Evaluation of Susceptibility of Walnut Genotypes to Sudden Cold and Frost Injury

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Abstract. Cold hardiness is a key factor limiting the distribution and productivity of perennial horticultural plants in the temperate regions of both hemispheres. Frequent frosts occurring during winter and spring in Khorasan, Iran cause serious damage to apricot, peach, nectarine, fig, olive, pomegranate, and walnut which necessitate the selection of resistant cultivars. Before introducing new genotypes, their capacity for adaptation to different conditions needs to be studied. Walnut production in Khorasan has been increasing through area expansion and the introduction of new genotypes. In this area, affective freeze occasionally damages walnut tree. The minimum temperature on winter of 2008 was about -22℃. The objective of this study was to evaluate cold hardiness in 12 walnut genotypes (K21B5/13, K21B5/15, K21B6/17, K21B6/18, K28B2/5, K28B2/6, K23B5/13, K23B5/14, K21B3/8, K21B9/25, K28B3/8, and K21B1/3) which were planted in 1986 on seedlings at the genotype collection (36° 17′ N and 59° 36′ E and 1,012 m altitude) of Khorasan Agricultural and Natural Sources Researches Center, Mashhad, Iran. The ionic leakages were evaluated at the Department of Horticulture at Ferdowsi University in Mashhad, Iran. One- and 2-year-old shoots of about 30 cm long were randomly collected on February, 2008 from mature trees. K21B5/13, K21B5/15, K21B6/18, K21B3/8, and K28B3/8 genotypes had more damage than the other genotypes, but K23B5/13, K23B5/14, and especially K21B9/25 showed resistance to -22℃. Linear correlation between ionic leakage and visual observation was found. Minimum ionic leakage was observed in K21B9/25 and visual observation confirmed its cold hardiness. Flower buds in K21B5/15 and vegetative buds in K21B3/8 were damaged completely. Sexual buds were the most sensitive organs of the tree. Annual shoots were damaged more severely than biennial shoots.

Additional key words: annual shoot, biennial shoot, cold hardiness, electrolyte leakage, flower bud, vegetative bud

Introduction

In high latitudes of both hemispheres, low temperature is a major environmental factor limiting the productivity and the geographical distribution of perennial horticultural crops. Low temperature is a dominant factor in the distribution, growth, and survival of woody plants (Grace, 1987, 1988). According to Kozlowski and Pallardy (1997), sudden exposure of unhardened plants to freezing temperatures typically results in injury to their shoots, cambia, and roots and often leads to death of the plants. Cold hardiness varies greatly among species, genotypes, and even different parts of the same plant. Skrøppa (1991) found differences in frost hardiness between two populations of *Picea abies* that originated only 60 km apart at the same altitude and latitude. In general, reproductive structures, roots, and young leaves are particularly sensitive to low temperature.

Environmental conditions that inhibit plant growth include low temperatures (Cannell and Shepherd, 1982; Christersson, 1978; Greer, 1983; Greer and Warrington, 1982; Gusta and Weiser, 1972; Howell and Weiser, 1970; Sakai and Larcher, 1987), short days (Bigras and D'Aoust, 1993; D'Aoust and Cameron, 1982; Greer and Warrington, 1982; Silim and Lavender, 1994), water deficiency (Chen et al., 1977; Timmis and Tanaka, 1976; Wildung et al., 1973; Yelenosky, 1979), and combinations of these conditions (Fuchigami et al., 1971), possibly inducing cold hardiness in some species. In woody temperate plants, cold hardiness develops gradually each year, long before onset of very low temperatures. Hardiness is induced in two sequential stages. In the first stage, accumulation of carbohydrates and lipids starts at temperatures between 10 and 20℃ during autumn. These compounds are substrates and energy sources for subsequent metabolic processes. The second stage, which is promoted by freezing temperatures, involves synthesis as well as structural changes of proteins and membrane lipids (Kozlowski and Pallardy, 1997). There is evidence that low but non-freezing temperatures induce accumulation of dehydrins and other non-dehydrin proteins in leaves, stems, and flowers (Muthalif and Rowland, 1994). Seasonal patterns of accumulation of dehydrins with acclimation to cold were confirmed in bark tissues of *Prunus persica*, *Malus domestica*, *Rubus* sp., *Populus* sp., *Salix babylonica*, *Cornus florida*, *Sassafras albidum*, and *Robinia pseudoacacia* (Wisniewski and Arora, 1992). However, the specific role of dehydrins during development of cold hardiness itself is not known (Wisniewski and Arora, 1992). Once dehardening started, temperature had the major influence on loss of cold hardiness (Greer, 1983). The role of photoperiod in development of cold

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hardiness in nature varies appreciably with different species. Species that set buds usually harden extensively in response to short days. Such treatment accelerates buds, induces early dormancy, and increases cold hardiness. Cold hardiness is often induced in part because water stress prevents growth of abnormal late-season shoots such as lammas and proleptic shoots (Kozlowski and Pallardy, 1997), which may be injured by autumn frosts.

