Phytochemistry, Vol. 28, No. 3, pp. 759–763, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

SEASONAL CHANGES IN CARBOHYDRATE CONTENT AND FROST RESISTANCE OF LEAVES OF *NOTHOFAGUS* SPECIES

MIREN ALBERDI* LUIS MEZA-BASSO†, JULIO FERNANDEZ, DARCY RIOS‡ and MAGDALENA ROMERO

Instituto de Botánica; †Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile, Casilla 567, Valdivia, Chile

(*Received* 10 *May* 1988)

Key Word Index—*Nothofagus dombeyi*; *Fagaceae*; cold hardiness; sugar accumulation.

Abstract—The annual course of frost hardiness and soluble carbohydrate content in leaves of woody *Nothofagus* species, located at different altitudes in Southern Chile, were studied. Frost resistance reached a maximum in the coldest period and a minimum in spring or summer. Leaves from individuals subjected to lower temperatures (higher altitudes) were more frost-resistant than those from warmer conditions (lower altitudes). Frost resistance was positively correlated with total sugar accumulation. In *N. dombeyi* sucrose was the only sugar whose content correlated with frost resistance. At higher altitudes a slight increase of fructose with respect to sucrose was found. However, the accumulation of the individual sugars measured accounted for only a small fraction of the increase in soluble total carbohydrates. Therefore the observed resistance is associated most likely with other factors. Artificial hardening at 0° for different periods of time caused changes in the carbohydrate levels of leaves. A rapid but small increase in the sugar concentration and frost hardiness after two hr of hardening were found. The nature of cold-induced metabolic adjustments and their ecophysiological significance are discussed.

INTRODUCTION

Frost hardiness of plants is an adaptative response to low temperatures [1–3]. Consequently plants subjected to climates with seasonal changes are more resistant to frost in winter than in spring or summer [4–9]. In mountain areas the temperature drop associated with rising altitude increases cold hardiness [10–12]. This pattern, however, was not found in the subalpine species investigated by Sakai *et al.* [13].

In some plants frost hardiness is associated with increased synthesis of low M_r metabolites, such as carbohydrates, amino acids and other related substances [4, 5, 14–17]. It is likely that these substances protect the protoplasm and membranes during cold stress [18–20].

The present work was undertaken to study concentration changes in soluble carbohydrates associated with seasonal and altitudinal fluctuations of frost resistance of leaves of woody evergreen *Nothofagus* species in south-central Chile. The effect of artificial hardening on frost resistance and on carbohydrates levels was also studied.

RESULTS AND DISCUSSION

A wide variation in temperature between winter and summer was found in the areas studied (Table 1). Frost resistance of leaves reached a maximum in the winter period (Figs 1–3). In this season, leaves of the *Nothofagus* species located at higher altitudes were more frost resistant than those located at a lower altitudes. *Nothofagus betuloides* and *N. dombeyii* were hardier (LT_{50} = 10.5 and −10° respectively) than *N. nitida* (LT_{50} = 8.5°). At the

Table 1. Temperature data of the investigated areas during the period April 1986 to April 1987

Locality and altitude (m) (a.s.l.)	Mean annual air temp.	Mean air temperature In the warmest month (January)	In the coldest month (July)	Mean annual* temp. range	Absolute minimum
Valdivia 9†	14.2°	22.2°	8.0	14.2°	−1.2°
A. Calientes 100†	9.1°	12.7°	1.9°	7.8°	−6.5°
Mallines 700	—	—	—	—	—
Antillanca 1.000‡	4.4°	12.2°	−2.0°	14.0°	−7.5°

*Temperature difference between the warmest and the coldest month.
†Data provided by the Climatological Station of Universidad Austral de Chile.
‡Based on personal climatological records.

---

*Author to whom correspondence should be addressed.

‡Present address: Facultad de Agronomia, Universidad de la Frontera, Temuco, Chile.

