Climatic Constraints Drive the Evolution of Low Temperature Resistance in Woody Plants

Walter LARCHER[†]

(Institute of Botany, University of Innsbruck, A – 6020 Innsbruck, Austria)

Abstract

Cold and frost are stress factors of widespread occurrence for plants. They damage woody plants due to chilling temperatures in the tropics, by freezing sensitive plants in regions with episodic frosts and temperatures down to –10 °C, and by freezing of even tolerant plants in regions with cold winters below –10 and –40 °C. The wide range of frost resistances in plants from different climatic zones indicates that the ability to become hardened to freezing has evolved in a stepwise process. There are opinions how an evolutionary acquisition of freezing tolerance could have taken place in the following way: First there is a differentiation of better adapted ecotypes and in the long term a series of functional plant groups develop along climatic gradients. Frequent climatic stress and climatic changes stimulate the selection of new genotypes resistant to climatic extremes. Furthermore, plants exposed to multiple stress factors in their habitat acquire a general cross–tolerance to climatic constraints.

The objective of this review is the understanding of low temperature resistance in woody plants, *e.g.* stabilization of biomembranes by lipid composition, deep supercooling, and ability to become freezing tolerant during winter dormancy. An over a hundred year long history of investigating frost stress and survival of different plant species has brought about an important understanding of basic processes. Recently antifreeze proteins which slow the freezing processes, and proteins, which protect the protoplasm against low temperature, frost and dehydration have been discovered. Findings that help to explain the mechanisms for coping with low temperature will be applicable to agriculture, horticulture, forestry and bioclimatology.

Key words: Acclimation, Evolutionary adaptation, Freezing–induced dehydration, Frost tolerance, Photoperiod, Protection proteins, Supercooling, Winter dormancy.

1. Introduction

Low temperature and drought are the most important wide reaching climatic factors affecting growth, production capacity and distribution of plants. Woody plants can produce phytomass and evolve phylogenetically most successfully in the humid tropics. Consequently, species phytodiversity is highest in the frost free tropical zone (approximately 1500 – 5000 different species per 10^4 km²; Barthlott *et al.*, 1996). The density of phytodiversity in climatic zones with episodic frosts and annual minimum temperatures down to – 10 °C (see Fig. 1) is already reduced to 1000 – 1500 species per 10^4 km². In the temperate zone where winter temperatures can be below – 20°C phytodiversity is only 500 – 1000, and in the boreal zone 200 – 500 per 10^4 km².

Low winter temperatures can be responsible for the latitudinal and altitudinal limits of woody plants. It is very probable that low winter temperatures are responsible for the northerly limits of distribution of some species of broad – leaved evergreen woody plants originating in subtropical and temperate climates (Sakai and Larcher, 1987). Spring frost has been considered a limiting factor in the distribution of various deciduous forest trees. Between the Tertiary and the Quaternary period the climate became cooler and from the Pleistocene onwards the selection pressure on plant species was enhanced. During this period many species became extinct. However, frequent climatic stress

and climatic change stimulate the selection of new genotypes resistant to climatic extremes.

The objective of this review is the understanding of basic processes of low temperature stress and the evolutionary adaptation of resistance in woody plants. 'Adaptation' is the response of plants to changing environmental conditions; it may be transitory (phenotypic) or permanent (genotypic). 'Evolutionary adaptation' is used here in the sense of genetically determined.

The first descriptive investigations of frost damage and freezing resistance in different plant species were carried out very early *e.g.*, Sachs (1860), Müller – Thurgau (1886), Molisch (1897). From the middle of the last century onwards intensive analytical investigations of cytological, biophysical and biochemical mechanisms were made on plant organs and tissues (*e.g.*, Asahina, 1956; Heber, 1968; Siminovitch *et al.*, 1968; Krasavtsev, 1972; Burke *et al.*, 1976), and general conclusions were drawn from these results (*e.g.*, Levitt, 1980; Tumanov, 1979). Recently our knowledge of low temperature stress and frost resistance has been advanced due to molecular biological methods. Nowadays certain polypeptides and proteins which trigger controlled freezing and which protect the protoplasm against low temperature, frost and dehydration are known. All findings that help to explain mechanisms for coping with low temperature will have applications in bioclimatology, agriculture, horticulture, forestry and breeding.

2. The Occurrence of Cold and Frost and Survival Capacity under Low Temperatures

Cold and frost are stress factors of widespread occurrence for plants. Low temperatures increase zonally with rising geographical latitude, regionally with distance from the coast, and altitudinally in mountains and highlands. The only places where air temperatures do not sink below +5 °C are the continuously warm, humid lowland regions of the Amazon Basin, the Congo Basin and parts of southeast Asia (Fig. 1). Only one third of the total land area is absolutely safe from frost. For 64% of the land mass the mean annual minimum air temperature is below 0 °C. This is made up of 15% where the minimum can be as low as – 10 °C, 15% where it can be as low as – 30 °C, and 34%
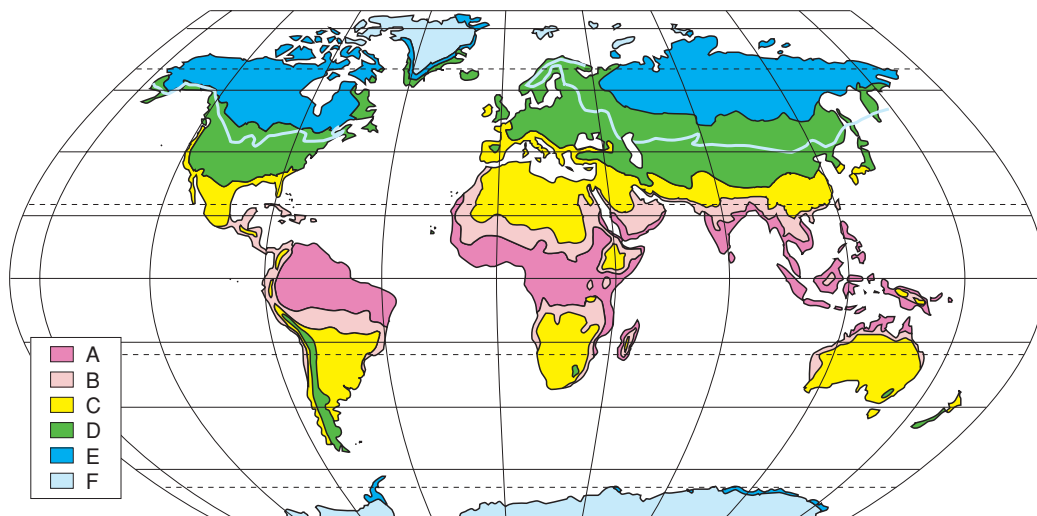

Fig. 1. Occurrence of low temperatures and frost on earth. *A* Annual minimum temperatures above +5 °C; *B* annual minimum subzero temperatures; *C* episodic frosts with temperatures down to – 10 °C; *D* regions with cold winters and mean annual minimum temperatures between – 10 and – 40 °C (white lines – 30 °C minimum isotherm); *E* mean annual minimum temperatures below – 40 °C; *F* polar ice. The zones correspond to the areas of plant species with different types of frost resistance. *Zone A* Chilling sensitive plants of the equatorial tropics; *zone B* extremely freezing sensitive plants; *zone C* freezing sensitive plants protected by effective supercooling; *zone D* plants with limited freezing tolerance and trees with wood capable of deep supercooling; *zone E* completely freezing tolerant plants. After Larcher (2003). For detailed regional frost zone maps of Europe see Heinze and Schreiber (1984) and of N. America see U. S. National Arboretum (1965); Ouellet and Sherk (1967); and DeGaetano and Shulman (1990).

where it can be below − 30 °C (Hoffmann, 1960). In E. Siberia, in inland Alaska and NW. Canada, where trees still grow, the air temperature regularly sinks to − 50 °C. The absolute temperature minimum is approximately − 70 °C in East Siberia.