Mitra et al. (1991) reported high sensitivity of walnuts to extremes in winter and summer temperatures as well as to its duration. The plant can tolerate up to -11℃ during deep dormancy without serious damage, but as soon as growth starts after dormancy, low temperature of even 2 or 3℃ severely injures leaves, shoots and flowers resulting in crop failure. In the same manner, plants which continue the growth till late fall are subjected to serious foliage damage by frost. However, frost tolerance varies with varieties. The Eureka group has been reported to be most susceptible to frost injury (Serr, 1969). Carpathian walnut race, grown in colder regions, is highly cold tolerant during dormancy but is less productive (Ramos et al., 1984). Several methods have been proposed for selecting frost tolerant genotypes in stone fruit species, such as visual observations (Szabó et al., 1996) and ionic leakage measurements (La Porta et al., 1994). These techniques successfully discriminated between frost-resistant and frost-sensitive walnut genotypes, but they were used on a limited number of genotypes. The subject is confined to aerial parts of walnut trees. Hence, the major climatic factor associated with walnut frost injury in northeast Iran is the severity of winter, i.e., low temperatures during January, February, and March.

The main objective of this research was to evaluate and identify cold hardiness of 12 walnut genotypes with a special emphasis on relative ionic leakage and visual observation for measuring hardiness characteristics.

Materials and Methods

Experiments were carried out using 12 walnut genotypes (K21B5/13, K21B5/15, K21B6/17, K21B6/18, K28B2/5, K28B2/6, K23B5/13, K23B5/14, K21B3/8, K21B9/25, K28B3/8, and K21B1/3) that were grafted in 1986 on seedling rootstocks at the genotype collection (36° 17′ N and 59° 36′ E and 1,012 m altitude) of Khorasan Agricultural Researches and Natural Sources Center, Mashhad, Iran. Plant materials were tested several weeks after winter injury (-22℃). Sixty buds per genotype from five trees were dissected and their injury rate determined and relative ionic leakage measured. In addition, 1- and 2-year-old shoots of about 30 cm long were randomly collected on February 1, 2008 from mature trees and the rates of injury were determined by ionic leakage.

Electrolyte leakage was measured using a digital conductivity meter (Jenway 4310, Tokyo, Japan) in a laboratory at Ferdowsi University of Mashhad, Iran. Ionic (K^+) distributions in cell material of different parts were also monitored. Ionic leakage percentage of different parts of walnut tree as reproductive buds, vegetative buds, and annual and biennial shoots were assayed in order to determine the correlation between ionic leakage and tissues browning. After removing the scales, buds and shoot (10 mm in length) pieces were separately shaken. Electrolytic conductivity (C1) was measured using a digital conductivity meter. Solutions and samples were then autoclaved. After cooling down the solution, conductance was again measured (C2). Ionic leakage (C %) was expressed as the percentage of the final reading (C % = C1/C2 × 100).

Signs of freezing injury could be detected by visual examination of thawed tissue samples or intact plants. The categories recorded were uninjured, partially injured, and injured. There are 3-5 primordia in flower buds of stone fruits. When not all of the primordia in a flower bud were brown, it was called 'partially injured'. Injured flowers were those which had brown pistils and or stamens as shown by Szabó et al. (1996). All genotypes were represented by ten fruiting shoots, which served to assess frost damage. The buds were dissected longitudinally and when examined that the whole primordium turned brown, it was judged to be frozen. In addition to weather conditions, soil characteristics have determinant role in survival of plants. The major soil characteristics of the studied orchard were analyzed to three depths from the surface (Table 1).

The statistical analysis and Duncan's multiple range test (DMRT) for electrolyte leakage were done using MSTAT-C software.

Results and Discussion

Climatic data related to December till March of three

Table 1. Physico-chemical characteristics of soil in tested orchard (Khorasan Agricultural and Natural Sources Researches Center, Mashhad, Iran).