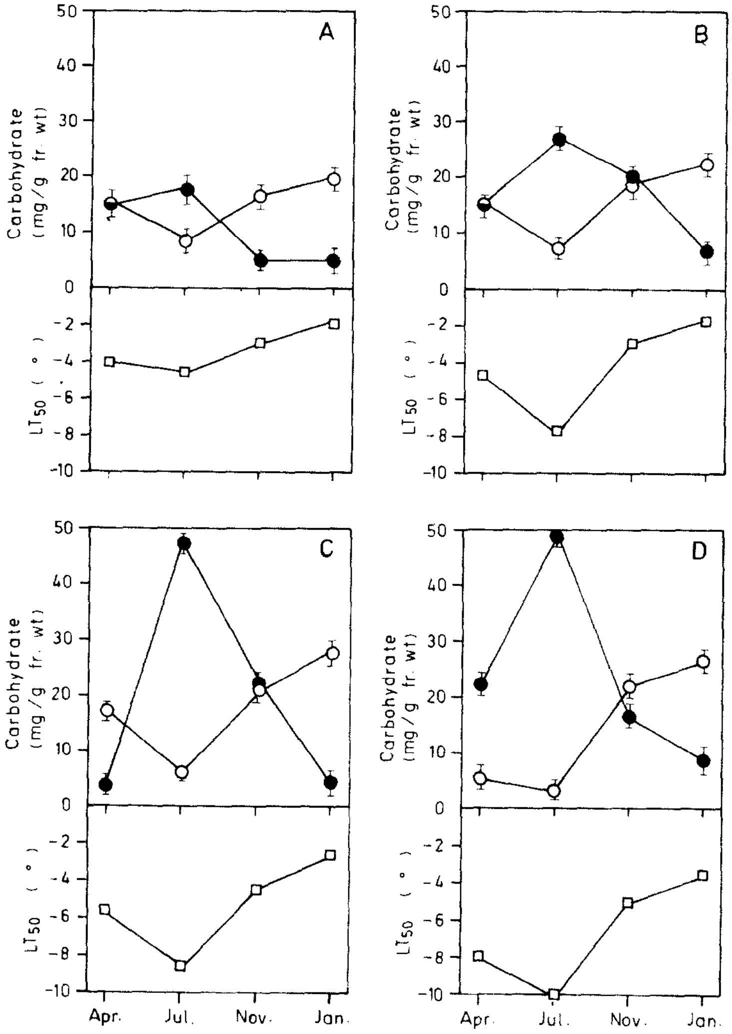

Fig. 1. Seasonal fluctuations of carbohydrate content and frost resistance of *N. dombeyi* leaves from different altitudes. (●) total soluble carbohydrate, (○) starch, (□) frost hardiness. Sugars and starch values are the average of 4 replicate assays, and frost hardiness of 10 replicate assays, of leaves collected at the same date each month. For LT_{50} the maximum standard deviation was ± 1.2°. Altitudes were 9 (A), 400 (B), 700 (C) and 1000 m a.s.l. (D).

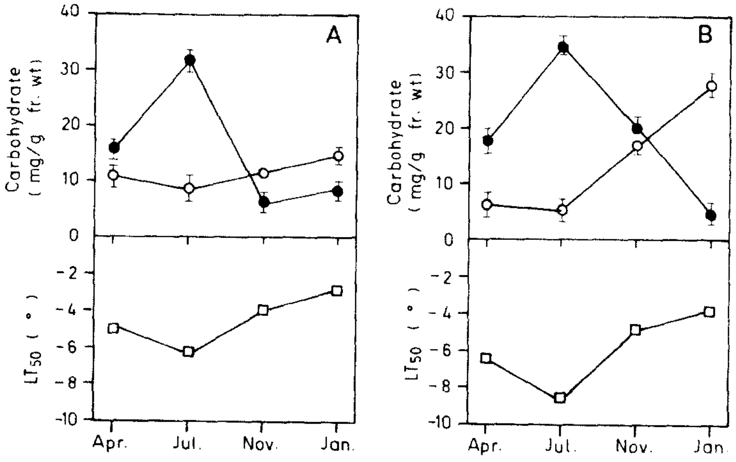

Fig. 2. Seasonal course of carbohydrate contents and frost resistance of *N. nitida* leaves from different altitudes. Symbols, sample collection conditions, and the value representations are the same as in Fig. 1. For frost resistance the maximum standard deviation was ± 1.0°. Altitudes were 9 (A) and 700 m a.s.l. (B).

