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Investigations of the potential for chilling injury during storage of chile peppers (Capsicum annuum L. and C. frutescens L.) /

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INVESTIGATIONS OF THE POTENTIAL FOR CHILLING INJURY DURING STORAGE OF CHILE PEPPERS (*Capsicum annuum* L. and *C. frutescens* L.)

A Thesis Presented

by

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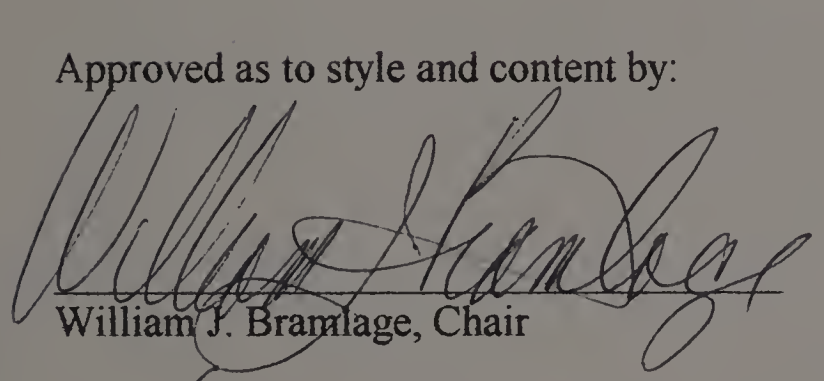

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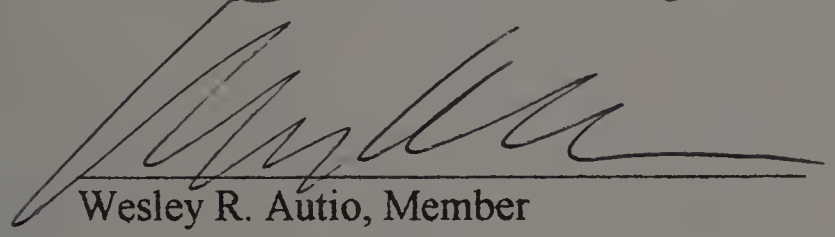

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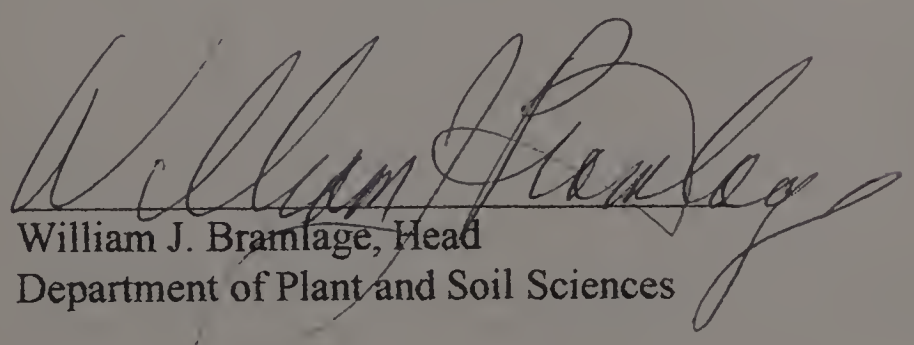

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ABSTRACT

INVESTIGATIONS OF THE POTENTIAL FOR CHILLING INJURY DURING STORAGE OF CHILE PEPPERS (*Capsicum annuum* L. and *C. frutescens* L.)

SEPTEMBER 2000

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Six cultivars, one with two ripening stages, of chile peppers (*Capsicum* spp.) were tested for differences in susceptibility to postharvest chilling injury (CI). Cherry 'Bomb', 'Cubanelle', 'Hungarian Wax' (HW), Poblano 'Ancho San Luis', 'Serrano', and mature green and full color (red) 'Jalapeño' fruit were stored at 2.5°C, 7°C, or ~15°C for 0 to 30 days, sampling every 4 days for the first 16 days. Ethylene (C_2H_4) evolution and internal C_2H_4 concentration were measured. Susceptibility to chilling varied among pepper types. HW, which produced the highest levels of C_2H_4, were the most susceptible, and 'Serrano', which had the lowest levels, were the least susceptible. Scald (a surface browning) appeared on HW and 'Cubanelle' fruit in addition to pitting, which occurred on all the cultivars. Poblano fruit stored at 2.5°C exhibited large, deep pits which appeared similar to freezing injury; they had small pits after 8 days at 7°C.

Red Cherry 'Bomb' peppers were harvested on three dates: 4 September, 16 September, and 2 October 1998. They were stored at 2.5°C, 7°C, or ~15°C and were sampled every 5 days for 15 days. CI symptom manifestation increased with later harvesting. Some peppers from the last harvest pitted after 10 days at 7°C.

Three cultivars of banana peppers, HW, 'Hungarian Yellow Wax' (HYW), and 'Sweet Banana' (SB), were stored at 2.5°C, 7°C, or ~15°C but sampled every 2 days. Scald was observed on all cultivars stored at 2.5°C, first as small, translucent pits then as

larger, irregular light brown pits. No peppers had CI symptoms after 8 days at 7°C. Ion leakage from pericarp tissue increased with duration of storage at all temperatures.

