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MEASURING COLD HARDINESS IN WOODY PLANTS

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ACADEMIC DISSERTATION

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ORIGINAL PUBLICATIONS

ABSTRACT

Cold hardiness is a key factor limiting the distribution and productivity of perennial horticultural plants in northern areas. Reliable methods for estimation of cold hardiness are needed in breeding and selection work as well as in studying the mechanisms of cold injury and low-temperature acclimation. In this thesis, plant cold hardiness is discussed with a special emphasis on methods for measuring hardiness characteristics. The main objective was to develop and evaluate computational and experimental techniques for determination of cold hardiness in deciduous trees and shrubs.

The logit model was used for analysis of qualitative freeze-survival data, based on visual examination of sample injuries. Determination of the lethal temperature on the basis of freeze-induced electrolyte leakage measurements was studied by applying linear interpolation and fitting logistic, Richards and Gompertz functions. Estimation of the lethal temperature was further explored using Monte Carlo simulation, linear interpolation and the Spearman-Kärber method. Logistic and Richards functions were also fitted to five real data sets and statistical resampling techniques were used. Use of artificial hardening/dehardening treatments and controlled freezing tests was examined in determination of cold hardiness characteristics of apple (*Malus domestica* Borkh.), mock orange (*Philadelphus lewisii* Pursh var. *lewisii* 'Waterton') and hydrangea (*Hydrangea paniculata* Sieb. 'Grandiflora'). Finally, statistical multivariate techniques were applied to identify the critical climatic factors associated with winter injury occurrences in Finnish apple orchards.

Qualitative freeze-injury data were successfully analysed using the logit model. The major benefits of the logit model are that the form of the sampling variation in the discrete response variable is taken into account, the lethal temperature is easily estimated with confidence intervals, and treatment effects can be directly evaluated. The main disadvantage is that the response data are assumed to be symmetric. In stem samples of red raspberry (*Rubus idaeus* L.), relating electrolyte leakage measurements to lethal temperature was best done by linear interpolation or by using the midpoint estimate of the logistic or the Gompertz function. The Richards function, in contrast, yielded largely deviating hardiness estimates and displayed a considerable lack of fit in several data sets. The simulation study on lethal temperature estimates produced by two non-parametric and two parametric methods revealed that they all performed equally well, when the response data were symmetric. For asymmetric conditions, linear interpolation is preferable to the Spearman-Kärber method and the logistic function. The benefits of the theoretically justified, asymmetric Richards function are often lost in practical applications, due to problems in fitting the complicated function to small data sets.

Application of controlled freezing tests and acclimation treatments revealed the level of actual, potential, and minimum cold hardiness in apple, mock orange and hydrangea. Under the climatic conditions of southern Finland, all three taxa remained in well-acclimated states until March, long after endodormancy release. Moreover, the three species displayed a relatively high level of minimum hardiness in late winter, indicating that they are able to manage warm periods during ecodormancy quite well.

The major climatic factor associated with apple winter injury in southwestern Finland was the severity of mid-winter, i.e. low temperatures during January, February, and March. Also, weather conditions during the preceding summer and fall proved relevant for successful overwintering, probably due to their impact on the annual cycle of vegetative growth. Consequently, mid-winter cold hardiness and timing of cold acclimation appeared as the most important hardiness characteristics to be assessed in breeding and selection of new apple cultivars.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Lindén, L., Rita, H. and Suojala, T. 1996. Logit models for estimating lethal temperatures in apple. HortScience 31: 91-93.

II Suojala, T. and Lindén, L. 1997. Frost hardiness of *Philadelphus* and *Hydrangea* clones during ecodormancy. Acta Agriculturae Scandinavica, Section B, Soil and Plant Science 47: 58-63.

III Lindén, L., Palonen, P. and Lindén, M. 2000. Relating freeze-induced electrolyte leakage measurements to lethal temperature in red raspberry. Journal of the American Society for Horticultural Science 125: 429-435.

IV Lindén, L. and Lindén, M. 2001. A comparison of four methods for estimating the lethal temperature in plant stress studies. Submitted manuscript.

V Lindén, L. 2001. Re-analysing historical records of winter injury in Finnish apple orchards. Canadian Journal of Plant Science 81: 479-485.

AUTHOR'S CONTRIBUTION

The contribution of the author of this thesis to the publications I-V is presented here. The following text has been adapted from the documents where each author's contribution to the above-mentioned publications was stated and signed.

In paper I , the author of this thesis was responsible for the planning and experimental part of the work. The data were analyzed and the manuscript was written by the author. Dr. Hannu Rita had a supervisory role and offered his advice and guidance during statistical analyses of the data and writing the manuscript. Dr. Terhi Suojala participated in the experimental work and preparation of the manuscript.

In paper II, the author was responsible for planning of the experiment, acted as a supervisor, participated in the experimental work and offered her advice and guidance throughout the work and manuscript preparation. Dr. Terhi Suojala carried out a major part of the experimental work, analyzed the results and was responsible for writing of the manuscript.

In paper III, the author was responsible for planning of the study, writing of the manuscript and part of the statistical analyses. Dr. Pauliina Palonen provided the experimental data, collaborated as a member of the research group for cold hardiness of woody plants and made revisions on the manuscript. Dr. Mikael Lindén carried out a major part of the data analyses and participated in manuscript preparation.

In paper IV, the author participated in planning of the study and provided part of the experimental data. The manuscript was written solely by the author. Dr. Mikael Lindén participated in planning of the study, carried out all data analyses and made revisions on the manuscript.

1 INTRODUCTION

In northern areas, low temperature is the major environmental factor limiting the productivity and the geographical distribution of horticultural plants. Low temperature decreases biosynthetic activity of plants, disturbs the normal function of physiological processes and may result in permanent injuries that finally bring about death. Cold hardiness, defined as the ability of plants to withstand sub-freezing temperatures without sustaining significant damage, is an important criterion for evaluation of the cultivation potential of a species or cultivar, and for breeding or selection work. The mechanisms of cold injury and low-temperature acclimation are a subject of lively research in plant biology as well as in agricultural, horticultural, and forestry sciences. Therefore, the ability to estimate the degree of cold hardiness in plants is of great value for both basic and applied studies. In this thesis, plant cold hardiness is discussed with a particular emphasis on methods for measuring hardiness characteristics. The subject is confined to aerial parts of deciduous trees and shrubs.

1.1 Cold hardiness in woody plants

Adaptation to seasonal changes in temperature is a precondition for woody plant life in temperate and boreal vegetation zones. The annual process of cold acclimation involves structural and metabolic adjustments that result in a transition from a lower to a higher level of cold hardiness. The ultimate survival of woody plants is dependent on not only the maximal capacity of cold hardening, but also on the timing and rate of both cold acclimation and deacclimation, the stability of cold hardiness, and the ability to reacclimate after unseasonably warm periods (Larcher 1968, Fuchigami et al. 1982). Hence, the successful performance of a woody species in a particular locality implies synchronization of the annual development of cold hardiness with the seasonal temperature changes.

The mechanisms that allow plants to withstand subfreezing temperatures are placed into two main categories; avoidance and tolerance of freezing (Levitt 1980). Both survival strategies are represented in woody plants (Sakai and Larcher 1987, Malone and Ashworth 1991). Tissues relying on freezing avoidance display deep supercooling in which some of the cellular water remains liquid far below the freezing point. The theoretical limit of supercooling is –38.1ºC for pure water (Rasmussen and MacKenzie 1972, ref. Gusta et al. 1983). Deep supercooling is enabled by an effective isolation of the protoplast from the nucleating effect of extracellular ice. Freezing of deep supercooled water can be observed as low temperature exotherms, i.e. the heat of fusion produced by the liquid to solid phase

change. In the 16 woody taxa examined by Gusta et al. (1983), initiation of low temperature exotherms occurred between -39ºC and -55ºC. Freezing avoidance occurs in certain tissues and organs of temperate trees: xylem ray cells of many hardwoods, flower buds of angiosperms and both shoot and floral primordia of conifers (Sakai and Larcher 1987).

Freezing tolerance involves water migration into intercellular spaces and a gradual growth of extracellular ice. At slow cooling rates, cold acclimated tissues undergo equilibrium freezing; i.e. diffusion of cellular water is rapid enough to maintain the chemical equilibrium between the protoplasm and the ice. In contrast, rapid cooling may result in a chemical disequilibrium and induction of intracellular freezing. Tolerance of extracellular freezing and the ensuing dehydration stress is the major survival mechanism of woody plants (Levitt 1980). The hardiest species of woody plants exhibit freezing tolerance; in a fully acclimated state they may survive experimental freezing to -196ºC (George et al. 1974).

1.1.1 Environmental control of cold hardening

Weiser (1970) described the seasonal cold acclimation of woody plants native to the temperate zones as a three-stage process. The first stage is strongly affected by photoperiod. In many woody plants short days induce growth cessation, which is a prerequisite for cold acclimation. The first stage, during which abundant organic substances are stored, depends mainly on photosynthesis and proceeds at relatively warm temperatures in autumn. Cells in the first stage of acclimation can survive temperatures well below 0ºC, but they are not fully hardened. The second stage of cold acclimation is induced by low temperature, especially subzero temperatures. During this stage plants undergo metabolic and/or structural changes, which lead to a considerable degree of cold hardiness. In many woody taxa, the maximum level of cold hardiness is obtained first after an exposure to low freezing temperatures (-30º to -50ºC). This can be defined as the third phase of cold hardening (Weiser 1970).

Thus, low temperature and shortening photoperiod are the two major factors triggering cold acclimation in woody plants. The sequence of these environmental cues is essential: short days should precede low temperatures for the whole acclimation capacity to be manifested (Irving and Lanphear 1967b, Bervaes et al. 1978). Furthermore, photoperiodic effects on growth and development can be modified by temperature (Junttila and Kaurin 1985, Westergaard and Eriksen 1997). According to Koski and Sievänen (1985), timing of growth cessation in seedlings of *Betula pendula* Roth is determined by the joint effect of photoperiod and heat sum. On the other hand, in second-year seedlings of *Pinus sylvestris* L.

a constant heat sum appeared to be characteristic of cessation of elongation growth, indicating a lack of joint effects (Koski and Sievänen 1985). Also water availability, mineral nutrition, and plant age can bring about alterations in the cold acclimation process (e.g. Rollins et al. 1962, Chen et al. 1977, Pellett and Carter 1981, McNamara and Pellett 2000). Finally, plants may have endogenous rhythms of cold acclimation, independent of environmental factors (Howell and Weiser 1970a, McKenzie et al. 1974, Siminovitch 1982).

The timing and capacity of cold acclimation are determined genetically, but modified by environmental cues. Within one species there can be marked differences in the maximum attainable hardiness between ecotypes and cultivars (e.g. Sakai and Weiser 1973, Mittelstädt and Murawski 1975, McNamara and Pellett 1998). However, this is not always true. Winter twigs of *Betula papyrifera* Marshall, *Populus tremuloides* Michx, *Populus balsamifera* L., and *Larix laricina* (Du Roi) C. Koch, collected from northern Wisconsin and Minnesota (45º to 50ºN lat.), were able to attain an equally high level of freezing resistance as twigs of the same species from College, Alaska (65ºN lat.) (Sakai and Weiser 1973). The rate of cold acclimation, as well as that of other hardiness characteristics may vary independently of the maximum hardiness attained (Lapins 1961, Scheumann 1968, Lindstrom and Dirr 1989).

1.1.2 Metabolic and structural changes during cold hardening

During cold acclimation, plant cells and tissues undergo a wide range of changes that allow cell functioning at low temperatures and enable survival of freezing stress. Cold acclimation is accompanied by reduced water content of tissues and accumulation of putative cryoprotective compounds, such as soluble carbohydrates and proteins (Levitt 1980, Guy 1990). The biochemical alterations of plasma membranes include qualitative changes in proteins concomitantly with an increase in membrane fluidity, phospholipid enrichment and fatty acid unsaturation (Yoshida and Uemura 1990). Cell structural changes involve e.g. an augmentation of cytoplasm and reduction in vacuole size (Pomeroy and Siminovitch 1971, Wisniewski and Ashworth 1986). In cells of the stem cortex of deciduous trees, the chloroplasts aggregate instead of being uniformly distributed throughout the cell (Kuroda and Sagisaka 1993).

1.1.3 Seasonal fluctuations of cold hardiness

In winter and spring, changes of cold hardiness are mainly dependent on ambient air temperature. Temperature has a direct effect on short-term changes in hardiness: the level of

hardiness increases as temperature drops and decreases as it rises (e.g. Proebsting 1963, Ketchie and Beeman 1973, Hong and Sucoff 1982). On the other hand, temperature contributes to the degree of hardiness in an indirect manner through its effects on the cycle of annual development (Fuchigami et al. 1982, Leinonen 1996, 1997). At the onset of winter, woody plants are more responsive to hardening stimuli than to dehardening temperatures. During deep dormancy, hardiness variation is reduced in both directions. In late winter, after dormancy release, plants are more easily dehardened and less easily rehardened. Even photoperiod can have a modifying effect on dormancy release and the following ontogenetic development, as shown for some northern deciduous trees by Heide (1993a, b).

Larcher (1985) describes the seasonal hardiness dynamics of plants using the concepts of actual, potential and minimum cold hardiness. The actual level of cold hardiness corresponds to the state of hardening at a given time, while potential cold hardiness refers to the maximum attainable level of hardiness. The minimum cold hardiness serves to indicate a specific base level of hardiness above which plant or tissue hardiness does not rise in spite of warm weather or an artificial dehardening treatment. The potential and minimum hardiness levels are dependent on the stage of annual and ontogenetic development. The difference between potential and minimum hardiness provides a measure of a plant's hardening/dehardening capacity at a given time.

The water content of a plant influences its cold hardiness through cell sap concentration and the degree of protoplasmic and cell wall hydration (Li and Weiser 1970, Chen and Li 1977, Anisko and Lindstrom 1996b). The water status may also have an indirect effect on cold hardiness mediated by a decreased respiratory consumption of cryoprotective sugars in dehydrated tissues (Ögren 1996). Exposure to drought can increase cold hardiness of woody plant stems and leaves by several degrees (Chen et al. 1977, Yelenosky 1979, Anisko and Lindstrom 1996b). On the other hand, dehardening appears to be closely associated with an increase in bud (Graham and Mullin 1976, Junttila et al. 1983) and stem (Pogosyan and Sakai 1969, ref. Sakai and Larcher 1987) water content. According to Bittenbender and Howell (1975), dehardening due to increased moisture content appears to be governed by a different mechanism than that induced by high temperature.

1.2 Laboratory testing of cold hardiness

Due to the importance of cold hardiness for winter survival of perennial plants, there has been considerable interest in developing methods for determining the level of plant cold hardiness.

Most of these methods are based on controlled freezing tests followed by evaluation of freezing injuries. However, for many years investigators have also searched for indirect indicators to predict the level of hardiness without freezing.

The content of dry matter or soluble carbohydrates, as well as carbohydrate composition, is often correlated with plant or tissue cold hardiness (e.g. Raese et al. 1978, Ichiki and Yamaya 1982, Kaurin et al. 1984, Flinn and Ashworth 1995, Imanishi et al. 1998). There are also numerous investigations showing a relationship between the quality or quantity of amino acids, proteins and lipids and the level of cold hardiness (e.g. Yoshida 1984, Khanizadeh et al. 1992, Arora and Wisniewski 1996, Arora et al. 1997). Additional methods used to determine cold hardiness without freezing include electrical impedance analysis (Coleman 1989, Repo et al. 1997, Repo et al. 2000, Väinölä and Repo 2000) and measurement of the ability to withstand plasmolysis (Siminovitch and Briggs 1953, Levitt 1980). The indirect hardiness indicators often work well for some plants or tissues, or under certain conditions, yet none of them can be trusted as a general measure of cold hardiness in all plants. Moreover, testing without freezing does not yield numerical hardiness estimates, unless the method is properly calibrated for the species and tissue examined.