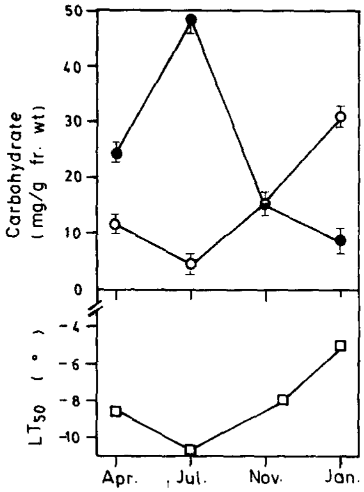

Fig. 3. Seasonal changes in sugars contents and frost hardiness of *N. betuloides* leaves. The altitude was 1000 m a.s.l. Symbols, sample collection conditions, and the value representations are the same as in Fig. 1. For frost resistance the maximum standard deviation was ±1.0°.

lowest altitude *N. nitida* was more frost resistant ($LT_{50} = 6°$) than *N. dombeyi* ($LT_{50} = 4.5°$). A greater frost hardiness was consistently correlated with an increase of total soluble carbohydrate ($r = -0.89$, $P < 0.01$) and a decrease of starch ($r = 0.75$, $P < 0.01$) in all species investigated (Figs 1–3). This carbohydrate increment could be explained by starch hydrolysis caused by the temperature drop [1, 2, 21, 22]. Likewise, it has been demonstrated in potato tuber that the differential sensitivity of phosphofructokinase to low temperature could lead to an accumulation of sucrose [23]. During the winter period, however, in broad-leaved evergreens, carbohydrate accumulation may occur when photosynthesis exceeds respiration [1]. Nevertheless, this correlation between frost hardiness and sugar accumulation was not found in other plants [24–26].

Seasonal changes in contents of glucose, sucrose, fructose and raffinose at various altitudes were determined in leaves of *N. dombeyii* (Fig. 4). The levels of these sugars showed the same annual course as the total sugars. They were generally higher in winter than in autumn and summer.The amount of individual sugars increased at higher altitudes. Fructose content was higher than the content of other sugars at 1000 m a.s.l. This suggests a more efficient breakdown of sucrose by the reverse sucrose synthase reaction [27] at colder temperatures [28]. On the other hand, fructose could be accumulated by the sucrose invertase reaction. It has been reported that the activity of this enzyme increases in plants subjected to low temperature [29]. There was a correlation ($r = -0.73$, $P < 0.01$) between sucrose content and frost resistance. Significant correlations were not found for other sugars. Pomeroy *et al.* [22] found a correlation between seasonal frost resistance and sucrose levels. In other species differ-

