9 Temperature Effects on Growth and Development of Plants

Plants actively grow in a relatively narrow range of temperatures, generally between about 0 and 45°C. Many species have an even narrower range in which they thrive and survival of nearly all plant species does not extend very far above 45°C. Some species can tolerate temperatures far below 0°C due to the remarkable physiological mechanisms of acclimation.

The ultimate cause of cell death at the upper limits of survival is the inability of plants to tolerate the cellular dehydration and the general disruption of metabolism that accompanies high temperatures. At the lower limits, acclimated plant cells can often survive both the dehydration that accompanies ultra-low temperatures and the rehydration that must occur upon thawing. Non-acclimated cells normally survive neither.

There are physiological reasons for the growth responses plants have to temperature and we will explore them in this chapter. We will also explore the physiology of low temperature stress resistance in plants as well as horticultural practices that can influence a plant's stress tolerance.

Temperature and Heat in the Environment

Measuring heat

Temperature is a measure of the heat content of a substance. In this chapter we are mostly interested in the temperature of the plant and its environment which includes the atmosphere and the soil. The temperature of the soil is well buffered because of its mass and water content, thus sudden and dramatic changes in soil temperature don't normally occur. On the other hand, air temperature may fluctuate wildly with huge diurnal and even hourly changes. The fastest temperature drop ever recorded on earth was 27.2°C (49°F) in 15 min in Rapid City, South Dakota, USA on 10 January, 1911 while the fastest rise in temperature ever recorded was 27°C (49°F) in 2 min in Spearfish, South Dakota, USA on 22 January, 1943 (Lyons, 1997). (Other rapid temperature changes are often cited but are not official records of a recognized meteorological authority.) Both are pretty remarkable.

Thermometers

Temperature is most often measured with thermometers, which come in all shapes and sizes, including bi-metal mechanical thermometers, Galileo thermometers, liquid crystal thermometers, and others. Regardless of type, all thermometers have two major components to them: (i) a temperature sensor; and (ii) a gauge or scale. Probably the most common non-digital thermometer is the liquid-in-bulb glass thermometer. This type of thermometer consists of a sensor (the bulb) filled with a liquid (usually alcohol or mercury) that is attached to a glass capillary (the gauge) with an expansion bulb sealing the end opposite the bulb. Enough alcohol or mercury is contained in the bulb and capillary to partially fill the capillary. The rest of the capillary and expansion bulb contain a mixture of nitrogen gas and alcohol or mercury vapor. As the temperature changes, the volume of alcohol or mercury changes and is reflected in the level of liquid filling the capillary that is read from an inscribed scale on the capillary. Of course the thermometer must be calibrated with a thermometer of known accuracy.

Min-max thermometer

A useful thermometer for horticulturists is the min-max mercury thermometer. This type of thermometer records the highest and lowest temperatures experienced by the thermometer between re-settings. The mercury filled glass tube is 'U' shaped rather than the typical linear shape. One arm of the U is the maximum scale and the other is the minimum scale. When the temperature increases, mercury is forced up the maximum scale, pushing a little wire and glass marker along with it. As the temperature

falls, the mercury retreats but the marker stays put, indicating the maximum temperature reached. In addition, the mercury in the minimum side rises, pushing the minimum marker along with it. The scale on the minimum side reads from low to high in a top to bottom direction while the scale on the maximum side reads from high to low from top to bottom like a normal thermometer. Once the temperature begins to rise again, the minimum marker stays put. Both minimum and maximum markers are reset with a magnet. Digital min-max thermometers are also available.

Thermocouples

Thermocouples are also widely used in horticulture for measuring temperature. A thermocouple consists of a sensor made of two dissimilar metals joined together. When the junction of the two metals warms or cools, a voltage proportional to the temperature is generated. This voltage is calibrated with known standards and the temperature of the junction can be displayed digitally. Different combinations of metals called calibrations are available for different temperature and environmental conditions. Thermocouples can be wired in series to construct a very sensitive device called a thermoelectric module which can be used in low-temperature stress-resistance studies. In these applications, the thermoelectric module is sensitive enough to detect the freezing of microscopic-sized droplets of water, for example the freezing of supercooled water in flower pistils of deciduous fruit trees.

Infrared thermometer

Infrared thermometers are also useful to horticulturists. All objects emit infrared radiation in proportion to their temperature, and an infrared thermometer detects this radiation and converts it into a digital temperature reading. Infrared thermometers are useful in situations where thermocouple sensors, digital electronic sensors or individual thermometers cannot be used to measure the temperature of an object. For example, infrared thermometers are useful for measuring plant canopy temperatures to provide an indirect measure of water stress.

Temperature scales

In order for the temperature readings to be useful, uniform scales must be used in describing measured temperatures. The International Temperature Scale of 1990 defines measurements of temperature from 0.65°K (–272.5°C, –458.5°F) to approximately 1358°K (1085°C, 1985°F). Most of us are familiar with the Celsius (C) and Fahrenheit (F) scales and equate our perception of heat relative to known values of water states at various temperatures. Water freezes (or more precisely, melts) at 0°C (32°F) (cold perception) and boils at 100°C (212°F) (heat perception). Actually, humans begin to perceive that something is hot at a temperature of about 30°C and cold around 5°C. Hot and cold are subjective terms, each individual has their own idea of cold and hot.

Movement of heat

Heat moves as electromagnetic energy, mostly in the infrared region of the spectrum, from an object at a higher temperature to an object at a lower temperature. It can move directly from one object to another or it can move from one object to another by passing through a gas or liquid. Changes in the heat content of an object are reflected in changes in temperature, which we can feel or measure. While heat movement can be explained using laws of physics, heat transfer will be discussed here in relation to its importance in horticulture.

Radiation

Radiation is the movement of heat between two objects that are not in contact with each other. The heat is transferred directly through the air in a straight line from a warmer object to a cooler object, with no change in the air temperature. The best example of this is when you go outside on a cool sunny day and feel quite warm standing in the sunshine. Heat energy is radiated from the sun to your body, and even though the air stays cool, you are warmed. Another example of this is when you sit close to a burning fire. The air between you and the fire is cold, but you intercept heat moving away from the fire and you are warmed.

Conduction

Conduction is the movement of heat through a solid body. The heat transfer occurs due to molecular agitation within the solid body, but the body itself doesn't necessarily move. A good example of this is when you stir a hot liquid with a metal spoon, the spoon heats up and you eventually feel it.

The molecules of the spoon at the hotter end are more agitated and have more energy than those at the cooler end. This energy is passed from more agitated molecules to less agitated molecules and the result is detected as a transfer of heat from the hot liquid to your hand.

Convection

Convection is the movement of heat in currents through a fluid such as air or water. The energy is transferred by actual motion of the fluid away from the source of heat. The fluid heating up over a source of heat expands and becomes more buoyant than cooler fluid around it and moves away from the source of heat. Cooler fluid is denser and less buoyant thus replaces the heated fluid. The entire volume of fluid exhibits currents of moving material as heat is transferred from the source of heat to the liquid or gas. An object placed in the fluid can be warmed by heat transfer from the source through the fluid. Think of potatoes in a pot on the stove. Heat from the stove warms the water in the pot which transfers heat energy to the potato, and voilà, dinner!

A horticultural example

Let's put this all together and evaluate heat movement in a typical production field. During the day, radiation from the sun warms the earth. As the soil, plants, and other objects in the field absorb the incoming solar radiation, they heat up and begin to pass some of that heat energy into the air via conduction. Once the air is heated by conduction, it begins to rise and is replaced by cooler, less buoyant air. Convective currents develop and heat that was captured by solid objects by absorbing solar radiation and passed into the air via conduction moves farther into the atmosphere via convection.

While the soil and other objects are absorbing radiation from the sun, they may also be re-radiating energy back out into space. During the day more energy is coming in than going out, thus the air heats up. At night, more energy is going out than coming in, thus the air cools. In addition, the air passes heat to the soil and plants via conduction and the air cools. As more heat is radiated to space by the soil and plants, more energy can be absorbed from the air and it gets cooler. Water vapor and CO_2 may absorb or reflect some of this outgoing radiation before it escapes into space, trapping the heat near the earth's surface. This is the well-known greenhouse effect. A cloudy night is particularly effective in trapping heat energy near the surface, thus it usually doesn't get as cool on a cloudy night as it does on a similar, but clear night.

Inversions

In general, the temperature of the atmosphere decreases with height. As the atmosphere cools at night a significant amount of cold air may accumulate at the earth's surface, resulting in an inversion of the normal temperature gradient from the earth's surface to space. Instead of decreasing with height, air temperature increases with height for some distance upwards before decreasing in a normal fashion. This layer of warmer air is used in some frost-protection methods.

Temperature and Plant Growth

Plant species normally have an optimum temperature range for growth and development, outside of which they suffer reduced productivity and quality. Most plant growth responses to temperature can be explained by looking at Q_{10} values. Temperature effects on growth and development are particularly important in greenhouse production schemes, as temperatures set incorrectly can have disastrous effects on the crop in question. First let's review the concept of Q_{10}.

Q_{10}

The Q_{10} value is the rate at which a biological reaction changes with a 10°C change in temperature. Many biological reactions have a Q_{10} of 2.0, meaning that the reaction rate doubles for each 10°C rise in temperature. Conversely, the reaction rate is halved with each 10°C decrease in temperature. Many chemical reactions and biological processes have published Q_{10} values. In addition there is a temperature range for every biological reaction, outside of which it will not occur. Thus any reaction has a curve which illustrates the relative rate at which it proceeds from 0 at a minimum temperature to 100% at an optimum/maximum temperature.

Each chemical or biochemical process has a temperature optimum which differs among species. Some species grow well at one temperature while others grow well at a very different temperature. This is because the temperature optimum for life processes evolved with species in their climate of origin and adaptation.

Suppose you were growing a cool-season greenhouse crop with a temperature optimum of 15°C. Photosynthesis, respiration, and most other growth processes are optimally balanced in a narrow temperature window around 15°C and all have a Q_{10} for our discussion. Suppose a faulty thermostat kept night temperatures 5°C higher than they should have been for this crop. You notice that your crop looks 'worn out' and you know that something is wrong (but remember, you don't know that the thermostat is broken). Fertility, light, photoperiod, and pest control all seem in order. So what's the problem? During the day, this crop has a hypothetical optimum photosynthetic rate of 10 (units don't matter here). Respiration occurs at a rate of 3 during the day and 4 at night for a total of 7, leaving you with a net of 3 photosynthetic units per day for growth, development, or storage. If the night-time temperature is 5°C above optimum, then respiration will occur at a rate of 8 units per night (1 (original respiration rate) + 1 (for an increase of 5°C and a Q_{10} of 2.0)). The daily balance under these conditions is 10 units of photosynthesis – 11 respiration units (3 daytime + 8 night-time respiration units) or a daily loss of 1 unit! Over time this deficit in photosynthetic products is apparent as reduced growth and poor quality. And all because the thermostat is off by 5°C.

A similar negative outcome would arise if you reduced the night temperature of a warm greenhouse crop in an attempt to save energy. A 5°C decrease in night-time temperature may not seem like that much and you might think reducing the night-time temperature would reduce respiration thereby making more photosynthetic products available for growth and development. However, reducing the night-time temperature has moved the crop away for its optimum temperature for growth and development as well. Reduced night-time temperatures of warm-season crops interfere with basic metabolism of growth, resulting in unacceptable production. Optimum growth temperatures are determined for a reason. It often takes several weeks after exposure to less than optimum conditions before symptoms appear. If optima are not adhered to, yield and quality will suffer.

Examples of the Q_{10} effect in horticulture

NIGHT TEMPERATURE AND FLOWER COLOR Flower color is often enhanced by decreasing the night-time temperature to just a few degrees cooler than optimum. Growers often do this a week or so before the end of the production cycle for bedding plants to improve quality and salability. By reducing night-time temperature, respiration is reduced just enough to allow more photosynthates to be used for anthocyanin production. Care must be taken not to decrease the temperature too much.

EASTER LILIES Careful timing of Easter lily growth is imperative for successful production. Plants reaching their prime too soon are not acceptable. This is especially troublesome in warmer climates where you're trying to grow a cool-season crop in a greenhouse in mid- to late spring when greenhouse temperatures can soar. If plants are growing too quickly, they can be placed in a cooler at 2°C for up to 20 days to slow their growth with no ill effects.

FALL BLOOMING MUMS Chrysanthemums are another seasonal flower crop where timing of bloom is critical. Consumers don't want them when it's still summer-like, but want them as soon as the weather turns cool. This transition in demand is often abrupt and mums may not be in full bloom when they are needed most. Increasing the night-time temperature can hasten bloom when needed. Again, care must be taken not to warm it up too much, as advanced senescence might occur or plants may bloom too soon.

Thermoperiod and DIF

Just as different proportions of light and dark in a 24 h cycle can elicit various responses in plants, so too can differences in the distribution and duration of temperature exposure during the same cycle. Probably the most well-known thermoperiodic response in plants is the DIF response.

DIF is the difference between the day temperature and the night temperature. If days are warmer than the nights, a positive DIF (+DIF) exists, and if the night is warmer than the day, a negative DIF (-DIF) exists. Stem elongation is enhanced with a more positive DIF, and plants remain short statured if the DIF is around zero or negative. By controlling DIF, growers can manipulate the size of their plants, but only to the extent that a species responds. Some species exhibit a large response to DIF and their height can be readily manipulated with DIF. These species include Easter, Oriental and Asian lilies (*Lilium* spp.), *Dianthus* spp., *Chrysanthemum* spp., tomato (*Solanum lycopersicum*), poinsettia

(*Euphorbia pulcherrima*), green bean (*Phaseolus vulgaris*), *Salvia* spp., watermelon (*Citrullus lanatus*), *Celosia* spp., sweet corn (*Zea mays*), *Fuchsia* spp., *Impatiens* spp., *Portulaca* spp., *Gerbera* spp., *Petunia* spp., snapdragon (*Antirrhinum* spp.), geranium (*Pelargonium* spp.), and rose (*Rosa* spp.). Species with little to no DIF response include squash (*Cucurbita* spp.), platycodon (*Platycodon grandiflorus*), French marigold (*Tagetes patula*), tulip (*Tulipa* spp.), hyacinth (*Hyacinthus orientalis*), *Narcissus* spp., and *Aster* spp. In species that respond to DIF, the response is observed when the plant is normally undergoing significant stem elongation. If a grower knows the crop growth characteristics well, they can time the DIF to occur only during the period of most significant stem elongation and not the entire growth cycle.

In general a DIF of -5°C is sufficient to induce shorter internode length and therefore shorter plants. If the DIF is too negative, undesirable responses such as chlorosis may occur. In addition, growers need to be cautious when using DIF as a method of growth regulation since the rate of crop development is affected by temperature as well. Any DIF treatment that results in an increase in the average daily temperature is likely to result in accelerated crop development and any treatment that reduced the average daily temperature would retard growth and development.

