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Testing the winter hardiness of selected chrysanthemum cultivars of Multiflora type

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Abstract

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Over the course of 2007–2013, 19 selected cultivars of *Chrysanthemum* × *grandiflorum* were tested. Testing was conducted during two seasons under field conditions and on potted plants in the freezing chamber at temperatures –8, –10 and –12°C. It was found that frost hardiness of chrysanthemums of Multiflora type can be reliably determined at a temperature intervention of –8°C. The field trials and tests in freezing chamber revealed that cv. Vlasta did not overwinter. In field trials, the most winter hardy cultivars were Zina, Berta, Mína, Dorota, Estela, Tereza and clone 912, which are possible to grow as short-lived perennials. Cvs Marika and Tereza showed the best frost resistance in the freezing chamber and the best survival results after simulating spring frost.

Keywords: *Chrysanthemum* × *grandiflorum*; frost hardiness; overwintering; regeneration; flowers

Small-blossoming chrysanthemums *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam have belonged among the most cultivated ornamental species in the Czech Republic in recent years. In 2006, their production exceeded a million units (Votruba 2006). According to Hanzelka (2008), they are becoming increasingly popular because they form naturally compact spherical bushes and can be conveniently used for planting in outdoor areas or grown as pot plants. The cultivation of these plants is not primarily targeted for overwintering, but some cultivars have winter hardiness spontaneously encoded in their genomes and can successfully overwinter in Czech Republic climatic conditions.

As stated by Bélanger et al. (2006), climatic conditions affect the overwintering of herbaceous perennials directly by environmental stress, and indirectly by the influence of plant winter hardiness. It is a complicated collection of adaptation processes and complex features including frost endurance, resistance to alternating freezing and thawing and mechanical organ damage, but also resistance to disease, ice cover and winter drought (Bláha et al. 2003). The winter hardiness of perennial herbaceous species is determined by their ability to tolerate a wide range of external stressors such as freezing temperatures, temperature fluctuations, high levels of soil moisture, ice cover, being partially pulled from the soil and pathogens operating at low temperatures (Andrews 1987). For this reason, the research focused on monitoring and testing the frost hardiness. The goal of this work was to detect winter hardiness cultivarss from new chrysanthemum assortment of the Silva Tarouca Research Institute for Landscape and Ornamental Gardening and develop simple methodology of testing frost hardiness suitable for another ornamental species in freezing chambers.

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MATERIAL AND METHODS

The first test was carried out between 2007 and 2009 in Slabce (Central Bohemia Region), under field conditions on a gentle slope with southeast exposure at an altitude of 419 m a. s. l. The climate is both slightly warm and slightly dry – with long, warm and dry summers, a short transition period with slightly warm spring and autumn seasons and a short, dry and moderately warm winter. Snow cover is maintained for an average of 50 days with a maximum layer height of 0.2 m. The average temperature is –2.4°C in January and 17.5°C in July. Average annual rainfall is 530 mm per year, of which 350 mm is in the growing season (Tolasz 2007). The pedological survey classified soil as brown soil, with loam soil in a level of 0–0.34 m. The soil reaction is neutral, with pH values of 6.6–7.2.

Further field trials were conducted in 2010–2012 at the Demonstration and Research Station of Czech University of Life Sciences Prague in Prague-Troja, on a gentle slope with western exposure at an altitude of 196 m a.s.l. The area has a moderately warm climate and the district is slightly warm and dry, mostly with mild winters. Snow cover is maintained for an average of 40 days, with a maximum snow depth of 0.15 m. The average temperature is –2.8°C in January and 16.3°C in July. Annual rainfall average is 500 mm per year, of which 350 mm is in the growing season (Tolasz 2007). The pedological survey detected the modal fluvisol soil type on non-calcareous alluvial with gravel subsoil terraces. At levels of 0–0.34 m is humic sandy loam soil with an addition of quartz pebbles up to 50 mm. This is a deep cultivated soil, significantly enriched by deeply sunk organic substances. It has a neutral soil reaction with pH values of 6.6–6.9.

