Cold Hardiness of *Ilex glabra* Cultivars from Field Trials and Laboratory Tests[1]

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Abstract

To evaluate the cold hardiness of *Ilex glabra* (L.) A. Gray (inkberry) cultivars and provide growth and cold-hardiness data for growers as references for production and marketing, field trials and laboratory tests were conducted in 2007–2008 and 2008–2009. Plant survival was 72 and 93% for the 2007 and 2008 planting, respectively. *Ilex glabra* 'Shamrock' was the most cold-hardy cultivar; *Ilex glabra* f. *leucocarpa*, 'Viridis' and 'Nigra' were the least cold-hardy cultivars; and *Ilex glabra* wild species and its cultivars including 'Compacta', 'Densa', 'Chamzin', and 'Pretty Girl' had intermediate cold hardiness. Based on controlled freezing tests of *Ilex glabra* cultivars, the temperature that results in 50% relative electrical conductivity (REC_{50}) of *Ilex glabra* cultivars ranged from –19.4 to –31.8C (–2.9 to –25.2 F) for January 2007 and –17.8 to –37.5C (–0.1 to –35.5F) for January 2008. The cold hardiness rates from field trials were significantly correlated with the REC_{50} values from laboratory tests. The factors influencing cold hardiness, including plant cultivar, tissue, temperature, deaacclimation, winter desiccation, and others (e.g. mechanical injury, snow pack) should be in consideration.

Index words: cold hardiness rate, inkberry, REC_{50} value, temperature.

Significance to the Nursery Industry

Ornamental plant field trials and laboratory tests provide important growth and cold hardiness data for growers to produce and market their plants. Our results suggested that cold hardiness of *Ilex glabra* varied with cultivars. *Ilex glabra* 'Shamrock' was the most cold-hardy among tested cultivars; while *Ilex glabra* f. *leucocarpa*, 'Viridis' and 'Nigra' were the least cold-hardy cultivars. Their field performance was significantly correlated with results from laboratory tests, suggesting laboratory test could be used to predict the cold tolerance of *Ilex glabra* cultivars.

Introduction

Ilex glabra (L.) A. Gray (inkberry) is a native evergreen shrub in the family *Aquifoliaceae* (holly family). It is an important foundation and hedge plant due to its environmental adaptability, ability to withstand heavy pruning, and resistance to diseases and insects (5, 6, 11). Native populations of *Ilex glabra* have been recorded along eastern US coast from Florida to Maine (25). In Maine, the single known population

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of this plant grew around the perimeter of a coastal sphagnum bog in Knox County (15). Unfortunately, we could not locate this native population. Although 27 cultivars were reported in the literature (5, 6), most cultivars are difficult to find or under-utilized in the nursery trade possibly because of susceptibility to winter injury.

The cold hardiness of other *Ilex* species and their cultivars had been investigated in field or laboratory studies. *Ilex opaca* Ait. (American holly), *Ilex opaca* 'Greenleaf' (greenleaf American holly), and *Ilex* × *attenuata* Ashe 'Foster's #2' ('Foster's #2' holly) were hardy to –30.0C (–22F); *Ilex latifolia* Thunb. (lusterleaf holly) and *Ilex* L.'Lydia Morris' (lydia morris holly) were less cold hardy, only surviving temperature as low as –17.8C (–0.1F); while *Ilex* × 'Nellie R. Stevens' (Nellie R. Stevens holly) and *Ilex* × *koehneana* Loes. 'Wirt L. Winn' (Wirt L. Winn Koehne holly) exhibited intermediate cold hardiness and survived a temperature of –21.1C (–6.0F) (7). Among the *Ilex* × *attenuata* cultivars, 'Foster's #2' was more tolerant than *Ilex* × *attenuata* 'Savanna' (Savannah holly) and *Ilex* × *attenuata* 'East Palatka' (East Palatka holly) (7).

Leaves of all *Ilex* taxa were generally less cold hardy than their stems. However, leaves of *Ilex* × *koehneana* 'Wirt L. Winn', *Ilex latifolia*, and *Ilex* 'Lydia Morris' were more cold-hardy than their stems (7). Extent of root injury of *Ilex crenata* Thunb. 'Green Island' was 33–100% when the substrate temperature decreased from –1.7 to –10.0C (28.9 to 14.0F) (20). The killing temperature of *Ilex crenata* 'Hetzi' roots, estimated using TTC (2, 3, 5-triphenyl tetrazolium chloride), ranged from –5.6 to –7.8C (21.9 to 18.0F) (10).

