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The Upside down Freezing Test – a new Method to Evaluate Frost Hardiness

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Summary

A new method to test plants for frost hardiness was developed by placing the top of container plants downwards in a computer-controlled freezer and keeping the roots upwards above an insulated cover – the upside down freezing test. Four cultivars of *Ilex* spp. were freeze tested 3 times during the autumn/winter 1999 at –10, –16, and –22 °C and compared with control plants kept at 4 °C. Electrolyte leakage from leaves of the freeze tested plants was measured and Index of Injury (I_t) was calculated. Afterwards the plants were placed in a greenhouse to observe plant performance, and the results were related to I_t.

The upside down freezing test was useful as the plant top was frozen without killing the roots. There were significant effects ($p<0.05$) of temperature, time, and cultivar and some of their second order interactions using I_t as variable. Plant performance under greenhouse conditions showed relatively good correlation with I_t. Plants registered as dead had high I_t and plants registered as not damaged had relatively low I_t.

It can be concluded that it is possible to use the upside down freezing test and the derived Index of Injury to estimate plant performance. Using these methods the testing time for selection of new frost hardy ornamental or landscape plants can be reduced.

Zusammenfassung

Der „Upside down freezing test" – eine neue Methode zur Frosthärtebestimmung. Es wurde eine neue Methode zur Frosthärte-Bestimmung bei Pflanzen, der „upside down freezing test", entwickelt, bei dem die Pflanzen in einer computerkontrollierten Gefriertruhe mit der Pflanzenkrone nach unten und den isolierten Wurzeln nach oben platziert werden. Im Herbst/Winter 1999 wurden vier *Ilex* spp. Sorten dem Gefriertest mit drei Wiederholungen und drei Temperaturstufen (–10, –16 und –22 °C) unterzogen und mit 4 °C-Kontrollpflanzen verglichen. Die Elektrolytverluste der gefrorenen Blätter wurden gemessen und daraus der Schadensindex (I_t) ermittelt. Die Pflanzen wurden im Anschluss ins Gewächshaus gebracht, um ihre Leistungsfähigkeit zu beobachten und die gewonnenen Ergebnisse mit dem I_t zu korrelieren.

Vorteil des „upside down freezing test" ist, dass die Pflanzenkrone eingefroren wird, ohne gleichzeitig die Wurzeln abzutöten. Durch die Verwendung von I_t als Variable war es möglich, Signifikanzen ($p<0.05$) hinsichtlich Temperatur, Zeitpunkt der Behandlung und Sorte sowie einiger Wechselwirkungen nachzuweisen. Zwischen der Leistungsfähigkeit der Pflanzen unter Gewächshausbedingungen und dem I_t bestand eine enge Korrelation. Pflanzen, die als „abgestorben" bonitiert wurden, hatten einen hohen und Pflanzen, die als „ungeschädigt" bonitiert wurden, einen relativ niedrigen I_t.

Abschließend kann festgestellt werden, dass der „upside down freezing test" in Kombination mit dem Schadensindex eine gute Möglichkeit darstellt, die Leistungsfähigkeit von Pflanzen zu schätzen. Die Anwendung dieser Methode kann die Dauer der Frosthärteselektion neuer Zier- und Baumschulpflanzen verkürzen.

Key words. electrolyte leakage method – frost hardiness – *Ilex aquifolium* – *Ilex* x *meserveae* – Index of Injury – selection of plants – upside down freezing test – whole plant freezing test.

Introduction

In order to introduce new garden and landscape plants from other climatic zones it is important to select plants that can grow under the new growing conditions. It is valuable to know the latitude, longitude and altitude of the origin of new plants in order to compare the climate of the native country and the new country where the plants are introduced. If in theory a plant is suitable for the new country it is time to test the plant (ARMITAGE and GREEN 2001) for whole plant performance, flower and foliar performance, health, and climate management. It is important that new plants can survive the climate of the country where they are introduced. New plants can be selected by growing them in different localities all around the country concerned. In

this way the plants' ability to grow in different type of soils and under different climatic influences will be expressed in the plant performance and plants damaged due to low temperatures, wind, etc. could be deselected.