In order to achieve a negative DIF, significant greenhouse heating at night is needed. However, lowering the greenhouse temperature below the night temperature for 2 h at sunrise (which creates a negative DIF) is just as effective as maintaining the negative DIF with heating for the entire night. This procedure is called the 'cool morning pulse'. It reduces the need for excessive heating during the night to maintain a negative DIF.

The DIF response may be a response to gibberellin production. Warmer days and cooler nights (+DIF) often stimulates internode elongation by enhancing gibberellin synthesis or action.

The Growing Season

Growing degree days (GDD)

Just as measuring the daily accumulation of light provides information regarding crop productivity, a plant's temperature history provides useful information in many crop production schemes, especially in field-oriented crops where temperature cannot be controlled. Since growth and development of both plants and plant pests (insects, disease organisms, and weeds) are all dependent on temperature, life stages can be monitored and modeled using growing degree days (GDD), also called heat units, a measurement of heat exposure. A GDD is defined as:

$$GDD = \frac{(Daily\ \max T^{\circ} + Daily\ \max T^{\circ})}{2} - Base\,T^{\circ}$$

GDD can be calculated using either degrees Celsius (°C) or Fahrenheit (°F) as long as appropriate conversions (5 GDDC = 9 GDDF) are made when consulting reference accumulations for specific stages of development. *Base T°* is the temperature at which growth commences in a species. It is often set to 50°F (10°C) (as in this example) but may be set lower or higher depending on the crop.

Modified GDD were adopted about 40 years ago to set limits on the minimum and maximum temperatures considered. All temperatures <10°C (50°F) are set to 10 (50) and all temperatures >30°C (86°F) are set to 30 (86). Therefore the maximum number of GDD possible every 24 h is 18 if using the Fahrenheit scale (i.e. assuming *Base T°* = 50°F, maximum GDD is [(86 + 50)/2] – 50 = 18) or 10 if using the Celsius scale.

Accumulated GDDs are calculated by summing daily GDDs over the growing season, starting the accumulation at some defined point, called the biofix, which might be at the fulfillment of the chilling requirement (covered later in this chapter), full bloom, day of seeding, or even a specific calendar date. Phenology models have been developed for specific crops and pests to help growers make management decisions based on crop or pest growth rather than calendar date.

GDDs are not only useful for monitoring growth stages of plants, insects and disease-causing organisms, but they are also useful in determining crop potential in local climates. If an estimate of GDD accumulation is available at a particular location, crops which may not accumulate enough GDD in a growing season can be avoided while those suited for the climate can be trialed. The GDDC requirements for some key developmental stages of several important world crops are presented in Table 9.1 (Neild and Newman, 1986; Miller *et al*., 2001; Kumar *et al*., 2008).

Another use for GDD is in timing herbicide applications for controlling annual weeds. The base

Table 9.1. Average GDDC (growing degree days calculated using degrees Celsius) needed to reach key developmental stages for four world crops.

Stage	Soybean (*Glycine max*)	Stage	Barley (*Hordeum vulgare*)	Wheat (*Triticum aaestivum*) (Hard Red)	Stage	Corn (*Zea mays*) mid-season hybrid
Seedling emergence	75	Seedling emergence	130	140	Seedling emergence	200
Unifoliate leaf	225	First tiller	330	400	Tassel formation	610
Flowering	978	Stem elongation	520	630	Ear formation	870
First pod	1150	Anthesis	840	850	Anthesis	1400
Final leaf	1430	Seed fill begins	1040	1120	Seed fill begins	1660
Seed mature	2400	Seed mature	1400	1600	Seed mature	2700

temperature for GDD calculation in these cases is usually adjusted to 2°C to reflect the growth of many early season weeds at lower temperatures. As in all examples using GDD, the biofix must be known in order to calculate GDD accumulation.

GDDs are used to time plantings for production of sweet corn (*Z. mays*) and peas (*Pisum sativum*) for processing. A steady supply of product is needed to keep the processing plant running through the season. The best way to ensure that there will be few gaps in production is to plant sequentially based on GDD accumulation.

Predictions of disease or insect outbreaks and the most appropriate times for specific control measures can be based on GDD accumulation.

The progression of bloom in fruit crops can be monitored using GDD accumulations in anticipation of the possible implementation of protective measures against frost injury during bloom. Additionally crop progression towards maturity can be monitored for many fruit crops, especially fruit trees, to schedule labor for harvest. Grape (*Vitis* spp.) harvest can be predicted based on GDD accumulation and the information combined with sugar and acid levels to help determine the best date for harvest.

High temperature stress

In many regions of the world heat stress caused by short-term or constantly elevated temperature results in significant reductions in yield and harvest quality. In general, heat stress is considered as any 10–15°C rise above normal ambient air temperature for a period long enough to induce irreparable damage to any aspect of plant growth and development (Wahid *et al.*, 2007). The rise in temperature is detected primarily by the plasma membrane (Saidi *et al.*, 2011) and is immediately reflected in influx of Ca^{2+} from the apoplast. Factors that influence the intensity of heat stress include: (i) temperature; (ii) duration of exposure; and (iii) the rate of temperature increase. The diurnal average temperature seems to regulate the development of heat stress symptoms over time rather than the absolute daytime or night-time temperature (Peet and Willits, 1998).

The heat-stress threshold is a daily average temperature above which a reduction in growth and productivity is observed. This threshold differs among species and is generally in the 25–35°C range (Wahid *et al.*, 2007). Productivity is often directly reduced with exposure to high temperatures during anthesis or seed fill. Heat stress during anthesis causes sterility and heat stress during seed fill causes photosynthates to be directed towards combating the heat stress rather than filling seeds with storage material.

PHYSIOLOGICAL EFFECTS OF HEAT STRESS Two important direct effects of high temperature are protein denaturation and increased membrane fluidity. Indirect injury is manifest as enzyme inactivation, inhibited protein synthesis, and loss of membrane integrity, which all eventually lead to slow growth, reduced ion exchange, and the production of ROS and other toxins. At moderately high temperatures, death occurs only after long-term exposure. If sudden, excessively high temperatures occur, cellular death may occur very quickly.

Heat stress can greatly reduce photosynthesis. The chemical reactions of photosynthesis in the thylakoid and the stroma are considered the primary site of photosynthetic inhibition (Wise *et al.*, 2004).

In addition, heat stress causes the formation of ROS which in turn cause degradation of chlorophyll a and b, thereby reducing light capture for photosynthesis. Since the plant is fixing less carbon via photosynthesis and respiration increases significantly with heat stress, an extremely negative carbon flow results.

High temperatures can cause leaf and twig scorching, sunburn on any aerial tissue, especially leaves and fruit, and general reduction of growth, all contributing to reduced yield and crop quality. Developmental alterations may also occur. Seeds exposed to abnormally high temperatures may suffer from reduced rates of germination, leading to poor stand establishment and reduced yield. Seeds which do germinate may grow very slowly if at all at high temperatures.

High temperatures may lead to poor seed set as a result of heat-induced sterility. High temperatures cause an extension of tomato styles beyond the anther cone which may result in the lack of self-pollination and result in poor fruit set (Wahid *et al*., 2007). Poor fruit set in tomato may also be the result of assimilate redirection away from reproductive tissue as a response to high temperatures (Kinet and Peet, 1997).

PLANT RESPONSES TO HEAT STRESS One of the first metabolic responses to heat stress is the production of specific proteins called heat-shock proteins. These proteins have a molecular mass from about 10–200 kDa and have chaperone-like activity (Schoffl *et al*., 1999). Chaperones are proteins that are involved in the non-covalent folding and the assembly or disassembly of macromolecules, but are not present when the macromolecules are involved in normal biological functions.

Many plants produce low molecular weight compounds called compatible osmolytes under stressful conditions such as heat stress. Compatible osmolytes are stable and not easily metabolized by the cells and have no effect on cell function, even at extremely high concentrations. Their cellular function is still relatively unknown but perhaps they function to prevent cellular dehydration under stressful conditions as heat stress normally increases transpiration and water demands. Compatible osmolytes include sugars, sugar alcohols, proline, tertiary and quaternary ammonium compounds, and tertiary sulfonium compounds (Sairam and Tyagi, 2004).

Plant hormone levels often respond to heat stress. Abscisic acid (ABA) increases under heat stress. This is not surprising since both heat and water stress often coincide and ABA increases in response to water stress. In some species ABA does not increase during the stress, but rather after the stress is over, suggesting some recovery role for the ABA signal (Maestri *et al*., 2002). Ethylene levels in plants increase with increases in temperature, however, at temperatures normally associated with heat stress, ethylene levels often decline. For the most part, increasing temperatures leads to reduced conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene. The temperature at which this accumulation of ACC occurs is species dependent. Salicylic acid (SA) can induce long-term thermotolerance by stimulating antioxidant activity and enhancing Ca^{2+} homeostasis in the cell. Both gibberellins and cytokinins decline under heat stress leading to reduced growth and yield. Brassinosteroids may confer thermotolerance to some species but not others (Dhaubhadel *et al*., 1999).

Phenolic compounds such as flavonoids and phenylpropanoids increase with heat stress. During heat stress, carotenoids, including xanthophyll, stabilize the lipid phase of thylakoid membranes helping to prevent heat-induced damage. Under high light levels, which often accompany heat-stress conditions, xanthophylls (violaxanthin and zeaxanthin) protect cells from excessive light levels. Zeaxanthin is found on the periphery of light-harvesting complexes where it prevents peroxidative damage to membrane lipids by ROS. Anthocyanins often accumulate in leaves under heat stress, even though levels of anthocyanin often decrease at warmer temperatures in flower petals and fruit. Accumulation in the leaf decreases leaf osmotic potential which may increase leaf water uptake and reduce water loss to transpiration.

Isoprenoids are low molecular weight, volatile secondary plant products. Under heat stress, plants that emit greater amounts of isoprenes from their leaves tend to have greater photosynthetic rates than those whose leaves do not emit large quantities of isoprenoids (Velikova and Loreto, 2005). Isoprenes may react directly with oxygen singlets and protect membranes from oxidation under heat stress. How well a plant tolerates heat stress is often determined by how well it detoxifies ROS like singlet oxygen.

Some species develop heat tolerance, physiological adaptations that diminish the negative consequences of exposure to high temperatures. The first physiological event associated with the development

of heat tolerance is increased fluidity of the lipid bilayer which activates enzymes responsible for the production of antioxidants and compatible osmolytes, both important in the development of heat tolerance. The compatible osmolytes protect the cell from dehydration often associated with heat stress while the antioxidants fight off the oxidative stress caused by the production of ROS under heat-stress conditions. Heat-shock proteins produced under heat stress work in concert with the osmolytes and antioxidants to reduce injury caused by heat stress.

Heat thermotolerance is the ability of a plant to grow and develop with little or no reduction in economic yield with subsequent high temperatures. Thermotolerance often develops within a few hours of an initial, brief exposure to a high, but sublethal temperature. It may also develop after a prolonged exposure to increasingly hotter temperatures via the interaction of heat-shock proteins, ABA, ROS, and SA. How these components confer thermotolerance is not clear.

There have been attempts to induce heat tolerance through foliar applications or a pre-sowing seed treatment with low concentrations of inorganic salts, osmoprotectants, hormones, or oxidants such as H_2O_2. Heat preconditioning of plants has also been attempted. Heat preconditioning has conferred greater subsequent heat tolerance to black spruce (*Picea mariana*), tomato (*S. lycopersicum*), and turfgrasses (Colclough *et al*., 1990; Morales *et al*., 2003; Xu *et al*., 2006). Pre-treating pearl millet (*Pennisetum glaucum*) seed at 42°C before sowing resulted in plants which were tolerant of overheating and dehydration (Tikhomirova, 1985).

IMPAIRED REPRODUCTION CAUSED BY HEAT STRESS Besides reduced photosynthesis coupled with increased respiration, a major reason yield is often drastically reduced with heat stress is that sexual reproduction is impaired at high temperatures. This impairment is reflected in fewer flower numbers per plant or greatly reduced fruit set of existing flowers.

Flowering and fruiting of cowpea (*Vigna unguiculata*) is a good example of just how sensitive the process is. If plants are exposed to 2 weeks of hot nights under long days during the first month after germination, flower production is greatly suppressed (Ahmed and Hall, 1993). The response is not observed under short days. Apparently, heat controls the process and photoperiod regulates the sensitivity to heat. At the next level of development, pod set is severely reduced under moderately high night temperatures due to high-temperature-induced male sterility (Warrag and Hall 1984a, b; Nielsen and Hall 1985a, b). Male sterility was observed only when the high temperatures were experienced in the latter half of the dark cycle and not if the elevated temperatures were applied during the first half of the dark cycle (Mutters and Hall, 1992) and this sensitivity appears to be under phytochrome control (Mutters *et al*., 1989). Pistils were not damaged by high night temperature. Additionally, neither pistils nor male fertility were affected by high daytime temperatures, even those higher than the night-time temperatures.

MANAGEMENT PRACTICES TO REDUCE HEAT STRESS AND ITS CONSEQUENCES One of the most obvious approaches to minimizing economic losses due to heat stress is selecting appropriate crops and cultivars for production. This includes selecting for geographic region as well as growing season. Don't try to grow cool-season crops in hot regions or during the summer in moderate climates.

A good example of crop manipulation for difficult production situations is with lettuce (*Lactuca sativa*). Lettuce is a cool-season crop that can be successfully grown in the spring and fall in warmer climates. If summer production is attempted in hotter regions, results can be catastrophic. Lettuce seed is very sensitive to temperature during the first 12 h of water imbibition. Exposure to temperatures above about 25°C leads to very poor germination and those seeds that do germinate produce weak, unacceptable seedlings (Fig. 9.1). For this reason, direct-seeded summer cropping is not usually attempted in warm to hot climates. Even though there are cultivars that have been developed that are more tolerant of the heat, leaf quality often suffers as leaves tend to be bitter and astringent when grown under hot, long days. Fall lettuce crops are normally seeded in late summer when soil temperatures can still be rather high. One way to minimize heat-induced reduced germination is by sowing seed late in the day and irrigating immediately after sowing. Seeds can then imbibe water from soil cooled by irrigation and seeds will have passed through the sensitive period by the time the soil heats up the next day. Another option is to use primed seed (see Chapter 12, this volume, for information on priming). Primed seed is often more expensive than non-primed seed and all cultivars are not always available as primed seed. Summer

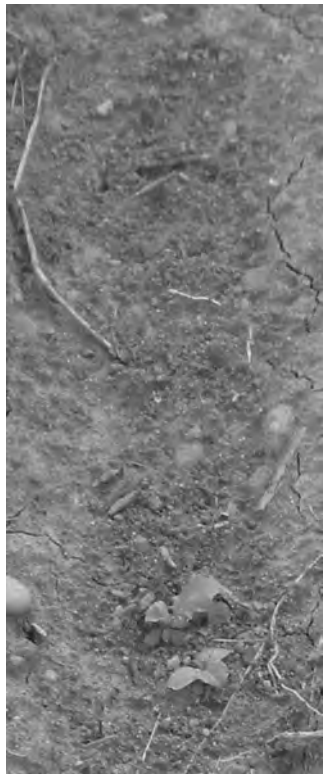

Fig. 9.1. Uneven stand of lettuce due to induced thermodormancy. Raw, non-primed seed was sown when soil temperature was >27°C.

lettuce production under a shaded high tunnel is another alternative. Seed could also be sown in flats of vermiculite, watered and put in a cold room overnight to avoid water imbibition at high temperatures. The additional labor expense of transplanting must also be considered. Ethylene can overcome the inhibition caused by high temperature thermoinhibition of germination (Matillaa and Matilla-Vázquez, 2008), however, no practical seed treatment with ethylene has been developed, and avoidance of thermoinhibition can be achieved by controlled imbibition at low temperatures.