Only observational data have been reported regarding the cold hardiness of *Ilex glabra* wild species and its cultivars. The white-fruit form, *leucocarpa*, which was collected from Florida could tolerate at least –26.0C (–14.8F). *Ilex glabra* 'Chamzin' survived winter temperatures ranging from –29.0 to –34.0C (–20.2 to –29.2F) (6). *Ilex glabra* 'Nigra' was not as cold hardy as *Ilex glabra* 'Compacta' and 'Viridis' (6). *Ilex glabra* was more cold-hardy than *Ilex crenata* at temperatures of –26.0 to –29.0C (–14.8 to –20.2F) in severe winters (5). *Ilex glabra* 'Compacta' and 'Nordic' survived well in Orono, ME (USDA Zone 5a) (4). The cold hardiness of *Ilex glabra* wild species and its cultivars have not yet been comprehensively examined. Therefore this information would increase its use as a native woody plant.

To systematically evaluate plant response to low temperatures in the field, more frequent assessments of cold tolerance rather than sporadic observations are required. Field trial is the standard method used to assess plant cold hardiness (21, 22). Entire above-ground portion of plant is exposed to frost in the natural environment until visible evidence of injury develops. This test is reliable, but the occurrence of freezing ambient temperatures in the field may be unpredicable. Moreover, field trial needs a larger plant population and often requires years to get results. However, tissue test for cold hardiness, based on physiological parameters that can be conveniently measured, provides results within one to forty-eight hours (1). Use a programmed freezer to expose plant tissues to a series of precisely controlled temperatures allows repeatable samplings of many plants in a short time. The reliability of tissue test depends on whether the results of tissue test match those of field trial. If there is a consistent relationship between field trials and one or more tissue tests, plant cold hardiness can be measured efficiently.

The short growing season and harsh winter leave gardeners in the northern United States with a relatively limited selection of broadleaf evergreen shrubs. Due to the glabrous dark green and fine textured foliage of *Ilex glabra*, it could be an important northern landscape plant if cold hardy cultivars are identified. The objective of this study was to evaluate the cold hardiness of *Ilex glabra* cultivars or accessions through a combination of field trials and laboratory leaf tissue tests. The growth and cold-hardiness data will help growers produce and market *Ilex glabra*.

Materials and Methods

Field trials. Field trials were conducted in the Lyle E. Littlefield Ornamentals Trial Garden at the University of Maine (Orono, ME; lat. 44°54'05"N, long. 68°39'36"W, elev. 44 m). The dominant soil is Orthods (26). The chemical characteristics of the soil were within the optimum range (data not presented). A 40 × 7.4 m^2 (131 × 24 ft^2) plot was completely tilled and prepared for planting. A total of 20 *Ilex glabra* cultivars (Table 1) were established on July 26, 2007. All plants were planted in a randomized complete block design (RCBD) with four rows as blocks and four plants for each cultivar. The distance between plants was 1.5 m (4.9 ft), with 2.4 m (7.9 ft) between rows. On June 30, 2008, another 22 *Ilex glabra*

Table 1. Growth characteristics and cold hardiness parameters of *Ilex glabra* accessions following the winter of 2007–2008. *Ilex glabra*, *I. glabra* 'Chamzin', *I. glabra* 'Compacta', *I. glabra* 'Densa', *I. glabra* f. *leucocarpa*, *I. glabra* 'Nigra', *I. glabra* 'Pretty Girl', and *I. glabra* 'Shamrock' were evaluated.

Accession and sources	Height (cm)	Canopy size (cm^2)	Cold hardiness rate[z]	Length of dead branch (cm)	Dead branch %
Ilex glabra AA200-2005/AA652-70-Mass/AA929-88	15.7 ± 1.1[y]	241.3 ± 26.3	2.6 ± 0.2	3.6 ± 0.4	50.6 ± 6.9
'Chamzin' LG1997-1435	14.8 ± 1.4	171.5 ± 23.0	3.3 ± 0.4	5.2 ± 1.5	25.0 ± 12.5
'Compacta' AA-179-2005, *Ilex glabra* Worest, MA	12.2 ± 1.0	166.6 ± 27.1	3.1 ± 0.2	9.2 ± 0.9	33.8 ± 4.8
'Compacta' AA-745-69 / AA1051-70, 'Jackson Compacta'	16.6 ± 0.9	257.9 ± 19.4	3.8 ± 0.1	4.0 ± 0.6	30.8 ± 4.1
'Compacta' LG1997-1423/UME, 'Cole's Compacta' WMN	15.5 ± 0.7	140.1 ± 7.3	2.2 ± 0.2	7.4 ± 0.7	61.2 ± 5.7
'Densa' LG1992-0298/PCF	15.4 ± 1.3	255.5 ± 38.5	3.6 ± 0.2	6.0 ± 0.9	36.0 ± 5.6
f. *leucocarpa* AA1489-82-Mass	15.1 ± 1.8	349.8 ± 52.2	1.5 ± 0.3	13.7 ± 0.8	51.2 ± 10.9
'Nigra' AA1464-82/GPN	14.4 ± 1.1	147.9 ± 19.0	1.3 ± 0.1	9.3 ± 0.6	85.4 ± 6.2
'Pretty Girl' LG1994-0494	12.7 ± 1.1	137.3 ± 11.9	2.8 ± 0.2	3.0 ± 0.5	38.8 ± 7.7
'Shamrock' GPN/LG1997-0973	16.0 ± 0.8	251.0 ± 18.8	4.0 ± 0.2	3.4 ± 0.8	5.7 ± 1.1