Controlled freezing tests involve exposure of plants or plant parts to a decreasing temperature gradient. Sometimes a single minimum temperature is used instead of a graded temperature series. The extent of freeze-induced damage is assessed after the low-temperature treatment. A quantitative measure of cold hardiness can be derived on the basis of injury data. Comparable results are to be expected only after careful standardization of the whole testing procedure.

A comprehensive determination of a plant's hardiness properties includes maximum cold hardiness of all organs and at all stages of development, the annual pattern of variability in hardiness as well as the acclimation capacity of various organs at different developmental stages (Larcher 1968, 1985). Because an exhaustive analysis of cold hardiness involves considerable time and effort, simple screening tests have been developed for comparative studies (e.g. Lapins 1962b, Mittelstädt and Murawski 1975, Fischer 1992, Hummer et al. 1995).

1.2.1 Plant material

Woody perennials are usually tested for cold hardiness either as whole plants (seedlings or rooted cuttings) or as detached plant parts (stem segments, buds, roots or leaves) collected

from plants growing in the field. In addition, *in vitro* cultures can be used as test material (e.g. Caswell et al. 1986, Zatylny et al. 1993, Palonen and Buszard 1998).

Whole-plant freezing tests allow investigation of freeze injuries in the entire plant and evaluation of recovery. Hence, the final extent of cold damage in the field can be approximated. In such tests, it is usually necessary to protect roots during low-temperature exposure due to their limited freezing tolerance. When using seedling material, the potential age-related differences in hardiness should be considered; younger plants may be less cold hardy than older specimens of the same species (Brown and Bixby 1976, Lim et al. 1999, McNamara and Pellett 2000). Using detached plant parts provides detailed information on the level of hardiness in different tissues and organs. The results have usually been in good agreement with field observations of natural cold injury (e.g. Lapins 1962a, Graham and Mullin 1976, Pellett et al. 1981). Relating the data to whole-plant survival requires knowledge of the relative importance of different tissues and of a plant's ability to recover.

Sample material collected in the field may deharden en route to the laboratory if precautions are not taken. Samples are usually maintained in a near-freezing environment during transport and storage (e.g. Holubowicz et al. 1982, Sakai et al. 1986, Cappiello and Dunham 1994). Proper handling ensures that test material is unaltered, with respect to hardening and phenology, from its field status prior to sampling.

1.2.2 Acclimation treatments

To obtain a complete picture of a plant's cold hardiness status under natural conditions, measurements must be made at frequent intervals throughout the year. However, artificial acclimation treatments provide an alternative approach for examination of hardiness characteristics including the potential and minimum level of hardiness as well as susceptibility to dehardening and capability of rehardening. The most appropriate temperature and duration of acclimation treatment vary according to species, provenance, and season and should be determined in preliminary experiments. According to Sakai and Larcher (1987), effective hardening in winter can be achieved by a two-step acclimation of 1 – 2 weeks, starting at a temperature of 0º to -3ºC then dropping to -5º to -10ºC. Proper dehardening can be attained by exposure for 1 – 3 d to temperatures above +15ºC in winter and above +20ºC in spring and autumn.

Since the initial stage of cold acclimation in many woody species is induced by short days, control of photoperiod is essential during hardening of non-acclimated samples.

However, use of artificial lighting may affect even the dehardening response in midwinter: when illuminated, green shoots of *Vaccinium myrtillus* L. dehardened at 10ºC more than when the shoots were kept in complete darkness (Ögren 1996). While using whole plants, acclimation treatments should be done in a way that allows differentiation between shoot and root zone temperatures. Methods developed for acclimation of forest tree seedlings, are described and discussed in a review by Keates (1990). For *in vitro* cultures, acclimation treatments may include manipulation of sugar and/or growth regulator levels in the culture medium (Caswell et al. 1986, Travert et al. 1997, Palonen and Buszard 1998).

1.2.3 Freeze testing

Sample exposure to freezing stress is usually done indoors, employing freeze chambers, controlled temperature liquid baths or temperature gradient bars. Mobile freezing units are sometimes used for testing intact trees or branches *in situ* (e.g. Scott and Spangelo 1964, Scott 1966). While using whole plants, all or any tissues may be subjected to test conditions. Seedlings and cuttings are usually tested in pots with their roots guarded from freezing by insulation or by heating coils.

Tissue samples tested in freeze chambers are protected against desiccation by enclosing them in plastic bags or aluminium foil (Arora et al. 1992, Kuroda and Sagisaka 1993, Carter and Hummer 1999). Placing the packed samples inside a vacuum flask (e.g. Howell and Weiser 1970b, Harrison et al. 1978a) retards temperature change and eliminates small temperature fluctuations during freezing. Tissue temperatures can be monitored with thermocouples inserted into the samples. When liquid baths are used, samples are normally placed in glass vials or stoppered test tubes (e.g. Blazich et al. 1974, Anisko and Lindstrom 1996a, Arora et al. 1997). Temperature gradient bar testing involves placing samples in holes along the length of a metal bar. A temperature gradient is established by maintaining the bar ends at different temperatures (Hodges et al. 1970, Timbers and Hocking 1971). Prior to freeze exposure, samples are equilibrated at a constant temperature.

To standardize the freezing protocol, Levitt (1980) proposed the following steps as basic requirements:

1. The plants must be inoculated to ensure freezing.
2. Cooling must occur at a standard rate.
3. A single freeze must be used for a standard length of time.
4. Thawing must occur at a standard rate of warming.

5. Post-thawing conditions must be standardized.

Excessive supercooling may cause increased freezing injury when ice formation is eventually initiated (Gusta and Fowler 1977, Levitt 1980). Freezing of detached samples can be ensured by placing small ice crystals into sample vessels, by touching samples with a spatula cooled in liquid nitrogen, by wrapping samples in moist paper towel, or by placing them in moist sand. According to Burke et al. (1976), large plants or plant samples are less likely to supercool extensively than small samples, since spontaneous ice nucleation is a chance event. On the other hand, the results of von Fircks (1993) indicate that the mechanism of supercooling is less effective in excised stem sections than in intact plants, because ice seeding may occur through cut surfaces. Cappiello and Dunham (1994) and Cappiello et al. (1997) stated that ice inoculation had no effect on the estimated hardiness value in woody plant stems.

The choice of cooling rate depends on the objectives of the hardiness test. Sakai and Larcher (1987) distinguish three practices: direct, gradual, and simulation cooling. In direct cooling, samples are brought to the test temperature quickly to avoid additional hardening during the freezing test. Direct cooling at a rate of 5 to 10ºC h^{-1} is used for determining actual and minimum levels of hardiness. In gradual cooling, the temperature is lowered stepwise and slowly enough to allow the samples to achieve their full hardening capacity. Thus the maximum hardiness potential is assessed. In simulation cooling, the temperature is lowered at a rate of 1 to 2ºC h^{-1} to imitate natural frost events. A common approach is to use a fixed rate of 2 to 6ºC h^{-1}. Even within this range the increased rate of 6ºC h^{-1} may cause lethal injury at higher temperature than cooling at 2ºC h^{-1} (Haynes et al. 1992, Anisko and Lindstrom 1996a). Sometimes samples are first frozen at a slow rate of 2 to 3ºC h^{-1} to -18ºC, after which a higher rate of 10ºC h^{-1} is employed (Kaurin et al. 1984, Junttila and Kaurin 1990). As shown by Harrison et al. (1978b), the rate of cooling is critical in the temperature range where the amount of unfrozen water is sufficient to cause injury. The critical temperature limit for hardy *Cornus stolonifera* Michx. was -15ºC; below it the cooling rate was unimportant due to the small amount of freezable water.

The low-temperature exposure must be long enough for thermodynamic equilibrium to establish. Generally, a longer exposure brings about increased damage (Rollins et al. 1962, Stoyanov 1973, Su et al. 1987). Larcher (1968) considered an exposure time of 4 to 6 h as a minimum. However, if a series of low temperatures is used, practical reasons may limit the duration of single exposures. In a multiple-temperature regime, subsamples are often removed from the freezer directly after reaching the predetermined test temperature (e.g.

Harrison et al. 1978a, Pellett et al. 1981). Alternatively, exposure times of 30 to 120 min are commonly applied (e.g. Blazich et al. 1974, Ketchie and Kammereck 1987).

The ideal method of thawing is to warm the samples at a constant rate in the freezing unit. If a single freezing unit is available, this may only be applied to the lowest test temperature of a stepwise decreasing temperature regime. Therefore, a common approach is to transfer samples from the freeze device directly to temperatures of 0ºC to 5ºC for a 16 to 24 h period of thawing (e.g. Holubowicz et al. 1982, McNamara and Pellett 1998).

1.2.4 Evaluation of freezing injury

To date, the overall understanding of freezing injury in plants remains far from complete. As the patterns of ice formation and injury depend upon freezing conditions, tissue type and state of hardiness, a single, general mechanism responsible for freeze-induced cell death can not be expected. It is, however, most probable that cellular membranes are the primary sites of freezing injury (Steponkus 1984, Palta and Weiss 1993).

Extracellular freezing subjects cell membranes to various types of stress, involving a physical effect of the low temperature *per se*, freeze-induced reduction in the surface area and solute concentration effects, freeze dehydration of the protoplasm, molecular packing of membrane constituents induced by cell shrinkage and/or a combination of these, as well as changes in pH and ionic strength (Sakai and Larcher 1987). These stresses may cause irreversible alterations in the structure and functioning of cell membranes and thus, bring about cell death.

During intracellular freezing, injury probably results from the mechanical stresses and dehydration imposed on cellular structures by ice (Burke et al. 1976). Membrane destruction is one of the most readily apparent manifestations of intracellular freezing. Formation of intracellular ice is considered responsible for cell death in deep supercooling tissues (Burke et al. 1976). Even freezing tolerant tissues may sometimes experience intracellular freezing in nature. Weiser (1970) stated that sunscald injury, which occurs on south-facing branches of many tree species, is caused by rapid cooling, which leads to intracellular freezing.

Generally, tissue viability is evaluated after a freeze-thaw cycle by measuring the consequences of primary injuries in plant membranes. A few techniques are able to indicate cell death during freezing, at the instance of lethal injury occurrence. The choice of evaluation method depends on the aims of the study, the type and the physiological state of

plant material tested, and the available facilities. More than one method can be used in parallel to confirm the results.

1.2.4.1 Visual observation

Signs of freezing injury can be detected by visual examination of thawed tissue samples or intact plants. Usually, freeze-injured tissues develop a brown or yellowish colour due to oxidation of polyphenols. On a macro level, the loss of cell membrane integrity may also be expressed as a soft, water-soaked appearance (Howell and Weiser 1970b). To allow the symptoms to develop, samples are kept in a relatively warm environment, protected from desiccation before injury evaluation. The length of the incubation period required varies from a few days to one week or even more, depending on tissue type and time of the year (Sakai and Larcher 1987). The visual method requires a minimum of instrumentation and is considered reliable, though subjective and qualitative in nature (Stergios and Howell 1973, Harrison et al. 1978a, Holubowicz 1978).

Visual symptoms of freeze injury vary between plant species and cultivars as well as between different organs and tissues (Lapins 1961, Malone and Ashworth 1991, Takeda et al. 1993). Hong et al. (1980) compared visual browning of xylem with low temperature exotherms recorded in eight hardwood species. In all species the death of ray cells and the start of xylem browning coincided with the start of freezing of deep supercooled water. In general, browning increased when temperature was further lowered. However, *Cercis canadensis* L. showed little browning when frozen to 7ºC below the start of the low temperature exotherm, whereas xylem of *Quercus rubra* L. was brown already at 3ºC below the start of the low temperature exotherm.

Visual evaluation of freeze injuries may be aided by using chemical compounds, such as neutral red or 2,3,5-triphenyltetrazolium chloride (TTC). Neutral red is a so-called vital stain, which penetrates living cells more deeply than nonliving cells (Parker 1953). TTC is reduced by enzyme systems of living cells forming red-coloured triphenyl formazan (Steponkus and Lanphear 1967). Colour reactions caused by neutral red and TTC have been used for distinguishing between living and dead cells (e.g. Hong et al. 1980, Malone and Ashworth 1991, Takeda et al. 1993). However, the validity of both tests has been questioned since neutral red may accumulate in partially injured cells (Palta et al. 1978) and dead cells may show a positive TTC test (Stergios and Howell 1973). Additionally, the penetration of TTC

into tissues can be a limiting factor since the reaction is most intense in cells near the surface or cut edges of the tissue (Larcher and Eggarter 1960).

Visual injury assessment is sometimes combined with observations on regrowth capacity, especially when testing intact plants (e.g. Holubowicz et al. 1982, Ketchie and Kammereck 1987, Embree and McRae 1991). Recently, Pellett and Heleba (1998) suggested callus growth on wounded stem pieces as a measure of freeze injury. Callus growth from the cambial zone of several deciduous woody taxa was easier to see than discoloration of the same tissues, providing a less subjective evaluation of injury. Regrowth tests require a lengthy incubation period in growing conditions, and the results may be hampered by endodormancy. Still, normal regrowth of the plant is the ultimate measure of survival and the most reliable method of assessing freeze injury. Visual observations and regrowth tests are often used as controls for more quantitative tests.

1.2.4.2 Electrolyte leakage test

The electrolyte leakage test is based on the principle that damage to cell membranes results in an enhanced leakage of electrolytes (mainly K^+) from the cell. Recording the amount of leakage will thus provide an estimate of tissue damage. Electrolyte leakage tests involve measurement of the electrical conductivity of pure water in which detached tissue samples have been placed after a freeze-thaw cycle. The electrolyte leakage method was first applied in cold hardiness research by Dexter et al. (1930, 1932). As there may be considerable variation in the total amount of electrolytes between different samples, Stuart (1939) introduced the idea of expressing the amount of leakage as a percentage of total electrolytes released from the sample after heat killing. Since the 1930s, the electrolyte leakage method has been used extensively, and further refinements to the technique continue to be made.

In the case of woody, deciduous plants, electrolyte leakage tests are mostly applied to stem tissues, but roots and buds can also be tested (Wilner 1961, Khanizadeh et al. 1989, Ryyppö et al. 1998). The test procedure is often started by rinsing the samples with deionized water to remove surface contaminants. Subsequently, the samples are sectioned to increase the rate of electrolyte diffusion and the sections are immersed in a small amount of deionized water for incubation at room temperature until electrolyte diffusion stabilizes. The length of the incubation period is usually 20 to 24 h, after which the initial leakage value is read with a conductivity meter. Electrolyte leakage can be promoted by shaking the samples during incubation or by infiltrating them under vacuum for a few minutes. After measuring the

initial leakage, samples are killed by autoclaving, boiling, or freezing, to release the remaining electrolytes. The final leakage is recorded after an additional 20- to 24-h incubation. The relative leakage is calculated as (initial leakage/final leakage) x 100 %.

Deans et al. (1995) examined the validity of the relative leakage method on assessing frost damage in leafless shoots of *Quercus petraea* (Matt.) Lieb. The results indicated that samples should be incubated longer than for the usual 20 to 24 h to permit the initial leakage to approach asymptotic values. In addition, killing samples by autoclaving at 121ºC for 15 min was found insufficient to release quickly all diffusible electrolytes. Consequently, the authors recommended an initial incubation period of 5 to 7 d at +4ºC to minimize microbial activity, followed by autoclaving for 90 to 120 min at 121ºC and allowing 24 h before measurement of the final leakage value.

Since considerable amounts of electrolytes are leached from unfrozen control samples, Flint et al. (1967) proposed transformation of relative leakage values to a scale where an unfrozen sample is given a value of zero. The leakage values thus scaled are termed indices of injury. Lim et al. (1998) took the data transformation one step further by adjusting the indices of injury for electrolyte leakage from totally freeze-injured samples, frozen to -80ºC. The resulting percentage-adjusted injury values range between 0 % and 100 %.

