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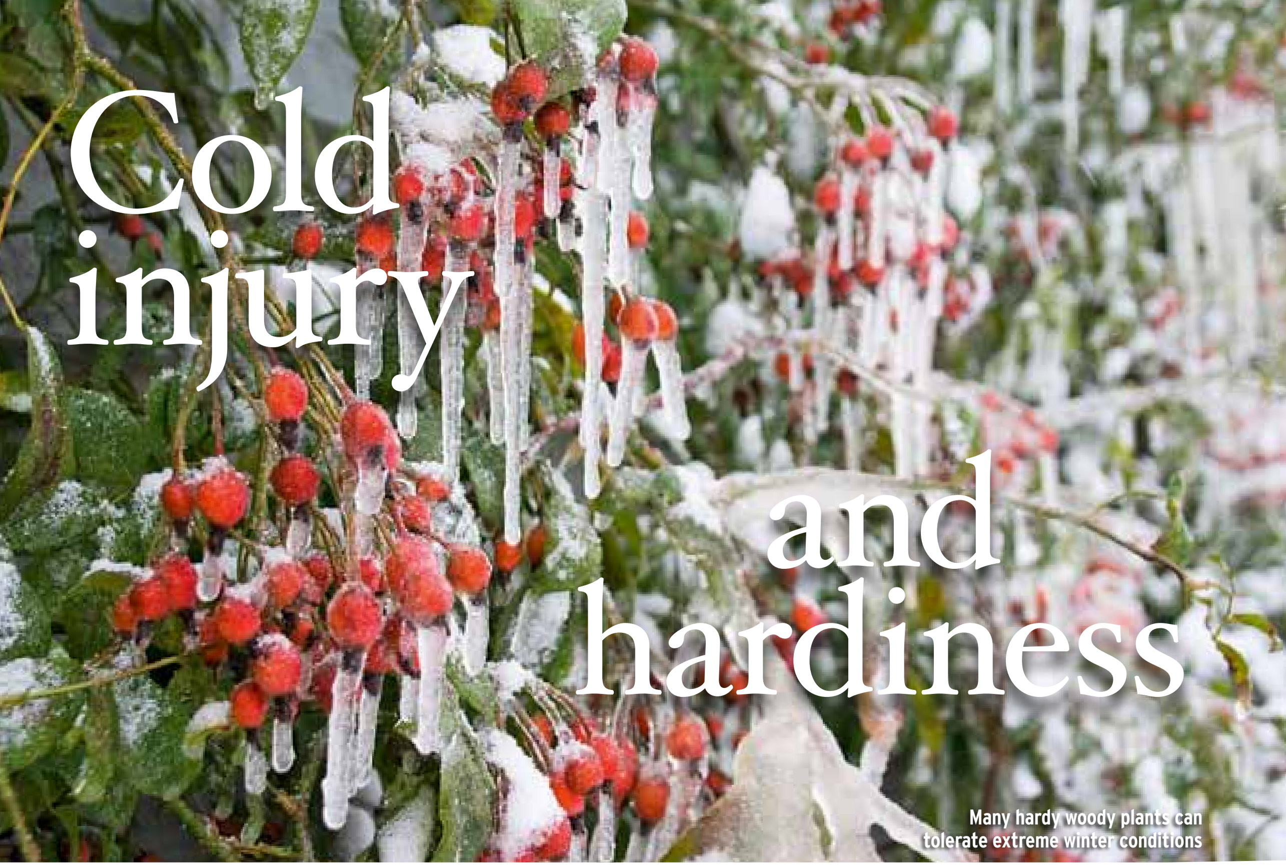


Cold injury and hardiness

Many hardy woody plants can tolerate extreme winter conditions

GEOFF DIXON summarizes recent research into plant hardiness and discusses how it may lead to more resilient plants

COLD INJURY is a term used by plant physiologists to describe the effects of an absence of plant hardiness. Hardiness is a genetic trait which expresses tolerance to environmentally induced stresses caused by low temperature and damage due to freezing.

Symptoms of cold injury are made worse by frozen soil which leads to water shortage both at plant roots and in transpiration streams. Injury is compounded further by desiccation from cold winds and scorching from the intense sunlight of bright, frosty, winter days. The syndrome of cold injury may also include stem and trunk splitting, bronzing and burning of foliage in evergreens, killing of dormant leaf and flower buds, and carbohydrate depletion where photosynthesis is interrupted (Anisko & Lindstrom 1995). Long-lasting freezes can also cause soil heave, which leads to fracturing and other physical damage to root systems.