'Serrano Chili' peppers were stored at 0°C for 0 to 30 days or 2.5°C for 0 to 15 days. C_2H_4 evolution was greater at 0°C than 2.5°C and increased with storage duration. After 30 days at 0°C, peppers were splotchy and soft with rot. Ion leakage increased with storage duration at both temperatures.

Recommendations for storage of peppers should be expanded to accommodate differences among cultivars. A temperature of 7°C cannot be considered safe and non-chilling for all pepper types.

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CHAPTER I

INTRODUCTION

In recent years there has been a strong increase in consumer demand for chile peppers in all regions of the United States. To meet this demand, peppers are shipped long distances, passed through marketing channels, and sometimes stored for short periods. To do this requires temperature management, and there is little guidance on appropriate temperatures for chile peppers.

The recommendations for postharvest storage of fresh chile pepper fruit (*Capsicum annuum* L. and *C. frutescens* L.) in the current edition of the United States Department of Agriculture (USDA) Agricultural Handbook 66 (48), the standard reference for postharvest practices, are to follow the recommendations given for fresh bell peppers as presented in the same volume. These recommendations are to store the peppers at a temperature no lower than 7°C to avoid chilling injury (CI) and rot caused by *Alternaria* species, and at a temperature no higher than 13°C to delay ripening and avoid the incidence of bacterial soft rot caused by *Erwinia* species. The recommended relative humidity is 90-95%. Precooling by forced-air, hydro-, or vacuum cooling is also recommended (48). However, there is no citation giving a scientific basis for recommending that all pepper types should be stored under the same conditions known to be safe for bell peppers, indicating that this is a presumption rather than an established fact.

Differences in chilling susceptibility among cultivars are common in plant species (3, 69), and it is my belief that the morphological differences among the many cultivars of chile peppers, as well as the differences between chile and bell peppers, raise distinct possibilities of variability in susceptibility to chilling temperatures or in development of symptoms of chilling injury. Due to the increase in consumer demand for chile peppers, these other cultivars have become of increased economic importance. Yet, their

postharvest requirements have attracted little attention. Furthermore, in other crops there is evidence that exposure to chilling temperatures in the field may affect the incidence of postharvest CI (2, 63), which is one of the reasons why it is unlikely that all peppers respond uniformly to postharvest temperatures. One of the experiments in this study explores this possibility, comparing red Cherry 'Bomb' peppers harvested at various times during the growing season.

The experiments described below have been designed to determine the extent of CI to chile pepper fruits by measuring C_2H_4 evolution, internal C_2H_4 concentrations, and ion leakage and observing the manifestation of CI symptoms. The results of these experiments can be used to expand the current recommendations regarding the storage temperatures and durations which are safe for these cultivars, as they pass through the rapidly expanding marketing channels.

The objectives of these experiments were: 1) to assess the manifestation of CI on chile pepper fruits stored at temperatures at, above, and below the established threshold temperature for bell peppers (7°C); 2) to determine physiological responses to low temperature stress for different durations of time; and 3) to assess the effect of harvest date on CI manifestation of Cherry 'Bomb' peppers.

CHAPTER II

LITERATURE REVIEW

A. Postharvest Physiology of Fruit

Botanically, a fruit is the mature ovary of a flowering plant. In common usage, however, other plant tissues are called fruit. An example of this is the strawberry, the fleshy parts of which are modified receptacles (14, 154). There is no such definition of the word vegetable. Many commodities which are commonly referred to as vegetables, such as tomatoes, peppers, and squashes, are actually fruit, and many true fruits are commonly thought of as vegetables. Examples of the latter are grains and legumes. Many vegetables are other plant organs such as roots (beets, carrots), stems (potatoes), and leaves (spinach, lettuce). True fruits can be fleshy (apple) or dry (wheat) at maturity (14). Most of the major crops of the world are fruit, especially grains.

The worldwide losses of harvested plant parts vary with the commodity, place of harvest, mode(s) of transportation, and final destination. Indeed, losses can occur at all steps from farm to market. Because of the variability of the factors affecting postharvest loss, estimates range from twenty to eighty percent annually (154) for all crops. Ten to thirty percent of most economically important crops are lost after harvest, enough food for hundreds of millions of people (65). In many cases, the loss of nutritionally important crops is much higher (112). It is obvious that the control of loss of food commodities already harvested should be a major area of study as most of the arable land is already cultivated and the increases in yield per unit of area have been essentially maximized (112).

Postharvest physiologists have long been concerned with the changes that take place in fruits which lead to an attainment and maintenance of an edible state. However, because the stages of development often overlap and are ill-defined, there has been

confusion regarding the appropriate terms of these stages. Watada et al. (151) have clarified this as follows: *Development* is the series of processes from the initiation of growth to death of a plant or plant part. *Growth* is the irreversible increase in characteristics of a developing plant or plant part. *Maturation* is the stage of development leading to the attainment of physiological maturity (when development will continue to ontogeny even if detached) or horticultural maturity (when the plant or plant part possesses the prerequisites for utilization by consumers for a particular purpose). *Ripening* is the composite of the processes from the latter stages of growth and development through the early stages of senescence. This results in the attainment of the edible stage due to changes in color, composition, or texture. These changes in the properties of the fruit are a result of changes in mRNA and protein synthesis (129). *Aging* is any increment of time which may or may not be accompanied by physiological change. *Senescence* is the composite of processes which follow maturity and lead to death (151).