[z]Cold hardiness rate was defined as follows: 5 = little or no damage; 4 = occasional tip dieback; 3 = regular tip dieback and occasional substantial dieback; 2 = typical moderate to severe dieback or dieback to the ground with suckering from the roots; 1 = entirely winter killed (4).

[y]Values are the means of 4, 8, or 12 plants with the standard error.

Table 2. **Growth characteristics and cold hardiness parameters of *Ilex glabra* accessions following the winter of 2008–2009. *Ilex glabra*, *I. glabra* 'Chamzin', *I. glabra* 'Compacta', *I. glabra* 'Densa', *I. glabra* f. *leucocarpa*, *I. glabra* 'Nigra', *I. glabra* 'Nova Scotia', *I. glabra* 'Pretty Boy', *I. glabra* 'Pretty Girl', *I. glabra* 'Shamrock', and *I. glabra* 'Viridis' were evaluated.**

Accession and sources	Height (cm)	Canopy size (cm^2)	Cold hardiness rate[z]	Length of dead branch (cm)	Dead branch %
Ilex glabra AA200-2005/AA652-70-Mass/AA929-88	19.9 ± 1.0[y]	399 ± 25	5.0 ± 0.0	0.0	0.0
'Chamzin' LG1997-1435	24.0 ± 1.2	400 ± 24	4.8 ± 0.1	0.0	0.0
'Cole's Compacta', 'Compacta' UME	34.3 ± 0.9	1427 ± 104	4.8 ± 0.1	0.0	0.0
'Compacta' AA-179-2005, *Ilex glabra* Worest, MA	21.0 ± 0.7	771 ± 32	4.1 ± 0.1	0.0	0.0
'Compacta' AA-745-69/AA1051-70	25.1 ± 1.3	676 ± 48	4.9 ± 0.1	0.0	0.0
'Densa' LG1992-0298/PCF	28.4 ± 0.5	812 ± 34	3.9 ± 0.2	3.1 ± 0.9	15.3 ± 6.1
f. *leucocarpa* AA1489-82-Mass	33.5 ± 1.0	1620 ± 43	3.0 ± 0.0	11.5 ± 2.6	24.0 ± 4.1
'Nigra' AA1464-82/GPN	19.0 ± 1.1	264 ± 22	2.3 ± 0.1	15.0 ± 0.5	52.9 ± 5.4
'Nova Scotia' RN	14.7 ± 0.8	458 ± 20	4.3 ± 0.2	0.5 ± 0.3	4.5 ± 2.3
'Pretty Boy' RN	20.2 ± 0.3	441 ± 7	4.0 ± 0.2	1.8 ± 0.9	12.5 ± 6.3
'Pretty Girl' LG1994-0494/RN	14.6 ± 0.2	229 ± 8	4.6 ± 0.1	0.0	0.0
'Shamrock' GPN/LG1997-0973	14.5 ± 0.5	298 ± 7	4.3 ± 0.1	2.0 ± 0.7	8.0 ± 2.7
'Viridis' AA1488-82	22.3 ± 0.6	218 ± 13	3.0 ± 0.2	10.5 ± 1.9	47.5 ± 8.5

[z]Cold hardiness rate was defined as follows: 5 = little or no damage; 4 = occasional tip dieback; 3 = regular tip dieback and occasional substantial dieback; 2 = typical moderate to severe dieback or dieback to the ground with suckering from the roots; 1 = entirely winter killed (4).