Whether frost occurs episodically or periodically in the course of the seasons is of considerable importance. Episodic frosts during brief spells of cold weather are frequent in the tropical semideciduous and montane forests and in subtropical regions. In the temperate climatic zone frosts may occur as late frosts in spring or as early frosts in autumn, usually reaching minimum temperatures around − 5 °C. These frosts can be dangerous for woody plants, which are caught unprepared at sensitive, unhardened phases in their life. In climatic zones with cold winters, the plants prepare for periodically cold temperatures by cessation of growth and by gradually starting the hardening process. Only in extremely cold winters, which occur at intervals of decades in the northern hemisphere, does the natural

vegetation suffer freezing damage. Cultivated plants which are often planted at the extreme limits for their survival (*e.g.*, fruit trees, vines, and particularly citrus and exotic fruits), frequently suffer quite extensive damage from frost.

Woody plants from various climatic zones vary in their susceptibility to cold and frost and have different strategies to survive low temperatures. In the tropical rainforests some species are damaged by exposure to low temperatures of above 0 °C, *e.g.*, tropical horticultural plants, mangroves and leaves of rain forest trees, shrubs and lianas (Biebl, 1964; Markley *et al.*, 1982; Bodner and Larcher, 1987). In contrast, the trees and shrubs in boreal conifer forests and subarctic woodlands survive winter temperatures of − 30 °C or below and remain frozen for 3 to 4 months or more, *e.g.*, exceptional species of *Betula*, *Populus*, *Salix*, *Larix*, *Abies*, *Picea* and *Pinus* (George *et al.*, 1974; Burke *et al.*, 1976; Sakai, 1983). Between these two extremes there is an enormous functional diversity of woody

Table 1. Frost resistance of woody plants from different climatic regions in the hardened state (threshold temperatures with injuries, in °C). These data are based on laboratory experiments and on controlled tests under field conditions. Modified after Larcher (2003).

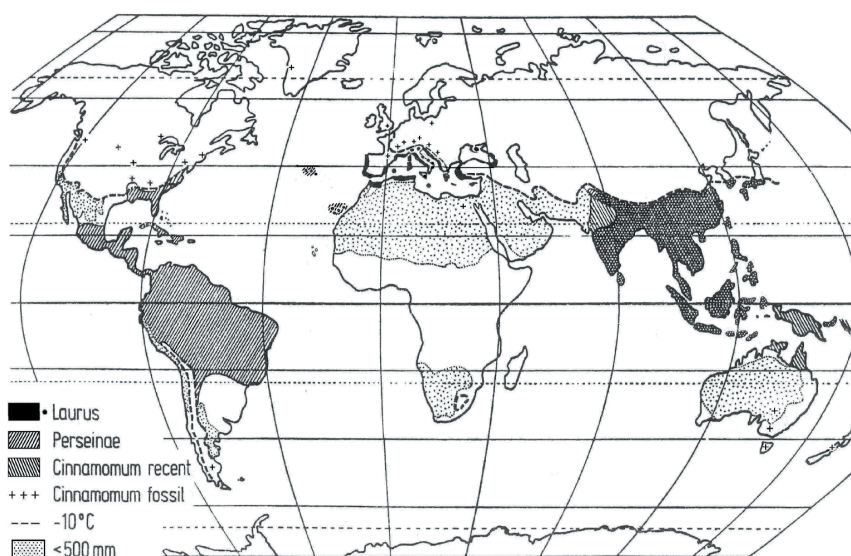| Climate zones and woody vegetation	Leaves	Shoot buds	Twigs and stems	Roots
Tropical climates				
Rainforests (lowland)[a]	> 0 to − 3	> 0 to − 3	> 0 to − 5	
Tropical mountain forests	− 3 to − 8 (− 12)	− 4 to − 6	− 5 to − 10	
Monsoon forests	− 5 to − 6	− 8 to − 10	− 15	
Palm leaves	− 3 to − 10 (− 14)			
Extra− tropical climates				
Evergreen (laurophyllous) trees	− 4 to − 7	− 5 to − 10	− 8 to − 12	
Sclerophyllous woody plants	− 6 to − 12	− 8 to − 15	− 10 to − 15 (− 20)	> − 5
Temperate climates with mild winters				
Evergreen broad− leaved trees	− 10 to − 15 (− 20)	− 10 to − 20	− 15 to − 25	− 5 to − 10
Southern hemisphere conifers	− 10 to − 20		− 15 to − 25	
Temperate climates with cold winters				
Conifers	− 20 to − 25	− 25 to − 30	− 25 to − 40	− 10 to − 20
Deciduous broadleaved trees		− 25 to < − 35	− 30 to − 50	− 15 to − 25
Boreal and alpine climates				
Conifers	− 40 to < − 70	− 40 to < − 70	− 50 to < − 70	− 20 to − 30
Deciduous trees and shrubs		− 30 to < − 50	< − 40	

[a] > 0°C = damage above freezing point

Fig. 2. All evergreen members of the Lauraceae are freezing – sensitive and restricted to regions with mild winters. Foliar frost resistances of *Cinnamomum spp.* are -7 to -10 °C, of *Laurus spp.* -9 to -12 °C; among the Perseine resistances of *Persea spp.* are -6 to -8 °C and *Machilus* -10 to -12 °C. Broken line: mean annual minimum temperature at -10 °C; dotted area: arid regions below 500 mm precipitation.

Table 2. Frost resistance after hardening of the genus *Nothofagus.*
Data by (A) Read and Hope (1989), (B) Sakai and Wardle (1978), (C) Alberdi *et al.* 1985.

