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Chilling sensitivity during tomato fruit ripening.

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CHILLING SENSITIVITY DURING TOMATO FRUIT RIPENING

A Dissertation Presented

By

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ABSTRACT

CHILLING SENSITIVITY DURING TOMATO FRUIT RIPENING

(May, 1985)

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Chilling injury is damage caused by suboptimal, above-freezing temperatures. Tomato (Lycopersicon esculentum Mill. cv Heinz 1350) fruits are sensitive to chilling temperatures, and in this study post-chilling ion leakage, respiratory activity, and ethylene biosynthesis were used to measure the degree of chilling injury of tomato fruits.

In the first experiment mature-green fruit were chilled for 0, 4, 8, 12, or 16 days at 2.5, 7.5, or 12.5 C, 12.5 being above the critical chilling temperature for tomatoes. As the chilling intensity or duration increased the level of ion leakage from chilled tissue increased. Ethylene biosynthesis was similarly stimulated but was more sensitive to the shorter chilling periods. Respiration was also stimulated similarly except at 7.5 C where there was no additional stimulation beyond 4 days.

In other experiments the effects of ripening on chilling sensitivity were observed. As the tomatoes began to ripen there was a decline in chilling sensitivity, as measured with ion

leakage, followed by an increase in chilling sensitivity during the later stages of ripening. The non-ripening mutant, nor, was used to assess chilling sensitivity in the absence of ripening, and it was found that chilling sensitivity increased during senescence. These data show that variation in chilling sensitivity during tomato ripening is biphasic, with a ripening-related decline followed by a senescence-related increase. The latter may be due to an increase in membrane viscosity which is a common feature of plant senescence.

Light and hormone treatments were found to have no effect on the chilling sensitivity of ripening tomatoes.

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INTRODUCTION

Many plant materials are injured or killed by temperatures that are suboptimal yet above freezing. The resulting damage was first described by Molisch in 1897 (70). He termed the damage "chilling injury". The critical temperature below which injury occurs may range from just above the freezing point of the tissue to 15-20 C (27). Many horticultural commodities of tropical or subtropical origin, e.g., avocados, bananas, grapefruits, mangoes, and tomatoes, are chilling-sensitive (18), as are several of temperate origin, e.g., apples, asparagus, cranberries, peaches, and plums (8).

Symptoms of chilling injury vary greatly from species to species but may include surface lesions, water-soaking, internal discoloration, tissue breakdown, abnormal ripening, accelerated senescence, increased decay, reduced storage life, compositional changes, or loss of growth capacity (72). Symptom intensity generally increases as the temperature is reduced or the duration of the chilling event is lengthened. Symptoms usually develop more rapidly if the chilled material is moved to nonchilling temperatures (52).

The importance of chilling injury to horticultural commodities is obvious. Plant material may be sensitive throughout development from germination to harvest. Chilling temperatures may prevent germination, alter seedling development, or affect reproductive growth (71). Chilling of fruit such as tomatoes prior to harvest can disrupt

normal ripening even at normal temperatures (71).

Chilling is of particular concern during the postharvest period. The need to reduce decay and slow ripening and senescence requires storage at as low a temperature as possible, but chilling sensitivity restricts storage below a commodity's critical chilling temperature. If the development of chilling injury as a result of cold storage was prevented the postharvest life of many commodities could be increased greatly.

This study utilized tomato fruit to study chilling sensitivity, because greenhouse-grown plants provide a year-round supply of fruit, and tomatoes are sensitive to relatively short chilling periods. The two major symptoms of chilling injury in tomatoes are an increased susceptibility to Alternaria rot (61) and a failure to ripen normally (62). An interesting aspect of chilling sensitivity in tomatoes is that it changes during ripening. Turning fruit (30% colored) are less sensitive to chilling than mature-green fruit (62). Suggested storage temperatures for mature-green fruit are between 13 and 18 C, whereas turning fruit may be stored at 10 C (102).

The major objective of this study was to characterize physiologically chilling sensitivity in tomatoes. This included determining the effects of temperature, duration of chilling, and ripening. Light treatments and hormone applications were performed to study endogenous hormone effects on chilling sensitivity. Also, the nonripening mutant, nor, was used to observe the effects of senescence, in the absence of ripening, on chilling sensitivity.

C H A P T E R II

LITERATURE REVIEW

Physiology of Chilling

The Importance of Membrane Phase Changes

It is well accepted that biological membranes are the site of the primary response to chilling (121). Potentially, any membrane system, e.g., mitochondria, chloroplasts, endoplasmic reticulum and plasmalemma, may be affected by chilling temperatures. The specific involvement of the membrane in chilling injury was first proposed by Lyons and Raison (54). Using mitochondria from chilling-sensitive and -resistant plant tissue they measured the effect of temperature on succinate oxidase activity. Arrhenius plots (log of the reaction rate vs. 1/T in K) of the data from chilling-resistant tissue gave straight lines from 1.5 to 25 C, but data from chilling-sensitive tissue exhibited a discontinuity at approximately the critical chilling temperature (see Figure 1). These data led Lyons and Raison (54) to propose a scenario for chilling injury using a membrane phase change as the primary response to chilling temperatures.

Lyons and Raison (54) suggested that the lipids of the mitochondria undergo a phase change in chilling-sensitive but not in chilling-resistant tissue. Above the critical temperature the lipids exist in a fluid or liquid crystalline state, but below the critical temperature they are in the more rigid solid gel state. Lyons and

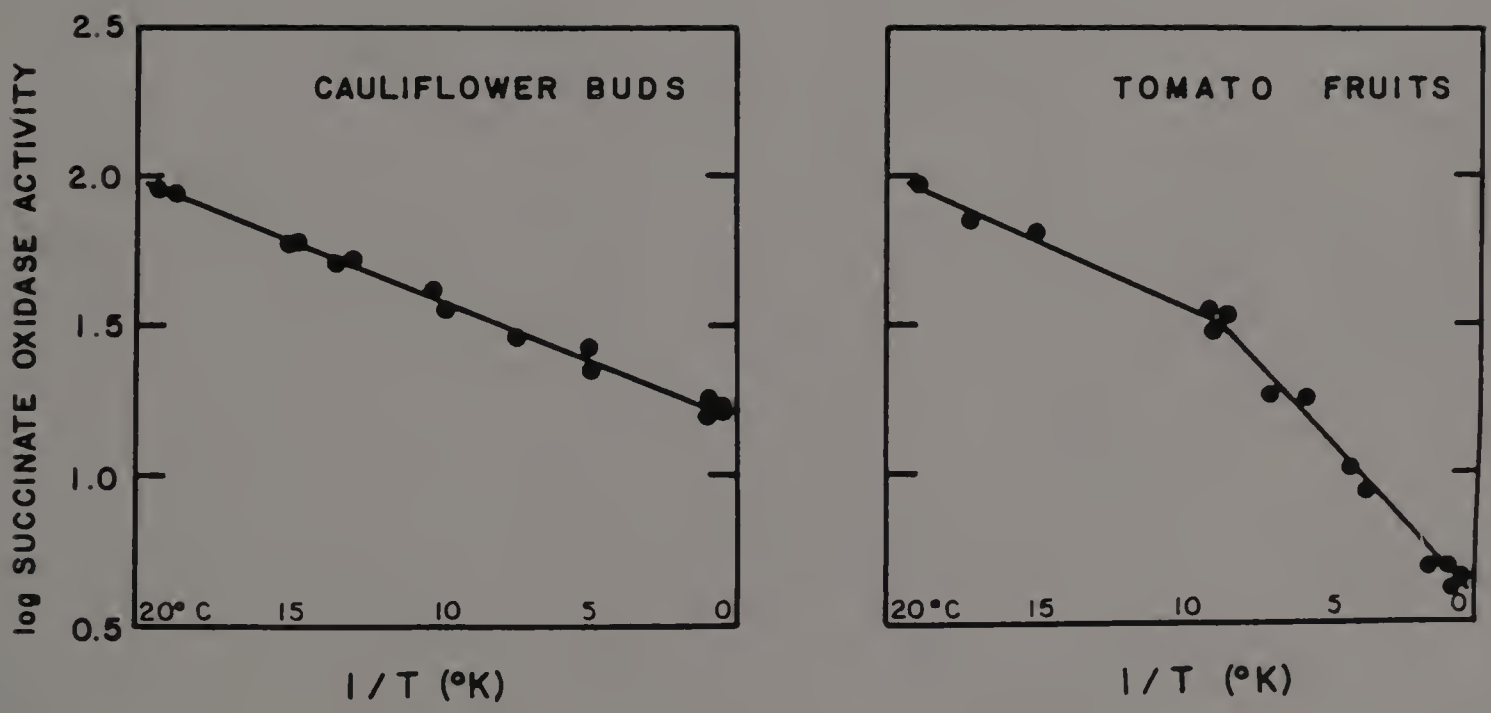

Figure 1: Arrhenius plots of succinate oxidase activity in mitochondria from chilling-resistant (cauliflower buds) and chilling-sensitive (tomato fruit) tissue. Adapted from Lyons and Raison (54).

Raison explain the discontinuity in the Arrhenius plot as a change in the oxidation rate possibly caused by reduced permeability to oxidizable substances when the membrane becomes more rigid. They further propose that reduced mitochondrial oxidation leads to the accumulation of metabolites and the reduction of ATP production, both of which could result in injury.

Raison et al. (94) used a membrane probe to study this problem in more detail. They used electron spin resonance spectroscopy (ESR), whereby a lipophilic, paramagnetic probe is introduced into a membrane fraction, and its mobility is measured throughout a temperature range. It was found that an apparent phase transition occurred in the mitochondrial membrane at a temperature corresponding to the temperature where the break occurred in the Arrhenius plot of succinate oxidase activity. This temperature was also the critical temperature for chilling injury. Wade et al. (120) found similar phase changes with ESR in protoplastids, glyoxysomes, and mitochondria. Both of these studies support the proposal of Lyons and Raison (54).

Several membrane systems and enzymes have been shown to respond similarly to chilling, e.g., cytochrome oxidase in mitochondria (127) and electron transport in thylakoids (75).

Lyons (52) outlined a more specific overall scheme for the development of chilling injury:

1) Chilling temperatures cause an overall phase transition of the cell membrane lipids.

2) a) The lipid phase change results in a conformational change in membrane-bound proteins, increasing their activation energies.

b) The phase change causes cracks to develop, increasing membrane permeability.

3) a) Increased permeability and increased activation energies of membrane-bound enzymes can cause imbalances in metabolism.

b) Increased activation energies can result in reduced ATP supplies.

c) Increased permeability can result in solute leakage.

4) If exposure is brief cells can recover, but if prolonged cell death can occur, leading to chilling injury symptoms.

Several studies have shown a correlation between the phase transition temperature, as observed by Arrhenius plots or ESR, and the chilling sensitivity of the tissue (e.g., 54, 93), but several inconsistencies also exist. McMurchie (65) observed the effects of temperature on K^+ stimulated Mg^{+2}-ATPases and found a break in the Arrhenius plot at about 10 C for both cucumber fruit (chilling-sensitive) and cauliflower buds (chilling-resistant). McGlasson and Raison (64) isolated mitochondria from several apple

cultivars with different chilling sensitivities. They measured the effects of temperature on succinate oxidase activity and ESR and found that all cultivars exhibited a break in the Arrhenius plot and showed an apparent phase transition, but there was no correlation between the temperature of the transition and chilling sensitivity.

The membrane phase transition theory of chilling injury as outlined by Lyons (52) has encountered several problems since its inception, primarily because of its oversimplification of the problem and its reliance on data which potentially have been incorrectly used. The problem with the data relates to the primary tool for detecting phase transitions, ESR. It was assumed that the probe partitions equally throughout the membrane, but it is more likely that it partitions into particular portions of the membrane (55). Changes in the mobility of the probe may actually be reflecting changes in discrete domains and not the entire membrane (55). Also, the ESR probe may be altering the properties of the membrane (5). These two concerns weaken the interpretation of the ESR data.

The major problem with the membrane phase transition theory is its oversimplification of the chilling response. It implies that a phase transition occurs throughout the entire membrane. However, the biological membrane is a heterogeneous system made of several lipid species and other components. As the temperature is lowered solidification may not occur throughout the membrane at the same temperature (28). Pike et al. (88) used a sensitive membrane probe, trans-parinnaric acid, which partitions only into solid phase lipids.

By studying fluorescence intensity and polarization the appearance of the first solid can be detected as a membrane vesicle is cooled. For a typical chilling-sensitive species solidification begins at about 10 C and is not complete until the temperature is below 0 C. Between the beginning and the end of solidification a phase separation exists.

The use of Arrhenius plots with straight lines is also in question. Bagnall and Wolfe (5) suggest that the use of straight lines with abrupt discontinuities is incorrect. In a complex membrane system several fluidities coexist at a given temperature. Different molecules of a specific protein may reside in regions of different fluidity (5, 134). As the temperature is reduced a gradual change in overall activation energy of particular enzyme should be seen through the region of phase separation, so Arrhenius plots should show curved lines through the phase separation temperatures (5).

Raison and Chapman (92) used 3 line segments (i.e., 6 parameters) to fit data on an Arrhenius plot. The first line is thought to describe the change in rate when the membrane is entirely liquid, the second when both phases exist, and the third when only solid phase exists. Bagnall and Wolfe (5) fit similar data to both a fifth order polynomial (6 parameters) and 3 straight lines and found a better fit with the curved relationship. The difference was not large enough to reject the 3-line model, but the curved relationship is more reasonable in terms of membrane physiology.

Visualizing membrane changes as gradual phase separations and Arrhenius plots as curves complicates the chilling picture

considerably. Specific temperatures cannot be chosen from Arrhenius plots as critical temperatures for chilling to occur. Even if the lipids surrounding a particular enzyme undergo a phase transition at the same temperature, the importance of that one change is questionable because of the complexity of the cellular system. Several different processes are probably altered by chilling and are likely sensitive to different temperatures.

Membrane Components and Chilling Sensitivity

As suggested earlier cell membranes are extremely complex systems containing several different components. Membrane composition is important in determining the physical and biological properties of the membrane including chilling sensitivity. The three major constituents of cell membranes are phospholipids, sterols, and proteins. Phospholipids and sterols will be discussed in terms of their effects on chilling sensitivity. Protein effects have been presented thoroughly in other reviews (11) and will not be discussed here.

Phospholipids. Phospholipids are the backbone of the cell membrane. They consist of a polar head group with attached fatty acyl chains. Each portion can affect the chilling sensitivity of the cell. It was noted many years ago that plants and animals native to warm climates have more saturated fats than similar organisms from cool climates (87). The significance of this observation was not fully understood until the structure of cell membranes became clear.

Richardson and Tappel (95) compared cold-blooded (fish) and warm-blooded (rat) animals and found that mitochondrial membranes from fish contained higher degrees of unsaturation. This property allows the membrane to remain fluid at lower temperatures, because the higher the degree of unsaturation of a fatty acid the lower the transition temperature.

Lyons et al. (56) compared mitochondria from several chilling-sensitive and -resistant plants and found that in general chilling-resistant plants had higher degrees of unsaturation in their mitochondrial membranes. Using an artificial system Lyons and Asmundson (53) showed that very small changes in the degree of unsaturation have large effects on the phase transition temperature of the lipids (see Figure 2).

Several studies have shown a correlation between chilling-resistance and the degree of unsaturation of fatty acids in the cell membranes, e.g., Tabacchi et al. (114) with tomato cultivars and Kimura et al. (42) with apple cultivars. Other studies have shown that treatments which alter the degree of unsaturation also alter chilling sensitivity.

The phase transition temperature of the mitochondrial membrane from sheep liver cells can be altered by changing the diet to cause different degrees of saturation in the membrane (52). Supplementing the growth medium of some microorganisms with saturated fatty acids can shift the phase transition temperature above the normal minimum growth temperature thus inhibiting growth (63).

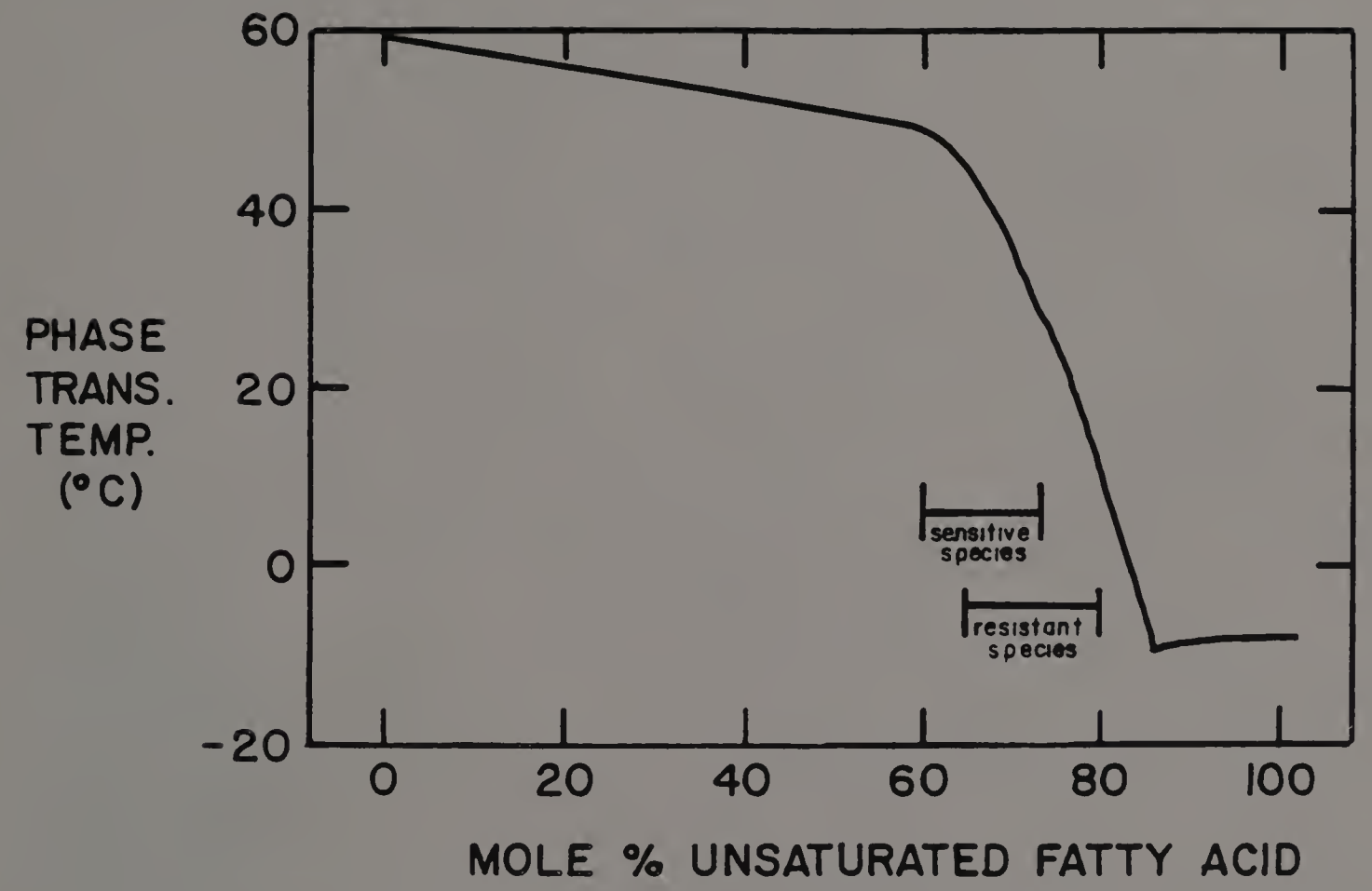

Figure 2: The phase transition temperature as affected by the degree of unsaturation. Adapted from Lyons and Asmundson (53).

Wang and Baker (124) found that treatment of cucumber and sweet pepper fruit with sodium benzoate or ethoxyquin reduced the degree of lipid saturation and also reduced chilling sensitivity. They also found that intermittent warming during chilling, which causes desaturation (121), reduced chilling injury. Treatment of tomato seedlings with ethanolamine-tween-oleate decreased chilling sensitivity, presumably due to a replacement of saturated C16:0 fatty acid with various 18 carbon fatty acids (126).

Chemical treatment can also increase chilling sensitivity. St. John and Christianson (111) treated cotton root tips with a pyridazinone analog which resulted in increased chilling sensitivity. They correlated this change with a shift from linolenic acid (C18:3) to linoleic acid (C18:2), i.e., saturation.

Another method by which chilling resistance may be increased is via cold hardening, i.e., keeping an organism at a temperature just above the critical chilling temperature. Generally, cold hardening causes desaturation (27). Wheat seedlings (67), rape plants (106), rape seeds, flax stems, flax seeds, sunflower seeds, and potato tubers (60) can all respond to cold temperatures by the desaturation of fatty acids. Several microorganisms (74, 83, 116) respond to cold temperatures in the same way.

The advantages of hardening to a plant are obvious. As the temperature drops, membranes become less fluid. It is advantageous for the plant to maintain a higher fluidity and that may be accomplished by reducing the degree of saturation in its fatty acids

(60). This response may be accomplished by increasing the biosynthesis of unsaturated fatty acids and the activity of membrane-bound desaturases (60). It has been suggested (60) that the change in fluidity is the trigger for increasing the activity of the appropriate enzymes. Chemical inhibitors of fatty acyl desaturation can prevent hardening (110).