[y]Values are the means of 4, 8, or 12 plants with the standard error.

cultivars (Table 2) were added to the existing plants. They were also arranged in a RCBD. The whole plot was mulched with newspaper for weed control. Ambient temperature at 30 cm (11.8 in) aboveground and 25 m (82 ft) away from the plot was monitored using type T thermocouples attached to CR10X Datalogger (Campbell Scientific Inc., Logan, UT). Plant height and width, measured at the east-to-west and north-to-south directions, were recorded at the beginning and end of every growing season. Plant cold hardiness rate was evaluated on May 7, 2008 and 2009, respectively, using the following rating scale: 5 = little or no damage; 4 = occasional tip dieback; 3 = regular tip dieback and occasional substantial dieback; 2 = typical moderate to severe dieback or dieback to the ground with suckering from the roots; 1 = entirely winter killed (4). The number of dead branches and mean length of all dead branches were also recorded.

Laboratory tests. Cold hardiness was determined by a modification of the frost-induced electrolyte leakage (FIEL) procedure (2, 3, 16). Twenty two-year-old plants of each *Ilex glabra* cultivar (Table 3) were grown in 3.3 liter (0.75 gal) plastic pots with a commercial substrate containing 35–45% bark, 20–30% coir, 10–20% Canadian sphagnum peat moss, 5–15% horticultural grade perlite, 5–15% processed bark ash, starter nutrient charge, dolomitic limestone, and wetting agent (Metro-Mix 560 Coir, Scotts-Sierra Horticultural Products Company, Marysville, OH). All plants were fertilized with controlled released fertilizer (Peters Professional 15 N-4.4 P-24.9 K, Scotts-sierra Horticultural Products Company, Marysville, OH). They were mulched during winter period. About 10 fully developed, non-senescent leaves of each plant were excised from the upper half of the current season's growth on January 15, 2008 and 2009. Leaves were immediately placed in a sealable plastic bag with wet paper towels and placed on ice in a cooler. Leaves were rinsed with distilled water to remove any electrolytes released by cells damaged during leaf excision. The detached leaves were prepared for freezing within 5 hr of collection. The leaves were cut into disk (28.7 ± 0.01 mm^2 and 0.095 ± 0.004 g) using a hole puncher. After all leaf discs (390 and 520 disks in 2008 and 2009, respectively) from each cultivar were pooled and mixed completely, ten discs were

Table 3. **Cold hardiness of *Ilex glabra* accessions based on laboratory tests on January 15, 2008 and 2009. *Ilex glabra* 'Chamzin', *I. glabra* 'Compacta', *I. glabra* 'Densa', *I. glabra* f. *leucocarpa*, *I. glabra* 'Nigra', *I. glabra* 'Nova Scotia', *I. glabra* 'Pretty Boy', *I. glabra* 'Pretty Girl', *I. glabra* 'Shamrock', and *I. glabra* 'Viridis' were tested.**

	REC_{50} (C)[z]	
Accessions	2008	2009
'Chamzin' LG1997-1435	–28.9 ± 1.6	–37.5 ± 1.4
'Cole's Compacta', 'Compacta' UME	–19.4 ± 0.9	—
'Compacta' AA-179-2005, *Ilex glabra* Worest, MA	–27.7 ± 2.4	–26.0 ± 1.0
'Compacta' AA-745-69/AA1051-70	–31.8 ± 1.1	–33.4 ± 0.6
'Densa' LG1992-0298/PCF	–27.0 ± 1.6	–32.8 ± 1.1
f. *leucocarpa* AA1489-82-Mass	—	–29.1 ± 0.8
'Nigra' AA1464-82/GPN	—	–17.8 ± 3.5
'Nova Scotia' RN	—	–33.3 ± 0.4
'Pretty Boy' RN	—	–26.9 ± 0.9
'Pretty Girl' LG1994-0494/RN	–24.4 ± 1.7	–30.7 ± 0.7
'Shamrock' GPN/LG1997-0973	–26.1 ± 0.5	–31.8 ± 0.5
'Viridis' AA1488-82	—	–24.7 ± 2.0

[z]Values are the means of 3 or 6 replications with the standard error for 2008, while that of 4 or 8 replications with the standard error for 2009. REC_{50} was defined as the temperature that results in 50% relative electrical conductivity (REC).