Species	*Distribution*	*Altitude*	*MTCM*	*Leaves*	*Buds*	*Author*
Evergreen species						
N. grandis	New Guinea 5°30'S	1950 m	11.0	-6.0^a		(A)
N. pullei	New Guinea 6°10'S	2300 m	9.1	-7.5^a		(A)
N. moorei	NE Australia 31°10'S	1050 m	-0.4	-8.5^a		(A)
N. moorei	NE Australia 31°50'S	1500 m	-1.6	-9.1^a		(A)
N. cunninghamii	S Australia 37°40'S	600 m	1.9	-10.4^a		(A)
N. cunninghamii	Tasmania 42°40'S	980 m	-1.2	-11.6^a		(A)
N. dombeyi	Chile 40°40'S	500 m		-8.5^a	-10	(C)
N. dombeyi	Chile 40°40'S	1020 m		-10.5^a	-13.5	(C)
N. fusca	New Zealand 46°30'S	230 m	-2.7	-8^b	-10	(B)
N. solandri	New Zealand 46°30'S	1200 m	-4.4	-13^b	-15	(B)
Deciduous species						
N. gunnii	Tasmania 42°S	1000 m			-17^b	(B)
N. pumilio	Chile 40°50'S	1040 m	-7.5		-18^a	(C)
N. antarctica	Chile 40°30'S	220 m	-3.0		-17.5^a	(C)
N. antarctica	Chile 40°30'S	1080 m	-7.5		-22^a	(C)

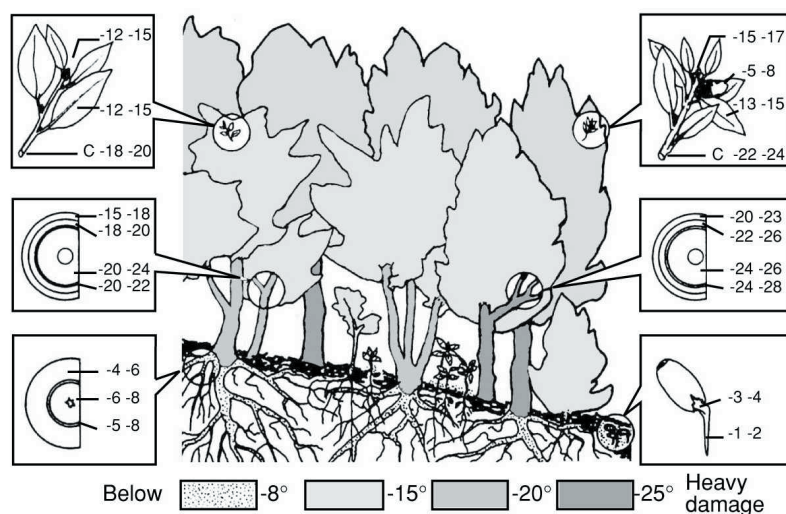MTCM: mean minimum temperature of the coldest month

[a] LT_{50} (at °C) = temperature at 50% lethality

[b] LT_i (at °C) = incipient killing temperature at which initial damage occurs

Table 3. Frost resistance (LTi at °C) of evergreen (E) and deciduous (D) species of the genus Quercus. From numerous authors, updated in Sakai and Larcher (1987).

Species	Leaves	Buds	Xylem	Northern limits of distribution
Evergreen oaks:				
Q. virginiana	− 8	− 8	− 8	Mississippi region [ca 32°N]
Q. suber	− 7	− 16	− 20	Mediterranean region [42°N]
Q.coccifera	− 9	− 13	− 20	Mediterranean region [42°N]
Q. ilex	− 12	− 15	− 25	Mediterranean region [44°N]
Q. gilva	− 10	− 10	− 13	Japan [35– 36°N]
Q. phillyraeoides	− 10 to − 13	− 13	− 15 to − 17	Japan [35°N; 700 m a.s.l.]
Q. myrsinaefolia	− 15	− 15	− 18	Japan [36°N]
Q. salicina	− 15	− 15	− 20	Japan [36°N]
Q. glauca	− 15	− 17	− 18 to − 20	Japan [36°N; 1000 m a.s.l.]
Q. acuta	− 15	− 17	− 20	Japan [36°N; 1200 m a.s.l.]
Deciduous oaks:				
Q. lyrata		− 20	− 20	Mississippi region [34°N]
Q. serrata		− 25	− 27	Japan [42°N; 1600 m a.s.l.]
Q. aliena		− 30	− 30	Japan [42°N]
Q. pubescens		− 25	− 35	S. Europa [48°N]
Q. robur		− 27		Central Europa [53°N]
Q. mongolica var. grosseserrata		− 30	− 30	Japan [45°N; 2300 m a.s.l.]
Q. mongolica		− 50	− 35	Vladivostok [ca 43°N]
Q. crispula		− 35 to − 50	− 35	Sakhalin [48°N]
Q. rubra			− 40 to − 45	NE. America [45°N]
Q. macrocarpa		< − 60	− 45	S. Canada [48°N]

Fig. 3. Frost resistance of a population of *Quercus ilex*. Numbers in the margin represent the threshold temperatures above which no damage occurs (number on the left) and below which 50% of frost damage is to be expected (numbers on the right). After Larcher and Mair (1969).

plants with survival capacity under low temperatures varying over latitudinal gradients (Table 1).

There are many plant families, such as palm species, whose members are confined to a certain climatic zone. Other taxa (families, genus, species or ecotypes) have advanced by adaptive radiation from their centres of distribution in the tropical regions to the subtropical regions with minimum temperatures of $-12\ °C$ (*e.g.*, Lauraceae; Fig. 2). The tropical genus *Ficus* can even be found in the warm temperate climatic zone (*e.g.*, *Ficus carica*; Hummel and Johnson, 1985). There are also genera such as *Nothofagus* (Table 2) or *Quercus* (Table 3) that are widely distributed through the tropical zone, over the low temperate and even into the boreal zone.

Differences in the frost resistance of the organs of a plant can be important (Fig. 3). After heavy frosts, foliage of some trees may be partly frozen whereas their buds and stems suffer little damage. These trees behave like deciduous species and completely renew their foliage at the end of winter.

3. Injury Patterns of Cold and Frost and Acquisition of Resistance to Low Temperatures in Different Plants

Low temperatures causes several patterns of damage in woody plants. Chilling – sensitive plants in the tropics suffer lethal injuries at low temperatures of only a few degrees above the freezing point (Fig. 4). In the case of chilling injury, it generally takes some days or even weeks for the full extent of the damage to develop. Cellular signs of chilling injury are chloroplast swelling, a distortion of thylakoids, a reduction in the size and number of starch granules, and the formation of small vesicles in the chloroplast envelope. The primary effect is the transition of the lipid components of the biomembranes from a fluid – crystalline to a gel – like state, and the separation of membrane proteins (Lyons 1973; Van Hasselt, 1974). Biomembranes are also damaged by photoinhibition and reactive oxygen species (Taylor and Rowley, 1971). The selectivity of permeation processes is decreased which leads to imbalances of metabolites and ions between the various cell compartments and to the diffusion of the cellular contents to the outside of the cell. As metabolic processes no longer occur and the energy yield is reduced toxic products accumulate. With the loss of cell compartmentalisation the cell deteriorates and dies.