The above discussion suggests that the degree of lipid saturation can be a very important determinant of chilling sensitivity, and in many cases it is, but there are also several exceptions to this hypothesis.

Sweet potatoes are much more chilling-sensitive than white potatoes but have less saturation in their mitochondrial membranes (136). Soybeans and peas have similar amounts of lipid unsaturation and greatly different chilling sensitivities (90). Chilling sensitivity in Passiflora species does not correlate with the degree of saturation (84).

Also, hardening does not necessarily cause desaturation (133). Natural acclimation of strawberry plants is not accompanied by desaturation (81). Wilson (132) showed that cold-hardening of beans resulted in desaturation of lipids and decreased chilling sensitivity, but drought-hardening also resulted in decreased chilling sensitivity without any change in the degree of saturation.

These exceptions question the importance of the degree of lipid saturation in determining the degree of chilling sensitivity, but it is important to note that these studies measured bulk fatty acid

concentrations, and this type of analysis does not adequately describe the character of the membrane (121). The character of specific domains within the membrane may be more important in governing chilling sensitivity than the overall character of the membrane (121).

Little is known regarding the effects of the phospholipid polar head groups on chilling sensitivity, but it has been shown that phosphatidylcholine (PC) has a lower phase transition termperature than phosphatidylethanolamine (PE) with the same fatty acyl chains (12, 20). It has also been shown that the polar head groups can affect membrane-bound enzyme activity (55). Work with apples (42) has suggested that chilling-sensitive cultivars have a higher percent PC and a lower percent PE, but the results were confounded with differences in degree of lipid saturation. More work will have to be done to assess fully the contribution of the polar head groups to governing chilling sensitivity.

Sterols. Sterols are another major component of biological membranes. Their effect on chilling sensitivity has not been assessed, but studies have shown that sterols affect the physical nature of membranes (21).

Gottlieb and Earnes (26) used erythracyte membrane lipids with the cholesterol removed to study the effects of increasing the cholesterol concentration on the phase transition temperature. With no cholesterol the lipids underwent a phase transition between 2 and 20 C. As the cholesterol concentration was increased to 7.3% the phase transition temperature dropped below -10 C. A change in the

sterol concentration of a membrane can drastically alter the phase transition temperature and potentially, chilling sensitivity. On the other hand, senescence studies (7, 113, 117) have suggested that increasing the sterol concentration of a membrane reduces membrane fluidity, and a less fluid membrane would be expected to be more sensitive to chilling injury. Even if sterols reduce the temperature of a phase change, a reduction in fluidity ,i.e., an increase in membrane rigidity, could have detrimental effects. These sterol properties may be important in altering chilling sensitivity.

Proteins and Chilling

Many proteins are affected by chilling temperatures, some directly and others via membrane phase changes. Graham and Patterson (27) thoroughly reviewed some of these effects including specific enzyme activity changes which lead to injury, alterations in Km's and Vmax's, and changes in the affinities of enzymes for activators and inhibitors. There are also several direct protein effects (27): dissociation of polymeric enzymes (22, 32, 46, 112, 118) and direct conformational changes (10). For a more detailed discussion see Graham and Patterson (27).

Hormonal Effects on Chilling

Hormones are involved with many plant processes. Chilling sensitivity, in many cases, can be altered with hormone treatments, but the effects are not well understood. Abscisic acid

(AbA) has probably the best documented effect on chilling sensitivity.

One of the symptoms of chilling injury in cucumber seedlings is wilting caused by a chilling-induced water stress. Rikin and Richmond (99) found that AbA treatments prior to chilling reduced chilling injury, presumably by causing stomatal closure and thus reducing the water stress. In a later study Rikin and Richmond (100) chilled cucumber seedlings in conditions which prevented the development of water stress and found that AbA was still able to reduce chilling injury. This study showed that AbA may be having a physiological effect on chilling sensitivity. Markhart (57) had similar findings when he chilled soybean roots. Root treatments of AbA do not affect stomatal closure but do reduce chilling injury. Markhart suggested that AbA increases root hydraulic conductivity possibly by altering membrane ion transport.

From a series of studies, Rikin et al. (97, 98) proposed that AbA may reduce chilling injury by secondarily protecting the membrane from oxidation. They found that cotton seedlings were sensitive to chilling. Darkness or treatment with dichlorophenyl dimethyl urea (DCMU), an inhibitor of photosynthetic electron transport, in the light increased chilling sensitivity, whereas AbA treatment in the light or with sucrose decreased sensitivity. They noted that release of tubulin from the membrane and the subsequent disassembly of microtubules accompanied chilling injury (97). AbA in the presence of light or sucrose reduced the release of tubulin and disassembly of microtubules. Rikin et al. (98) also noted that NADPH levels

correlated with cold hardiness, and the loss of reduced glutathione (GSH) was an early response to chilling. These two points tie directly into their theory. They suggest that AbA causes the closing of stomates and prevents CO_2 entry. Light or sugar result in an accumulation of NADPH which maintains high levels of GSH, which can protect the membrane by scavenging oxidizing compounds. Unfortunately, this theory does not account for AbA's effect without stomatal closure or in callus suspensions. The common factor may be the alteration of ion balances across membranes.

The chilling-resistance of several plant materials can be increased with AbA, e.g., rye and wheat cell cultures (13), tobacco callus (6), grapefruit (39), and potato leaves (14).

In one case AbA increased chilling sensitivity. Wills and Scott (131) found that injection of AbA into apples increased the incidence of low temperature breakdown (LTB), a type of chilling injury. Also, treatments which increase the endogenous AbA content increased the incidence of LTB (27). Why apples are affected this way by AbA is unknown, but it may relate to the nature of the injury. LTB forms after long periods of chilling, and this type of injury may be completely different from the short term effects seen with chilling-sensitive seedlings.

Other hormones alter chilling sensitivity, but the systems have not been studied as thoroughly as with AbA.

Gibberellins (GA) are known to affect many plant processes. Most work with chilling sensitivity has been with pome fruits.

Dipping of Jonathan apples in GA solutions reduced the incidence of LTB after storage at -1 C (27, 129, 130, 131). Also, inhibitors of isoprenoid metabolism increased LTB, presumably by reducing GA biosynthesis (129). Also, in Bartlett pears GA can prevent chilling-induced premature ripening (125).

As with AbA, there are inconsistencies in the response of chilling sensitivity to GA treatment. Flesh browning is a chilling injury symptom found in Delicious apples stored at -0.5 C. GA dips have no effect on this injury (66). It is possible that GA was not taken into the cells.

The effects of ethylene on chilling sensitivity are extremely varied. Chilling injury is increased by ethylene treatment of avocados (11, 140) and grapefruit (33) and reduced by treatment of sweet potatoes (9) and muskmelons (51). In mature-green and breaker tomatoes ethylene appears to have no effect on chilling injury (37).

Auxin also can increase or decrease chilling sensitivity. IAA treatment to sorghum seedling reduced chilling-induced wilting (115). Tajima and Shimazu (115) suggest that in those species where auxin is reducing chilling injury IAA may be increasing 3',5'-cAMP and maintaining membrane stability. Omran (80) noted that IAA oxidase increased during chilling of cucumber seedlings. If IAA levels fall as a result of oxidation, chilling injury may be allowed to occur. Auxins may also increase chilling sensitivity as with LTB in apples, where IAA or NAA injections resulted in significantly more chilling injury (131). In grapefruit postharvest applications of 2,4-D

increased chilling injury, but preharvest treatments reduced chilling injury (36).

Few studies have assessed the effects of cytokinins on chilling sensitivity. Ismail and Grierson (36) found that BA reduced chilling sensitivity if applied preharvest and increased sensitivity if applied postharvest. Thiabendazole and benomyl, which have cytokinin-like properties (104), reduced chilling injury when applied in the wax coating of grapefruit (103). This apparent difference could be due to the treatment method or the chemical used.

The effects of hormones other than AbA on chilling sensitivity are so poorly understood that it is difficult to suggest any specific involvements with chilling sensitivity. Observed alterations in sensitivity could be from secondary effects. Inconsistencies could be due to variations in the chemicals used, concentrations, timing, or any number of differences in treatment methods. Plant hormones potentially may be valuable for reducing chilling sensitivity, but more research must be done to assess fully the responses.

Assessing Chilling Sensitivity

The development of chilling injury symptoms is one method by which chilling sensitivity can be assessed, but the difficulty with quantification and the lack of consistency make the collection of good data difficult. Quantifiable physiological changes are much more reliable tools for measuring sensitivity. Membrane alterations are

undoubtedly the major or primary response to chilling. Several secondary responses are also known and potentially could be used to assess chilling injury. These include (121): stimulation of ethylene biosynthesis, increased respiration, slowing of protoplasmic streaming, depression of photosynthesis, alteration of energy production, disturbances of cell structure, increased activation energies of membrane-bound enzymes, and increased membrane permeability. In this study ethylene production, respiration, and ion leakage were used to assess chilling sensitivity. Their involvement with chilling will be discussed.

Membrane Permeability

Leakage of cellular components from chilled tissue due to increased membrane permeability has been observed in several chilling-sensitive tissues (e.g., 15, 43, 48, 49, 77, 85, 86). The degree of leakage can be correlated with chilling sensitivity. Lewis and Workman (48) showed that chilled tomatoes (chilling-sensitive) had much higher levels of ion leakage than fruit which had not been chilled, whereas chilled cabbage leaves (chilling-resistant) showed no increase in ion leakage. Patterson et al. (85) correlated ion leakage in response to chilling with the chilling sensitivity of several *Passiflora* species. The leakage from tomato leaves (86) and fruit (43) in response to chilling has also been correlated with the sensitivity of particular cultivars.

Increased leakage of cellular components can occur during the

chilling treatment (2, 15, 25, 77, 85, 86) or only after warming (43, 48, 49, 135). Tomato fruit tissue, for example, did not exhibit any increased leakage during storage at 2 C (77), but after warming leakage increased greatly (43, 48). Tomato leaf tissue, on the other hand, showed increased leakage within 1 hour of being placed in the chilling treatment (86).

If membrane permeability is increased immediately upon chilling the formation of cracks or unstable zones in the membrane could cause the increase (135). This view is supported by work with cotton seedling radicles (15) where calcium treatment can prevent the chilling-induced enhancement of leakage. Calcium is known to stabilize the membrane and may prevent the formation of unstable areas in the membranes of cotton radicles.

The formation of cracks or unstable regions cannot explain delayed increases in leakage or increases only occurring after warming. A delay in enhanced leakage can be explained by a simple scenario involving ATP production (27). Chilling may be disrupting ATP production by altering the enzymes of electron transport in the mitochondria. As the ATP levels drop the ionic gradients normally maintained are disturbed, so reduced ATP could result in passive ion leakage. This may also explain the post-chilling leakage response. Warming may increase several metabolic activities utilizing much of the available ATP. Electron transport may not be able to recover prior to an increase in passive leakage.

Yoshida et al. (138) proposed that leakage from the vacuole

could be a major chilling response. Their scheme could explain some systems which do not respond immediately to chilling. They suggest that chilling causes perturbations of the vacuolar membrane resulting in the release of salts and protons. This release disrupts mitochondrial function which reduces ATP production. The rough endoplasmic reticulum counters the release to some degree by uptake of the components, but ATP may be utilized for the uptake. Prolonging the chilling treatment results in further release of vacuolar contents including lytic enzymes. The lytic enzymes may play an active role in reducing plasmalemma integrity resulting in leakage. Also, reduction of ATP supply may cause passive leakage. This scenario is intriguing but has not yet been fully substantiated.

Regardless of the mechanism, ion leakage can be used to assess the degree of chilling injury in plant tissue.

Respiration

Respiration is the process which oxidizes sugars to yield CO_2 and ATP. It is the sum of 3 systems: glycolysis, the tricarboxylic acid cycle, and oxidative phosphorylation (47). Glycolysis is generally thought to occur in the cytoplasm (47). The TCA cycle is located in the mitochondrial matrix, and oxidative phosphorylation occurs through the inner membrane of the mitochondria (47). Chilling may affect any of these processes, but this discussion will concentrate on general effects of chilling on respiration. Specific effects on oxidative phosphorylation will also be discussed.

Many studies have shown that chilling may induce changes in respiratory activity particularly when the data are plotted on an Arrhenius plot. For chilling-sensitive tissue the plot is not linear (see Figure 1). Whether it is a curve or a series of straight lines is in question (5), but these plots suggest an alteration in activity.

In an early study with cucumber fruit Eaks and Morris (24) showed that respiration, as measured by CO_2 production, was actually stimulated during the early stages of chilling. When these fruit were warmed there was an even greater stimulation of respiration. Other studies with corn seedlings (19), banana pulp (76), and lemon fruit (23) have shown a similar post-chilling burst in respiratory activity. Creencia and Bramlage (103) suggested that chilling results in some perturbation of oxidative phosphorylation.

Oxidative phosphorylation is an electron transport system located in the inner membrane of mitochondria (47). The general flow of electrons is as follows: substrate to NAD to Fp to Coenzyme Q (CoQ) to Cyt b to Cyt c to Cyt aa_3 to O_2 (47). The conversion of ADP to ATP is coupled to the pathway at 3 points: one prior to CoQ and the other two after CoQ (47). An alternate electron transport system has CoQ giving its electrons to another flavoprotein which in turn releases them to O_2 (107). It can be seen that the alternate pathway is coupled to only one phosphorylation (107). Cyanide can trigger the alternate pathway by blocking the cytochrome system between Cyt aa_3 and O_2 (47). The alternate pathway is commonly called cyanide-insensitive respiration. When triggered it would yield

similar respiratory quotients to the cytochrome system, but the respiratory control ratio (RCR=ADP/O) is drastically reduced depending on the completeness of the cytochrome pathway block.

To study the contribution of the alternate pathway salicylhydroxamic acid (SHAM) may be used to block electron flow through the alternate pathway (107). Two studies will be discussed here, one to assess the contribution of the alternate pathway during chilling and one to determine its contribution after chilling.

Yoshida and Tagawa (139) used a chilling-sensitive callus (Cornus) and a chilling-resistant callus (Sambucus). Arrhenius plots of succinate oxidation showed no breakpoint for either callus. When SHAM was added to both, the Sambucus again showed no breakpoint, but Cornus callus gave a definite breakpoint at 13 C. They suggested that alternate respiration was engaged below 13 C in the Cornus callus. SHAM inhibited respiration of Cornus callus at all temperatures greater that 13 C, and as the temperature was reduced below 13 C the inhibition increased concomitantly. Similar results were obtained for both intact callus and isolated mitochondria. The temperature of this breakpoint coincided with the critical chilling temperature.

This study presented clear evidence that in a chilling-sensitive plant the alternate electron transport system accepts an increased portion of the electrons with chilling. Respiration in the presence of KCN (i.e., alternate respiration) gave a linear Arrhenius plot. Respiration in the presence of SHAM (i.e., cytochrome pathway) showed a clear break in the Arrhenius plot. The authors stated that this

observation is evidence that the 2 systems occur in portions of the membrane which are affected differently by temperature. Since CoQ is a small, hydrophobic molecule it may be able to move between the two systems within the membrane.

Purvis (91) showed that SHAM can inhibit respiration during chilling of grapefruit (chilling-sensitive), whereas it had no effect on respiration of oranges (less chilling-sensitive). These data support the suggestions of Yoshida and Tagawa (139).

The post-chilling respiratory burst is generally greater as chilling sensitivity increases and may be used as an index of chilling injury (24). Kiener and Bramlage (41) studied the nature of this burst. They chilled 8-day-old cucumber seedlings and measured hypocotyl respiration (O_2 consumption) in the presence of carbonyl cyanide chlorophenyl hydrazone (CCCP,an uncoupler of oxidative phosphorylation), SHAM, and KCN during recovery at 25 C. Respiration rose to a maximum of 140% of the control in 3 hours, after which it dropped gradually. CCCP was less effective at the peak of the burst and became more effective during the 24 hour recovery period. SHAM was most effective at the peak and had virtually no effect after 24 hours. KCN seemed to be equivalently effective throughout the recovery period. These data suggest, first of all, that there is some degree of uncoupling at the respiratory peak, which may account for some of the burst. Also, the authors suggest that alternate respiration is at its maximum during and accounts for a large portion (50%) of the respiratory burst. They also calculated the percentage

of the capacity of the alternate pathway used without chilling, during the burst of respiration, and after recovery. The largest portion of the capacity was used during the burst, and virtually none of it was used in unchilled tissue and after recovery.

These data are evidence that the post-chilling respiratory burst is at least in part due to alternate respiration. Kiener and Bramlage (41) suggest that membrane phase changes may be the reason for the shift from the cytochrome pathway to the alternate pathway. Their reasoning relates to the number of members of each, in that the cytochrome pathway has many more members then does the alternate pathway. It may be easier for electrons to pass through the alternate pathway when membrane phase changes occur. They also speculate on the reasons for the alternate pathway's existence. The first is that the alternate pathway provides a means for electron flow when the cytochrome system is depressed, allowing TCA cycle activity without the accumulation of glycolysis products. Another reason relates to the thermogenic properties of the alternate pathway which may increase the temperature of the mitochondria and avoid membrane phase changes. The actual role of alternate respiration in chilling is unknown but definitely is active both during and after chilling.

Measurement of respiration provides a relatively easy method to measure the degree of chilling injury.

Ethylene Biosynthesis

Several studies have shown that ethylene biosynthesis increases

after a plant is stressed (137). Hanson and Kende (31) and Natti and Loy (78) showed that wounding caused increased ethylene production Apelbaum and Yang (4) showed that water stress could also cause increased ethylene production. These studies used aminoethoxyvinylglycine (AVG), an inhibitor of ethylene production from methionine, to demonstrate that methionine was the source of increased ethylene biosynthesis. Chilling of sensitive plants also causes increased ethylene biosynthesis (17). Kiener and Bramlage (41) with cucumber seedlings and Wang and Adams (122) with cucumber fruit found that AVG blocked the increase in ethylene production after chilling. These results confirm that methionine is the source of chilling-induced ethylene production.

The function of the post-chilling increase in ethylene biosynthesis is unknown. It was postulated by Solomos (107) that ethylene functions to alter the flow of electrons through the cytochrome system so as to increase the flow through the alternate pathway. Kiener and Bramlage (41) treated cucumber seedlings with AVG and found that chilling did not stimulate ethylene biosynthesis but still increased the flow of electrons through the alternate pathway. These results show that ethylene does not function as Solomos (107) proposed.

In most plant tissues ethylene biosynthesis follows the pathway: methionine to S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylate (ACC) to ethylene (3). As mentioned above chilling stimulates flow through this pathway. Wang and Adams

(122, 123) studied ethylene biosynthesis in relation to chilling. They chilled cucumber fruit at 2.5 C for varying lengths of time and found a post-chilling burst in ethylene biosynthesis, peaking 7 hours after the termination of chilling. In this tissue ACC stimulated ethylene production suggesting that ACC is the limiting component. Data from treatments with transcription and translation inhibitors suggest that chilling triggers the production of mRNA for ACC synthase (which catalyses the conversion of SAM to ACC) but does not allow translation. During warming the message is rapidly translated ultimately increasing ethylene production. How chilling stimulates mRNA synthesis is unknown, but it is interesting to see a possible effect on gene expression.

The post-chilling stimulation in ethylene biosynthesis provides a very rapid means to measure the degree of chilling injury.

Tomatoes

Tomato Fruit Ripening

Tomato fruit, once mature, may be harvested any time during ripening. For transport they are normally harvested at a "mature-green" stage. Many physiological and biochemical changes occur during ripening, with the most prominent being red color development. Characteristically, tomatoes show a loss of chlorophyll along with an increase in carotenoid pigments, particularly lycopene (40). Color development has been used to describe specific stages of

ripening (102). "Mature-green" (MG) is the stage where a fruit is physiologically mature but has no sign of color other than green. When the fruit begins to color at the blossom end and less than 10% is light-yellow, pink, or red it is said to be at the "breaker" (BR) stage. At the "turning" (TU) stage 10 to 30% is colored. "Pink" (PI) is 30 to 60% colored. "Light-red" (LR) has between 60 and 90% color, and "red" (R) has greater that 90% of the fruit colored. This system provides an easily identifiable index of ripening.

Tomatoes are a climacteric fruit, exhibiting a rapid rise, peak, and fall in respiratory rate during ripening (96). The climacteric peak occurs approximately at the TU stage. Paralleling the respiratory climacteric is a rise and fall in ethylene biosynthesis. Reported changes in ascorbate, soluble solids, polygalacturonases, firmness, starch, and pH are shown on Figure 3. Other biochemical changes include a rise in membrane phospholipids throughout ripening (38) and a decline in membrane sterols until the PI stage, with a subsequent rise (59).

All the major classes of hormones vary during ripening. Figure 4 shows the levels of ethylene, auxin, cytokinin, GA, and AbA throughout ripening. Also, levels of AbA and possibly GA can be altered by ripening under different wavelengths of light (40). Figure 5 shows the effects of red and far-red light on AbA concentration.