randomly sampled and transferred into one of twelve 14 mL polypropylene round bottom tubes (Becton Dickinson Labware, Franklin Lakes, NJ) containing 10 ml of deionized water. Samples were subjected to 12 temperatures [–2, –6, –10, –14, –18, –22, –26, –30, –34, –38, –42, –46C (28.4, 21.2, 14, 6.8, –0.4, –7.6, –14.8, –22, –29.2, –36.4, –43.6, –50.8F)] plus a control [3.9 ± 1.6C (39.0 ± 2.9F)]. This procedure was repeated three to four times. The test tubes with 10 ml of deionized water (electrical conductivity = 0.002 ms) were prepared in advance and frozen overnight in the refrigerator (Thermo Electron Corporation, Asheville, NC). Before leaf discs were loaded, test tubes were thawed, leaving a piece of ice floating as a nucleation core. All test tubes, except the control, were kept overnight in a 40–9.4 super cold freezer (ScienTemp Corporation, Adrian, MI), preset at the temperature of 0C (32F). Controls were kept on ice in a cooler during the whole freezing program. The temperature, monitored by type T thermocouples attached to a datalogger (CR-10, Campbell Scientific, Logan, UT), was lowered at a rate of $4C{\cdot}hr^{-1}$ ($7.2F{\cdot}hr^{-1}$) to each of the desired target temperatures within the tubes. Three or four test tubes corresponding to the target temperature were thawed on ice in a cooler overnight after they were held at the target temperature for 30 min. Another three or four sets of leaf discs were immersed in liquid nitrogen [–196C (–320.8F)] for inducing total cell lysis. After the tissues were fully thawed, all test tubes were agitated on a shaker at 150 rpm for 24 hr at a temperature of 16.5 ± 0.9C (61.7 ± 1.6 F) and the electrical conductivity (EC) was measured using an EC meter (Amber Science Inc., Eugene, OR). From these data, the relative electrical conductivity (REC) was calculated by the following formula: $REC = 100 \times [(EC_{frozen}) - (EC_{control}) / (EC_{liquid\text{-}nitrogen})]$. Plots of REC versus temperature were constructed and non-linear curve based on the growth/sigmoidal model from the Boltzmann equation were fitted using the OriginPro 7.0 (OriginLab Corporation, Northampton, MA). The Boltzmann sigmoidal equation is expressed as $y = a + (b - a) / (1 + e^{(c-x)/d})$, where the parameters a and b represent the probability at the minimum and maximum plateaus, c represents the *X*-value when the response is halfway between a and b, and d represents the slope of the curve. Fitted regression curves were used to calculate the temperature that results in 50% REC was determined and denoted as REC_{50}.

Experimental design and statistical analysis. Since the influence of plant sources of *Ilex glabra* cultivars on cold hardiness was not significant (2007: $P = 0.48$; 2008: $P = 0.91$), all data were pooled for the following data analysis. The results presented are the means of 4, 8, or 12 replications with the standard error. Data transformation was performed as necessary. A completely random design was employed for the laboratory tests. Results from laboratory tests showed that all REC values fit well for Boatman's model and no significant difference was observed among plant sources of *Ilex glabra* (2008: $P = 0.47$; 2009: $P = 0.93$). Data from different plant sources were therefore pooled for further data analysis. The results presented are the means of 4 or 8 replications with the standard error.

All data including height, cold hardiness rate, length and percentage of dead branches, canopy size, and REC_{50} were analyzed using analysis of variance in the Statistical Analysis Systems 9.1 (SAS Institute, Inc., Cary, NC). Student-Newman-Keuls test at $P \leq 0.05$ was applied for means separation. Correlation and linear regression analysis was also conducted using data of both field trials and laboratory tests.