The climate in Denmark is very changeable and no two winters are alike. Sometimes hard frost occurs each winter, other times 10 years may pass without hard frost in the winters. In the spring there is a very high risk of frost at night (DMI 2003). In the daytime high temperature and sun promote plant growth whereas frost during the night may lead to plant damages. At the introduction of new plants into such a climate it is especially important that the plants can survive these temperature fluctuations.

Another climatic problem in Denmark is due to the geographical position where coastal climate is normal but sometimes the climate can be more continental. This can result in a cold January, a mild February and then again a cold March, which is a bad situation for many plants as a mild period may lead to early growth.

When testing new plants it is necessary to grow them for several years to ensure exposure to frost in order to select the most hardy cultivars of the species, especially in the case of trees and shrubs, frost tolerance is crucial. This testing and further selection can go on for years before the most frost hardy plant for the country concerned is found. It is a very time- and money-consuming project.

Therefore, it is desirable to develop a test method that can give a quick answer whether the plants are frost hardy or not.

Plants in the field are exposed to frost at the shoot tips, twigs and stems and for evergreen plants also the leaves, but the roots are mostly protected by the soil depending on the duration of the frost period and the temperature. However, roots are sensitive to frost (HAVIS 1976; STUDER et al. 1978; SMIT-SPINKS et al. 1985; LINDSTRÖM 1986; LINDSTRÖM and NYSTRÖM 1987; MCEVOY and MCKAY 1997) and they are more sensitive than the above ground parts of the plants (PELLETT and WHITE 1969; CALMÉ et al. 1994; COLOMBO et al. 1995). Therefore, an investigation of the frost tolerance of the plant top necessitates protection of the roots against frost damage.

Several methods are described for such investigations. One method used is freezing whole plants without freezing the roots by insulating the roots of the whole plant with a bed of vermiculite in the freezer and comparing this test with visually evaluated damage (RIETVELD and TINUS 1987; FISKER et al. 1995). GREEN and WARRINGTON (1978), COLOMBO et al. (1982) and BURR et al. (1990) compared the whole plant freezing test with the electrolyte leakage method and found good correlation between the electrolyte leakage method and the visually evaluated damage.

The electrolyte leakage method (WILNER 1955; COLOMBO et al. 1984; BURR et al. 1990; MCKAY 1992) was used on leaves from the frozen plants to test the frost hardiness level of the plants.

In this experiment evergreen holly (*Ilex* spp.) was used as model plant because it is popular among Danish gardening people, but often die back occurs due to the changeable Danish climate during wintertime. A new procedure to freeze plants was tested, the upside down freezing test. This experimental design should resemble plants standing in their natural environment.

The purpose of this study was to evaluate the upside down freezing test in combination with the electrolyte leakage method as a reliable method to select frost hardy plants in order to reduce the testing time in selection programs.

Materials and Methods

Plant material

The experiment was conducted in autumn/winter 1999 based on results from pilot tests performed during the previous two years (data not shown in this paper). 1-yr- and 2-yr-old containerised plants of holly (*Ilex* spp.) were propagated and grown at the Department

