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DSC STUDIES OF RHODODENDRON HYBRIDS FROST RESISTANCE

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The DSC method was used to study phase transitions in *Rhododendron* L. leaf tissues caused by temperatures below the freezing point of water. The curves show several stages of water crystallisation, demonstrating that these processes do not occur simultaneously in various types of cell organelles. Temperatures and enthalpies of the phase transitions were determined and significant changes were found in the DSC curves when the sample was repeatedly subjected to sub-zero temperature cooling and heating. Also, frost resistance of the same rhododendron taxons was studied by conductometric analysis and the DSC results were compared with the data from other laboratory studies.

Keywords: *DSC, frost resistance, Rhododendron hybrids*

Introduction

Frost resistance of rhododendrons is a criterion in selecting these ornamental plants for breeding in Polish climate conditions. During severe winters many rhododendron cultivars suffer serious frost damage, especially in eastern and southern Poland. Breeding new plants calls for quick verification of the frost resistance of the produced cultivar population. It is a lengthy and costly process in field conditions and this is why laboratory methods of frost resistance of evergreen plants are of great importance. One of the methods frequently employed involves conductometric study of leachates obtained from frost-damaged tissues in laboratory conditions [1, 2]. Also studied was the effect of flavonoid content [3] and the dehydrin group proteins [4] on rhododendron frost resistance. So far, calorimetric methods were rarely used in studying frost resistance of rhododendron flower buds and leaves [1, 5, 6]. Study of exothermal processes in tissues of other woody plants was conducted earlier to determine geographical limits of plants [7], ice formation [8] or deep supercooling of plant tissues [7, 9].

In this study, DSC analysis of rhododendron leaf tissues was used to determine frost resistance of specimens of rhododendron hybrids, and the results were compared with the frost resistance data obtained by the conductometric method. Up to now, the DSC method has been applied to study the freezing of bound water in living tissues of animals [10] and in cellulose–water systems [11, 12]. The role of bound water in biological membranes is thoroughly discussed by Nimtz [13].

Experimental

Materials and methods

Calorimetric studies were conducted in Pyris 1 DSC Perkin Elmer in the 2004/2005 winter season. The leaves for testing were picked on the day of measurements during the winter resting period in February and March. Before the measurements, the leaves were kept at a uniform temperature of 276 K. Just before testing, a disk of 5 mm diameter was cut out of the leaf and placed, after weighing, in the calorimetric cell. Repeated cyclic temperature scanning in the 293 to 223 K range was employed, at a constant cooling rate of 20 K min^{-1}. The tests were performed on 9 years old rhododendron taxons originating from Piotr Muras' garden located in Tomaszkowice in the neighbourhood of Wieliczka. In this study, maternal plants and F_1 hybrids of series 375 of *Rhododendron aureum* × 'Tomasz Wojciech' as well as hybrids of series 383 of 'Hachmann's Charmant' × *Rhododendron purdomii* were used. Hybrid plants were selected for DSC studies so that they represented the least and most frost-resistant ones, based on the criteria of comparative analysis made by conductometric method in the same winter period.

Comparative analysis of rhododendron taxons was performed in the laboratory by conductometric study of the electrolyte (leachate) obtained from tissues damaged by freezing. In winter 2005, samples were taken from plants selected for the study, which consisted of 5 discs with tissue diameters of 11 mm. The samples were subjected to the action of low tem-

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perature 238 K in a freezer and then extracted with water using a shaker. Some samples were extracted without prior freezing, as a control sample. All the samples were prepared in the same way and the leachates and conductometric measurements with a CC311 Elmatron conductometer and EPS-2ZE electrode were conducted in the same conditions [3]. The leachates and the tissues were then temperature treated in an autoclave and next the measurement of electric conductivity was repeated to obtain the maximum value as a reference.

Results and discussion

The DSC curves obtained during cooling of leaf tissues taken from different taxons have different shapes corresponding to phase transition (Figs 1–4). The greatest differences have been found among the first cycle curves, both in the cooling and heating sweeps. The curves representing the first cooling have a complex structure, characteristic of each hybrid. The scanning done on other samples cut off from the same leaf show good reproducibility of cyclic scanning curves. The curves representing the first cooling sweep for different plants have various slopes at minima corresponding to phase transitions. In the studied hybrids, the number of identifiable components of phase transitions in the 263 to 248 K range was also different, and in some plants additional, low-enthalpy phase transitions appeared in a lower temperature range (Figs 1 and 3). The values of temperatures at which minima of heat flow occur for the studied rhododendrons are compiled in Tables 1 and 2.