Results and Discussion

Field trials. All *Ilex glabra* cultivars in the 2007 planting were fairly uniform. No significant differences were observed for their height ($P = 0.59$) and canopy size ($P = 0.19$) (Table 1). They grew to about 15 cm (5.9 in) [12–17 cm (4.7–6.7 in)] high with a canopy size of about 196 cm^2 (30.4 in^2) [137–258 cm^2 (21.2–40.0 in^2)]. They were covered by snow for most of the winter season (January 15 to March 14, 2008). The lowest temperature recorded was –25C (–13F) on March 4, 2008 (data not presented). Plant survival was 72% (57/ 80) in 2008. All surviving plants were still alive in 2009 (data not presented). There was a significant difference of cold hardiness rates among cultivars ($P = 0.0012$). *Ilex glabra* 'Shamrock' (GPN/LG1997-0973), *I. glabra* 'Compacta' (AA745-69/1051-70/Jackson Compacta), and *I. glabra* 'Densa' (LG1992-0298/PCF) were the most cold-hardy cultivars, which corresponded to their low cold hardiness rates of 4.0, 3.8, and 3.6, respectively. In contrast, *Ilex glabra* f. *leucocarpa* (AA1489-82-Mass) and *I. glabra* 'Nigra' (AA1464-82/GPN) were not cold hardy in Orono, ME. Their cold hardiness rates were 1.5 and 1.3, respectively. But they were not statistically different from *Ilex glabra* (AA200-2005/652-70-Mass/929-88), *I. glabra* 'Chamzin', *I. glabra* 'Compacta' AA179-2005/*Ilex glabra* Worest, MA, *I. glabra* 'Compacta' (LG1997-1423/UME/WMN) and *I. glabra* (LG1994-0494), whose cold hardiness rates ranged from 2.2 to 3.3. The length of damaged branches was similar among most of the cultivars tested; only *Ilex glabra* f. *leucocarpa* (AA1489-82-Mass) showed excessive damage, but it was not significantly different from *I. glabra* 'Compacta' AA179-2005/*Ilex glabra* Worest, MA, *I. glabra* 'Compacta' (LG1997-1423/UME/WMN), 'Nigra' (AA1464-82/GPN). There was a high percentage of dead branches, 85%, for 'Nigra' (AA1464-82/GPN), while the percentage of the dead branches of shamrock was less than 6%. Among the rest of the cultivars tested, there was such a large variation in percentage of dead branches (25–62%), but the values were not significantly different.

For the 2008 planting, there were much higher variations in cultivar height and canopy size (Table 2). Their height was about 23 cm (9.1 in) [14–34 cm (5.5–13.4 in)], while canopy size was about 644 cm^2 (99.8 in^2) [218–1620 cm^2 (33.8–251.1 in^2)]. These variations resulted from the different growth habit or different length of growth in greenhouse and/or nursery. However, all plants were still completely covered with snow starting from December 21, 2008, until March 19, 2009. The lowest temperature recorded was –30.5C (–22.9F) on March 1, 2009 (data not presented). Plant survival was 93% (81/87) during this winter. Cold hardiness rates differed among cultivars ($P < 0.0001$) (Table 2). *Ilex glabra* (AA200-2005/AA652-70-Mass/AA929-88) and *Ilex glabra* cultivars including 'Chamzin', 'Compacta', 'Densa', 'Nova Scotia', 'Pretty Boy', 'Pretty Girl', and 'Shamrock' were the most cold-hardy cultivars. For these cultivars, less than 15% of their branches were damaged and the length of damaged branches was less than 3 cm which was, on average, about 14% of the plant height. However, there was no difference among these cultivars. On the other hand, *Ilex glabra* f. *leucocarpa* (AA1489-82-Mass), *Ilex glabra* 'Nigra' (AA1464-82/GPN), and 'Viridis' (AA1488-82) were the least cold hardy among all cultivars tested. The damaged branches accounted for 24, 48, and 53% for *Ilex glabra* f. *leucocarpa*, 'Viridis' and 'Nigra', respectively. A length of 10.5 to 15 cm (4.1–5.9 in) of branch was damaged, which accounted for 34–79% of the height of whole plant.

Based on data from the two growing seasons, *Ilex glabra* 'Shamrock' was the most cold-hardy cultivar, followed by *I. glabra* 'Compacta', *I. glabra* 'Densa', *I. glabra* 'Chamzin', *I. glabra* 'Pretty Girl', *I. glabra*, then *I. glabra* f. *leucocarpa*, *I. glabra* 'Viridis' and *I. glabra* 'Nigra'.