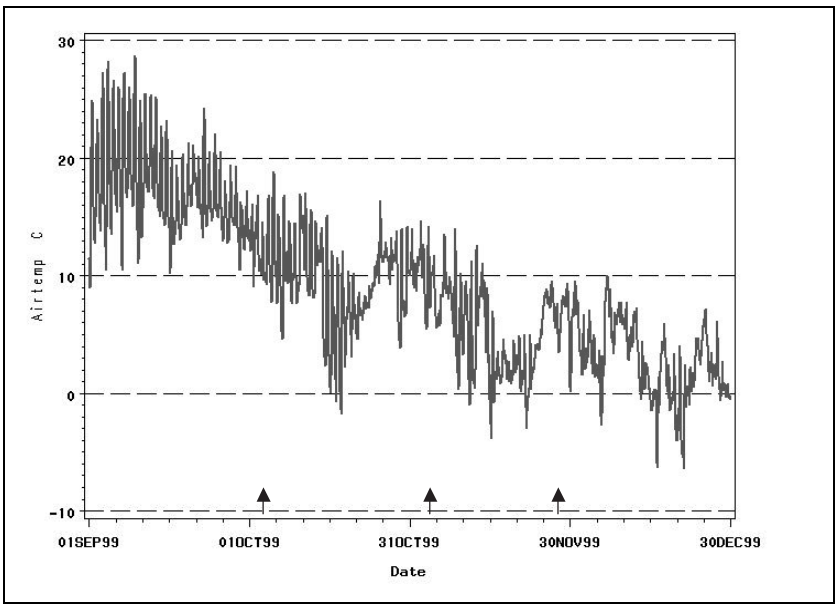


Fig. 1. Air temperature (°C) measured 1.5 m above the ground during the period September 1999–January 2000. Arrows show when plants were sampled for freeze tests Oct. 4 (week 40), Nov. 4 (week 44) and Nov. 29 (week 48) 1999.

of Horticulture, Aarslev, Denmark (latitude 55 °18 ´N, longitude 10 °26 ´E). Four different cultivars of evergreen *Ilex* were used as model plants, *I. aquifolium* 'Alaska' (1-yr old), *I.* x *meserveae* 'Blue Prince' (1- and 2-yr old), *I. aquifolium* 'Ils' (1- and 2-yr old plants), and *I. aquifolium* 'Mildegull' (1-yr old).

Plants were grown outdoors in one-litre containers in peat substrate containing clay (Pindstrup 3, Denmark, pH 5.5) fertilized with liquid fertilizer containing macro- and micronutrients (Hornum and Pioner, Brøste, Denmark, 1–1.5 ‰). The air temperature was measured 1.5 m above the ground during the period September–January (Fig. 1). Plants were kept outdoors so that the frost hardening processes occurred naturally until the plants were used for freezing tests.

Freezing tests

The plant top was exposed to frost and the roots kept above an insulating cover by placing the plants upside down in a freezer, Fig. 2, called the upside down freezing test. In this way the roots were not killed and the plants were able to continue to grow after the frost treatment.



Fig. 2. The upside down freezing test design with plants placed in a computer-controlled freezer with the top downwards and the roots in containers upwards above an insulated cover.

Freezing tests of plants were conducted three times during October to December (week 40, week 44 and week 48) each including three different temperature treatments below zero, –10, –16, and –22 °C and a control treatment at 4 °C. For each freezing test six plants per cultivar and temperature were randomly selected. Plants were placed upside down in a computer–controlled freezer, the roots including container and growing medium above an insulated cover (Fig. 2). This protected the roots against frost damage. The temperature measured in the root ball never fell below 15 °C even though the top was exposed to –22 °C. The freezer cooled at a rate of 5 °C per hour to 0 °C and subsequently at 2 °C per hour until the selected temperature was reached. The test temperature was maintained for three hours and after this period the plants were removed from the freezer. Control plants were placed in a cold storage at 4 °C during the freezing test period.

Electrolyte leakage

Fully developed leaves from two different positions were used to investigate if leaves at different positions on the plant differed in frost hardiness. Position one was leaf number one and two from the shoot tip and position two was leaf number 10 and 11 from the shoot tip. The leaves were cut longitudinally in 1.5 cm pieces, which were rinsed five times in de-ionised water by immersion to wash away ions coming from the cut. One sample consisted of two 1.5 cm leaf pieces. The samples were weighed and put into 20 ml plastic bottles. The samples weighed between 0.1 and 0.5 g. 20 ml de-ionised water was added to each test bottle to allow electrolyte leakage. The electrical conductivity (EC) was measured using a temperature-compensating conductivity meter (CDM 80, Radiometer Copenhagen). After 24 hr incubation at room temperature the samples were measured for EC_{frozen}. Hereafter the bottles were capped and placed in an autoclave for 1 hour at 121 °C to allow maximum leakage of ions. Removed from the autoclave the bottles were placed at 4 °C overnight and the next day placed at room temperature until the water in the bottles reached room temperature. $EC_{autoclave}$ was measured within 24 hours from autoclaving. The electrical conductivity of de-ionised water (EC_{water}) was measured to give the zero level of EC.