Tim Sandall / RHS

Cold injury can lead to stem splitting

Weather conditions

Traditionally, British winters are thought of as times of freezing temperatures and snow, but this is now only infrequently the case. Our maritime climate generally provides warm and wet weather interspersed occasionally with sharply cold, easterly winds and severe frosts. Milder autumns, it seems, are now extending into December (Simons 2014), followed by brief periods of severe cold from the New Year into

Frost can damage young leaves of winter-flowering shrubs such as *Viburnum farreri*

Andy Hellman / RHS

Geoff Dixon

February and early March, possibly interspersed with longer spells of rainfall. Plants are therefore subjected to rapid cycling of mild and cold conditions. Such variable weather patterns exacerbate winter damage, especially in woody plants such as shrubs and trees. In these circumstances, species which are normally hardy may be damaged (Polle *et al*. 1996).

Coping with cold

British horticulturists have long sought cold hardiness in plants by experimenting with new selections, or hybridizing with recently introduced species. Breeding for hardiness is not always a straightforward objective. Selections that thrive in one garden through severe weather may fail in relatively mild conditions only a few miles away. Site, soil, elevation and husbandry are well recognized as factors contributing towards cold hardiness. Tolerance of low temperature, however, remains the key factor.

On the RHS Hardiness Ratings scale, hardiness is defined by a direct relationship with temperature (Gardiner 2013). For example, plants with an H2 rating are tender, meaning they are only likely to survive down to 1°C and winter survival is ensured only by providing artificial protection. Plants rated as H3 are half-hardy and may withstand temperatures down to -5°C, but tolerance varies substantially depending on factors such as geographic origin. Plants in the tender and half-hardy categories develop blackening and shrivelling symptoms as temperatures fall. Plants in categories H4 to H7 can withstand freezing conditions down to -10, -15, -20 and below -20°C respectively.

Other hardiness rating schemes are available. For example, the United States Department of Agriculture Plant Hardiness Zone Map is used to determine which plants are likely to survive at a particular location. Most schemes equate hardiness with tolerance to low temperatures.

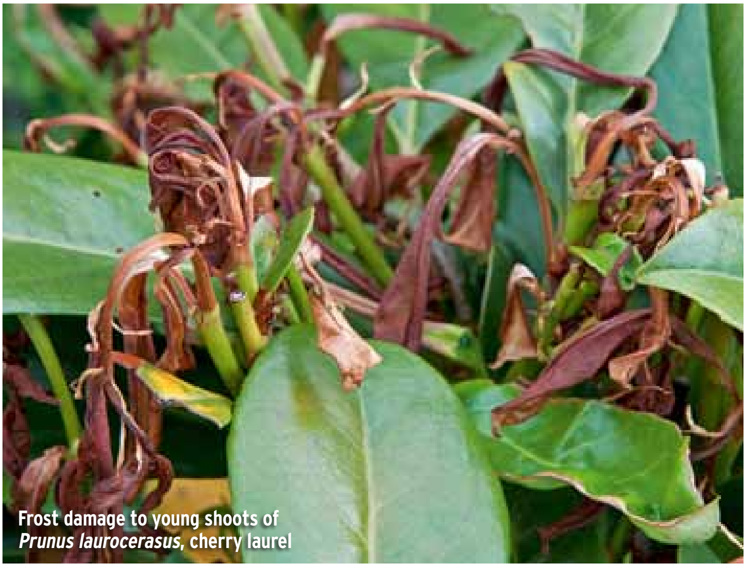
Assessing cold hardiness of garden plants was begun in the UK by James Barnes at Bicton garden in Devon. He compared weather conditions and plant survival (Barnes 1850). Similar assessments continue today (e.g. David 2011), keeping gardeners updated on the tolerance of particular species and cultivars to cold.

Components of hardiness

The terms acclimatization, dormancy and cold tolerance are used in defining hardiness. Molecular biology has revealed that, at each stage in an organism's seasonal cycle, different sets of genes take control of growth and reproduction

(Coen 1999). As the growing season of woody plants draws towards closure, so genes which guide preparations for winter are activated. Before abscising the leaves change colour as pigments other than green chlorophyll become visible. The buds which will form leaves, flowers and fruit in the following season go through preparations for dormancy as temperatures decline. This process is known as acclimatization and was described by Weisser (1970) as a response to shortening day-length or photoperiod. During these processes buds and woody tissues gain abilities which make them capable of withstanding damaging low temperatures.

During acclimatization the buds are not fully dormant and may resume growth should warmer temperatures return. Cold acclimatization results in changes in the protein, carbohydrate and lipid composition of plant tissues. In these processes, for example, there are changes in the structure of lipid molecules that form cell membranes (Biggs 1996). Studying *Rhododendron bureavii* growing at Younger (now Benmore) Botanic Garden, Argyll, he showed that cell membranes consist of different lipids during winter than they do during summer.