Laboratory tests. Cold hardiness based on REC_{50} values differed significantly among tested *Ilex glabra* cultivars (2008 and 2009: $P < 0.0001$) (Table 3). Similar results had been observed for other *Ilex* species or cultivars (7). From the 2008 laboratory test, *Ilex glabra* 'Compacta' (AA-745-69/AA1051-70) was one of the more cold-hardy cultivar with a REC_{50} of –31.8C (–25.2F) while *I. glabra* 'Cole's Compacta'/'Compacta' (Umaine) was the least cold-hardy cultivar with a REC_{50} of –19.4C (–2.9F). Umaine accession of 'Compacta' was produced via cuttings from the plants which were located under the canopy in the Lyle E. Littlefield Ornamentals Trial Garden at University of Maine, Orono. These plants may have adapted to the specific understory microclimates. They might have been subjected to photoinhibition under low temperature and high light irradiation, and thus sensitive to the low temperature (14, 23). 'Cole's Compacta' came from the rooted cuttings at the Western Maine Nursery, but we don't know the exact origin. It is reasonable to speculate that they were from a more southern source. *Ilex glabra* 'Chamzin', *I. glabra* 'Compacta' (AA-179-2005)/ *Ilex glabra* (Worest, MA), *I. glabra* 'Densa' (LG1992-0298/ PCF), and *I. glabra* 'Shamrock' (LG1997-0973/GPN) were more cold-hardy with a REC_{50} value of –28.9, –27.7, –27.0, and –26.1C (–20.0, –17.9, –16.6, and –15.0F), respectively. Our experimental result with *Ilex glabra* 'Chamzin' are in agreement with the observations of Dirr and Alexander (6). They found that *Ilex glabra* 'Chamzin' survived winter temperatures ranging from –29.0 to –34.0C (–20.2 to –29.2F). *Ilex glabra* 'Pretty Girl' (LG1994-0494/RN) was the intermediate cold-hardy cultivar. From 2009 laboratory test, REC_{50} values of *Ilex glabra* 'Chamzin', *I. glabra* 'Compacta' (AA-745-69/ AA1051-70), *I. glabra* 'Densa' (LG1992-0298/PCF), *I. glabra* 'Nova Scotia', and *I. glabra* 'Shamrock'(GPN/LG1997-0973) were higher than –31.8C (–25.2F).They can be considered the most cold-hardy cultivars. REC_{50} values of *Ilex glabra* 'Pretty Girl', *I. glabra* f. *leucocarpa*, *I. glabra* 'Pretty Boy', *I. glabra* 'Compacat' (AA-179-2005, Worest, MA), and *I. glabra* 'Viridis' ranged from –24.7 to –30.7C (–12.5 to –23.3F). They were the intermediate cold hardy cultivars. The least cold hardy cultivar with a REC_{50} value of –17.8C (–0.1F) was *Ilex glabra* 'Nigra'. The 2008 and 2009 laboratory tests suggest that *Ilex glabra* 'Chamzin', *I. glabra* 'Compacta', *I. glabra* 'Nova Scotia' were the most cold hardy cultivars, followed by *I. glabra* 'Densa', *I. glabra* 'Shamrock', *I. glabra* 'Pretty Girl', *I. glabra* f. *leucocarpa*, *I. glabra* 'Pretty Boy', *I. glabra* 'Viridis', and *I. glabra* 'Nigra'.

The cold hardiness rates from field trials were significantly correlated with REC_{50} value from laboratory test in 2008 ($P = 0.0002$, $r = –0.87$) and 2009 ($P = 0.0011$, $r = –0.80$) (Fig. 1). These results suggested that data from field trials and laboratory tests were corroborative and either one reliable for the measurement of *Ilex glabra* cold hardiness. High degree of correlation supports the use of laboratory tests to predict

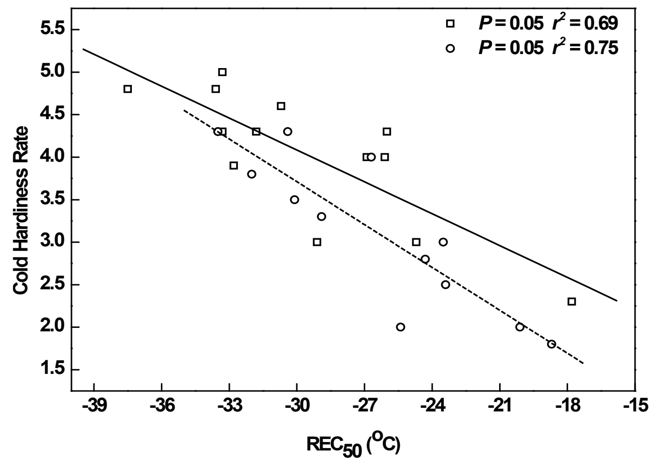

Fig. 1. The temperature that results in 50% relative electrical conductivity (REC_{50}) of *Ilex glabra* accessions in January 2008 (open circle) and 2009 (open square) relating their cold hardiness rate in 2007–2008 and 2008–2009 field trials. Linear regression analyses revealed the following relationships, 2008: y = –0.17x – 1.35 (dash line); 2009: y = –0.13x + 0.12 (solid line).

cold tolerance of *Ilex glabra* cultivars in the field. Both data were then analyzed using a linear regression model to establish the equation for the prediction [2008: y = –0.17x – 1.35 ($P = 0.05$, $r^2 = 0.75$); 2009: y = –0.13x + 0.12 ($P = 0.05$, $r^2 = 0.69$)] (Fig. 1). Cold ambient temperature in Orono, ME could account for over 70% of the observed damage to field plants. In addition, snow pack, stem injury from insects, and/or physiological changes such as deacclimation, winter desiccation and photoinhibition could enhance damage in the field trials.

