CHILLING INJURY IN PLANTS

James M. Lyons
Department of Vegetable Crops, University of California, Davis

INTRODUCTION

Tropical and subtropical plants exhibit a marked physiological dysfunction when exposed to low or nonfreezing temperatures below about 10° to 12°C. This dysfunction is referred to as chilling injury and has been of great concern for many years with harvested plant parts because lowered storage temperatures are generally an effective means of extending the postharvest life of fruits and vegetables. The phenomenon has not been well understood or recognized for its importance even though plant sensitivity to low temperature has been recorded for centuries.

Copyright 1973. All rights reserved
Molisch (117) cited several early studies demonstrating that a number of plant species were killed at low temperatures above the freezing point. He suggested (118) that this physiological harm should be referred to as "chilling injury" (Erkältung) to differentiate it from freezing damage (Erfrieren). Such injury to susceptible plants (or plant parts) has also been referred to as "low-temperature injury" (48), as "cold injury" (135), and in apples as "low-temperature breakdown" (193). Chilling injury appears to be the preferable term (148) because it is not easily confused with freezing injury or with phenomena related to cold or winter hardiness (78, 190). Chilling-sensitive plant species appear to have a commonality of temperature response, with the critical temperature below which injury occurs being most often around 10° to 12°C. This generalization does not apply in all cases, however, and species vary somewhat in tolerance with their region of origin. For example, the lower temperature limit is 0° to 4°C for temperate fruits such as apples, around 8°C for subtropical fruits such as citrus, avocado, and pineapple, and around 12°C for the more tropical banana (193).

As in many fields of plant physiology, instrumentation and techniques developed in the last decade have provided the means of developing new insight on this elusive problem, leading to a more integrated concept of chilling injury. The most important recent discussions of chilling injury are by Levitt (78), in a book on environmental stresses, and by Fidler (48), in a review. General considerations of the effects of temperature on biological systems can be found in reviews by Bělehrádek (7,8), Kayser (70), Langridge (77), and Luyet & Gehenoio (90). Chilling injury has also been considered in conjunction with other topics in reviews on the storage and postharvest physiology of fruits and vegetables by Biale (10-12), Hansen (51), Miller (109, 111), Pentzer & Heinze (133), Ulrich (176), Wardlaw (184, 185, 187), and in a text by Ryall & Lipton (148). Additional discussion is found in general treatments of the biochemistry and physiology of specific commodities such as citrus (13, 109, 171), avocado (14), banana (128, 183, 186), mango (61), pineapple (37), and tomato (56). Reviews on freezing injury in plants include some aspects of low-temperature effects in the range just above freezing (97, 98).

This review summarizes some of the striking horticultural manifestations of chilling injury, with particular emphasis on harvested crops, and relates these to the physiology of the tissue involved. The discussion considers how temperatures below around 10° to 12°C can so abruptly and dramatically affect physiological function in sensitive plant species.

**HORTICULTURAL ASPECTS OF CHILLING INJURY**

**Symptoms**

The symptoms of chilling injury vary with the plant tissue and the severity of injury, and they usually develop more rapidly if the tissue is transferred to a non-chilling temperature. Some generalities will be provided here without describing symptoms for all susceptible commodities. Specific information on various crops
is given by Lutz & Hardenburg (89), Ryall & Lipton (148), and papers on the postharvest behavior of specific commodities. Although chilling injury has been recognized for many years, its severity is difficult to define quantitatively, being estimated essentially only qualitatively in terms of visual observations. Katz & Reinhold (69) experimented with changes in electrical conductivity for estimating injury in Coleus before external symptoms developed, but this method has not been extended widely to other plant material.

Perhaps the most apparent symptoms of general concern are surface pitting, necrotic areas, and external discoloration. As early as 1896, Molisch (117) described a brown spotting on sensitive tropical plants after brief exposure to chilling temperatures. The symptomology of chilling injury has since been observed and described for almost all commercially important horticultural commodities sensitive to low temperatures. If bananas, for example, are exposed to mild chilling when green, the peel develops a smoky or dull-yellow appearance (186), and with more severe chilling it turns dark brown or black. Cucumbers give an excellent example of typical surface pitting (42). The pitting results from injury and collapse of subsurface cells, followed rapidly by invasion of decay organisms. Such visible pitting can be related to rate of water loss in some commodities (120), and pitting has been reduced by maintaining a high relative humidity or waxing the fruit to decrease water loss. Some tissues become discolored both internally and externally as a result of chilling. Sweet potatoes, for example, develop a brown discoloration if the roots are cut and exposed to air for a short period after chilling (81).

Mature-green fruits commonly fail to ripen normally after chilling. Biale (9) reported that avocado fruits stored at chilling temperatures neither ripened normally nor developed the typical climacteric rise in respiration associated with ripening. Despite a lack of climacteric rise in respiration at 5°C, there was a cycle of ethylene production (as measured by the pea bioassay), though not until the fruit had been at the chilling temperature for 57 days (141). When avocado fruits were given a chilling treatment (5°C for 34 or 43 days) and then transferred to a warm temperature (20°C), CO₂ production rose but without the normal corresponding rise in ethylene (15). Abnormal patterns of ethylene evolution have also been shown in chilled bananas (121) and grapefruit, sweet oranges, avocados and leaves (29, 30). Because tomato fruits and bananas are harvested and shipped at a mature-green stage, their ripening behavior at various storage temperatures has been studied extensively (e.g. 79, 121, 147, 183–185).

Apple scald (superficial scald), a brown discoloration giving the skin a cooked appearance, is a symptom of chilling injury in some varieties of apples upon long-term storage at 0° to 4°C (108, 193). Low-temperature breakdown of apples, also caused by storage at such temperatures, occurs in the cortex, and in certain varieties involves most of the flesh (62, 193, 198). "Wooliness" of peaches and plums refers to a mealy texture and discolored appearance of the flesh caused by chilling injury in storage at 0° to 4°C (17, 34, 35).

