where leaf length was less than 2 cm (0.75 in), whole leaves were used. In Experiment 2, four to six leaf discs were sampled from a minimum of 20 leaves per plant. Leaf discs were placed on ice in 50 ml centrifuge tubes, sealed with twist-on caps and placed in a computer controlled Neslab (Portsmouth, NH) ULT-80 alcohol cooling bath. The bath was programmed to cool at 1C (1.8F)/hr from 0C (32F) to –20C (–4F), then to automatically warm to 0C (32F) at 1C (1.8F)/hr.

After removal from the centrifuge tubes, the leaf discs were placed on moist Perlite in transparent beakers covered with parafilm. They were held for three weeks at room temperature and bright indirect natural light before classification as being either green or discolored. If more than 50% of a leaf disc showed discoloration which appeared to be permanent, the disc was classified as discolored (not green). While this type of classification may not truly represent dead or live foliage, we believe it more accurately represents the perception of life and death ('functional evergreeness') which is the important marketing factor for the grower.

A Chi-square test was used to determine if the taxa differed in the proportion of brown and green leaf discs at each sampling period for each experiment. Differences were considered statistically significant at the $\alpha = .05$ level.

Field observations were made for Experiment 1 on February 7 and February 24, 1994. This followed two consecutive nights (January 19–20 1994) with lows which corresponded to the minimum low in the laboratory (–20.6C (–5F)). On February 7, the percentage of the foliage not discolored on each plant and on the tips of each plant were subjectively scored by two persons and averaged. On February 24, five branch tips from the outside of the plant and five from the inside were randomly sampled from each of the taxa. Leaves were classified as: (1) green, (2) bronzed or browned (but alive based upon green tissue on the underside of the leaf), and (3) dead.

Field observations for Experiment 2 were made on March 14 and 30, 1994. On March 14, four to eight branch tips 12 cm (4.5 in) long were randomly sampled from each plant and each leaf was scored as to the percentage of cold injury (discoloration). On March 30, plants were subjectively scored by two persons for percentage of cold injury (discoloration) on the plant and percentage of cold injury on the tips.

Correlation coefficients were estimated for field and laboratory scores with the SAS CORR procedure (9). In addition, an exact two-tailed binomial test was performed to estimate the exact probability that the field leaf samples on March 14, 1994 and the December 1993 laboratory samples came from the same binomial distribution. Branch-to-branch variation was examined using analysis of variance using the

Table 1. **Number of leaf discs sampled, percent green after freezing at –20C, percent leaves alive and green for inside and outside branches, and subjective field score for percent undamaged foliage in March 1994. Experiment 1.**

	December 1992		January 1993		March 1994				
					Outside		Inside		
Taxa	Number	% Green	Number	% Green	% Live	% Green	% Live	% Green	Field score[z]
Pyracantha 1	10	0			56	22	78	72	38
Pyracantha 2	13	15			75	32	59	30	32
Pyracantha 3	19	0			22	2	39	22	10
Pyracantha 4	17	18	30	0	28	3	8	0	8
Pyracantha 5	23	44	23	78	91	45	98	85	90
Pyracantha 6	28	7			51	37	76	63	42
Pyracantha 7	39	44	46	37	91	65	89	65	62
Pyracantha 8	15	0			81	49	65	34	62
Pyracantha 9	25	0			80	50	74	51	58
Pyracantha 10	23	0			30	8	0	0	20
Pyracantha 11	33	3			70	35	60	26	15
Pyracantha 12	16	25	24	12	0	0	46	15	30
Pyracantha 'Mohave'	17	0	29	0	78	38	99	84	40
Pyracantha 'Navaho'	26	0			48	0	40	11	30
Pyracantha 'Apache'			36	0	52	22	37	18	5
Viburnum 'Conoy'	19	32	22	64	100	14			95
Viburnum 'Eskimo'	19	5	25	48	40	20			40
Viburnum 'Cree'	22	32	26	100	100	100			100

[z]Field score is the average of two persons scoring percent undamaged foliage.

field leaf data from Experiment 2. The SAS VARCOMP procedure (9) was used to estimate the leaf-to-leaf, branch-to-branch, and among taxa variance components.

Results and Discussion

The percent of leaf discs which were still green after the –20C (–4F) freezing for Experiment 1 ranged from 0 to 44% in December 1992 and from 0 to 100% in January 1993 (Table 1). Chi-square tests showed that there were statistically significant differences among the taxa at both test times. The *Viburnum rhytidophyllum* 'Cree', *Viburnum* 'Conoy', and two pyracantha selections (5 and 7) were consistently evergreen at all sampling times.