The extent of winter injury depends on taxa (species, cultivars, and/or individual plant) (4, 5, 6, 7). In our studies, *Ilex glabra* 'Shamrock' was the most cold-hardy cultivar, while *I. glabra* f. *leucocarpa*, *I. glabra* 'Viridis' and *I. glabra* 'Nigra' were not cold hardy cultivars in Orono, ME.

For *Ilex*, variation in cold hardiness among tissues has been reported (7). During the winter season from 2006 to 2007, we observed that 100% of our *Ilex glabra* plants were killed when placed outside without any protection. However, when plants were placed in a white plastic winter house without any supplementary heat, where the lowest temperature recorded was –20.5C (–4.9F), all roots turned brown, while the aboveground plant parts still looked healthy, suggesting that the leaves and stems of were more cold-hardy than roots. Regan et al. (20) reported that roots of *Ilex* were less cold-hardy than shoots and the hardiness of *Ilex* mature root tissue was –6.3C (20.7F) while that of shoots was –32.3C (–26.1F) and Dirr and Lindstrom (7) reported that leaves of *Ilex* are generally less cold hardy than stems.

Temperature has been identified as the major environmental factor that affects plant cold hardiness (14). The distribution of *Ilex aquifolium* L. (English holly) and its habitat preference suggest sensitivity to low temperature (23). It is absent from areas where the mean temperature of the coldest month falls below –0.5C (31.1F) (12). In our field studies, most *Ilex glabra* cultivars could tolerate –25.0C (–13.0F). Subzero temperatures in plants usually lead to the freezing of tissue water, which entails ice growth, cell dehydration, osmotic concentration, and complex freeze-induced cell-volume changes (19). This can result in the death of plant tissues or even whole plants. The cellular sap concentration in current year leaves of natural stand English holly increased, while osmotic potential of the cellular sap decreased and cell volume reduced when the minimum ambient temperature decreased (23).

Plant cold hardiness varies seasonally. A gradual increase in cold resistance of English holly leaves from about –9C (15.8F) in late summer to –24C (–11.2F) in mid-winter when the minimum air temperature decreased to 0C (32F) or below has been reported (23). Usually, the maximum frost hardiness occurs in the fall season when the temperature gradually decreases and subsequently it is lost when temperatures become favorable for growth in the spring. We found that the maximum frost hardiness of *Ilex glabra* 'Densa', *I. glabra* f. *leucocarpa*, and *I. glabra* 'Shamrock' occurred in mid to late January (data not presented). This is similar to the previous studies (7), in which most of the tested *Ilex* species reached their maximum cold hardiness in mid-January. In midwinter, plants can quickly deacclimate to lose their cold hardiness to some extent when they are exposed to warmer ambient temperature (18). Our previous experiments indicated that deacclimation significantly reduced cold hardiness of *Ilex glabra* and its effect varied with cultivars (24). A total of 33.5 and 17.2% of cold hardiness reduction were observed for *Ilex glabra* 'Densa' and *I. glabra* 'Shamrock', respectively, when they were deacclimated for 9 days in a greenhouse [20.8 ± 1.93C (69.4 ± 3.5 F)].

Other factors (such as snow pack, animal and insect damage, and mechanical injury) and winter desiccation might affect to some degree the cold hardiness of *Ilex glabra*. Buried beneath snow, plants are exposed to mild temperatures close to 0C (32F) and protected from excessive irradiation and winter desiccation. In our winter protection practice, a total of 17.7% plants experienced mechanical injury, such as branch and bark damage, in a snow pack area, 7.6% in a less snow covered area (such as a canopy), while less than 3.2% in other snow-free places (e.g. cold storage, fabric cover, microfoam, and plastic winter house) (data not presented).

Based on both field trials and controlled laboratory tests of *Ilex glabra* cultivars, *I. glabra* 'Shamrock' was the most cold-hardy cultivar; *I. glabra* f. *leucocarpa*, *I. glabra* 'Viridis' and *I. glabra* 'Nigra' were the least cold-hardy cultivars; while *I. glabra* 'Compacta', *I. glabra* 'Densa', *I. glabra* 'Chamzin', *I. glabra* 'Pretty Girl', *I. glabra* had intermediate cold hardiness. Laboratory tests were strongly correlated with winter damage in field trials and could be used to predict the cold tolerance of *Ilex glabra* cultivars in the field. Many factors contribute to cold hardiness, including: plant cultivar, tissue, temperature, deaacclimation, winter desiccation, and others (e.g. snow pack, mechanical injury).

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