Whitlow et al. (1992) suggested a further modification of electrolyte leakage protocol, which takes into account the sample surface area through which diffusion occurs and the electrochemical gradient between the cell sap and the bathing solution. The modified electrolyte leakage parameter was termed tissue ionic conductance. Applying this modification to electrolyte leakage values measured from drought-stressed *Malus ioensis* (A. Wood) Britt. and senescing *Quercus rubra* L. leaves provided a more reliable measure of membrane integrity than did the traditional relative leakage, or index of injury values (Whitlow et al. 1992). Manley and Hummel (1996) compared tissue ionic conductance with index of injury values measured from freeze-stressed leaves of *Brassica oleracea* L. The two parameters produced almost similar rankings of tissue freezing tolerance and thus, the use of the simpler index of injury value was recommended. As pointed out by Manley and Hummel (1996), calculation of tissue ionic conductance may still be essential for sample material that responds to sublethal temperatures with low levels of electrolyte leakage.

Several authors (Zhang and Willison 1987, Murray et al. 1989, Deans et al. 1995) suggested that the rate of electrolyte leakage based on repeated measurements, rather than a single measurement, should be used as an indication of freezing injury. According to Deans et al. (1995), measuring the rate of electrolyte leakage from stem tissues of *Quercus petraea*

revealed small differences in freezing injury more accurately than single measurements. However, because the leakage rate method is more laborious, the authors considered measurement of relative leakage as an acceptable compromise, provided that samples are incubated long enough, and that an appropriate method of autoclaving is used to achieve complete release of electrolytes.

The electrolyte leakage technique allows for simultaneous spectrophotometric measurement of leached phenolic compounds, which can also be used as a measure of freezing injury (Chalker-Scott et al. 1989, Dunning et al. 1994, Anisko and Lindstrom 1995). Some researchers prefer measuring the concentration of potassium ions (K^+) (Stoyanov 1973, Pukacki and Pukacka 1987), or the amounts of amino acids and other ninhydrin-reacting substances (Siminovitch et al. 1964) in extracts from freeze-injured tissues rather than recording the electroconductivity. Data on the release of phenolics, K^+, or amino acids are transformed into relative values or indices of injury similarly as records on electrolyte leakage. According to Taulavuori et al. (1996) the pH of the cell effusate is likewise indicative of the response of cell membranes to freezing.

The electrolyte leakage test is well suited for measuring freeze-induced damage as it is based on alterations in cell membranes, i.e. in the locus of initial injury. Furthermore, the test is fairly simple and rapid, yields quantitative data and requires only small amounts of plant material. However, certain concerns limit the validity of the technique. The test is not able to differentiate between injury among various tissues. Siminovitch et al. (1964) cautioned that the sensitivity of the test diminishes in proportion to the contribution made by nonliving tissues. Similar concerns were raised by Lapins (1961) and Anisko and Lindstrom (1995). The anatomical complexity and rigidity of woody stems results in a more gradual increase of leakage in stems than in leaves (Anisko and Lindstrom 1995). Furthermore, the rate of leakage changes in woody plants along with cold acclimation, indicating differential membrane permeability with changing physiological status (Sutinen et al. 1992, Anisko and Lindstrom 1995, Deans et al. 1995). Consequently, a single critical value discriminating between live and dead tissues cannot be found, but the electrolyte leakage test has to be calibrated against another measure of cold hardiness to ascertain the level of leakage that parallels lethal injury.

1.2.4.3 Other approaches

Among the numerous methods developed for determination of tissue viability visual observation and the electrolyte leakage test are the most common ones. Additional techniques used for deciduous woody plants include differential thermal analysis, observations on cellular fluorescence and on cell plasmolysis/deplasmolysis, release of hydrogen cyanide gas, changes in xylem pressure potential, as well as measurement of electrical impedance (Calkins and Swanson 1990).

Differential thermal analysis is based on measuring the heat released when different fractions of water freeze within the tissue. The technique can be used for determining the killing point of deep supercooled cells (Quamme et al. 1972, Ketchie and Kammereck 1987). It allows for rapid measurement of cold hardiness in floral and xylem tissues of several deciduous fruit crops (Montano et al. 1987, Quamme 1991). Observations on cellular fluorescence and plasmolysis as well as recording xylem pressure potential and release of hydrogen cyanide gas have gained very limited use. Measurements of electrical impedance on plant stems before and after freezing have been used to quantify tissue damage since the 1960s (e.g. Wilner 1961, Blazich et al. 1974). Changes in tissue impedance parameters reflect membrane alterations in freeze-injured cells (Ryyppö et al. 1998). The method is rapid and non-destructive, yet many factors can complicate the interpretation of impedance measurements (Calkins and Swanson 1990). However, recent development of techniques for measurement and analysis (e.g. Repo and Zhang 1993, Privé and Zhang 1996, Repo et al. 1997, Repo et al. 2000) indicate that the method has ample potential for measuring cold hardiness both with and without freezing tests.

1.2.5 Analysis of data

Raw data on freezing injury can be used to determine relative hardiness differences due to e.g. genotypic or treatment effects. However, it is often desirable to express cold hardiness in a standardized, quantitative form. The most common measure of cold hardiness is $LT_{50,}$ the temperature at 50 % lethality. Other comparable measures include the lowest survival temperature (LT_0) (Quamme et al. 1972), the temperature interval between 10 % and 90 % injury (LT_{10} to LT_{90}) (Ketchie et al. 1972) and the ultimate killing temperature (LT_{100}) (Howell and Weiser 1970a). The quantitative measures of cold hardiness can be derived by linear interpolation, the Spearman-Kärber method, the logit and the probit model, as well as by fitting nonlinear functions. The choice of the method depends partly on the nature of

injury data. The logit and probit models, as well as the Spearman-Kärber method apply to binary responses, e.g. visual observations on freeze injury recorded as living or dead. Linear interpolation and nonlinear functions presume quantitative response data such as percent lethality or electrolyte leakage quantities.

1.2.5.1 Linear interpolation

Linear interpolation involves graphic estimation of the LT_{50} value on the basis of a freeze injury vs. test-temperature plot. The two mean injury values that parenthesize the 50 % lethality are connected by a straight line and the temperature associated with the 50 % level is read from the x-axis. Temperatures for any percentage damage can be obtained similarly.

When freeze responses are recorded as lethality values, linear interpolation is straightforward. When handling electrolyte leakage data, the level of leakage corresponding to 50 % lethality has to be determined. Sometimes the 50 % level of relative leakage, or index of injury, is simply equated with 50 % sample lethality (e.g. Arora et al. 1992, Grossnickle 1992, Boorse et al. 1998). In woody plant tissue, leakage may change according to the physiological state of the plant and thus, the use of any single leakage value as a general indicator of LT_{50} is unsound. Accordingly, Sutinen et al. (1992) estimated the critical level of leakage as the arithmetic midpoint between non-injured and lethally injured samples. The lethal temperature estimate based on the midpoint level of leakage reliably predicted freezing stress resistance of *Pinus resinosa* Ait. and *Pinus nigra* Arnold needles for most of the year (Sutinen et al. 1992). A similar approach was used by Rajashekar et al. (1982) for determination of the killing temperature in stem tissues of *Pyrus* spp.

Linear interpolation is a robust, quick, and easy graphical method for estimation of lethal temperature values. It assumes that freeze injuries increase monotonically with decreasing temperatures. If monotonicity is violated, adjacent mean injury values are pooled until a set of monotonically increasing means is constructed. Linear interpolation also assumes that the temperature-response pattern is linear between adjacent test temperatures. As this assumption may not always hold true, and because of the graphical nature of the method, the derived lethal temperature estimates may be biased. In addition, no estimate for the degree of confidence associated with the lethal temperature value is readily available.

1.2.5.2 The Spearman-Kärber method

The Spearman-Kärber method is a nonparametric statistical procedure for estimating the median effective dose in binary data. Bittenbender and Howell (1974) suggested application of the Spearman-Kärber method for computing LT_{50} of cold stressed flower buds. The method requires that the test temperature range include 0 % and 100 % lethality responses. If this condition is not met, the next dose in the series of test temperatures, though untested, may be assumed to have given the desired response of total and/or no lethality (Bittenbender and Howell 1974). However, such use of data is without any theoretical basis. Though often it may do little harm, it could be seriously misleading if applied uncritically (Finney 1964).

The Spearman-Kärber method is easy to use, and the variance for the LT_{50} estimate can be readily approximated (Finney 1964). Interpretation and validity of a single-point estimate is clearly improved by providing a measure of variation for it. Statistical investigations of the properties of the Spearman-Kärber estimate have shown that it is an efficient, unbiased maximum likelihood estimator of a discretized mean in symmetric data distributions (Brown 1961, Miller 1973). In cold hardiness research the method has been occasionally used for estimation of LT_{50} in flower buds (Bittenbender and Howell 1974, Johnson and Howell 1981, Takeda et al. 1993, Carter and Hummer 1999) and stem tissues (Hummer et al. 1995).

1.2.5.3 The logit and the probit model

An alternative approach to handling binary data is based on the construction of a statistical model. In the logit model, the dependency of freeze-death probability on the temperature treatment is analysed in the context of generalized linear models by using the logistic transformation (Collett 1991). The transformation ensures that the fitted probabilities will lie between zero and one. The transformed function is symmetric and essentially linear between probabilities of 0.2 and 0.8; outside this range the function becomes markedly nonlinear. Confidence intervals for the estimated LT_{50} values can be constructed on the basis of standard errors and covariances of the logit model parameters (Collett 1991). In addition to treatment temperature, the logit model can be extended to include other numerical or categorical explanatory variables.

The probit model represents another type of statistical model for studying binary responses, first adapted to cold hardiness data by Proebsting and Fogle (1956). The logit and probit functions yield quite similar curves, but the probit transformation relies on normal distribution of the data. The logistic transformation is computationally more convenient, and

allows for a more direct interpretation of model parameters than the probit transformation (Finney 1964, Collett 1991). To date, the logit and probit models have been occasionally applied for analysis of cold hardiness data (Brown et al. 1977, Gudleifsson et al. 1986, Fowler et al. 1989, Deans et al. 1995, Pellett and Heleba 1998).

1.2.5.4 Fitting of nonlinear functions

Curves describing plant response to freezing stress follow a sigmoid shape bounded by a lower and an upper asymptote (Repo and Lappi 1989, von Fircks and Verwijst 1993). Accordingly, nonlinear regression is often used for estimating the lethal temperature, then defined as the inflection point of the dose-response curve. Most researchers have employed the symmetric, logistic function with an inflection point located exactly halfway between the asymptotes, at the midpoint, or median, of the curve (e.g. Khanizadeh et al. 1989, Palliotti and Bongi 1996, Repo et al. 1996). However, von Fircks and Verwijst (1993) showed that freezing injury data were better fitted with the flexible Richards function, which is able to describe asymmetric responses, than with the logistic function. The main virtue of using an asymmetric function is that it allows for determining the inflection point, where the highest rate of change in plant response occurs, independently of the midpoint level of response. It remains to be determined which of the two is a better measure of cold hardiness.

The Richards function inflection point failed to estimate properly the critical level of electrolyte and phenolic leakage in stems and leaves of five woody plant taxa studied by Anisko and Lindstrom (1995). Instead, the lethal temperature range generally coincided with the initial increase in leakage caused by freezing. Therefore, the authors preferred to estimate the lethal temperature by using a point of interception of the lower asymptote with a line tangential to the inflection point of the Richards curve.

Lim et al. (1998) applied the asymmetric Richards and Gompertz functions to electrolyte leakage data measured from freeze-stressed *Rhododendron* leaves. No significant difference was found between the two functions, yet the Gompertz function was preferred due to its slightly better fit and smaller number of parameters. Neither was there any significant difference between the midpoint and the inflection point measures of leaf freezing tolerance. The authors chose temperature at the inflection point as the quantitative measure of cold hardiness because they considered it physiologically more descriptive than the temperature at the midpoint level of leakage.

Nonlinear models represent an efficient tool for analysing freeze injury data, provided that the model corresponds with the data. While using the logistic function, a standard error for the inflection point estimate is easily calculable (e.g. Ingram and Buchanan 1984). For asymmetric sigmoid functions, variation of the point estimates can be approximated using asymptotic statistical methods (e.g. Morgan 1992, Appendix A), or by statistical resampling techniques (von Fircks and Verwijst 1993, Lim et al. 1998). The main disadvantage is the complexity of the curve-fitting process. Practical restrictions often limit the size of experiments and the resulting data sets, which may cause additional problems in estimation of function parameters.

2 AIMS OF THE STUDY

The main objective of the present study was to develop and evaluate computational and experimental techniques for determination of cold hardiness in deciduous trees and shrubs. More specifically, the following objectives were included:

1) to evaluate methods for analysing freeze injury data and for determining the lethal temperature (I, II, III, IV),
2) to examine application of acclimation treatments and controlled freezing tests for determination of cold hardiness characteristics in apple, mock orange and hydrangea (I, II) and
3) to facilitate the design of controlled freezing tests by characterization of critical weather factors accounting for winter injury occurrences in apple trees under Finnish climatic conditions (V).

3 MATERIALS AND METHODS

Materials and methods are described briefly. More detailed information can be found in the original publications (I-V).

3.1 Plant materials and acclimation treatments

The plant material used in controlled freezing tests included two cultivars of apple (*Malus domestica* Borkh.) (I), three clones of both mock orange (*Philadelphus lewisii* Pursh var. *lewisii* 'Waterton') and hydrangea (*Hydrangea paniculata* Sieb. 'Grandiflora') (II) as well as three cultivars of red raspberry (*Rubus idaeus* L.) (III).

Sample twigs of apple were collected on 16 March 1993 from the orchard of the Department of Applied Biology, University of Helsinki (60º10'N, 25º00'E). Mock orange and hydrangea, growing in field trials at Agrifood Research Finland MTT, Horticulture, in Piikkiö (60º23'N, 22º33'E), were sampled for freeze-testing on 4 March, 1993. Raspberry canes were sampled on six dates during the winter 1996-97 (7 October, 7 November, 1 December, 3 February, 3 March, and 10 April), from a cultivar trial at Agrifood Research Finland MTT, Häme Research Station (61º20'N, 24º13'E). All sample material consisted of previous year's growth.

The level of actual cold hardiness was determined for all sample lots. To assess the minimum and potential level of hardiness in late winter (I, II), samples of apple, mock orange and hydrangea were subjected to four different acclimation treatments: hardening at -15ºC for 3 d (i), or for 7 d (ii), and dehardening at 14ºC (apple) or at 12ºC (mock orange and hydrangea) for 3 d (iii), or for 7 d (iv). During hardening, samples were preserved in double polyethylene bags, while dehardening was performed by keeping sample twigs with their basal ends in tap water. The acclimation treatments were carried out in the dark.

3.2 Controlled freezing tests and evaluation of freeze injuries

Samples were frozen in a walk-in controlled-climate chamber (Weiss 2600/45…5 Du-Pi, Weiss Umwelttechnik, Reiskirchen, Germany) to seven test temperatures at 5ºC intervals and at a rate of 5ºC h^{-1}. The test temperatures ranged from –20 to -50ºC (I, II), from –5 to -35ºC (III), or from –15 to -45ºC (III). The duration of each test temperature was 30 minutes. Before freezing, raspberry canes (III) were wrapped in moist paper towels to ensure ice nucleation. All samples were packed in polyethylene bags. While measuring actual cold hardiness,

lowering of air temperature started from 2ºC (I), 0ºC (II), or from prevailing outdoor air temperature (III). When assessing the minimum and potential hardiness levels (I, II), freezing started from the temperature of each respective acclimation treatment. Samples were thawed at 2ºC overnight (I, II) or at 0ºC for 15 to 24 h (III).

Freeze injury on stem tissues (I, II, III) and buds (II) was assessed visually after an incubation period. Apple shoots were placed in sand on a mist propagation bench under daylight conditions for 14-21 d at 20-25ºC. Sample twigs of mock orange and hydrangea were incubated with their cut bases in water at 25ºC under a 16 h photoperiod for 14-17 d, while raspberry canes were kept in polyethylene bags under daylight conditions at room temperature for 7 d. After incubation each sample was evaluated for freeze injury under a dissecting microscope. Injury rating was based on the degree of discoloration and firmness of tissues. A two-class rating (alive/dead) was used for buds as well as for stems of apple and raspberry. Stems of mock orange and hydrangea were judged using a four-class scale.