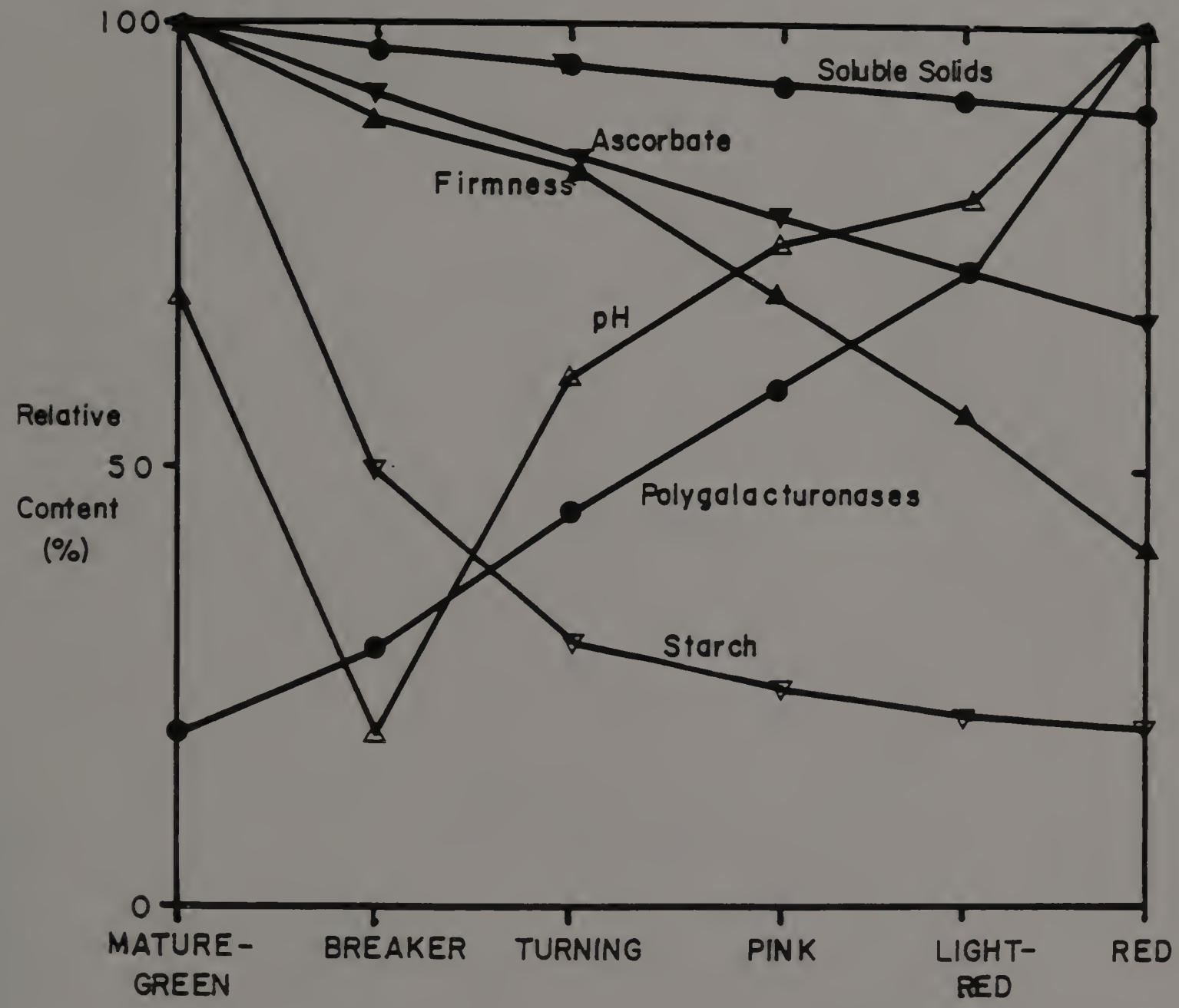

Figure 3: Biochemical and physical changes associated with tomato fruit ripening. Adapted from Rick (96).

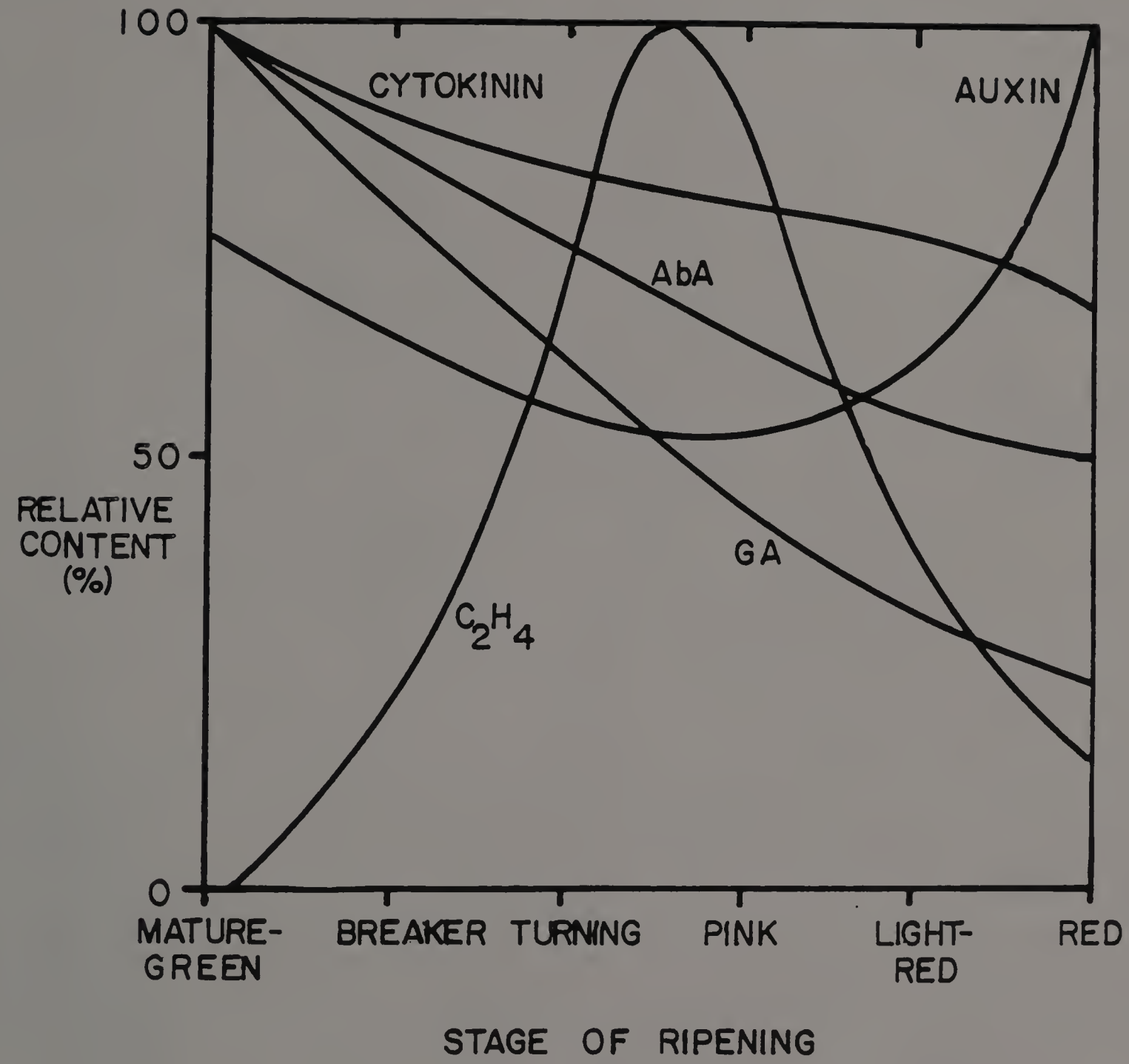

Figure 4: Hormonal changes associated with tomato fruit ripening. Ethylene measurements are from our own work. AbA, auxin, cytokinin, and GA levels were adapted from Abdel-Rahman (1).

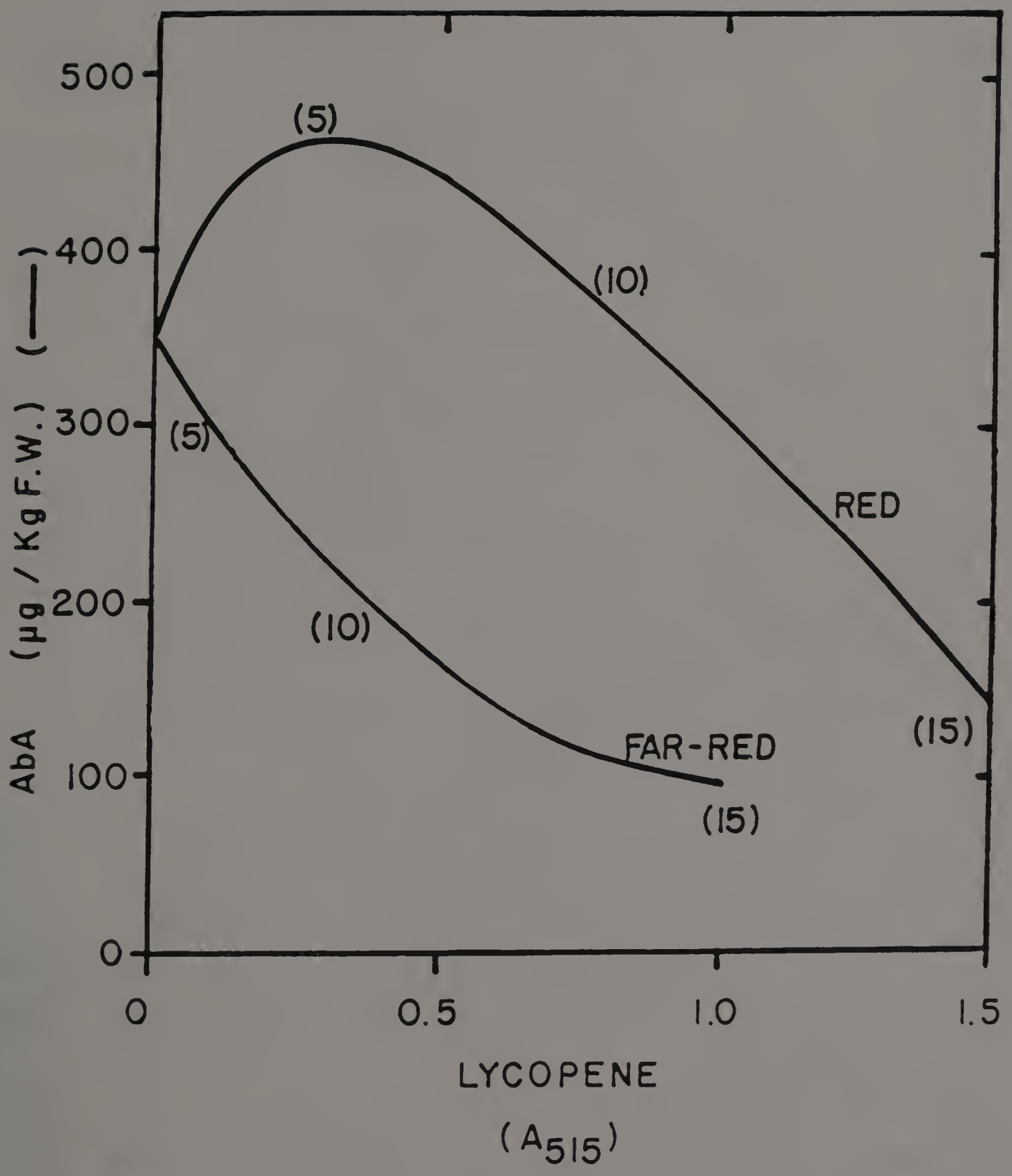

Figure 5: The effects of red and far-red light on the AbA content of ripening tomato fruits (40). Both red and far-red treated fruit produced similar amounts of ethylene as they colored. Numbers in parentheses are days after harvest.

Tomato Chilling

The two major symptoms of chilling injury in tomato fruit are increased susceptibility to Alternaria rot and failure to ripen normally. Several studies (e.g., 16, 61, 62) have noted an increase in decay caused by Alternaria after the chilling of tomatoes. McColloch and Worthington (61) quantified the effect by showing that the diameter of lesions caused by Alternaria increased as the temperature of storage was decreased or the length of the storage at 0 C was increased.

Abnormal ripening includes less consistent ripening among fruits, slower ripening, and uneven color development when ripe (62). The effects of chilling on ripening were quantified by Rosa (101). He found that MG fruit, after 25 days at 8 C, required 9.2 days to ripen at 20 C, whereas control fruit ripened in 7 days. Also, only 32% of the fruit were able to ripen at all. If the temperature was reduced to 0 C no fruit were able to ripen after storage. Other studies (16, 126) have shown similar results. Hall (30) showed that only 1 day at 4.4 C could reduce the ultimate lycopene contentof fruits.

Moline (69) found that one of the first observable ultrastructural changes (after 10 days) in MG tomatoes held at 2 C was the interference with the conversion of chloroplasts to chromoplasts. This alteration may be the reason for abnormal color development. After 15 days at 2 C mitochondria and plastids swell and degenerate, and after 21 days organelles are barely discernible. Increased susceptibility to Alternaria rot may be in some way related to general

membrane degradation.

An interesting aspect of tomato chilling sensitivity is that it decreases during ripening (102). McColloch et al. (62) showed that the effect of chilling on abnormal ripening and Alternaria rot declined from the MG to the TU stage. Fifty-six % of MG fruit chilled for 2 weeks at 0 C were able to color and 82% developed Alternaria rot after 12 days at 20 C, whereas under the same conditions 80% of PI fruit were able to color and only 42% developed Alternaria rot. Stenvers and Stork (109) have shown a similar relationship throughout ripening. Hobson (34) presented data to the contrary, where he showed that MG fruit could ripen better than TU fruit after chilling at 2 C and holding at 20 C. Hobson's results are questionable because of the subjectivity of his data and the small number of fruit used.

This apparent change in chilling sensitivity is particularly interesting, because some aspect of the fruit's physiology which alters chilling sensitivity is changing during ripening. One of the goals of this dissertation was to study why this change occurs. Obviously, endogenous hormone levels may be responsible for changes in chilling sensitivity. Specifically, the effects of AbA and GA on the chilling sensitivity of tomato fruit were studied. Also, a proposal using changes in membrane viscosity to account for alterations in chilling sensitivity, will be discussed.

C H A P T E R III

MATERIALS AND METHODS

Plant Materials

During the course of this study tomato fruit were obtained from both field-grown and greenhouse-grown plants. Heinz 1350 was the cultivar used throughout all experiments except those using the nonripening mutant, *nor*. *Nor* seeds were obtained from E. C. Tigchelaar, Purdue University, Lafayette, IN. All greenhouse plants were grown in a 2:1:1 mixture of soil, peatmoss, and perlite in 7.5 l plastic pots. They were fertilized weekly using $Ca(NO_3)_2$ solution (600 ppm Ca) alternating with a soluble 20-20-20 fertilizer (about 700 ppm N). Field plants were set on June 1 and fertilized with a 10-10-10 commercial preparation as needed. All plants were pruned to a single stem and maintained on 1.5 m wooden stakes.

Sensitivity Measurement

To measure the degree of injury after a chilling treatment three different types of analyses were performed. In all experiments ion leakage was measured, and in most experiments respiration and ethylene biosynthesis were also measured. The physiological bases for the use of these methods are given in the Literature Review.

A set of experiments was conducted using tissue disks from the

outer pericarp wall of tomatoes. The tissue was kept in citrate buffer (58) or distilled water during chilling. Ion leakage, respiration, and ethylene biosynthesis were measured after the termination of the chilling treatment. The potential advantages of this system include a more rapid measurement of response to chilling because of the small tissue units, and the ability to introduce chemicals easily. The inherent variability of the system rendered it useless, so whole fruit were used for all experiments.

Ion Leakage

After removal from the chilling treatment fruit were quartered and seeded. Ten 8 mm disks (3-4g) were cut from the outer pericarp wall of each fruit with a cork borer. The disks were then placed into approximately 50 ml of tap water for about 2 minutes. Next, they were rinsed for 4 seconds in running tap water and rinsed once in deionized-distilled water. After the rinsing process the 10 disks were placed into 100 ml of leakage solution. An osmoticum was used to avoid potential osmotic problems. One experiment included deionized-distilled water as a leakage solution. Figure 6 shows a comparison of different concentrations of sucrose and manitol. Sucrose at 0.3 M was chosen for these experiments.

Ion leakage after 4 hours at room temperature was quantified by measuring the electrical conductivity of the solution using a Markson Electromark Analyzer equipped with a no. 1100 conductivity cell. After the conductivity was determined samples were frozen for 48 hours

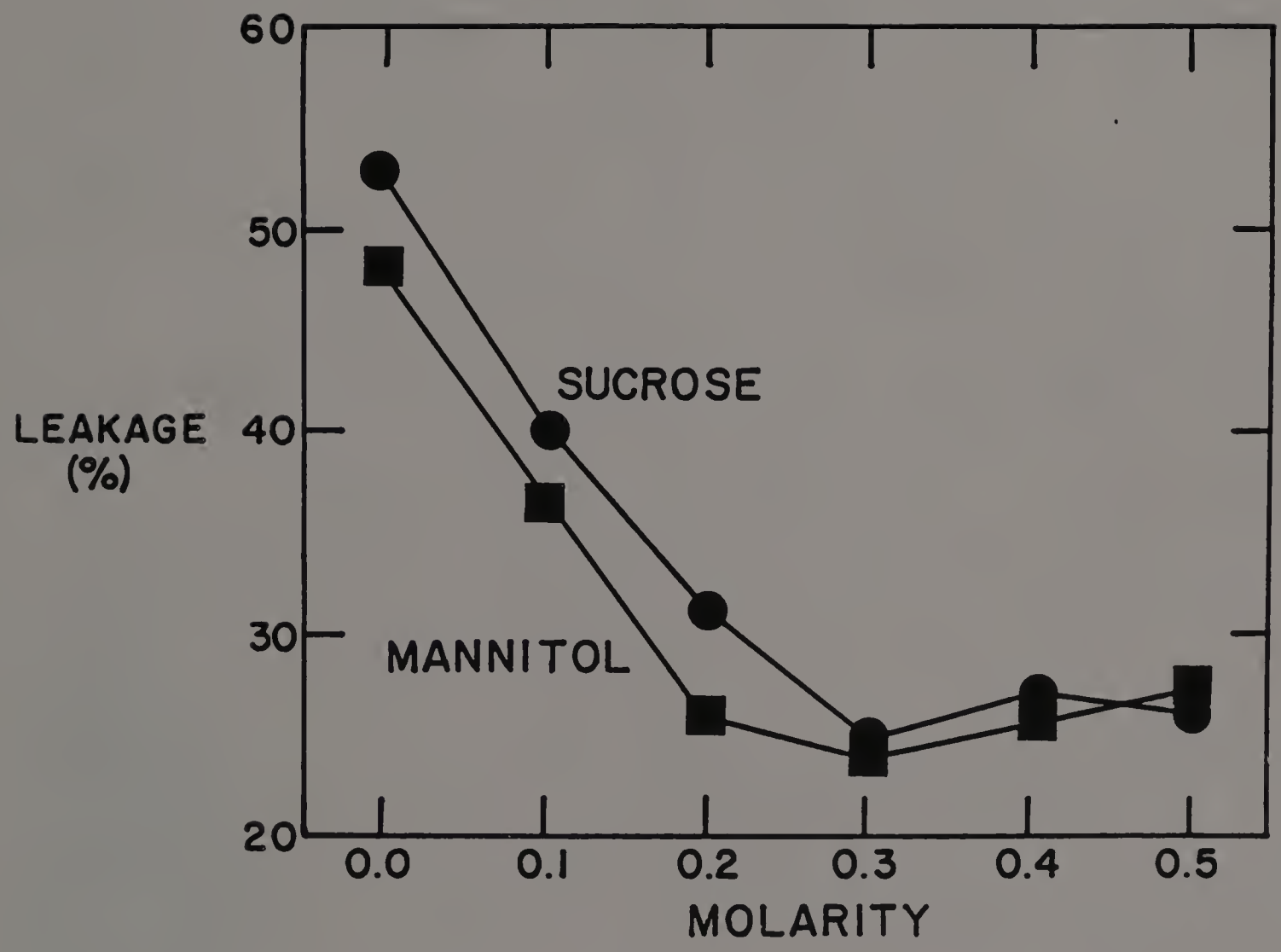

Figure 6: Ion leakage from mature-green fruit held 3 hours in different sucrose and mannitol solutions.

at -15 C to destroy cellular integrity. After thawing the conductivity of the solution represents the total possible leakage. All leakage data are presented as the 4 hour reading expressed as a percent of the total leakage from frozen tissue.

Respiration and Ethylene Biosynthesis

Respiration was assessed by measuring the rate of CO_2 production from single fruit during the post-chilling period. After removal from chilling fruit were placed individually into 500 ml jars equipped with serum caps in their lids. The jars were kept at 20 C or 28 C, depending upon the experiment, and were sealed at various times during the post-chilling period. At the end of a sealed period 1 ml of the head gas from each jar was removed and injected into a Varian 920 gas chromatograph with a thermal conductivity detector. CO_2 production is presented in mmoles/(KgHr). A second 1 ml gas sample was removed from each jar to measure ethylene concentration. The 1 ml sample was injected into a Varian 2700 gas chromatograph with a flame ionization detector. Ethylene biosynthesis is presented in nmoles/(KgHr).