Fruit have been classified as climacteric or non-climacteric (14). The climacteric period is the period in the development of some fruits and cut flowers (10) which involves biochemical changes associated with a rise in respiratory rate and autocatalytic ethylene (C_2H_4) production. It consists of the preclimacteric, the preclimacteric minimum, the climacteric rise, the climacteric peak, and the postclimacteric stages. The most important observation of postharvest change is the measurement of changes in respiration rate, as evidenced by changes in carbon dioxide (CO_2) evolution or oxygen consumption over time. Respiration is the process by which sugars are oxidized to yield CO_2 and usable energy in the form of adenosine triphosphate (ATP) using the systems of glycolysis, the tricarboxylic acid cycle, and oxidative phosphorylation. The distinguishing rise in respiration rate and C_2H_4 production is coincident with the onset of ripening, and a subsequent decline in respiration rate usually occurs. Examples of this type of fruit include apple, tomato, and banana (14). Non-climacteric fruit, such as cucumber and orange, show a continual, gradual decline in respiration over time (13). The respiration

rate and height of the climacteric peak were first shown to be affected by storage temperature by Kidd and West (66).

Because the onset of ripening is coincident with the cessation of growth, it is obvious that this is also a time of changes in hormone concentrations. Gibberellins decrease, and auxins and abscisic acid increase (32). Ethylene has been called the ripening hormone in plants and has long been known to promote senescence (21, 62) and abscission (21). Its concentration increases in response to a wide variety of stresses (157), such as pathogen infection (53), insect infestation (133), wounding (including harvesting), and exposure to suboptimal temperatures (including chilling) (8, 86). S-adenosylmethionine (SAM) is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) in the presence of ACC synthase. ACC is then converted to C_2H_4 in the presence of ACC oxidase. The first of these reactions is thought to be the rate-limiting step (34). Ethylene biosynthesis has been classified into two parts, System 1 and System 2 (95, 110). The latter is initiated by ethylene produced by the former in climacteric fruit. Non-climacteric fruit do not have System 2. Many ACC synthase genes are activated by System 2 C_2H_4; synthesis of ACC oxidase is not as dependent on System 2 C_2H_4 (110). Multi-gene families code for both enzymes, and in tomato, many of these genes have been identified. Some genes which code for each enzyme are induced by C_2H_4 and some are not (107, 110). Ethylene hastens ripening (37) and increases the respiration rate by increasing glycolysis (137). Ethylene and its analogs, such as ethephon, have been long been applied postharvest to control timing of ripening (134). It has been shown that endogenous and exogenous C_2H_4 stimulate synthesis of polygalacturonase (43, 61, 134) and lycopene and invertase in tomatoes (134).

1. The Physiology of Chilling Injury

The term chilling injury (CI) was coined in the late nineteenth century by Molisch (103) to distinguish it from freezing injury. Fruit injured by exposure to freezing

temperatures have large, deep, water-soaked areas. In contrast, the lesions which occur after exposure to chilling temperatures are numerous with diameters of 3-10 mm and a depth of 1 mm (86). Other effects of chilling on plant organs other than fruit, such as sweetening of potatoes (155), have also been observed. Both chilling and freezing can occur in the field or in storage. Chilling injury can occur on the whole plant, parts of whole plants, or harvested plant organs when subjected to chilling temperatures above the temperature at which freezing occurs (86).

Although crops of tropical and sub-tropical origin are understandably most susceptible to CI, some species of temperate origin are also chilling-sensitive (18). The extent of CI is affected by the temperature and the duration of exposure. Other variables which affect chilling include the harvest season and the field temperature (63, 158). Tomato fruit harvested in the summer have been shown to be more resistant to storage chilling than those from a winter harvest (2). Purvis et al. (121) have demonstrated that midseason grapefruit, which have a higher concentration of reducing sugars, are more resistant to CI than fruit harvested at either the beginning or the end of the season. Purvis has also shown that resistance to CI is correlated with high proline concentration (120). The degree of ripening has also been shown to affect CI of tomatoes (7, 69) and bell peppers (76). Cultivar differences in susceptibility to CI have been shown in cucumbers (6), snap beans (152), bananas (3), and *Passiflora* species (114). Eaks has shown that the concentration of oxygen in the atmosphere also has an effect on the incidence of CI (35). Grapefruit harvested from the exterior canopy are more susceptible to chilling stress than fruit harvested from the interior of the same tree (119).

The physiological mechanisms of chilling injury manifestation have not been traced back to a single event which 'turns on' the other processes involved. It is known, however, that a membrane phase change is a primary event which is reversible if the plant material is exposed to chilling temperatures for only a short duration of time (87). Changes in plant cell membranes have long been recognized as major effects of chilling.