In addition to visual assessment, an electrolyte leakage test was used for examining the degree of injury in stem tissues of mock orange (II), hydrangea (II), and red raspberry (III). Three internodal 1-cm-long sections of each sample were placed in a test tube containing 8 ml of deionized (II) or ultra-pure (III) water. The number of replicate samples was three per clone (II) or cultivar (III) and freezing temperature. Samples were incubated at room temperature and measured for electrolyte leakage using a conductivity meter (Jenway 4020, Jenway, Felsted, England). Raspberry samples were rinsed before the test, and incubated for 21 h under constant shaking at 160 rpm. Twigs of mock orange and hydrangea were not rinsed and the samples were incubated for 23 h with only a light manual shaking immediately before conductivity measurement. All samples were killed by autoclaving at 120-121ºC for 15 minutes. The final conductivity was measured after re-incubation for 21 h under constant shaking (III), or for 23 h and a manual shaking (II). The results were expressed as relative electrolyte leakage (II) or as percentage-adjusted injury values (III) (Table 1).

Table 1. Calculation of electrolyte leakage indices.

Relative electrolyte leakage at temperature T: $REL_T = (EL_T / EL_{AUTOCL}) \times 100\ \%$
Index of injury at temperature T: $I_T = [(REL_T - REL_{CONTR}) / (100 - REL_{CONTR})] \times 100\ \%$
Percentage-adjusted injury at temperature T: $(I_T / I_{TLOWEST}) \times 100\ \%$

EL_T = sample electrolyte leakage after exposure to temperature T
EL_{AUTOCL} = sample electrolyte leakage after autoclaving
REL_{CONTR} = the mean relative electrolyte leakage of control samples
$I_{TLOWEST}$ = the mean index of injury of samples exposed to the lowest test temperature

3.3 Statistical analyses of freeze injury data

The logit model (Table 2) was applied for analysing visual injury ratings on stem samples (I, II, III). The initial four-class scores for mock orange and hydrangea (II) were transformed to a binary scale. Raspberry data from each sampling time were analysed separately. The freeze-death probability was regressed on freezing temperature, cultivar, acclimation treatment, and treatment duration (I), freezing temperature, clone, and acclimation treatment (II), or freezing temperature and cultivar (III). The software used for estimation of logit model parameters was BMDP logistic regression (LR) procedure (Dixon et al. 1990) (I, II) or the GENMOD procedure of SAS (SAS Institute Inc. 1993) (III). LT_{50} values with 95 % confidence intervals were calculated using logit model parameters as well as their respective standard errors and covariances.

The electrolyte leakage data measured from stems of mock orange and hydrangea (II) were subjected to two-way analyses of variance using BMDP procedure 7D (Dixon et al. 1990). The main effects of freezing temperature and clone and interactions between the two were tested separately for each acclimation treatment. Tukey's test was used for comparison of means when appropriate.

Electrolyte leakage measurements from raspberry canes (III) were used for a comparison between the graphic and curve-fitting methods of determining the lethal temperature. Raspberry leakage data were analysed separately for each cultivar and sampling time. The critical amount of leakage, corresponding to lethal injury, was determined by linear interpolation and by the midpoint and inflection point estimates given by the logistic, Richards and Gompertz functions (Table 2). In addition, two correction procedures were applied to the inflection point estimates produced by the Richards and Gompertz functions. The ten different hardiness indices, thus derived, were compared to the results of visual evaluation by calculating the mean and absolute differences between each respective leakage-based index and the visual LT_{50}, and by conducting a partial correlation analysis (holding sampling time constant). The three nonlinear functions were fitted to the leakage data using Gauss 3.2 software (Aptech Systems 1992). The partial correlation coefficients were calculated using SAS CORR procedure (SAS Institute Inc. 1989).

Table 2. Calculation of lethal-temperature estimates LT_{50} (temperature at 50 % lethality) and LT_{max} (temperature at the highest rate of change in the response variable).

The logit model (I, II, III): Logit $\left[\pi(T)\right] = \ln\left(\frac{\pi(T)}{1-\pi(T)}\right) = \alpha_1 ... + \alpha_n + \beta T + \gamma$, $LT_{50} = -\left(\frac{\alpha_1 ... + \alpha_n}{\beta}\right)$

π is the freeze-death probability,
parameters $\alpha_1 ... \alpha_n$ describe the effects of categorical explanatory variables,
parameter β describes the magnitude of the effect of freezing temperature T
and γ describes the effects of interactions.

Nonlinear regression (III, IV*)

The logistic function: $\frac{100}{1+\exp(b-cT)}$ $LT_{50} = \frac{b}{c}$ $LT_{max} = \frac{b}{c}$

The Richards function: $\frac{100}{\left[1+\exp(b-cT)\right]^{1/d}}$ $LT_{50} = \frac{b-\ln(2^d-1)}{c}$ $LT_{max} = \frac{b-\ln(d)}{c}$

The Gompertz function: $100\exp\left[-b\exp(-cT)\right]$ $LT_{50} = -\frac{\left[\ln\ln(2)-\ln(b)\right]}{c}$ $LT_{max} = \frac{\ln(b)}{c}$

b, *c* and *d* are function parameters,
exp indicates exponential,
T is freezing temperature.

The Spearman-Kärber method (IV): $LT_{50} = \sum_{i=1}^{k-1}(p_{i+1} - p_i)(T_i + T_{i+1})$, assuming that $p_i = 0$ and $p_k = 1$

p_i denotes lethality frequency at freezing temperature T_i.

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*In the simulation study (IV) the logistic and Richards functions were applied in a slightly modified form.

3.4 Simulation study

The different methods for estimating the lethal temperature were further explored by Monte Carlo simulation under a range of sampling conditions (IV). The relative merits of linear interpolation, the Spearman-Kärber method and fitting of the logistic function were evaluated on the basis of data generated by the Richards function, here considered as the true model for lethality frequencies at different temperatures. Data sets were generated using two numbers of doses (i.e. test temperatures), and for both dose numbers, five different patterns of skewness in response data. For each of the ten conditions, 1000 simulated data sets were drawn from the Richards distribution. An LT_{50} estimate was determined for all Monte Carlo replications using linear interpolation, the Spearman-Kärber method, the logistic and the Richards function (Table 2). The Monte Carlo sample mean and standard deviation were calculated for each respective LT_{50} estimator. The four methods were evaluated by considering the accuracy and precision of the Monte Carlo means. All calculations were done using Gauss 3.2 software (Aptech Systems 1992).

The four estimation methods were also applied to five real data sets (IV), representing small experiments typical in cold hardiness research. The first set of data originated from a controlled freezing test on apple trees carried out in the Department of Applied Biology in 1997 (L. Lindén, unpublished data). The subsequent two data sets, from a study by Palonen and Junttila (1999) show the effect of exogenously applied sucrose on the cold hardiness of *in vitro* red raspberry plants. Finally, the four estimation techniques were applied to two published sets of data on the cold hardiness of blueberry (*Vaccinium australe* Small) flower buds (Bittenbender and Howell 1974). In all experiments, the effect of freezing temperature was assessed by visually rating samples as living or dead.

The lethal temperature for each of the five data sets was estimated by linear interpolation, the Spearman-Kärber approach and fitting of the logistic and Richards function. Confidence intervals were analytically derived for the Spearman-Kärber, the logistic and the Richards estimators. The performance of all four LT_{50} estimators was further examined by a bootstrap resampling technique. Each data set was resampled with replacement for 1000 times; samples where lethality declined with lowering temperature were discarded. The lethal temperature was estimated for each bootstrapped data using the four methods. The bootstrap LT_{50} means were computed and confidence intervals for them were derived using the 95 percentiles of bootstrap distributions. The methods were evaluated on the basis of the LT_{50} estimators as well as their analytical and empirical confidence intervals.

3.5 Winter injury occurrences and climatic data

A re-analysis of historical records on winter injury (V) was undertaken to determine which characteristics of cold hardiness are critical for winter survival of apple trees under Finnish climatic conditions. The scope of the study covered 71 years (1927-1998), out of which 12 years were associated with wide-spread winter injury. Records of winter injury occurrences were mainly based on observations from apple orchards in southwestern Finland, collected through the Agricultural Research Centre of Finland.

The weather data for 1927-1998 were acquired from the Finnish Meteorological Institute, Climate Service. The data consisted of records on the monthly minimum, maximum, and mean air temperature and the monthly precipitation for each year as well as records on the length of growing season and the cumulative growing degree days for years 1928-1998. To study the frequency and character of winter temperature fluctuations, six additional weather variables were derived on the basis of daily minimum and maximum temperatures. Measurements of air temperature and precipitation had been made at Piikkiö (60º23’N, 22º33’E), while data on the length of growing season and growing degree days were based on observations made at the weather station of Turku (60º31’N, 22º16’E).

3.6 Statistical analyses of climatic and winter injury data

The critical weather factors associated with winter injury occurrences (V) were determined by subjecting two sets of 24 climatic variables to discriminant and cluster analyses. The first data set, based on a preliminary exploration of scatterplots and frequency distributions, included monthly mean temperature from September to March, monthly minimum temperature from November to March, monthly maximum temperature from December to March, monthly precipitation from June to November, length of the growing season, and growing degree days. To make the results comparable with a Canadian study conducted by Coleman (1992), a second set of variables identical with those employed by Coleman was composed. The second set consisted of monthly mean, minimum, and maximum temperature from October to March, monthly precipitation for October and November and four variables derived from occurrences of daily temperature drops from above zero to below -20ºC. Both data sets were subjected to similar analyses.

The discriminant analyses were first carried out with the 24 weather variables as independent and the historical records of winter injury as dependent variables. Stepwise discrimination was employed for selection of the most relevant variables from amongst the

24. The years under study were further explored by cluster analysis to investigate if they could be partitioned into two or more clusters such that years within one cluster were similar and years in different clusters dissimilar with respect to the 24 weather variables. The impact of the 24 weather variables on the clustering was studied by further discriminant analyses using the chosen number of clusters as dependent variables. Clustering was done employing Späth's medoid partitioning algorithm in the program NCSS 2000 (Hintze 1998). For discriminant analyses both procedure DISCRIM in SAS (SAS Institute Inc. 1989) and NCSS 2000 were used.

4 RESULTS AND DISCUSSION

4.1 Evaluation of methods for analysing freeze injury data and for determining the lethal temperature

4.1.1 The logit model (I, II, III)

The qualitative freeze-injury data, recorded through visual assessments, were analysed using the logit model. Effects of freezing temperature (I, II, III), cultivar (I, III), and acclimation treatment (I, II) on the freeze-death probability could be directly assessed by model coefficients and the corresponding odds ratios. The lethal temperature values (LT_{50}s) were easily estimated using logit model parameters. Corresponding measures of cold hardiness can be determined for any probability level desired (e.g. LT_0, LT_{10}, LT_{90}), with the aid of parameter estimates (Collett 1991). Calculation of confidence intervals for the lethal temperature values (I, II) renders the hardiness estimates more informative than single-point estimates. If freezing injuries are recorded using a multi-class rating, the resulting data can be analysed by employing the ordinal logit technique (e.g. Liao 1994). Alternatively, the ratings can be re-classified into two categories: living (expected to recover) and dead (irreversibly injured), as was done for mock orange and hydrangea (II).

The main disadvantage of the logit model is that the freeze-death probability curves are assumed to be symmetric. Von Fircks and Verwijst (1993) compared freeze-response curves produced by the symmetric logistic function and the asymmetric Richards function for *Salix viminalis* L. shoots. The results revealed that freeze-response may be inherently asymmetric and thus, fitting the data with a symmetric model may lead to biased lethality estimates. However, in their study the bias was largest for point estimates near the tails of the logistic function (LT_5 or LT_{95}), while the LT_{50} estimates given by the symmetric and the asymmetric function were quite similar.

4.1.2 Comparison of linear interpolation and fitting of nonlinear functions (III)

For raspberry canes, determination of the lethal temperature on the basis of freeze-induced electrolyte leakage was most successful using linear interpolation or by applying the midpoint estimate of the logistic or the Gompertz function. The LT_{50} values thus obtained were well correlated with hardiness estimates based on visual evaluation of freeze injuries. Even the inflection point of the Gompertz function yielded lethal-temperature estimates fairly well in accordance with those obtained visually, but both the midpoint and the inflection point estimate of the Richards function deviated largely from visual LT_{50}s.

The simple linear interpolation method suggested by Sutinen et al. (1992) performed reasonably well in relating electrolyte leakage measurements to lethal temperature in red raspberry. The method is easily applicable and independent of data transformation. The LT_{50} estimate given by linear interpolation was comparable to the estimates obtained by the midpoint of both the symmetric, logistic function and the asymmetric Gompertz function. In contrast, the Richards function, recommended for analyses of freeze-injury data by von Fircks and Verwijst (1993), failed in lethal temperature estimation. The Richards function inflection point overestimated cold hardiness in all data sets analysed. Similar results were obtained by Anisko and Lindstrom (1995) while applying the Richards function to leakage data from stems and leaves of five woody plant taxa. However, Lim et al. (1998) reported a close agreement between visual LT_{50}s and leakage-based critical-temperature estimates obtained from both the Richards and the Gompertz function.

Anisko and Lindstrom (1995) achieved an improvement in interpretation of leakage data by using a correction method in connection with the Richards function. In the current study neither their method, nor the related, but more general correction procedure introduced by us brought about any substantial improvement in lethal temperature prediction as compared with using the original Richards and Gompertz functions.

The Richards function is highly nonlinear in comparison with the logistic and Gompertz functions. The nonlinearity in parameters makes fitting of the Richards function difficult. In this study, the electrolyte leakage data were transformed into percentage-adjusted injury values, spread between 0 and 100 %, as suggested by Lim et al. (1998). The data transformation made curve fitting easier, yet fitting the Richards function to leakage data from raspberry canes failed in several data sets, whereas the logistic and Gompertz functions fitted the data well in most cases. Also Lim et al. (1998) obtained a better fit for the Gompertz than for the Richards function. Taking into consideration the previous findings and the results of the current study, the Gompertz function is suggested for modelling freeze-induced electrolyte leakage data.

For raspberry canes, the midpoint estimate of the Gompertz function was better correlated with visual LT_{50} than the inflection point estimate. The midpoint of leakage was always found at a lower temperature than the highest rate of change in leakage. However, Lim et al. (1998) considered the inflection point physiologically more descriptive than the midpoint; in their study the two point estimates predicted freezing tolerance equally well. As indicated by von Fircks (1994), the most valid hardiness index would be the point at which the threshold for irreversible freezing damage is located. Because recovery of plants has been

observed at temperatures below the inflection point, it probably does not represent the threshold (von Fircks 1994). In the present study, the highest rate of electrolyte leakage was found at a temperature that was on average 3.4ºC higher than the visually estimated LT_{50}. Hence, the results correspond with von Fircks's view: the inflection point of electrolyte leakage does not seem to represent the threshold for irreversible damage. However, considering both the midpoint and the inflection point estimate may be useful e.g. in cultivar comparisons.

In our study, curves of electrolyte leakage vs. freezing temperature were flattened in the course of cold acclimation and dormancy development, rendering curve fitting more difficult. Poor leaching of electrolytes from samples of well-acclimated woody plants has been reported in several earlier papers (Sutinen et al. 1992, Anisko and Lindstrom 1995, Deans et al. 1995, Repo et al. 1996). Changes in the electrolyte leakage test technique suggested by Deans et al. (1995) (a longer incubation and autoclaving time) might improve the release of electrolytes and the quality of data. Furthermore, in the present study, it would have been beneficial to increase the number of test temperatures at the low-temperature end. Thus the low-temperature asymptote of the leakage curve would have been probably better covered. Also changes in the freezing test technique, e.g. a longer exposure time and controlled thawing at a constant rate, might alter the shape of leakage curves. Briefly, every effort to improve the quality of freeze-induced electrolyte leakage data should be made to ascertain a proper interpretation of leakage vs. test temperature curves. The meaningfulness of a methodological comparison in such imperfect data like ours may be questioned. However, we sought to establish a reliable method for determination of the LT_{50} for use in practical situations, under the restrictions of time, labour and equipment available.