At subzero temperatures, ice forms first in those parts of the plant that cool down fastest and freeze most readily, these are peripheral tissues and the vascular bundles. Water that has condensed in the intercellular spaces also freezes. Biomembrane rupture is the cause of damage by freezing (Sikorska and Kacperska, 1982; Hincha *et al.*, 1987). The freezing behavior of tissues and cells varies (Pearce, 2001) depending on whether lethal ice – nucleation occurs after previous undercooling ("freezing – sensitive") or gradually by extracellular ice formation and freezing – induced cellular dehydration ("freezing – tolerant"). While ice is crystallizing, it has the same effect as dry air because the vapor pressure above ice is lower than that above a cell solution. The process of freezing continues until a thermodynamic equilibrium between the ice and the cell sap is reached; *e.g.*, at a temperature of $-5\ °C$ tissue water potential is $-6\ MPa$, and at $-10\ °C$ it is roughly $-12\ MPa$. Thus, water is withdrawn from the cell, which may shrink to two thirds of its volume. Cell walls partially resist the collapse in cellular volume, creating a divergence from equilibrium and reducing the extent of dehydration (Zhu and Beck, 1991). During dehydration of the protoplasm, ions and organic acids in the remaining unfrozen solutions reach abnormally high concentrations and exert a toxic effect, inactivating enzymes. In the end, there is so much water withdrawn from the cell that, at a certain level of dehydration, the fine structure of the protoplasm is irreversibly destroyed.

Any acclimation (*i.e.*, the nonheritable modification of traits caused by exposure of plants to changes in environmental conditions; Kramer, 1980) to lower temperatures has to guarantee the protection of biomembranes. Membranes are the target of temperature extremes (deHayes *et al.*, 1997; Sung *et al.*, 2003). Calcium influx during cold stress induces Ca^{+2} to bind to calcium – dependent proteins, which interact with other cellular processes and structures, including the cytoskeleton. These Ca^{+2} – bound proteins may stimulate a cascade of proteolytic enzymes that cleave key proteins. Ca^{+2} might also regulate gene expression by acting as a secondary messenger in a signal transduction process (Thomashow, 1999; Kratsch and Wise, 2000; *see* Fig. 6). To render plants chilling – tolerant the composition of lipid molecular species and the ratio between proteins and lipids has to be adequate for membrane fluidity (Palta and Li, 1978; Senser and Beck, 1982). Various cultivated plants with tropical origins have been successfully conditioned to the cold (*e.g.*, Wilson

Fig. 4. Cold damage (spotted) in the exposed cambium of a 3 – year – old stem of *Coffea arabica* after chilling at +1 °C for 2 days.

Fig. 5. Scattered necrosis in a leaf of *Cinnamomum camphora* after undercooling and subsequent freezing. Sclerenchymatic girders surrounding mesophyll islands act as barriers to the propagation of ice crystallization.

and Crawford, 1974; St John and Christiansen, 1976; Ramalho *et al.*, 2003).

Transient undercooling may protect frost – sensitive plants for a few hours against nocturnal reradiation frosts of − 3 to − 5 °C. Persistent supercooling can be maintained for a considerable time. Leathery and sclerophyllous leaves (Fig. 5), palm leaves with siliceous cell walls (Larcher *et al.*, 1991), and xylem ray parenchyma cells in tropical and subtropical stems (Kuroda *et al.*, 1997) can persist in supercooling down to − 10 and − 12 °C. In tissues with densely lignified or cutinized barriers, the propagation of ice is delayed or inhibited, and the cell – wall rigidity can lead to increased cell tension and reduced cell dehydration (Wisniewski and Davis, 1989; Rajashekar and Burke, 1996). Deep supercooling in some flower primordia of winter buds is possible down to − 20 to − 25 °C (*e.g.*, Kaku *et al.*, 1980; Ishikawa and Sakai, 1981), in shoot primordia down to − 20 to − 30 °C, and in wood parenchyma and xylem rays of many forest and fruit trees and shrubs of the temperate zone down to − 30 to − 50 °C (*e.g.*, Quamme *et al.*, 1972; George *et al.*, 1974; Gusta *et al.*, 1983). When the supercooling threshold is crossed, however, the metastable state collapses spontaneously and ice crystals form inside the cells (homogeneous nucleation; Rasmussen and MacKenzie, 1972). The ability to perform persistent supercooling has the advantage of maintaining meta-bolically active cells due to the lack of dehydration stress (Larcher, 2000).

Frost hardening is a system of protection whose development is triggered by a single stress factor but which offers protection against other stress factors (Levitt, 1958; Siminovitch and Cloutier, 1983). One protective factor is antifreeze proteins which inhibit the growth of ice that forms in intercellular spaces (Griffith *et al.*, 1992; Griffith and Yaish, 2004). Below freezing the antifreeze proteins adsorb onto the surfaces of ice and form many small ice crystals. The degree of freezing – tolerance depends on the protection of biomembranes against freezing – induced dehydration (Fig. 6). This is achieved both by changes in membrane lipid composition and by accumulation of substances in the surrounding cytosol. Low – molecular compatible compounds accumulate during acclimation, including soluble carbohydrates, proline and betaines. These compounds stabilize water molecules in case of freezing – induced dehydration and prevent ions from accumulating in the membrane surface (Heber *et al.*, 1979). The acquisition of cross – tolerance in over – wintering plants is based on general stress – related gene products, which induce the synthesis of protection proteins (Thomashow, 1999). Extremely hydrophilic polypeptides (dehydrins; Close, 1996) from the group of late – embryogenesis – abundant proteins
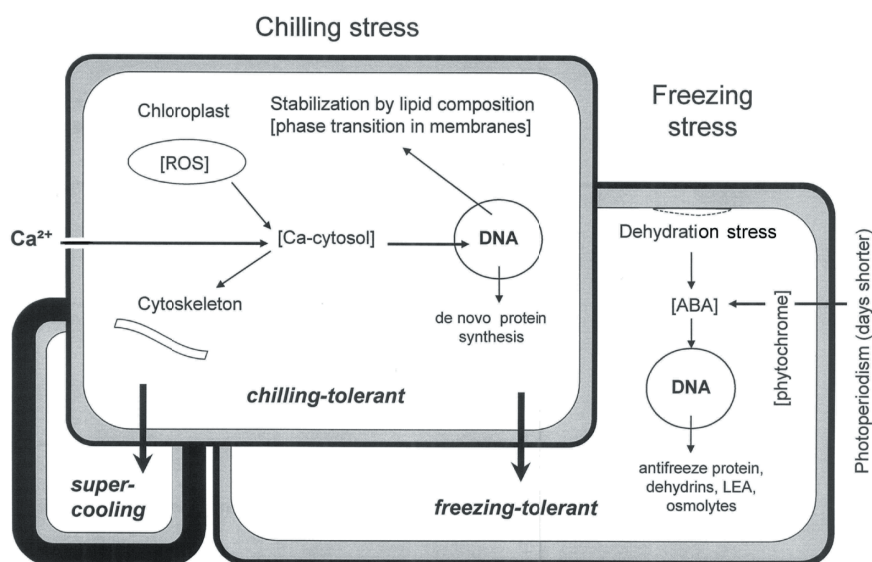
Fig. 6. Schematic representation of acquisition of tolerance to low temperature. Decreasing temperature and shortening days in autumn, and messengers for signal transduction (in square brackets) may induce incorporation of unsaturated fatty acids into biomembranes and synthesis of protection proteins by gene expression. ABA = abscisic acid; Ca^{2+} – cytosol = calcium pools; ROS = reactive oxygen species. Taken from Kratsch and Wise (2000) and Sung *et al.* (2003).

(LEA) accumulate. These dehydrins bind to biomembranes and displace low – molecular solutes.