Susceptibility to decay is increased by surface pitting, necrotic areas, and general weakening of the tissue. Injured tissue is readily infected by decay organisms such
as alternaria rot (57, 99-104). Even without such ensuing decay, storage life would be much reduced by the pitting, necrosis, and lack of ripeness, so abbreviated storage life in itself can be considered a symptom of chilling injury in horticultural commodities (42). It is important that the symptoms described above are not necessarily specific to chilling injury. Surface pitting typical of chilling injury can be induced at high temperatures by low-oxygen atmospheres (120, 124, 125), which is relevant to the proposal of Nelson (124) that chilling injury is caused by the inability of tissue to obtain or use enough oxygen for normal respiration. Since, however, low oxygen can induce similar symptoms in both chilling-sensitive and chilling-resistant species (124), low oxygen does not appear to be a cause of chilling injury per se. Similarly, cucumbers at warm temperatures can become pitted as a result of mechanical injury and desiccation in the absence of chilling (3).

Symptoms of chilling in vegetative organs vary with the tissue involved, but under severe conditions the ultimate result is impairment of function and death. For example, if cotton seedlings are chilled at time of seed hydration, there will be abortion of the radicle tip, whereas chilling applied after germination but during early seedling growth will result in damage to the root cortex (20). These latter symptoms have also been described for muskmelon and pepper seedlings (53). Chilling of sensitive grass species has been shown to cause a reduction in photosynthesis (169) and changes in chloroplast ultrastructure (114, 167). Common symptoms of chilling injury in developing vegetative tissues are necrotic lesions, increased susceptibility to decay organisms, cessation of growth, and ultimately death.

**Susceptibility**

Chilling injury is a characteristic of plants of tropical or subtropical climates, although plants of the temperate zone can also have a sensitivity. Early works of Sachs (149), Ewart (44), and Molisch (117) described the influence of chilling temperatures on a number of species of tropical and semitropical plants. Sellschop & Salmon (153) demonstrated the sensitivity to chilling temperatures of a number of crop plants, including cotton, cowpea, peanut, corn, and rice. When these species were subjected at various stages of growth to chilling temperatures, 2° to 4°C for periods as short as 12 hours induced injury in some. Seible (151) described symptoms of chilling in several species exposed to low temperatures for only a few hours. Similarly, Spranger (163) described the symptoms of chilling injury on vegetative tissues from a number of ornamental plants. Since those early reports, susceptibility has been described in many horticultural crops of economic importance, and Lutz & Hardenburg (89) and Ryall & Lipton (148) have listed many of the susceptible commodities, their symptoms, and their safe low-temperature limits.

Variatel differences in susceptibility to chilling injury have also been reported. For example, Smith & Millet (160) showed that the average period for sprouting at 10°C ranged between 18 and 46 days among 10 tomato cultivars. Apeland (3) demonstrated varietal differences in cucumber responses to a 4-day chilling treatment at 5°C: decline to poor quality at 12.5°C took widely varied periods, from
10 days for 'Ohio M.R. 200', to 47 days for 'Marketeer'. Watada & Morris (188, 189) demonstrated that the symptoms and pattern of senescence were similar among 9 snap bean cultivars but that their storage lives differed significantly. Likewise, their rates and patterns of respiration were similar although they differed in severity of injury symptoms and reduction of storage life. Christiansen (26) demonstrated marked differences between 'Delta' and 'Acala' cotton selections in response to chilling injury during seed hydration, with the results suggesting that variation in resistance to chilling injury is a heritable factor.

Physiological age at chilling also affects susceptibility to injury. A good example is found in seedling growth and development. Although seedlings of chilling-sensitive species are susceptible, dry seeds of these same species experience no harm when stored for extended periods at chilling temperature. Wheaton (191) found that fully imbibed corn seeds (12 hours in aerated water) and 1-day-old seedlings were uninjured by 4 days at 1°C but that sensitivity appeared during the following 2 days and reached a maximum by the third day following imbibition. This transition in germinating seeds has also been studied in lima bean seeds (139, 140) and in cotton seeds (22, 23, 170). These studies indicated two critical periods of sensitivity: 1. Chilling applied coincident with imbibition as the initial step in germination causes an injury that can be avoided if the seed has been first allowed a brief hydration at elevated temperature before the chilling. 2. A second period of sensitivity appears sometime after 24 hours of germination. A precise determination of the metabolic events coincident with this transition in chilling sensitivity would be of immense value to an understanding of chilling injury in general. Physiological age certainly plays a role in the susceptibility of plant tissues at other stages of growth as well. The less mature apical "hands" of bananas, for example, are more susceptible to chilling than the more mature basal hands (129).

Preharvest environment can influence susceptibility. Fidler (48) describes the influence on chilling sensitivity of the climate of origin. 'Cox's Orange Pippin' apples, for example, can be stored at 1.5°C when grown in mainland Australia, 2°C when in Tasmania, 2.5° to 3°C when in New Zealand, and 3° to 4°C when grown in the United Kingdom. Apeland (3) reported that greenhouse-grown cucumbers are more sensitive to 10°C than are similar cultivars grown under field conditions. Similarly, Palmer (128) cited studies indicating that banana fruits maturing at higher field temperatures are more susceptible than those maturing in a cooler climate.