4.1.3 Simulation study of linear interpolation, the Spearman-Kärber approach, and fitting of the logistic and the Richards function (IV)

The simulation study was aimed at comparing the statistical properties of LT_{50} estimates produced by two nonparametric and two parametric methods under different sampling conditions. To the best of our knowledge, Monte Carlo simulation has not previously been applied to problems of lethal dose estimation in temperature stress studies. Instead, Monte Carlo studies are frequently used in the field of e.g. econometrics, to shed light on the small-sample properties of competing estimators. As presented by Kennedy (1998), the general idea behind a Monte Carlo study is to (1) model the data-generating process, (2) generate several sets of artificial data by computer simulation, (3) employ these data and an estimator to create

several estimates, and (4) use these estimates to assess the sampling distribution properties of that estimator. The results are viewed in the context of the specific conditions studied, i.e. under the restrictions of the model chosen to produce the data.

In this study, the Richards function was assumed to be the true model for lethality frequencies at different temperatures. The true lethal temperature value was controlled, as the data sets were generated from the Richards distribution function with *a priori* set parameter values. The bias and precision of the different estimators could thus be evaluated directly. The results indicated that all four methods perform equally well, when the response data are symmetric. In asymmetric situations, linear interpolation was superior to the Spearman-Kärber method and the logistic function in accuracy and precision. The Spearman-Kärber method was particularly sensitive to negatively skewed response patterns. As expected, the Richards estimator was the least biased and variable.

The superiority of linear interpolation for skewed distributions can be explained by the fact that the method is not affected by tail observations, as it utilizes only the two mean responses on both sides of the median level. In contrast, fitting the logistic function and the Spearman-Kärber method employ all data. Furthermore, the Spearman-Kärber approach yields a discretized estimator of the mean (Brown 1961, Miller 1973), which is equal to the median (i.e. LT_{50}) only if the underlying tolerance distribution is symmetric. The present results indicate that the Spearman-Kärber method does not perform well in asymmetric conditions.

The real data applications demonstrated the drawbacks of the Richards method: fitting of the complex function to small experimental data sets can be particularly difficult. In each of the five actual data sets studied, the Richards function yielded the most deviating lethal temperature estimate, while the other three estimators were rather similar. The bootstrap resampling, however, indicated that, notwithstanding fitting problems, the small-sample properties of the Richards estimator were comparable to those of estimators obtained by linear interpolation or the logistic function. The bootstrap is a special Monte Carlo method for estimating the distribution of an estimator or a test statistic by resampling data. The basic idea is to take the sample of interest and regard it as a population and then by resampling create a new (bootstrap) sample, which is used to compute the desired quantity. Provided that the sample is a good approximation of the population, bootstrapping will render a good approximation of the sampling distribution of the estimator or test statistic (Efron and Stein 1981).

Contrary to the Richards function, the Spearman-Kärber point estimate performed quite well in the five actual data sets. However, bootstrapping the method produced more deviating and variable lethal temperature estimates than the other procedures. It appears that the Spearman-Kärber approach is less reliable than the other methods when applied to small samples where there is asymmetric contamination or where the doses are not symmetric around the median response. Linear interpolation and fitting of the logistic function yielded robust lethal temperature estimates calculable in all examples studied.

The empirical confidence intervals, derived by bootstrap resampling, indicated that the average precision of estimators obtained by linear interpolation, the logistic, or the Richards function were similar. In temperature stress studies, resampling procedures have previously been applied only for determining the standard error of the Richards estimator (von Fircks and Verwijst 1993, Lim et al. 1998). Bootstrap resampling proved useful and justified as the empirical confidence intervals were nearly always wider than those obtained analytically. However, the most obvious problem with bootstrapping relates to small sample size. If the sample distribution function is not a good approximation of the population distribution function, the bootstrapped estimate of the sampling distribution will be inaccurate (Mooney and Duval 1993). Since all the five data sets bootstrapped in our study were small, the results must be considered with caution: the empirical confidence intervals may be inaccurate due to the small size of the original data sets.

As stated by Hamilton (1979), the method for routine analyses of the median effective dose ED50 – analogous to LT_{50} – must have the following characteristics: (a) be reasonably accurate and precise; (b) be relatively unaffected by a small fraction of anomalous responses; and (c) be calculable for all experimental outcomes. Concerning practical applications, the list could be continued with a fourth criterion: (d) be quick and feasible. The present study indicated that in small samples, estimation of LT_{50} by simple linear interpolation fulfils these characteristics equally well or even better than the two parametric methods examined.

4.2 Application of acclimation treatments and controlled freezing tests for determination of cold hardiness characteristics in apple, mock orange and hydrangea (I, II)

The actual stem cold hardiness of apple cv. Antonovka was -46ºC and that of cv. Samo -43ºC (Table 3). The actual cold hardiness estimates for mock orange and hydrangea stems were -38ºC and -37ºC, respectively (Table 3), while buds of both species were hardy to below -50ºC. Artificial hardening at -15ºC for 3 to 7 d had no detectable effect on the level of cold hardiness in stems of any of the three taxa. Control twigs of all three species, when forced at

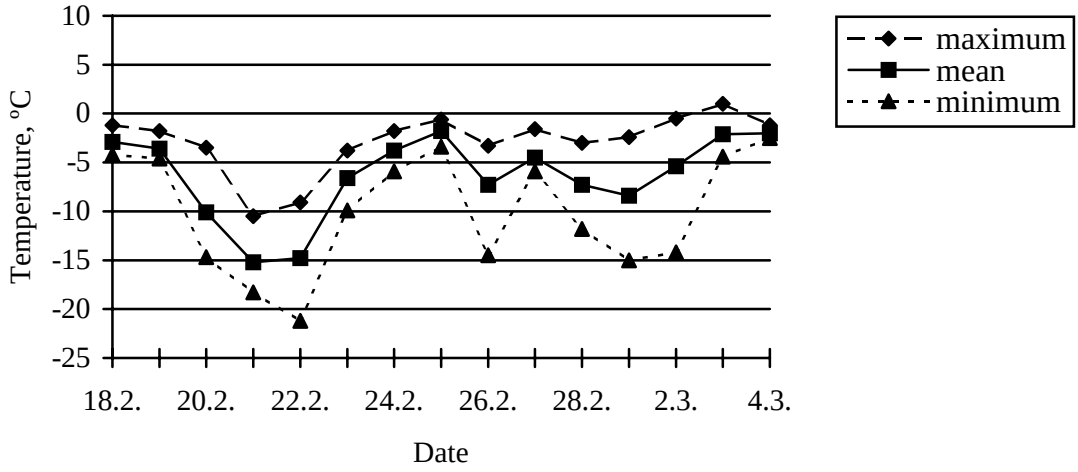
room temperature, broke bud within 2 to 3 weeks, indicating that endodormancy was released at the time of sampling. The results are in accordance with those presented earlier for apple (Tjurina 1968, Quamme et al. 1972), *Betula pubescens* Ehrh. (Sakai 1973) and *Amelanchier alnifolia* Nutt. (Junttila et al. 1983): under natural conditions the trees retain a high level of cold hardiness until late March, long after the release of endodormancy.

Table 3. Cold hardiness characteristics (LT_{50} with 95 % confidence intervals) in stems of two apple cultivars, mock orange and hydrangea as indicated by direct freezing and application of four acclimation treatments (hardening at -15ºC, dehardening of apple at 14ºC, dehardening of mock orange and hydrangea at 12ºC). Estimates are based on visual ratings analysed using the logit model.

	LT_{50}, ºC				
	Direct freezing	Hardening for 3 d	Hardening for 7 d	Dehardening for 3 d	Dehardening for 7 d
Apple cv. Antonovka	-46	-44	-42	-34	-31
	(-42.5, -48.8)	(-40.5, -47.1)	(-39.1, -45.6)	(-29.9, -37.8)	(-27.4, -33.6)
Apple cv. Samo	-43	-41	-40	-31	-28
	(-39.7, -46.1)	(-37.7, -44.6)	(-36.5, -42.8)	(-27.7, -34.5)	(-24.6, -30.9)
Mock orange	-38	-38	-38	-22	-22
	(-36.4, -39.5)	(-36.9, -39.9)	(-36.4, -39.5)	(-20.0, -23.1)	(-20.0, -23.1)
Hydrangea	-37	-36	-36	-28	-26
	(-35.0, -38.8)	(-34.5, -38.2)	(-34.5, -38.2)	(-25.6, -29.4)	(-24.6, -28.2)

The ultimate limit of cold hardiness for apple trees, which display deep supercooling of xylem ray cells, lies between –40ºC and -45ºC (Quamme 1976). The trees under study were thus at or close to the state of their maximum attainable cold hardiness still in mid-March. The average of two weeks' mean temperatures (Fig. 1a) prior to sample collection was –1.7ºC, being close to the long-term average temperature of March in Helsinki (-2.1ºC) (Ilmatieteen laitos 1993b). During the two weeks, air temperature fluctuated a few degrees around zero until two days before sampling, when the temperature remained continuously above zero. Hence, the short natural thaws did not bring about dehardening in apple shoots.

a)



b)

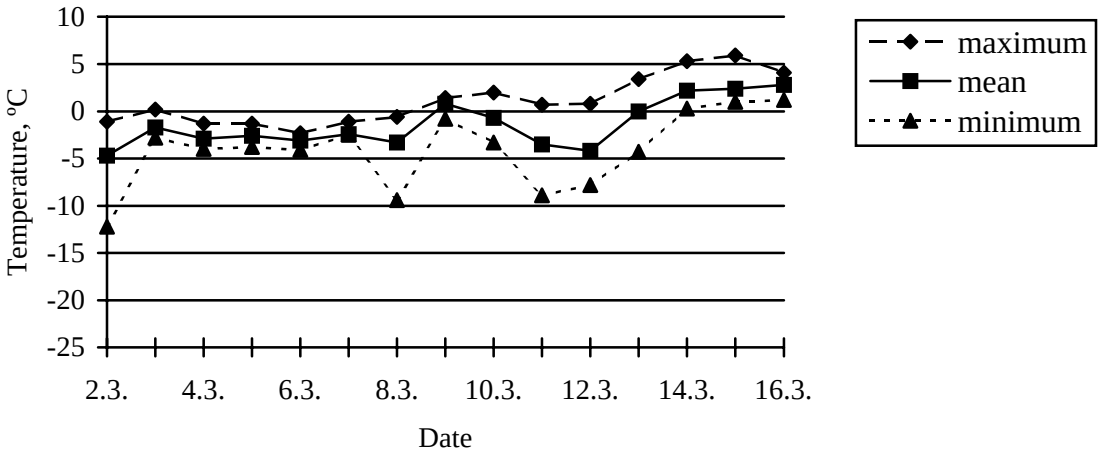


Figure 1. Daily mean, minimum and maximum temperature during two weeks prior to sample collection (a) at Piikkiö and (b) in Helsinki (Ilmatieteen laitos 1993a, b).

No previous reports on controlled freezing of either mock orange or hydrangea were found. During two weeks prior to sampling, the average of daily mean temperatures (Fig. 1b) in Piikkiö was –6.7ºC, which is close to the site's long -term (1960-1990) average in February (-6.3ºC) (Ilmatieteen laitos 1993a). Until one day before sample collection, the daily maximum temperatures remained below zero, keeping the plants at a high level of cold hardiness.

The two apple cultivars lost 12ºC of their initial cold hardiness during three days' dehardening at +14ºC. Dehardening at +12ºC for 3 d diminished the cold hardiness of mock orange and hydrangea stems by 16ºC and 10ºC, respectively. Prolonging the dehardening

treatment from 3 to 7 d had no significant effect on stem cold hardiness in any of the respective species. Thus, the minimum level of stem cold hardiness in late winter was –28ºC to -34ºC for apple, -22ºC for mock orange and -26ºC to -28ºC for hydrangea (Table 3).

After dehardening, buds of mock orange were injured at the same temperature as stem tissues, i.e. their level of cold hardiness decreased from below -50ºC to -22ºC in three days. Hydrangea buds were more resistant to dehardening; the three days' treatment did not affect their cold hardiness, while after seven days the buds survived -30ºC. Previous studies have shown that after endodormancy release artificial exposure to warm temperatures brings about rapid dehardening in twigs of *Acer*, *Viburnum*, and *Cornus*, as well as in buds of *Amelanchier* (Irving and Lanphear 1967a, Litzow and Pellett 1980, Kaurin et al. 1984). On the other hand, twigs of extremely hardy *Betula*, *Salix*, and *Populus* lost little or no cold hardiness when exposed to +2ºC, +10ºC, or +18ºC for three days (Sakai 1973).

As the sample twigs were kept with their bases in water during the dehardening treatments, the observed loss of cold hardiness might not be solely due to warm temperature. An increase in tissue water content is closely correlated with dehardening (Junttila et al. 1983, Kaurin et al. 1984, Sakai and Larcher 1987). High water content has both a direct effect on cold hardiness and an indirect effect mediated by increased respiratory consumption of cryoprotective sugars (Ögren 1996). Hence, a more realistic dehardening effect would have been obtained, if samples used for determining actual cold hardiness had also been allowed to gain full water status prior to the freezing test, or by excluding rehydration from the dehardening treatment.

The concepts of actual, potential and minimum cold hardiness are here used as suggested by Larcher (1985). Actual cold hardiness refers to the momentary level of hardiness at the time of sample collection. The potential and minimum levels of hardiness provide a measure for the plant's hardening and dehardening capacity at the time of sampling, i.e. in the prevailing stage of annual development. Hence, the acclimation treatments indicated that apple, mock orange and hydrangea were in a fully hardened state in the first half of March. At the same time, the dehardening capacity of apple, mock orange and hydrangea was 15ºC, 16ºC and 11ºC, respectively.

Hydrangea clones dehardened less than clones of mock orange, which may reflect the maritime origin of the species. Maritime plants are not as susceptible to deacclimation in spring as continental species and ecotypes (Scheumann 1968, Larcher 1985). Yet, all respective clones of both species, and the two apple cultivars, seemed to manage warm

periods during ecodormancy quite well, as the 7-d dehardening treatment did not diminish the level of cold hardiness in comparison with the 3-d treatment.

The current study proved apple cv. Antonovka more cold hardy than cv. Samo. 'Antonovka' is an old Russian cultivar, regarded as extremely hardy on the basis of both field performance and laboratory tests (e.g. Emmert and Howlett 1953, Vuorinen 1956). It is recommended for the whole Finnish apple-growing area (hardiness zones I-V), while the newly bred Finnish cv. Samo is considered intermediate in cold hardiness (zones I-III) (Lehmushovi et al. 2001).

On the basis of visual assessments, the three clones of mock orange and hydrangea did not differ from each other with respect to actual, potential, or minimum cold hardiness. The electrolyte leakage tests revealed slight interclonal differences in both species, yet the clone effect was minimal in comparison with the effect of freezing temperature. The clones originated from selected hardy, true-to-type mother plants growing in established ornamental plantations in different parts of Finland. In long-term field trials, the clones have not displayed any major differences in winterhardiness (Juhanoja et al. 1998, 2001). Relative to genotypic hardiness differences, the present results are thus in accordance with observations on field performance, for apple as well as for mock orange and hydrangea.

The cold hardiness characteristics of the three taxa were examined by artificial acclimation treatments and controlled freezing tests. The results thus obtained are inevitably bound to the methods applied, i.e. application of different acclimation times or temperatures as well as changes in the freezing protocol might yield different results. The acclimation and freezing methods of the present study seemed to work relatively well for the three taxa studied.

Kaukovirta (1967) studied overwintering of woody ornamentals in different parts of Finland in field experiments comprising 37 deciduous and 12 coniferous taxa. The results indicated that the plants were more often injured by longer periods of fairly cold weather (<-20ºC) than by short, very cold spells. Hence, it might be useful to test potential plant introductions by subjecting them to prolonged freezing at a moderately low temperature, rather than to a severe, short-duration cold stress as in the present study. Preliminary results on prolonged freezing of apple (L. Lindén, unpublished data) and red raspberry (Palonen et al. 2001) shoots at –20 to -35ºC for up to 15 d indicate that the method works well for assessing varietal hardiness differences.