Experiments with potted plants were held in 2012 and 2013 in the freezing chamber of Selgen in Stupice (Central Bohemian Region). This was a ventilated room, with dimensions of 12.5 × 2.5 × 3.5 m with a cooling device (it was tailored made for Selgen) from the Frigera 21 a.s. (Kolín, Czech Republic), with a temperature adjustable to –22°C and an accuracy of 0.5°C.

In the field trials 12 plants of each of 19 cultivars were evaluated and 18 plants from one cultivar were assessed in the freezing chamber (6 plants for each of three frost temperatures). The cuttings, sized 2.5–4 cm, were collected in the spring from mother plants in the Silva Tarouca Research Institute for Landscape and Ornamental Gardening in Průhonice. Then they were rooted in the greenhouse. After ten days of rooting, the plants were supplementary fertilized by Kristalon (concentration 0.1% – 10 g Kristalon/ 10 l water) from AGRO CS (Česká Skalice, Czech Republic). It is a universal fertilizer, 100% water-soluble with N-P-K (19-6-20), 3% Mg, 7,5% S and trace elements B, Mo, Fe, Cu, Mn and Zn.

In 4 weeks the rooted cuttings were transplanted into plastic pots (0.1 m in diameter), filled with Professional Peat Cultivation Substrate RKS II from AGRO CS. It is prepared from quality peat, bark humus and clay, NPK fertilizer with trace elements was added and pH reaction 5.5–6.5 is adapted with dolomitic limestone. N content is 250–350 mg/l, P content in the form P_2O_5 is 200–250 mg/l, K content is 300–400 mg/l in the form K_2O. On the test plots the young plants were planted in late May (Troja) and June (Slabce) in a random arrangement (randomised block design, 3 repetitions with 4 plants, spacing 0.9 × 0.8 m), in order to eliminate the influence of the plot itself. The plants were pinched twice and prematurely forming buds were removed according to Votruba (2004). Supplementary fertilizing of the plants was carried out in mid-August at fortnightly intervals with Kristalon (0.1% concentration). The plot was weeded manually. The cultivation of the soil around the plants was hoed by hand in order to prevent soil crust. During the growing season, plants were watered with well water according to need. At the end of November, the plants were cut to a height of 50 mm, and a layer of spruce pine twigs were laid upon them. In early spring, when soil and weather conditions allowed, the twigs were removed and plants were cleared of the dead parts. They were regularly irrigated, fertilized (from mid-May with Kristalon 0.1%) and manually weeded. An evaluation of overwintering was carried out in April and May according to the actual onset of spring. In early September 2011 white rust was found on cv. Stáza in Troja. Therefore, all plants of that cultivar were removed and the remaining crop was repeatedly treated through a manual pressure sprayer with fungicides (Baycor 25 WP with 0.15% concentration and Horizon 250 EW with 0.2% concentration).

The plants for testing in the freezing chamber were obtained in the same manner as the field-growing plants, but they were grown in plastic containers of a diameter of 0.15 m in a substrate

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Table 1. Point scale of plant regeneration after cold intervention

Points	Plant condition
1	dead plant without regeneration
2	5% of shoots regenerated
3	20% of shoots regenerated
4	40% of shoots regenerated
5	50% of shoots regenerated
6	60% of shoots regenerated
7	80% of shoots regenerated
8	95% of shoots regenerated
9	new shoots in full

composed of four parts Professional Peat Cultivation Substrate (RKS II from AGRO CS) to one part horticultural compost. During the growing season, the containerised plants were placed in the outdoor areas in Troja. The base for the containerised plants was flattened and then coated with a black non-woven fabric. Plants were stationed at a distance of 0.4 × 0.4 m. In late June, supplementary fertilizing was initiated using Kristalon (0.2% concentration), carried out at weekly intervals. Before moving to the freezing chamber, Rovral Aquaflo (AgroBio, Opava, Czech Republic) was used as a preventive treatment against grey mould.

In late November, the plants were transferred to Selgen freezing chamber; where one part of the test plants were placed in the freezing chamber for two days and exposed to hardening temperatures of 0 and –5°C. Only then was the temperature lowered to the first intervention level of –8°C; the next set at –10°C and the third test set to –12°C. Upon completion of tests in the freezing chamber (3 days for –8°C, 3.5 days for –10°C and 4 days for –12°C), plants were placed in an unheated, interspaced and ventilated room with 0–5°C temperatures, where they were thawed. After that plants were transferred for regeneration to a 5°C temperature-controlled greenhouse, where they were watered regularly. The freezing chamber experiments were repeated with the second part of containerised plants in early January.