Index of Injury

The relative electrolyte leakage (REL) was estimated as the relation between the conductivity before and after autoclaving. The conductivity of de-ionised water (EC_{water}) was subtracted before estimating REL. The values of REL for a frozen and a control sample were used to calculate Index of Injury (I_t) to the temperature t according to FLINT et al. (1967):

$I_t = 100 \times (REL_F - REL_C) / (1 - REL_C)$, where

$REL_F = (EC_{frozen}) / (EC_{f\text{-}autoclave})$

$REL_C = (EC_{c\text{-}24hour}) / (EC_{c\text{-}autoclave})$

EC_{frozen} = electrical conductivity of frozen sample

$EC_{f\text{-}autoclave}$ = electrical conductivity of frozen sample after autoclaving

$EC_{c\text{-}24hour}$ = electrical conductivity of control sample after 24 hour

$EC_{c\text{-}autoclave}$ = electrical conductivity of control sample after autoclaving

Plant performance

After frost treatment the plants were placed in a frost-free greenhouse (minimum temperature 3 °C, windows open by 10 °C) to observe survival. In March and June 2000 the plants were categorised as not damaged, damaged or dead. Plants categorised as not damaged had no injury to the top and leaves whereas damaged plants had injury to the top and leaves.

Statistical analysis

The average of the estimated variable REL for the six plants in each cultivar was used for calculating the Index of Injury (I_t). The resulting I_t-values were analysed using a mixed linear normal models (proc mixed procedure in SAS (SAS Inst. 1999)), with the treatment factors temperature, cultivar and time, and their second order interactions as fixed effects. Visual evaluation of the distribution of the residuals indicated that the assumption of normal distribution and variance homogeneity was valid. Preliminary analysis showed second and third order interactions involving leaf sample positions. Thus, it was decided to perform the analysis for each leaf position separately. The model was reduced by sequentially removing non-significant interactions (at the 0.05 level).

Results

Leaf position

The samples taken from position two, leaf number 10 and 11 from the shoot tip, were surprisingly more damaged by the frost treatments – according to the statisti-

Table 1. Index of Injury (I_t) at sample position one and two for different cultivars of *Ilex* spp. at different freezing temperatures for each week number the plants were tested.

Cultivar	Temperature (°C)	Sample position one Week no.				Sample position two Week no.			
		40	44	48	Mean[a]	40	44	48	Mean
I. aquifolium 'Alaska'	–10	56.2	18.1	2.6	25.6 efgh	62.2	4.5	3.2	23.3
	–16	66.8	38.1	15.6	40.2 b	78.4	25.3	11.0	38.2
	–22	69.4	62.4	35.9	55.9 a	68.5	63.4	33.3	55.1
	Mean[b]	64.1	39.5	18.0		69.7 a	31.1 d	15.8 fghi	
I. x *meserveae* 'Blue Prince' 1-yr-old	–10	62.2	4.9	2.9	23.3 fgh	46.6	2.0	1.5	16.7
	–16	52.7	21.7	6.1	26.8 efg	56.2	24.6	9.1	30.0
	–22	58.5	33.6	17.4	36.5 bcd	53.8	40.5	17.4	37.2
	Mean[b]	57.8	20.1	8.8		52.2 bc	22.4 ef	9.3 ij	
I. x *meserveae* 'Blue Prince' 2-yr-old	–10	45.2	0.9	1.9	16.0 ij	44.4	–1.1	–0.9	14.1
	–16	53.9	8.1	5.6	22.5 fghi	61.6	12.8	5.7	26.7
	–22	49.4	25.2	13.6	29.4 ef	50.7	33.1	17.8	33.9
	Mean[b]	49.5	11.4	7.0		52.2 bc	14.9 fghij	7.5 j	
I. aquifolium 'Ils' 1-yr-old	–10	47.4	14.3	2.0	21.2 ghi	52.8	2.9	2.2	19.3
	–16	57.4	20.5	8.0	28.7 ef	63.1	14.8	16.6	31.5
	–22	57.5	36.9	22.5	39.0 bc	56.4	36.5	28.6	40.5
	Mean[b]	54.1	23.9	10.8		57.4 b	18.1 fgh	15.8 fghi	
I. aquifolium 'Ils' 2-yr-old	–10	45.6	8.1	3.2	19.0 hij	34.9	6.1	1.3	14.1
	–16	48.9	22.9	7.5	26.4 efg	53.2	30.7	13.1	32.3
	–22	60.2	42.5	21.0	41.2 b	53.1	41.5	24.3	39.6
	Mean[b]	51.6	24.5	10.6		47.1 c	26.1 de	12.9 ghij	
I. aquifolium 'Mildegull'	–10	37.9	1.5	2.3	13.9 j	44.5	0.8	1.4	15.6
	–16	61.7	22.7	7.3	30.6 de	64.1	26.5	8.0	32.9
	–22	55.3	24.3	17.0	32.2 cde	62.1	33.2	25.0	40.1
	Mean[b]	51.6	16.2	8.9		56.9 b	20.2 efg	11.5 hij	