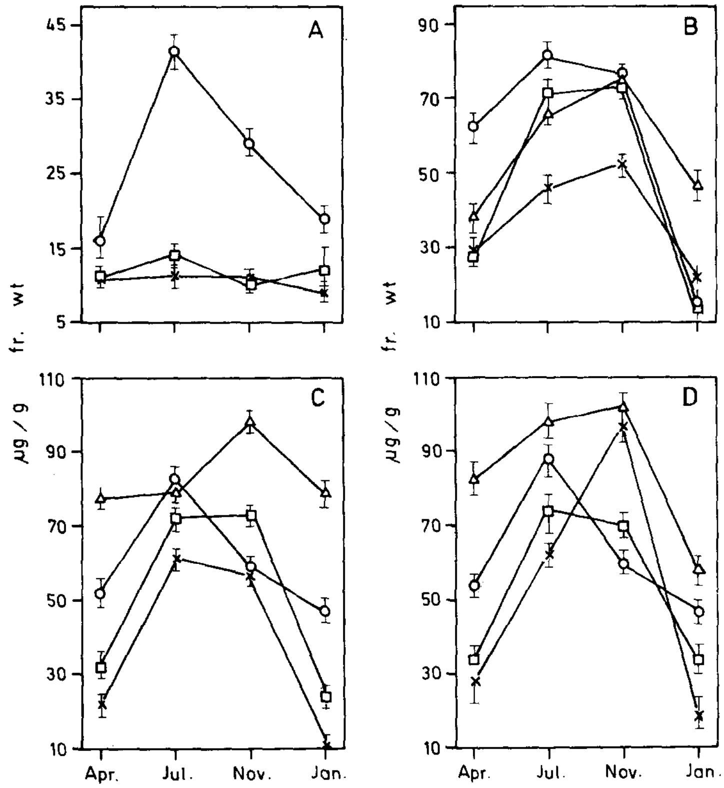

Fig. 4. Annual course of content of individual carbohydrates in leaves of *N. dombeyi* at different altitudes. (○) sucrose; (□) raffinose; (△) fructose; (x) glucose. Values are averages of 4 repetitions per trial. Altitudes were 9 (A), 400 (B), 700 (C) and 1000 m a.s.l. (D).

ent soluble sugars (raffinose and glucose) have been found to predominate 2, 4, 5, 6, 30]. This suggests that no specific sugar is consistently related with frost hardiness, and that the kind of carbohydrate accumulated in winter is dependent on the particular metabolism of the plant. Moreover, in *N. dombeyi* the changes observed in invidual sugars can account for only a small fraction of the increase in total soluble carbohydrates. Therefore, their contribution to frost hardiness is most likely small. It would be interesting to analyse which other sugars are involved in the increase of total soluble carbohydrates of this species. On the other hand, sucrose was a more efficient cryoprotective agent of thylakoid membranes of leaves of *N. dombeyi* than any other sugar tested [16].

Changes in the level of total soluble carbohydrate can be also experimentally induced (Fig. 5). Leaves of *N. dombeyi* from different altitude were subjected to artificial hardening at 0° for various periods of time. After four hr of treatment the carbohydrate content of leaves for both altitudes reached a steady state level, that was *ca* 20% higher than controls kept at room temperatures. Subsequently a consistent decrease of these substances was observed. The rapid increase in carbohydrates coincides with the maximum changes in frost resistance.

The increase of frost hardiness of *N. dombeyi* leaves is not associated only with an increase of sugars, but also with the levels of free proline [15]. In some herbaceous plants sugars are required for proline accumulation, because they inhibit its oxidation [31]. An increase of these substances appears to be related to effects of stress on tissue water balance in plants [1, 2]. The winter desiccation capacity (drought tolerance) and heat resistance of *N. dombeyi* leaves is very high [32, Alberdi, M., unpublished results]. Also, a cryoprotective polypeptide was isolated in seedlings of *N. dombeyi* exposed to frost [16]. These results could explain the pioneer capacity of *N. dombeyi* in the 'Lahares' (areas without vegetation formed by volcanic scoria exposed to strong insolation and frost even in the warmer season) [33]. In response to abrupt temperature changes, plants could quickly accumulate cryoprotective substances which increase the thermal and drought tolerance. The simultaneous winter maximum of resistance to frost, heat and drought were also found in woody Proteaceae in Chile [34]. These findings support Levitt's hypothesis [35] of a general physiological resistance to climatic stresses.