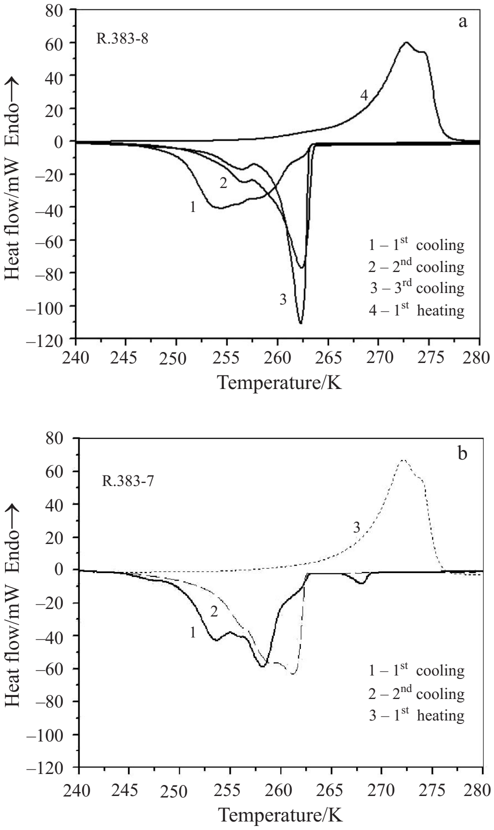

Fig. 1 DSC cooling and heating curves obtained for two selected, more frost-resistant Rhododendron hybrids of series 383 of ‘Hachmann’s Charmant’ × *Rhododendron Purdomii*

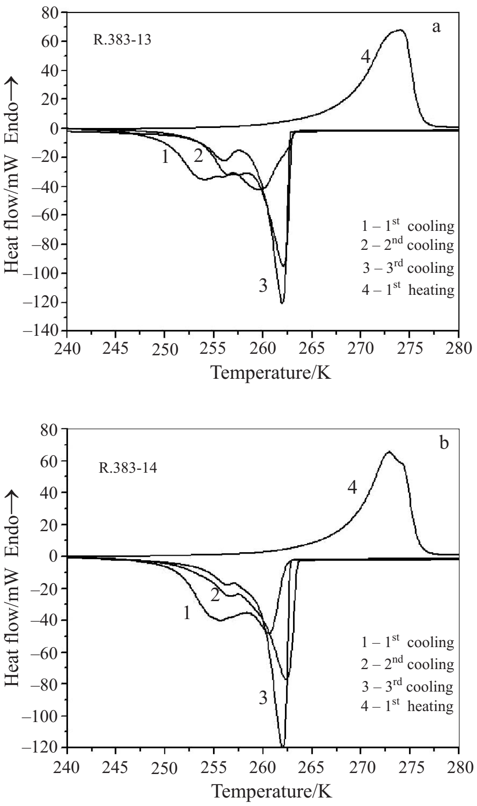

Fig. 2 DSC cooling and heating curves obtained for two selected, less frost-resistant Rhododendron hybrids of series 383 of ‘Hachmann’s Charmant’ × *Rhododendron purdomii*

The structure of subsequent cooling curves in DSC charts (Figs 1–4) is less complex in the range of temperatures below the freezing point of water. The broadest and most complex structures of phase transitions occur during the first cooling sweep, in contrast to the second and third sweeps. The curves representing the second and third cooling sweeps are similar. It may mean that during the first cooling the cells become disrupted and the cell membranes form liposome-like structures [13] surrounded by free water or ice in sub-zero temperatures. Much smaller differences between subsequent cycles have been observed in the heating curves. The calculated differences between temperatures (ΔT) of heat flow minima corresponding to the first phase transition in subse-