For tomatoes, both resiration and ethylene biosynthesis rise to a peak and fall during the post-chilling period. In the experiment assessing the interaction of duration and intensity of chilling, data from shortly after removal from chilling to just after the peak in respiration or ethylene biosynthesis are presented. In all other experiments the given values are for a specific time after chilling.

Chilling Conditions

Chilling treatments were performed at various temperatures and for different lengths of time depending upon the experiment. The relative humidity was approximately 60% during chilling, and in all cases, chilling was performed in the dark in growth chambers.

Chilling Temperature and Duration

MG fruit were harvested from the field and chilled at 2.5, 7.5, or 12.5 C for 0, 4, 8, 12, or 16 days. Ion leakage, respiration, and ethylene biosynthesis were assessed after chilling. Fruit receiving 0 days chilling were harvested 1 day prior to measurement of respiration and ethylene biosynthesis to avoid a response due to picking. For ion leakage the experiment was replicated 10 times within each temperature and for respiration and ethylene biosynthesis it was replicated 8 times within each temperature. Only fruit which had not begun to ripen during chilling were used for analysis.

Characterizing Chilling Sensitivity During Ripening

In the first experiment to assess the effects of ripening on chilling sensitivity fruit were harvested from the greenhouse at the MG, BR, TU, PI, and LR stages and chilled for approximately 12 days at

2 C. For measurement of respiration and ethylene biosynthesis control fruit were harvested one day prior to the termination of chilling and kept at 20 C. Because this experiment was done prior to the decision to use only 0.3 M sucrose as a leakage solution, measurements of ion leakage were done both in deionized-distilled water and 0.3 M sucrose. Leakage experiments were replicated 9 times, and respiration and ethylene measurements were replicated 10 times.

In another experiment silver thiosulfate (STS), an inhibitor of ethylene action, was used to inhibit ripening of portions of tomato fruits. A small section of stem tissue above each fruit was removed, and 20 ul of 254 mM STS (5 umoles) was placed on the cut surface (see Figure 7). The STS presumably is taken into the fruit and ripening is inhibited in those portions serviced by the severed vascular bundles. Fruit which were R on one side and MG on the other were chilled for 14 days at 5 C. Ion leakage was measured from both sides of control and chilled fruit. The experiment was replicated 7 times.

The non-ripening mutant, nor, was used to observe the effects of senescence on chilling sensitivity without the confounding effects of ripening. Flowers were tagged at anthesis to provide fruit of known ages. Six-, ten-, and fourteen-week-old fruit were chilled for 14 days at 5 C. At 6 weeks the fruit appeared to be mature, but by 14 weeks they were definitely senescent. Control fruit were harvested the day of leakage measurements and the day prior to respiration and ethylene measurement. Leakage, respiration, and ethylene measurements were repeated 14, 7, and 6 times, respectively.

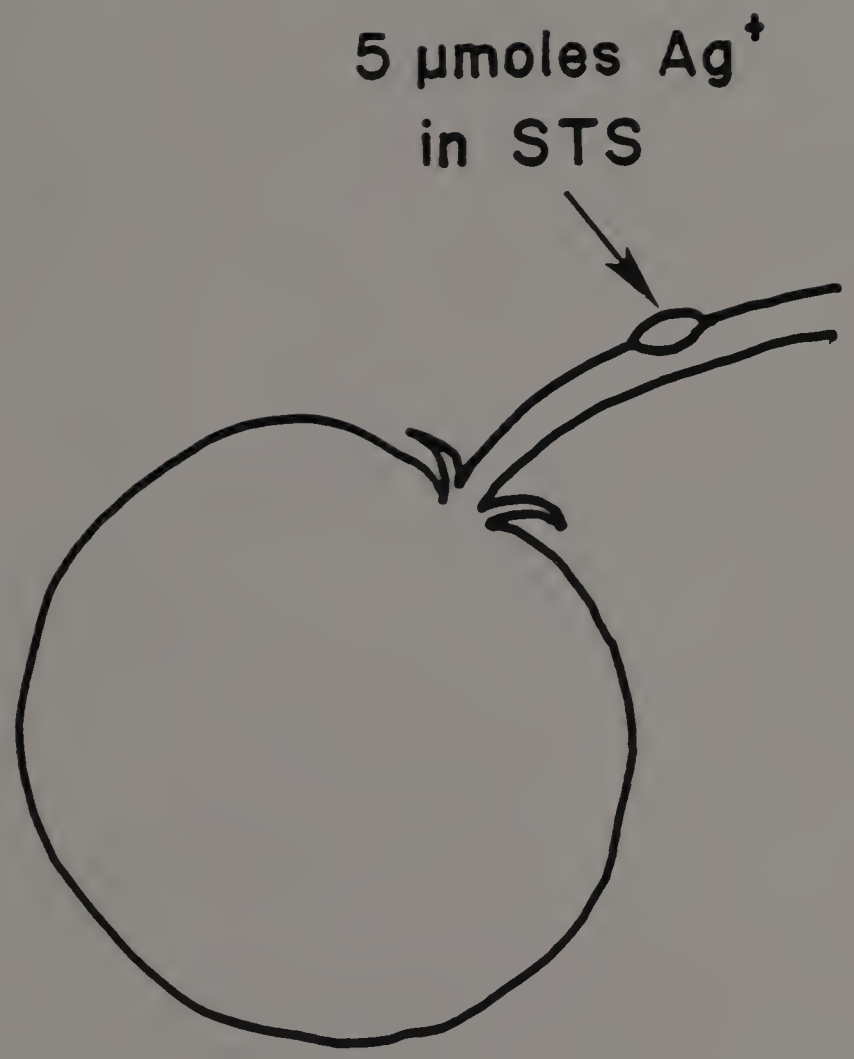

Figure 7: Treatment of tomatoes with silver thiosulfate (STS): 5 umoles of STS were placed onto the pedicel of an attached fruit as shown, following the removal of a portion of pedicel tissue.

Light Experiments

Because of the potential effects of light quality on endogenous hormone concentrations, an experiment was initiated to assess the effects of light quality on chilling sensitivity throughout ripening. All fruit were harvested from the field at the MG stage and then ripened at 20 C in 50x50x20 cm wooden boxes equipped with a far-red filter (720 nm, FRF 700, Westlake Plastics, Lenni, PA 19052), a red filter (660 nm, Roscolene 823 polyvinylacetate, Rosco Laboratories, Port Chester, NY 10573), clear glass, or an opaque sheet in the cover. Light was given 24 hours per day from eight 20 watt fluorescent tubes plus two 25 watt incandescent bulbs (50 cm from the fruit surface). Fruit were chilled for 14 days at 5 C. The experiment was replicated 4 times within each stage: MG, BR, TU, PI, and R. Only ion leakage determinations were made.

Early experiments suggested that light intensity during ripening may alter chilling sensitivity, so this premise was tested by ripening fruit under 3 light intensities (0, 2.5, and 17.5 watts/m^2). Fruit were handled similarly to those in the light quality experiment except all boxes had clear covers, and the fruit were harvested from the greenhouse. Fruit were chilled for 14 days at 5 C. Control fruit were harvested the day of leakage measurements and the day prior to respiration and ethylene measurement. Leakage, respiration, and ethylene measurements were repeated 9, 4, and 4 times, respectively,

within each stage.

Hormone Treatments

Because of thir known effects on chilling sensitivity and strong positive correlations with reported changes in tomato chilling sensitivity, GA and AbA were chosen for direct fruit treatments. Fruit were vacuum infiltrated with GA or AbA solutions using the procedure of Ismail (35). Hormone solutions (1ml/100g F.W.) were placed on the stem scar of freshly harvested fruit, and a vacuum of 100 torr was drawn and held for 5 minutes. The vacuum was released over a 1 minute period, and the hormone solutions were drawn into the fruit.

Three separate experiments were conducted. The first used MG fruit and 0, 200, and 400 ppm AbA (in 0.1% aqueous methanol). After infiltration the fruit remained at room temperaure for 1 day prior to chilling (14 days at 5 C). Control fruit were infiltrated the day prior to the termination of chilling and were kept at room temperature. Ion leakage determinations were performed, and the experiment was replicated 15 times.

The second experiment utilized fruit which ranged from TU to LR. They were infiltrated with 0 or 1000 ppm AbA (in 1% aqueous methanol) under the same conditions as the first experiment. Fruit were chilled for 14 days at 2.5 C. Ion leakage was used to assess chilling-injury, and the experiment was replicated 13 times.

In the last experiment MG fruit were infiltrated with 0 or 1000 ppm GA_3 (in 3% aqueous isopropanol) under the same conditions as the first experiment. Fruit were chilled for 14 days at 5 C. The experiment was replicated 8 times.

Statistical Analysis

Analyses of variance were conducted on all experiments using programs available on the University Computing System. In those experiments where significant interaction terms existed, sums of squares were partitioned into units consisting of a main effect nested within one level of the other main effect involved in the interaction (108). In some cases sums of squares were partitioned using orthogonal polynomials. A log transformation was used on all ethylene data prior to analysis.

Error bars shown on certain figures depict one standard error of a particular mean on either side of that mean.

C H A P T E R IV

RESULTS

Chilling Temperature and Duration

To determine the effects of chilling duration and intensity MG tomatoes were chilled for 0, 4, 8, 12, or 16 days at 2.5, 7.5, or 12.5 C. The 12.5 C treatment is not considered to be in the chilling range for tomatoes. In Figure 8 the ion leakage for the 5 durations and 3 intensities of chilling is shown. The analysis of variance appears in Table 9 (Appendix). The major response to chilling occurred when fruit were chilled more than 8 days. Temperature had a highly significant linear effect on ion leakage after 12 and 16 days of chilling. These data correspond well to data reporting the development of symptoms after chilling, where more than 6 days of chilling was required to significantly increase *Alternaria* rot or abnormal ripening (62).

Post-chilling respiratory activity, in the form of CO_2 evolution, is given in Figures 9 and 10. The analysis of variance appears in Table 10 (Appendix). Figure 9 gives the mean respiration for each time (4.5, 6, 7.5, and 9 hours) during the post-chilling period, whereas Figure 10 reports only the means for the peak activity of individual fruits. The data used for Figure 10 were also used for the statistical analysis. Generally, as the intensity of chilling increased the respiratory response increased, and as the duration

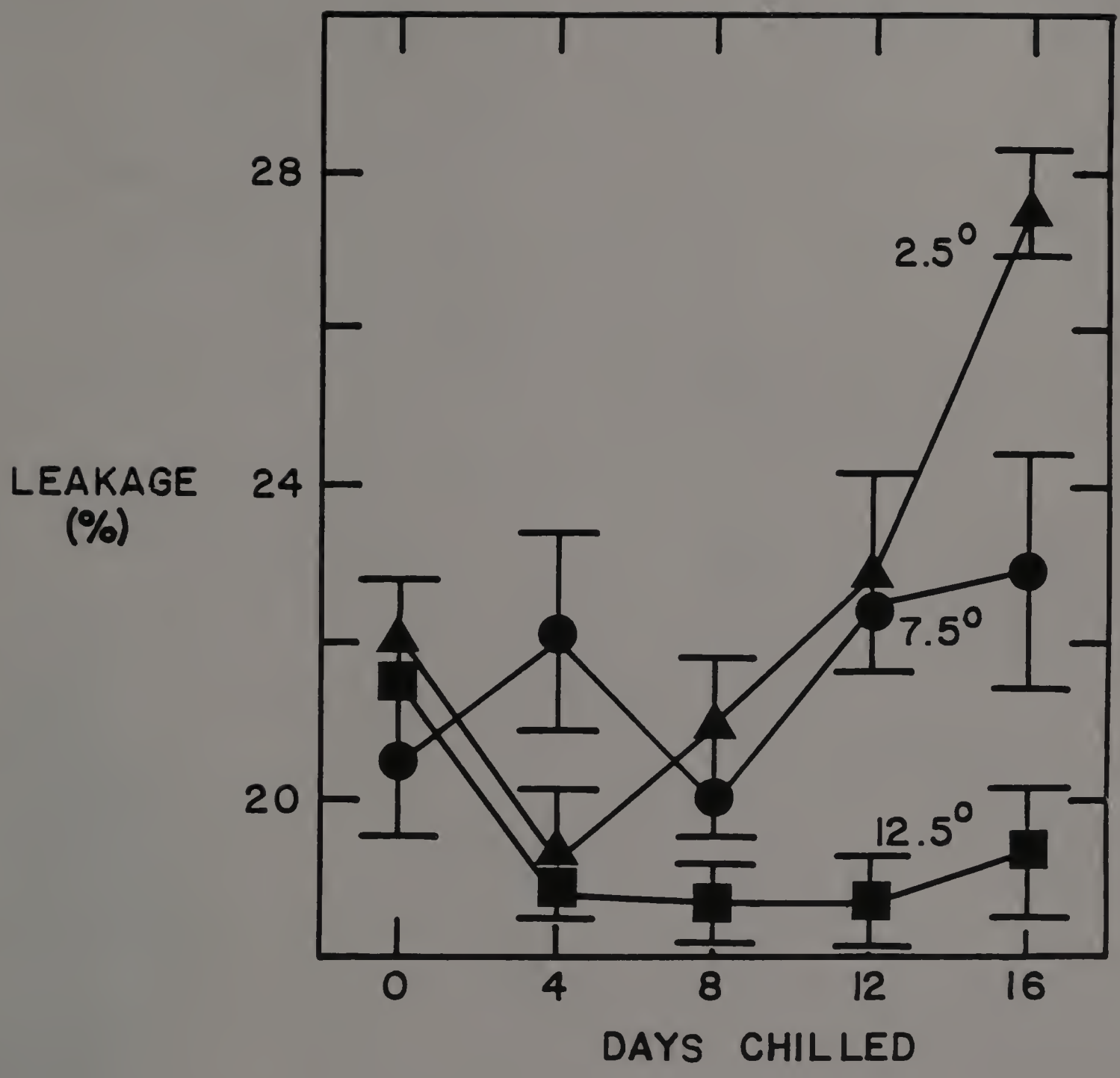

Figure 8: Ion leakage from mature-green fruit after chilling for 0, 4, 8, 12, or 16 days at 2.5, 7.5, or 12.5 C.

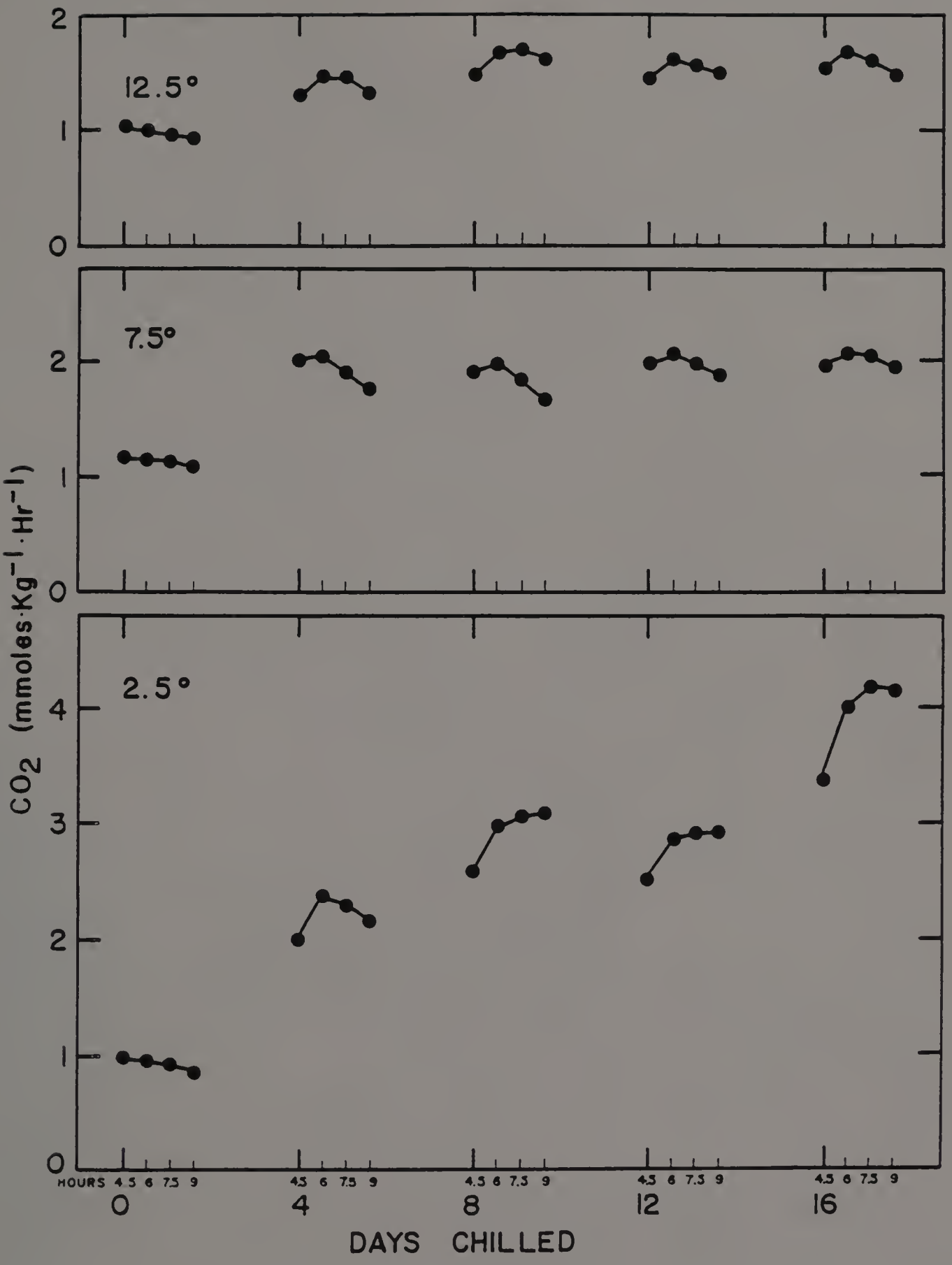

Figure 9: CO_2 evolution during the post-chilling period from mature-green fruit chilled for 0, 4, 8, 12, or 16 days at 2.5, 7.5, or 12.5 C. Fruit were kept at 28 C after removal from chilling.

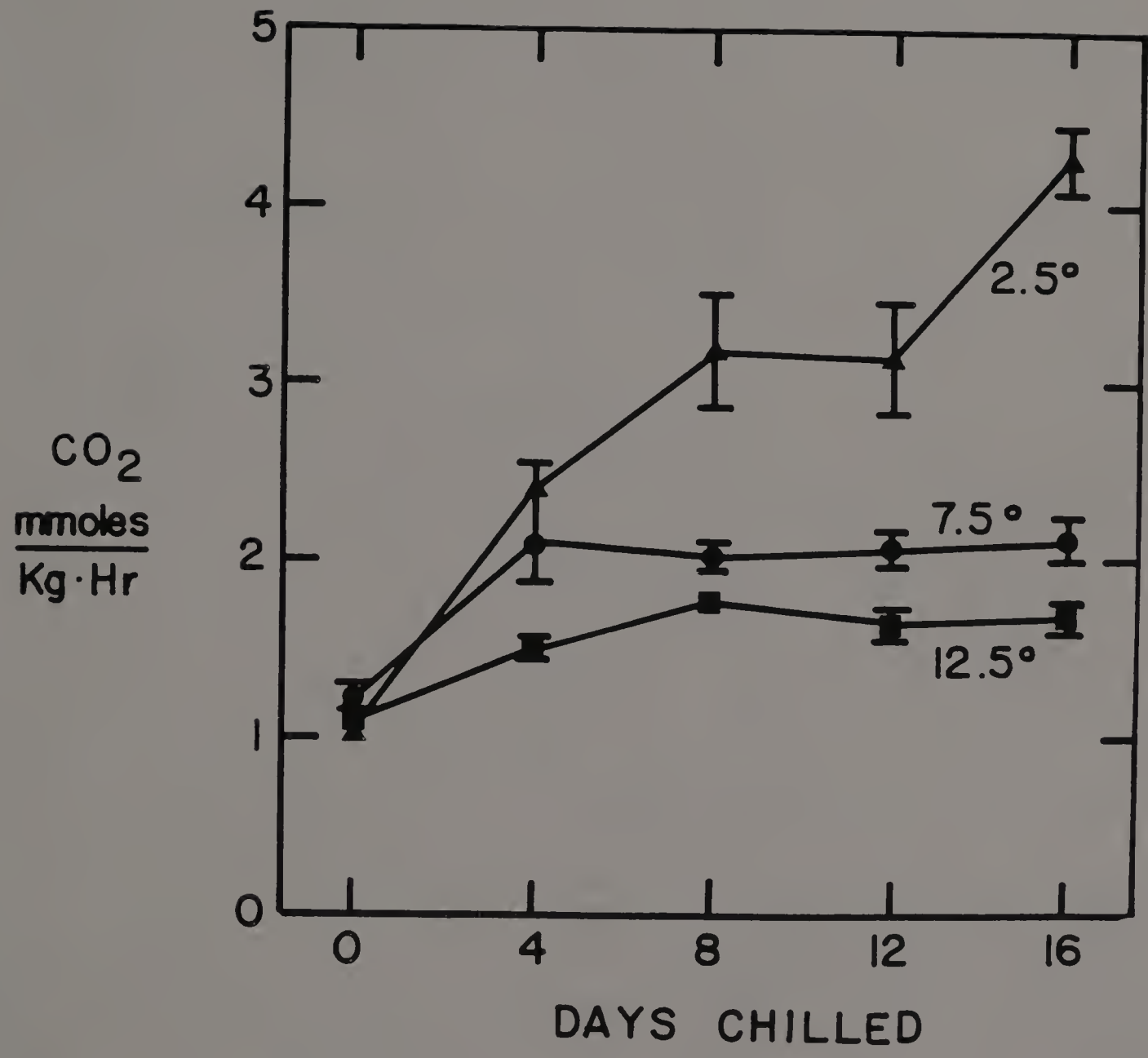

Figure 10: Mean peak CO_2 evolution from mature-green fruit after chilling for 0, 4, 8, 12, or 16 days at 2.5, 7.5, or 12.5 C.

increased the response increased at 2.5 C, but at 7.5 C there was no additional response to increasing the chilling duration beyond 4 days. The specific effect of intensity varied. After 4, 8, 12, and 16 days of chilling the relationship between ion leakage and temperature was a highly significant linear, a highly significant linear and significant quadratic, a highly significant linear,and a highly significant linear and quadratic one, respectively.