Another management approach to avoiding heat stress is to time production of crops to coincide with the lowest chance of high temperatures during any particularly sensitive stage of development, particularly flowering, pollination, and fertilization. These three stages of development in most species are often extremely sensitive to high temperatures.

Some fruit, tomatoes (*S. lycopersicum*) and citrus (*Citrus* spp.) for example, are particularly sensitive to sunscald and damage by high temperature. Training and pruning techniques can be optimized to provide maximum foliar shading of fruit to minimize injury. In trees where bark temperatures may be excessive, causing injury to the cambium underneath, painting the trunk with a white reflective material can substantially reduce bark and cambium temperature.

As our understanding of how plants tolerate high temperatures improves, stress-resistant cultivars can be developed to reduce the economic losses associated with heat stress.

Dormancy and Crop Production

Many perennial species leave the growing season and enter into a state of visible inactivity most horticulturists call dormancy. This phase of a plant's yearly cycle allows it to survive an often hostile environment in which survival might not normally be possible. While little visible growth is occurring during this period, many things are happening on the cellular level. In fact, regrowth and crop production the following growing season depend on the many physiological changes during the dormant season. When chilling requirements are fulfilled, regular and synchronous flowering occurs supplying the first ingredient for a good crop.

Many buds on a plant remain 'inactive' even during the growing season. It is good that they do not grow, for fields might be overcome with vegetative growth if that were to happen. These quiet buds are also a source of regrowth should something happen to a plant's normal vegetative growth during the growing season, for example some sort of pest infestation which consumes many of the plant's stems and leaves. Plants have evolved a set of finely tuned signaling mechanisms to control bud growth during inhospitable periods or when sufficient growth already exists to support a full crop and further growth is not necessary and might even be detrimental.

Dormancy, rest, and quiescence

The names dormancy, rest, and quiescence have been given to this period of suspended bud growth, resulting in much confusion in the literature and among horticulturists. The problem lies in the specific definition of each term and the inconsistent use of these terms in describing the dormancy phenomenon.

Rather than list the many different definitions that are associated with each term, a more useful approach is to explore the terminology that defines, without confusion, this phenomenon of plant physiology.

A universal nomenclature for dormancy

In the late 1980s, a group of researchers suggested a universal nomenclature for the phenomenon of dormancy that could be used without confusion by laymen and researchers alike (Lang *et al*., 1987). The terms endodormancy, ecodormancy, and paradormancy were suggested as terms that fully described a particular type of bud dormancy in deciduous plants.

Endodormancy describes that type of dormancy that is located within the dormant tissue itself, usually a bud. A physiological condition within the bud makes growth even under optimum conditions highly unlikely. This is the type of dormancy that requires bud chilling before active growth will return in the spring.

Ecodormancy describes dormancy caused by some factor located outside of the plant, usually environmental, that prevents bud tissues from growing. Usually this factor is temperature, but it might also be light or water.

Paradormancy is dormancy caused by a factor within the plant but outside of the affected tissue. A great example of this type of dormancy is dormancy of lateral buds caused by apical dominance of the terminal bud (Fig. 9.2). The lateral buds will not grow, even in an optimum environment, because auxin is being translocated from the terminal bud to lateral buds, preventing their growth. If the terminal bud is removed, the source of auxin is removed and lateral buds will begin to grow.

Fig. 9.2. Raspberry (*Rubus* spp.) lateral buds beginning to grow after removal of terminal meristem and release of lateral buds from paradormancy.

Even though we often describe a particular example of dormancy with one of these terms, the tissue in question may in fact be experiencing more than one type at any given time.

The cell cycle and dormancy

Once released from dormancy, vegetative bud growth requires cell division. Changes in the expression of specific cell-cycle genes have been observed with the onset of bud growth following release from dormancy in many species (Horvath *et al*., 2003). The cell cycle is a series of phases in cell activity involved in cellular reproduction. Cells in the G1 phase are preparing for DNA replication that occurs in the S phase. After the S phase, cells enter the G2 phase to prepare for mitosis in the M phase. Cells in vegetative buds are arrested in the G1 phase just before the S phase, or in other words, at the G1-S phase.

Dormancy breaking results in the release of cells from this arrested phase. Histones and D-type cyclins (CYCD) are involved with this release. Histones are proteins involved in DNA replication and packaging in the nucleus and cyclins are proteins that help regulate the cell cycle by activating kinases, enzymes that phosphorylate other enzymes to activate them. Different CYCDs are expressed when buds are exposed to different signaling agents such as cytokinins, gibberellins, brassinosteroids, and sugar. Once the release from the G1-S phase is signaled by one or more of the above-mentioned agents, cell division moves into the G2-M phase, where it can be arrested due to the action of hormonal signals or by the lack thereof. Movement out of G2-M requires B-type cyclins, which are induced by auxin, cytokinin, and gibberellin.

Physiology of dormancy

Endodormancy

Light and temperature are the two main environmental signals that regulate endodormancy induction and release in plants. When plants perceive either or both signals, their response is the production of a cascade of physiological signals that eventually result in either the induction of or release

from dormancy. These signals are often amplified and transferred within the plant as changes in hormone levels which regulate the many metabolic and anatomical changes that occur.

In some species, such as poplar (*Populus* spp.), birch (*Betula* spp.), red osier dogwood (*Cornus sericea*), and wild grape (*Vitis* spp.), short days alone can induce endodormancy. Other species, such as domesticated grape (*Vitis* spp.), heather (*Calluna vulgaris*), and leafy spurge (*Euphorbia esula*), require both cold temperatures and short days (Chao *et al*., 2007). Few species enter endodormancy utilizing only a low temperature signal.

The short-day response is a response mediated by phytochrome. Endodormancy induction occurs when a sufficiently low P_{fr}:P_r ratio exists after the dark cycle. If the long dark period of a short day is interrupted with red light, dormancy is not induced (Howe *et al*., 1996), and the effect of red light can be nullified if far-red light immediately follows the red light. Cold temperatures enhance endodormancy induced by short days.

A common idea in plant physiology is that bud formation leads to the induction of dormancy or that dormancy leads to the formation of buds. Bud formation and dormancy induction, both initiated by phytochrome-regulated short days are independent processes as mutated plants can become dormant without setting buds (Rohde and Bhalerao, 2007).

Once dormancy is induced, it is maintained by unknown mechanisms. Exposure to low temperature then becomes the key environmental signal leading to a release from endodormancy (Fig. 9.3), however, in many species, this is not a strict requirement as chemical treatments with hydrogen cyanamide can replace the requirement for low temperature exposure (Chao *et al*., 2007). Bud growth may not occur immediately after release from endodormancy due to low-temperature-induced ecodormancy. Once temperatures increase to a sufficiently warm level, ecodormancy will fade and bud growth will commence. After ecodormancy fades, the dormancy-regulating mechanism is reset, probably by increasingly long days and production of dormancy-regulating genes.

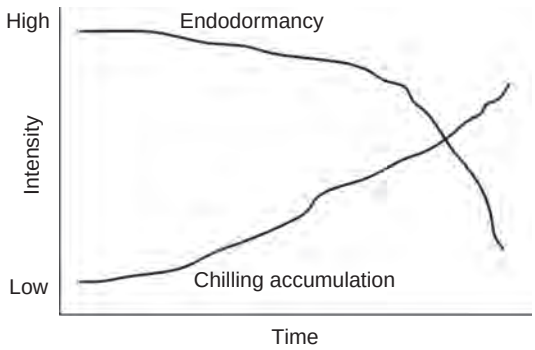

Fig. 9.3. The relationship between release from endodormancy and chilling.

Flowering and endodormancy may be interregulated as genes promoting flowering delay induction of dormancy and genes inhibiting flowering induce dormancy (Chao *et al*., 2007). Senescence is often associated with a decrease in basipetal transport of auxin and this decline in auxin levels associated with senescence may provide a signal for lateral buds to exit paradormancy and enter endodormancy.

Paradormancy

Paradormancy of lateral buds is due to the inhibition of cytokinin production or activity in stem tissue adjacent to lateral buds by auxin. Cytokinin is required for production of cyclin-D. Once auxin levels are reduced enough to allow cytokinin production and passage through the G1-S phase, gibberellins may then trigger the progression into the M phase. Sugar is required for cyclin-D production in some species, but has been shown to inhibit growth of paradormant buds of other species.

Ecodormancy

Ecodormancy is enhanced by high levels of ABA. Factors inducing ecodormancy such as temperature or water stress, lead to elevated levels of ABA.

Chemical messengers

Once the temperature or light signal is perceived by the plant, these environmental signals must be converted to chemical messengers within the plant. These chemical messengers include sugars and the phytohormones. Even though a tremendous amount of research has focused on these messengers, information on how these messengers regulate all three types of dormancy is fairly limited. In addition, while specific regulatory roles are given to different substances, there are myriad interactions among them that occur, both known and unknown.

Sugars (glucose and sucrose) are important in maintaining paradormancy and also the transition from paradormance to endodormancy. Sugars interfere with gibberellin perception and enhance ABA perception (Chao *et al*., 2007). Elevated levels of ABA are associated with endodormancy and ethylene is need for ABA accumulation. Short days inhibit the synthesis of gibberellic acid (GA) and reduced GA levels are often associated with induction of endodormancy. GA is also important for stimulating bud growth following the release from endodormancy, but seems to have limited involvement in endodormancy maintenance.

Chilling and bud endodormancy

We may not know how dormancy release is regulated physiologically, but we certainly have enough evidence that exposure to low temperatures for an extended time is required for release from endodormancy. While it is not a strict requirement since chemical treatments with hydrogen cyanamide can replace the requirement for low temperature exposure (Chao *et al*., 2007), natural release from endodormancy relies on exposure to cold temperatures. Use of endodormancy-releasing chemicals is limited to instances where insufficient natural chilling occurs for adequate bud break and crop production.

Mathematical modeling of the release of buds from endodormancy has been the subject of countless research articles. Much of the work has been with fruit and nut trees. Regardless of which model is used, they all quantify the relationship between low temperature and release of buds, both vegetative and floral, from endodormancy. Such models are extremely helpful tools for horticultural management. Of the many models presented in the literature, three stand out as the most widely used and accepted models for horticultural crops: (i) the chilling hour model (Weinberger, 1950); (ii) the Utah model and modifications (Richardson *et al*., 1974); and (iii) the dynamic model (Fishman *et al*., 1987).

The chilling hour model

The chilling hour model is the simplest model. It assumes that the release of buds from endodormancy proceeds in a linear fashion as plants are exposed to temperatures between 0 and 7°C. Below 0°C and above 7°C, no chilling accumulates and chilling is not reversible. One major drawback of the chilling hour model is that it doesn't account for the negative effect elevated winter temperatures have on chilling accumulation. In addition, the date on which chilling accumulation begins is often arbitrary or based on a specific calendar date. To be effective, a model must account for the chilling a plant receives from the time endodormancy is initiated until it is complete. Experiments needed to determine these points are time consuming and often not performed for different species and cultivars.

The Utah model

In order to account for the negative influence elevated winter temperatures can have on chilling accumulation, the Utah model was developed. This model assumes that chilling accumulation occurs within the temperature range of 1.5–12.5°C. Outside this range accumulation is either negative or zero. The measure of chilling in the Utah model is the chill unit (CU). CU values for different temperatures are presented in Table 9.2. The date chilling begins to accumulate in the fall is determined via an iteration of the model, beginning around 1 August to determine the date on which the greatest number of negative units have accumulated. Normally, in temperature regions, a run of the model for the months August through to November will reveal the date for the start of chilling accumulation each fall. In more tropical climates, this model may continue to accumulate negative chilling units well after November, and a suitable date for chilling accumulation is hard to define or must be arbitrarily set. To overcome this major problem with the Utah model, the dynamic model was developed.

A variation of the Utah model, which is often called the positive Utah model, is when all negative CU values are eliminated from the model. This model

Table 9.2. Chill unit (CU) values for 1 h exposure to different temperatures (after Richardson *et al.*, 1974).

Temperature (°C)	Chill unit value
<=1.4	0
1.5–2.4	0.5
2.5–9.1	1
9.2–12.4	0.5
12.5–14.9	0
15–18	–0.5
>18	–1

provides a better fit to data in subtropical situations; however, the dynamic model is a better choice.

The dynamic model

The dynamic model takes into consideration the impact of high temperatures on chilling accumulation. In this model chilling is accumulated as chill portions (CP) in two separate stages. In the first stage, a hypothetical metabolite is synthesized and accumulates with chilling but may be metabolized and removed from the accumulated pool if temperatures are warm. Once a certain amount of the metabolite is accumulated, it enters a second stage where it is converted with relatively warmer temperatures into a stable, irreversible compound which represents the CP. Stage one is reset to zero to begin accumulating the metabolite again. An adjustment is made to the model for temperatures below 4°C. At these temperatures, only a part of the metabolite is transformed into the CP and stage one is not reset to zero, but rather to a level above zero, the specific value depending on temperature. One CP is equal to 28 h at 6°C or more than 28 h at less effective temperatures (6.7–12.8°C). The mathematics of the model are complex and beyond the scope of this text, however, a spreadsheet is available online (http://ucanr.org/sites/fruittree/How-to_Guides/Dynamic_Model_-_Chill_Accumulation/) for calculating CP values using hourly temperature data. Values for CP accumulation are much lower than those calculated using chilling hours or the Utah model, thus published chilling requirements must be used carefully ensuring that the correct units for chilling accumulation are being utilized.

The dynamic model works well in predicting the stage of endodormancy for those species that have been extensively studied. Rigorous studies are needed to generate the needed information to equate CP to phenological stages for more species (and even cultivars within species) under each climatic zone of interest. As more work is done on this aspect of the dynamic model, probably it will be used more and may become the model of choice for most if not all chilling studies.