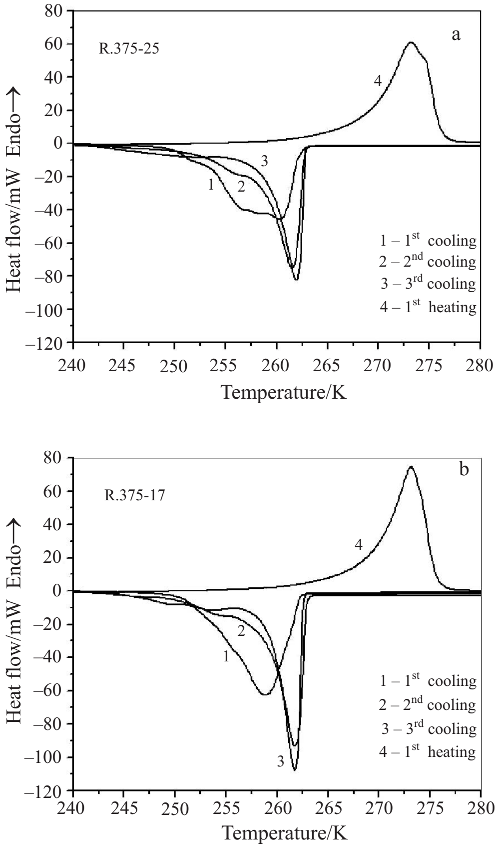

Fig. 3 DSC cooling and heating curves obtained for two selected, more frost-resistant Rhododendron hybrids of series 375 of *Rhododendron aureum* × 'Tomasz Wojciech'

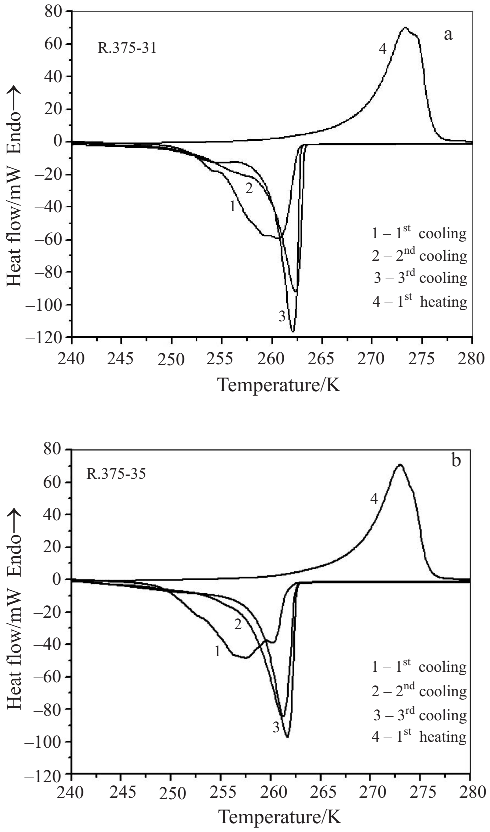

Fig. 4 DSC cooling and heating curves obtained for two selected, less frost-resistant Rhododendron hybrids of series 375 of *Rhododendron aureum* × 'Tomasz Wojciech'

quent cooling sweeps are compiled in Tables 1 and 2. The enthalpies of phase transitions during cooling, calculated for both rhododendron series, are compiled in Tables 3 and 4.

The data on frost resistance of rhododendron taxons obtained by conductometric analysis of the leachate from tissues damaged by freezing allowed calculation of the percentage of damage, as the amount of electrolyte leached from damaged tissues was greater than that from undamaged tissues. Information about the maximal diffusibility of the electrolyte was obtained by subjecting the tissues to the action of high temperatures in an autoclave. The plots made it possible to arrange the taxons and maternal plants in ascending order according to their frost resistance (Fig. 5).

Comparison showed that the agreement between data obtained from calorimetric and conductometric studies is basically good. In the hybrid series under study, greater leaf damage caused by freezing in conductometric studies corresponds to lower temperatures of main phase transitions and greater ΔT values between cooling curves 1 and 2. To check these de-

Table 1 Temperatures of heat flow minima corresponding to phase transition (water crystallisation), and $\Delta T_{\min}$ values corresponding to $T_{\min}$ shifts in subsequent cooling cycles for samples of F1 hybrids (series 375) of *Rhododendron aureum* × 'Tomasz Wojciech' and parent plants