The percentage of undamaged foliage from subjective field scoring of whole plants in March 1994 was significantly correlated with both the December 1992 (r = 0.56) and January 1993 (r = 0.90) lab data from the previous year (Table 2). Correlations between the percent green leaves from individual leaf scoring in March 1994 and the previous winter's lab results were not significant (Table 2). The poor correlations with the individual leaf scoring could be attributable to a number of reasons: the leaf discs may not have been good predictors; the year-to-year variation in cold hardiness due to differences in climate may have caused the cold hardiness ranking among taxa to differ between years; and/or there may not have been sufficient degrees of freedom to detect the small correlations.

Table 2. **Correlations among lab results from December 1992 and January 1993 sampling dates and the percent green and alive foliage measured from same plant position as lab sample and with the subjective field score of percent damaged foliage in March 1994.** (α level in parenthesis).

Field observations	Laboratory results	
	December 1992 Lab (df = 15)	January 1993 Lab (df = 7)
Percent green	.40 (.12)	.64 (.06)
Percent live	.47 (.06)	.67 (.05)
Field score	.56 (.02)	.90 (.001)

It should be noted that the storing of the leaves may have altered their cold hardiness. Freezing resistance can be increased in stem material by using prechilling treatments; temperatures below 0C (32F) being most effective (8). Without further research it would be impossible to determine whether any hardening/dehardening had occurred since the temperature at which the leaves were stored was above 0C (32F) and the effect of prechilling on leaves has not been documented.

The range of percent 'green' leaf discs in Experiment 2 varied from 0 to 98% in December 1993 and from 0 to 100% in January 1994 (Table 3). Similar ranges were shown for the field scores. Chi-square tests indicated that significant taxa differences were present for all traits. A number of selections proved evergreen: *Camellia oleifera* 'Lu Shan Snow', the *Illicium anisatum* selection, *Viburnum* 'Conoy', the *Quercus acuta* and *Q. myrsinifolia* selections, and the *Daphniphyllum macropodum* var. *humile* selection. Those selections which were not evergreen were: the *Raphiolepis umbellata* and *R. indica* selections, the *Daphniphyllum macropodum* selection, the *Quercus saliciana, Q. glauca,* and *Q. laurifolia* selections, and the *Itea oldhami* selection.

Correlations between lab and field results for Experiment 2 were significant for all comparisons (Table 4). For 'Conoy' viburnum, the January 1993 data was substituted for the January 1994 laboratory data in the correlations because the January 1994 reading was considered anomalous. The January lab correlations with the field results were lower than the

Table 3 **Results of laboratory testing in December 1993 and January 1994, subjective field scoring plants and tips for cold damage in March 1994, and percent of leaves scored damaged from individual leaf scoring in March 1994.**

Taxa	December 93 Lab n	December 93 Lab % green	January 94 Lab n	January 94 Lab % green	Tips[z]	Plant[y]	Leaves[x]	Fishers exact[w]
Camellia oliefera 'Lu Shan Snow'	136	98	100	100	88	95	89	0.011
Illicium anisatum	161	96	95	82	98	98	94	0.466
Viburnum 'Conoy'	134	87	87	30	90	90	75	0.098
Quercus acuta	125	79	108	58	88	94	75	0.433
Daphniphyllum macropodum var. *humile*	68	78	95	87	82	82	79	1.000
Quercus myrsinaefolia	175	51	101	27	60	68	73	0.014
Raphiolepis indica	75	47	98	44	5	5	0	<0.001
Quercus saliciana	132	33	76	1	16	47	47	0.051
Quercus laurifolia	117	28	95	7	0	3		<0.001
Lithocarpus henryi	175	23	98	5	8	15	45	0.003
Raphiolepis umbellata	60	23	103	5	2	35	5	0.023
Daph. macropodum	119	18	87	18	15	10	20	0.651
Quercus glauca	157	1	99	0	0	0	0	0.498
Itea oldhami	67	0			0	0	0	1.000
Sycopsis sinensis	123	0			15	80	52	<0.001
Camellia japonica A			92	93	72	78	62	<0.001[v]
Camellia japonica B			107	31	63	63	33	0.698[v]
Gardenia 'Klines Hdy'			42	5	0	0	0	0.494[5]
Gardenia 'Daisy'			42	5				
Illicium mexicana			69	87				