Post-chilling ethylene biosynthesis rate is given in Figures 11 and 12. The analysis of variance appears in Table 11 (Appendix). Figures 11 and 12 report the data in a similar manner to Figures 9 and 10 for respiration. As with respiration, the response increased as the duration or intensity increased. For the 4, 8, 12, and 16-day treatments temperature had a highly significant linear effect on ethylene biosynthesis rate. Eight days included a highly significant quadratic component

Both post-chilling respiratory activity and ethylene biosynthesis rate respond to chilling quicker than does ion leakage, i.e., only 4 days of chilling was required to elicit a significant respiratory or ethylene response, whereas more than 8 days was needed to increase ion leakage. The leakage response corresponded more closely than the ethylene or respiratory response to symptom development.

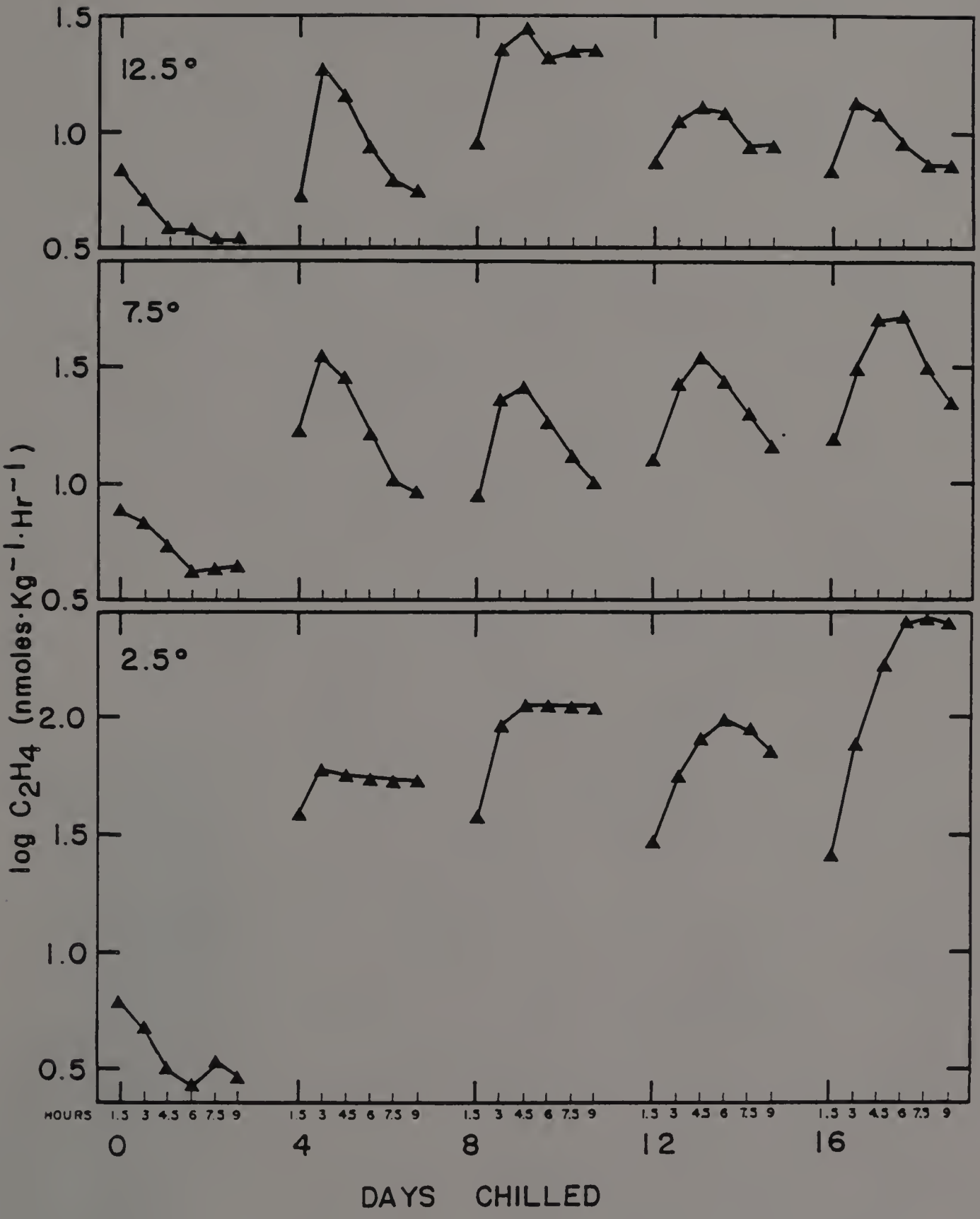

Figure 11: Ethylene biosynthesis during the post-chilling period of mature-green fruit chilled for 0, 4, 8, 12, or 16 days at 2.5, 7.5, or 12.5 C. Fruit were kept at 28 C after removal from chillling.

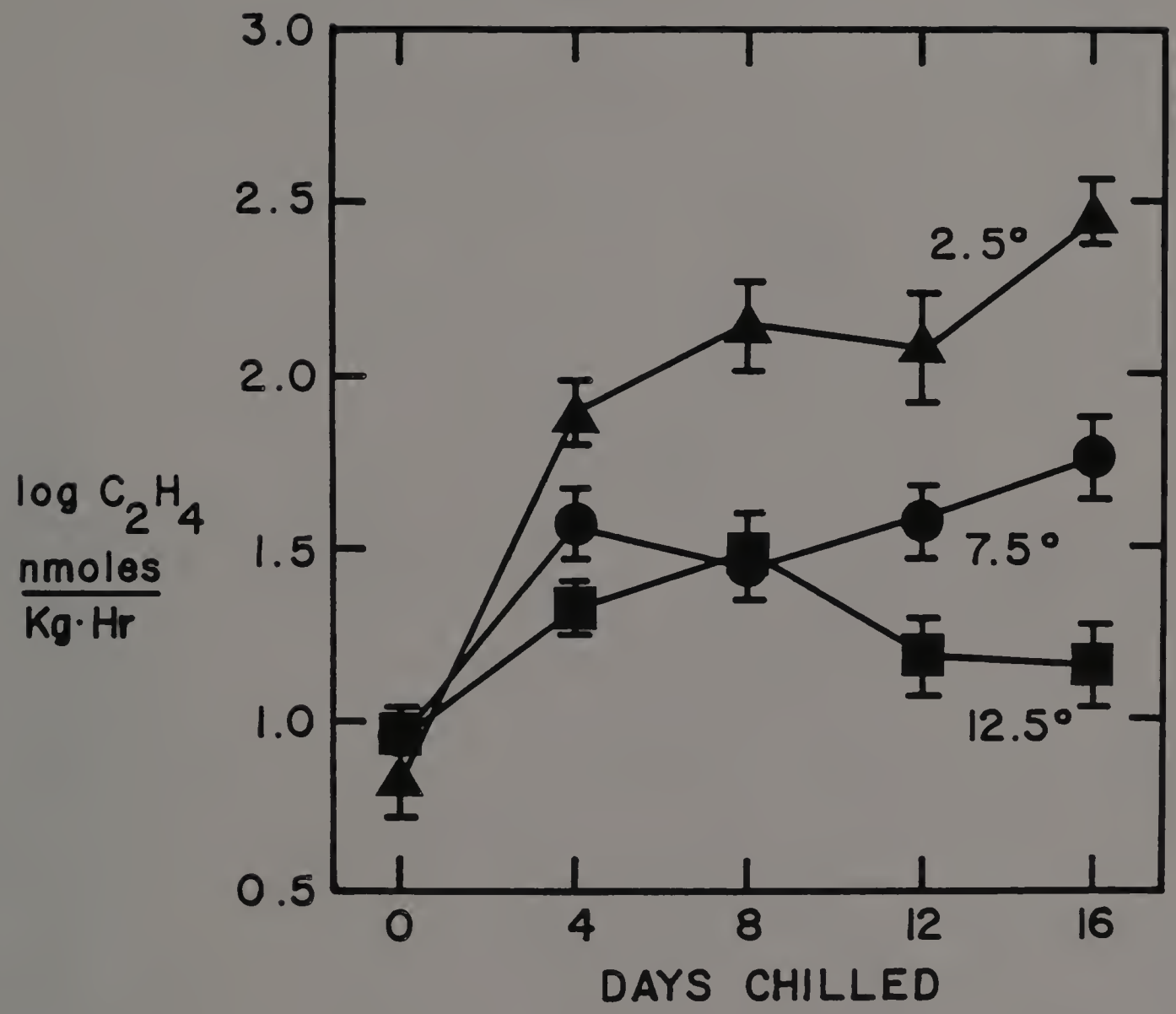

Figure 12: Mean peak ethylene biosynthesis of mature-green fruit after chilling for 0, 4, 8, 12, or 16 days at 2.5, 7.5, or 12.5 C.

Chilling Sensitivity During Ripening

In the first experiment to assess the effects of ripening on chilling sensitivity, fruit were harvested at the MG, BR, TU, PI, and LR stages and chilled for approximately 12 days at 2 C. Ion leakage was measured both in deionized-distilled water and 0.3 M sucrose. Data are presented in Table 1, and the analysis of variance is given in Table 12 (Appendix). Ripening had no significant effect on the response due to chilling, but in 0.3 M sucrose there appeared to be a larger leakage stimulation in MG fruit than in other stages. This difference was not significant, but a difference may have been masked by the high variances. Data showing significant effects of ripening on ion leakage after chilling will be presented in a later section.

Figure 13 shows the respiratory rate 5.5 hours after removal from chilling, and the analysis of variance is given in Table 13 (Appendix). Chilling significantly enhanced respiration in MG and BR fruit but had no effect later in ripening. Figure 14 shows the ethylene biosynthesis rate 5.5 hours after removal from chilling. Chilled fruit were producing significantly more ethylene at the MG stage but significantly less at the BR and TU stages with no differences later in ripening. It is possible that the reduction in BR and TU fruit occurred because the peak of ethylene biosynthesis had not been reached after 5.5 hours.

Chilling stimulated ethylene biosynthesis and respiration of MG fruit, and the response decreased as the fruit ripened (Figures 13 and

Table 1: Mean ion leakage from control and chilled tomato tissue at various stages of ripening.

Leakage solution	Stage of ripening	Ion leakage (%)	
		Control	Chilled
H2O	MG	48.0	50.8
	BR	47.3	52.9
	TU	54.0	67.0
	PI	63.8	79.0
	LR	75.5	85.6
0.3M Sucrose	MG	22.5	30.9
	BR	22.9	24.9
	TU	27.3	29.1
	PI	28.4	32.5
	LR	33.3	35.9

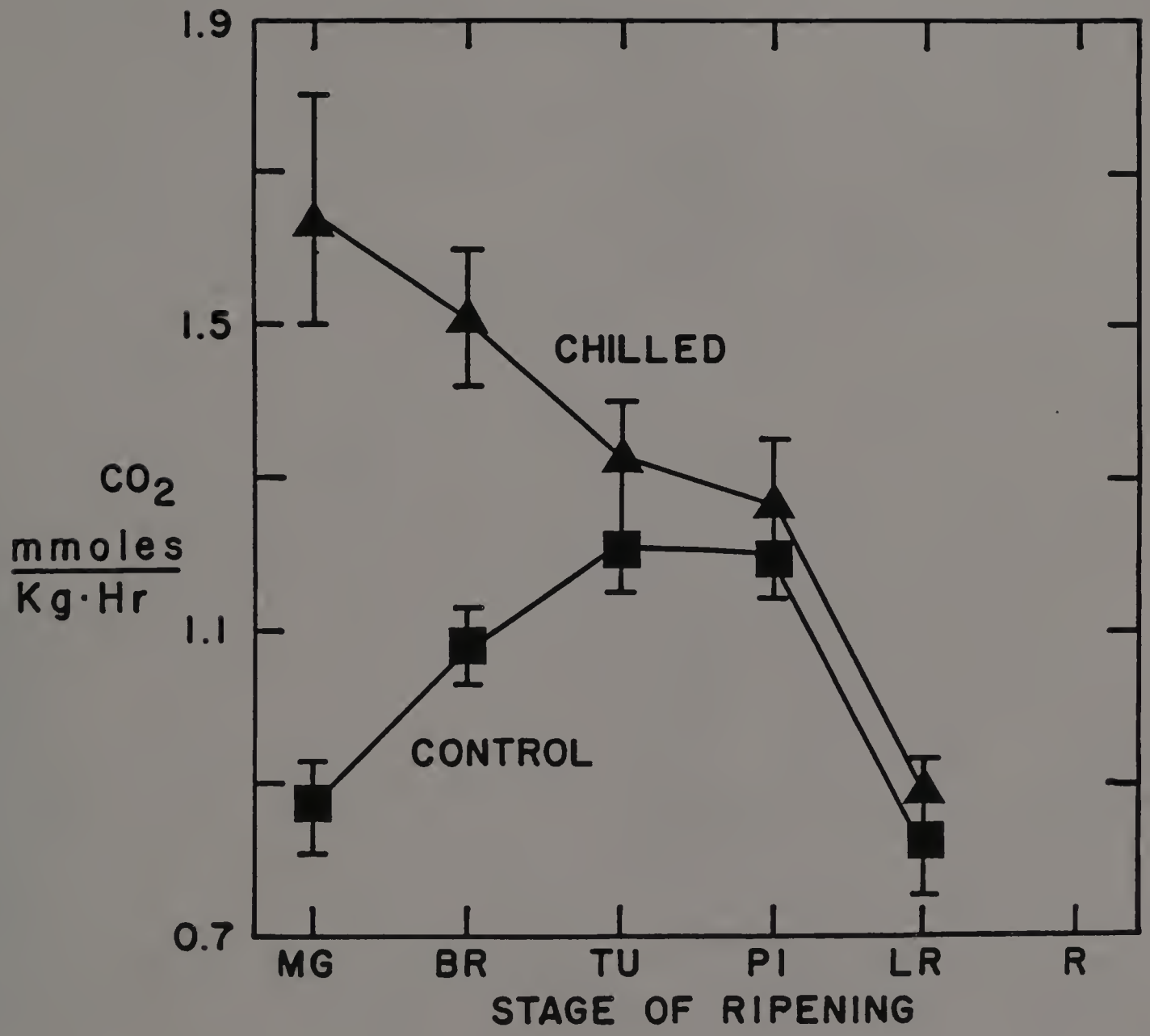

Figure 13: CO_2 evolution 5.5 hours after removal from chilling (2 C for 12 days). Fruit were kept at 20 C during the post-chilling period. The stages of ripening used were mature-green (MG), breaker (BR), turning (TU), pink (PI), and light red (LR).

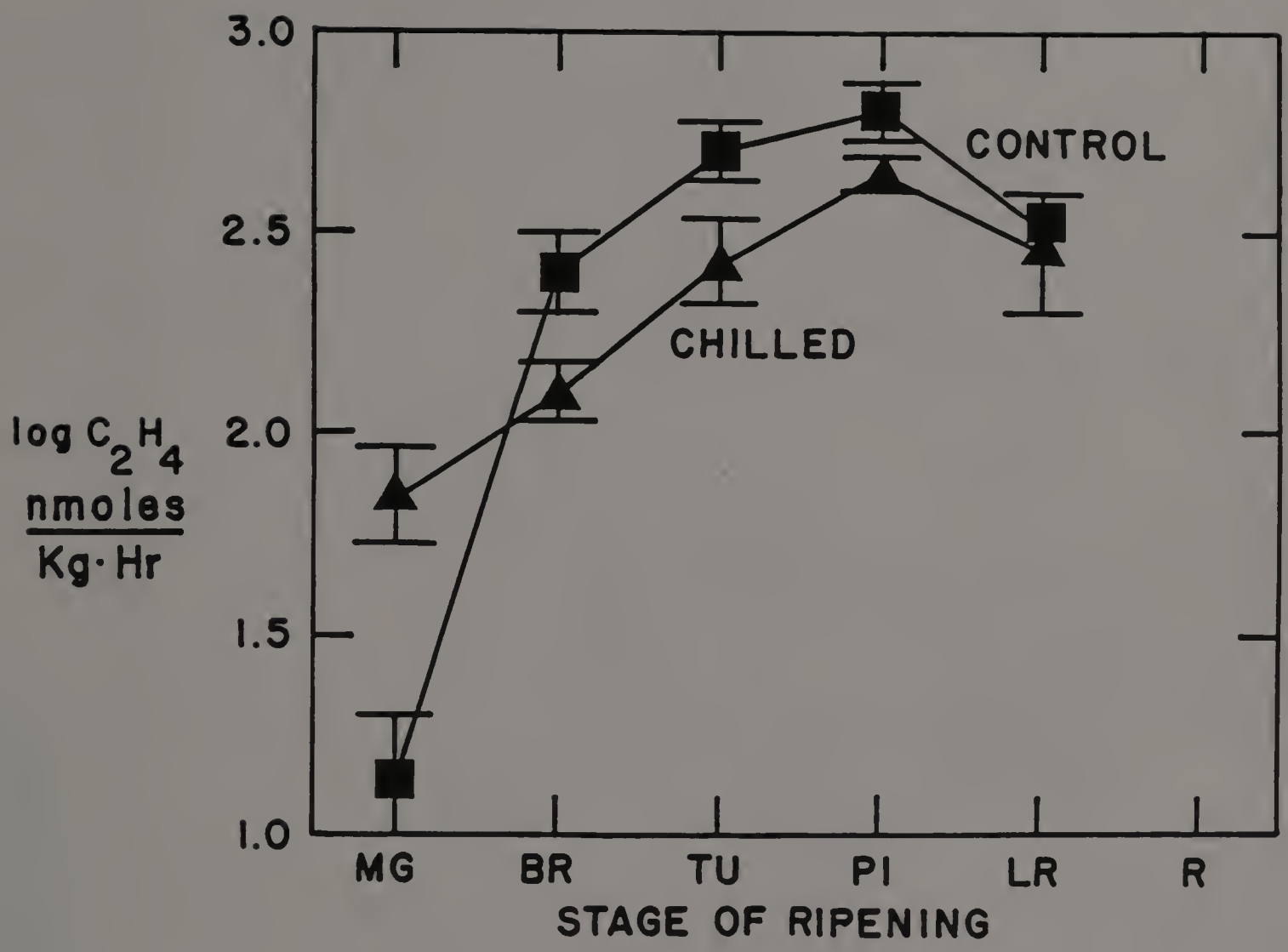

Figure 14: Ethylene biosynthesis 5.5 hours after removal from chilling (2 C for 12 days). Fruit were kept at 20 C during the post-chilling period. The stages of ripening used are the same as those listed in Figure 13.

14). These data may be interpreted to mean that chilling sensitivity decreases as fruit begin to ripen, but the chilling response may be confounded with the ethylene and respiratory climacteric. This problem limits the use of ethylene biosynthesis and respiration to measure chilling injury in ripening fruit.

The data from both light experiments showed a highly significant chilling-ripening stage interaction (Tables 15 and 16, Appendix). Figure 15 presents the means of all light quality experiments when leakage was measured from MG, BR, TU, PI, and R fruit. Fruit were chilled for 14 days at 5 C. The effect of chilling was highly significant at the MG, PI, and R stages but nonsignificant at the BR and TU stages. The percents of the sum of squares associated with the difference between control and chilled fruit within each stage are 18, 0, 1, 13, and 68% for MG, BR, TU, PI, and R fruit, respectively. These data suggest that chilling sensitivity drops as fruit begin to ripen and increases during the later stages of ripening. Figure 16 shows the data from the light intensity experiment where ion leakage was measured from BR, TU, PI, LR, and R fruit. These fruit were also chilled for 14 days at 5 C. In this experiment chilling significantly enhanced leakage in all but PI fruit. The effect of chilling in BR, TU, PI, LR, and R fruit accounted for 4, 6, 2, 6, and 82% of the sum of squares associated with chilling within each stage. This supports the suggestion that chilling sensitivity increases during the later stages of ripening. The increase late in ripening may be a senescence phenomenon.