Research also included a simulation of spring frost on already regenerating plants. In this case containerised plants were placed in an unheated greenhouse in Troja in early December – where they began to gradually regenerate due to the higher temperatures. The plants were transferred to the Selgen freezing chamber in early February, where the hardening temperature was dropped in order to replicate the sudden drop in temperature in natural conditions. The plants were exposed to temperatures of –5, –8 and –10°C. A point scale of 1–9 was chosen in order to evaluate the plant regeneration (Table 1). The measured values were statistically analysed with ANOVA method using the Statistica CZ program (version 12.0 software system from Stat Soft CR), and Fisher's LSD test was used to determine homogenous groups at $P < 0.05$.

RESULTS AND DISCUSSION

The evaluation of climatological conditions (October–March) for individual experiments in Ruzyně, Prague, the closest reference site, is shown in Table 2. This table was created based on data from the Czech Hydrometeorological Institute (CHMI), Kožnarová and Klabzuba (2002) and Květoň (2001). As shown in Table 2, the climatological conditions observed in cold periods of the year mostly mirrored the normal course of temperatures. There were only lower-than-normal temperatures in October 2010 and February 2012, which were assessed as cold, and as very cold in December 2010. Rainfalls were mostly normal throughout the period, with the exceptions of October 2008, January 2010, November 2011 and January 2012 which were classed as wet; January 2009, February 2012 and March 2012, which was dry; and February 2011, which was very dry. The lowest winter air temperature in Slabce was 9.5°C in January 2007, –9.7°C in February 2008, –13°C in January 2009. Snow cover 0.02 m was here in November 2007, 0.05 m in January 2008, 0.09 m in November 2008 and 0.07 m in December 2010 and January 2010. The lowest winter temperature in Troja was –17.1°C in December 2010, –9.6°C in February 2011 and –14.3°C in February 2012. Snow cover 0.3 m was here in December 2010 and 0.15 m in January 2011. The winter 2011–2012 was without snow cover in Troja.

The percentages of overwintering plants in the field trials are summarised in Table 3. Cv. Vlasta overwintered in 8% and was excluded from the second experiment. Cv. Estela showed the best overwintering (100%) in all observed periods and cv. Tereza survived the first winter in 100% and 25% in the second winter. More than 71% of cvs Světla,

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Table 2. Evaluation of selected meteorological conditions in 2007–2012 according to Normal (1961–2000) in Ruzyně, Prague