Again, the date for initiating chilling accumulation in the fall must be set, and the criteria for setting this may vary from site to site and study to study. A universal phenological stage such as 75% leaf drop should be agreed upon for the initiation of chilling accumulation in the fall.

Determining chilling requirement

When the chilling requirement for a specific cultivar or a lesser known species is not available in the literature, the chilling requirement can be determined experimentally. Besides academic reasons, why would you even want to determine chilling requirement and/or stage of endodormancy? In climates that are marginal for chilling accumulation, it is imperative to have this type of information for scheduling rest-breaking treatments. While estimating chilling requirement demands considerable work, it is not a difficult task.

One assumption of the approach described here, is that typical chilling temperatures will be considered and precise estimates of temperature requirements for breaking rest are beyond the scope of this procedure. In addition, the use of standard chilling temperatures reflects typical field conditions. A physiological marker for establishing the end of rest must be chosen for vegetative, floral, or mixed buds. A standard mark for indicating the termination of endodormancy is growth of 50% of the buds on excised shoots held at an appropriate temperature (15°C) for a specific length of time (3 or 4 weeks) with their cut bases in water. A temperature of 15°C is a good choice since it neither contributes to additional chilling nor negates any accumulated chilling (Dennis, 2003). An alternative indicator would be measuring the length of time it takes for buds to reach a specific stage of development once moved from the chilling environment to the forcing one. A definition of what constitutes growth must also be made, such as greening of bud scales, at least 2 mm growth, etc. One of the models described above must be selected to determine the chilling requirement. If you are located in the temperate zone, either the Utah model or the dynamic model should be used. If you are working in the subtropics, you should use the dynamic model. Recent work suggests that the dynamic model is the best model in all climates (Luedeling *et al.*, 2009). Finally the experimental unit used for making observations must be selected. Small potted trees should be used if available as they represent the closest approximation to orchard trees. Care must be given to small potted specimens in the field to ensure that they do not become desiccated or frozen. Individual shoots with multiple buds or individual nodes with a single bud can be used, but all three have their limitations (Dennis, 2003). Shoot bases should be cut every couple of days to prevent plugging of the xylem.

In general, the procedure is to collect specimens, either potted trees or shoots, periodically from fall through to spring, from the field where they have received natural exposure to chilling temperatures. The samples are then held at a warm temperature (15°C) and periodically the percentage of buds that grow in a specific length of time are determined, or the buds are observed over time and how long it takes them to reach a specific stage is calculated. Determining the percentage of buds that grow in a specific length of time is preferred to observing how long it takes for buds to reach a specific stage as the latter may reflect exhaustion of the buds' food supply rather than a lack of sufficient chilling, especially with cuttings.

Once data are collected, a graph can be drawn with chilling accumulated along the *x*-axis and percentage bud break along the *y*-axis. The chilling needed to reach a certain percentage bud growth can then be estimated. Ideally the work should be repeated for several years and if possible at several locations.

Vernalization

Vernalization is the transition of meristems from the vegetative state to the floral state in response to prolonged exposure to low temperatures. It is required by many biennials including cabbage (*Brassica oleracea* Capitata Group), beets (*Beta vulgaris*), carrots (*Daucus carota*), and winter annuals such as winter rye (*Secale cereale*), and winter wheat (*Triticum* spp.). Vernalization is similar to endodormancy in the sense that both rely on cold temperatures to proceed. However, while endodormancy usually occurs in meristems where cell division has essentially ceased, vernalization requires actively dividing cells to proceed (Wellensiek, 1962). In tissue culture of vernalized *Lunaria biennis* plants, only tissue-cultured plants obtained from actively dividing tissues (meristems) flowered. Plants cultured from non-dividing tissue from vernalized plants did not flower (Sung and Amasino, 2004).

A fascinating aspect of vernalization is that plants retain a permanent cellular memory of their vernalization (Michaels, 2009) such that cuttings of *L. biennis* taken from vernalized plants develop into flowering plants while cuttings taken from non-vernalized specimens develop vegetative plants (Wellensiek, 1962) (Fig. 9.4). When vernalized plants of the biennial *Hyoscyamus niger* are grown under non-inductive photoperiods they do not flower. Even after they are grown for a very long time under non-inductive conditions, the plants 'remember' that they were vernalized and will flower once placed under an inductive photoperiod. This cellular memory is not passed to the next generation since sexually reproduced plants must be vernalized for flowering.

In many species, flowering requires a photoperiodic signal, often a long day, following vernalization. In most of these long-day plants, treatment with GA can replace the requirement for cold treatment. However, in short-day plants, GA cannot replace the cold treatment. Thus the flowering response after vernalization is complex. Many scientists have studied this complex interaction of vernalization and photoperiod, but only recently has a fairly clear picture of what happens at the molecular level developed.

The molecular regulation of vernalization

The incredible plant *Arabidopsis thaliana* has provided enormous clues towards understanding flowering in plants. Besides being a small plant with a rapid generation time and sequenced genome, flowering is controlled by both photoperiod (it's a long-day plant) and vernalization. In many plants, strains of both summer and winter annuals exist in the same species. Many laboratory strains of *Arabidopsis* are rapid-flowering summer annuals. 'Normal' *Arabidopsis* plants are winter annuals that flower under long days after receiving sufficient vernalization. This winter annual trait and the need for vernalization is controlled by a single dominant gene called FRIGIDA (FRI). In addition, the control of flowering by vernalization, requires the FLOWERING LOCUS C (FLC) gene, which represses flowering. When a dominant allele of FRI is present, FLC expression is at a high enough level to inhibit flowering (Sung and Amasino, 2004).

The cellular memory of vernalization

The vernalization process which requires 30–40 days for maximum response, leads to permanent epigenetic repression of the FLC gene for the life of the plant, which allows flowering to occur under an appropriate photoperiod. This cellular memory of vernalization is caused by chromatin restructuring of the FLC which suppresses its expression. The down-regulation of FLC is removed in progeny

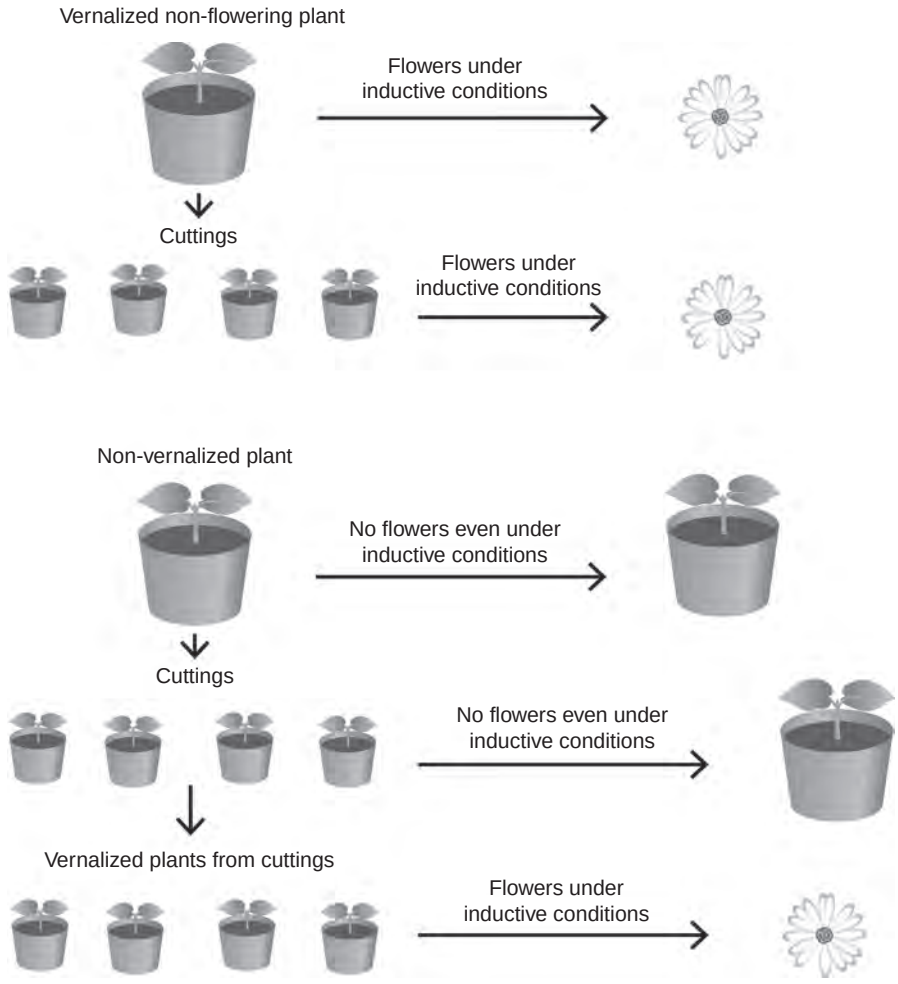

Fig. 9.4. Vernalization is 'remembered' by plants. (Plant and flower symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science, ian.umces.edu/symbols/.)

from sexual reproduction, thus they must experience vernalization in order to flower under long days.

The initial establishment of the vernalized condition is regulated by a gene called VIN3. Expression of VIN3 is induced by exposure to cold, but quickly disappears if the plant is exposed to warm temperatures. The permanent state of vernalization is due to chromatin remodeling of FLC which is regulated by VRN1 and VRN2. VIN3 initially represses the FLC and makes it susceptible to histone modifications triggered by VRN1 and VRN2. (Histones are the protein components of chromatin around which DNA is wrapped. Adding or removing methyl groups from specific amino acids in the histones, confers changes in protein structure which effectively turns the DNA off (adding a methyl group) or on (removing a methyl group).) Repression of FLC by VIN3 is not permanent. Only after methylation of the histones at the FLC locus, which turns off the FLC gene, is FLC permanently repressed, allowing the plant to cellularly remember that it is vernalized (Sung and Amasino, 2004). The FLC gene seems to act by inhibiting the expression of a group of floral activators, also called floral integrators. These are genes coding for proteins that promote flowering. The genes FT and SOC1 are such integrators in *Arabidopsis*.

Flowering after vernalization

The photoperiodic component of flowering in *Arabidopsis* is controlled by the CONSTANS (CO) gene, which is considered a floral promoter. CO expression is low early in the day and increases remarkably 8–10 h after dawn (Michaels, 2009). The protein product of CO peaks about 16 h after dawn (Wigge, 2011) and is stabilized by light and degraded in the dark. Since CO peaks in the light of long days and in the dark of short days the CO protein accumulates and promotes flowering only under long days, since it is not stable in the dark (Michaels, 2009).

CO transcription is regulated by the circadian clock. One protein required for CO transcription is CDF1 which is produced early in the day. It binds to the CO promoter and suppresses CO transcription. Later in the day, two other genes, GI and FKF1, degrade CDF1 and removes the suppression of CO. White, blue, or far-red light promote CO protein accumulation while red light or darkness promote its degradation. The genes responsible for this response have been identified as PHYB, PHYA, VRY1, and CRY2. PHYB promotes the degradation of CO early in the day while PHYA, CRY1, and CRY2 stabilize it later in the day (Michaels, 2009).

Under inductive long days, there is an increase in the activity of the CO gene which in turn activates FT and SOC1. The genes LEAFY and APETALA1, which direct floral organ production at the meristem, are then activated by FT and SOC1. Until FLC is repressed by VRN1, VRN2, and VIN3 genes following vernalization, photoperiod is ineffective in inducing flowering: FLC is repressing the floral integrators! (Amasino, 2005).

Florigen, vernalization, and endodormancy

The protein produced by FT is considered the universal flowering hormone, florigen (Zeevaart, 2008), and evidence indicates that FT is a highly conserved gene across many species (Wigge, 2011). The FT gene has been transplanted into a number of unrelated species, and when overexpressed has led to premature flowering even under non-inductive conditions. Thus FT regulates flowering under both vernalization and photoperiodic control. Not surprisingly, FT is also involved in endodormancy regulation.

In poplar (*Populus* sp.), the onset of dormancy is triggered by short days and cold temperatures with a concomitant down-regulation of FT. Transcription of the CO gene (which activates FT) peaks at the end of a long day or during the night of a short day. As previously mentioned, the product of the CO gene is extremely labile in the dark and only accumulates under long days. Under long days, the CO product up-regulates the FT gene and promotes flowering. When plants are transferred from long day to short day, FT expression quickly decreases and plants set buds. When FT genes are down-regulated using RNA interference (no FT produced) plants are much more sensitive to short-day-induced bud set. However, if FT genes are overexpressed, short-day-induced growth cessation is inhibited (Böhlenius *et al*., 2006) and plants do not set buds (Wigge, 2011). Daylength is sensed by phytochrome, which regulates the CO gene, which in turn regulates the FT gene, thereby regulating both floral and dormancy status of meristems (Rohde and Bhalerao, 2007). After vernalization, there is intense up-regulation of FT expression in embryonic leaves within the bud, suggesting FT's involvement in spring bud growth regulation (Wigge, 2011).

Vernalization and flowering of geophytes

Geophytes are plants in which their perennial buds are located on underground storage organs such as rhizomes, tubers, tuberous roots, bulbs, or corms. Many geophytes are used for their beautiful flowers, both in and out of season. Even though there are about 60 taxa that are regularly used for flowering, the bulk of commercial production worldwide relies on only six taxa: (i) *Tulipa*, 39%; (ii) *Narcissus*, 20%; (iii) *Lilium*, 19%; (iv) *Gladiolus*, 8.5%; (v) *Hyacinthus*, 4%; and (vi) *Iris*, 3% (Gross *et al*., 2002).

Many of these geophytes exhibit endodormancy that must be broken with chilling. In addition, many of them require vernalization for flower formation. Even though endodormancy and vernalization are often discussed separately, the two processes are linked and under similar physiological control. Since many of these species are forced in greenhouses, adequate chilling for breaking endodormancy or fulfilling vernalization requirements is imperative for success. Correct procedures for handling these propagules after harvest in the field until forcing in the greenhouse are easily accessed on the Internet. With respect to chilling requirements, it is absolutely essential to understand: (i) when flower initiation occurs; (ii) how long it takes for

development from initiation to anthesis; and (iii) the temperatures regulating the different phases of flowering. Unlike with tree fruit buds, models are not needed to estimate chilling of geophytes since chilling can be precisely controlled in storage.

Stratification and After-ripening of Seeds

Another important effect of temperature on plant growth and development is its effect on removing dormancy from seeds allowing them to germinate. While there are a number of different types of seed dormancy (including morphological, physical, physiological, morphophysiological, and combinational dormancy; see Chapter 6, this volume) stratification and after-ripening are connected with releasing seeds from physiological and the physiological component of morphophysiological dormancy, allowing them to germinate.