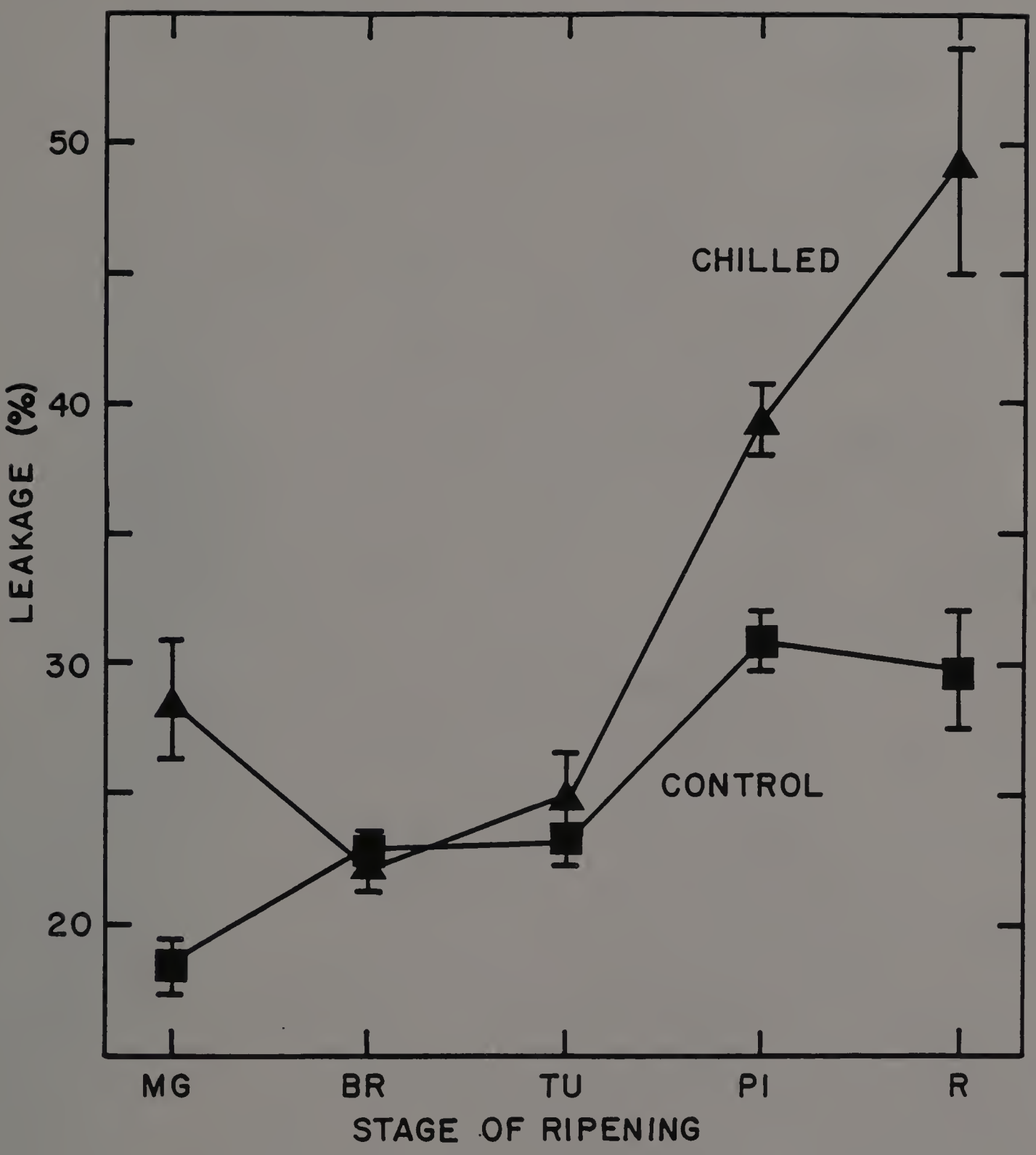

Figure 15: Ion leakage after chilling for 14 days at 5 C: means from the light quality experiment. The stages of ripening used were mature-green (MG), breaker (BR), turning (TU), pink (PI), and red (R).

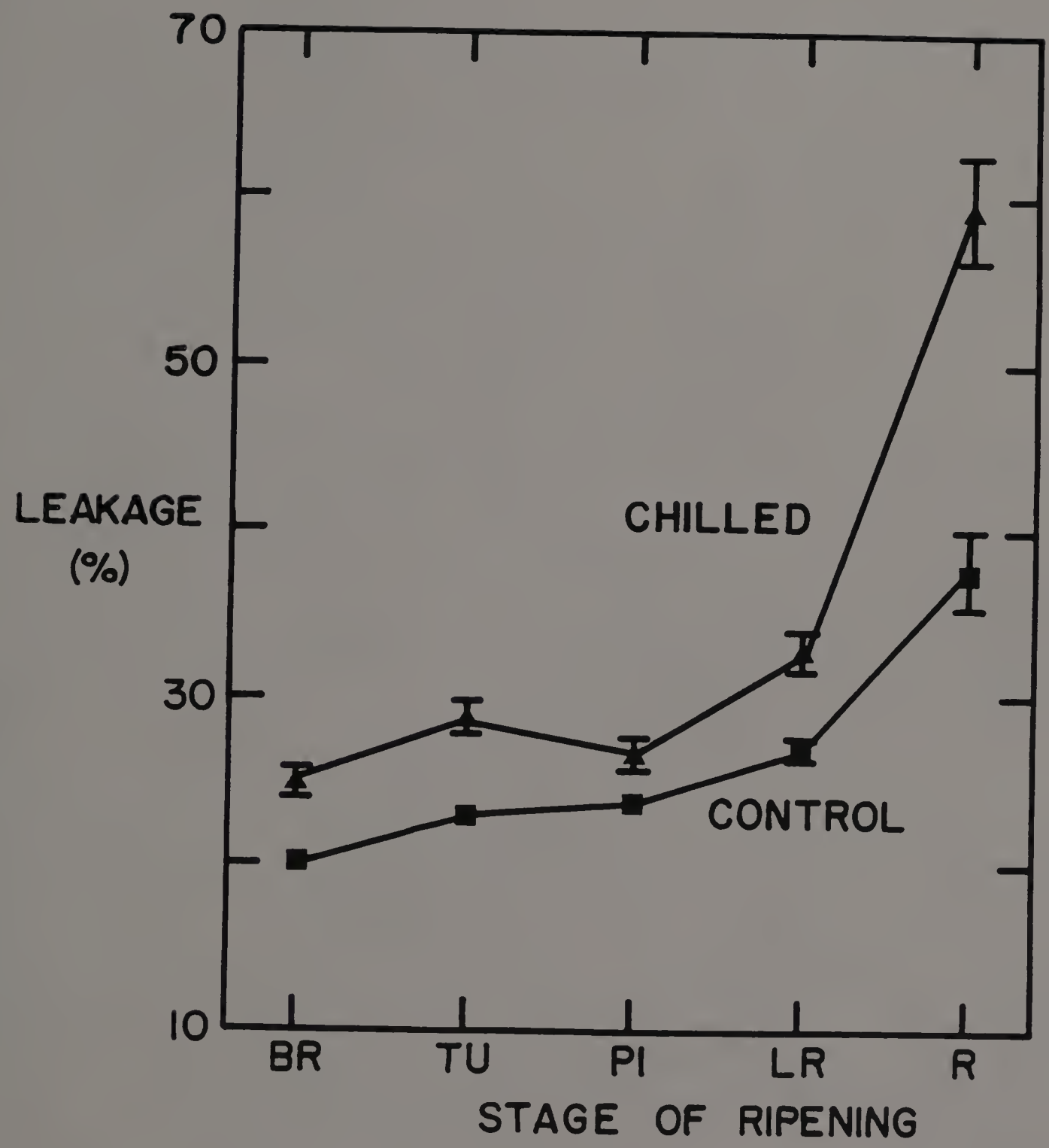

Figure 16: Ion leakage after chilling for 14 days at 5 C: means from the light intensity experiment. The stages of ripening used were breaker (BR), turning (TU), pink (PI), light red (LR), and red (R).

To study the effect of senescence, the non-ripening mutant, nor, was used. Six-, ten-, and fourteen-week-old fruit were chilled for 14 days at 5 C. Ion leakage data are graphed in Figure 17, and the analysis of variance is given in Table 17 (Appendix). Chilling enhanced ion leakage only in 14-week-old fruit. Fourteen-week-old fruit are senescent, so these data support the idea of a senescence related increase in chilling sensitivity.

Respiration 6.25 hours after the removal from chilling is plotted in Figure 18. The analysis of variance is given in Table 18 (Appendix). Respiration was enhanced in all ages, but the response appeared to decline as fruit aged. As fruit senesce their ability to respond to chilling may decline. Ethylene biosynthesis rate 6.25 hours after chilling is given in Figure 19, and the analysis of variance is in Table 19 (Appendix). The rate was enhanced in 6- and 10-week-old fruit but not in 14-week-old fruit. After 14 weeks control fruit began to produce small amounts of ethylene. This level was the same as for all chilled fruit and may be the maximum production rate in nor fruit, explaining the lack of stimulation due to chilling in the older fruit.

Silver thiosulfate (STS) treatments inhibited ripening of portions of tomato fruit. These fruit were chilled for 14 days at 5 C. Leakage data are given in Table 2, and the analysis of variance is given in Table 20 (Appendix). Chilling enhanced ion leakage similarly in both sides of STS-treated fruit, as would be expected if chilling sensitivity is highest early and late in ripening.

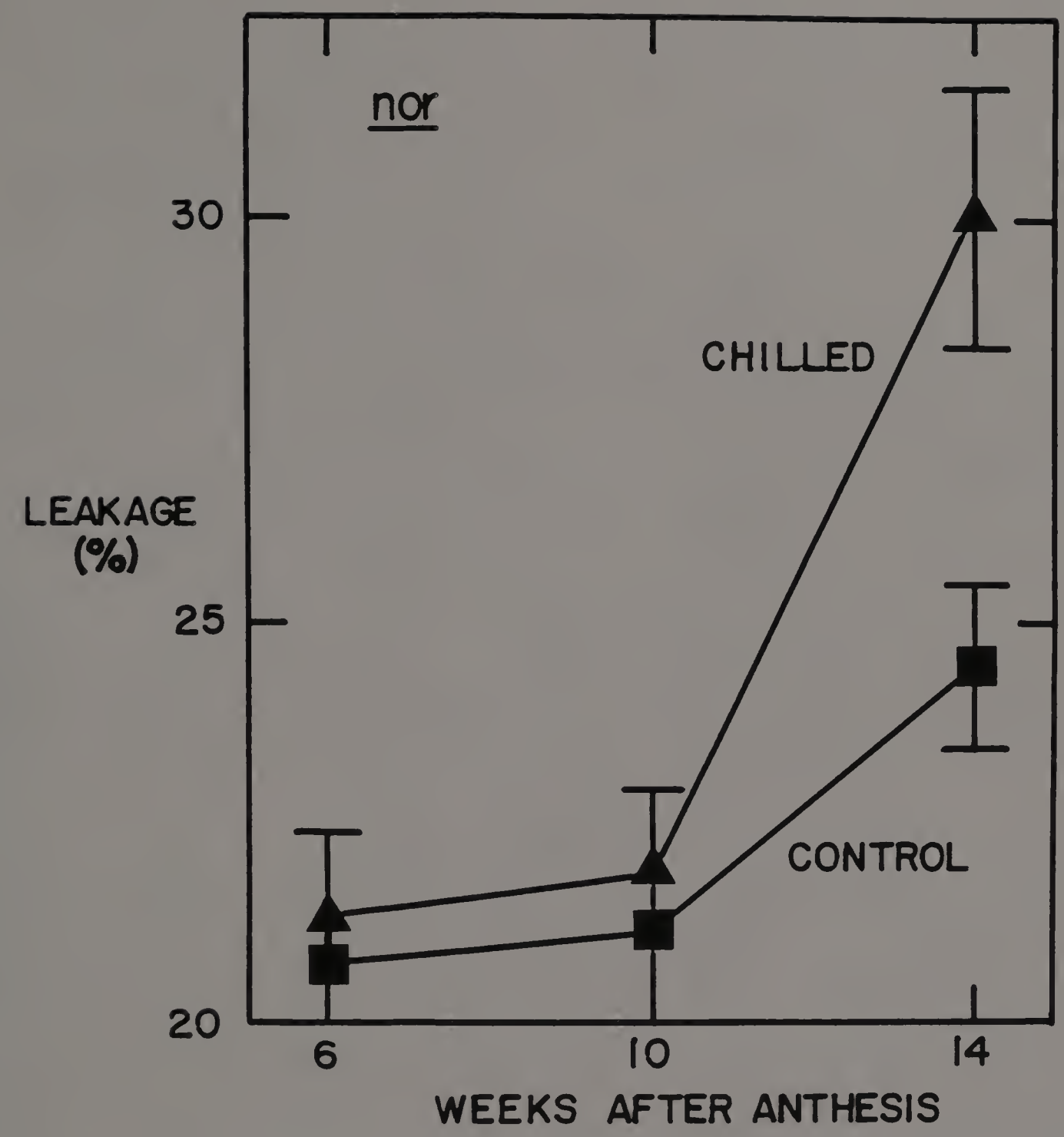

Figure 17: Ion leakage from 6-, 10-, and 14-week-old *nor* fruit after chilling for 14 days at 5 C.

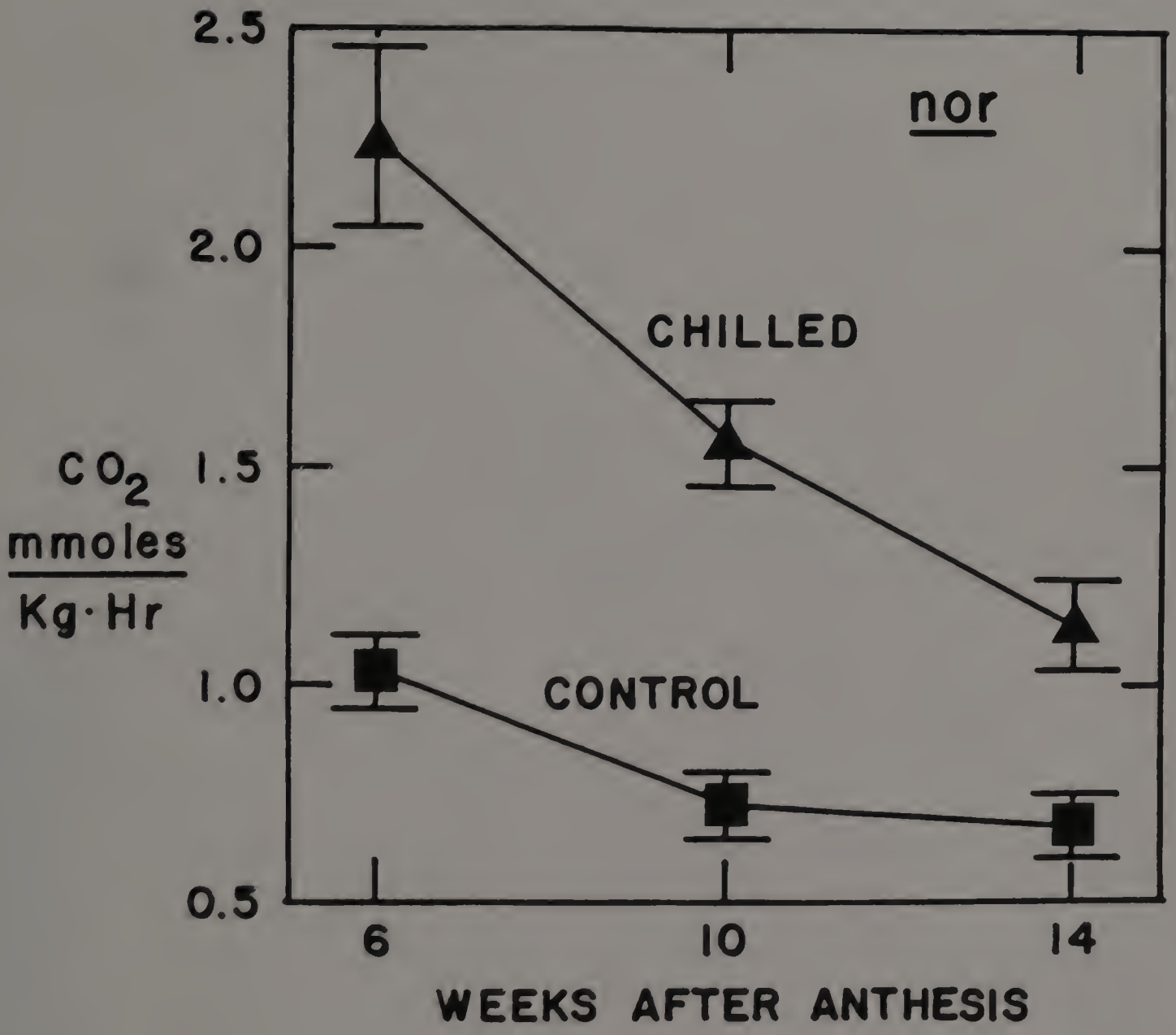

Figure 18: CO_2 evolution from 6-, 10-, and 14-week-old nor fruit 6.25 hours after removal from chilling (14 days at 5 C). Fruit were kept at 20 C during the post-chilling period.

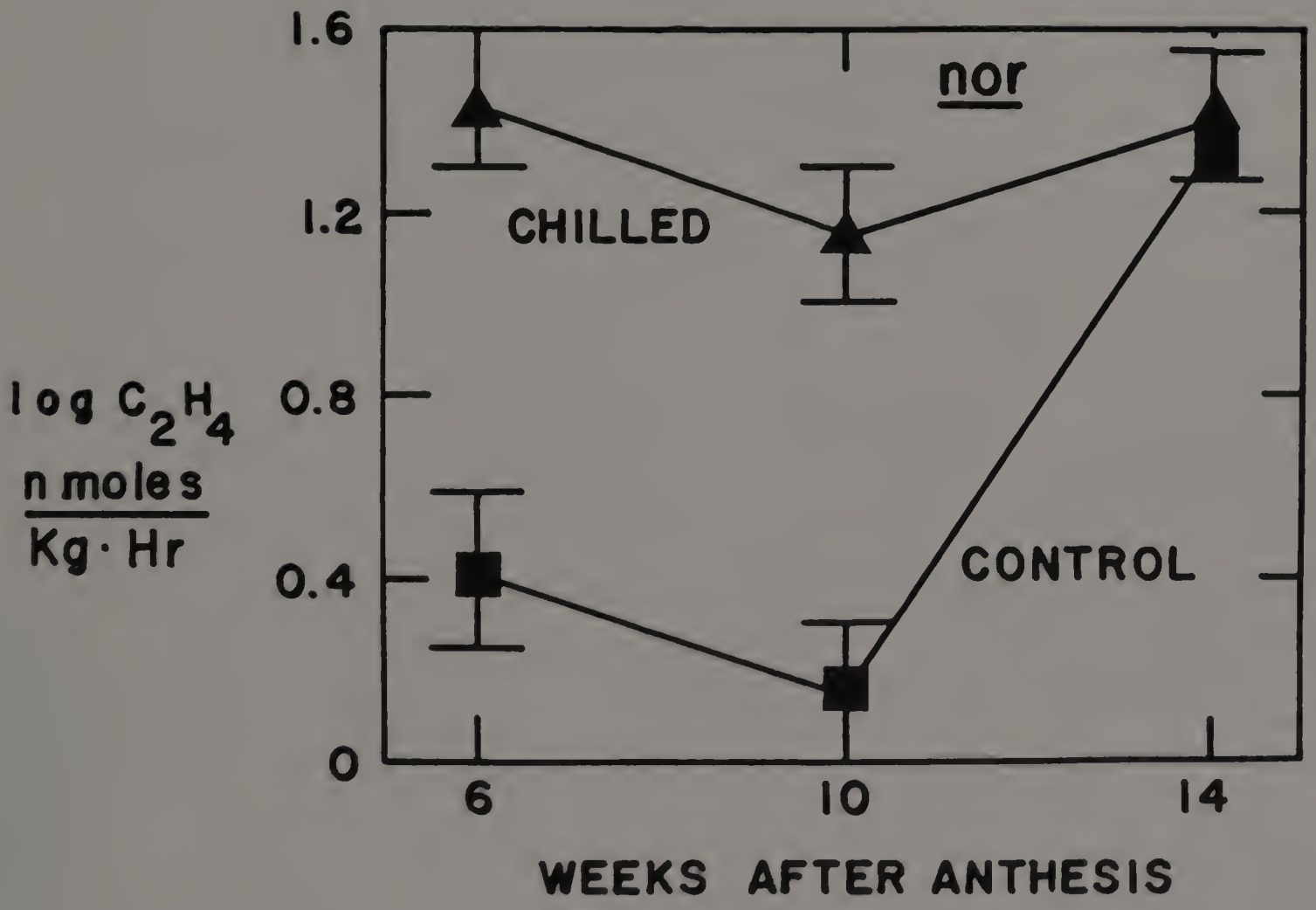

Figure 19: Ethylene biosynthesis of 6-, 10-, and 14-week-old nor fruit 6.25 hours after removal from chilling (14 days at 5 C). Fruit were kept at 20 C during the post-chilling period.