4. Seasonality of Freezing Tolerance in the Cold Climate Zones

In regions with a seasonal climate, plants switch periodically between low and high levels of resistance. During the most intensive phase of growth, woody plants are extremely sensitive to low temperatures. High levels of freezing tolerance in trees and shrubs can only occur during winter dormancy (Tumanov, 1979; Sakai and Larcher, 1987). However, herbaceous plants, spring geophytes and high mountain plants in particular, can tolerate -6 to -12 °C while growing (Tyurina, 1957; Beck *et al.*, 1982; Taschler and Neuner, 2004).

Seasonal cold acclimation involves a sequence of processes in which each stage is a prerequisite for the next (Table 4). Onset of hardening is induced by short days and lower temperatures of slightly above zero for several days to weeks (Moschkov, 1935; Tumanov *et al.*, 1965; Biebl, 1967; Weiser, 1970; Fig. 7). An increase in concentration of abscisic acid (ABA) triggers gene expression for the synthesis of stress proteins (Powell, 1987; Thomashow, 1999). ABA and dehydrins play an essential role in the photoperiodic

induction of winter dormancy and the acquisition of frost tolerance in the prehardening stage (Bertrand *et al.*, 1997, Welling *et al.*, 1997; Rinne *et al.*, 1998; Fig 8). If freezing tolerance of the protoplasm is regarded as the ability to survive water withdrawal resulting from extracellular ice formation in tissues, it is likely to be coupled with desiccation (dehydrin gene induced drought and freezing tolerance; Robertson *et al.*, 1994). In various species of woody plants freezing tolerance is linked with desiccation tolerance during the dormant phase (Fig. 9).

The protoplasm is now ready for frost hardening. The early stage of acclimation is promoted by daily subzero minimum temperatures for a week (*e.g.*, Tyurina *et al.*, 1978). A higher level of hardening is achieved by uninterrupted exposure to temperatures between -5 and -15 °C. The highest level of hardening is achieved by slow cooling for one month or more at temperatures from -10 to -60 °C. Prolonged exposure to temperatures in this range causes twigs to acquire a state of hardiness which may not commonly be attained in nature; willows, white birches, pines and black currants were artificially hardened by this method and became hardy even at -196 °C or -253 °C (Tumanov *et al.*, 1959; Sakai, 1956, 1960).

Table 4. Stages of the frost hardening process of woody plants from regions with cold winters. After DeHayes *et al.* (1997), Lachaud (1989), Larcher (1971), Sakai and Larcher (1987), Samygin 1974, Siminovitch (1981), Sung *et al.* (2003), Tumanov (1979), Weiser (1970).

Induction of cold acclimation (prehardening stage)

Readiness for cold acquisition: cessation of intensive shoot elongation and cambium growth, accumulation of photosynthates.

Stimulus for acclimation: short–day and gradual temperature decrease.

Cell responses: changes of phytohormones (increase of ABA), differential gene activity, increase in cytoplasmic RNA, synthesis of proteins (dehydrin, antifreeze protein), reduced amount of membrane–located carriers and receptors, wall thickening.

Results: transition to winter dormancy.

Early stages of the hardening process

Readiness for hardiness: winter dormancy has to be accomplished.

Stimulus for acclimation: frosts around $-5°C$.

Cell responses: changes in cytoplasm ultrastructures (biomembrane stability, cytoskeleton), increase in compatible organic compounds (proline, polyols).

Results: depression of freezing point of cell sap and tissue supercooling, acquisition of increased freezing tolerance.

Final stage of the hardening process

Readiness for full hardiness: cells have to show freezing tolerance.

Stimulus for full acclimation: plants exposed repeatedly or continually to temperatures of about $-10°C$ or below.

Cell responses: increased extracellular ice formation and cell shrinkage, mechanisms to stabilize the cell structures, acquisition of tolerance to dehydration.

Results: species–specific maximum level of freezing tolerance.

The ultimate survival mechanism is genetically determined dormancy. Programmed dormancy of woody plants in temperate and cold climate zones is an endogenously regulated and environmentally influenced interruption of growth. There are many different forms and stages of dormancy (Lang, 1996). The typical series of events leading to winter dormancy begins with predormancy. Towards the end of summer, the tips of shoots are transformed into winter buds before the onset of winter. During the transition to winter dormancy the cells become less turgid and the central vacuole splits up into many small vacuoles (Lachaud, 1989; Ristic and Ashworth, 1994). Cell cycle activity is reduced and the mitotic index declines to low levels (Colombo *et al.*, 1989; Mellerowicz *et al.*, 1995). In cold–winter regions of the northern hemisphere complete endodormancy is attained by November or December. At this phase of dormancy, the plants can no longer be activated by temporary warming. The inability of plants to emerge prematurely from their resting state once they have entered complete dormancy is an important ecological factor in resistance, given the unpredictability of the weather.

5. Opinion: Evolution of Freezing Tolerance in Woody Plants

The wide range of frost resistances in from different climatic zones indicates that the ability to become hardened to freezing has evolved in a stepwise fashion along a series of plant types (Larcher, 1971; 1981; Sakai, 1983; Sakai and Larcher, 1987).

Evolutionary adaptation could have occurred in the following way. The first step in the evolution must have been the adaptation of biomembranes, of initially chilling–sensitive plants of the humid tropics, to tolerate lower temperatures above zero (*see* Fig. 6). The next step could have been an improvement in persistent supercooling so temperatures of below zero could be tolerated. This would have allowed the plants to survive moderate frosts down to about -10 to -12
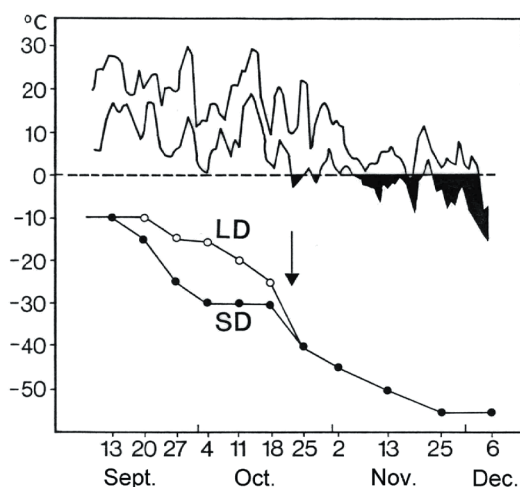
Fig. 7. Seasonal hardening patterns of young apple trees at different day – lengths in the field. SD natural short days in autumn, LD long – day treatment (photoperiod 18h using additional incandescent light). Air temperatures are daily maxima and minima. Arrow: First leaf – killing frost. After Howell and Weiser (1970).
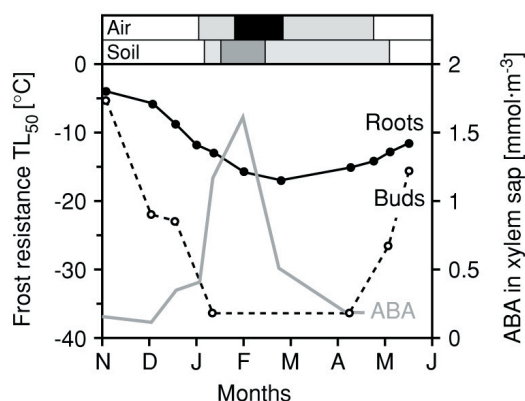

Fig. 8. Time course of frost resistance in buds and roots and increase in abscisic acid (ABA) concentration in the xylem sap during the hardening phase of young *Acer saccharum* plants. Grey bars: Period of temperature minima of the air below 0 °C; black bars: frequent frost events below – 20 °C. Soil at a depth of 20 cm between 0 °C and – 3 °C (light grey) and between – 3 and – 7 °C (dark grey). After Bertrand *et al.* (1997).