**Chilling Treatment**

Exposure to chilling temperatures must be relatively long before cells of most sensitive plants are injured. The injuries observed most often require days or weeks of chilling temperatures, although Seible (151) described injury in several species (*Episcia, Achimines*, and *Gloxinia*) after only a few hours at 1° to 5°C, and bananas can be injured after a brief exposure below 10°C (183). In general, the severity of injury of sensitive plant tissues increases as temperature is lowered or as exposure is extended at any chilling temperature.
Since a reliable quantitative measure is lacking, chilling injury must be evaluated by qualitative visible symptoms as described above. It is a function of both physiological injury per se and rate of symptom development in the particular plant tissue. For example, van der Plank & Davies (182) reported that injury in plums and peaches was greater at 3°C than at 0°C. They explained these results on the basis of an "equilibrium" factor, the temperature increment below that critical for injury, and a "kinetic" factor which regulated the rate of chemical change and hence symptom expression. These factors operate in opposing directions as the temperature is lowered, and at some point (3°C for plums and peaches) the kinetic factor allows a more rapid development of symptoms even though injury is ultimately greater at 0°C. Further, evaluating the injury that occurs during continued chilling (182) differs from that which develops at some elevated temperature (e.g. 25°C) after chilling for various periods (42, 79, 188). The latter is important as an experimental method since it not only standardizes the conditions for symptom expression, but reflects the usual practical situation, with commodities held at low temperatures during storage and transport followed by several days of higher temperatures during the marketing period. The injury that occurs during chilling is usually not immediately conspicuous but may very rapidly become visible during 2 or 3 days at the elevated temperature.

Much concern with chilling phenomena relates to the storage of fruits and vegetables, where chilling is most easily recognized as a problem. Chilling in the natural environment can be of equal concern, however. For example, chilling in the field as the season extends into the fall can cause tomato fruits to fail to ripen into marketable condition, even if held at proper temperature after harvest (119). And, of course, field temperatures will be of concern where chilling would prevent germination (52) or influence seedling development and growth after germination (20–22, 24, 53, 160), reduce photosynthetic rate (16, 169), and even affect reproduction in some temperate species (172).

Amelioration of Chilling

TEMPERATURE CONDITIONING Acclimation and hardening are terms used to describe a change in woody species from a susceptible to a winter-hardy or resistant condition capable of withstanding freezing temperatures to around —60°C (190). Similar "hardening" toward chilling injury is not possible, although a few experiments indicate that the sensitivity of some plant material can be reduced by exposure to temperatures slightly above the chilling range (3, 88, 101, 192). Wheaton & Morris (192) showed that exposing 5-day-old tomato seedlings grown at 25°C to a conditioning temperature of 12.5°C for as little as 3 hours provided some protection from a subsequent 2-day exposure to 1°C; maximum protection required 48 hours of such exposure. The protection was effective only against slight to moderate chilling, however, and none of the seedlings survived a 7-day exposure to 1°C. In similar attempts with sweet potatoes, conditioning treatments influenced respiration rates slightly but did not reduce chilling injury as measured by visible symptoms (192). The sensitivity of cucumber fruits chilled at 5°C for 4 or 6 days was decreased somewhat by a conditioning period at 12.5°C.
Bananas transferred directly from 21°C to 5°C showed more chilling injury than bananas whose temperature was lowered by steps of 3°C at 12-hour intervals (129). As with the tomato seedlings and sweet potatoes cited above, however, conditioning of cucumbers and bananas is effective only against slight chilling.

**Alternating Temperatures**

It was demonstrated that injury in plums could be ameliorated by interrupting the chilling treatment with a warm (above 20°C) period of 2 or 3 days (158, 161). The basis of this system is the assumption that the cause of injury is accumulation of a toxic compound which can be metabolized, or depletion of a critical metabolite which can be restored at the warm temperature. This avoidance of chilling injury by applying a warm period has been observed in a number of plant tissues. Decreased ascorbic acid and increased chlorogenic acid in sweet potato roots during chilling could be reversed by a warm period at 15°C following 2 weeks’ exposure to 7.5°C but not 4 weeks or longer (84). Cacao seed germination is completely destroyed by exposure to 4°C for as little as 10 minutes (64), but immediate immersion in 37°C water for 10 minutes restored germination to 50% (63, 65). In cacao seeds, the chilling exposure at 4°C that still allowed reversibility was 10 to 20 minutes. In cotton seedlings, a chilling-induced decrease in ATP levels was reversible after a 24-hour exposure to 5°C but not after a 48-hour exposure (164). In corn seedlings, visual leaf injury appeared in 36 hours at 0.3°C, but upon transfer to 21°C the leaves returned to normal and the leaf symptoms disappeared within 72 hours (33). Chilling injury in some of the seedlings could be partially reversed after 48 to 60 hours at 0.3°C, but not after 72 hours.

Thus, chilling injury can be avoided in many tissues if they are returned to a warming temperature before degenerative changes occur.