Frost damage to young shoots of *Prunus laurocerasus*, cherry laurel

Tim Sandall / RHS

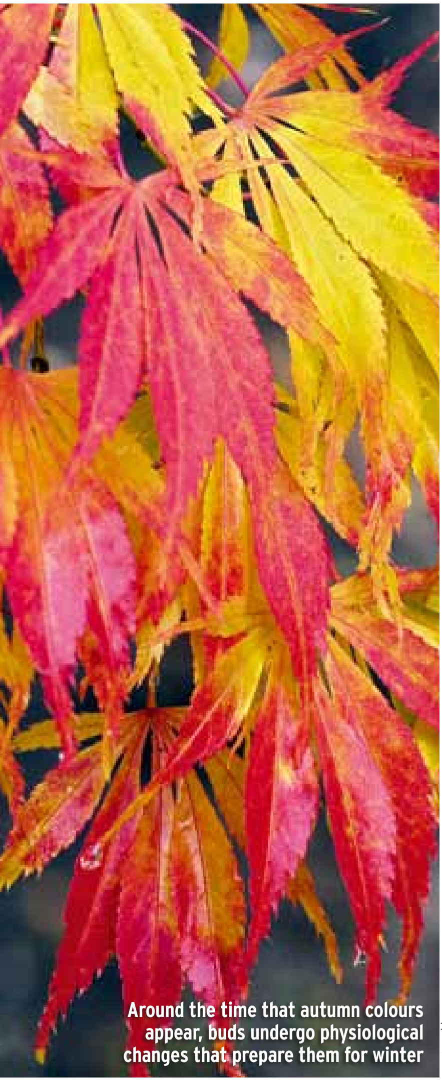
Fruit crops such as pears can be devastated by late frosts

Tim Sandall / RHS

These changes in lipid composition are, however, very sensitive to prevailing temperature. A short period of higher temperature, for instance in late autumn, may reverse acclimatization and break dormancy. This frequently results in increased winter damage when colder conditions return (Kuroda *et al.* 1993). Once cooler conditions return, the structure of the membrane lipids reverts to that which is suited to colder conditions (Dixon & Biggs 1996). Similar results have been reported by Liu *et al.* (2011) who studied the relationship between the changes of membrane fatty acid in *Spiraea* and cold temperatures. The unsaturated fatty acid components of membranes in *S. japonica* 'Gold Mound' changed significantly under low temperatures.

Environmental adaptability in plants, their capacities for withstanding freezing temperatures, and the activation of many genes was described by Wisniewski *et al.* (2014). Proteins known as transcription factors play major roles in sensing low temperature, initiating the process of cold acclimatization, and inducing the expression of a large group of cold-regulated genes. These genes probably provide protection against cold damage by controlling the withdrawal of water from tissues and thereby the process of ice formation in and between cells.

As acclimatization deepens plants move towards a period of maximum rest, culminating in full dormancy. This is defined as a state where organisms are alive but inactive;



Around the time that autumn colours appear, buds undergo physiological changes that prepare them for winter

Carol Sheppard / RHS

Freezing conditions have damaged the cells inside the branch of this *Ilex*, holly, leading to a brown ring of dead tissue

Pathology / RHS

their metabolisms have slowed and growth has ceased. In woody plants, dormancy is broken by the return of rising temperatures and lengthening days in spring. Then buds start opening, growth restarts, and leaves and flowers emerge. These processes are guided by different genes to those responsible for guiding cold acclimatization and dormancy.

Assessing cold tolerance

Visual estimation is the quickest and simplest way of assessing cold tolerance. Under experimental conditions, whole plants or separate leaves and flowers are exposed to temperatures at or below 0°C and the resultant damage recorded on a numerical scale against standardized drawings. This approach is valuable where large numbers of plants are being assessed, as in breeding programmes. The drawback of this system is that it provides no understanding of the physical, chemical or biological events happening within plant tissues.

Achieving deeper scientific precision is essential for raising plants with more stable and longer lasting cold tolerance.

The extent of cold damage inside plant tissues is assessed by examining disruption caused to cell structure. As tissues freeze ice crystals form and these rupture cell membranes, causing leakage of their contents. These cell fluids can conduct minute electrical charges. Hence, comparing the electrical conductivity of fluids from cold damaged tissues with standard values obtained by total disruption, achieved by boiling the plant tissues (Cameron 1993), provides numerical measurements of damage. Using this technique, Cameron & Dixon (1997, 2000) followed the rates of acclimatization and attendant reductions in cold damage in *Acer*, *Ceanothus*, *Euphorbia*, *Magnolia* and *Rhododendron*.