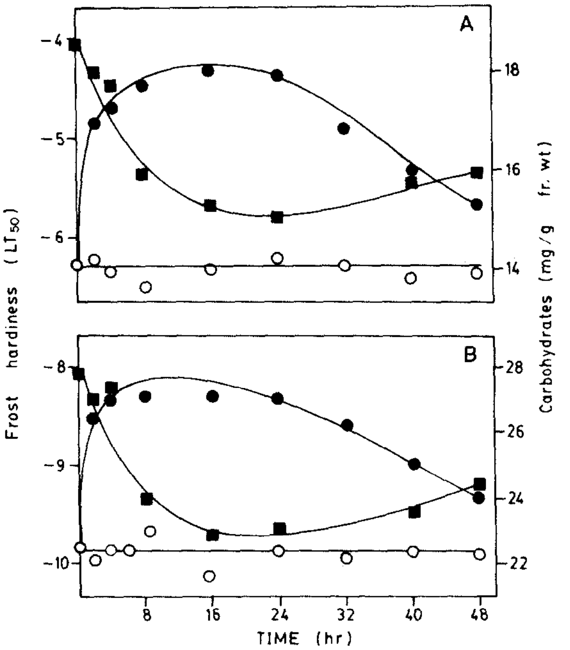

Fig. 5. Time course of frost hardiness and total soluble carbohydrate contents of *N. dombeyi* leaves from different altitude. Leaves were exposed to 0° for various periods of time. Unhardened plants kept at room temperature served as control. Determinations were made in autumn 1986. Altitudes were 9 (A) and 1000 m a.s.l. (B). Symbols are: frost hardiness (■), total soluble carbohydrate (●) and control (○).

EXPERIMENTAL

Materials. The evergreen species of *Nothofagus* (*N. dombeyi* (Mirb.) Oerst., *N. nitida* (Phil.) Krasser and *N. betuloides* (Mirb.) Oerst. from an altitudinal and thermal gradient in the Andes of South Central Chile (X Region) (Table 1) were studied. Twigs of adult trees with current year leaves were harvested 3–4 hr after sunrise, from autumn 1986 through to summer 1987, and taken to the laboratory in insulated containers. Soluble carbohydrate levels of leaves were determined and compared with its frost hardiness.

Carbohydrate analysis. Leaves were frozen in liquid N_2 and then crushed and homogenized in water using a Sorvall Omni-Mixer at full speed for 30 sec. The homogenate was extracted twice with H_2O at 80° for 30 min. The combined extracts were passed through a cation exchanger (Dowex W-X4) as described by Purvis *et al.* [36]. The immediate eluate was used for sugar determinations. Total soluble carbohydrates were determined by the phenol–H_2SO_4 method as described in ref. [37]. Sucrose, raffinose, glucose and fructose were determined by TLC using cellulose plates (water–ethylacetate–pyridine, 5:20:7 [38]. The retained fraction was dried at 80° and extracted twice into boiling water followed by hydrolysis in 4.6 N $HClO_4$. The combined supernatants were analysed subsequently for starch, using glucose as standard [39].

Determinations of frost hardiness. The plant material (10 repetitions per trial) was placed in polythylene bags and kept in controlled low temperature regimes for 190 min as previously described [12]. After treatment, the samples were thawed in the air at 1°. The lower parts of the shoots were kept in water for 7 days and then freezing injury (browning) was measured [40]. The temperature that damaged 50% of the leaf (LT_{50}) was used as a measure of frost hardiness [1].

Cold hardening treatment. Unhardened twigs of *N. dombeyi* were kept at 0° for various periods of time. After hardening, frost resistance and carbohydrate levels of the leaves were measured.

Acknowledgements—This work was supported by Fondo Nacional de Ciencias, Chile (Grants N° 1229, 0897 and 0898 and Universidad Austral Chile, Valdivia (Grants RS-86–30). The authors wish to thank Dr Luis Corcuera (Facultad de Ciencias, Universidad de Chile, Santiago) for helpful criticism of the manuscript and Mr Risnel Bustos for secretarial assistance.

REFERENCES

1. Levitt, J. (1980) in *Responses of Plants to Environmental Stresses* (Kozlowski, T. T., ed.) Vol. 1, p. 497. Academic Press, New York.