Seed dormancy is an innate characteristic of seed that blocks developmental progress from sexual reproduction to germination. It is controlled by genetics and the environment, in particular, light and temperature. Physiologically it is a very difficult process to measure as it can only be measured by the lack of germination (Finch-Savage and Leubner-Metzger, 2006). Germination is an 'all-or-none' process, it either occurs or it doesn't. Dormancy, on the other hand, is a continuum in the state of readiness to germinate, ranging from not ready at all to fully ready. Dormancy can be controlled by the embryo (physiological dormancy), endosperm, seedcoat, or any combination of the three. Our discussion will focus on physiological seed dormancy, contained within the embryo, that may or may not respond to temperature, GA, or light treatments to remove dormancy and promote germination.

Differences in opinion concerning whether or not a particular factor influences dormancy or germination or both often arise due to differences in how an author defines where dormancy ends and germination begins (Kucera *et al*., 2005; Finch-Savage and Leubner-Metzger, 2006; Tsiantisa, 2006). In this discussion, the viewpoint presented by Finch-Savage and Leubner-Metzger (2006) has been adopted. Any factor that increases the environmental window wherein seeds can germinate is involved in regulating dormancy rather than germination (Finch-Savage and Leubner-Metzger, 2006). For example, many researchers suggest that temperature controls both dormancy and germination while light controls only germination. Since red light alters the seed via phytochrome, rendering it capable of germinating in the dark, it should be considered the last step in breaking dormancy rather than the first step in germination (Finch-Savage and Leubner-Metzger, 2006).

In order for a new seedling to develop, dormancy release and germination cues must be supplied in the proper order. In most cases this consists of a temperature exposure followed by a light treatment. The temperature exposure is usually a long-term process and the response is a slow and gradual shift towards germination. The light exposure is short, as little as a few seconds and the results are instantaneous. As soon as the red light converts P_r to P_{fr}, the switch has been set to the on position and germination can commence.

Plants whose seeds require stratification are said to be in deep physiological dormancy following the classification scheme of seed dormancy proposed by Baskin and Baskin (2004). They require either cold temperatures (subtype a) or warm temperatures (subtype b) during stratification in order to be susceptible to light stimulation of germination and GA will not substitute for the temperature treatment.

The majority of plants produce seeds that are in the 'non-deep physiological dormancy' class. Treatment of seeds with GA can break dormancy, or dormancy can be broken with warm or cold stratification (depending on species) or after ripening. After ripening is storage of seed at room temperature for up to several months in order to break dormancy and promote germination. Physiological changes during the release from dormancy are reflected in seeds germinating at a wider range of temperatures as dormancy is released and an increase in their response to GA and light promotion of germination.

Dormancy is regulated by a balance between GA and ABA in the embryo and the embryo's sensitivity to both hormones. ABA induces dormancy and ABA synthesis occurs in imbibed dormant seeds but not imbibed non-dormant seeds. The ABA production seems to be what maintains the seed in a dormant state. The dormant state is often characterized by ABA synthesis and GA degradation. When GA is applied to dormant seeds, ABA increases, indicating some sort of feedback mechanism to keep the ABA:GA ratio high until dormancy is broken. As dormancy is broken there is a degradation of ABA or a decrease in its biosynthesis with a concomitant increase in GA biosynthesis. GA stimulates germination

and is not really involved in dormancy release. It is the decline in ABA level that signals the loss of dormancy (Finch-Savage and Leubner-Metzger, 2006). During the transition from dormant to non-dormant, there is also a decrease in sensitivity to ABA and an increase in sensitivity to GA. Both cold and light stimulate GA production, gearing the seed up for germination, but until the ABA levels are sufficiently low enough to break dormancy, germination will not occur. The response to cold and light is not really an increase in GA per se since biologically inactive GA9 and GA20 are always formed in the seed (Finch-Savage and Leubner-Metzger, 2006). The increase in GA following cold treatment is regulated by a transcription factor (a protein that regulates the transcription of the message coded in DNA to RNA) called SPATULA (SPT). Prior to dormancy release, this protein prevents expression of the gene responsible for conversion of inactive GAs and GA precursors to GA (Penfield *et al*., 2005).

Different theories have been proposed to explain the after-ripening phenomenon in seeds such as removal of germination inhibitors or changes in membrane structure, however, the physiology of after ripening for the most part has not been documented.

Some species have a dual stratification requirement, for example ash (*Fraxinus excelsior*) (Finch-Savage and Leubner-Metzger, 2006). Warm stratification for 16 weeks followed by 16 weeks of cold stratification are required to remove dormancy and enhance germination. The warm stratification leads to a decline in ABA levels breaking dormancy while the cold stratification increases GA content and stimulates germination. If the cold stratification is given at a constant temperature, germination will proceed slowly, however, exposure to alternating 3 and 25°C leads to hastened germination, but only if the warm period does not exceed the cold period in any 24 h cycle. If seeds receive more warm than cold temperatures during this chilling phase, seeds will enter into a secondary dormancy. This suggests that dormancy breaking is still occurring during the chilling treatment and separating temperature effects on seeds into specific categories is not always justified.

Low Temperature Stress in Plants

In many regions of the world plants may be vulnerable to various forms of injury due to low temperatures. Low temperature stress to plants is often divided into stress caused by low, but above-freezing temperatures (normally called chilling injury) and below-freezing temperatures (freezing injury). Both types of injury can result in substantial economic losses and at times, may be lethal to the entire plant. In this section, both types of low temperature injury and their physiological implications will be explored.

Chilling injury

Many fruits, vegetables, and ornamental crops, particularly those of subtropical or tropical origin, are sensitive to low temperatures (between 0 and 15°C). The more tropical the species, the higher the temperature at which the injury will occur. The injury sustained by exposure to temperatures in this range are very different from injuries due to temperatures below freezing and chilling injury often occurs at a temperature well above freezing. Chilling injury is often associated with injury to the cell membrane, resulting in a physiological abnormality that produces a toxin or fails to produce a necessary metabolite. Freezing injury is often due to direct injury from ice crystals or due to cellular dehydration and an inability to rehydrate upon thawing.

Crops from the temperate zone often experience chilling injury in a much narrower range of temperature (0–5°C). Either the entire plant or a specific tissue may suffer injury. The injury may appear immediately upon exposure to chilling temperatures or may not become evident until much later. Some chilling injury may not be observed until the affected plant or tissue is exposed to warm temperatures.

Chilling injury often reduces or eliminates the market value of many crops, thus this discussion will mainly focus on chilling injury to harvested commodities. Keep in mind that chilling exposure can accumulate, for example in the field, in transit, and in storage, and once a certain intensity is reached, injury will be seen. Crops that are sensitive to chilling injury usually have a short storage life since low temperature cannot be used to slow metabolism and senescence. In addition, symptoms may not be external.

The three main factors determining the extent of injury are: (i) temperature; (ii) duration of exposure; and (iii) plant species. With tropical species, a shorter duration at a higher temperature can lead to substantial injury compared with more temperate crops. Temperate crops can be exposed to lower

temperatures for a longer period before injury will occur. If the intensity of exposure (temperature × exposure time) is low, the damage may be reversible, while at higher intensities, injury is irreversible. In the case of fruits and vegetables, maturity and ripeness also influence injury.

Symptoms of chilling injury

There are many symptoms of chilling injury. A general discussion with examples for injury associated with specific plant parts follows. For symptoms, remedies, and handling procedures for specific commodities, see Gross *et al*. (2002).

Regardless of organ affected, chilling injury is often accompanied by some general symptoms. Desiccation is a classic symptom of chilling injury. This is the direct result of membrane injury affecting its permeability. Chilling injury often alters the lipids in the membrane bilayer which renders it unable to regulate water diffusion out of the cell. Discoloration, water-soaking or pitting is often externally visible. Internal tissues often appear discolored and disorganized, with unacceptable texture or flavor. Injured tissues often experience accelerated senescence and increased decay from microorganisms. Shelf life is often greatly reduced in chilled commodities and they may fail to ripen normally after removal from storage.

FRUITS (BOTANIC) Fruits, in the botanical rather than horticultural context, may suffer substantial chilling injury and a wide variety of symptoms develop. In thick-skinned fruit such as citrus (*Citrus* spp.) and cucumbers (*Cucumis sativus*), sunken areas of discoloration or pits appear on the surface of the skin. In fruit with thinner skins, the symptoms appear as water-soaked areas.

In apples (*Malus domestica*), chilling injury results in low temperature breakdown, brown core, and internal browning. All disorders tend to become more severe with longer storage times. In addition, a cold, wet growing season tends to make susceptible cultivars even more susceptible to chilling injury during storage. Low temperature breakdown is observed as brown discoloration of vascular bundles, flesh browning, and a ring of unaffected tissue just underneath the skin. Brown core is another malady resulting in browned flesh beginning near the core and advancing through the cortex. It is often hard to distinguish between low temperature breakdown and brown core. Internal browning is not browning per se, but rather a distinct graying of the flesh.

Some fruit suffer chilling injury within a very specific temperature range. Apricots (*Prunus armeniaca*) experience 'gel breakdown' when exposed to temperatures between 2 and 8°C, which leads to the formation of water-soaked areas in the flesh that quickly turn brown and become spongy or gel-like. If stored between 0 and 2°C, gel breakdown does not occur.

Avocados (*Persea americana*) can suffer two different types of chilling injury, each caused by different temperatures. Internal chilling injury is caused by exposure to temperatures around 6°C and is characterized by gray-brown discoloration of flesh at the base of the fruit (opposite the stem end) around the seed. External chilling injury, which occurs at temperatures below 3°C, appears as dark, patchy discoloration of the skin.

Banana and plantain (*Musa* sp.) fruit are susceptible to chilling injury at temperatures as warm as 13°C. Symptoms appear on the peel as brown or black streaks along with a grayish cast to the flesh. Fruit may also fail to ripen properly.

Green beans (*P. vulgaris*) experience chilling injury if stored at 5°C or lower. The general symptoms include a general opaque discoloration of the entire bean and sometimes pitting on the surface. At slightly warmer temperatures (5–8°C) rust-colored lesions which are very susceptible to pathogen attack appear on the surface. If held at warmer temperatures to avoid chilling injury, undesirable seed development, yellowing, and desiccation can occur.

ROOTS Jicama (*Pachyrhizus erosus*) roots are crisp, white, and somewhat sweet tasting. Chilling injury may develop after storage at 10°C or lower for as little as 1 week but no injury is observed at roots stored at 12.5°C. Externally, injury appears as decay with flesh discoloration and loss of crispness appearing internally. Roots may even become rubbery in severe cases.

LEAVES AND STEMS Basil (*Ocimum basilicum* L.) is a culinary herb with extreme sensitivity to chilling. If exposed to temperatures lower than about 12°C, severe blackening and necrosis of leaves and stems will occur.

While not necessarily considered a form of injury, potatoes (*Solanum tuberosum*) are very sensitive to storage temperature with profound changes in starch and sugar ratios occurring with

changes in temperature. This can have major effects on the suitability of the tubers for different culinary uses. After harvest, potatoes are cured at 20°C for 1 or 2 weeks to stimulate suberization, wound healing, and to reduce respiration. If cured, potatoes can be stored for up to 1 year. At temperatures above freezing but below 10°C, starch in the tuber is readily converted to sugars which may give the potato an unpleasantly sweet taste and darken during cooking. Tubers for fries or chips are stored at 10–15°C, depending on cultivar. Many 'chipping' cultivars will accumulate excess sugar if stored at <15°C and these sugars readily burn during frying. Most chipping cultivars are stored at 15–20°C which makes them more susceptible to decay and attack by pathogens. Besides irreversible changes in carbohydrate composition at temperatures below 10°C, a mahogany discoloration of the flesh can occur with exposures to 1 or 2°C for prolonged periods.

Asparagus (*Asparagus officinalis*) is harvested for its succulent stems (spears) just as they emerge from the soil. Spears lose their bright and shiny appearance and spear tips become gray after about 10 days at 0°C. Dark spots or streaking near spear tips may occur under severe chilling conditions.

Ginger (*Zingiber officinale*) harvested for its pungent rhizome may lose skin color and experience pitting of skin if held below 12°C. Internal breakdown may also occur.

FLOWERS Flowers with a tropical origin such as *Anthurium*, bird of paradise (*Strelitzia* spp.) and ginger (*Z. officinale*) may suffer chilling injury at temperatures below 10°C. The symptoms of injury include water-soaked petals followed by blackened leaves and petals. A major exception to this general rule is orchids (family *Orchidaceae*). Many orchids are not sensitive to chilling injury and they can be safely stored between 0 and 12.5°C, depending on cultivar.

FOLIAGE PLANTS Many foliage plants are injured by temperatures below 15–18°C. To prolong usefulness, many foliage plants are shipped at 10–13°C, but this is the absolute lowest that foliage specimens should experience as some injury may occur at these temperatures. Many growers acclimate plants before shipping by reducing water, light, fertilizer, and temperature for 2–4 weeks before shipment. This makes the specimens more tolerant of environmental stresses likely to be encountered on their journey from grower to consumer.

FLOWERING POTTED PLANTS Chilling-sensitive potted flowering plants that are injured below 10–15°C include African violet (*Saintpaulia* spp.), *Bougainvillea, Browallia*, Christmas cactus (*Schlumbergera*), *Clereodendron, Crossandra, Cymbidium*, Easter cactus (*Hatiora gaertneri*), *Exacum, Gloxinia, Hibiscus*, poinsettia (*E. pulcherrima*), and *Streptocarpus*.

BEDDING PLANTS AND SEEDLINGS Vegetable and flower bedding plants are often subjected to stressful conditions during transport and storage at retail outlets. Two types of plants are often considered for marketing: (i) plugs; and (ii) finished plants. Plugs are more compact and are easier to ship and store than finished plants while finished plants are often hardier than plugs. Injury to plugs or plants may include minor cosmetic injury, delayed or advanced flowering, stunted growth or even death. There are three general groups with respect to chilling sensitivity:

- The most sensitive species should never experience temperatures below 15°C including balsam (family *Balsaminaceae*), fibrous begonia (*Begonia* × *semperflorens-cultorum*), *Celosia*, celery (*Apium graveolens*), coleus (*Solenostemon*), cucumber (*C. sativus*), eggplant (*Solanum melongena*), *Kochia*, muskmelon (*Cucumis melo*), pepper (*Capsicum annuum*), pumpkin (*Cucumis* spp.), squash (*Cucumis* spp.), tomato (*S. lycopersicum*), *Vinca rosea*, watermelon (*Citrullus lanatus*), and *Zinnia*.
- Moderately sensitive species should never go below 10–13°C including *Ageratum*, aster (family *Asteraceae*), broccoli (*Brassica oleracea* Italica Group), *Browallia*, Brussels sprouts (*Brassica oleracea* Gemmifera Group), cabbage (*Brassica oleracea* Capitata Group), Cauliflower (*Brassica oleracea* Botrytis Group), collards (*Brassica oleracea* Acephala Group), *Centaurea cyanus, Dahlia, Dianthus*, dusty miller (*Centaurea cineraria*), geranium (*Pelargonium*), *Impatiens*, lettuce (*L. sativa*), marigold (*Tagetes* spp.), *Nierembergia*, onion (*Allium cepa*), *Petunia, Phlox, Portulaca, Salvia*, and *Verbena*.
- Relatively insensitive species can be subjected to temperatures as low as 7–10°C without injury, including alyssum (*Lobularia maritima*),

Calendula, carnation (*Dianthus caryophyllus*), larkspur (*Delphinium*), *Lobelia*, pansy (*Viola* × *wittrockiana*), and snapdragon (tall and dwarf; *Antirrhinum*).