**Hypobaric Storage**

Burg & Burg (18) described a system of fruit storage under reduced atmospheric pressures which greatly extended storage life at nonchilling temperatures. They (personal communication) have also shown that low-pressure storage at 50 to 200 mm Hg with continuous air changes to remove volatiles could relieve symptoms of chilling injury in avocados, bananas, grapefruit, peppers, and tomatoes. Tolle (173) applied the term “hypobaric” to the storage at subatmospheric pressure (low-pressure storage). Pantastico et al (129) showed that when bananas were held at 5°C and 220 mm Hg pressure, the green color of the fruit was maintained for about a month and symptoms of chilling injury were reduced. Pitting symptoms of chilling injury from storage of limes and grapefruit (130) at 4.5°C were lower under 220 mm Hg than under atmospheric pressure: 0.0% vs 65.4% in limes held 4 weeks, and 4.4% vs 23.5% in Marsh grapefruit held 7 weeks. Holding grapefruit at only 380 mm Hg did not give similar amelioration (49). Oudit et al (127) found no alleviation of chilling injury from hypobaric storage at 220 mm Hg in bananas, avocados, limes, cucumbers, and tomatoes. All the same, hypobaric storage appears able to alleviate symptoms of chilling injury under at least some conditions and therefore deserves further study.
MODIFIED ATMOSPHERES The analogy between symptoms of injury induced by chilling temperatures and those induced by low oxygen (120, 124) has prompted investigations of the effect of modified atmospheres on chilling injury. Kidd & West (71) reported that increased CO₂ in modified-atmosphere storage increased the susceptibility of apples to low-temperature breakdown. Eaks (38) showed that atmospheres of compositions differing from 0% to 100% O₂ had very little influence on injury of cucumber fruits exposed to 5°C for 8 days, but that increased CO₂ concentrations intensified symptoms of chilling injury. Similarly, Tomkins (174) has shown that increased CO₂ in the storage atmosphere increases damage from chilling treatments in the tomato. Miller (109), in his review of citrus storage, described a number of studies in which modified-atmosphere storage was reported to decrease pitting and other symptoms of chilling injury in citrus. Despite some success reported for relatively high percentages of CO₂, such treatment generally resulted in some type of rind injury to citrus. Vakis et al (179) reported that 10% CO₂ in the storage atmosphere reduced chilling injury in grapefruit and avocados. Waxing of fruit and storage in pliofilm packages modified the internal atmosphere of citrus fruits and alleviated chilling injury in grapefruit (49) but aggravated it markedly in limes (55, 130).

OTHER TREATMENTS Some evidence exists that chemical treatments can alleviate chilling injury. Recent studies in Australia have shown that superficial scald in apples, a chilling injury occurring after prolonged storage at 0°-4°C, was caused by injurious conjugated triene hydroperoxides, oxidation products of α-farnesene (58-60). It was further shown that oxidation of α-farnesene could be inhibited by the antioxidant diphenylamine, either as a coating on the apple skin or in wrappers around the fruit. Pantastico et al (130) could induce scald (an advanced stage of pitting) in limes by treatment with acetaldehyde at chilling temperatures, and this scald could be reduced approximately 12 to 50% by diphenylamine treatment. Another antioxidant which inhibits this scald (162) is ethoxyquin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline). The fungicide thiabendazole (TBZ) has been shown to reduce pitting of grapefruit significantly during prolonged storage at around 8°C (150). It was concluded that TBZ possibly had some physiological effect on amelioration of chilling in addition to its effect as a fungicide. Amin (1) found that applications of IAA and vitamins increased the vegetative growth of chilled cotton plants over that of untreated controls.

Waxes applied to the surface of chilling-sensitive tissues have alleviated chilling symptoms in some instances. Waxing reduced surface pitting on chilling-sensitive fruits held at 0.5°C and 70% relative humidity, with the reduction in pitting related to slower water loss than in unwaxed fruits under similar conditions (120). Waxing was suggested by Platenius (137) as a method for reducing chilling injury. In contrast, Mack & Janer (96) observed that pitting was more severe on waxed than on unwaxed cucumber fruits held for one week at 2.2° to 3.3°C and 95% relative humidity. These results are difficult to assess since wax could slow desiccation, which has been associated with pitting (3, 120), or it could modify the internal atmosphere of a commodity (depending on the type of wax and thickness
of coating). Which of these factors is operating in a given set of experimental conditions is not yet resolved.

Another disorder of apples called low-temperature breakdown has been shown to be caused by the accumulation of acetic acid during prolonged storage at low temperatures (195–199). Treatments that promoted water loss reduced acetic acid in the tissue by accelerating its removal as acetate esters (or the free acid), thereby reducing susceptibility to breakdown. Hypobaric storage or alternating temperatures (discussed above) could presumably accelerate removal or metabolism of acetic acid or its esters, thereby reducing low-temperature breakdown.

PHYSIOLOGICAL MANIFESTATIONS

Protoplasmic Streaming

Perhaps one of the more spectacular effects of chilling temperature on sensitive plant tissues is the effect on protoplasmic streaming. As early as 1864, Sachs (149) observed that protoplasmic streaming ceased at about 10°–12°C in root hairs of cucumber and tomato plants, and other workers of the time indicated that streaming continued down to or near 0°C in chilling-resistant species. Lewis (80) examined this differential response in a number of plant species. He found that streaming ceased or was just perceptible after 1 or 2 minutes at 10°C in petiole trichomes of chilling-sensitive plants and invariably ceased promptly at 5° or 0°C. If exposure to 0°C exceeded 24 hours, streaming failed to resume in most trichomes upon return to a warmer temperature. In contrast, streaming in chilling-resistant plants proceeded at temperatures down to 0°–2.5°C. Wheaton (191) confirmed these results with five sensitive and four resistant species.

The exact mechanism of protoplasmic streaming has not been delineated, but it is clear that the process requires energy and is dependent on the physical properties of the protoplasm and subcellular membrane system (68, 152). If, as discussed later, the ATP supply is greatly reduced in chilling-sensitive species, and the lipoprotein membranes undergo a phase transition from a flexible to a solid structure as an immediate effect of chilling, it follows that streaming would very quickly be greatly impeded or cease. Some evidence for the energy relationship can be found in the observation by Ewart (45) that streaming ceased at a higher temperature if the tissue was cooled under anaerobic rather than aerobic conditions. The impact of cessation of streaming on the events leading to injury is difficult to evaluate because the exact role of streaming in normal metabolism is little understood (152); however, it is likely that normal metabolism would be greatly upset by a complete cessation of the process.