4.3 Characterization of critical weather factors by multivariate statistical techniques (V)

Exploring the historical records of winter injury in Finnish apple orchards by multivariate analyses revealed low winter temperatures as the major factor associated with winter injury occurrences in the climate of south-western Finland. The result is in accordance with the findings of Caprio and Quamme (1999). In the Okanagan Valley of British Columbia, low temperature during November, December, and February is the main climatic factor limiting apple production.

In addition to mid-winter severity, the current study proved weather conditions during the preceding summer and fall relevant for successful overwintering at high latitudes. A decrease in growing degree days, drought in August, and abundant rainfall in September were associated with winter-kill years. On the northern edge of apple production, accumulation of growing degree days is critical for completion of the annual growth cycle. Insufficient growing time reduces the hardening capacity of apple trees (Tumanov et al. 1972). Drought in late summer and a high level of precipitation in September may alter timing of growth cessation late in the season. As termination of apical elongation growth is a prerequisite for cold acclimation, late-growing trees may not acclimate properly. In Central Europe, a delay in growth cessation due to abundant rainfall is recognized as being one of the factors that sensitises apple trees to winter injury (Kolbe 1985, Heinze 1999).

Rapid temperature fluctuations in mid-winter are associated with apple winter injury occurrences in Canada (Coleman 1992, Caprio and Quamme 1999). Kaukovirta and Syri (1985) regarded mild spells during December followed by a rapid drop in temperature as a typical feature of winter-kill years in Finland. Therefore, they suggested that breeding apple for Finnish conditions should be aimed at improving the stability of cold hardiness and the rate of rehardening after thaw periods. However, in the present study the hypothesis on the adverse effects of mild spells could not be verified.

The results indicated that in the Finnish climate, mid-winter cold hardiness is the most important trait for winter-survival of apple trees. Achievement of full acclimation capacity presumes timely growth cessation, which is ensured by choosing early-maturing cultivars as well as by using appropriate rootstocks and cultural practices.

5 CONCLUSIONS

Experimental measurement of cold hardiness provides a useful means for examination of different hardiness characteristics in plants, and for defining the impact of environmental factors on the level of hardiness. Results from laboratory tests can thus be used to supplement and accelerate field experimentation. The main emphasis of this study was on the methods used for laboratory measuring of cold hardiness.

Laboratory testing of cold hardiness involves evaluation of freeze injuries. The qualitative and discrete method of visual assessment is commonly applied, at least as a control for more quantitative tests. On the basis of the present results, qualitative freeze-injury data can be successfully analysed using the logit model. The major benefits of the logit model are: 1) the form of the sampling variation in discrete response data is taken into account, 2) the lethal temperature, with confidence intervals, can easily be estimated, and 3) treatment effects can be directly evaluated. The main disadvantage is that the freeze-response data are assumed to be symmetric. However, while determining the temperature at 50 % lethality, the effect of the potential asymmetry is insignificant.

The electrolyte leakage test is one of the quantitative methods for evaluation of tissue injury after controlled freezing tests. In cold acclimating woody plants, a single critical value of freeze-induced electrolyte leakage, discriminating between living and dead samples, can not be established. The present study indicated that in woody stem samples, relating electrolyte leakage measurements to the lethal temperature is best done by linear interpolation or by the midpoint estimate of the logistic or the Gompertz function. Linear interpolation is easily applicable and independent of data transformation. Fitting of nonlinear functions is facilitated by transforming the relative leakage data between 0 % and 100 %. The Gompertz function is preferable to the logistic function as it allows for asymmetry in leakage response and determination of two point estimates for cold hardiness: the midpoint and the inflection point of electrolyte leakage. Consideration of both point estimates might be useful in e.g. cultivar comparisons.

The standard measure of cold hardiness, temperature at 50 % lethality (LT_{50}), can be derived using a range of computational techniques. The simulation study on LT_{50} estimates produced by linear interpolation, the Spearman-Kärber method, and fitting of the logistic or the Richards function revealed that all four methods performed equally well, when the response data were symmetric. For asymmetric conditions, linear interpolation is preferable to the Spearman-Kärber method and the logistic function. Previously, the Richards function

was recommended for analyses of quantitative temperature-stress data. On the basis of current results, the superiority of the Richards function may often be lost in practical situations due to problems in fitting the complicated function to small data sets. The simple linear interpolation is a robust, quick and feasible method, which yields reasonably accurate and precise LT_{50} estimates.

Application of controlled freezing tests and acclimation treatments revealed the level of actual, potential and minimum cold hardiness in apple, mock orange and hydrangea. The results indicated that under the climatic conditions of southern Finland, all three taxa remain in a well-acclimated state till March, long after endodormancy release. Furthermore, the three species seem to manage warm periods during ecodormancy quite well, since they were able to maintain a relatively high level of minimum cold hardiness under deacclimating conditions.

To be effective, laboratory testing of cold hardiness should be directed towards those hardiness characteristics that are the most crucial under respective individual climatic conditions. On the basis of the current study, the severity of mid-winter (January through March) is the major climatic factor associated with apple winter injury in southwestern Finland. Weather conditions during the preceding summer and fall are also relevant for successful overwintering at high latitudes, due to their impact on the annual cycle of vegetative growth. Consequently, mid-winter cold hardiness and timing of cold acclimation are the most important hardiness characteristics to be assessed when screening new apple genotypes for their cultivation potential under Finnish conditions.

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REFERENCES

Anisko, T. & Lindstrom, O.M. 1995. Applying the Richards function in freezing tolerance determination with electrolyte and phenolic leakage techniques. Physiologia Plantarum 95: 281-287.

Anisko, T. & Lindstrom, O.M. 1996a. Survival of water-stressed Rhododendron subjected to freezing at fast or slow cooling rates. HortScience 31: 357-360.

Anisko, T. & Lindstrom, O.M. 1996b. Cold hardiness and water relations parameters in Rhododendron cv. Catawbiense Boursault subjected to drought episodes. Physiologia Plantarum 98: 147-155.

Aptech Systems Inc. 1992. The Gauss system, version 3.0. Vol. 1 and 2. Maple Valley, WA, Aptech Systems, Inc. 391 p. and 592 p.

Arora, R., Rowland, L.J. & Panta, G.R. 1997. Chill-responsive dehydrins in blueberry: Are they associated with cold hardiness or dormancy transitions? Physiologia Plantarum 101: 8-16.

Arora, R. & Wisniewski, M. 1996. Accumulation of a 60-kD dehydrin protein in peach xylem tissues and its relationship to cold acclimation. HortScience 31: 923-925.

Arora, R., Wisniewski, M. & Scorza, R. 1992. Cold acclimation in genetically related (sibling) deciduous and evergreen peach [Prunus persica (L.) Batsch]. 1. Seasonal changes in cold hardiness and polypeptides of bark and xylem tissues. Plant Physiology 99: 1562-1568.

Bervaes, J.C.A.M., Ketchie, D.O. & Kuiper, P.J.C. 1978. Cold hardiness of pine needles and apple bark as affected by alteration of day length and temperature. Physiologia Plantarum 44: 365-368.

Bittenbender, H.C. & Howell, G.S., Jr. 1974. Adaptation of the Spearman-Kärber method for estimating the T_{50} of cold stressed flower buds. Journal of the American Society for Horticultural Science 99: 187-190.

Bittenbender, H.C. & Howell, G.S. 1975. Interactions of temperature and moisture content on spring de-acclimation of flower buds of highbush blueberry. Canadian Journal of Plant Science 55: 447-452.

Blazich, F.A., Evert, D.R. & Bee, D.E. 1974. Comparison of three methods of measuring winter hardiness of internodal stem sections of Forsythia intermedia 'Lynwood'. Journal of the American Society for Horticultural Science 99: 211-214.

Boorse, G.C., Bosma, T.L., Meyer, A.-C., Ewers, F.W. & Davis, S.D. 1998. Comparative methods of estimating freezing temperatures and freezing injury in leaves of chaparral shrubs. International Journal of Plant Science 159: 513-521.

Brown, B.W., Jr. 1961. Some properties of the Spearman estimator in bioassay. Biometrika 48: 293-302.

Brown, G.N. & Bixby, J.A. 1976. Relationship between black locust seedling age and induction of cold hardiness. Forest Science 22: 208-210.

Brown, G.N., Bixby, J.A., Melcarek, P.K., Hinckley, T.M. & Rogers, R. 1977. Xylem pressure potential and chlorophyll fluorescence as indicators of freezing survival in black locust and western hemlock seedlings. Cryobiology 14: 94-99.

Burke, M.J., Gusta, L.V., Quamme, H.A., Weiser, C.J. & Li, P.H. 1976. Freezing and injury in plants. Annual Reviews in Plant Physiology 27: 507-528.

Calkins, J.B. & Swanson, B. T. 1990. The distinction between living and dead plant tissues – Viability tests in cold hardiness research. Cryobiology 27: 194-211.

Cappiello, P.E. & Dunham, S.W. 1994. Seasonal variation in low-temperature tolerance of Vaccinium angustifolium Ait. HortScience 29: 302-304.

Cappiello, P.E., Wachter, J.F. & Libby, B. 1997. Low-temperature tolerance of testing woody ornamental plants: a comparison of ice-seeded vs. nonseeded methodologies. HortScience 32:508.

Caprio, J.M. & Quamme, H.A. 1999. Weather conditions associated with apple production in the Okanagan Valley of British Columbia. Canadian Journal of Plant Science 79: 129-137.

Carter, J. & Hummer, K.E. 1999. Gooseberry mite infestation decreases the cold hardiness of dormant black currant flower buds. HortScience 34: 218-220.

Caswell, K.L., Tyler, N.J. & Stushnoff, C. 1986. Cold hardening of in vitro apple and saskatoon shoot cultures. HortScience 21: 1207-1209.

Chalker-Scott, L., Fuchigami, L.H. & Harber, R.M. 1989. Spectrophotometric measurement of leached phenolic compounds as an indicator of freeze damage. Journal of the American Society for Horticultural Science 114: 315-319.

Chen, P.M. & Li, P.H. 1977. Induction of frost hardiness in stem cortical tissues of Cornus stolonifera Michx. by water stress. II. Biochemical changes. Plant Physiology 59: 240-243.

Chen, P.M., Li, P.H. & Burke, M.J. 1977. Induction of frost hardiness in stem cortical tissues of Cornus stolonifera Michx. by water stress. I. Unfrozen water in cortical tissues and water status in plants and soil. Plant Physiology 59: 236-239.

Coleman, W.K. 1989. Electrical impedance and freezing injury in apple shoots. Journal of Horticultural Science 64: 249-257.

Coleman, W.K. 1992. A proposed winter-injury classification for apple trees on the northern fringe of commercial production. Canadian Journal of Plant Science 72: 507-516.

Collett, D. 1991. Modelling binary data. London, Chapmann & Hall. 369 p.

Deans, J.D., Billington, H.L. & Harvey, F. 1995. Assessment of frost damage to leafless stem tissues of Quercus petraea: A reappraisal of the method of relative conductivity. Forestry 68: 25-34.

Dexter, S.T., Tottingham, W.E. & Graber, L.F. 1930. Preliminary results in measuring the hardiness of plants. Plant Physiology 5: 215-223.

Dexter, S.T., Tottingham, W.E. & Graber, L.F. 1932. Investigations of the hardiness of plants by measurement of electrical conductivity. Plant Physiology 7: 63-78.

Dixon, W.J., Brown, M.B., Engelman, L. & Jennrich, R.I. 1990. BMDP statistical software manual. Vol. 1 and 2. Berkeley (CA), University of California Press. 1385 p.

Dunning, C.A., Chalker-Scott, L. & Scott, J.D. 1994. Exposure to ultraviolet-B radiation increases cold hardiness in Rhododendron. Physiologia Plantarum 92: 516-520.

Efron, B. & Stein, C. 1981. The jackknife estimate of variance. Annals of Statistics 9: 586-596.

Embree, C.G. & McRae, K.B. 1991. An exploratory study of reciprocal apple rootstock and scion hardiness with two methods of assessment. HortScience 26: 1523-1525.

Emmert, F.H. & Howlett, F.S. 1953. Electrolytic determinations of the resistance of fifty-five apple varieties to low temperatures. Proceedings of the American Society for Horticultural Science 62: 311-318.

Finney, D.J. 1964. Statistical method in biological assay. 2nd ed. London, Charles Griffin & Comp. 668 p.

Fircks, H.A. von 1993. Exothermic responses of dormant Salix stems during exposure to subzero temperatures. Physiologia Plantarum 87: 271-278.

Fircks, H.A. von 1994. Frost resistance in Salix. D. Ph. Thesis. Swedish University of Agricultural Sciences, Department of Ecology and Environmental Research, Report No. 67: 1-78.

Fircks, H.A. von & Verwijst, T. 1993. Plant viability as a function of temperature stress. The Richards function applied to data from freezing tests of growing shoots. Plant Physiology 103: 125-130.
Fischer, M. 1992. Frostresistenzuntersuchungen an Malus-Artbastard-Unterlagen. Erwerbsobstbau 34: 194-197.
Flinn, C.L. & Ashworth, E.N. 1995. The relationship between carbohydrates and flower bud hardiness among three Forsythia taxa. Journal of the American Society for Horticultural Science 120: 607-613.
Flint, H.L., Boyce, B.R. & Beattie, D.J. 1967. Index of injury – A useful expression of freezing injury to plant tissues as determined by the electrolytic method. Canadian Journal of Plant Science 47: 229-230.
Fowler, D., Cape, J.N., Deans, J.D., Leith, I.D., Murray, M.B., Smith, R.I., Sheppard, L.J. & Unsworth, M.H. 1989. Effects of acid mist on the frost hardiness of red spruce seedlings. New Phytologist 113: 321-335.
Fuchigami, L.H., Weiser, C.J., Kobayashi, K., Timmins, R. & Gusta, L.V. 1982. A degree growth stage (ºGS) model and cold acclimation in temperate woody plants. In: Li, P.H. & Sakai, A. (eds.). Plant cold hardiness and freezing stress. Vol. 2. New York, Academic Press. p. 93-116.
George, M.F., Burke, M.J., Pellett, H.M. & Johnson, A.G. 1974. Low temperature exotherms and woody plant distribution. HortScience 9: 519-522.
Graham, P.R. & Mullin, R. 1976. A study of flower bud hardiness in azalea. Journal of the American Society for Horticultural Science 101: 7-10.
Grossnickle, S.C. 1992. Relationship between freezing tolerance and shoot water relations of western red cedar. Tree Physiology 11: 229-240.
Gudleifsson, B.E., Andrews, C.J. & Bjornsson, H. 1986. Cold hardiness and ice tolerance of pasture grasses grown and tested in controlled environment. Canadian Journal of Plant Science 66: 601-608.
Gusta, L.V. & Fowler, D.B. 1977. Factors affecting the cold survival of winter cereals. Canadian Journal of Plant Science 57: 213-219.
Gusta, L., Tyler, N.J. & Chen, T.H.-H. 1983. Deep undercooling in woody taxa growing north of the -40ºC isotherm. Plant Physiology 72: 122-128.
Guy, C.L. 1990. Cold acclimation and freezing stress tolerance: role of protein metabolism. Annual Review in Plant Physiology and Plant Molecular Biology 41: 187-223.
Hamilton, M.A. 1979. Robust estimates of the ED50. Journal of the American Statistical Association 74: 344-345.
Harrison, L.C., Weiser, C.J. & Burke, M.J. 1978a. Environmental and seasonal factors affecting the frost-induced stage of cold acclimation in Cornus stolonifera Michx. Plant Physiology 62: 894-898.
Harrison, L.C., Weiser, C.J. & Burke, M.J. 1978b. Freezing of water in red-osier dogwood stems in relation to cold hardiness. Plant Physiology 62: 899-901.
Haynes, C.L., Lindstrom, O.M. & Dirr, M.A. 1992. Cooling and warming effects on cold hardiness estimations of three woody ornamental taxa. HortScience 27: 1308-1309.
Heide, O.M. 1993a. Daylength and thermal time responses of budburst during dormancy release in some northern deciduous trees. Physiologia Plantarum 88: 531-539.
Heide, O.M. 1993b. Dormancy release in beech buds (Fagus sylvatica) requires both chilling and long days. Physiologia Plantarum 89: 187-191.
Heinze, W. 1999. Winterhärte von Gehölzen. Beiträge zur Gehölzkunde 1999: 70-78.
Hintze, J.L. 1998. NCSS 2000 user's guide. Vol. 2. Kaysville, UT, Number Cruncher Statistical Systems. 716 p.