SEEDS AND POLLEN Seeds of many tropical species are chilling sensitive and should be kept at a temperature above 15°C. There are no reports of direct chilling injury to pollen. Keep in mind that most species have an optimum temperature range for pollen germination and growth and low temperatures may reduce pollination and fertilization.

Avoiding chilling injury

Obviously the best protection against chilling injury is avoidance of exposure to inappropriate temperatures. Unfortunately the temperature to which commodities are exposed are not always under our control. In the field, nature controls the thermostat. In transit and storage we control the temperature, however, faulty equipment and human inefficiency may lead to exposure of commodities to injurious temperatures.

There are some approaches to reducing chilling injury. Some approaches are universal, others are crop specific. Consult a good reference such as Gross *et al*. (2004t) for specifics. When selecting cultivars for production, investigate whether or not chilling-resistant cultivars are available and whether there are production practices, such as calcium treatments to stabilize membranes, available that might help avoid injury. If chilling conditions occur, minimize how long a product is exposed to the chilling temperature. Also, in some crops preconditioning consisting of stepwise cooling can allow the commodity to adapt to cooler and cooler temperatures, minimizing injury. Intermittent warming of some commodities allows for the metabolic removal of toxins which can reduce injury. Care must be used with intermittent warming procedures since premature softening may occur and decay may increase due to moisture condensation on the product. If possible, try to store fruit that is more ripe, since it often is less susceptible to chilling injury. Controlled atmosphere storage may eliminate adverse effects on product metabolism by slowing metabolic processes down via decreased oxygen availability, for example in nectarines and peaches (*Prunus persica*), okra (*Abelmoschus esculentus*), and avocado (*P. americana*). Be careful with controlled atmosphere storage since some commodities may experience increased chilling injury under controlled atmosphere conditions, for example cucumbers (*C. sativus*), tomatoes (*S. lycopersicum*), asparagus (*A. officinalis*), and citrus (*Citrus* spp.).

Freezing injury

In the previous section plant injury caused by low but above-freezing temperatures was examined. In this section, injury caused by exposure to temperatures at or below 0°C will be explored. Many species are not able to tolerate any exposure to freezing temperatures while others are quite resistant to freezing injury. Still other species exhibit a remarkable avoidance mechanism, supercooling, to avoid injury.

Frosts versus freezes

Freezing injury to plants can be caused by either frosts or freezes. Many people do not make a distinction between the two, since they both cause similar damage. However, the differences between the two are important since whether or not protective measures to avoid injury are successful depend on which type of episode occurs (Perry, 1998).

Radiational frost

A radiational frost is generated locally, on site by cold air generation through radiational cooling. Radiational frosts occur under clear, calm conditions usually during the fall and spring. The depth of the layer of cold air in the atmosphere usually ranges from as little as 10 m up to 60 m. Cold air may drain to lower regions in a field and frost pockets may develop. Generally, the dew point temperature (the temperature at which the air becomes saturated with water and it begins to condense out of the air) is low during a radiational frost. If enough moisture is in the air, visible hoar frost will occur, if not, a black frost (no visible ice crystals) will be seen. Radiational frosts can occur even if the air temperature is above freezing. Object temperatures regulate whether or not frost forms, not the air temperature. With a radiational frost, there is a good chance for successful crop protection.

Advective freeze

An advective freeze is caused by the movement of cold air at or below freezing into a region. It is

often accompanied by windy conditions with or without clouds. The depth of cold air in the atmosphere may range from 150 to 1500 m with no warm air inversion. Cold air drainage does not occur and no frost forms. Crop protection options are extremely limited with an advective freeze.

Evaluating freezing injury

In order to understand the horticultural implications of frost or freezing injury, methods for evaluating injury caused by such stresses have been utilized for many years. Generally tissues are divided into vegetative tissue or reproductive tissue (flower buds, flowers, and young fruit) for damage assessment.

VEGETATIVE TISSUE Visual evaluation of freezing injury to vegetative tissue is relatively easy to do and requires no special equipment. The vascular cambium is the tissue most often evaluated in vegetative tissues. The cambium is evaluated for browning either immediately or 1–2 weeks after the freezing event. The tissue is cut with a razor blade and observations made ranking any injury as none (no tissue browning) to severe (most tissue is brown). It is very subjective, but none the less, it provides a reasonable estimate for the degree of injury.

Another fairly easy assessment of injury involves collecting samples following a freeze, holding them at room temperature for 1–2 weeks and monitoring bud growth. Fewer buds will grow following injury. The assumption must be made that any chilling requirement has been fulfilled prior to forcing after freezing. Late-season assessments can be performed in the field simply by monitoring bud break as growth commences in the spring.

A more rigorous evaluation examines the leaching of electrolytes out of cells following a freezing event. The assumption is made that membrane damage occurs with freezing and the extent of injury can be estimated by how much electrolyte leakage occurs. Non-frozen control samples are handy as a baseline for 'no injury'. Small segments of tissue are incubated in a controlled volume of distilled water for a fixed time at a fixed temperature. The electrical conductivity of the water solution is measured. The same samples are then heat killed in the same water they were incubated in, allowed to incubate for another fixed time and temperature and conductivity again measured. The extent of injury can be expressed as a ratio of conductivity after injury: conductivity after killing and expressed as a percentage.

REPRODUCTIVE TISSUE Evaluation of flower buds or flowers usually involves observing the pistil for signs of injury. In most species, the pistil is either alive or dead, there's no in-between. Thus visual evaluations of pistils following a freeze are very reliable measures of injury. In the bud stage, flowers are cut along the equator and the pistil(s) examined for browning. Live pistils are green. A percentage injury can easily be calculated from pistil counts. Similarly, open flowers can be evaluated, even in the production field. Field assessment of freezing injury is quite common in fruit production regions susceptible to freezing injury. Samples can also be collected and evaluated for growth following a freeze, but visual pistil assessment is quicker and often easier.

SUPERCOOLING AND FREEZING IN PLANTS Supercooled water is water remaining liquid below 0°C. Water droplets in clouds are often supercooled, freezing when they come in contact with aircraft, causing icing. Some plant tissues also contain water capable of supercooling. Water in pistils and xylem ray parenchyma of many species will often supercool, avoiding physical injury caused by ice crystals and the dehydration associated with their extracellular formation. However, if supercooled water in a pistil freezes, it is lethal. This is an important consideration. Supercooling is a freeze-avoidance mechanism, but only to the point at which the supercooled water freezes. The temperature at which supercooled water freezes is called the supercooling point and varies depending on: (i) species; (ii) tissue; (iii) previous air temperatures; and (iv) stage of endodormancy.

It is also important to consider that all pistils, for example, on the same plant or in the same field are not at the exact same stage of development and are not all at the same level of hardiness. If many buds are examined from a population of buds, an average killing temperature could be calculated. This is often expressed as a lethal temperature (LT). LT_n is the temperature needed to kill a specific percentage of the pistils in a population where n is the percentage killed. Values are often calculated for LT_{10}, LT_{50}, and LT_{90}. An LT_{10} would indicate the temperature at which little damage is done, while an LT_{90} indicates the temperature at which severe injury occurs.

In order to understand freezing in plants, an underlying knowledge of water and freezing is necessary. For pure water to freeze its temperature must be at 0°C or lower and a nucleus for ice crystal formation must be present. Nuclei for ice crystal formation might be other ice crystals, dust particles, bacteria with specific proteins on their coats or chemical crystals such as silver iodide. In the absence of a suitable nucleus, water may remain liquid between 0 and –40°C. At –40°C, the spontaneous nucleation temperature of water, water crystallizes whether a nucleator is present or not. Thus water in plant tissues may remain liquid in this temperature range. However, ice crystals form even in supercooled plant tissue at temperatures somewhat warmer than –40°C. The temperature at which the water freezes is called its nucleation temperature.

When a solute such as sugar, salt or protein is dissolved in water, the freezing point of water is lowered in direct relation to solute concentration resulting in freezing point depression. Freezing point depression is not supercooling. The freezing point of water is maximally depressed to –18°C via addition of a solute. Due to concentrations achievable in plant tissues, the freezing point depression observed in plants is generally on the order of 3–5°C, thus most plant tissue freeze at –3 to –5°C. Supercooled plant cells may not freeze until –40°C.

Ice formation in plant cells always begins extracellularly due to the freezing point depression of the cell sap compared with the depression of the extracellular solution. Once formed, extracellular ice draws water out of the cell, concentrating the cell sap even more. In many cases, ice crystals do not puncture the cell membrane, thus the damage done by water freezing is not due to a puncture of the cell membrane. The damage occurs during thawing, as we shall see in a later discussion of acclimation in this chapter.

Supercooling and bud hardiness has been extensively studied in *Prunus*. The water content of the vascular traces and bud primordia and not the whole bud establish the degree of supercooling in *Prunus* flower buds. Other factors such as soluble sugar content (particularly sucrose and sorbitol) are involved since flower buds with similar vascular trace and primordia water content but from different cultivars, do not supercool to the same level (Quamme and Gusta, 1987). The loss of the ability to supercool during the spring is associated with bud development, which is concomitant with increased size, decreased sugar content, and increased water content of the pistil as well as vascular development from the twig to the pistil (Ashworth, 1982, 1984; Ashworth and Rowse 1982; Callan, 1990; Durner, 1990b). As buds exit endodormancy, the ability to supercool is lost. This loss of supercooling ability may be directly related to chilling accumulation and release from endodormancy and not just circumstantially associated with the other changes occurring over time. When CU accumulation is accelerated, so is the rate of loss in the ability to supercool (Callan, 1990). There is a short period during bloom when *Prunus* flowers are frost tolerant, but this period is short, and frost injury can result in significant damage (Andrews *et al*., 1983).

When water freezes, it releases heat that can be directly measured. This property has been used to study freezing in plants with exotherm analysis and differential thermal analysis. Before discussing acclimation and hardiness, let's look at these two methods. Both methods utilize water's heat of fusion for measuring the freezing point of plant tissues during studies of plant cold hardiness.

EXOTHERM ANALYSIS Heat given off during freezing is called an exotherm. When living plant tissues freeze, two freezing events are often detectable via exotherm analysis: (i) the high temperature exotherm(s); and (ii) low temperature exotherm(s). High temperature exotherms are associated with the non-lethal freezing of extracellular water at relatively warm temperatures (0 to –10°C). Low temperature exotherms are associated with the often lethal freezing of supercooled intracellular water at very low temperatures (–10 to –40°C). As previously mentioned, pistils and xylem ray parenchyma are two plant tissues that routinely supercool. The temperature to which they supercool is easily measure by recording tissue temperature over time during a freeze and examining the time-temperature profile for sudden increases in temperature associated with the freezing of water.

The freezing of supercooled water in pistils has been shown to be directly associated with pistil death in many species, including peach (*P. persica*), apricot (*P. armeniaca*), cherry (*Prunus avium*), and *Forsythia*. By analyzing a sample of flower buds during controlled freezing, a clear assessment of the average lethal temperature for buds can be made at any time during the winter. In this way, the effect of

specific horticultural practices on hardiness can be evaluated quite accurately. Do not merely assume that low temperature exotherms are directly linked to tissue death if studies have not been undertaken to establish the fact. Substantial work is required to link the two events. Also, samples must be evaluated for the number of living pistils prior to testing, since dead flower buds may supercool for several weeks after they are dead (Kadir and Proebsting, 1993).

Exotherm analysis is often performed on woody tissue to examine the lethal temperature for xylem ray parenchyma as death of these cells is often correlated with significant injury.

DIFFERENTIAL THERMAL ANALYSIS Differential thermal analysis is similar to exotherm analysis except that the freezing curve of the live sample is compared to a freezing curve for a known dead sample, hence the term differential.

Dormancy, Acclimation, and Hardiness

The ability of plant tissue(s) to resist or tolerate exposure to low temperatures without injury is given the general term 'hardiness'. While some summer annual plants may have a certain amount of hardiness associated with different stages of their growth, hardiness is of particular interest for individuals concerned with winter annuals, biennial, and perennial plants. In this section the process of acclimation, the physiology of cold hardiness and their interaction with dormancy will be explored.

Seasonal aspects of hardiness in plants

As days become shorter and cooler in the fall, winter annuals, biennial, and perennial plants in temperate regions often acquire a resistance to low temperature injury. This process, called acclimation, is linked to another previously discussed survival mechanism, dormancy. The daylength and temperature triggers change a plant's physiology rendering it less susceptible to low temperature injury. Some of the changes include: (i) increased carbohydrate content; (ii) reduced water content; and (iii) alterations to lipid composition of membranes. Changes in membrane lipid composition are accompanied by changes in membrane sugar and water content as well. These changes during acclimation allow the membrane to recover during rehydration following the dehydration associated with freezing. In non-acclimated plants, these membrane alterations do not occur and membranes are not able to accommodate the rehydration during thawing (Gordon-Kamm and Steponkus, 1984). Thus it is normally the rehydration upon thawing that causes the observable damage of a freezing event.

The process of acclimation is a lengthy one that starts in the fall in response to shortened days and cooler temperatures. The combination of the two triggers is advantageous in that daylength changes are constant from year to year while temperature decreases in the fall are often unpredictable and vary from year to year. Even during an unusually warm fall, acclimation will commence due to the photoperiodic signal. Hardiness will continue to increase over time until a plant has reached its minimum hardiness level (MHL). Once the MHL has been reached, that species will be at least that hardy until dormancy ends. Exposure to cold temperatures normally enhances hardiness beyond the MHL. This gained hardiness may be lost with subsequent exposure to warm temperatures, but the level of hardiness will not be less than the MHL regardless of temperature (Fig. 9.5). Once the end of dormancy is reached, hardiness fluctuates with air temperature and may be substantially less than the MHL after even short exposure to warm temperatures. Reacclimation can occur with exposure to cold temperatures. As dormancy release progresses, most plants' ability to reacclimate is inhibited more and more until reacclimation no longer occurs once dormancy release is complete.