Chilling causes the membrane lipids to change from a flexible liquid-crystalline phase to a solid gel phase (87, 123). The critical temperature, below which membrane phase changes and chilling injury occur, are demonstrated by Arrhenius plots. These are the logarithm of the reaction rate vs. 1/T in Kelvin. Lyons et al. (87) have shown a phase transition at the critical temperature. The slope of Arrhenius plots of succinate oxidase (87) and ATPase (68) show a discontinuity, or "break", at the critical temperature. Exposure to these temperatures can cause the membranes to "crack", which is measured by solute leakage or ion accumulation. The membrane lipids of chilling-sensitive plants usually have a higher ratio of saturated to unsaturated fatty acids, which may help account for the degree of susceptibility to cold temperatures (87). Nuclear magnetic resonance has been used to study the rupturing of vesicles during phase transition (118). Although most research has been with the tonoplast and the mitochondrial membranes, chloroplast membranes have also been studied (87). Phase changes in mitochondria from chilling-sensitive plants have been detected with spin labeling at the critical temperature (124). The catalytic properties of proteins embedded in the membranes can be adversely affected by these phase changes (122). The activation energy of membrane-bound enzymes is increased (125) which leads to an accumulation of potentially toxic compounds such as pyruvate, acetaldehyde, and ethanol (37). The difference in chilling sensitivity between cultivars may be at least, in part, a result of difference in the ability of these cultivars to metabolize these compounds (37). However, the above theory disregards the complexity of membrane systems. Biological membranes contain far more than one lipid species, and the properties of lipids associated with proteins vary from those only associated with other lipids (9).

Like the primary event of membrane lipid phase transition, secondary events are dependent on duration of exposure to chilling stress as well as temperature. They are also usually reversible for some period of time (123). These secondary responses to chilling include an increase in C_2H_4 production and the respiration rate, changes in metabolism,

alterations in cell structure resulting in the visible symptoms of CI, and decreases in protoplasmic streaming and photosynthesis (113, 147). As with other stresses, exposure to chilling has a positive effect on C_2H_4 biosynthesis (29, 148). The pathway of C_2H_4 synthesis in chilling is the same as that in ripening: Methionine → SAM → ACC → C_2H_4. Some fungi produce C_2H_4 but by a different pathway than that used by plants (158). Chilling often stimulates ACC synthase, which is not associated with the membrane, resulting in an increase in ACC (148). In contrast, ACC oxidase, which converts ACC to C_2H_4, is membrane-bound and its activity is sometimes reduced by chilling (157). Osmotic shock may result in an inhibition of auxin-induced C_2H_4 production due to damage to the membrane systems (58).

There is a rapid increase in respiration when chilled fruit are returned to room temperature. Lyons (86) has reviewed changes in detached organs, tissues, and isolated mitochondria. The Respiration Quotient (RQ) is a measurement of respiration rate as a function of CO_2 produced / O_2 consumed (154). The RQ has been used to measure the extent of CI by Eaks (37). Internal gas samples of lemon fruit had an elevated RQ seven hours after returning to room temperature after four weeks at a chilling temperature. After twenty-four hours at room temperature, the RQ was the same as that of control fruit which had not been chilled. This demonstrates the reversibility of slight damage from chilling. This reversibility of CI has also been demonstrated in corn seedlings (30). In contrast, lemon fruit that were in cold storage for longer periods of time suffered irreversible chilling damage, perhaps due to an increase in oxidizable intermediates (37).

It has been proposed that the alternate respiration pathway, also known as the cyanide-insensitive respiration pathway, is engaged during chilling (67). It is thought that chilling results in a reduction in usage of the cytochrome (cyt) pathway and an increase in respiration using the alternate pathway due to membrane changes (19). Kiener and Bramlage (67) found that up to half of the postchilling increase in respiration of cucumber hypocotyls could be accounted for by the alternate pathway. Low temperature

changed the electron flow to the alternate pathway from the cyt pathway. The alternate pathway may survive chilling better than the cyt pathway because the former has fewer components. In addition, it has been shown that chilling decreases the phosphorylative capacity of tomato fruit, which may cause an energy deficit (73).

The effects of chilling on metabolism are varied. Kozukue and Ogata (70) have studied metabolism changes in chilled pepper fruit and found an increase in a-keto acids in fruit during storage at 1°C and after returning to room temperature. Malic acid and citric acid production was greatly increased during storage at 1°C. Chlorogenic acid, which is found in brown seeds of bell pepper fruit, increased in storage at 1°C for seven days then decreased sharply (70). In addition, the shikimic acid pathway is induced in chilled pepper fruit, which may result in the accumulation of phenylpropanoids, including chlorogenic acid (70).

The symptoms of CI vary greatly with the plant tissue and severity of injury. Chilling affects seed germination by causing necrosis of the radicle (49). Cotyledons are also susceptible to chilling (57). Symptoms of chilling on fruit can include surface pitting, necrosis, external and internal discoloration (86), and an increased susceptibility to decay by microorganisms such as *Alternaria* species (92). Chaplin and Scott (26) have observed that chilling injury is first manifested on the distal end of avocado fruit and appears later on the proximal end. Surface and subsurface discoloration are symptoms of CI on muskmelons (77). Disorders called low temperature breakdown and scald, a browning of the skin, are common symptoms of CI on apples (154). "Wooliness" of peaches and plums are also symptoms of CI (48). Sheet pitting has been described on bell peppers (90). The calyx has been shown to be the part of the bell pepper fruit most sensitive to chilling (70, 159). Browning of seeds is also a symptom of chilling injury of pepper fruits (70). All of these may manifest themselves after the plant material is returned to a warmer, non-chilling temperature for a few days (86).