Hodges, H.F., Svec, L.V. & Barta, A.L. 1970. A technique to determine cold hardiness in plants. Crop Science 10: 318-319.
Holubowicz, T. 1978. Survival test as a method of frost injury estimation. Acta Horticulturae 81: 119-122.
Holubowicz, T., Cummins, J.N. & Forsline, P.L. 1982. Responses of Malus clones to programmed low-temperature stresses in late winter. Journal of the American Society for Horticultural Science 107: 492-496.
Hong, S.-G. & Sucoff, E. 1982. Temperature effects on acclimation and deacclimation of supercooling in apple xylem. . In: Li, P.H. & Sakai, A. (eds.). Plant cold hardiness and freezing stress. Vol. 2. New York, Academic Press. p. 341-356.
Hong, S.-G., Sucoff, E. & Lee-Stadelmann, O.Y. 1980. Effect of freezing deep supercooled water on the viability of ray cells. Botanical Gazette 141: 464-468.
Howell, G.S. & Weiser, C.J. 1970a. The environmental control of cold acclimation in apple. Plant Physiology 45: 390-394.
Howell, G.S. & Weiser, C.J. 1970b. Fluctuations in the cold resistance of apple twigs during spring dehardening. Journal of the American Society for Horticultural Science 95: 190-192.
Hummer, K., Fuchigami, L.H., Peters, V. & Bell, N. 1995. Cold hardiness in Rubus. Fruit Varieties Journal 49: 52-58.
Ilmatieteen laitos. 1993a. Kuukausikatsaus Suomen ilmastoon 1993/02: 1-8.
Ilmatieteen laitos. 1993b. Kuukausikatsaus Suomen ilmastoon 1993/03: 1-8.
Ichiki, S. & Yamaya, H. 1982. Sorbitol in tracheal sap of dormant apple (Malus domestica Borkh) shoots as related to cold hardiness. In: Li, P.H. & Sakai, A. (eds.). Plant cold hardiness and freezing stress. Vol. 2. New York, Academic Press. p. 181-187.
Imanishi, H.T., Suzuki, T., Masuda, K. & Harada, T. 1998. Accumulation of raffinose and stachyose in shoot apices of Lonicera caerulea L. during cold acclimation. Scientia Horticulturae 72: 255-263.
Ingram, D.L. & Buchanan, D.W. 1984. Lethal high temperatures for roots of three citrus rootstocks. Journal of the American Society for Horticultural Science 109: 189-193.
Irving, R.M. & Lanphear, F.O. 1967a. Dehardening and the dormant condition in Acer and Viburnum. Proceedings of the American Society for Horticultural Science 91: 699-705.
Irving, R.M. & Lanphear, F.O. 1967b. Environmental control of cold hardiness in woody plants. Plant Physiology 42: 1191-1196.
Johnson, D.E. & Howell, G.S. 1981. The effect of cane morphology and cultivar on the phenological development and critical temperatures of primary buds on grape canes. Journal of the American Society for Horticultural Science 106: 545-549.
Juhanoja, S., Aaltonen, M., Aflatuni, A. Heinonen, A., Kemppainen, R., Paasikivi, P., Sorvari, K., Vehkalahti, M. & Virtanen, A. 2001. Tutkittuja kasveja viherrakentamisen tarpeisiin. KESKAS-kantavalintakokeet MTT:ssä vuosina 1988-2000. Abstract: Clone selection of woody ornamentals in Finland in 1988-2000. MTT Publications A 98. 60 p. + 11 app.
Juhanoja, S., Heikkilä, M. & Virtanen, A. 1998. Clone selection of woody ornamentals in Finland. Acta Agriculturae Scandinavica, Section B, Soil and Plant Science 48: 113-127.
Junttila, O. & Kaurin, Å. 1985. Climatic control of apical growth cessation in latitudinal ecotypes of Salix pentandra L. In: Kaurin, Å., Junttila, O. & Nilsen, J. (eds.). Plant production in the north. Tromsø, Norwegian University Press. p. 83-91.
Junttila, O. & Kaurin, Å. 1990. Environmental control of cold acclimation in Salix pentandra. Scandinavian Journal of Forest Research 5: 195-204.

Junttila, O., Stushnoff, C. & Gusta, L.W. 1983. Dehardening in flower buds of saskatoon-berry, Amelanchier alnifolia, in relation to temperature, moisture content, and spring bud development. Canadian Journal of Botany 61: 164-170.

Kaukovirta, E. 1967. Eräiden puuvartisten koristekasvien talvehtimisesta Viikissä, Jyväskylässä, Kauhajoella ja Rovaniemellä. Summary: Overwintering of some woody ornamentals at Viik, Jyväskylä, Kauhajoki and Rovaniemi. Journal of the Scientific Agricultural Society of Finland 39: 216-228.

Kaukovirta, E. & Syri, P. 1985. An attempt to reanalyze winter injury of apple trees in Finland during this century. Acta Horticulturae 168: 73-83.

Kaurin, A., Stushnoff, C. & Junttila, O. 1984. Cold acclimation and dormancy of Amelanchier alnifolia. Journal of the American Society for Horticultural Science 109: 160-163.

Keates, S.E. 1990. Assessing cold hardiness in conifers: A problem analysis and discussion paper. FRDA Report 106: 1-20.

Kennedy, P. 1998. A guide to econometrics. 4th ed. Oxford, Blackwell Publishers Ltd. 468 p.

Ketchie, D.O. & Beeman, C.H. 1973. Cold acclimation in 'Red Delicious' apple trees under natural conditions during four winters. Journal of the American Society for Horticultural Science 98: 257-261.

Ketchie, D.O., Beeman, C.H. & Ballard, A.L. 1972. Relationship of electrolytic conductance to cold injury and acclimation in fruit trees. Journal of the American Society for Horticultural Science 97: 403-406.

Ketchie, D.O. & Kammereck, R. 1987. Seasonal variation of cold resistance in Malus woody tissue as determined by differential thermal analysis and viability tests. Canadian Journal of Botany 65: 2640-2645.

Khanizadeh, S., Buszard, D., Fanous, M.A. & Zarkadas, C. G. 1989. Effect of crop load on seasonal variation in chemical composition and spring frost hardiness of apple flower buds. Canadian Journal of Plant Science 69: 1277-1284.

Khanizadeh, S., Buszard, D. & Zarkadas, C. G. 1992. Effect of crop load on hardiness, protein and amino acids content of apple flower buds at the wintering stage and the beginning of the growth. Journal of Plant Nutrition 15: 2441-2455.

Kolbe, W. 1985. Auswirkungen der Winterfröste im Januar/Februar 1985 auf Obstbau und Pflanzenschutz im Vergleich zu strengen Wintern früherer Zeiten. Erwerbsobstbau 27: 286-296.

Koski, V. & Sievänen, R. 1985. Timing of growth cessation in relation to the variations in the growing season. In: Tigerstedt, P.M.A., Puttonen, P. & Koski, V. (eds.). Crop physiology of forest trees. Helsinki, University of Helsinki, Department of Plant Breeding. p. 167-193.

Kuroda, H. & Sagisaka, S. 1993. Ultrastructural changes in cortical cells of apple (Malus pumila Mill.) associated with cold hardiness. Plant and Cell Physiology 34: 357-365.

Lapins, K. 1961. Artificial freezing of 1-year-old shoots of apple varieties. Canadian Journal of Plant Science 41: 381-393.

Lapins, K. 1962a. Cold hardiness of young apple trees originating from the juvenile and adult zones of seedlings. Canadian Journal of Plant Science 42: 521-526.

Lapins, K. 1962b. Artificial freezing as a routine test of cold hardiness of young apple seedlings. Proceedings of the American Society for Horticultural Science 81: 26-34.

Larcher, W. 1968. Die Temperaturresistenz als Kostitutionsmerkmal der Pflanzen. Deutsche Akademie für Landwirtschaftswissenschaft, Berlin, Tagungsberichte 100: 7-21.

Larcher, W. & Eggarter, H. 1960. Anwendung des Triphenyltetrazoliumchlorids zur Beurteilung von Frostschäden in verschiedenen Achsengeweben bei Pirus-Arten, und Jahresgang der Resistenz. Protoplasma 51: 595-619.

Larcher, W. 1985. Frostresistenz. In: Rademacher, B. & Richter, H. (eds.) Handbuch der Pflanzenkrankheiten, Bd. 1, Teil 5. Berlin und Hamburg, Parey. p. 177-259.
Lehmushovi, A., Tahvonen, R. & Hietaranta, T. 2001. Ammattiviljelyn ja kotipuutarhan hedelmä- ja marjalajikkeet. Puutarhakalenteri 60: 116-119.
Leinonen, I. 1996. A simulation model for the annual frost hardiness and freeze damage of Scots pine. Annals of Botany 78: 687-693.
Leinonen, I. 1997. Frost hardiness and annual development of forest trees under changing climate. D. Sc. (Agr. and For.) thesis. University of Joensuu, Faculty of Forestry, Research Notes. p. 1-42.
Levitt, J. 1980. Responses of plants to environmental stresses. Vol. 1. Chilling, freezing and high temperature stresses. 2nd ed. New York, Academic Press. 497 p.
Li, P. H. & Weiser, C. J. 1970. Increasing cold resistance of stem sections of Cornus stolonifera by artificial dehydration. Cryobiology 8: 108-111.
Liao, T.F. 1994. Interpreting probability models: logit, probit, and other generalized linear models. Sage University Paper series on Quantitative Applications in the Social Sciences, 07-101. Thousand Oaks, California, SAGE Publications, Inc. 88 p.
Lim, C.C., Arora, R. & Townsend, E.C. 1998. Comparing Gompertz and Richards functions to estimate freezing injury in Rhododendron using electrolyte leakage. Journal of the American Society for Horticultural Science 123: 246-252.
Lim, C.C., Krebs, S.L. & Arora, R. 1999. A 25-kDa dehydrin associated with genotype- and age-dependent leaf freezing-tolerance in Rhododendron: a genetic marker for cold hardiness? Theoretical and Applied Genetics 99: 912-920.
Lindstrom, O.M. & Dirr, M.A. 1989. Acclimation and low-temperature tolerance of eight woody taxa. HortScience 24: 818-820.
Litzow, M. & Pellett, H. 1980. Relationship of rest to dehardening in red-osier dogwood. HortScience 15: 92-93.
Malone, S.R. & Ashworth, E.N. 1991. Freezing stress response in woody tissues observed using low-temperature scanning electron microscopy and freeze substitution techniques. Plant Physiology 95: 871-881.
Manley, R.C. & Hummel, R.L. 1996. Index of injury compared to tissue ionic conductance for calculating freeze damage of cabbage tissues. Journal of the American Society for Horticultural Science 121: 1141-1146.
McKenzie, J.S., Weiser, C.J. & Burke, M.J. 1974. Effects of red and far red light on the initiation of cold acclimation in Cornus stolonifera Michx. Plant Physiology 53: 783-789.
McNamara, S. & Pellett, H. 1998. Cold hardiness of weigela cultivars. Journal of Environmental Horticulture 16: 238-242.
McNamara, S. & Pellett, H. 2000. Cold hardiness of Phellodendron sachalinense Friedr. Schmidt seedlings increases with age. HortScience 35: 304-305.
Miller, R.G. 1973. Nonparametric estimates of the mean tolerance in bioassay. Biometrika 60: 535-542.
Mittelstädt, H. & Murawski, H. 1975. Beiträge zur Züchtungsforschung beim Apfel. XVII. Untersuchungen zur Frostresistenz an Apfelsorten. Archiv für Züchtungsforschung 5: 71-81.
Montano, J.M., Rebhuhn, M., Hummer, K. & Lagerstedt, H.B. 1987. Differential thermal analysis for large-scale evaluation of pear cold hardiness. HortScience 22: 1335-1336.
Mooney, C.Z. & Duval, R.D. 1993. Bootstrapping: a nonparametric approach to statistical inference. Sage University Paper series on Quantitative Applications in the Social Sciences, 07-095. Newbury Park, California, SAGE Publications Inc. 73 p.
Morgan, B.J.T. 1992. Analysis of quantal response data. London, Chapman & Hall. 511 p.

Murray, M.B., Cape, J.N. & Fowler, D. 1989. Quantification of frost damage in plant tissues by rates of electrolyte leakage. New Phytologist 113: 307-311.
Ögren, E. 1996. Premature dehardening in Vaccinium myrtillus during a mild winter: a cause for winter dieback? Functional Ecology 10: 724-732.
Palliotti, A. & Bongi, G. 1996. Freezing injury in the olive leaf and effects of mefluidide treatment. Journal of Horticultural Science 71: 57-63.
Palonen, P. & Buszard, D. 1998. In vitro screening for cold hardiness of raspberry cultivars. Plant Cell, Tissue and Organ Culture 53: 213-216.
Palonen, P. & Junttila, O. 1999. Cold hardening of raspberry plants in vitro is enhanced by increasing sucrose in the culture medium. Physiologia Plantarum 106: 386-392.
Palonen, P., Lindén, L., Hovi, T., Hytönen, T., Keinänen, L. & Lilja, P. 2001. Vadelman ja mansikan talvenkestävyyden parantaminen lajikevalinnan ja viljelytekniikan keinoin. Summary: Improvement of raspberry and strawberry winter hardiness through cultivar selection and cultural management. Helsingin yliopisto, soveltavan biologian laitoksen julkaisu n:o 7: 1-92.
Palta, J.P., Levitt, J. & Stadelmann, E.J. 1978. Plant viability assay. Cryobiology 15: 249-255.
Palta, J.P. & Weiss, L. S. 1993. Ice formation and freezing injury: An overview on the survival mechanisms and molecular aspects of injury and cold acclimation in herbaceous plants. In: Li, P.H. & Christersson, L. (eds.). Advances in plant cold hardiness. Boca Raton, CRC Press. p. 143-176.
Parker, J. 1953. Criteria of life: Some methods of measuring viability. American Scientist 41: 614-618.
Pellett, H. & Carter, J.V. 1981. Effect of nutritional factors on cold hardiness of plants. Horticultural Reviews 3: 144-171.
Pellett, H., Gearhart, M. & Dirr, M. 1981. Cold hardiness capability of woody ornamental plant taxa. Journal of the American Society for Horticultural Science 106: 239-243.
Pellett, N.E. & Heleba, D.A. 1998. Comparing callus growth with discoloration and electrical conductivity as measures of stem injury after freezing woody plants. Journal of the American Society for Horticultural Science 123: 826-831.
Pogosyan, K.S. & Sakai, A. 1969. Freezing resistance in grape vines. Low Temperature Science Series B 27: 125-142.
Pomeroy, M.K. & Siminovitch, D. 1971. Seasonal cytological changes in secondary phloem parenchyma cells in Robinia pseudoacacia in relation to cold hardiness. Canadian Journal of Botany 49: 787-795.
Privé, J.-P. & Zhang, M.I.N. 1996. Estimating cold stress in ‘Beautiful Arcade’ apple roots using electrical impedance analysis. HortTechnology 6: 54-58.
Proebsting, E.L. 1963. The role of air temperatures and bud development in determining hardiness of dormant Elberta peach fruit buds. Proceedings of the American Society for Horticultural Science 83: 259-269.
Proebsting, E.L., Jr. & Fogle, H.W. 1956. An apparatus and method of analysis for studying fruit bud hardiness. Proceedings of the American Society for Horticultural Science 68: 6-14.
Pukacki, P. & Pukacka, S. 1987. Freezing stress and membrane injury of Norway spruce (Picea abies) tissues. Physiologia Plantarum 69: 156-160.
Quamme, H.A. 1976. Relationship of the low temperature exotherm to apple and pear production in North America. Canadian Journal of Plant Science 56: 493-500.
Quamme, H.A. 1991. Application of thermal analysis to breeding fruit crops for increased cold hardiness. HortScience 26: 513-517.