[a] Means in a column in sample position one followed by the same letter are not significantly different at $p<0.05$.
[b] Means in a row in sample position two followed by the same letter are not significantly different at $p<0.05$.

Table 2. Results from the statistical analysis with Index of Injury (I_t) as calculated variable.

	F Value I_t at sample position one	Significance	F Value I_t at sample position two	Significance
Cultivar	19.50	***	8.84	***
Temperature	96.69	***	116.17	***
Week	546.95	***	420.93	***
Temperature × week	10.25	***	17.93	***
Cultivar × week	2.24	NS	2.54	*
Temperature × cultivar	2.75	*	0.92	NS

NS = non significant, * = significant at $p<0.05$, ** = significant at $p<0.01$ and *** = significant at $p<0.001$.

cal analysis - than samples taken from position one, leaf number one and two from the shoot tip, but looking at the different cultivars and week numbers the differences were very small (Table 1).

Development of frost hardiness

Ilex spp. develop frost hardiness during autumn and winter as shown in Table 1 where I_t is decreasing from week 40 to week 48. Some cultivars developed frost hardiness quicker than others. Apparently 'Alaska' and 'Ils' developed frost hardiness more slowly than the other cultivars, especially 'Alaska', compared for instance with 'Blue Prince'.

Plant reaction to the freeze tests

The statistical analysis showed significant effects of temperature, week and cultivar as single factors on Index of Injury (Table 2) but also second order interactions. A significantly higher level of I_t was found in week 40 compared with week 44 and 48 in all temperatures (average over cultivars, data not shown). The lowest temperature, –22 °C, resulted in a significantly higher degree of frost damage to all cultivars in week 44 and 48 than both –16 and –10 °C (average over cultivars, data not shown). At –10 °C there were no significant differences in frost damage between week 44 and 48 (average over cultivars, data not shown). The tolerance to lower temperatures, –16 and –22 °C, was better in week 48 than in week 44, and the same damage, expressed as I_t, was found in week 44 at –16 °C as in week 48 at –22 °C (Table 1).

The interaction between cultivar and week was only significant for sample position two (Table 2) showed that each cultivar had significantly different I_t within the weeks, with most damage in week 40 and least damage in week 48. E.g. 'Alaska' showed most damage in week 40 at sample position two (Table 1, means[b]) but did not show most damage in week 48. Likewise 'Blue Prince', 2-yr-old, was least damaged in week 48 at sample position two but was not least damaged in week 40.

The interaction between temperature and cultivar was only significant for sample position one (Table 2) and showed different damages to each cultivar according to the different temperature treatments (Table 1, means[a]).

The statistical analysis showed no significant differences ($p<0.05$) between 1-yr- and 2-yr-old plants of 'Ils' and 'Blue Prince', respectively (data not shown). In this statistical analysis only data from the two cultivars concerned were used.