Many plants that acclimate in the fall remain quite hardy and resistant to temperature changes during the winter until endodormancy is broken. Part of the acclimation process intimately linked to a plant's tolerance of low temperature is its ability to tolerate the desiccation associated with freezing in plant tissues. In fact, the difference between acclimated and non-acclimated plants is often a difference in desiccation tolerance. Once enough chilling has accumulated to release the plant from endodormancy, plant hardiness can fluctuate substantially with prevailing air temperature. Loss of hardiness is called deacclimation. Deacclimation is primarily regulated by prevailing air temperature. Many species retain an ability to reacclimate with environmental conditions, particularly with re-exposure to colder temperatures. On the other hand, most species reach a point in their development during the late winter and early spring where they are no longer able to reacclimate. After this

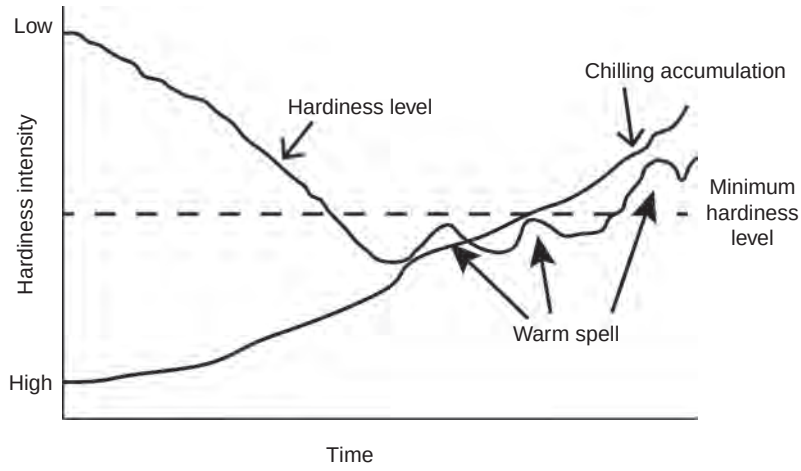

Fig. 9.5. Fluctuations in hardiness in response to prevailing air temperature.

point, exposure to low temperatures is often lethal to at least some part of the plant.

General hardiness of all species is important, however, hardiness of flower buds, particularly those of biennial and perennial fruit crop species, is of tremendous economic importance. Hardiness during dormancy through to full bloom can greatly impact yield in many years when plants are subjected to extremely low temperatures during endodormancy or to frost and freeze events during bloom. The timing of low temperature stress is important. Little injury occurs if the stress occurs when buds or flowers are hardy. However, if it occurs following deacclimation in late winter or spring, results are disastrous.

Maximum acclimation normally requires several weeks to months to occur. Deacclimation may occur within hours of exposure to warm temperatures. In many species, deacclimation proceeds rapidly upon initial exposure to warm temperatures but the rate of deacclimation declines with time. On the other hand, some species deacclimate at a fairly constant rate over time. Reacclimation normally requires substantially longer exposure to cold temperatures, days not hours, to reach the level of acclimation the plant was at prior to deacclimation. The differences in rates of acclimation compared with deacclimation suggest that acclimation and reacclimation require substantial up-regulation of genes for the synthesis of metabolic materials required for the processes (such as sugars, different lipids) while deacclimation results from down-regulation of genes and requires little in the way of metabolites or metabolic energy. Reacclimation is more rapid than initial acclimation probably due to a lack of total deacclimation to pre-acclimation levels. In other words, plants don't deharden all the way upon deacclimation, thus they don't have to start at the very beginning as with initial acclimation. A plant's capacity to reacclimate depends on its previous acclimation and deacclimation history. Naturally acclimated plants may not respond with respect to deacclimation and reacclimation the same as artificially acclimated plants. This indicates that the environment of acclimation directly affects deacclimation and reacclimation more than simply the degree to which a plant is hardened.

Midwinter hardiness and resistance to deacclimation are often independent attributes. A species that is very hardy (*Vitus labrusca*) may deacclimate more rapidly than a less hardy similar species (*Vitus vinifera*) when exposed to warm temperatures. On the other hand, maximum hardiness in rabbiteye blueberries (*Vaccinium ashei*) is observed in cultivars with the greatest deacclimation resistance. Similarly, plants that acclimate more rapidly may not necessarily become hardier than those that acclimate more slowly. It seems that plants that are most resistant to deacclimation gained this capacity through evolution in a climate with large temperature changes during the dormant season (Kalberer *et al*., 2006). Those species with a fairly constant rate of deacclimation evolved in a climate with fairly consistent temperatures during dormancy. Another possibility is that deacclimation resistance

is highest in plants that develop later in the spring or have a deeper ecodormancy following release from endodormancy.

Several mechanisms for cold hardiness are apparent in apple (*M. domestica*). Xylem ray parenchyma and pit tissue avoid freezing injury by supercooling while bark and flower buds do not supercool (Ashworth *et al.*, 1988). Keep in mind that the tissues that avoid injury by supercooling only do so if the temperature does not go below the temperature to which they will supercool. If the temperature goes below this, supercooled water will freeze and injury will result.

Since apple buds do not supercool, they must avoid injury due to desiccation. Hardy buds have a reduced cellular water content, yet somehow maintain enough intracellular water to avoid desiccation injury. Another piece of evidence showing how intimately endodormancy and cold hardiness are linked is gleaned from the fact that during endodormancy, much of the water in buds is bound to other molecules. This bound water helps prevent desiccation injury. As endodormancy is released by chilling, more and more of this bound water is released, probably for metabolic use. As more of the water in a bud becomes free, the bud becomes more susceptible to freezing injury.

As apple cortical cells acclimate, the volume of cytoplasm in the cells increases along with the transient increase in the number of organelles needed for protein synthesis. Starch granules disappear during acclimation, probably being converted into soluble sugars for their cryoprotective effect. Hardiness of apple shoots and buds has been positively correlated with high levels of sorbitol and raffinose family oligosaccharides. Activity of plasma-membrane-associated ATPase increases during acclimation corresponding to the increased metabolic energy requirements associated with acclimation.

When warm temperatures induce growth along with deacclimation, the deacclimation is usually irreversible. This may be due to a reallocation of metabolites away from rehardening towards growth, as well as changes in cell water content and cell structure associated with tissue growth. It could also be due to changes in gene expression involved in growth rather than metabolite and water re-allocation during growth.

Deacclimation is accelerated under long days either through a photoperiodic (relying on responses to phytochrome) or photosynthetic enhancement of growth under long days (Kalberer *et al.*, 2006). During deacclimation, water often moves from frost-tolerant tissues into frost-sensitive tissue. An example of this is observed in *Prunus* (Ashworth, 1992). As flower buds deacclimate, water moves from bud scales into the pistil, rendering the pistil more susceptible to injury. If reacclimation occurs, water moves from the pistil back out to the bud scales, thereby increasing the pistil hardiness. Concomitant with increased water content of susceptible tissues during dehardening is a reduction in soluble sugars, particularly in the raffinose family (stachyose, raffinose). These sugars probably protect membranes from dehydration stress during freezing, thus their reduction during deacclimation is likely to render membranes more susceptible to desiccation stress. However, changes in sugar content may or may not be associated with changes in hardiness. They may simply change due to enzyme activity and its sensitivity to temperature. A specific group of proteins called dehydrins is known to accumulate with acclimation and decline with deacclimation (Kalberer *et al.*, 2006). Dehydrins protect cellular components from the desiccation damage caused by freezing. Blueberry flower buds exhibit an accumulation of dehydrin proteins during acclimation (Muthalif and Rowland, 1994).

Horticultural practices that influence hardiness

Many horticultural practices influence the level of winter injury (Fig. 9.6). In this section, some of these are briefly examined and discussed.

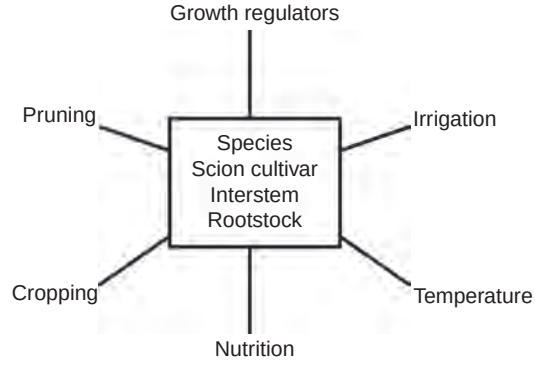

Fig. 9.6. Factors affecting plant cold hardiness.

Pruning

Late summer or fall pruning may stimulate growth of ectodormant buds. Shoots arising from these buds are likely to be very intolerant of low temperatures and significant damage may result. Additionally, active growth may delay acclimation making the entire plant more susceptible to freezing injury.

Pruning stimulates bud growth during endodormancy. Dormant pruning reduces peach flower-bud hardiness by stimulating bud development. Increased bud development leads to increased dehardening during warm weather and reduced rehardening with the return of cold weather (Durner, 1990b, 1995).

Dormant pruning of ornamental specimens and fruit crops is postponed until as late in the winter or early spring as possible in order to assess the amount of winter injury present before pruning cuts are made. In this way, damaged tissue can be removed first, followed by adjustment of specimen form. Additionally, an assessment of potential crop load is made for tree fruit crops by examining flower buds for injury. Since one of the purposes of pruning tree fruits is to reduce excessive crop load, it is important to know what the potential crop load is before pruning commences. In fruit crops particularly susceptible to frost injury, such as peaches and apricots, pruning is often delayed as far into the spring as possible to wait for any 'natural thinning' by frosts to occur before buds are removed with pruning. Unfortunately, growers often have to prune earlier than desired due to the volume of trees that must be pruned in the spring.

Rootstock

Tree fruit crop production often utilizes rootstocks, separate genetically distinct tissues to which the desired cultivar is grafted. Rootstocks influence scion flower-bud hardiness in a number of ways (Brown and Cummins, 1988; Durner and Rooney, 1988; Durner, 1990a; Durner and Goffreda, 1992; Westwood, 1993). Rootstocks can exert a direct influence on flower-bud cold hardiness as hardiness of buds of the same cultivar differ with rootstock after test winters or controlled freezing experiments. Rootstocks also influence the time and rate of bloom in the spring. Buds on rootstocks that induce earlier bloom are more likely to be injured by spring frosts compared with those on rootstocks that induce a delay in bloom. The difference in time of bloom is probably related to differences in chilling and heat requirements of both the rootstock and the scion. The scion may also directly influence rootstock hardiness (Kalberer *et al*., 2006).

Irrigation

Water stress and low temperature stress are related. Conditions that induce water stress are likely to lead to enhanced low temperature stress resistance, if the plant survives the water stress. Plants subjected to water stress during the growing season are often observed to have greater winter hardiness than similar plants that received adequate or excessive water during the growing season.

One method of avoiding injury from frost is by delaying bloom with evaporative cooling using overhead irrigation or under-tree misters. As water that has been applied to trees evaporates, energy is consumed from the surrounding buds with a concomitant decrease in heat units accumulated. In general this method is expensive, requires just the right conditions for use, and often reduces the intrinsic hardiness of buds and flowers, even though their development is delayed (Bauer *et al*., 1976; Strang *et al*., 1980).

Nutrition

Excessive or late-season fertilization can lead to the stimulation of succulent growth which in turn leads to reduced cold hardiness.

Cropping

Since tissue carbohydrate content influences cold hardiness, it is not surprising that crop load can influence cold hardiness (Byers and Marini, 1994). Excessive cropping leads to a drain on the carbohydrate supply of the plant, leaving less available for tissues remaining on the plant after harvest. This carbohydrate deficit leads to reduced cold hardiness of buds and twigs. Light crop loads do not remove excessive amounts of carbohydrates, leaving adequate reserves for cold hardiness to develop in tissue remaining after harvest.

Growth regulators and protectant sprays

Sprays of various materials such as polyvinylpyrolidone K-30, glycerol, ethylene glycol and dimethyl sulfoxide, calcium cyanamid, Frost-Free® (Plant Products, Vero Beach, Florida), and Vapor Gard®

(Miller Chemical & Fertilizer, Hanover, Pennsylvania) have been evaluated for increasing frost resistance or delaying bloom of many tree fruit species. In general, most are ineffective at best and some actually render buds and flowers more susceptible to frost injury (Ketchie and Murren, 1976; Durner and Gianfagna, 1988; Aoun *et al*., 1993).

Probably the most documented and effective treatment to reduce frost injury is the fall application of the growth regulator ethephon to *Prunus* species, particularly peach. A fall application of ethylene increases peach pistil hardiness, delays bloom, and increases yield (Durner and Gianfagna, 1988). Ethephon works by prolonging dormancy and increasing the chilling requirement which delays deacclimation and bloom (Durner and Gianfagna, 1991a). In addition, ethephon-treated pistils exhibit enhanced supercooling and an increased number of pistils that supercooled after deacclimation. Concomitant with this enhanced hardiness were: (i) smaller pistils; (ii) decreased pistil water content; (iii) increased pistil sucrose and sorbitol content; and (iv) slower growth rates during bloom (Durner and Gianfagna, 1991b).

Whitewashing trees delays bloom slightly, but not enough to make it an economic option (Durner and Gianfagna, 1990, 1992). Pre-bloom application of dormant oil may be used in an attempt to reduce heat accumulation and oxygen entry into buds and thereby delay bloom. Such an application may delay bloom but it decreases blossom hardiness which may result in decreased yield (Durner and Gianfagna, 1992).

Crop Protection from Frosts and Freezes

Before embarking on an attempt to alleviate or avoid injury from frosts, it is wise to ascertain whether or not frost protection is feasible and worth the effort. Usually with an advective freeze, protective measures to avoid injury are quite limited. This is especially true if the advective freeze is accompanied by windy conditions (and most advective freezes are). The chances of protecting crops from injury are much better with radiational frosts. Besides the type of freezing event anticipated, consideration must be given to: (i) the specific crop; (ii) its stage of development; (iii) its hardiness characteristics; and (iv) the economic implications involved in a protective attempt. It makes no sense to spend more money than a crop is worth in trying to protect it.

In considering protective measures, two major methods for protection are available, passive and active protective measures. Passive methods are preventative measures taken long before a frost is at hand and involves mostly relatively low-cost, biological and ecological measures that may help reduce the need for active measures. Passive measures include such things as: (i) site and cultivar selection; (ii) cold air drainage management; (iii) plant nutrition; (iv) pruning; (v) methods to delay bloom; (vi) plant covers; (vii) irrigation; and (viii) tree-trunk painting or wrapping. Active measures are temporary and immediate, physical, energy-requiring methods used to replace the energy lost during the frost event and are often expensive and labor intensive. Active measures include: (i) heaters; (ii) wind machines; (iii) helicopters; and (iv) overhead sprinkler irrigation.