Susceptibility to chilling injury is measured in many ways. The extent of injury symptoms is often hard to quantify, and the characteristic lesions of most fruit do not manifest until after the fruit has been returned to a non-chilling temperature. For this reason, it has been desirable to measure changes that take place before the outward symptoms of CI are visible. Kamps et al. (64) have reviewed assays of CI of tomato leaflets including ion leakage, visual rating, and chlorophyll fluorescence. Of these, the latter was found to have the highest correlation with CI. Delayed light emission has also been useful in measuring CI of green plant tissue (1).

Procedures used to ameliorate chilling injury are varied, and Wade (144) gives a review of methods that have been employed. Genetic selection for resistance to chilling has long been employed and continues to be an important strategy (33). In addition, manipulation of harvested fruit by intermittent warming (24, 31) and temperature conditioning by exposure to both chilling and warming temperatures (97, 153) have been shown to be of benefit. Film wrapping of harvested fruit has also reduced the incidence of CI symptoms (100). The application of chemicals postharvest has helped reduce CI. Methyl jasmonate reduced CI in zucchini (150), and the application of free radical scavengers decreased the severity of CI on cucumber and bell pepper fruits (149).

B. Peppers

Capsicum species plants are of the family Solanaceae. They originated in South America and were introduced into Central America, Mexico, the southwest United States, and the Caribbean through trade and the migrations of birds. Archeological evidence shows that peppers were eaten as early as 8,000 years ago (5). Fruit found at Huaca Prieta are clearly larger than wild fruit and show that pepper was domesticated and cultivated at least 4,500 years ago (51). It was Christopher Columbus who initiated the spread of what he called "pepper" throughout the world (5). He had, of course, been

searching for a new sailing route to Asia in order to obtain black pepper (the berries of *Piper nigrum*) and other spices. Instead, he discovered a New World where the fresh and dried fruit of the indigenous *Capsicum* plants were important culinary staples. The common name, pepper, has remained, although the plants are unrelated. There has also been confusion regarding the common name chile and its various spellings. The current convention is to use the word chili when referring to a stew made with peppers and beans or meat. Chile refers to the fresh or dried fruit of the plant (46, 99). All spellings of the word come from the Nahuatls who lived in Central America and southern Mexico in the fifteenth century and called the plant chilli. Adding to the confusion, many chiles have more than one common name, and the same name is used for different cultivars that are similar in appearance. In addition, many cultivars have different common names in the fresh and dried states. For example, Poblano peppers are called Poblano when fresh and ancho when dried, although it is common to see fresh Poblanos labeled ancho (5, 51).

The spread of chiles through the world was fostered by the Spanish and Portuguese explorers and missionaries who encouraged agriculture and brought seed back to Europe and around the world with them. Peppers quickly became important to the cuisines of Southeast Asia, Hungary, India, and Africa (51). Since the earliest cultivation of peppers, plants have been selected for larger fruit with a pendulate habit, which facilitates harvesting. The wild type *C. annuum aviculare* has small berries which are borne upright on the stem. From this, the elongate, flattened, conical, and globose forms have evolved (5). Some dwarf varieties of pepper retain the characteristic of small upright fruit and are grown as ornamentals (51). Variations in form have been achieved by crossing different cultivars (39). In the sixteenth century, the plants were classified as *Capsicum* by herbalists. Tournefort and Linnaeus then adopted this name for the genus (52). The origin of this word are unclear. Some have said it comes from the Latin "capsa" meaning "box", referring to the shape of the fruit. Others have postulated it comes from the Greek "kapto" which means "to bite", referring to the characteristic pungency of

these fruit (51). Since that time, several classifications have been made at the species level (40, 52, 136). Other than habañeros and scotch bonnets, which are both of the species *C. chinense*, most economically important fresh peppers are classified as *C. annuum* or *C. frutescens* (5).

Chile peppers are unique in being the only known source of a family of molecules called capsaicinoids (126, 138). Capsaicinoids, of which capsaicin (N-vanillyl-methyl nonanamide) is the most prevalent, are the active ingredients in chile peppers. The content of capsaicin, which determines the relative hotness of the cultivars, is measured in Scoville units, named for Wilber L. Scoville who devised the Scoville Organoleptic Test in 1912 for Parke-Davis (108). Capsaicin purified in the laboratory has 12,000,000 Scoville units. The units are a measure of the volume of water needed to dilute the capsaicin to levels barely perceptible by human subjects. Capsaicin contents of chile peppers are given as ranges because of differences among fruit from the same cultivar and the subjectivity of the test. The degree of pungency is recorded differently by different people as capsaicin is the only "hot" molecule to which humans can develop a tolerance, unlike those found in black pepper, ginger, and mustard. It had been thought that there was a genetic predisposition to tolerance of chile peppers. However, it is now understood that this tolerance is merely a result of exposure (5). The amount of capsaicin varies greatly among cultivars. For example, sweet bell peppers have no capsaicin. Jalapeños have 2,500 to 5,000 units, and Serranos can have 5,000 to 15,000 units. Habañeros, the hottest peppers of them all, have 100,000 to 300,000 units (5, 108). Pungency increases with ripening and has been shown to be higher in summer fruit than autumn fruit (11). Capsaicinoids are excreted through spherical structures called receptacles, which are 0.3-1 mm in diameter, found on the interlocular septa. Secretory cells are found just below the receptacles, and the capsaicin is secreted when the thin cuticle layer of the receptacle cracks (111). The placenta is the hottest part of the chile fruit, and the pericarp and seeds become pungent only due to association with the

placenta (5, 111, 144). Indeed, capsaicin content of parthenogenic fruit has been shown to be comparable with normal fruit (111). Capsaicin content has been shown to be higher in fruit which are small and in fruit with thin pericarps (138).