°C without injury. In regions with frequent nocturnal frosts throughout the year, especially the tropical highlands and the subtropics, rapid supercooling is the best
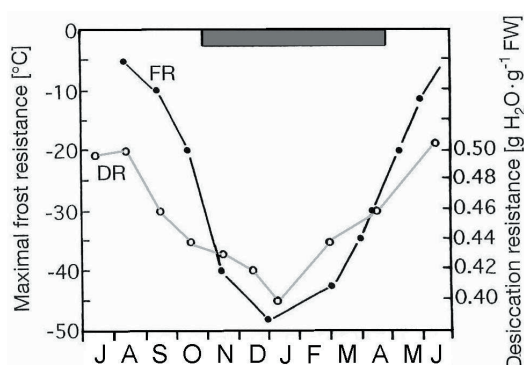

Fig. 9. Synchronous course of frost resistance and resistance to desiccation in needles of *Pinus cembra* growing at the alpine treeline. (FR) frost resistance: temperature at which 5 – 10% of needles are killed. (DR) desiccation resistance: water content of the needles causing 1 – 2% damage to the mesophyll. Bar: period during which frost occurs on more than 15 days per month. After Pisek and Larcher (1954). The coupled acquisition of the two tolerances are programmed during winter dormancy.

compromise between survival and adequate dry matter production during the cold season.

The last step towards achieving freezing tolerance could have been the acquisition of increased dehydration tolerance. Such preadaptations could have occurred in tropical monsoon forests where rainy and dry seasons alternate. As trees in tropical forests with regular drought are deciduous species (Borchert, 1999; Borchert and Pockman, 2005), desiccation during the leafless state appears to enhance frost resistance.

In the intermediate or higher latitudes life – cycles are controlled by photoperiodic and thermoperiodic signals. These ensure the plant is ready for hardening at the right time, so that a high level of freezing tolerance is reached before serious danger from winter cold can be expected.

Recently a genetic analysis has demonstrated that there is an evolutionary separation of freezing behavior in *Cornus* species; all phylogenetically early dogwood species exhibited supercooling behavior and species distributed into the northernmost ranges are freezing and desiccation tolerant (Karlson *et al.*, 2004). This example shows that climatic stress and climatic changes stimulate the development of better adapted ecotypes, varieties, or species along climatic gradients. If long – term changes in climate, woody plants are

able to survive in harsher environments.

Acknowledgments

I am indepted to Prof. Dr. Rolf Borchert (University of Kansas, Lawrence) for valuable suggestions.

References

Alberdi, M., Romero, M., Rios, D., and Wenzel, H., 1985: Altitudinal gradients of seasonal frost resistance in Nothofagus communities of southern Chile. *Acta Oecologica – Oecol. Plantarum*, **6 (20)**, 21 – 30.

Asahina, E., 1956: The freezing process of plant cell. *Contr. Inst. Low Temp. Sci. Sapporo*, **10**, 83 – 126.

Barthlott, W., Lauer, W., and Placke, A., 1996: Global distribution of species diversity in vascular plants: towards a world map of phytodiversity. *Erdkunde*, **50**, 317 – 327.

Beck, E., Senser, M., Scheibe, R., Steiger, H. M., and Pongratz, P., 1982: Frost avoidance and freezing tolerance in Afroalpine "giant rosette" plants. *Plant Cell Environ.*, **5**, 215 – 222.

Bertrand, A., Robitaille, G., Castonguay, Y., Nadeau, P., and Boutin, R., 1997: Changes in ABA and gene expression in cold – acclimated sugar maple. *Tree Physiol.*, **17**, 31 – 37.

Biebl, R., 1964: Temperaturresistenz tropischer Pflanzen auf Puerto Rico. *Protoplasma*, **59**, 133 – 156.

Biebl, R., 1967: Kurztag – Einflüsse auf arktische Pflanzen während der arktischen Langtage (with English summary). *Planta*, **75**, 77 – 84.

Bodner, M. and Larcher, W., 1987: Chilling susceptibility of different organs and tissues of *Saintpaulia ionantha* and *Coffea arabica*. *Angew. Bot.* **61**, 225 – 242.

Borchert, R., 1999: Climatic periodicity, phenology, and cambium activity in tropical dry forest trees. *IAWA J.*, **20**, 239 – 247.

Borchert, R. and Pockman, W. T., 2005: Water storage capacitance and xylem tension in isolated branches of temperate and tropical trees. *Tree Physiol.*, **25**, 457 – 466.

Burke, M. J., Gusta, L. V., Quamme, H. A., Weiser, C. J., and Li, P. H., 1976: Freezing and injury in plants. *Ann. Rev. Plant Physiol.*, **27**, 507 – 528.

Close, T. J., 1996: Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. *Plant Physiol.*, **97**, 289 – 290.

Colombo, S. J., Glerum, C., and Webb, D. P., 1989: Winter hardening in first – year black spruce (*Picea mariana*) seedlings. *Physiol. Plant.*, **76**, 1 – 9.

DeGaetano, A. T. and Shulman, M. D., 1990: A climatic classification of plant hardiness in the United States and Canada. *Agr. Forest Met.*, **51**, 333 – 351.

DeHayes, D. H., Schaberg, P. G., Hawley, G. J., Borer, C. H., Cumming, J. R., and Strimbeck, G. R., 1997: Physiological implications of seasonal variation in membrane – associated calcium in red spruce mesophyll cells. *Tree Physiol.*, **17**, 687 – 695.

George, M. F., Burke, M. J., Pellett, H. M., and Johnson, A. G., 1974: Low temperature exotherm and woody plant distribution. *HortScience* **9**, 519 – 522.

Griffith, M. M. and Yaish W. F., 2004: Antifreeze proteins in overwintering plants: a tale of two activities. *Trends Plant Sci.*, **9**, 399 – 405.

Griffith, M. M., Ala, P., Yang, D, S, C., Hon, W. Ch., and Moffatt, B. A., 1992: Antifreeze protein produced endogenously in winter rye leaves. *Plant Physiol.*, **100**, 593 – 596.

Gusta, L. V., Tyler, N. J., and Chen, H. H., 1983: Deep undercooling in woody taxa growing north of the – 40 °C isotherm. *Plant Physiol.*, **72**, 122 – 128.

Heber, U., 1968: Freezing injury in relation to loss of enzyme activities and protection against freezing. *Cryobiology*, **5**, 188 – 201.

Heber, U., Volger, H., Overbeck, V., and Santarius, K. A., 1979: Membrane damage and protection during freezing. In *Proteins at Low Temperatures* (ed. by Fennema, O. R., Powrie, W. D. and Marth, E. H.). Marcel Dekker, New York, pp. 159 – 189.