Soil depth (cm)	pH	EC ($dS \cdot m^{-1}$)	O.C	Sand	Silt	Clay	N	P	K	Fe
			%					ppm		
0-20	7.7	1.16	0.19	49	39	12	0.021	5.2	73	4.40
20-40	7.7	1.21	0.21	52	36	12	0.015	5.2	67	4.42
40-60	7.7	1.77	0.52	61	25	14	0.045	19.2	197	3.64

consecutive years from 2006 to 2008 in Khorasan, Iran are shown in Fig. 1. The minimum temperature on winter of 2008 was about -22℃. The freeze-injured buds were characterized by both relative ionic leakage and visual observation compared to non-injured samples. Data are presented separately for reproductive and vegetative buds and annual and binnial shoots in 12 walnut genotypes. The incidence of winter injury varied with genotypes in 2008. The number of male and female flower buds containing brown primordia was high in K21B5/15.

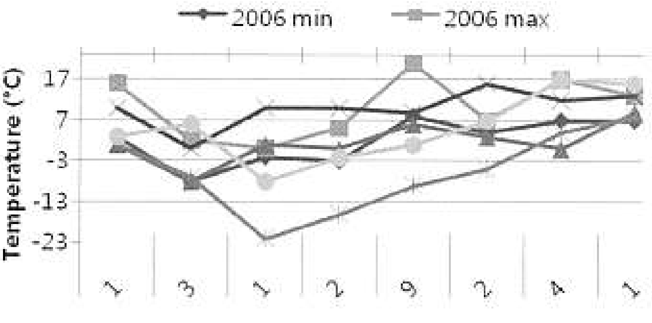

Fig. 1. Minimum and maximum daily temperatures during December to March of three consecutive years from 2006 to 2008 recorded in Khorasan Agricultural Researches Center in Mashhad, Iran.

The damaged female flower buds were determined by visual observations (Table 2). In all tested genotypes, visual symptoms of frost injury were observed. Maximum percentage of injured flower buds was 90% in female flower buds. Relative ionic leakage of female flower buds in K21B9/25, K23B5/14, and K23B5/13 were significantly lower than those in other genotypes. Highest relative ionic leakage was observed in K21B5/15 (Fig. 2A). With the results of ionic leakage and visual observation of freeze injury, it can be concluded that female flowers in K21B5/15, K21B5/13, K21B6/17, K21B6/18, and K21B1/3 were much more susceptible to freeze condition than the other genotypes, while K21B9/25, K23B5/14 and K23B5/13 were much more resistant to this condition.

In male flower buds, the lowest relative ionic leakages were observed in K21B9/25, K23B5/13, and K23B5/14, and while the highest ionic leakages were observed in K21B5/15