2. Sakai, A. and Larcher, W. (1987) *Frost Survival of Plants* (Billings, W. D., Golley, F., Lange, O. L., Olson, J. S., Remmert, H., eds), p. 321. Springer, Berlin.
3. Larcher, W. (1987) *Naturwissenschaften* **74**, 158.
4. Parker, J. (1959) *Bot. Gaz.* **121**, 46.
5. Parker, J. (1959) *Forest Sci.* **5**, 56.
6. Sakai, A. (1960) *Low temp. Sci.*, Ser **8**, 1.
7. Sakai, A. (1966) *Plant Physiol.* **41**, 353.
8. Kimball, S. L., Bennett, B. D. and Salisbury, F. B. (1973) *Ecology* **54**, 168.
9. Alberdi, M. and Rios, D. (1983) *Oecol. Plant.* **4**, 3.
10. Sakai, A. (1970) *Ecology* **51**, 485.
11. Palta, J. P. and Li, P. H. (1979) *Crop Sci.* **19**, 665.
12. Alberdi, M., Romero, M., Rios, D., and Wenzel, H. (1985) *Oecol. Plant.* **6**, 21.
13. Sakai, A., Paton, D. M. and Wardle, P. (1981) *Ecology* **62**, 563.
14. Purvis, A. C. and Yelenosky, G. (1983) *Plant Physiol.* **73**, 887.
15. Meza-Basso, L., Guarda, P., Rios. D. and Alberdi, M. (1986) *Phytochemistry* **25**, 1843
16. Rosas, A. Alberdi, M. Delseny, M. and Meza-Basso, L. (1986) *Phytochemistry* **25**, 2497.
17. Rios, D., Meza-Basso, L., Guarda,., Peruzzo, G. and Alberdi, M. (1988) *Acta Ecologica/Oecol. Plant.* **9**, 135.
18. Heber, U., Tyankova, L. and Santarius, K. (1971) *Biochem. Biophys. Acta* **241**, 578.
19. Santarius, K. and Bauer, J. (1983) *Cryobiology* **20**, 83.
20. Santarius, K. A. and Giersch, Ch. (1983) in *Effects of Stress on Phytosynthesis* (Mercelle, R., Clysters, H. and van Poucke, eds) pp. 201–210. Martinus Nijhoff/Dr. W. Junk Publishers, The Hague.
21. Biebl, R. (1962) in *Hd. der Protoplasma Forschung. Protoplasmatische Oekologie der Pfanzen. Wasser und Temperatur* (Alfert, M., Bauer, H. and Harding, C. V., eds) p. 344. Springer, Wien.
22. Pomeroy, M. K., Siminovitch, D. and Wightman, F. (1970) *Can. J. Botany* **48**, 953.
23. Dixon, L. W., Franks, F. and Rees ap T. (1981) *Phytochemistry* **20**, 969.
24. Piseck, A (1950) *Protoplasma* **39**, 129.
25. Larcher, W. (1954) *Planta* **44**, 607.
26. Green, D. E. and Ratzlaff, C. D. (1975) *Can. J. Botany* **53**, 2198.
27. Preis, J. (1982) *Annu. Rev. Plant. Physiol.* **33**, 431.
28. Pontis, H. (1985) *Arch. Biol. Med. Exp.* **18**, 99.
29. Graham, D. and Patterson, B. D. (1982) *Annu. Rev. Plant. Physiol.* **33**, 347.
30. Breckle, W. and Kull, V. (1971) *Flora* **160**, 43.
31. Stewart, C. (1978) *Plant Physiol.* **61**, 775.
32. Weinberger, P., Romero, M. and Oliva, M. (1973) *Vegetatio* **28**, 75.
33. Mc Queen, D. K. (1977) *Tuatara* **22**, 223.
34. Alberdi, M., Oyarzún, M., Romero, M. and Ríos, D. (1983) *Rvsta. Brasil. Bot.* **6**, 67.
35. Levitt, J. (1956) in *Handb. d. Pfanzenphysiol. II*, p. 638. Springer, Berlin.
36. Purvis, A. C.; Kawada, K. and Grierson, W. (1979) *Hort. Sci.* **14**, 227.
37. Dubois, M., Guilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956) *Methods Enzymol* **8**, 93.
38. Raadsveld, C. W. and Klomp, H. (1971) *J. Chromatogr.* **57**, 99.
39. O'Neill, S. Q. (1983) *Plant Physiol.* **72**, 983.
40. Larcher, W. (1968) *Tagunsbericht* **100**, 7.