<table>
<tr><th>Year</th><th>Month</th><th>Average monthly temperature (°C)</th><th>Evaluation according to average monthly temperature</th><th>Average monthly rainfall (mm)</th><th>Evaluation according to monthly rainfall</th></tr>
<tr><td rowspan="3">2007</td><td>10</td><td>8.1</td><td rowspan="3">normal</td><td>17.4</td><td rowspan="6">normal</td></tr>
<tr><td>11</td><td>2.1</td><td>35.1 (sc)</td></tr>
<tr><td>12</td><td>0.2</td><td>15.3</td></tr>
<tr><td rowspan="6">2008</td><td>1</td><td>2.2</td><td>very warm</td><td>22.1</td></tr>
<tr><td>2</td><td>3.5</td><td>extraordinary warm</td><td>12.5</td></tr>
<tr><td>3</td><td>3.7</td><td rowspan="2">normal</td><td>20.0</td></tr>
<tr><td>10</td><td>8.6</td><td>46.2</td><td>wet</td></tr>
<tr><td>11</td><td>4.6</td><td>very warm</td><td>23.7 (sc)</td><td rowspan="2">normal</td></tr>
<tr><td>12</td><td>1.0</td><td rowspan="5">normal</td><td>29.1 (sc)</td></tr>
<tr><td rowspan="6">2009</td><td>1</td><td>–3.6</td><td>12.3 (sc)</td><td>dry</td></tr>
<tr><td>2</td><td>–0.3</td><td>16.2</td><td rowspan="4">normal</td></tr>
<tr><td>3</td><td>4.1</td><td>36.1</td></tr>
<tr><td>10</td><td>8.0</td><td>33.9</td></tr>
<tr><td>11</td><td>6.4</td><td>extraordinary warm</td><td>32.2</td></tr>
<tr><td>12</td><td>–0.8</td><td rowspan="4">normal</td><td>51.1</td><td>very wet</td></tr>
<tr><td rowspan="6">2010</td><td>1</td><td>–4.4</td><td>30.2</td><td>wet</td></tr>
<tr><td>2</td><td>–1.6</td><td>9.5</td><td>dry</td></tr>
<tr><td>3</td><td>3.7</td><td>15.0</td><td rowspan="2">normal</td></tr>
<tr><td>10</td><td>6.8</td><td>cold</td><td>12.5</td></tr>
<tr><td>11</td><td>4.8</td><td>very warm</td><td>50.7</td><td>wet</td></tr>
<tr><td>12</td><td>–5.2</td><td>very cold</td><td>36.8 (sc)</td><td rowspan="2">normal</td></tr>
<tr><td rowspan="6">2011</td><td>1</td><td>–0.9</td><td rowspan="5">normal</td><td>23.0 (sc)</td></tr>
<tr><td>2</td><td>–1.2</td><td>4.6</td><td>very dry</td></tr>
<tr><td>3</td><td>4.8</td><td>29.0</td><td rowspan="2">normal</td></tr>
<tr><td>10</td><td>8.7</td><td>25.4</td></tr>
<tr><td>11</td><td>2.9</td><td>11.1</td><td>very dry</td></tr>
<tr><td>12</td><td>2.9</td><td rowspan="2">warm</td><td>29.6</td><td>normal</td></tr>
<tr><td rowspan="3">2012</td><td>1</td><td>1.0</td><td>37.0</td><td>wet</td></tr>
<tr><td>2</td><td>4.3</td><td>cold</td><td>8.3</td><td rowspan="2">dry</td></tr>
<tr><td>3</td><td>6.4</td><td>very warm</td><td>10.0</td></tr>
</table>

sc – snow cover

Zina, Věra, Berta, Mína, Dorota and clone 912 overwintered successfully. The detailed statistical evaluation found that there was a statistical difference on the level of $\alpha = 0.05$ in the overwintering percentage of group of cvs Vlasta, Celie, Zoja bronzová, Flavie, Kordula, Radana, 523 and 534 clones, with the average percentage of overwintering 4–42% and the group of cvs Světla, Zina, Věra, Berta, Mína, Dorota, Marika, Estela, Stáza, Tereza and clone 912 with the average percentage of overwintering 67–100%.

Comparing the results of field tests shown in Table 3 with the climatological conditions in Table 2, it is apparent that the overwintering test cultivars responded negatively to the cold and dry February 2012 without snow cover, which followed the warm December 2011 and January 2012. The current frost hardiness of wintering plants was reducing after a long period of high temperatures and most cultivars did not survive the effects of low temperatures with the absence of snow cover. Similarly, the win-

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Table 3. Evaluation of chrysanthemum overwintering in field trials

Cultivar	Overwintering plants in Slabce (%)			Overwintering plants in Troja (%)		Whole experiment average (%)
	07/08, 2-year experiment	08/09, 2-year experiment	08/09, 1-year experiment	10/11, 2-year experiment	11/12, 2-year experiment	
Vlasta	8	0	nt	nt	nt	4[a]
Celie	nt	nt	nt	33	0	17[ab]
Světla	nt	nt	nt	100	42	71[b–e]
Zoja bronzová	nt	nt	nt	83	0	42[a–d]
Flavie	nt	nt	nt	83	0	42[a-d]
Zina	83	90	92	nt	nt	88[cde]
Věra	92	82	100	nt	nt	91[de]
Berta	100	92	92	nt	nt	95[e]
Kordula	nt	nt	nt	83	0	42[a–d]
Mína	83	90	83	nt	nt	85[cde]
Radana	nt	nt	nt	75	0	38[abc]
Dorota	100	92	100	nt	nt	97[e]
Marika	67	75	58	nt	nt	67[b–e]
Estela	100	100	100	nt	nt	100[e]
Stáza	nt	nt	nt	83	*	83[b–e]
523	nt	nt	nt	42	0	21[b–e]
534	nt	nt	nt	50	0	25[ab]
Tereza	100	100	100	100	25	85[cde]
912	100	100	82	nt	nt	94[e]