All pepper fruit are good sources of vitamins A and C, and a large bell pepper contains as much vitamin C as an orange or a tomato of comparable size (5, 46, 51, 71). Orange and red peppers are also a source of lycopene (5). Fresh peppers have not been shown to be high in calories, potassium, or sodium (54). Peppers are often used fresh in cooking, and the sweet cultivars are often eaten raw. Larger peppers, especially bells and Poblanos, are often stuffed and baked (*chiles rellenos*). The dried pods of many varieties are often ground into spices such as cayenne and paprika, while many other fresh chiles are used to make condiments and sauces (51). The tabasco pepper of the species *C. frutescens* L. is grown solely for this purpose (108). Peppers are also commercially available canned, frozen, and pickled (*chiles en escabeche*) (59).

Before Columbus, both fresh and dried chiles were used by the Aztecs, Incas, Mayans, Olmecs, and Toltecs. The fruit were dried for seasoning and food preservation. In ancient Peru the pods of pepper plants were used as a medium of exchange in the marketplace (5). The Mayans often used chiles as punishment or torture by rubbing powder or cut fruit into the victim's wounds. Maidens caught flirting had chiles rubbed into their eyes; if they were unchaste, chiles were rubbed into their "offending parts". The cannibalistic Caribs burned and cut their captives then rubbed chiles into the wounds before they were cooked and eaten. It is unclear if this practice was intended as torture or seasoning (5, 51). South American natives burned peppers and used the smoke as a gas in warfare with the invading Spanish. In 1963 in South Vietnam during a dispute with the government Buddhist monks armed themselves with spray guns filled with a mixture containing chile powder (51). In modern times, capsaicin is used in self-defense "pepper sprays". Throughout time, chiles have also been used as remedies for ailments varying from epilepsy and malaria to arthritis and stomach problems (5, 51, 99, 108).

Although pepper plants are perennial in warmer climates, they are treated as annual plants in temperate areas, which accounts for the misnomer *annuum* (5). Today, Mexico is the world's largest grower of chile peppers. New Mexico, California, Texas, and Arizona grow most of the chiles for the fresh market in the United States (5). However, many cultivars of peppers are successfully grown in other parts of the United States in the summer season. Pepper plants are usually started in a greenhouse from seeds which are sometimes pregerminated. When seedlings are six to eight weeks old, they are planted almost to the cotyledons twelve inches apart in full sun. Leggy seedlings may be planted up to the first set of true leaves as adventitious roots will form from the stem. They do best in well-drained, fertile soil with a pH of 5.5-6.8. The seedlings should be fertilized when transplanted and sidedressed with a high nitrogen fertilizer when the plants have begun to set flowers. Peppers are usually harvested by hand, starting in July in the United States (5).

Common pests of peppers include aphids (*Aphis* spp.) and European corn borers (*Pyrausta nubilalis*). Pepper maggots (*Zonosemata electra* Say) commonly infest red Cherry peppers. Rodents (Order Rodentia) sometimes eat seeds and seedlings. Diseases of pepper include bacterial leaf spot, leaf spot caused by *Cercospora capsici*, southern blight caused by *Sclerotium rolfsii*, damping off caused by *Rhizoctonia* and *Pythium* species, and phytophthora blight caused by *Phytophthora capsici*. Mosaic viruses can be vectored by aphids and leafhoppers and spread through mechanical means. Blossom-end rot is the most significant physiological problem of peppers and is a result of poor calcium uptake due to fluctuations in watering and concentrations of fertilizer (74).

The classification of many cultivars of most peppers as *C. annuum* L. belies the tremendous variety in their morphologies and pungency. There is great variation in size, shape, pericarp thickness, and color when ripe. There are also differences in the stage of ripening when consumed. The smallest chiles are the chiltepìn and pequìn, which retain the upright character of fruit growth, and the largest are the bells and Poblanos. Most

pepper fruit are green while immature (5), and many are harvested and utilized at the mature green stage. Fruit which are more than 95% green are considered mature green (MG), while fruit which are less than 5% green are considered full color (FC) (76). During ripening, many changes take place including a decrease in chlorophyll content and an increase in anthocyanins and carotenoids. In New Mexican chiles, ethylene increases during rapid fruit growth and color change (16). When ripe, the fruit can be yellow, orange, red, purple, or brown. The latter have retained chlorophyll, which, combined with the red pigments, causes the peppers to appear brown (5). Many pepper cultivars are harvested after they have turned color, and some, such as bells and Jalapeños, are used at both the MG and FC stages (5, 99, 108). Banana type peppers, harvested when yellow, will reach a red, inedible state if allowed to further develop (5). Fruit firmness decreases with an increase in the activity of b-galactosidase and other degradative enzymes (16). There is also a decrease in non-cellulosic neutral sugars (44). Most of these ripening-related changes take place fifty-four to sixty-nine days after flowering (5).