Heinze, W. and Schreiber, D., 1984: Eine neue Kartierung der Winterhärtezonen für Gehölze in Europa. *Mitt. Dtsch. Dendr. Ges.*, **75**, 11 – 56.

Hincha, D. K, Höfner, R., Schwab, K. B., Heber, U., and Schmitt, J. M., 1987: Membrane rupture is the common cause of damage to chloroplast membranes in leaves injured by freezing or excessive wilting. *Plant Physiol.*, **83**, 251 – 253.

Hoffmann, G., 1960: Die mittleren jährlichen und absoluten Extremtemperaturen der Erde. II. Ergebnisse. *Meteorol. Abh.* **8**, **(3)**, Reimer, Berlin.

Howell, G. S. and Weiser, C. J., 1970: Similarities between the control of flower initiation and cold acclimation in plants. *HortScience*, **5**, 18 – 20.

Hummel, R. L. and Johnson, C. R., 1985: Freezing tolerance in the genus *Ficus*. *HortScience*, **20**, 287 – 289.

Ishikawa, M. and Sakai, A., 1981: Freezing avoidance

mechanisms by supercooling in some Rhododendron flower buds with reference to water relations. *Plant Cell Physiol.*, **22**, 953 – 967.

Kaku, S., Iwaya, M., and Kunishige, M., 1980: Supercooling ability of Rhododendron flower buds in relation to cooling rate and cold hardiness. *Plant Cell Physiol.*, **21**, 1205 – 1216.

Karlson, D. T., Xiang, Q－Y., Stirm, V. E., Shirazi, A. M., and Ashworth, E. N., 2004: Phylogenetic analyses in *Cornus* substantiate ancestry of xylem supercooling freezing behaviour and reveal lineage of desiccation related proteins. *Plant Physiol.*, **135**, 1654 – 1665.

Kramer, P. J., 1980: Drought, stress, and the origin of adaptations. In *Adaptation of Plants to Water and High Temperature Stress* (ed. by Turner, N. C. and Kramer, P. J.). Wiley, New York, pp. 7 – 20.

Krasavcev, O. A., 1972: *Kalorimetriya rastenii pri temperaturakh nizne nulya.* Izdat. Nauka, Moskva.

Kratsch, H. A. and Wise, R. R., 2000: The ultrastructure of chilling stress. *Plant Cell Environ.*, **23**, 337 – 350.

Kuroda, K., Ohtani, J., and Fujikawa, S., 1997: Supercooling of xylem ray parenchyma cells in tropical and subtropical hardwood species. *Trees*, **12**, 97 – 106.

Lachaud, S., 1989: Participation of auxin and abscisic acid in the regulation of seasonal variations in cambial activity and xylogenesis. *Trees* **3**, 125 – 137.

Lang, G. A., 1996: *Plant dormancy.* CAB International, Wallingford.

Larcher, W., 1971: Die Kälteresistenz von Obstbäumen und Ziergehölzen subtropischer Herkunft (with English summary). *Oecol. Plantarum*, **6**, 1 – 14.

Larcher, W., 1980: La posizione delle piante sempreverdi mediterranee nella evoluzione della resistenza al freddo. *Atti Ist. Veneto Sci., Lett., Arti, Cl. Sci.*, **138**, 103 – 111.

Larcher, W., 1981: Resistenzphysiologische Grundlagen der evolutiven Kälteakklimatisation von Sprosspflanzen (with English summary). *Plant Syst. Evol.*, **137**, 145 – 180.

Larcher, W., 2000: Temperature stress and survival ability of Mediterranean sclerophyllous plants. *Plant Biosyst.*, **134**, 279 – 295.

Larcher, W., 2003: *Physiological Plant Ecology*, 4th ed. Springer, Berlin.

Larcher, W. and Mair, B., 1969: Die Temperaturresistenz als ökophysiologisches Konstitutionsmerkmal. 1. *Quercus ilex* und andere Eichenarten des Mittelmeergebietes (with English summary). *Oecol. Plantarum*, **4**, 347 – 376.

Larcher, W., Meindl, U., Ralser, E., and Ishikawa, M., 1991: Persistent supercooling and silica deposition in cell walls of palm leaves. *J. Plant Physiol.*, **139**, 146 – 154.

Levitt, J., 1958: *Frost, drought, and heat resistance.* Springer, Wien.

Levitt, J., 1980: *Responses of plants to environmental stresses*. 1. *Chilling, freezing and high temperature stresses*, 2nd ed. Academic Press, New York.

Lyons, J. M., 1973: Chilling injury in plants. *Annu. Rev. Plant Physiol.*, **24**, 445 – 466.

Markley, J. L., McMillan, C., and Thompson, G. A., 1982: Latitudinal differentiation in response to chilling temperatures among populations of three mangroves, Avicennia *germinans, Laguncularia racemosa, and Rhizophora mangle*, from the western tropical Atlantic and Pacific Panama. *Can. J. Bot.*, **60**, 2704 – 2715.

Mellerowicz, E. J., Riding, R. T., and Greenwood, M. S., 1995: Nuclear and cytoplastic changes associated with maturation in the vascular cambium of *Larix laricina. Tree Physiol.*, **15**, 443 – 449.

Molisch, H., 1897: *Untersuchungen über das Erfrieren der Pflanzen.* Fischer, Jena.

Moschkov, B. S., 1935: Photoperiodismus und Frosthärte ausdauernder Gewächse. *Planta*, **23**, 775 – 803.

Müller－Thurgau, H., 1886: Über das Gefrieren und Erfrieren der Pflanzen. *Landw. Jahrbücher*, **15**, 435 – 610.

Ouellet, C. E. and Sherk, L. C., 1967: Woody ornamental plant zonation. 3. Suitability map for the probable winter survival of ornamental trees and shrubs. *Can. J. Plant Sci.*, **47**, 351 – 358.

Palta, J. P. and Li, P. H., 1978: Cell membrane properties in relation to freezing injury. In *Plant Cold Hardiness and Freezing Stress* (ed. by Li, P. H. and Sakai, A.). *Vol. 1*, Academic Press, New York, pp. 93 – 115.

Pearce, R. S., 2001: Plant freezing and damage. *Ann. Bot.*, **87**, 417 – 424.

Pisek, A. and Larcher, W., 1954: Zusammenhang zwischen Austrocknungsresistenz und Frosthärte bei Immergrünen. *Protoplasma*, **44**, 30 – 46.

Powell, L. E., 1987: Hormonal aspects of bud and seed dormancy in temperate－zone woody plants. *HortScience*, **22**, 845 – 850.

Quamme, H. A., Stushnoff, C., and Weiser, C. J.,

1972: The relationship of exotherms to cold injury in apple stem tissues. *J. Amer. Soc. Hort. Sci.*, **97**, 608 – 613.

Rajashekar, C. B. and Burke, M. J., 1996: Freezing characteristics of rigid plant tissues. *Plant Physiol.*, **111**, 597 – 603.

Ramalho, J. C., Quartin, V. L., Leitao, E., Campos, P. S., Carelli, M. L, C., Fahl, J. I., and Nunes, M. A., 2003: Cold acclimation ability and photosynthesis among species of the tropical *Coffea* genus. *Plant Biol.*, **5**, 631 – 641.