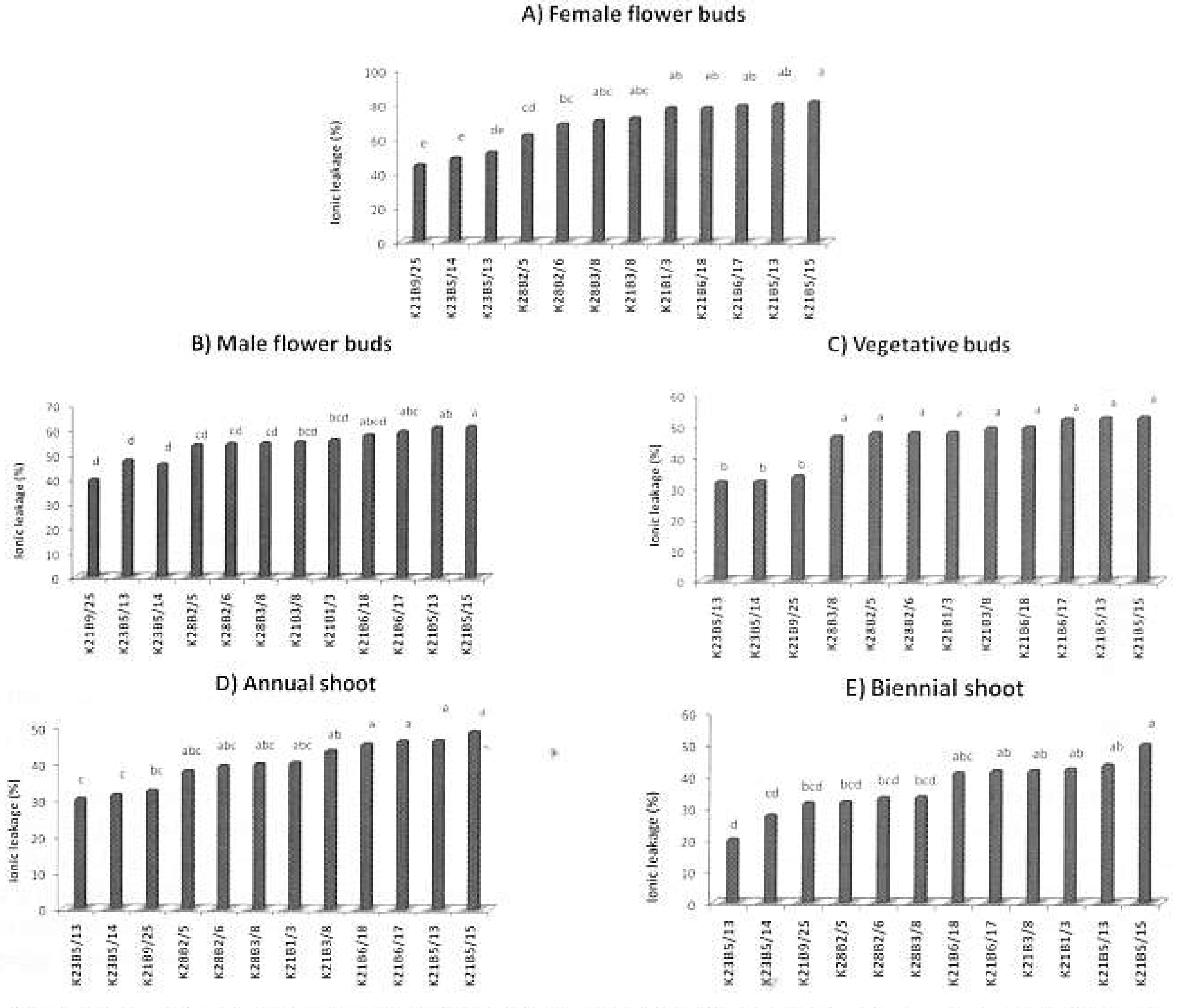

Fig. 2. Relative ionic leakage of female (A) and male flowers (B), vegetative buds (C), and annual (D) and biennial shoots (E) of 12 walnut genotypes. Mean separation within genotypes by DMRT at $P \leq 0.05$.

Table 2. Frost injury on female and male flower and vegetative buds of 12 walnut genotypes. The injury was visually estimated on February 1, 2008.

Genotype No.	Frost injury (%) on female flower buds	Frost injury (%) on male flower buds	Frost injury (%) on vegetative buds
K21B5/13	60.2 ab[z]	60.4 abc	50.4 ab
K21B5/15	90.0 a	90.0 a	70.2 a
K21B6/17	60.2 ab	70.0 ab	50.2 ab
K21B6/18	60.2 ab	70.0 ab	60.2 ab
K28B2/5	40.4 bc	20.6 de	30.4 bc
K28B2/6	40.4 bc	30.6 cde	30.4 bc
K23B5/13	10.8 c	10.8 e	1.0 c
K23B5/14	10.8 c	10.8 e	1.0 c
K21B3/8	60.2 ab	70.0 ab	50.2 ab
K21B9/25	10.8 c	1.0 e	1.0 c
K28B3/8	60.2 ab	80.0 ab	40.2 ab
K21B1/3	60.2 ab	50.0 bcd	60.0 ab

[z] Mean separation within columns by DMRT at $P \leq 0.05$.

and K21B5/13. Significant difference was observed in ionic leakage of male flower buds among the genotypes (Fig. 2B). Maximum percentage of injured male flower buds was observed in K21B5/15 and K28B3/8, while minimum percentage at K23B5/13, K23B5/14, K28B2/5, and K21B9/25 (Table 2).

Vegetative buds of several walnut genotypes may withstand -22℃ during the deep dormancy period if trees were planted in a favorable site and are in a good healthy condition. In K21B9/25, K23B5/13, and K23B5/14, ionic leakages were similar to each other, but were significantly lower than the rest genotypes tested (Fig. 2C). Maximum percentage of injured buds was measured in K21B5/15, K21B6/18, and K21B1/3 (Table 2).

Maximum and minimum ionic leakages of annual shoots were observed in K21B5/15 and K23B5/13, respectively. In spite of annual shoots which showed higher resistance than reproductive and vegetative buds, they were also damaged by low temperature. For example, K21B5/15 had high relative ionic leakage (Fig. 2D). In the woody parts (xylem) of shoots and branches, ice formation occurred, whereas in the bark tissue (phloem), dehydration killed the cell (Ashworth et al., 1983). Symptoms of chilling injury may include surface lesions, tissue breakdown and ion leakage (Bramlage and Meir, 1990; Morris, 1982).