[z]Percent of foliage damamged on branch tips subjectively scored in March 1994.
[y]percent of foliage damamged on total plant subjectively scored in March 1994.
[x]percent of foliage from branch tips scored damaged from individual leaf assessment March 1994.
[w]Exact binomial comparison of December 1993 lab test with x above.
[v]No December 1993 lab data available; January 1994 data substituted

December lab correlations with field results; however, the differences were not statistically significant. Any marginal improvement in the December correlations may have been because the December lab data was collected immediately after the leaves were sampled, while the January sample was collected before the freeze of January 19th and stored until February 4, when lab freezing was conducted. The same caveats as previously mentioned hold here. However, it should be noted that where differences were observed, in 9 of 11 taxa, the stored leaves were less hardy, indicating prolonged storage of leaves taken in midwinter or later may lead to dehardening.

The exact two-tailed binomial test comparison of the December 1993 lab data and the individual leaf assessment of plants in the field (March 1994) yielded additional support for the leaf disc method. In 9 of 16 taxa, the level of observed damage in the field matched that in the lab exactly ($\alpha > .05$, ie no difference). In 15 of 16 taxa, if leaf discs indicated damage to be greater than 50% then field injury was also greater than 50%. This rather crude 50% injury point is the normal standard in the field, whether measuring electrolyte leakage, tetrazolium reduction, or the more rigorous regrowth test.

The correlations of the lab data with individual leaf scoring were more significant in Experiment 2 than Experiment 1. This would be expected since Experiment 1 was a comparison between two dissimilar years, while Experiment 2 was data from the same year.

These correlations demonstrate that the laboratory technique corresponds very well with field results in the same year. Even the off year correlation between the January 1993 lab and the 1994 field scores was high despite the fact that the winter of 1992/93 was warmer than average and the winter of 1993/94 was colder than average. For both experiments it should be noted that when the January laboratory results had very high percentages of green leaf discs (> 75%), the plants remained evergreen and when the percentage was extremely low (< 25%) the plants were not evergreen. Those plants with January green disc percentages between 25 and 75% were more difficult to classify as evergreen in the field. The fact that some of the 'non-evergreen' taxa from the December 1992 sampling date had relatively good field resistance in January 1994 suggests that taxa such as pyracanthas #8 and #9 may continue to significantly harden off into January.

The analysis of variance for the field leaf data showed that the largest component of variation was the among taxa

Table 4. **Correlations among lab and field scores for percent green foliage for Experiment 2. (level in parenthesis).**

Field scores	Laboratory scores: December 1993	Laboratory scores: January 1993
Field[z]	.82 (.001)	.76 (.01)
Tips[y]	.92 (.0001)	.88 (.0001)
Plants[x]	.76 (.001)	.81 (.001)

[z]Average percent undamaged from scoring individual leaves in March 1994.
[y]Percent undamaged foliage subjectively scored for branch tips in March 1994.
[x]Percent undamaged foliage subjectively scored for entire plant in March 1994.

component (48% of the total variation). The branch-to-branch component made a significant contribution to the overall variation in 'evergreeness' (22% of the total variation). The branch-to-branch could lead to variable results if one was to only sample a small number of branches. It should be noted that the branch-to-branch variation in the field is a function of both the physiology of the leaves and their microenviroment. Most plants suffered more damage on the exposed (wind facing) side of the plant, implying some of this variation is environmental. Certain plants in both experiments had significant differences between cold hardiness of foliage on the outside (tips) and on the inside of the plant. The difference was not predictable. Both pyracantha 12 in Experiment 1 and the *Sycopsis* in Experiment 2 appeared to have significantly more hardy interior foliage; but pyracantha 10 in Experiment 1 had more hardy exterior foliage. Although plant-to-plant variation within clone was not investigated, it is probable that plant-to-plant variation is significant.

In summary, leaf discs were an effective means of assessing foliage cold hardiness for the taxa sampled. It allows for a more thorough sampling of a plant because a large number of samples can be obtained and the laboratory results correlate well with field results. In using leaf discs one should thoroughly sample the plant (or plants), being sure the whole canopy is represented. For best results, controlled freezing of the discs should begin the same day or soon thereafter. After thawing and incubating in a moist environment at room temperature for three weeks the discs can be visually scored on the basis of discoloration (< 50% discoloration = green). Taxa which have over 75% green leaf discs should prove to be evergreen and those with less that 25% green leaf discs are likely not to be evergreen. It is important that one test over the winter so that timing and rates of acclimation are addressed (6).

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