Respiration

STUDIES WITH DETACHED ORGANS Anomalous respiratory behavior during or after the chilling of sensitive plant tissues has been reported by a number of investigators (40, 41, 67, 71, 81, 121, 126, 129, 138, 188, 207). Except during the ripening of fruit of the climacteric type (10), the normal pattern of respiration is
a gradual decline with time after harvest; and such is the case with chilling-resis-
tant plant tissues at all temperatures (e.g. 148) and with chilling-sensitive plant
 tissues held above chilling temperatures. In contrast, Kidd & West (71) noted
 that respiration at 1°C was faster in apples injured by low-temperature break-
down, and similar reports of faster respiration (following an initial brief decline)
 have been made for sweet potato roots (81), tomatoes (79), cucumbers (41),
 peppers (126), and a number of other commodities. The faster respiration during
 storage at chilling temperatures usually precedes any external visible symptoms
 of injury (41, 81, 188). This respiration pattern was clear when data of Eaks &
 Morris (41) for cucumber fruits were presented as an Arrhenius plot (93). Their
 initial respiratory rates, at 24 hours of storage, indicated a discontinuity or
 “break” in the Arrhenius plot, with a higher activation energy at the chilling
 than the nonchilling temperatures. The plot revealed an increased respiration
 after 5 days of storage in fruits stored at 0°C but not at 5° or 10°C, which cor-
relates with the fact that upon transfer to a warm temperature for 3 days injury
 was observed in the 0°C fruit but not in the 5° or 10°C fruit. Upon 10 days of
 storage, injury was observed at 5° and 10°C as well as 0°C and respiration had in-
creased at all three temperatures. This must reflect an injury phenomenon which
 has upset metabolism and induced accelerated respiration.

Discussions relative to the effect of temperature on Q10 values must consider
 the relationship between time and temperature. For example, if Q10 values were
 calculated at the 24-hour period for the cucumber data of Eaks & Morris (41)
 above, the Q10 values would be higher at the lower temperatures than at higher
 temperatures—the situation generally observed with most plant species, whether
 chilling-sensitive or not. In contrast, Q10 calculated from the respiration rates
 after 10 days would give values similar over the entire temperature range.

Another respiratory phenomenon of importance in relation to chilling injury
 is the greatly exaggerated respiration rate observed at warm temperatures after
 transfer from a chilling treatment. This respiratory stimulation upon transfer has
 been observed for many plant species (4), but with chilling-sensitive species it is
 greatly amplified and can be used as an index of the severity of chilling injury. If,
 for example, cucumber fruits are transferred to 25°C after 4 days at 5°C, there is
 a peak in respiration, but the rate quickly returns to that of fruits held continuously
 at 25°C, indicating that injury is not yet permanent (41). With chilling for 8 or 12
 days, in contrast, a similar peak in respiration upon transfer to 25°C occurred but
 the rate did not return to a normal level. Further, symptoms of injury become
 apparent in the 8-day and 12-day treatments but not in the 4-day treatment. Re-
sponses have been similar in chilling-sensitive pepper (126), citrus (39), tomatoes
 (79), snap beans (188), and bananas (121). Thus, in evaluating the impact of chill-
ing treatments on the respiratory process, it is important that the initial reversible

A more complete discussion of the usefulness of presenting data as an Arrhenius
 plot, as well as the thermodynamic basis for such a plot, is found in reviews by Raison
 (142, 143) and Lyons (91).
response to brief exposures be distinguished from the response when chilling time has been long enough to cause irreversible degenerative changes in the tissue.

The respiratory quotient (RQ) at chilling temperatures has been examined in an attempt to demonstrate altered metabolism in sensitive plant tissues at chilling temperatures. Platenius (138) studied the effect of chilling temperatures on the RQ of a number of plant species but found no differences which could be related to chilling sensitivity. Eaks & Morris (41), on the other hand, demonstrated that the RQ for cucumbers held at 0°C was less than unity for the first 7 days of storage and then rose abruptly above unity. One can attempt to interpret RQ values in terms of the substrate available for the respiratory process, but, regardless of the inferences drawn, the sudden shift from less than unity to above unity correlates with rather severe injury and does reflect a major upset in normal metabolism of the tissue. In contrast, cucumbers held at the nonchilling temperature of 15°C exhibited an RQ of unity throughout the 14-day storage period.

Anomalous respiration of seedling tissues exposed to chilling temperatures has also been shown. For example, respiration in cucumber leaves (76, 200) declined for short periods at chilling temperatures but increased markedly upon transfer to warm temperatures. As with the other plant material studied, the magnitude and course of the respiratory pattern depended on the severity of previous chilling.

Tissue-level studies Observation of the anomalous respiratory behavior associated with chilling injury has naturally led to a questioning of the impact of chilling on cellular respiration and oxidative phosphorylation. In one approach to this question, slices and segmented tissues are utilized to facilitate the use of respiratory inhibitors. Shichi & Uritani (154) prepared disks from sweet potato roots and showed that the tissue lost the ability to respond to 2,4-dinitrophenol (DNP) at 20°C after 10 to 15 days at 0°C. This led them to propose that the respiratory mechanism was uncoupled and ATP formation retarded as a result of the chilling treatment. Lewis & Workman (82) demonstrated a marked decline in the incorporation of 32p at 20°C in tissue slices from mature green tomatoes following chilling at 0°C, an observation which also suggests a reduced capacity for oxidative phosphorylation. Wheaton (191), on the other hand, found that root segments from chilling-sensitive corn were highly responsive to DNP after 3 hours at 5°C. He did not extend the chilling treatment to the point where injury caused degenerative cellular changes, and his data suggest that the loss of phosphorylative capacity is not the primary step in chilling injury but follows upon some irreversible physiological breakdown. Creencia & Bramlage (33) demonstrated that DNP could stimulate respiration in segments of corn leaves if the tissue was tested before injury from chilling was irreversible. Upon irreversible injury the capacity to respond to DNP was lost and the respiration rate increased. Similarly, malonate inhibited the respiration of healthy banana slices but not that of chill-injured slices (121, 122). Tissue segments from cotton seedlings had a higher respiration rate at 25°C following chilling treatments up to 24 hours at 2.8°C (2). Respiratory activity was similar in tissue slices from both bananas and
limes (180). Respiration of tissue slices from pepper fruits increased markedly after 7 weeks at 1°C, was relatively constant at 6°C, and declined slightly at 18°C (126).