The lowest ionic leakage was observed in biennial shoots in all tested genotypes except K21B5/15, which showed higher relative ionic leakage than the others (Fig. 2E).

The freezing tolerance of woody plants, as measured by ionic leakage after a freeze-thaw event, is influenced by the freezing temperature, cultural environment before test, cultivar, and test time (Eugénia et al., 2003). In our experiment, good relation was found between ionic leakage measurements and visual evaluations, which confirms the use of ionic leakage measurements as a method to analyze freeze tolerance of woody crops. Comparison of relative ionic leakage in various genotypes and in various parts of the walnut trees indicates that the most sensitive organ of plants are female buds in the next order male buds, vegetative buds, annual shoots, and finally biannual shoots. Phenological development is considered to be the most important factor contributing to the hardiness of woody plant buds (Rodrigo, 2000). It seems that development possess in female flower buds is faster than male and vegetative buds before the sudden cold occurred on February 1, 2008.

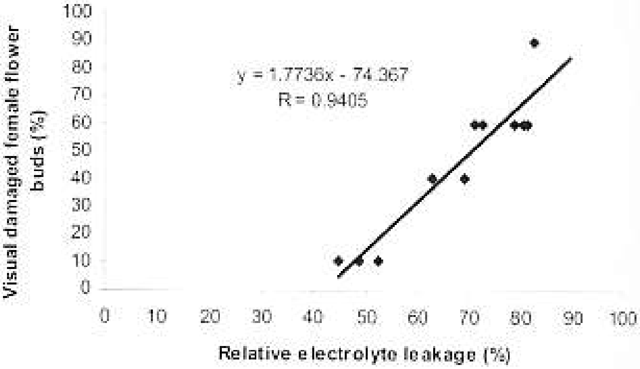

Fig. 3. Correlation between relative electrolyte leakage and damaged flower buds in 12 walnut genotypes (R = 0.94).

The correlation between electrolyte leakage values and visual observation of damaged flower buds (Fig. 3) was determined with a correlation coefficient (R = 0.94 ± 0.08) and the coefficient of determination ($R^2 = 0.88$) indicating a strong relation between electrolyte leakage values and visual observation. More damaged female flower buds showed more percentage of relative ionic leakage.

Conclusion

We identified frost resistant walnut genotypes within the studied genotypes. They were K21B9/25, K23B5/13, and K23B5/14. We suggest that to achieve a more comprehensive determination of the cold hardiness, trials will be needed in at least three growing areas over a period of at least 3 years. In some instances, the percentage cold damage was highly variable among trees within the same genotype. This variability in damage percentages may be due to different conditions of trees, and/or the microclimate of the particular locations.

The sudden cold caused reproductive and vegetative buds injury and loss in some walnut genotypes. Flower buds were the most sensitive part and had the most damage in comparison with other organs. There was a direct correlation between increasing ionic leakage and sensitivity to cold. Finally, environmental factors such as temperature inhibit plant growth and its survival.

Literature Cited

Ashworth, E.N., D.J. Rowse, and L.A. Billmyer. 1983. The freezing of water in tissues of apricot and peach and the relationship to freezing injury. J. Amer. Soc. Hort. Sci. 108:299-303.

Bigras, F.J. and A.L. D'Aoust. 1993. Influence of photoperiod on shoot and root frost tolerance and bud phenology of white spruce seedlings (*Picea glauca*). Can. J. For. Res. 23:219-228.

Bramlage, W.J. and S. Meir. 1990. Chilling injury of crops of temperate origin. p. 37-49. In: C.-Y. Wang (ed.). Chilling injury of horticultural crops. CRC Press, Boca Raton, FL, USA.

Cannell, G.F. and L.J. Shephard. 1982. Seasonal changes in the frost hardiness of provenances of *Picea sitchensis* in Scotland. Forestry 55:137-153.

Chen, P.M., P.H. Li, and M.J. Burke. 1977. Induction of frost hardiness in stem cortical tissues and water status in plants and soil. Plant Physiol. 59:236-239.

Christersson, L. 1978. The influence of photoperiod and temperature on the development of frost hardiness in seedlings of *Pinus sylvestris* and *Picea abies*. Physiol. Plant. 44:288-294.