Most postharvest studies have focused on either sweet bell peppers or only one selected cultivar of hot pepper. 'Changjiao' peppers have been shown to be non-climacteric (82). At this time, only 'Chooraehong', a Korean cultivar, has been shown to undergo a respiratory climacteric. The CO_2 production measured at the peak of this climacteric was ~130 mg kg^{-1} hr^{-1}. The ethylene peak was only 0.7 µl kg^{-1} hr^{-1}, which is much smaller than those measured in other climacteric fruits (45). MG bell peppers responded like non-climacteric fruit in response to exogenous C_2H_4 and propylene (135). Postharvest changes in pepper fruit include weight loss and a decrease in firmness coincident with a decrease in insoluble pectins and an increase in water-soluble pectins. Patterns were similar for the cells of red and green fruit (84). Other changes include an increase in membrane permeability. Microviscosity, sterols, and abscisic acid all increase initially and then decrease with time (83).

Desiccation, chilling injury, and heat injury are the primary physiological problems encountered postharvest by peppers. Other postharvest losses are due to bacterial soft rot by *Erwinia* species, anthracnose, alternaria rot, botrytis rot, and viruses vectored by mites and thrips (90, 91, 93). McColloch (90) found the lowest safe storage temperature for bell peppers to be 7°C, while Yao et al. (158) have determined the critical temperature to be 9°C. When kept at temperatures below this, CI is manifested in the form of pitting (including sheet pitting) and black lesions on the surface of pepper fruit after they have been returned to room temperature. Another symptom of CI in peppers is increased susceptibility to alternaria rot (90). Peppers stored at and below 4.5°C are susceptible to botrytis rot (93). In addition, CI has been shown to be influenced by field temperature and harvest season. Bell peppers harvested in the summer, which were not exposed to chilling in the field, were more susceptible to CI than those harvested in the autumn, which had been exposed to some chilling temperatures (159). Storage of bell peppers in high CO_2 at 13°C decreased wall softening and ripening but caused calyx injury and increased decay (146). Treatment of chile pepper plants (135) and of mature green pimiento peppers (78) with C_2H_4 analogs has been shown to induce color change.

Factors that affect water loss postharvest include water content at harvest, surface area to volume ratio, cuticle weight, and epicuticular wax content (80). Methods studied to reduce water loss in stored bell peppers include packaging in perforated polyethylene (4, 145), foam polystyrene trays overwrapped with polyethylene film (23), cardboard trays overwrapped with polyethylene film, and corrogated cardboard boxes (145). Application of a thin coat of wax has reduced postharvest water loss (50). Ascorbic acid concentration in pepper fruit was unaffected by polyethylene packaging (4). Film wrapping of individual fruit (12), which does help reduce desiccation in storage, does not lower the incidence of CI (100). Heat treatment prior to storage (97) and intermittent warming (149) have proven to reduce the incidence of CI. Also, treatment with sodium

benzoate or ethoxyquin, which are free radical scavengers and increase the degree of saturation in the lipid membranes, has been shown to alleviate chilling injury (146). The stage of ripening of the fruit has been shown to be a factor in the incidence of CI. mature green bell peppers are much more susceptible to chilling temperature than full color bell peppers (76). In another study yellow peppers were more susceptible to CI at 5°C than were green or red fruit (96).

The experiments described below help determine differences in susceptibility to chilling injury among several types of economically important chile peppers, including differences between Jalapeños harvested both green and red and among three cultivars of banana-type peppers. In addition, the effect of harvest date on the chilling sensitivity of red Cherry 'Bomb' peppers is evaluated.

CHAPTER III

MATERIALS AND METHODS

A. Plant Material

All plants were seeded in Bowditch Hall Greenhouse #6 at the University of Massachusetts at Amherst. The seedlings were transplanted into fields at the University of Massachusetts Experimental Station in South Deerfield, MA. The rows were oriented east-west in 1998 and north-south in 1999. The seedlings were planted one foot apart in rows three feet apart with a two foot alley between each replication. For each experiment there were five replications arranged in a Randomized Complete Block design. For the experiments with more than one type of pepper, it was desired that the neighboring types be varied.

1. Experiment 1: Differences among Chile Pepper Types

Seven pepper types of economic importance and representative of morphological differences in fruit size and shape (pod-types), stage of ripening at harvest, and pod wall thickness were chosen and are described as follows:

Cherry 'Bomb' (*Capsicum annuum* L. var. Cerasiforme Group) - Lot 158 1046, Petoseed Co., Saticoy, CA - 60-65 days, 3-4 cm in diameter, globose, red, thick-walled

'Cubanelle' (*C. annuum* L.) - Lot 1, The Chas. C. Hart Seed Co., Wethersfield, CT - 60-68 days, 15-20 cm long, 5 cm in diameter, elongate flattened, light green, thin-walled

'Hungarian Wax' (*C. annuum* L.) - Lot 1, W. Atlee Burpee and Co., Warminster, PA - 70-75 days, 12-15 cm long, 2½-4 cm in diameter, elongate conical, yellow, thin-walled

'Jalapeño' mature green (MG) (*C. annuum* L.) - Lot 2, W. Atlee Burpee and Co., Warminster, PA - 70-75 days, 5-7 cm long, 2½-4 cm in diameter, elongate conical, green, thick-walled