Taxa	$T_{\min}$/K			$\Delta T_{\min}$/K		
	1st cooling	2nd cooling	3rd cooling	(2nd–1st)	(3rd–1st)	(3rd–2nd)
R. aureum	259.43	261.38	262.15	1.95	2.73	0.78
'Tomasz Wojciech'	256.11	260.98	260.84	4.87	4.74	–0.13
R. 375-25	260.24	261.54	261.95	1.31	1.18	0.41
R. 375-17	258.81	261.75	261.76	2.94	2.94	0.01
R. 375-31	261.21	262.31	262.04	1.09	0.82	–0.27
R. 375-35	257.47	261.19	261.63	3.72	4.16	0.45

Table 2 Temperatures of heat flow minima corresponding to phase transition (water crystallisation), and $\Delta T_{\min}$ values corresponding to $T_{\min}$ shifts in subsequent cooling cycles for samples of series 383 hybrids of 'Hachmann's Charmant' × *Rhododendron purdomii* and parent plants

Taxa	$T_{\min}$/K			$\Delta T_{\min}$/K		
	1st cooling	2nd cooling	3rd cooling	(2nd–1st)	(3rd–1st)	(3rd–2nd)
'Hachmann's Charmant'	253.97	261.12	262.18	7.15	8.21	1.06
R. purdomii	261.43	262.52	262.23	1.09	0.79	–0.30
R.383-8	254.27	262.33	262.26	8.06	8.00	–0.07
R.383-7	253.56	261.16		7.60		
R.383-13	259.69	262.09	262.01	2.39	2.32	–0.08
R.383-14	260.55	261.95	261.98	1.40	1.43	0.03

Table 3 Calculated enthalpies of phase transitions in subsequent cooling and heating cycles for F_1 hybrids (series 375) of *Rhododendron aureum* × 'Tomasz Wojciech'

Breeding hybrids	Sweep number and type	ΔH/J g^{-1}	ΔH_1/J g^{-1}	ΔH_2/J g^{-1}	ΔH_3/J g^{-1}	ΔH_4/J g^{-1}
R.375-25	1st cooling	–106.15	–9.21	–0.21	–2.61	–0.29
	2nd cooling	–98.91	–54.63	–0.77	–0.23	
	3rd cooling	–95.10	–66.68	–3.88	–0.97	
	1st heating	111.68	9.97	0.99		
	2nd heating	112.52	112.52			
R.375-17	1st cooling	–115.76	–115.76	–1.20	–0.32	
	2nd cooling	–115.86	–79.51	–1.82	–0.74	
	3rd cooling	–110.80	–74.75			
	1st heating	122.33	122.33			
	2nd heating	120.08	120.08			
R.375-31	1st cooling	–115.36	–12.34	–5.05	–0.46	
	2nd cooling	–117.64	–65.73	–0.91		
	3rd cooling	–103.43	–77.59	–24.21		
	1st heating	131.34	131.34			
	2nd heating	132.53	132.53			
R.375-35	1st cooling	–112.34	–4.53	–1.40	–1.22	–0.13
	2nd cooling	–106.96	–106.96	–0.80		
	3rd cooling	–103.59	–74.37			
	1st heating	111.98	111.98			
	2nd heating	115.01	115.01			

pendencies quantitatively, correlation between the two methods was plotted. The results showed agreement for ΔT values (Fig. 6), which indicates that cyclic scanning of leaf tissues makes it possible to observe the dynamics of tissue structure damage and provides more information about the plant's resistance to the action of low temperatures, which reached 223 K during the DTA analysis. The several distinct phase transitions identified during the first cooling can indicate that initially water crystallizes in different cell and tissue structures. The disappearance of separate phase transitions in repeated scanning indicates structural damage, which leads to mixing of various cell solutions. The lower susceptibility to frost damage should result in smaller changes in the second cooling curve and smaller difference between the second and third cooling curves, which can be observed in the DSC charts (Figs 2, 4). In addition, the smaller slope of the cooling curve indicates slower crystalization of water in tissues during freezing, which is advantageous to plants and results in less damage.

The results of conductometric analysis, which show the extent of frost damage to leaves depend, however, on plant acclimation during winter, and some changes in the sequence of relative frost resistance were observed [3]. This shows that the pattern of winter temperatures influences the results obtained in that season. Consequently, frost resistance studies in breeding gardens take many years and any additional information can be useful. That is why it is important to determine the calorimetric curves for hybrid plants, which can help to determine their frost resistance. Further study of differences between hybrids obtained in breeding gardens will provide more insight into differences in their predisposition to sub-zero temperature stress.