Quamme, H., Stushnoff, C. & Weiser, C.J. 1972. The relationship of exotherms to cold injury in apple stem tissues. Journal of the American Society for Horticultural Science 97: 608-613.

Raese, J.T., Williams, M.W. & Billingsley, H.D. 1978. Cold hardiness, sorbitol, and sugar levels of apple shoots as influenced by controlled temperature and season. Journal of the American Society for Horticultural Science 103: 796-801.

Rajashekar, C., Westwood, M.N. & Burke, M.J. 1982. Deep supercooling and cold hardiness in genus Pyrus. Journal of the American Society for Horticultural Science 107: 968-972.

Rasmussen, D.H. & MacKenzie, A.P. 1972. Effect of solute on ice-solution interfacial free energy; calculation from measured homogeneous nucleation temperature. In: Jellnek, H.H.G. (ed.). Water structure at the water polymer interface. New York, Plenum Publishing Corporation. p. 126-145.

Repo, T., Hiekkala, P., Hietala, T. & Tahvanainen, L. 1997. Intracellular resistance correlates with initial stage of frost hardening in willow (Salix viminalis). Physiologia Plantarum 101: 627-634.

Repo, T., Hänninen, H. & Kellomäki, S. 1996. The effects of long-term elevation of air temperature and CO_2 on the frost hardiness of Scots pine. Plant, Cell and Environment 19: 209-216.

Repo, T. & Lappi, J. 1989. Estimation of standard error of impedance-estimated frost resistance. Scandinavian Journal of Forest Research 4: 67-74.

Repo, T. & Zhang, M.I.N. 1993. Modelling woody plant tissues using a distributed electrical circuit. Journal of Experimental Botany 44: 977-982.

Repo, T., Zhang, G., Ryyppö, A. & Rikala, R. 2000. The electrical impedance spectroscopy of Scots pine (Pinus sylvestris L.) shoots in relation to cold acclimation. Journal of Experimental Botany 51: 2095-2107.

Rollins, H.A., Jr., Freeman, S.H. & Emmert, F.H. 1962. Factors affecting apple hardiness and methods of measuring resistance of tissue to low temperature injury. Ohio Agricultural Experiment Station, Research Bulletin 901: 1-71.

Ryyppö, A., Repo, T. & Vapaavuori, E. 1998. Development of frost hardiness in roots and shoots of Scots pine seedlings at non-freezing temperatures. Canadian Journal of Forest Research 28: 557-565.

Sakai, A. 1973. Characteristics of winter hardiness in extremely hardy twigs of woody plants. Plant & Cell Physiology 14: 1-9.

Sakai, A., Fuchigami, L. & Weiser, C.J. 1986. Cold hardiness in the genus Rhododendron. Journal of the American Society for Horticultural Science 111: 273-280.

Sakai, A. & Larcher, W. 1987. Frost survival of plants. Responses and adaptation to freezing stress. Ecological Studies, vol. 62. Berlin, Springer Verlag. 321 p.

Sakai, A. & Weiser, C.J. 1973. Freezing resistance of trees in North America with reference to tree regions. Ecology 54: 118-126.

SAS Institute Inc. 1989. SAS/STAT® user's guide, version 6, 4th ed. Vol. 1. Cary, NC, SAS Institute Inc. 943 p.

SAS Institute Inc. 1993. SAS® technical report P-243, SAS/STAT® software: The GENMOD procedure, release 6.09. Cary, NC, SAS Institute Inc. 88 p.

Scheumann, W. 1968. Die Dynamik der Frostresistenz und ihre Bestimmung an Gehölzen in Massentest. Deutsche Akademie für Landwirtschaftswissenschaft, Berlin, Tagungsberichte 100: 45-53.

Scott, K.R. 1966. Use of liquid nitrogen in a winterhardiness test chamber for fruit trees. Canadian Journal of Plant Science 46: 691-693.

Scott, K.R. & Spangelo, L.P.S. 1964. Portable low temperature chamber for winter hardiness testing of fruit trees. Proceedings of the American Society for Horticultural Science 84: 131-136.

Siminovitch, D. 1982. Major acclimation in living bark of Sept. 16 black locust tree trunk sections after 5 weeks in the dark – evidence for endogenous rhythms in winter hardening. In: Li, P.H. & Sakai, A. (eds.). Plant cold hardiness and freezing stress. Vol. 2. New York, Academic Press. p. 117-128.

Siminovitch, D. & Briggs, D.R. 1953. Studies on the chemistry of the living bark of the black locust in relation to its frost hardiness. III. The validity of plasmolysis and desiccation tests for determining the frost hardiness of bark tissue. Plant Physiology 28: 15-34.

Siminovitch, D., Therrien, H., Gfeller, F. & Rheaume, B. 1964. The quantitative estimation of frost injury and resistance in black locust, alfalfa, and wheat tissues by determination of amino acids and other ninhydrin-reacting substances released after thawing. Canadian Journal of Botany 42: 537-649.

Steponkus, P.L. 1984. Role of the plasma membrane in freezing injury and cold acclimation. Annual Review of Plant Physiology 35: 543-584.

Steponkus, P.L. & Lanphear, F.O. 1967. Refinement of the triphenyl tetrazolium chloride method of determining cold injury. Plant Physiology 42: 1423-1426.

Stergios, B.G. & Howell, G.S., Jr. 1973. Evaluation of viability tests for cold stressed plants. Journal of the American Society for Horticultural Science 98: 325-330.

Stoyanov, S.M. 1973. An improved method for the determination of winter hardiness in fruit trees. Tidsskrift for Planteavl 77(1): 13-18.

Stuart, N.W. 1939. Comparative cold hardiness of scion roots from fifty apple varieties. Proceedings of the American Society for Horticultural Science 37: 330-334.

Su, W.A., Mi, R.Q., Wang, W.Y. & Wang, H.C. 1987. A relationship among stress intensity, duration, and strain. In: Li, P.H. (ed.). Plant cold hardiness. New York, Alan R. Liss, Inc. p. 141-153.

Sutinen, M.-L., Palta, J.P. & Reich, P.B. 1992. Seasonal differences in freezing stress resistance of needles of Pinus nigra and Pinus resinosa: evaluation of the electrolyte leakage method. Tree Physiology 11: 241-254.

Takeda, F., Arora, R., Wisniewski, M.E., Davis, G.A. & Warmund, M.R. 1993. Assessment of freeze injury in 'Boskoop Giant' black currant buds. HortScience 28: 652-654.

Taulavuori, K., Taulavuori, E., Niinimaa, A. & Laine, K. 1996. Frost resistance and pH of cell effusate in needles of artificially deacclimated Scots pine (Pinus sylvestris). Physiologia Plantarum 96: 111-117.

Timbers, G.E. & Hocking, R.P. 1971. A temperature gradient bar for seed germination and cold hardiness studies. Canadian Journal of Plant Science 51: 434-437.

Tjurina, M.M. 1968. Untersuchungen zur Frostresistenz von Holzgewächsen bei künstlicher Frostung. Deutsche Akademie für Landwirtschaftswissenschaft, Berlin, Tagungsberichte 100: 76-85.

Travert, S., Valerio, L., Fourasté, I., Boudet, A.M. & Teulières, C. 1997. Enrichment in specific soluble sugars of two eucalyptus cell-suspension cultures by various treatments enhances their frost tolerance via a noncolligative mechanism. Plant Physiology 114: 1433-1442.

Tumanov, I.I., Kuzina, G.V., Karnikova, L.D. & Khvalin, N.N. 1972. Effect of vegetation time on ability of woody plants to increase their frost resistance during the process of hardening. Soviet Plant Physiology 19: 31-39.

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

Westergaard, L. & Eriksen, E.N. 1997. Autumn temperature affects the induction of dormancy in first-year seedlings of Acer platanoides. Scandinavian Journal of Forest Research 12: 11-16.

Whitlow, T.H., Bassuk, N.L., Ranney, T.G. & Reichert, D.L. 1992. An improved method for using electrolyte leakage to assess membrane competence in plant tissues. Plant Physiology 98: 198-205.

Wilner, J. 1961. Relationship between certain methods and procedures of testing for winter injury of outdoor exposed shoots and roots of apple trees. Canadian Journal of Plant Science 41: 309-315.

Wisniewski, M. & Ashworth, E.N. 1986. A comparison of seasonal ultrastructural changes in stem tissues of peach (Prunus persica) that exhibit contrasting mechanism of cold hardiness. Botanical Gazette 147: 407-417.

Väinölä, A. & Repo, T. 2000. Impedance spectroscopy in frost hardiness evaluation of Rhododendron leaves. Annals of Botany 86: 799-805.

Vuorinen, J. 1956. Omenalajikkeiden talvenkestävyydestä. Koetoiminta ja Käytäntö 13 (10): 30.

Yelenosky, G. 1979. Water-stress-induced cold hardening of young citrus trees. Journal of the American Society for Horticultural Science 104: 270-273.

Yoshida, S. 1984. Chemical and biophysical changes in the plasma membrane during cold acclimation of mulberry bark cells (Morus bombycis Koidz. cv Goroji). Plant Physiology 76: 257-265.

Yoshida, S. & Uemura, M. 1990. Responses of the plasma membrane to cold acclimation and freezing stress. In: Larsson, C. & Møller, I.M. (eds.). The plant plasma membrane. Berlin, Springer-Verlag. p. 293-319.

Zatylny, A.M., Proctor, J.T.A. & Sullivan, J.A. 1993. Screening red raspberry for cold hardiness in vitro. HortScience 28: 740-741.

Zhang, M.I.N. & Willison, J.H.M. 1987. An improved conductivity method for the measurement of frost hardiness. Canadian Journal of Botany 65: 710-715.

SELOSTUS

Puuvartisten kasvien kylmänkestävyyden mittaaminen

Kylmänkestävyys on yksi tärkeimmistä puuvartisten kasvien menestymistä ja sadontuottoa määräävistä tekijöistä pohjoisessa ilmastossa. Kylmänkestävyyttä ja kasvien ilmastollista sopeutuneisuutta voidaan tutkia altistamalla tutkittavat kasvit tai kasvinosat keinotekoiselle pakkaskäsittelylle. Altistuksen jälkeen määritetään solukoiden kylmässä saamat vauriot ja kuvataan kylmänkestävyyden tasoa jonkin numeerisen tunnuksen avulla.

Tämän tutkimuksen tavoitteena oli kehittää puuvartisten kasvien kylmänkestävyyden mittaamisen menetelmiä. Tutkimuksen kohteena olivat erityisesti kylmävaurioiden analysoinnin ja kylmänkestävyystunnusten estimoinnin menetelmät. Lisäksi arvioitiin kylmänkestävyyden eri osatekijöiden mittaamisen menetelmiä ja noiden osatekijöiden merkitystä Suomen ilmastossa. Havaintoaineistona käytettiin tarhaomenapuulla, tähtijasmikkeella, syyshortensialla, vadelmalla ja pensasmustikalla tehtyjen kylmänkestävyyskokeiden tuloksia sekä omenapuiden talvivaurio- ja säähavaintoja vuosilta 1927-1998.

Kylmävaurioita silmävaraisesti mitattaessa havaintoaineisto jaetaan usein kahteen luokkaan: elävä (toipumiskykyinen) ja kuollut (parantumattomasti vaurioitunut). Käsillä olevassa tutkimuksessa osoitettiin logit-mallin käyttökelpoisuus tällaisen kaksiluokkaisen mittausaineiston analysoinnissa. Kvalitatiivisesta mittausaineistosta voidaan logit-mallin avulla helposti laskea kylmänkestävyystunnuksena yleisimmin käytetty LT_{50}-arvo (lämpötila, jossa puolet näytteistä kuolee) luottamusväleineen. Logit-mallilla voidaan tutkia samanaikaisesti useiden muuttujien vaikutusta kylmänkestävyyden tasoon. Lisäksi logit-mallia käytettäessä kvalitatiiviselle vastemuuttujalle tyypillinen otosvaihtelu tulee oikein huomioiduksi. Logit-mallin suurin puute on, että sitä käytettäessä kuolemistodennäköisyys oletetaan symmetriseksi lämpötilan suhteen. Symmetriaoletuksen tuottama harha on kuitenkin pienimmillään logit-mallin kuvaajan keskikohdassa, jonka perusteella LT_{50}-arvo lasketaan.

Kylmävaurioiden kvantitatiivisista mittausmenetelmistä tavallisin on ionivuototesti, jossa näytteiden vaurioitumisen astetta ennustetaan niistä solukalvojen rikkoutumisen seurauksena vuotavien ionien suhteellisen määrän perusteella. Tässä tutkimuksessa ionivuototestin tulosten tulkintaa kehitettiin vertaamalla lineaarisen interpoloinnin ja kolmen epälineaarisen funktion (logistinen, Richardsin ja Gompertzin funktio) antamia

kylmänkestävyystunnuksia vadelman versoaineistossa. Vertailuarvoina käytettiin samasta aineistosta silmävaraisesti mitaten saatuja kylmänkestävyystunnuksia. Lineaarisen interpoloinnin sekä logistisen ja Gompertzin funktion keskikohdan (50 %:n pisteen) avulla lasketut kylmänkestävyystunnukset vastasivat parhaiten silmävaraisen mittaamisen tuloksia. Richardsin funktio tuotti kaikkein poikkeavimpia kestävyystunnuksia ja sen sovittaminen pieniin, käytännön koetilanteissa syntyneisiin havaintoaineistoihin oli usein vaikeaa.

Kylmänkestävyystunnuksen estimoinnin menetelmiä tutkittiin edelleen Monte Carlo –simulointikokeiden avulla ja käyttämällä lineaarista interpolointia, Spearman-Kärberin menetelmää sekä logistisen ja Richardsin funktion sovittamista viidessä kuolleisuusfrekvenssiaineistossa. Simulointiaineisto luotiin epäsymmetrisen Richardsin funktion avulla. Tulokset osoittivat, että kaikki neljä menetelmää toimivat yhtä hyvin, jos havaintoaineisto on lämpötilan suhteen symmetrinen. Epäsymmetrisessä aineistossa lineaarinen interpolointi antoi tarkempia ja harhattomampia estimaatteja kuin Spearman-Kärberin menetelmä ja logistinen funktio. Vertailtujen neljän menetelmän soveltaminen todellisiin kuolleisuusaineistoihin paljasti, että Richardsin funktion sovittaminen ei käytännön tilanteissa usein onnistu mallin monimutkaisuuden takia.

Tarhaomenapuun, tähtijasmikkeen ja syyshortensian kevättalvista kylmänkestävyyttä, karaistumiskykyä ja karaistuneen tilan purkautumisalttiutta tutkittiin keinotekoisten lämpötilakäsittelyiden ja pakkasaltistusten avulla. Tulosten perusteella kaikki kolme lajia näyttävät säilyttävän korkean kylmänkestävyyden tason pitkälle kevättalveen Etelä-Suomen ilmastossa. Mikään lajeista ei myöskään osoittautunut herkäksi korkean lämpötilan karaistunutta tilaa purkavalle vaikutukselle.

Omenapuiden talvivaurioiden syitä selvitettiin tutkimalla vanhoja vaurio- ja säähavaintoaineistoja erottelu- ja ryhmittelyanalyysin avulla. Tärkeimmäksi laajoihin talvivaurioihin johtavaksi tekijäksi osoittautui keskitalven kylmyys: tammi-, helmi- ja maaliskuun alhaiset lämpötilat. Myös edeltävän kesän ja syksyn sääoloilla näytti olevan yhteyttä talvivaurioiden syntyyn, todennäköisesti siitä syystä, että ne vaikuttavat puiden tuleentumiskehitykseen. Tuloksista voidaan päätellä, että riittävä karaistumiskyky ja karaistumiskehityksen oikea ajoittuminen ovat omenapuiden kylmänkestävyyden tärkeimmät osatekijät Etelä-Suomen ilmastossa. Uusien lajikkeiden valinnassa ja testaamisessa on hyödyllistä keskittyä näihin, talvehtimisen kannalta ratkaiseviin ominaisuuksiin.