nt – not tested in the given period; *liquidation due to disease (white rust); values followed by the same letter are not significantly different at α = 0.05

ter 2011–2012 gave an example in garlic growers, where most crops died as a result of the weather conditions (Prášil 2014). Tábor (2012) informed about the damage of evergreen rhododendrons and deciduous azaleas in 2011–2012 winter. Results of our experiments showed that even covering crops with evergreen spruce in the absence of snow cover did not protect plants from death. As Baadshaug (1973) stated, snow provides excellent insulation from fluctuations in air temperature. The temperature of the low layer of snow remains at a level close to the temperature of air. Leep et al. (2001) showed that a 0.1 m layer of snow keeps crops at a temperature of around 0°C. In our experiments, plants were prepared for winter by being shorn to a height of 50 mm and covered by evergreens. Böhm (1991) or Jarolímková (2004) recommended uncut crop wintering, where the retained plant detritus acts as heat insulation.

Field trials are the most widely-used method of testing winter hardiness of herbaceous perennials such as the chrysanthemum (Wildung 1979; Anderson et al. 2001; Anderson, Gesick 2004). As highlighted by Blum (1988), Tcacenco et al. (1989) and Anderson and Gesick (2004) these methods have disadvantages, especially in needing multi-annual tests in multiple locations. Therefore, field trials that followed in 2012 to 2013 tested selected cultivars in the freezing chamber. The results in terms of surviving plant % are given in Table 4. These include plants with a regeneration score of 3 or more. Cv. Vlasta did not survive these tests, corresponding to its results in the field trials. The highest survival in freezing chamber showed cv. Tereza, with an average of 87% survived, and 100% at –8°C. Cv. Marika showed an average of 64% survived. The detailed statistical evaluation found that there was a statistical difference on the level of α = 0.05 in the survival percentage of group of cvs Vlasta, Estela and Stáza, with the average survival 19%, from cv. Marika with average survival 64%. In the statistical valuation cv. Tereza

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Table 4. Evaluation of plant survival during tests in freezing chambers

Cultivar	Surviving plant at the temperature (%)						Average survival (%)
	–8°C Test 1	–8°C Test 2	–10°C Test 1	–10°C Test 2	–12°C Test 1	–12°C Test 2	
Vlasta	0	0	0	0	0	0	0[a]
Flavie	50	32	50	16	32	0	30[b]
Marika	50	84	32	84	68	68	64[c]
Estela	16	16	0	32	16	16	16[ab]
Stáza	50	16	50	0	0	0	19[ab]
Tereza	100	100	84	68	84	84	87[d]

values followed by the same letter are not significantly different at α = 0.05

appears to be best, with an average survival 87%. A comparison of Tables 3 and 4 shows that the average survival percentage of cultivars tested in the field trials corresponds to the mean survival of the cultivars tested in the freezing chambers in Vlasta (4% and 0%), Flavia (42% and 30%), Marika (67% and 64%) and Tereza (85% and 87%). Tests in the freezing chamber did not confirm results in the higher average survival of plants from field trials in cv. Estela (100% and 16%) and Stáza (83% and 19%).

The results of plant regeneration after the freezing in the chamber are expressed on a point scale in Table 5. The highest regeneration score, 4.5 points showed cv. Tereza and 3.9 points cv. Marika. Plant death was confirmed for cv. Vlasta. Results in Table 5 confirmed that cultivar's differences in regeneration are already apparent at the temperature of –8°C. Upon detailed statistical evaluation it was found that there was a statistical difference at the level of α = 0.05 in group of cvs Vlasta and Estela, where average regeneration is expressed as 1.0 and 1.5; and group of cvs Marika and Tereza, where 3.9 and 4.5 regeneration points are set. Marika and Tereza can be evaluated as cultivars with high frost resistance and significantly statistically different from Vlasta, Flavie, Estela and Stáza, which can be regarded as cultivars susceptible to frost killing.