Table 4 Calculated enthalpies of phase transitions in subsequent cooling and heating cycles for series 383 hybrids 'Hachmann's Charmant' × *Rhododendron purdomii*

Breeding hybrids	Sweep number and type	ΔH/J g^{-1}	ΔH_1/J g^{-1}	ΔH_2/J g^{-1}	ΔH_3/J g^{-1}	ΔH_4/J g^{-1}
R.383-8	1st cooling	–113.53	–0.86	–0.35	–0.10	–1.47
	2nd cooling	–115.30	–58.74	–0.66	–0.53	
	3rd cooling	–106.33	–65.98	–3.64	–1.07	
	1st heating	119.38	0.63	1.05		
	2nd heating	125.31	125.31			
R.383-7	1st cooling	–137.31				
	2nd cooling	–130.85				
	3rd cooling					
	1st heating	137.99				
	2nd heating	139.09				
R.383-13	1st cooling	–115.99	–27.006	–0.30	–6.06	
	2nd cooling	–116.22	–55.14	–0.02	–1.79	
	3rd cooling	–110.84	–70.45	–5.86	–1.28	
	1st heating	122.90	122.90			
	2nd heating	128.92	128.92			
R.383-14	1st cooling	–122.02	–11.45	–5.51		
	2nd cooling	–120.75	–91.93	–0.73		
	3rd cooling	–103.93	–72.26	–1.23	–0.56	
	1st heating	118.59	2.96	0.15		
	2nd heating	128.46	128.46			

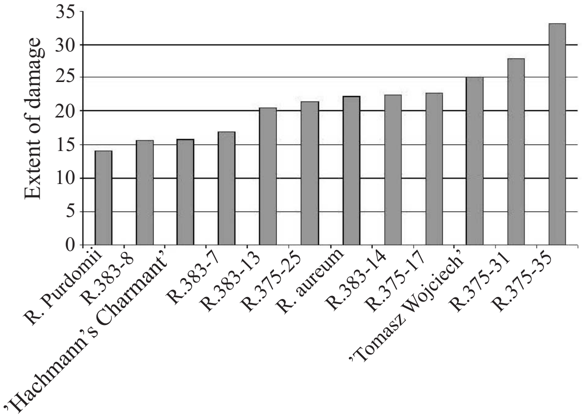

Fig. 5 Percentage of frost damage in F$_1$ Rhododendron hybrids and the parent plants; determined by conductometric measurements

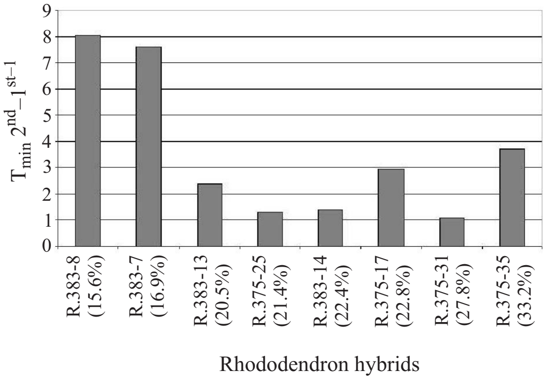

Fig. 6 Dependence of ΔT_{min}(2$^{\text{nd}}$–1$^{\text{st}}$) on frost damage in F$_1$ Rhododendron hybrids series

Conclusions

The DSC studies and the obtained data on phase transitions and temperatures of water crystallisation in leaf tissues obtained by DSC analysis make it possible to learn about changes occurring in studied plants in sub-zero temperatures. Cyclic temperature scanning provides more information about irreversible tissue damage than does a single DSC scan, thus providing a basis for comparison of rhododendron garden-grown hybrids. As found in this study, the cell membranes become damaged during the first cooling. Calorimetric study adds to the knowledge of plant resistance to frost damage and combined with data obtained by other laboratory methods based on conductometric analysis enable a more complete characteristics of differences among studied plants and the selection of specimens that are valuable for further breeding.

Nomenclature

ΔH_1–ΔH_4	enthalpies of successive phase transitions
T_{min}	temperatures of heat flow minima during cooling
ΔT_{min}	difference between T_{min} values in subsequent cooling cycles
R.375-25	breeding hybrid F$_1$ (the number following the dash denotes the number of hybrid in series 375 obtained from the same parent plants)

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