**MITOCHONDRIAL STUDIES** Changes in mitochondrial physiology have been of interest because of the central role of these organelles in the respiratory process. Lieberman et al (84) attempted further elucidation of the mechanism of chilling effects on the respiratory process by studying mitochondria isolated from sweet potato roots. Their basic approach was to compare activity at 25°C of mitochondria derived from sweet potato roots stored at chilling (7.5°C) and nonchilling (15°C) temperatures. They found essentially no difference resulting from the first 4 weeks of storage. After the fifth week, however, activity began to decline in the mitochondria from the chilled roots, and by the tenth week the chilling treatment yielded completely inactive mitochondria. Similarly, Minamikawa et al (115) found a decrease in oxidative activity at 25°C of mitochondria isolated from sweet potato roots chilled at 0°C. Uritani et al (177) also compared activity at a high temperature (28°C) of mitochondria from unchilled and chilled sweet potato roots and found that oxygen uptake and respiratory control (RC) were lower in the chilled tissues. If the sweet potato roots were chilled only slightly, cytochrome c added to the reaction mixture could restore the respiration rate to that of mitochondria from unchilled tissues. When the roots were severely injured, restoration of the rate by added cytochrome c was only partial, and they suggested that cytochrome c was released from the mitochondrial membranes during the chilling treatment. They also presented data indicating that malate dehydrogenase activity was impaired during storage of the roots at 0°C. Electron micrographs of mitochondria derived from the chilled sweet potato roots showed a large proportion in an extremely swollen form not observed in mitochondria from healthy tissue (203, 204). The swollen appearance resulted from degradation of mitochondria and the release of phospholipid from both the inner and outer membranes during storage at chilling temperatures (202). Furthermore, the capacity to bind added phospholipid (after removal by aqueous acetone treatment) was greatly reduced in mitochondria from chill-injured sweet potato roots in comparison to those from healthy roots (205). Pantastico et al (130) isolated mitochondria from limes and grapefruit after chilling treatment and found that the energy transfer system was impaired. Because of the many complicating factors operating during the procedures for isolation of plant mitochondria (144), distinguishing direct effects of chilling from indirect effects due to the preparation of mitochondria from injured tissue is most difficult.

In contrast to the approach used above, Lyons & Raison (93) isolated mitochondria from healthy plant tissues and studied the effect of a wide range of temperatures on oxidative activity of the healthy mitochondria. When the effect of temperature on oxidative activity was presented as an Arrhenius plot, the mitochondria from chilling-resistant plant species each exhibited a linear plot with a constant activation energy (Ea) over the entire temperature range from near 1°C to 25°C. In contrast, mitochondria from chilling-sensitive species each exhibited
a discontinuity in plot at 10° to 12°C, with a marked increase in $E_a$ below the break temperature, indicating that an immediate and direct effect of low temperature on these species is to suppress mitochondrial respiration. Their data also clearly showed that phosphorylative efficiency was not influenced at any temperature for either the chilling-sensitive or the chilling-resistant plant species, and therefore, although the rate of production would be decreased concomitantly with the suppressed mitochondrial respiration, the efficiency of energy production is not altered by low temperature.

**Metabolic Changes**

Numerous attempts have been made to correlate changes in cellular constituents or related enzyme activities with chilling injury. Tissue given a chilling treatment is invariably compared with healthy tissue, but this approach has not contributed significantly to an understanding of the mechanism of chilling injury. Cordner & Mathews (31) found essentially no change in sucrose, total sugar, acid-hydrolyzable substances, or starch in squash fruit after 8 days in storage at $2°$-$4°C$. Lorenz (87) similarly found that all major components in summer squash remained practically constant during storage at chilling temperatures. Jones (67) found a slight decrease in hydrolysis of sucrose in chilled papaya fruits, with a concomitant increase in soluble solids. Roots of sweet potatoes injured by chilling decreased in ability to synthesize carotenoid pigments (46, 47) and had an accelerated loss of ascorbic acid (46, 47, 85). Accelerated loss of ascorbic acid as a result of chilling was observed also in pineapple (110, 112) and bananas (123), but not in guava (157) or tomato (32, 79). Barnell & Barnell (6) reported that the pulp of chilled banana fruit contained higher levels of tannins, and suggested that oxidation of these compounds gave rise to the dark color symptomatic of chilling injury in that fruit. Similarly, Murata & Ku (122) observed increased levels of tyrosine and dopa in chilled banana tissue which could produce the dark pigment upon polymerization and oxidation. Lieberman et al (85) showed an increased accumulation of chlorogenic acid in chilled sweet potato roots, a fact most probably instrumental in the decreased activity of mitochondria prepared from such tissues (83, 84). Both chlorogenic acid and total polyphenols in pepper seeds increased initially and then gradually decreased during storage at chilling temperatures (72). Compounds closely associated with intermediary metabolism—acetaldehyde, ethanol, and the keto acids—have been shown to increase in several tissues injured by chilling (113, 121, 122, 126, 130, 206). Chilling altered the amino acid content of sensitive grass species causing a sharp decrease in the content of those amino acids closely related to intermediates of the $C_4$-photosynthetic pathway within 1.5 days (168). As pointed out earlier, some primary responses to chilling temperatures must be clearly distinguished from events which are the result of degenerative tissue injury; many of the chemical changes listed above result from the latter event. Similarly, many enzyme systems isolated from chilling-injured tissue have shown an altered activity. For example, as a result of chilling treatments of sensitive tissues increases have been reported in the activity of invertase (19), polygalacturonase (181), phenylalanine ammonia-lyase and tyrosine am-
monia-lyase (73), catalase (122), pyruvate decarboxylase, alcohol dehydrogenase, isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, and phosphoenolpyruvate carboxykinase (206). Likewise, decreases have been observed in a number of systems including malate dehydrogenase (177), pectinmethylesterase (181), and amylase (19).