Table 2: The effects of silver thiosulfate (STS) on ion leakage from control and chilled fruit. STS treatments inhibited the ripening of certain sections resulting in fruit which were MG on one side and R on the other. These fruit were chilled for 14 days at 5 C.

Portion of fruit	Ion leakage (%)	
	Control	Chilled
MG	28.7	37.9
R	48.7	64.6

The data presented in this section show that chilling sensitivity, as measured by ion leakage, changes during ripening. As fruit begin to ripen from the MG to the BR stage chilling sensitivity drops. As the fruit reach the later stages of ripening chilling sensitivity increases. This increase may be a senescence phenomenon.

Light Experiments

Light treatments were used to attempt to alter chilling sensitivity of ripening tomatoes. In the first experiment fruit were ripened under continuous red light, far-red light, white light, or darkness until they reached specific stages of ripening. Fruit were chilled 14 days at 5 C. Figure 20 graphs ion leakage for fruit treated with red light, far-red light, white light, and darkness. The analysis of variance is given in Table 15 (Appendix). Light quality had no effect on chilling sensitivity. There was a chilling-ripening stage interaction which was discussed earlier. As mentioned in the Literature Review, red light can enhance endogenous AbA levels if given during tomato ripening (120). In this experiment, either the light treatments did not alter endogenous AbA levels or AbA did not affect the chilling sensitivity of tomatoes. To determine which scenario is correct direct treatments of tomatoes with AbA solutions were conducted. The data will be discussed in a later section.

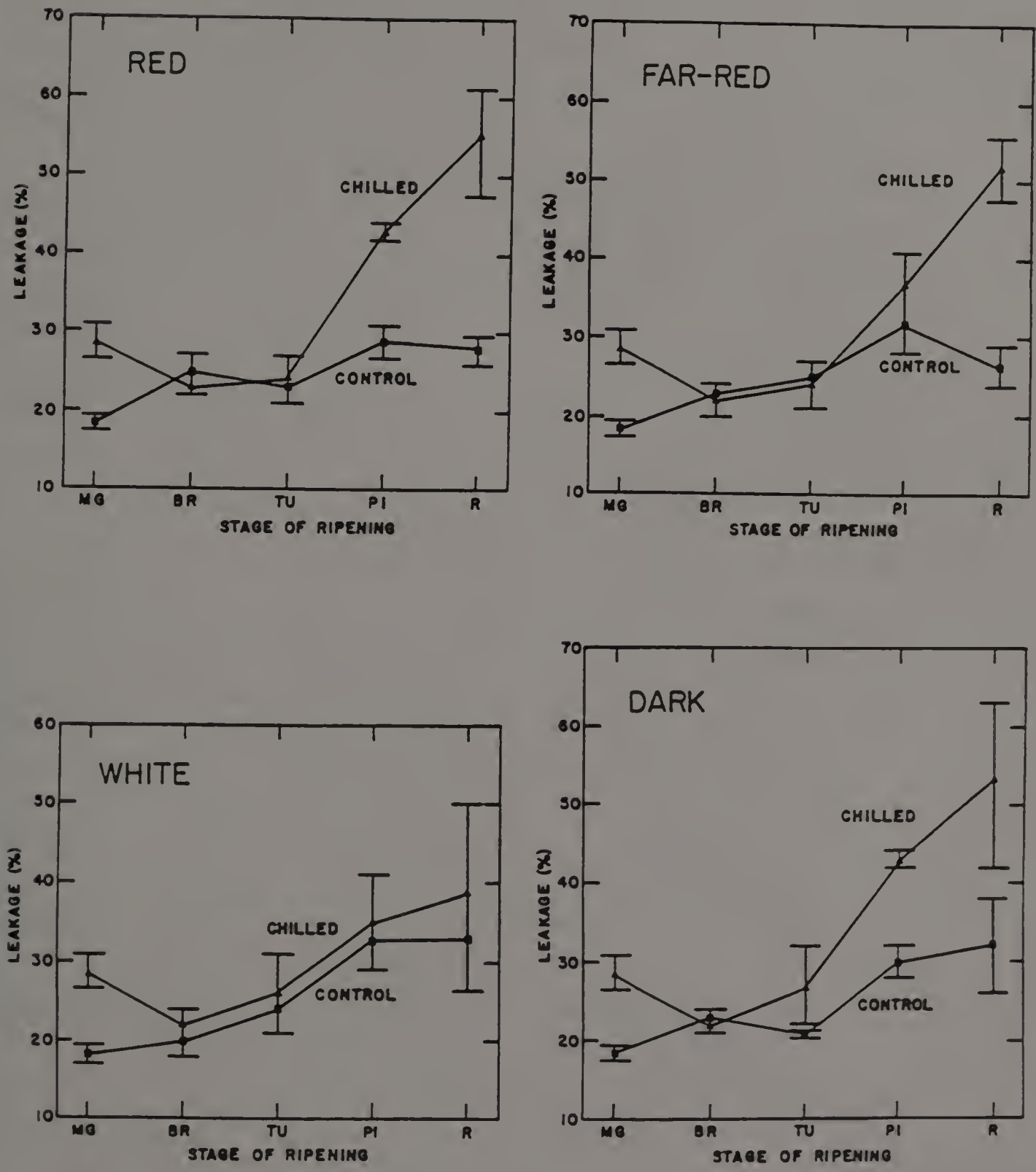

Figure 20: Ion leakage from fruit ripened under red light, far-red light, white light, or darkness and chilled for 14 days at 5 C. Stages of ripening used are the same as those listed in Figure 15.

Since there appeared to be a reduction in chilling sensitivity during the later stages of ripening under white light, albeit nonsignificant, a second light experiment was performed to determine the effects of light intensity during ripening on chilling sensitivity. Fruit were ripened under darkness, low (2.5 watts/m^2), or high (17.5 watts/m^2) intensity light until they reached specific stages of ripening. Fruit were chilled for 14 days at 5 C. Ion leakage data are presented in Figure 21. The analysis of variance is given in Table 16 (Appendix). Light intensity had no effect on chilling sensitivity as measured with ion leakage. Post-chilling respiratory rate and ethylene biosynthesis are given in Table 3, and the analyses of variance are given in Tables 21 and 22 (Appendix). Light treatments had no effect on chilling-induced changes in respiratory rate or ethylene biosynthesis.

Hormone Treatments

In the first hormone experiment MG fruit were vacuum infiltrated with 0, 200, or 400 ppm AbA and were chilled for 14 days at 5 C. Means are reported in Table 4, and the analysis of variance is given in Table 23 (Appendix). AbA had no effect on control fruit but had a significant linear and quadratic effect on ion leakage from chilled fruit, suggesting an increase in chilling sensitivity caused by AbA. These data are suspect because of the abnormally low levels of leakage from chilled fruit which received no AbA. The experiment was repeated

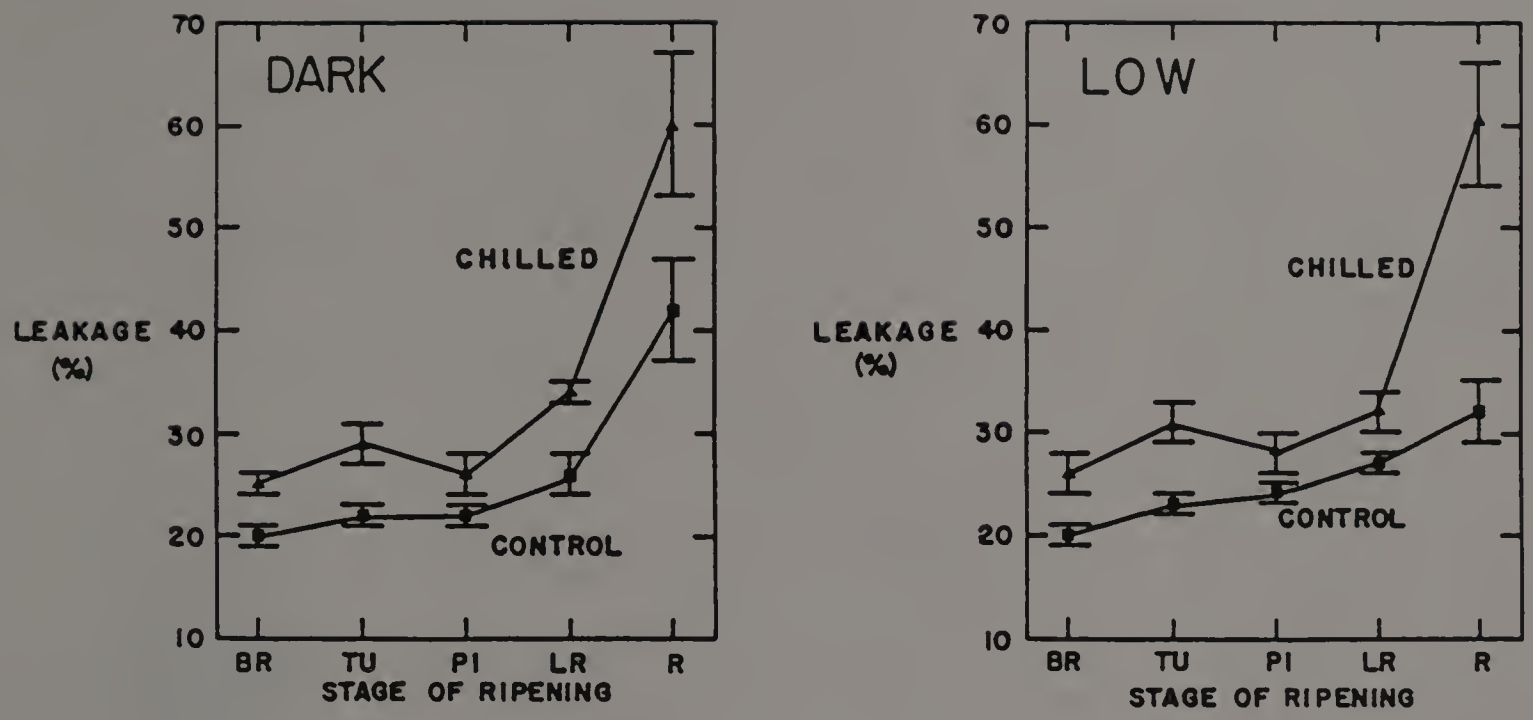

Figure 21: Ion leakage from fruit ripened in darkness, under low intensity (2.5 watts/m^2) light, or under high intensity (17.5 watts/m^2) light and chilled for 14 days at 5 C. Stages of ripening used are the same as those listed in Figure 16.

Table 3: The effects of light intensity on chilling-induced alterations in respiration and ethylene biosynthesis.

Ripening stage	Light	CO_2 mmoles/(KgHr)		log C_2H_4 nmoles/(KgHr)	
		Control	Chilled	Control	Chilled
BR	Dark	1.24	1.72	1.39	1.42
	Low	1.01	1.99	0.84	1.67
	High	0.86	1.56	0.49	1.28
TU	Dark	1.63	1.59	2.81	2.56
	Low	1.68	2.02	2.74	2.70
	High	1.59	1.67	2.57	2.40
PI	Dark	1.70	1.76	2.67	2.86
	Low	1.59	1.60	2.72	2.69
	High	1.48	1.53	2.55	2.30
LR	Dark	1.64	1.27	2.95	2.77
	Low	1.35	1.24	2.53	2.68
	High	1.82	1.33	2.82	2.69
R	Dark	1.26	1.36	2.75	2.67
	Low	1.21	1.46	2.62	2.79
	High	1.14	1.23	2.56	2.71

Table 4: The effects of AbA and GA_3 pretreatments on ion leakage.

Concentration	Ion leakage (%) Control	Chilled
Experiment One--Mature-green		
0 ppm AbA	18.9	23.0
200 ppm	19.0	27.1
400 ppm	20.1	26.1
sig.	ns	l*q*
Experiment Two--Ripening fruit		
0 ppm AbA	29.2	41.5
1000 ppm	30.8	37.6
sig.	ns	ns
Experiment Three--Mature-green		
0 ppm GA_3	20.9	31.3
1000 ppm	20.6	32.4
sig.	ns	ns

in a manner potentially to intensify the response. Fruit which are naturally less sensitive to chilling, i.e., ripening fruit, were chosen. They were infiltrated with 0 or 1000 ppm AbA and chilled for 14 days at 2.5 C. Means are given in Table 4. The analysis of variance appears in Table 24 (Appendix). AbA had no effect on ion leakage from control or chilled fruit, suggesting that AbA does not significantly alter tomato chilling sensitivity.

MG fruit were also infiltrated with 0 or 1000 ppm GA_3. The data are given in Table 4, and the analysis of variance is given in Table 25 (Appendix). GA had no significant effect on ion leakage from control or chilled fruit, suggesting that GA is not important in governing tomato chilling sensitivity, or endogenous GA is saturating a GA response.

C H A P T E R V

DISCUSSION

Measurement of Chilling Sensitivity in Tomatoes

To measure chilling sensitivity in plant tissue it must be possible to measure a response to a chilling treatment. The obvious responses to measure are visual symptoms. In tomatoes the two major symptoms are abnormal ripening (62) and an increased incidence of *Alternaria* rot (61). Both symptoms are difficult to quantify and require keeping fruit at room temperature for several days after chilling before determinations can be made. One of the objectives of this study was to determine what quantifiable, physiological measurement could be used to assess chilling injury in tomatoes.

The first measurement used to assess injury was ion leakage from chilled tissue (22). Chilling induced an increase in the quantity of ions leaked from tomato fruit tissue (Figure 8). Increased leakage from tomato tissue occurred only after return to nonchilling temperatures (43, 48, 77), suggesting that the damage caused by chilling is not direct physical damage to the membrane but may be related to disruption of ATP production or an increase in utilization (27). The alteration of ATP levels could cause the malfunction of membrane-bound ion pumps allowing the passive leakage of ions (27).

The ion leakage data from this study correlate with the reported data measuring symptom development. McColloch et al. (62) presented

data suggesting that more than 6 days of chilling was required to increase significantly the development of Alternaria rot or abnormal ripening in MG tomatoes after chilling. The data from this study show increased ion leakage due to chilling at 2.5 or 7.5 C occurring after chilling periods of greater than 8 days. Measurement of the post-chilling respiratory burst (Figure 9) and burst of ethylene biosynthesis (Figure 10), on the other hand, show a response after only 4 days of chilling at 2.5 or 7.5 C. This high degree of sensitivity is advantageous, but measurements of respiration and ethylene biosynthesis are difficult to interpret once the fruit have begun to ripen. The ethylene and respiratory climacterics may alter the capacity of the response where there is no real difference in chilling sensitivity. The results of this study suggest that ion leakage after chilling is a more reliable measure of chilling injury in ripening tomatoes. The data are easily interpreted throughout ripening, and they correlate with reported symptom development in MG tomatoes.

Ripening and Chilling Sensitivity

Ripening-related changes in chilling sensitivity are not uncommon among fruit species. Lipton (50) showed that the chilling sensitivity of Honey Dew muskmelons decreased as they ripened from the mature, unripe stage 1 to the half-ripe stage 2. There was no change in sensitivity between stage 2 and the fully ripe stage 3.

Mangoes exhibit a similar reduction in chilling sensitivity during the early stages of ripening which allows lower storage temperatures once ripening has begun (73). Green banana fruit are more chilling sensitive than yellow ones, but the difference is not great enough to be utilized commercially (18). Chilling sensitivity in grapefruit correlates with green color (29, 82), and chilling sensitivity in papayas also declines with ripening (79).

A reduction in chilling sensitivity as fruit ripen appears to be the most common relationship, but some fruit act differently. Avocados are most sensitive to chilling, as measured by the appearance of surface scald and flesh browning, during the climacteric rise and peak, and are less sensitive during the pre- and postclimacteric periods. (44, 45). Apples are somewhat more complex. Brown core, a chilling injury symptom, is more prevalent if the fruit are immature when chilled (105). Table 5 shows the effect of holding McIntosh apples at room temperature prior to chilling at 0 C. As the fruit ripen their sensitivity to brown core drops. The same relationship is apparent if fruit are picked at different times (Table 6), i.e., later harvests are less sensitive to brown core. Another chilling injury in apples, low temperature breakdown, is most prevalent in fruit chilled during the climacteric rise (128). A third symptom of chilling in apples, soggy breakdown, is most prevalent if fruit are chilled during the later stages of ripening (89). Table 7 shows the effect of holding Golden Delicious apples at room temperature prior to chilling. The data show that it is definitely a chilling injury and is much more

Table 5: Influence of picking date on brown core in McIntosh apples kept at 0 C. Adapted from Smock (105).

Harvest date	% Brown core		
	1941-42	1942-43	1943-44
Sept 3	12	100	78
Sept 10	10	89	36
Sept 17	2	96	19
Sept 24	0	37	32
Oct 1	0	31	14
Oct 8	0	31	26

Table 6: Effect of delayed storage on brown core in McIntosh apples after storage at 0 C. Adapted from Smock (105).

Days after harvest	% Brown core	
	1937-38	1938-39
0	84	67
5	42	44
10		37
15	8	35
20		12

Table 7: Effect of storage temperature and delayed storage on soggy breakdown in Golden Delicious apples. Adapted from Plagge (89).

Days after harvest	Storage Temp (C)			
	-1.1	0	1.1	2.2
1	0	0	0	0
7	12	0	0	0
14	74	26	2	0
21	58	42	23	0

common in older fruit.

The above discussion makes it clear that ripening-related changes in chilling sensitivity are common. Table 8 shows data from McColloch et al. (62) depicting the effect of chilling for 14 days at 0 C on post-chilling tomato fruit quality. Fruit were held for 10 to 12 days at room temperature prior to the assessment of color and Alternaria rot. The chilling effects on color development and Alternaria rot are greatly reduced as the fruit ripen from the MG stage to the TU stage. Another study (16) has shown that R fruit can be held much longer at 0 C than MG fruit. One study disagrees with the idea of a gradual decrease in chilling sensitivity as tomatoes ripen. Hobson (34) showed that TU fruit could only survive 4 days at 2 C, whereas MG fruit could survive 7 days. He proposed that the highest sensitivity is at the climacteric peak, but low sample numbers and subjective measurements make his data suspect.

This study, using ion leakage from chilled tissue, shows that chilling sensitivity not only declines during the early stages of ripening but increases later in ripening. The increase in chilling sensitivity during the later stages of ripening has not been noted in the literature, probably because of the symptoms used to assess chilling injury. If a fully ripe tomato is chilled, abnormal ripening will not be a problem, and it may deteriorate faster than the development of Alternaria rot. One study has shown an effect of chilling on R fruit but did not compare fruit of different stages. Hall (30) reported that keeping red tomatoes at 2 C could cause a

Table 8: Chilling injury of tomatoes after storage for 14 days at 0 C. Adapted from McColloch et al. (62).

Stage of ripening	% Sound fruit	% Color development	% Alternaria rot
MG	15	56	82
TU	18	78	50
PI	21	80	42

reduction in red color after as little as 4 days. This shows that red fruit do indeed respond to chilling, but their symptoms are not as visible.

Why does chilling sensitivity change during tomato ripening? In tomatoes the change has 2 phases, one that appears to be related to ripening and the other related to senescence.

An obvious choice of a factor which may affect chilling sensitivity in tomatoes is endogenous hormone concentration. All the major classes of hormones fluctuate during tomato ripening (Figure 4). One objective of the dissertation was to study the effects of particular hormones on tomato chilling sensitivity. Auxins and cytokinins were eliminated, because of the limited evidence for their involvement in chilling sensitivity of other plant tissues. Ethylene was also eliminated because Kader and Morris (37) showed that ethylene treatment or removal of ethylene via hypobaric storage had no effect on chilling injury in tomatoes. AbA and GA were chosen because of their involvement with chilling sensitivity in other plant tissues and their strong positive correlations with the reported changes in tomato chilling sensitivity. AbA, in most cases, reduces chilling sensitivity, as in cucumber seedlings (99, 100), cotton seedlings (97, 98), soybean seedlings (57), rye and wheat cell cultures (13), tobacco cell cultures (6), grapefruit (39), and potato leaves (14). However, in apples it increases chilling sensitivity (27, 131). If changes in AbA levels caused changes in the chilling sensitivity of tomatoes it would have to act to increase the

sensitivity because of the natural changes in endogenous levels.

GA is known to reduce chilling sensitivity in apples (27, 129, 130, 131) and pears (125), but if it is important during tomato ripening it would have to increase chilling sensitivity because of the natural changes in endogenous levels.

The first technique used to alter endogenous AbA level was reported by Khudairi (40). He found that AbA biosynthesis appeared to be phytochrome related, in that fruit given red light treatments had higher levels of endogenous AbA than those given far-red light treatments (Figure 5). This technique provides a very simple, nondestructive method whereby AbA levels may be altered. In this study fruit were ripened under darkness, red, far-red, or white light. The light treatments had no effect on chilling sensitivity as measured with ion leakage. (Figure 20). These results suggest two possibilities: either the light treatments did not alter AbA levels or AbA has no effect on chilling sensitivity in tomatoes.