Assessment of low temperature intervention on plants already regenerating after the cold season is shown in Table 6. Surviving plants of each cultivar, which have a point score of 3 or more, are recorded as a percentage after intervening temperatures of –5, –8 and –10°C. The results of plant regeneration after simulating spring frost are expressed on a point scale. It is evident from – that cv. Tereza survived the simulated spring frosts the best (in average survival 44%) although no statistically significant differences were found. Plants of most cultivars survived temperatures of –5°C with 68% or more, whereas at –8°C and –10°C percentage of the survival rate dropped to 16–0%. The reactions of these cultivars corresponded with field trials in 2011 and 2012, where a cold and dry February followed a warm December and January.

The statistical evaluation did not show significant differences between cultivars in the regeneration. However there was a noticeable difference in plant regeneration between individual cultivars at –5°C temperature and also difference in plant regeneration at –5 and –8°C temperatures. The worst re-

Table 5. Plant regeneration scoring after freezing chamber tests

Cultivar	Temperature intervention						Average
	–8°C Test 1	–8°C Test 2	–10°C Test 1	–10°C Test 2	–12°C Test 1	–12°C Test 2	
Vlasta	1.0	1.1	1.1	1.0	1.0	1.0	1.0[a]
Flavie	2.4	2.4	3.4	2.1	2.9	2.0	2.5[c]
Marika	4.4	4.8	2.9	4.1	3.5	3.6	3.9[d]
Estela	1.7	1.7	1.0	1.8	1.3	1.4	1.5[ab]
Stáza	3.3	2.9	2.3	1.6	1.3	1.9	2.2[bc]
Tereza	6.8	5.1	4.4	2.9	4.5	3.1	4.5[d]

values followed by the same letter are not significantly different at α = 0.05

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Table 6. Evaluation of spring frost simulation

Cultivar	Surviving plants* (%)			Average survival (%)	Scoring of plant regeneration			Average
	–5°C	–8°C	–10°C		–5°C	–8°C	–10°C	
Vlasta	0	0	0	0[a]	1.0	1.0	1.0	1.0[a]
Flavie	16	0	0	5[a]	1.3	1.0	1.0	1.1[a]
Marika	100	0	0	33[a]	5.0	1.7	1.8	2.8[a]
Estela	84	0	0	28[a]	2.7	1.0	1.2	1.6[a]
Stáza	68	16	0	28[a]	2.8	1.6	1.2	1.9[a]
Tereza	100	16	16	44[a]	5.0	1.7	1.9	2.9[a]

*plants with point score of 3 or more; values followed by the same letter are not significantly different at α=0.05

sults of regeneration were again seen in cv. Vlasta (died). The most resistant cultivar was again Tereza (average score 2.9) and Marika (average score 2.8). These results correspond with the field trials in late 2011 and 2012, when only 25% of cv. Tereza and 42% of cv. Světla plants overwintered. As stated by Procházka et al. (1998), frost resistance is strongly seasonal and not permanently kept as a constitutional character. With temperatures close to zero, herbs will usually last for a few days.

Similar conclusions were reached by Hanson (1988) about alfalfa. Unacclimated alfalfa cannot tolerate even mild frost of around –5°C in summer, but after a period of acclimatisation in autumn it can survive winter temperatures of –15 to –20°C. Also interesting is the effect of photoperiod on the induction of frost resistance of timothy (Eagles et al. 1997). The timothy adapted to northern Norway maintained a high level of hardiness when exposed to 10°C under a short photoperiod (8 h), whereas it de-hardened significantly at temperatures above 4°C under a long photoperiod (16 h). Temperatures above 0°C have less impact on the loss of winter hardiness when they occur early in winter. This is in agreement with our results in 2009 and 2010.

In the freezing chamber fully-grown annual plants were tested to best simulate the real conditions in the winter. Kim and Anderson (2006) compared laboratory freezing methods to establish cold tolerance of detached rhizomes and intact crowns in garden chrysanthemums. The question here is whether the results obtained correspond to the situation in field tests, as according to Procházka et al. (1998) sufficient assimilation is an important condition to frost resistance. Such data are certainly important in the breeding process, but ultimately field testing must be carried out. Thus, tests carried out in the freezing chamber with intact plants represent a compromise between field tests and laboratory tests with separated plant parts. Future detailed evaluation in freezing chambers (correct temperature) with intact plants can develop methodology which will simulate field conditions more exactly.

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