Studies of chilling effects on seed germination and seedling development have revealed the extreme sensitivity and short periods required for changes. For example, Guinn (50) showed that chilling decreased RNA, protein, and lipid-soluble phosphate in young cotton seedlings, and Stewart & Guinn (164, 165) showed a very early decline in ATP and other nucleotides as well. Some of these changes in the phosphorylating system were observed as early as 6 hours after exposure to chilling temperatures. Similarly, studies of enzymes isolated from cotton seed exposed to chilling temperatures have shown isocitratase levels sharply depressed but little or no effect on malate synthetase (116, 159). Christiansen (25), in a recent review of his own and co-workers' studies on germinating cotton (20-23, 27, 28), presented a table listing a number of aberrations in the metabolism of cotton seed subjected to chilling temperatures. This comparative analysis indicated that chilling changed the entire metabolic system of the cell, with some processes recovering quickly and others only slowly. He concluded that the initial impact of chilling temperatures was a rapid physical change in membranes to a freely permeable state which allowed secondary events to alter metabolism (this is discussed in more detail in the next section).

Membrane Phenomena

Permeability Change in membrane permeability in response to chilling temperatures has often been investigated as a possible cause of chilling injury. Some studies to develop information on permeability covered the uptake and translocation of water and solutes by intact plants in response to temperature. Kramer (74) investigated the effects of soil temperature on water absorption by cool- and warm-season crops, and showed that low temperature reduced water absorption in all species but much more in the chilling-sensitive warm-season crops. He concluded that the reduced water uptake was caused by an increased viscosity and a decreased permeability in response to low temperature. Studies on the transport of water and ions through chilling-sensitive plants (36, 66, 200) indicated that transport also was reduced in response to low temperatures. Similarly, Hartt (54) reported that translocation in sugar cane ceased completely at 5°C. Although it is evident that these water uptake and translocation phenomena are greatly decreased by chilling temperatures, it is difficult to derive specific knowledge on membrane permeability from these studies.

Studies in which permeability changes are measured by solute leakage or ion accumulation have provided some direct evidence for an increased membrane permeability in response to chilling. Lewis (79) reported no difference between chilled and healthy tomato tissue disks in electrolyte leakage into tap water. In contrast, Lieberman et al (84) showed that ion leakage at 20°C was five times as great from chilled sweet potato root tissue as from healthy tissue. Wheaton (191)
observed increased leakage of potassium ions in beans and corn root tips at chilling temperatures but no increased leakage in chilling-resistant pea or wheat. These differences were apparent in as little as 3 to 6 hours after exposure to 1°C. This increased leakage of electrolytes has also been shown for Coleus (69), orange, grapefruit, and lime (130), cotton roots (27), cotyledons (50), and cucumber leaves (200). Christiansen et al (27) found an increased exudation from roots of cotton seedlings in response to chilling temperatures, but also showed that the exudation could be completely prevented (or even reversed, once started) by adding calcium or magnesium. These studies were also extended to show that comparison of root exudation among several genetic lines at chilling temperatures revealed differences which may provide basis for genetic selection to reduce chilling injury in cotton (26).

**PHASE TRANSITIONS** Early workers noted that plants (and animals) originating in warm climates tended to have more saturated fatty acids in their lipids (132), and it was suggested that solidification of the protoplasmic lipids could account for observed death or injury at low temperatures (above 0°C) (131, 166). This concept was extended in recent studies focused on the mitochondria (75, 92, 93, 95, 145, 146), clearly showing that the membranes did undergo a physical-phase transition from a flexible liquid-crystalline to a solid-gel structure at 10° to 12°C—which correlates precisely with the temperature below which injury occurred in the sensitive species of tropical origin. Electron spin resonance (ESR) studies using spin-labeled compounds with intact mitochondria and with extracted phospholipids indicated that the lipids controlled the physical state of the membranes (107, 145). This change in the physical state of the membrane lipids imparts a conformational change in membrane-bound enzymes and can account for the discontinuities observed in the Arrhenius plots of mitochondrial oxidation discussed earlier (93); for discontinuities in phosphoenolpyruvate carboxylase in chilling-sensitive C₄ species (134), and tomato and corn chloroplasts (143, 155); and for ¹⁴C-incorporation by microsomes (175) and ATPase systems (106) in warm-blooded animals. None of these phase transitions of membranes or membrane-bound enzymes are observed in chilling-resistant species. It was also recently reported that mitochondria from different apple cultivars undergo phase transitions at temperatures ranging from 3° to 10°C (105). Several of these cultivars, though not all, are susceptible to low-temperature breakdown during storage at these temperatures, and the existence of the phase transition indicates the same mechanism for low-temperature breakdown and chilling injury in sensitive species of tropical origin.

Studies on the physical phase transition stemmed from the observation that membrane lipids from chilling-sensitive plant species tended to have a higher proportion of saturated to unsaturated fatty acids than do their resistant counterparts (95). The correlation between fatty acid composition and chilling sensitivity is not precise (92, 178, 201), but it has been shown for microorganisms that the fatty acid composition can determine the existence of a temperature-induced phase transition (43). In higher plants and animals this has been more difficult to
elucidate; nevertheless it has been shown that dietary modifications can alter the fatty acid composition of mitochondrial membranes but have little effect on shifting the temperature of the phase transition in rat liver mitochondria (194), though it did shift the transition temperature considerably in sheep liver mitochondria (J. K. Raison, unpublished data). It is not yet completely clear whether the fatty acid composition of the membrane lipids determines the physical state in response to decreasing temperatures, as originally proposed, or whether other membrane components, such as sterols, exert an additional influence, as has been shown in model membranes for cholesterol (86).