Plant performance in relation to I_t

Registration of plant performance in the greenhouse showed relatively good correlation with I_t. Plants registered as dead had a high I_t and plants registered as not damaged had relatively low I_t. Example is shown in Fig. 3 for plants frozen to –10 °C in week 40, 44 and 48. The average of cultivars is connected with the registration of plant performance in Table 3. Another example is shown in Fig. 4 with 'Mildegull' as this plant showed the same performance for all plants within the same treatment (e.g. all plants were not damaged in week 48 at treatment –10 °C and all plants were dead in week 40 at –16 °C).

Discussion

Development of frost hardiness

Plants develop frost hardiness during autumn and winter due to shorter photoperiod and lower temperature (ALEXANDER and HAVIS 1980; SMIT-SPINKS et al. 1985; CALMÉ et al. 1993; CAMERON and DIXON 2000; LI et al. 2003). As the daylength gets shorter and the air temperature approaches 0 °C or drops below 0 °C, the plants develop more frost hardiness (CAMERON and DIXON 2000). It is presumed that these mechanisms control the frost hardiness development in *Ilex,* but more detailed information is not yet clarified.

Plants used in the present experiment stood outside so the hardening processes occurred naturally. Plants freeze tested in week 40 were not exposed to natural frost (Fig. 1), whereas plants freeze tested in week 44 and 48 were exposed to natural frost several times correlating with increased frost hardiness in week 44 and 48 expressed by lower value of I_t. There was no significant difference in frost damage between plants exposed to –16 °C in week 44 and plants exposed to –22 °C in week 48, showing that the plants developed more frost hardiness during time and could tolerate more frost in week 48 than in week 44.

The different *Ilex* cultivars differed in frost hardiness development and 'Alaska' was in general most susceptible to frost damage and 2-yr-old 'Blue Prince' least susceptible as shown by the upside down freezing test.

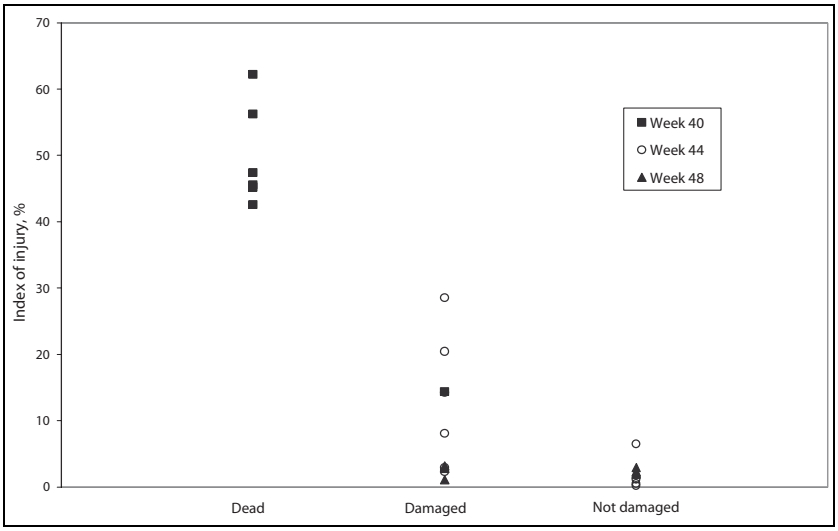


Fig. 3. Plant performance of *Ilex* spp. six months after freeze testing compared with Index of Injury (I_t). The plants were frozen to –10 °C in week 40, 44 and 48, 1999. The plants were categorised in the greenhouse as dead, damaged or not damaged. Values represent average over cultivars in each week (refer to Table 3).

Table 3. Plant performance six months after freeze testing for different cultivars of *Ilex* spp. at different freezing temperatures for each week number the plants were tested.