The second technique used to alter levels of AbA was vacuum infiltration. Specific quantities of AbA were introduced into tomatoes. Preliminary tests suggested an increase of chilling sensitivity in MG tomatoes from AbA treatments. However, when the experiment was repeated using ripening fruit, higher AbA concentrations, and lower chilling temperatures, conditions which should have intensified any effect of AbA, there were no differences between fruit receiving AbA and those which did not.

Fruit were also vacuum-infiltrated with GA, but it too had no

effect on the degree of injury after chilling. It would appear that changes in endogenous GA or AbA are not the reason for changes in tomato chilling sensitivity.

Changes in tomato chilling sensitivity may be directly related to membrane viscosity. The most interesting aspect of chilling sensitivity in tomatoes is the increase during the later stages of ripening. The data from nor fruit suggest that this is a senescence related phenomenon. It is well known that membrane viscosity increases during senescence (7, 117). A senescent membrane may be more sensitive to chilling, because of this increase in viscosity. In a senescent membrane, chilling may further increase the viscosity and thus be more detrimental. it is logical to assume that a more viscous membrane would be more sensitve to chilling.

Membrane viscosity was not measured in this study, but it has been shown in senescing carnation petals (117) and in senescing rose petals (7) to be directly related to the sterol-to-phospholipid ratio of the membrane. As carnation petals senesce they exhibit a symptom called inrolling, and this corresponds very closely with a loss of phospholipids and an increase in sterols accompanied by an increase in membrane viscosity (117). Rose petals leak anthocyanins from their cells in increasing quantitiies as they senesce (7). This leakage is accompanied by a loss in phospholipids, with sterols staying constant, and an increase in membrane viscosity (7). Borochov et al. (7) also showed the relationship between the sterol-to-phospholipid ratio and membrane viscosity. Figure 22 shows the data from an artificial

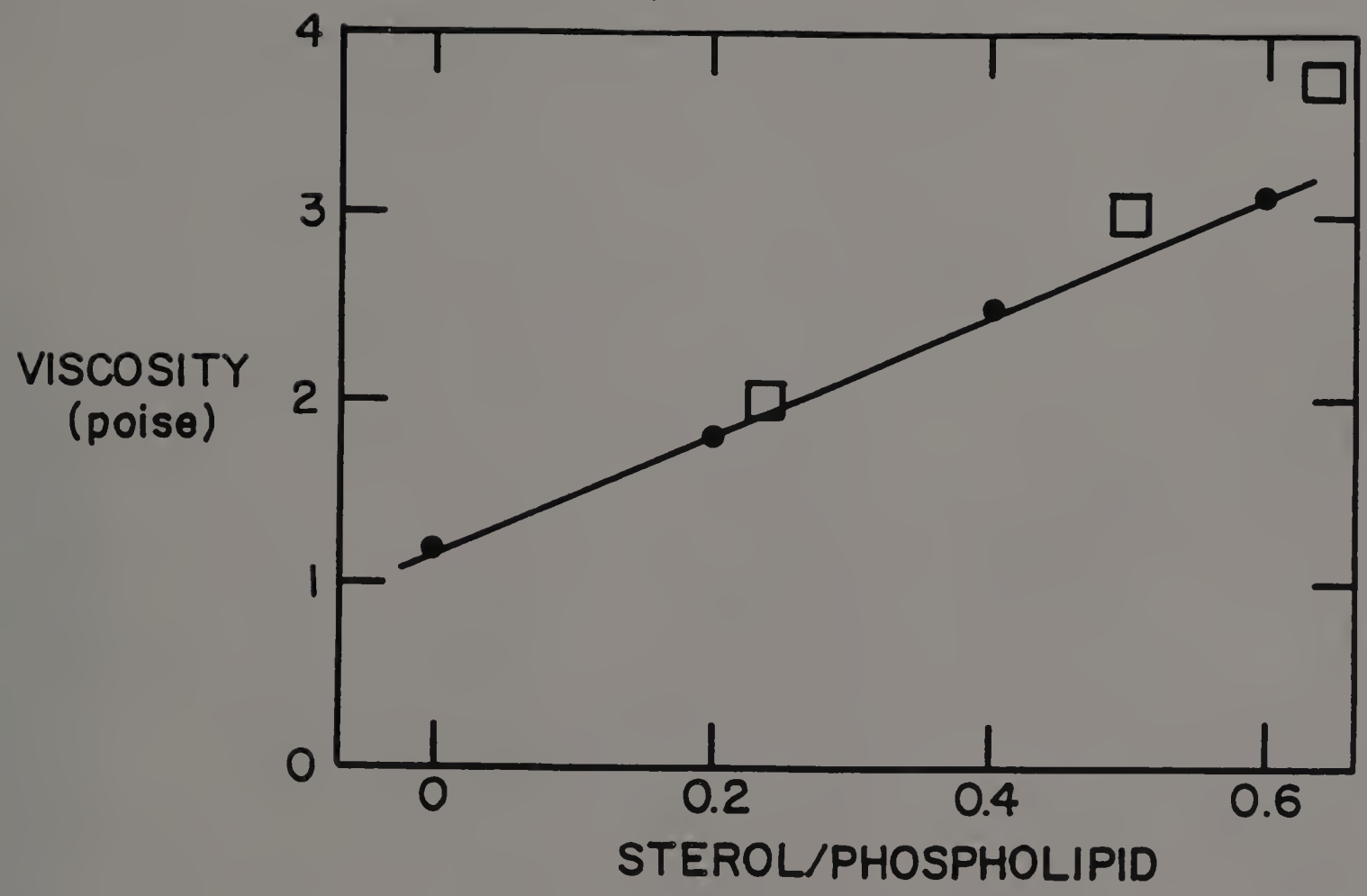

Figure 22: The effect of the sterol-to-phospholipid ratio on membrane viscosity. Closed circles are for vesicles of egg lecithin with different amounts of sterol to vary the sterol-to-phospholipid ratio. Open squares represent the values obtained from senescing rose petals. Adapted from Borochov et al. (7).

system of sterols plus egg lecithin. As the molar ratio of sterols increased, the viscosity increased linearly. Also plotted on Figure 22 are the values for the senescing rose petals (open squares) which correspond very closely to the plotted line.

It would appear that the sterol-to-phospholipid ratio is a primary determinant of membrane viscosity. Ueda et al. (119) have measured the sterol content of tomato fruit during ripening, and Minamide et al. (68) have measured the phospholipid content. A sterol-to-phospholipid ratio was calculated from their data and is plotted in Figure 23. It is recognized that these data are from two separate studies and may not be directly comparable, but they suggest that as fruit mature from the immature-green (IG) stage to the PI stage there is a decline in the sterol-to-phospholipid ratio suggesting a drop in membrane viscosity. After the PI stage there is a rapid increase in the sterol to phospholipid ratio suggesting a rapid increase in membrane viscosity. This change is likely a senescence-related increase in membrane viscosity. Actual measurements of membrane viscosity would have to be made to verify this relationship, but these data may explain increases in chilling sensitivity during the later ripening stages and possibly the decrease as fruit begin to ripen.

An increase in chilling sensitivity related to senescence may be common to ripening fruits or any plant tissue. It appears that apples can respond more severely if chilled during senescence, as was seen with soggy breakdown. Measurement of chilling sensitivity in other

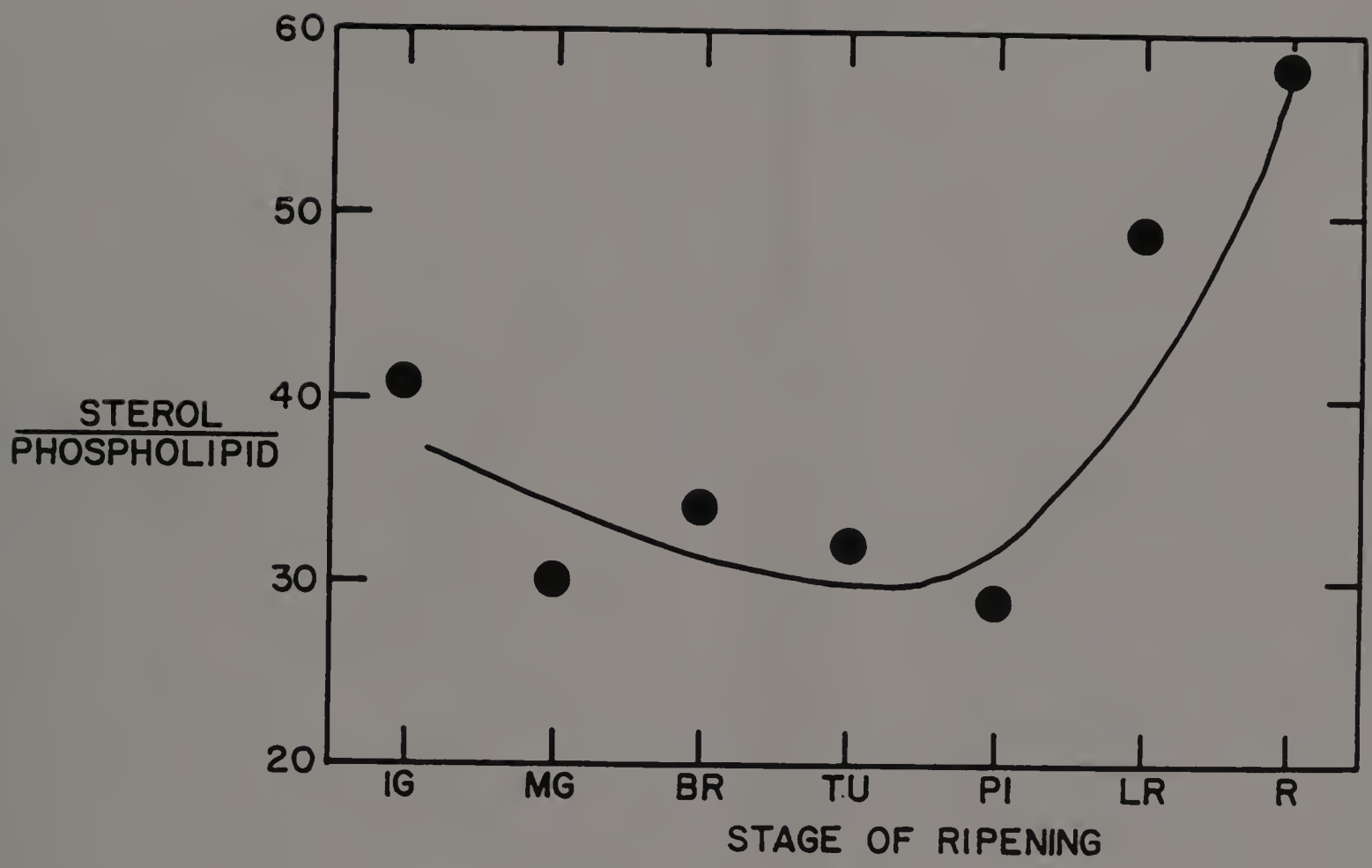

Figure 23: Sterol-to-phospholipid ratio during tomato ripening. Sterol contents (mg/100g F.W.) are from Ueda et al. (119). Phospholipid contents (%F.W.) are from Minamide et al. (68). Stage designations have been altered from the original papers to more closely correspond to those used in this study. IG refers to immature-green fruit.

fruit tissues, where it has been found to decrease, during ripening, may not have been made when the fruit had actually begun to senesce. The critical question is: When does senescence begin? It is often suggested that senescence begins at harvest, but it may be more accurate to say that it begins when the membranes begin to deteriorate or when the membrane viscosity begins to increase.

Importance of This Study

The results and conclusions made from this dissertation are of little importance to tomato growers and handlers. Changes as fruit begin to ripen are already well known. Knowledge of the increase later in ripening is of limited value, because the life of a red tomato is very short regardless of chilling injury. This work may be more important to other fruit crops such as apples. In crops where fruit have long storage periods and long shelf lives after storage changes in chilling sensitivity late in ripening may be much more critical. Ripening and senescence during the storage period itself may alter sensitivity, so that fruit may not be sensitive at harvest but become so during storage. Gradually raising the storage temperature may be advantageous with these fruits.

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APPENDIX

Table 9: Analysis of variance for ion leakage data from the Duration/Intensity experiment (Figure 8).

Source	df	MS	F
Whole Plot			
Temperature	2	132.21	15.3**
Rep:T	27	8.62	
Split Plot			
Duration	4	53.98	7.5**
DT	8	37.06	5.1**
DR:T	108	7.22	
T:D1	2	8.4	1.1ns
T:D2	2	31.7	4.2*
T:D3	2	13.5	1.8ns
T:D4	2	53.8	7.2**
l	1	88.2	11.8**
q	1	19.3	2.6ns
T:D5	2	173.3	23.1**
l	1	344.5	45.9**
q	1	2.0	0.3ns
pooled error	92	7.5	

Table 10: Analysis of variance for CO_2 data from the Duration/Intensity experiment (Figure 10).

Source	df	MS	F
Whole Plot			
Temperature	2	169602	71.9**
Rep:T	21	2360	
Split Plot			
Duration	4	86844	49.1**
DT	8	23566	13.3**
DR:T	84	1770	
T:D1	2	843	0.4ns
T:D2	2	17123	9.1**
l	1	33124	17.5**
q	1	1121	0.6ns
T:D3	2	45971	24.3**
l	1	80656	42.7**
q	1	11285	6.0*
T:D4	2	47048	24.9**
l	1	88804	47.0**
q	1	5292	2.8ns
T:D5	2	152851	81.1**
l	1	266256	141.0**
q	1	39445	20.9**
pooled error	71	1888	

Table 11: Analysis of variance for ethylene data from the Duration/Intensity experiment (Figure 12).

Source	df	MS	F
Whole Plot			
Temperature	2	4.53	53.9**
Rep:T	21	0.08	
Split Plot			
Duration	4	2.98	31.7**
DT	8	0.62	6.6**
DR:T	84	0.09	
T:D1	2	0.04	0.5ns
T:D2	2	0.63	7.2**
l	1	1.25	14.3**
q	1	0.01	0.1ns
T:D3	2	1.27	14.5**
l	1	1.80	20.4**
q	1	0.75	8.5**
T:D4	2	1.63	18.5**
l	1	3.24	36.8**
q	1	0.02	0.2ns
T:D5	2	3.44	39.1**
l	1	6.86	78.0**
q	1	0.02	0.2ns
pooled error	71	0.09	

Table 12: Analysis of variance for ion leakage data from the ripening experiment (Table 1).

Source	df	MS	F
Leak. sol.	1	48446	384.4**
Chilling	1	1799	11.9**
Stage	4	2816	63.6**
Rep	8	150	
LC	1	116	1.6ns
LS	4	940	20.9**
LR	8	126	
CS	4	12	0.2ns
CR	8	151	
SR	32	44	
LCS	4	67	1.7ns
LCR	8	75	
LSR	32	45	
CSR	32	69	
LCSR	32	39	

Table 13: Analysis of variance for CO_2 data from the ripening experiment (Figure 13).

Source	df	MS	F
Chilling	1	17689	9.9**
Stage	4	7204	14.3**
Rep	9	1378	
CS	4	6187	18.2**
CR	9	1792	
SR	36	505	
CSR	36	341	
C:S1	1	31920	50.6**
C:S2	1	9548	15.1**
C:S3	1	551	0.9ns
C:S4	1	144	0.3ns
C:S5	1	245	0.4ns
pooled error	31	631	

Table 14: Analysis of variance for ethylene data from the ripening experiment (Figure 14).

Source	df	MS	F
Chilling	1	0.004	0.1ns
Stage	4	4.747	62.1**
Rep	9	0.151	
CS	4	0.857	10.2**
CR	9	0.035	
SR	36	0.076	
CSR	36	0.084	
C:S1	1	2.506	33.9**
C:S2	1	0.435	5.9*
C:S3	1	0.384	5.2*
C:S4	1	0.085	1.1ns
C:S5	1	0.022	0.3ns
pooled error	31	0.074	

Table 15: Analysis of variance for ion leakage data from the light quality experiment (Figures 15 and 20).

Source	df	MS	F
Whole Plot			
Stage	4	1963	10.1**
Rep:S	15	195	
Split Plot			
Chilling	1	2441	133.0**
Light	3	36	0.5ns
CL	3	66	1.9ns
SC	4	496	27.0**
SL	12	44	0.7ns
SCL	12	63	1.9ns
CR:S	15	18	
LR:S	45	67	
CLR:S	45	34	
C:S1	1	820	45.6**
C:S2	1	3	0.2ns
C:S3	1	26	1.5ns
C:S4	1	570	30.7**
C:S5	1	3023	167.9**
error	15	18	

Table 16: Analysis of variance for ion leakage data from the light intensity experiment (Figures 16 and 21).

Source	df	MS	F
Whole Plot			
Stage	4	5932	60.1**
Rep:S	40	99	
Split Plot			
Chilling	1	4621	67.3**
Light	2	18	0.5ns
CL	2	45	0.7ns
SC	4	803	11.7**
SL	8	36	1.0ns
SCL	8	31	0.5ns
CR:S	40	69	
LR:S	80	36	
CLR:S	80	66	
C:S1	1	303	4.4*
C:S2	1	462	6.7*
C:S3	1	128	1.8ns
C:S4	1	451	6.5*
C:S5	1	6294	91.2**
error	40	69	

Table 17: Analysis of variance for ion leakage data from the nor experiment (Figure 17).

Source	df	MS	F
Age	2	390.0	32.5**
Chilling	1	133.0	17.7**
Rep	15	44.9	
AC	2	66.7	3.5*
AR	30	12.0	
CR	15	7.5	
ACR	30	19.2	
C:A1	1	3.1	0.2ns
C:A2	1	4.5	0.3ns
C:A3	1	258.8	16.9**
pooled error	25	15.3	

Table 18: Analysis of variance for the CO_2 data from the nor experiment (Figure 18).

Source	df	MS	F
Age	2	2.04	17.0**
Chilling	1	7.38	369.0**
Rep	6	0.17	
AC	2	0.50	4.9*
AR	12	0.12	
CR	6	0.02	
ACR	12	0.10	
C:A1	1	5.21	74.4**
C:A2	1	2.44	34.9**
C:A3	1	0.75	10.7**
pooled error	10	0.07	

Table 19: Analysis of variance for ethylene data from the nor experiment (Figure 19).

Source	df	MS	F
Age	2	1.73	24.7**
Chilling	1	4.52	18.1**
Rep	5	0.14	
AC	2	0.80	11.4**
AR	10	0.07	
CR	5	0.25	
ACR	10	0.07	
C:A1	1	3.18	24.5**
C:A2	1	2.94	22.6**
C:A3	1	0.04	0.3ns
pooled error	8	0.13	

Table 20: Analysis of variance for ion leakage data from the STS experiment (Table 2).

Source	df	MS	F
Chilling	1	1094	16.0**
Stage	1	3819	72.9**
Rep	6	76	
CS	1	79	1.7ns
CR	6	68	
SR	6	52	
CSR	6	47	

Table 21: Analysis of variance for CO_2 data from the light intensity experiment (Table 3).

Source	df	MS	F
Whole Plot			
Stage	4	6882	6.2**
Rep:S	15	1123	
Split Plot			
Chilling	1	6308	6.3*
Light	2	1337	2.3ns
CL	2	1683	1.7ns
SC	4	8670	8.7**
SL	8	1409	2.5*
SCL	8	307	0.3ns
CR:S	15	1000	
LR:S	30	576	
CLR:S	30	999	
C:S1	1	32561	32.6**
C:S2	1	963	1.0ns
C:S3	1	96	0.1ns
C:S4	1	6273	6.3*
C:S5	1	1291	1.3ns
error	15	1000	

Table 22: Analysis of variance for C_2H_4 data from the light intensity experiment (Table 3).

Source	df	MS	F
Whole Plot			
Stage	4	10.72	30.6**
Rep:S	15	0.35	
Split Plot			
Chilling	1	0.19	4.5ns
Light	2	0.64	8.6**
CL	2	0.19	1.8ns
SC	4	0.45	11.0**
SL	8	0.12	1.6ns
SCL	8	0.11	1.1ns
CR:S	15	0.04	
LR:S	30	0.07	
CLR:S	30	0.10	
C:S1	1	1.75	43.8**
C:S2	1	0.15	3.8ns
C:S3	1	0.01	0.3ns
C:S4	1	0.02	0.5ns
C:S5	1	0.04	1.0ns
error	15	0.04	

Table 23: Analysis of variance for the AbA experiment with MG fruit (Table 4).

Source	df	MS	F
Chilling	1	840	46.7**
AbA	2	46	3.8*
Rep	14	47	
CA	2	30	3.4*
CR	14	18	
AR	28	12	
CAR	28	9	
A:C1	2	7	0.6ns
A:C2	2	70	6.4**
l	1	74	6.7*
q	1	67	6.0*
pooled error	28	11	

Table 24: Analysis of variance for AbA experiment with ripening fruit (Table 4).

Source	df	MS	F
Chilling	1	1192	31.5**
AbA	1	16	0.3ns
Rep	12	96	
CA	1	103	2.2ns
CR	12	38	
AR	12	71	
CAR	12	46	

Table 25: Analysis of variance for GA experiment (Table 4).

Source	df	MS	F
Chilling	1	968.0	21.8**
Ga	1	2.0	0.3ns
Rep	7	66.8	
CG	1	4.5	0.2ns
CR	7	44.5	
GR	7	6.9	
CGR	7	21.4	