D'Aoust, A.L. and S.E. Cameron. 1982. The effect of dormancy induction, low temperatures, and moisture stress on cold hardening of containerized black spruce seedlings. Proc. Can. Containerized Tree Seedling Symp. p. 153-156.

Eugénia M., S. Nunes, and G.R. Smith. 2003. Electrolyte leakage assay capable of quantifying freezing resistance in rose clover. Crop Sci. 43:1349-1357.

Fuchigami, L.H., D.R. Evert, and C.J. Weiser. 1971. A translocatable cold hardiness promoter. Plant Physiol. 47:164-167.

Grace, J. 1987. Climatic tolerance and distribution of plants. New Phytol. 106 (Suppl.):113-130.

Grace, J. 1988. Temperature as a determinant of plant productivity. p. 91-107. In: S.P. Long and F.I. Woodward (eds.). Plants and temperature. Dept. of Zool., Univ. of Cambridge, Cambridge, UK.

Greer, D.H. 1983. Temperature regulation of the development of frost hardiness in *Pinus radiata*. Austral. J. Plant Physiol. 10:539-547.

Greer, D.H. and I.J. Warrington. 1982. Effect of photoperiod, night temperature, and frost incidence on development of frost hardiness in *Pinus radiata*. Austral. J. Plant Physiol. 9:333-342.

Gusta, L.V. and C.J. Weiser. 1972. Nucleic acid and protein changes in relation to cold acclimation and freezing injury of Korean boxwood leaves. Plant Physiol. 49:91-96.

Howell, G.S. and C.J. Weiser. 1970. The environmental control of cold acclimation in apple. Plant Physiol. 45:390-394.

Kozlowski, T.T. and S.G. Pallardy. 1997. Growth control in woody plants. Academic Press, San Diego, CA, USA.

La Porta, N., M. Zacchini, S. Bartolini, R. Viti, and G. Roselli. 1994. The frost hardiness of some clones of olive cv. Leccino. J. Hort. Sci. 69:433-435.

Mitra, S.K., D.S. Rathore, and T.K. Bose. 1991. Temperate fruits. Horticulture and Allied Publ., Chakraberia Lane, Calcutta, India.

Morris, L.L. 1982. Chilling injury of horticultural crops: An overview. HortScience 17:161-162.

Muthalif, M.M. and L.J. Rowland. 1994. Identification of dehydrin like proteins responsive to chilling in floral buds of blueberry (*Vaccinium*, section *Cyanococcus*). Plant Physiol. 104:1439-1447.

Ramos, D.E., G. McGranaham, and L. Hendricks. 1984. Walnuts. Fruit Var. J. 38:112-120.

Rodrigo, J. 2000. Spring frosts in deciduous fruit trees: Morphological damage and flower hardiness. Sci. Hort. 85:155-173.

Sakai, A. and W. Larcher. 1987. Frost survival of plants: Responses and adaptation to freezing stress. Springer-Verlag, Berlin, Germany.

Serr, E.F. Jr. 1969. Persian walnuts in the western states. p. 240-263. In: R.A. Jaynes (ed.). Handbook of North American nut trees. Northern Nut Growers Association, Knoxville, TN, USA.

Silim, S.N. and D.P. Lavender. 1994. Seasonal patterns and environmental regulation of frost hardiness in shoots of seedlings of *Thuja plicata*, *Chamaecyparis nootkatensis*, and *Picea glauca*. Can. J. Bot. 72:309-316.

Skroppa, T. 1991. Within-population variation in autumn frost hardiness and its relationship to bud-set and height growth in *Picea abies*. Scand. J. For. Res. 6:353-364.

Szabó, Z., M. Soltész, and J. Nyéki. 1996. Frost injury to flower buds and flowers of cherry varieties. Acta Hort. 410:315-321.

Timmis, R. and Y. Tanaka. 1976. Effects of container density and moisture stress on growth and cold hardiness of Douglas-fir seedlings. For. Sci. 22:167-172.

Wildung, D.K., C.J. Weiser, and H.M. Pellett. 1973. Temperature and moisture effects on hardening of apple roots. HortScience 8:53-55.

Wisniewski, M. and R. Arora. 1992. Adaptation and response of fruit trees to freezing temperatures. p. 299-320. In: A.R. Biggs (ed.). Cytology, histology, and histochemistry of fruit tree diseases. CRC Press, Boca Raton, FL, USA.

Yelenosky, G. 1979. Water-stress induced cold hardening of young citrus trees. J. Amer. Soc. Hort. Sci. 104:270-273.