Passive methods of frost protection

Site selection

The most fundamental measure one can take to avoid injury from frosts is site selection. If the crop is already selected and a choice of site is possible, an appropriate site can almost always be found with enough investigation. When searching for a production site, talk to local agricultural advisors and growers for their input. When the site is 'preselected', these same individuals can help guide the selection of crop, cultivar, and production methods.

The topography of any site should be evaluated for potential frost pockets and low areas where cold air might drain. This is true at both a regional and a local scale. Regionally, valleys are usually colder than elevated sites, especially during the frost season. Locally, any low spot has the potential for being a frost pocket. An easy way to identify potential frost pockets is to look for locations where ground fog readily forms. Ground fogs due to radiational cooling are most prevalent in the fall. Elevated sites or sites with slopes not directly facing the sun delay spring growth reducing the chance of frost damage.

An evaluation of the soil and its drainage characteristics should be conducted. Sandy, dry soils hold less heat than clayey, wetter soils and therefore crops grown on them are more prone to radiational frosts. Organic or peat soils hold less heat, wet or dry, than either sandy or clay soils, and are therefore not a good choice if frost-sensitive crops are planned for the site.

If weather records are available for the site, they should be reviewed for evidence of frosts that may impact production. If site records are not available, regional climate data are normally always available from the local climate office or from an Internet source. In addition, anecdotal evidence may be gleaned from other growers in the area with regard to frost potential.

Sites where cold air will drain either on a local or regional scale should be avoided even if an effective screen to cold air drainage such as a wooden fence or dense planting to protect the field is planned. Cold air will pool behind the screen which would protect the production field. However, adjacent land parcels are bought and sold, buildings and roads built and demolished, thus the air drainage around any site is subject to change.

Crop selection

Only crops adapted to a specific site should be cultivated if successful production is expected. If inappropriate crops or cultivars are selected for a susceptible site, most attempts to avoid frost injury will be futile. Besides selecting spring hardy crops, care must be given to ensure that the growing season is long enough to allow the crop to mature to reach the point of harvest subsequent to plant acclimation. For example, 'Granny Smith' apple (195 days to maturity) is not an appropriate cultivar in a region with a growing season of 170 days while 'Spur Red Delicious' (150 days to maturity) is a totally acceptable choice.

Canopy trees

Planting an overstory tree is an effective way to provide a small degree of frost protection to the understory crop. Citrus growers in Southern California often interplant date palms (*Phoenix dactylifera*) as an overstory to the *Citrus* crop (Snyder *et al*., 2005). The date trees help hold in some of the heat radiating out into space and the chance of frost is reduced. Several other examples include an overstory of pine trees (*Pinus* spp.) for the satsuma mandarin (*Citrus unshiu*) crop in Alabama and shade tree overstories for coffee (*Coffea arabica*) in Brazil.

Plant health

While it may sound like common sense, healthy plants are more resistant to frost than unhealthy ones. Proper care throughout the rest of the year may add some resistance to frost in the spring. Proper nutrient management, pruning, irrigation, and pest control all support healthy plants. Another aspect of this is that frost-injured plants are often more susceptible to pest pressure, thus turning the problem into a vicious circle.

In general, excessive nitrogen increases plant susceptibility to frost injury by enhancing the growth of succulent tissue. In fact, any management practice that encourages new growth late in the season should be avoided, as new growth is likely to acclimate to a lesser degree than older growth. Two other nutrients often cited for improving frost resistance are phosphorus and potassium. However, there is no clear cut evidence that either improves frost resistance other than that exhibited to well-grown, healthy specimens.

Bloom delay

Evaporative cooling from overhead irrigation can be used to retard the accumulation of heat units in the late winter and early spring, delay bloom and thereby reduce the chances of frost injury. Since the chances of a spring frost decline rapidly over time, even a delay of several days might make the difference between no crop and a full crop. This method of bloom delay depends substantially on the weather in that air is only cooled to its dew point temperature, thus in a humid climate, little cooling below the air temperature might be accomplished with this method. During warm springs in a drier climate delays of up to 2 weeks can be achieved. While this method is included under passive measures, it actually requires substantial grower commitment of time, energy, and money to implement such a venture.

Fall applications of the growth regulator ethephon can delay bloom in peach (*P. persica*) and cherry (*P. avium*) by up to 2 weeks in a cool spring and for several days in a warm spring. An additional benefit of fall-applied ethephon is an increase in the intrinsic hardiness of peach and cherry flower buds both in the winter and during bloom.

Row covers

Synthetic row covers made of spun-bonded polypropylene are often used to increase downward long-wave radiation at night to protect from frost (Fig. 9.7). These covers are lightweight, opaque to

Fig. 9.7. A row cover such as this provides several degrees of protection against frost injury for sensitive species. The amount of protection is determined by weight of the row cover, with heavier weights providing greater protection.

long-wave radiation and allow air, water, and light to pass freely. However, they are expensive and are easily blown about even in light wind and must be secured with sandbags or other heavy weights. Straw offers an alternative; however, it must be removed to allow light exposure. Many other forms of covers are utilized depending on region and availability. Many are relatively inexpensive, however, all materials including straw and synthetic row covers require substantial labor for installation and removal.

Soil cultivation, moisture, and row middle management

When a frost is anticipated, soil should not be cultivated to maximize heat retention during the day and heat transfer to crops at night. Cultivated soil is aerated and holds less heat than non-cultivated soil. If possible, soil should be irrigated prior to a frost event. Water holds a tremendous amount of heat, thus a wet soil will store more heat during the day than a dry soil. That stored heat can then be released at night, reducing the chances of frost injury. Row middles should be mown as short as possible prior to a frost. Excess vegetation reflects solar energy and increases removal of soil moisture via transpiration. Thus crops with row middles of longer vegetation are more likely to suffer from frost injury than crops with short row middles. Grasses and weeds often have a high population of ice nucleation-active (INA) bacteria associated with them, thus mowing overgrown middles might remove some of the INA bacteria. However, bacteria will still be on the mowing litter and many frost-prone species have intrinsic nucleating agents in them. Thus reducing INA bacteria populations does not guarantee a reduced risk of frost. Removing the entire row middle down to bare soil might reduce the chances of spring frost but would make the field more prone to wind and water erosion.

By covering the soil surface with plastic sheeting heat storage during the day is increased. Clear plastic is a better choice than black plastic as more energy is stored in the soil under clear plastic. Wetting the soil before plastic application also improves heat storage. Organic mulches should not be used as they reduce the transfer of solar energy to the soil. However, midwinter organic mulching may provide protection from soil heaving.

Tree-trunk painting

The trunks of deciduous trees are often covered with a wrap or white latex paint to reduce temperature fluctuations on sunny winter days. There can be as much as a 20°C difference in trunk versus air temperature on a sunny, cold day. If the sun is suddenly blocked by clouds, trunk temperature may plummet and bark cracking can occur. Cracked trunks expose tissue to disease and insect pests, as well as creating a wound that the plant must heal. Trunk painting or wrapping has also been reported to delay bloom in apples (*M. domestica*) by a few days.

Insulated trunk wraps are often used in *Citrus* production to provide as much as 8°C protection to injury to young trunks from frosts. It is critical that the air spaces in the wrap are not filled with water during irrigation or rain, as they will lose their protective nature and may also increase the chances of injury when wet. Soil mounding can be used to protect young trunks from injury; however, the level of protection soil mounds afford is quite variable. In addition, disease pressure is often higher with soil mounds compared with trunk wraps.

Controlling INA bacteria

As previously mentioned, many plants have intrinsic ice nucleators, thus controlling populations of

INA bacteria does not guarantee reduced chances of frost injury. The main INA bacteria are *Pseudomonas syringae*, *Erwinia herbicola* and *Pseudomonas fluorescens* (Lindow, 1983). In crops that do not necessarily have internal ice nucleators present, controlling populations of INA bacteria may afford some protection from frost. Populations of INA bacteria can be reduced with bacteriocides or by increasing pressure from enhanced populations of non-INA bacteria. Again, this type of frost control is expensive.

Frost-protecting sprays

While there are many commercially available sprays that claim to reduce injury from frost, no reputable reports exist confirming their effectiveness. In particular, chemicals which reportedly reduce frost injury by preventing desiccation ignore the fact that injury is from the failure of desiccated cells to rehydrate upon thawing, not from transpiration-induced desiccation. In fact, chemicals that protect against freezing often render blossoms more susceptible to freezing injury.

Active methods of frost protection

Some of the methods listed under passive methods of frost protection could be included in this section, especially those requiring substantial labor and money investments. However, this section will be limited to those methods employed on the night of a frost in an attempt to reduce or avoid frost injury.

Wind machines and helicopters

If a radiational frost is the result of a loss of radiant heat from the earth to the atmosphere, what better way to reverse the situation than by bringing some of that lost heat back to earth? This is precisely what wind machines and helicopters do.

Wind machines are essentially large fans operating at about 600 rpm with two or four 3–6 m blades mounted on a tower 10 m above the field floor that rotate around the tower every 5 min while drawing air aloft towards the surface at about a 7° angle (almost horizontal). In order to be effective, the fan must have warmer air (i.e. an inversion) to draw towards the surface. Besides drawing warm air in from aloft, wind machines can blow colder air out of low spots in the field. Wind machines are environmentally friendly except for the noise pollution they cause.

Helicopters push warm air aloft towards the surface, but only if there is an inversion present. They need to fly over the field under protection once every 30–60 min to be effective. In general, helicopters can increase the surface air temperature by about 4°C with a strong inversion. Helicopters are expensive to use and are normally only used in emergencies.

Heaters

Since frosts are the result of a loss in heat energy, one way to combat their occurrence is by replacing the lost energy through field heaters. The heat released by burning any one of a number of different fuels will directly heat plants through radiant transfer and also heat the surrounding air by conduction and convection. For heaters to be effective the night must be calm with an inversion layer above the field. Heaters will often be used in combination with wind machines or helicopters to stir the heated air up and to force it back to the proximity of the crop after it rises in the atmosphere. If the inversion is strong enough, it will act as a blanket, keeping much of the heat in the air surrounding the plants. It is extremely important that heaters are placed correctly and burnt at the appropriate level so that holes aren't punched in the inversion layer creating chimneys in which most of the heat will escape to the upper layers of the atmosphere. Heaters can be units purchased for explicit use in production fields or they may be open fires in the field.

Heaters are expensive to purchase and operate and less environmentally friendly than other methods of frost protection. They are, however, a dependable form of protection for high value crops. Most of the heat released by heaters is in the form of hot gasses which rise and cool until they reach the ambient temperature when it then cools, spreads out, and begins to sink, creating a circulation pattern. Very little heat energy is moved by radiant transfer directly to plants. The energy generally required to prevent a frost is somewhere around 20–40 W/m^2; heater output is usually around 140–280 W/m^2, depending on fuel, burning rate, and the number of heaters involved. Most of the energy released during burning is lost, thus the process is extremely inefficient.

Heaters are more efficient with stronger inversions (low ceiling) because they have to heat a smaller volume of air. More heaters are needed along the edges of a field to account for cold air being drawn in from outside the field area by the rising air above the heated field. Smoke does not contribute to heat release or retention from heaters, regardless of fuel or burner type. Local regulations should be carefully reviewed before purchasing or using any type of heater.

Sprinkler irrigation

Sprinkler irrigation can be used to effectively and economically protect crops from frost. Many growers already have the irrigation equipment, thus there are only time, labor, and fuel investments with this type of protection. Labor is needed to set up irrigation equipment and also to ensure there is no ice build up on the sprinkler heads during operation. Some drawbacks to this method include: (i) fuel and water costs; (ii) labor requirements; and (iii) potential soil waterlogging.

Frost protection with water relies on the same principles regardless of the application method used. Water contains a large amount of heat energy. That heat can be transferred to the soil or to plants, raising their temperature and reducing the chance of a frost. While water adds heat energy to the environment, it can also remove heat energy through evaporation. Thus one must consider the energy added with water application plus the energy lost due to evaporation. Additionally, water releases heat when it freezes and this heat is transferred to whatever object the water is freezing on. When liquid and solid water are commingled, their temperature cannot go above or below the freezing point (0°C) until a complete phase change (all liquid or all ice) occurs. Thus if a film of liquid water is maintained on ice, the temperature will stay at 0°C. This is the key to frost protection with water. If enough water is not being applied or the system is turned off too soon in the morning, no liquid–solid interface will be maintained and damage will occur, often more than if no protection were even attempted. Additionally, if the system is not started soon enough in the evening, evaporative cooling may cause temperatures to drop sharply and cause extensive damage. Evaporation of water at 0°C removes about 2500–2800 kJ/kg from the surrounding environment, depending on whether the evaporation is from liquid water or ice (2501 and 2825.5 kJ/kg, respectively). When the same amount of water freezes 418.3 kJ/kg are released, thus it takes six times the amount of water freezing as evaporating just to break even! This is especially crucial when the air is dry (which it often is during a radiational frost) and considerable evaporation is occurring.

Overhead sprinkler irrigation systems attempt to maintain a layer of liquid water on the ice forming on plant and soil surfaces, and thereby maintain a temperature around 0°C. This system can provide up to 7°C protection under ideal conditions. Overhead systems are used on low-growing crops and larger specimens that can support the weight of ice that builds up during protection. In some cases sprinkling over the structure of covered crops (crops in unheated greenhouses or high/low tunnels) may be employed to keep the temperature inside the structure at 0°C.

Under-plant sprinklers are designed to maintain a layer of liquid water on ice forming on the soil surface and ground cover. It is used on any crop that benefits from heat movement from soil to plant during a radiational cooling event and on crops requiring only a few degrees of protection.

Computer programs are available to assist with decisions regarding on-off times and application rates based on air temperature, dew point, wind speed, and crop being protected.

Surface irrigation or flooding

Supplying water to a field via trench or furrow irrigation provides heat from the water as it cools. Similarly flooding entire fields with water provides protection from frost during radiational cooling. If the water cools enough to start freezing, any standing water will freeze from the top down due to the density properties of water. Water is most dense at 4°C, thus the warmer water will sink as the colder water rises to the surface and freezes. If a layer of ice forms, a barrier of heat transfer from the warmer, deeper water and soil develops and the ice surface and adjacent air temperature can cool to dangerous levels.

Artificial fog

High pressure lines with specialized nozzles in an irrigation system can create an artificial fog to

protect against frost. The small droplets absorb long-wave radiation and re-emit them downwards providing protection from frost. Light wind and high humidity are required for this method to work and generally work best for moderate frost events. The cost of foggers is high but the operational costs are 20% or less than other conventional heating or sprinkler systems.

Controlling INA bacteria

Controlling the population of INA bacteria is not an effective means to reduce frost injury as most woody species have intrinsic ice nucleators that initiate frost formation with or without INA bacteria present (Gross *et al.*, 1984; Ashworth *et al.*, 1985; Proebsting and Gross, 1988).