Rasmussen, D. H. and MacKenzie, A. P., 1972: Effect of solute on ice – solution interfacial free energy; calculation from measured homogeneous nucleation temperatures. In *Water structure at the water – polymer interface* (ed. by Jellinek, H. H. G.). Plenum, New York, pp. 126 – 145.

Read, J. and Hope, G. S., 1989: Foliar frost resistence of some evergreen tropical and extratropical Australasian Nothofagus species. *Aust. J. Bot.*, **37**, 361 – 373.

Rinne, P., Welling, A., and Kaikuranta, P., 1998: Onset of freezing tolerance in birch (*Betula pubescens* Ehrh.) involves LEA proteins and osmoregulation and is impaired in an ABA – deficient genotype. *Plant Cell Environ.*, **21**, 601 – 611.

Ristic, Z. and Ashworth, E. N., 1994: Response of xylem ray parenchyma cells of red osier dogwood (*Cornus sericea* L.) to freezing stress. Microscopic evidence of protoplasm contraction. *Plant Physiol.*, **104**, 737 – 746.

Robertson, A. J., Weninger, A., Wilen, R. W., Fu, P., and Gusta, L. V., 1994: Comparison of dehydrin gene expression and freezing tolerance in Bromus inermis and Secale cereale grown in controlled environments, hydroponics, and the field. *Plant Physiol.*, **106**, 1213 – 1216.

Sachs, J., 1860: Krystallbildungen bei dem Gefrieren und Veränderung der Zellhäute bei dem Aufthauen saftiger Pflanzenteile. *Ber. Verh. kgl. sächs. Ges. Wiss. Leipzig, Math. – phys. Kl.*, **12**, 1 – 50.

Sakai, A., 1956: Survival of plant tissue of super – low temperatures. *Low Temp. Sci. B*, **14**, 17 – 23.

Sakai, A., 1960: Survival of the twigs of woody plants at – 196 °C. *Nature*, **195**, 393 – 394.

Sakai, A., 1983: Comparative study on freezing resistance of conifers with special reference to cold adaptation and its evolutive aspects. *Can. J. Bot.*, **9**, 2323 – 2332.

Sakai, A. and Larcher, W., 1987: *Frost Survival of Plants.* Springer, Berlin.

Sakai, A. and Wardle, P., 1978: Freezing resistance of New Zealand trees and shrubs. *New Zealand J. Ecol.*, **1**, 51 – 61.

Samygin, G, A., 1974: *Priciny vymerzaijy rastenii*. Nauka, Moskva.

Senser, M. and Beck, E., 1982: Frost resistance in spruce (*Picea abies* (L.) Karst.): V. Influence of photoperiod and temperature on the membrane lipids of the needles. *Z. Pflanzenphysiologie*, **108**, 71 – 85.

Sikorska, E. and Kacperska, A., 1982: Freezing – induced membrane alterations: injury or adaptation? In *Plant cold hardiness and freezing stress* (ed. by Li, P. and Sakai, A.). Academic Press, New York, pp. 261 – 272.

Siminovitch, D., 1981: Common and disparate elements in the processes of adaptation of herbaceous and woody plants to freezing – a perspective. *Cryobiology*, **18**, 166 – 185.

Siminovitch, D. and Cloutier, Y., 1983: Drought and freezing tolerance and adaptation in plants: some evidence of near equivalences. *Cryobiology*, **20**, 487 – 503.

Siminovitch, D., Rheaume, B., Pomeroy, K., and Lepage, M., 1968: Phospholipid, protein, and nucleic acid increases in protoplasm and membrane structures associated with development of extreme freezing resistance in black locust tree cells. *Cryobiology*, **5**, 202 – 225.

St.John, J. B. and Christiansen, M. N., 1976: Inhibition of linolenic acid synthesis and modification of chilling resistance in cotton seedlings. *Plant Physiol.*, **57**, 257 – 259.

Sung, D – Y., Kaplan, F., Lee, K – J., and Guy, C. L. 2003: Acquired tolerance to temperature extremes. *Trends Plant Sci.*, **8**, 179 – 187.

Taschler, D. and Neuner, G., 2004: Summer frost resistance and freezing patterns measured in situ in leaves of major alpine plant growth forms in relation to their upper distribution boundary. *Plant Cell Environ.*, **27**, 737 – 746.

Taylor, A. O. and Rowley, J. A., 1971: Plants under climatic stress. 1. Low temperature, high light effects on photosynthesis. *Plant Physiol.*, **47**, 713 – 718.

Thomashow, M. F., 1999: Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **50**, 571 – 599.

Tumanov, I. I., 1979: *Fiziologiya zakalivaniya i morozostoikosti rastenii*. Izdat. Nauka, Moskva.

Tumanov, I. I., Krasavtsev, O. A., and Khvalin, N. N., 1959: Promotion of resistance of birch and black currant to − 253 °C, temperature by frost hardening. *Transl. USSR Acad. Sciences*, **127**, 6, 1301 – 1303.

Tumanov, I. I., Kuzina, G. V., and Karnikova, L. D., 1965: Vliyanie fotoperiodov na morozostoikost abrikosa i chernoi smorodiny (with English summary). *Fiziol. rast.*, **12**, 665 – 682.

Tyurina, M. M., 1957: *Issledovanie morozostoikosti rastenii v usloviyakh vysokogorii Pamira*. Izdat. Nauk Tadshik., Dushambe.

Tyurina, M. M., Gogoleva, G. A., Jegurasdova, A. S., and Bulatova, T. G., 1978: Interaction between development of frost resistance and dormancy in plants. *Acta Hort.*, **81**, 51 – 60.

U.S.National Arboretum, 1965: Plant hardiness zone map. *U.S.D.A., A.R.S. Misc. Publ.* 814, Washington.

Van・Hasselt, Ph. R., 1974: Photo – oxidative damage to the ultrastructure of cucumis chloroplasts during chilling. *Proc. Koninkl. Nederl. Akad. Weternsch. Amsterdam, Series C*, **77 (1)**, 50 – 56.

Weiser, C. J., 1970: Cold resistance and injury in woody plants. *Science*, **169**, 1269 – 1278.

Welling, A., Kaikuranta, P., and Rinne, P., 1997: Photoperiodic induction of dormancy and freezing tolerance in Betula pubescens. Involvement of ABA and dehydrins. *Physiol. Plant.*, **100**, 119 – 125.

Wilson, J. M. and Crawford, R. M. M., 1974: The acclimatization of plants to chilling temperatures in relation to the fatty – acid composition of leaf polar lipids. *New Phytol.*, **73**, 805 – 820.

Wisniewski, M. and Davis, G., 1989: Evidence for the involvement of a specific cell wall layer in regulation of deep supercooling of xylem parenchyma. *Plant Physiol.*, **91**, 151 – 156.

Zhu, J. J. and Beck, E., 1991: Water relations of *Pachysandra* leaves during freezing and thawing. Evidence of negative pressure potential alleviating freeze – dehydration stress. *Plant Physiol.*, **97**, 1146 – 1153.