THE MECHANISM OF CHILLING INJURY

A number of mechanisms have been proposed to accommodate the physiological and biochemical changes associated with chilling injury. Since a review of these changes indicates that chilling disrupts the entire metabolic and physiological process, however, it would almost appear futile to explain the various phenomena by some primary single change or "master reaction" controlling chilling injury (7, 26). Nevertheless, a single controlling response is found in the evidence that cellular membranes in sensitive plants undergo a physical-phase transition from a normal flexible liquid-crystalline to a solid gel structure at the temperature critical for chilling injury (93, 143, 145).

Figure 1 (modifying a previous presentation by Lyons & Raison (94) and incorporating some proposals by Levitt (78)) presents in schematic fashion the consequences resulting from the phase transition in cellular membranes and how this accommodates the known events in chilling. As the temperature is lowered in chilling-sensitive species, the membrane lipids solidify at the critical temperature (e.g. 10°-12°C in sensitive species of tropical origin), and the change in state would be expected to bring about a contraction that causes cracks or channels, leading to increased permeability. This immediate effect on permeability would lead to an upset in ion balance as well as account for the ion leakage that results from chilling in some tissues. The phase transition not only increases permeability, but also increases the $E_a$ of membrane-bound enzyme systems, leading to a suppressed reaction rate and establishing an imbalance with nonmembrane-bound enzyme systems. For example, as temperature is decreased the rate of reaction of the soluble enzyme systems such as glycolysis will decrease with a constant $E_a$ and a $Q_{10}$ of about 2. Similarly, the rate of reaction of the membrane-associated enzymes of mitochondrial respiration will decrease with a $Q_{10}$ of about 2 until the critical temperature at which the phase transition occurs. Below this critical temperature the membrane-bound enzyme system exhibits a marked increase in $E_a$, establishing a major imbalance in the two systems. Then metabolites such as pyruvate, acetaldehyde, and ethanol would be expected to accumulate at the interface between glycolysis and the mitochondrial system, and these compounds do indeed accumulate very early in chilling (121, 126, 130). Similar events can be projected for the chloroplast, where the phase transition leads to suppressed activity (155) and changes in metabolites after brief chilling (168). The external
symptoms of injury and ultimate death of the tissue would reflect the cell's inability to withstand increasing concentrations of these metabolites as a function of time. Apparent differences among species or cultivars in chilling sensitivity could be explained by different tolerances in withstanding or metabolizing these toxic compounds, even though the primary response is the same. For example, Watada & Morris (188) found that a number of snap bean cultivars were similar in respiratory pattern and were all injured by chilling temperatures but differed widely in visible symptoms. Similarly, McGlasson & Raison (105) showed a phase transition in all apple cultivars studied although some failed to develop symptoms, apparently being resistant to injury.

Also, a greatly reduced energy supply accompanying the suppressed mitochondrial respiration, along with the possibility of altered activity of the membrane-bound ATPase system (106), would greatly upset the normal energy balance of the cell. This altered energy supply, coupled with the rigidity of the membrane system following the phase transition, very easily accounts for the cessation of protoplasmic streaming observed in trichomes of sensitive species. It is also prob-
ably a critical factor in germination and seedling injury under chilling stress.

The temperature-induced phase change in the lipid portion of the membranes is completely reversible, though the effect on the whole organism is reversible only until the system incurs some degenerative injury. Thus, with a short chilling treatment followed by a warmer temperature, respiration increases sharply but only transiently, with the normal metabolism soon reestablished. If chilling temperatures continue long enough for degenerative changes to occur, however, the respiration rate remains elevated, reflecting a disrupted metabolism. These effects are the basis of the success that intermittent warming has given in ameliorating chilling effects, as discussed previously.

It is of interest that the symptoms of chilling injury are not specific to chilling but are identical to symptoms induced by suboxidation (124). It was suggested that suboxidation injury resulted from the accumulation of toxic products from disrupted metabolism, and Barker & Mapson (5) found an accumulation of fermentation products, including acetaldehyde and ethanol, in plant tissues held under anaerobic conditions. Thus, the visible symptoms of chilling and suboxidation injury are similar because they are both caused by the same mechanism—an accumulation of toxic metabolites—even though the accumulation stems from two entirely different effects.

The mechanism for chilling injury described here encompasses several elements operating independently or simultaneously: imbalances in metabolism, accumulation of toxic compounds, and increased permeability. Many reports (90, 135, 136, 153, 182) have focused on the formation of toxic substances, differential effects of temperature on the rate of vital enzymatic processes, and disequilibrium of reactions and changes in the velocities of interrelated chemical reactions. Levitt (78) proposed that all types of chilling injury can be the result of a change in cell permeability, and Christiansen (25) concluded that the initial impact of chilling on cotton is a rapid physical change in membranes to a freely permeable state. Similarly proposed as the mechanism of injury has been reduced ATP supply (97, 164, 165) or possibly a breakdown in the ATP-ADP transfer system leading in some cases to an accumulation of unavailable energy (130, 179).

These concepts can all be accommodated by the common event of a temperature-induced phase transition in the cellular membranes as the primary response in chilling injury.
Literature Cited


163. Spranger, E. 1941. *Gartenbauwissenschaft* 16:90–128
165. Ibid 1971. 48:166–70
185. Ibid 1938. 15:171–73
189. Ibid, 375–80
193. Wilkinson, B. G. 1970. See Ref. 61, 1:537–54
203. Ibid, 795–805
205. Yamaki, S., Uritani, I. *Plant Physiol.* In press