Cold acclimatization increases soluble sugars such as raffinose and stachyose in bark tissue and buds of temperate woody plants (Labeke & Volckaert 2010). In their experiments, shifts in sugar concentrations provided means of measuring winter hardiness in deciduous trees species such as *Crataegus monogyna*, *Prunus avium* and *Quercus rubra*. Declining sugar concentrations in spring correlated with increased frost susceptibility.

Two further methods can be used to measure cold tolerance. One is to treat damaged and undamaged tissues with a stain called 2,3,5-triphenyl tetrazolium chloride. This will indicate the extent of cell death because it only colours areas of tissue undergoing active respiration. Colour changes can be measured accurately using a spectrophotometer. The second method is to measure changes in the emission of fluorescence from chlorophyll. With other researchers, I used both these methods to understand cold stress resistance in *Rhododendron ponticum* (Dixon *et al.* 2003, Dixon & Biggs 1996).

As dormancy develops there are changes in water content and water binding and mobility in woody plants. Magnetic resonance imaging (MRI), as used in human medicine, has shown that dormancy in *Populus* is associated with reduced water mobility during the early stages of dormancy (Tanino *et al.* 2010).

Science is steadily demonstrating parallels between plants and animals in their physiological and biochemical responses to environmental stresses. Early work by Burdon (1993) showed that damaging, highly reactive oxygen molecules accumulate in stressed cells. Heggie (1999) related this to molecular processes controlling the promotion and inhibition of acclimatization in *Rhododendron*. Her research was extended by Arora (2013) who showed how cycles of cold acclimatization and its erosion are important for winter survival in woody plants.

It is apparent that temperate woody plants have evolved sophisticated means for winter survival, and these enable them to adapt to environmental changes (Ko *et al.* 2011). Genes related to cold hardiness, defence, carbohydrate biosynthesis and cell transport are especially important. It is now well established that stresses caused by pathogens and by environmental sources, such as cold damage, result in the stimulation of similar defence mechanisms in plants.

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Ribes nigrum 'Ben Alder' is one of a range of blackcurrant cultivars bred for their later flowering, allowing them to avoid frost

Mike Sleigh / RHS

Artificial control of winter damage

Physical protection using insulation is the gardener's first line of defence against winter damage. Woody plants established in the open ground may be protected by straw and fleece wrapping. More tender, containerized plants are safest if protected inside glasshouses or under plastic tunnels (Pottage 2014).

The development of synthetic chemicals, generally termed cryoprotectants, has not yet resulted in commercial products for gardeners to use. However, Percival & Barnes (2008) showed that some types of calcium fertilizer, experimentally sprayed onto *Quercus ilex* and apple 'Golden Crown', increased freezing tolerance in twigs, leaves, and roots. The most effective were calcium hydroxide, calcium nitrate borate, and a commercial product known as Metalosate Calcium. They detected a positive correlation between increased freezing tolerance and calcium content of tissue. For this purpose, calcium sprays are best applied during late summer and autumn.

Organic seaweed extracts sprayed onto the foliage of woody plants are also associated with enhanced acclimatization and increased cold tolerance. Another method used to reduce risks from overnight frost damage to tree fruit crops is to spray irrigation water over trees, a method used by commercial fruit growers. As the water freezes it releases minute amounts of latent heat which are sufficient to prevent foliar damage.

Plant breeders are developing new cultivars of woody plants with increased freezing tolerance, later flowering and longer dormancy periods. A classic example from recent years is the development of the 'Ben' range of blackcurrant cultivars by Malcolm Anderson of the Scottish Crop Research Institute (now The James Hutton Institute). He achieved later flowering and subsequent rapid crop maturity so that the crops avoided losses from frost damage.

This type of approach demands long-term studies, such as those described by Arora *et al*. (2003). They showed that leaf freezing tolerances and variations in hardiness in F_2 and backcross populations derived from a *Rhododendron* cross (*R. catawbiense* x *R. fortunei*) are controlled by three genes with strong additive effects. Differences in freezing tolerance were primarily due to variations in acclimatizing ability among the progeny. Results indicated that in *Rhododendron*, a woody perennial with prolonged juvenile or pre-flowering phases, the physiological age strongly influences cold tolerance. There was a significant increase in freezing tolerance of 5–6°C per year as seedlings aged from two to five years old. Heat-stable, glycine-rich, stress proteins, known as dehydrins, are closely associated with differences in freezing tolerance of several *Rhododendron* species. Similar results were reported by Rowland *et al*. (2004), working with blueberry, in which dehydrins increased with cold acclimatization and decreased with de-acclimatization.

Conclusion

Gradually, horticultural science is increasing our understanding of the basic biological processes which contribute towards cold hardiness. This should lead to making the gardener's task of coping with winter damage easier.

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