Cultivar	Temperature (°C)	Week no. 40			Week no. 44			Week no. 48		
		dead	damaged	not damaged	dead	damaged	not damaged	dead	damaged	not damaged
I. aquifolium 'Alaska'	–10	6	0	0	0	5	1	0	4	2
	–16	6	0	0	1	5	0	0	6	0
	–22	5	1	0	6	0	0	2	4	0
I. x *meserveae* 'Blue Prince' 1-yr-old	–10	6	0	0	0	1	5	0	0	6
	–16	6	0	0	4	1	1	0	0	6
	–22	6	0	0	2	4	0	0	5	1
I. x *meserveae* 'Blue Prince' 2-yr-old	–10	6	0	0	0	1	5	0	0	6
	–16	6	0	0	0	0	6	0	1	5
	–22	6	0	0	6	0	0	0	1	5
I. aquifolium 'Ils' 1-yr-old	–10	6	0	0	0	6	0	0	1	5
	-16	6	0	0	5	1	0	1	5	0
	–22	6	0	0	5	1	0	2	4	0
I. aquifolium 'Ils' 2-yr-old	–10	6	0	0	0	6	0	0	6	0
	–16	6	0	0	1	5	0	0	6	0
	–22	6	0	0	5	1	0	1	5	0
I. aquifolium 'Mildegull'	–10	5	1	0	0	2	4	0	0	6
	–16	6	0	0	1	3	2	0	0	6
	–22	6	0	0	6	0	0	0	6	0

Leaf positions and damage

The significant difference between sample position one and two concerning frost hardiness showed that position two was most damaged even though it is difficult to explain the frost hardiness differences between the two positions. It was expected that the uppermost leaves of the plants would be the most frost sensitive area but there seem to be exceptions. COLOMBO et al. (1995) showed in *Picea mariana* seedlings a frost hardiness gradient from shoot tip to root tip, where the terminal bud had the highest degrees of frost hardiness and positions away from the bud towards the container surface were more frost sensitive. This is also the case in this investigation even though the differences between the two positions are very small. The results indicate that position of the leaf should be taken into consideration when samples are taken for frost hardiness test.

1-yr- and 2-yr-old plants

There was no difference in frost hardiness between 1- and 2-yr-old containerised plants of evergreen *Ilex*. The

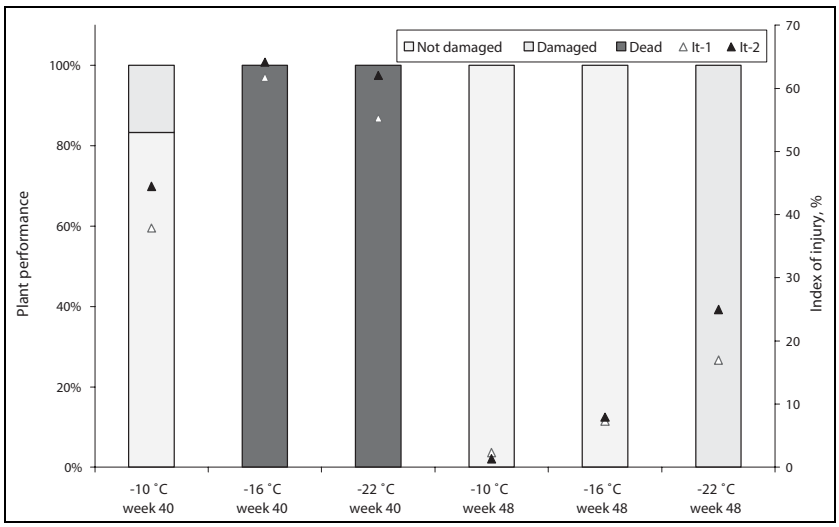


Fig. 4. Plant performance of *Ilex aquifolium* 'Mildegull' six months after freeze testing compared with Index of Injury (I_t). The plants were categorised in the greenhouse as not damaged, damaged, or dead. It-1 is I_t at sample position one (leaf number one and two from the shoot tip) and It-2 is I_t at sample position two (leaf number 10 and 11 from the shoot tip).

leaf samples used to test frost hardiness were taken from the leader shoot so that leaves were at the same physiological age and the difference in the age of the plants had no influences in this case.

The upside down freezing test

The method of freezing the top of whole plants without freezing the roots was in combination with the calculated I_t a useful tool to separate frost hardiness between different *Ilex* cultivars. The value of I_t increased significantly the lower the temperature. The higher I_t values indicate that the plasma membranes of the leaf cells are more damaged by higher frost temperature (PUKACKI and PUKACKA 1987; MCKAY 1992; MCEVOY and MCKAY 1997). The temperatures used in this experiment were satisfactory for selection of Holly plants. The highest temperature used, –10 °C, was not too close to 0 °C as this treatment caused damage to some of the cultivars in week 48 where the frost hardiness should be highest. The lowest temperature used, –22 °C, was not too low or too high as some plants of the cultivars survived and others died in week 48.

The value of I_t was significantly different from week to week, showing the highest value of I_t in week 40 where the plants had the lowest frost hardiness. Development of frost hardiness during time results in lower values of I_t.

In the upside down freezing test the plants were removed from the freezer after three hours at the selected test temperatures without heating up the freezer as other papers describe (COLOMBO et al. 1982; PUKACKI and PUKACKA 1987; COLOMBO et al. 1984; FISKER et al. 1995; DEANS and HARVEY 1996). This process could be harmful to the membranes (PUKACKI and PUKACKA 1987; MCEVOY and MCKAY 1997) but as I_t showed good correlation with plant performance in the greenhouse our test method is useful.

Others have used freezing of whole plants by insulating the roots of the whole plant with a bed of vermiculite in the freezer (COLOMBO et al. 1982; RIETVELD and TINUS 1987; BURR et al. 1990; FISKER et al. 1995). Our experimental design by putting the plants upside down and keeping the roots in containers above an insulated cover ensured that the roots were not exposed to frost as temperature measured in the root balls never fell below 15 °C even though the top was exposed to –22 °C.

However, the work by ALEXANDER and HAVIS (1980) showed that the frost hardiness of roots and lower stems of *Rhododendron* plants was less when roots were kept warm rather than cool, while at the same time the shoots were exposed to cold temperatures. This might indicate that the level of frost hardiness measured in our experiment could be higher if the root balls were kept cool, but as it is possible to deselect frost sensitive plants using the upside down freezing test together with the electrolyte leakage method, our experimental design seems useful.

Plant performance in greenhouse and I_t

The results showed good correlation between I_t and plant performance in the greenhouse. Plants with high values of I_t died during the plant performance test and plants not damaged had a relatively low value of I_t.

The value of I_t could fluctuate according to the plants' condition when they were freeze tested, but mostly this was influenced by the frost hardiness development during time as observed for the cultivar 'Mildegull' where the I_t value fluctuates for the plant registered as dead.

Looking across all the cultivars, the level of I_t decreased the higher the temperature and this corresponded well with a higher survival rate of these plants. GREEN and WARRINGTON (1978), COLOMBO et al. (1982) and BURR et al. (1990) also found a good relationship between freeze-induced electrolyte leakage and the whole-plant freeze test.

Evaluating the upside down freezing test for practical use

The usefulness of the method of freezing whole plants followed by measuring electrolyte leakage and calculating I_t is supported by the good correlation with plant performance in the greenhouse. It has been shown that the survival of plants is increasing the higher the temperature and the better developed the frost hardiness.

In week 40 and 44 (data not shown) the number of dead plants was higher than in week 48 irrespective of freezing temperature. The increase in the survival of plants in week 48 is an expression of the developed frost hardiness in the plants.

Segregation of frost hardy cultivars is possible with the laboratory measurement I_t and the plant performance in the greenhouse supported this segregation. The method is a useful tool to make the first broad selection of frost hardy plants and to test the plants' conditions concerning frost hardiness, but the method cannot yet give numerical values for frost hardiness that can be compared with other species. The method has to be more developed to be used for further detailed selection.

In practice 'Blue Prince' is very frost hardy and more frost hardy than 'Alaska' as this experiment also showed.

It can be concluded that the upside down freezing test on whole plants and calculation of the Index of Injury relates very well to plant performance afterwards. Using these methods the testing time for selection of new frost hardy ornamental or landscape plants can be reduced.

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