'Jalapeño' full color (FC) - same as above but red at harvest

Poblano 'Ancho San Luis' (*C. annuum* L.) - Lot 3B, Shepherd's Garden Seeds, Felton, CA - 60-65 days, 10-12 cm long, 3½-4 cm in diameter, conical, dark green, thin-walled

'Serrano' (*C. frutescens* L.) - 75-80 days, 2½-5 cm long, 1¼-2 cm in diameter, elongate conical, dark green, medium-walled

One hundred and twenty-five plants of each type were seeded on 10 April 1998 and transplanted into a 135' x 21' (4.1 x 6.4 m) field on 1 June 1998. There were twenty-five plants in each replication. The plot design is shown below (Figure 1A). The flowers were tagged at anthesis by tying a tag marked with the tag date on the peduncle of each open flower. Because pepper plants flower continuously during the season, tagging was done every few weeks. At a point in the season when it appeared as if enough fruit were ready at the same time, four hundred and twenty-five (425) fruit of each type of similar size and shape, bearing the same tag, and free from injury were hand-harvested and subjected to the storage treatments described below. Jalapeños which were >95% green were considered mature green; those which were <5% green were considered full color. A total of two thousand, nine hundred and seventy-five (2975) fruit were utilized in this experiment.

2. Experiment 2: Effects of Harvest Date

One hundred and twenty-five plants of Cherry 'Bomb' were seeded on 22 April 1998 and transplanted into a 27' x 15' (8.2 x 4.6 m) field on 1 June 1998. There were twenty-five plants in each replication. The flowers were tagged at anthesis by tying a tag marked with the tag date on the peduncle of each open flower. Because pepper plants

flower continuously during the season, tagging was done every few weeks. At three points in the season, when it appeared as if enough fruit were ready at the same time, three hundred and seventy-five (375) full color (red) fruit of similar size and shape, bearing the same tag, and free from injury were hand-harvested and subjected to the storage treatments described below. In order to have groups of peppers which had experienced different field conditions, the third harvest was after there had been a few nights with low temperatures of 37°F (2.8°C). The dates of harvest were: 4 September, 16 September, and 2 October 1998. A total of seven hundred and twenty (720) fruit were utilized in this experiment.

3. Experiment 3: Differences among Banana Pepper Cultivars

Three cultivars of banana peppers (*Capsicum annuum* L.) of economic importance and similar shape and size were chosen and are described as follows:

'Hungarian Wax' - Lot 2, W. Atlee Burpee and Co., Warminster, PA

'Hungarian Yellow Wax' - Lot 1, NK Lawn and Garden Co., Chattanooga, TN

'Sweet Banana' - Lot 1, W. Atlee Burpee and Co., Warminster, PA

One hundred and five plants were seeded on 19 April 1999 and transplanted into a 15' x 64' (4.6 x 19.5 m) field on 22 June 1999. There were twenty-one plants in each replication. The plot design is shown below (Figure 1B). Three hundred (300) fruit of each type of similar size, shape, and color and free from injury were hand-harvested once during the season and subjected to the storage treatments described below. A total of nine hundred (900) fruit were utilized in this experiment.

Figure 1. Randomized Complete Block Design of field plots.
A. Experiment 1

2	3	5	1	7	6	4
5	1	7	6	4	3	2
3	6	5	1	2	7	4
4	7	3	6	5	1	2
6	1	4	7	3	2	5

1 - Cherry 'Bomb'
2 - 'Cubanelle'
3 - 'Hungarian Wax'
4 - 'Jalapeño' - MG
5 - 'Jalapeño' - FC
6 - Poblano 'Ancho San Luis'
7 - 'Serrano'

B. Experiment 3

2	3	1	2	3
1	2	3	1	2
3	1	2	3	1

1 - 'Hungarian Wax'
2 - 'Hungarian Yellow Wax'
3 - 'Sweet Banana'

4. Experiment 4: Verification of Low Susceptibility to Chilling of Serrano Peppers

A cultivar of Serrano (*Capsicum frutescens* L.) peppers different than the one used in 1998 (experiment 1) was chosen and grown as follows:

'Serrano Chili' - Lot 9, NK Lawn and Garden Co., Chattanooga, TN

Seventy-five plants were seeded on 19 April 1999 and transplanted into a 15' x 14' (4.6 x 4.3 m) field on 21 June 1999. There were fifteen plants in each replication. Two hundred (200) fruit of similar size and shape and free from injury were hand-harvested once during the season and subjected to the storage treatments described below. A total of two hundred (200) fruit were utilized in this experiment.

B. Treatments

Storage rooms were first analyzed for ethylene concentration using a Shimadzu GC-8A gas chromatograph equipped with a 50 cm alumina column, and there was no measurable concentration. Fruit were gently wiped with a towel to remove surface dirt and moisture. They were placed in polyethylene bags perforated with holes one half centimeter in diameter and five centimeters apart, and put in storage rooms. Experimental units consisted of five fruit for experiment 1, four fruit for experiments 2 and 3, and seven fruit for experiment 4. Five experimental units were pulled randomly from the bags on the designated days and subjected to the measurements described below.

1. Experiment 1:

Peppers were placed in storage rooms at temperatures of 2.5°C, 7°C, and ~15°C). Five experimental units were taken randomly from the bags after durations of 0, 4, 8, 12, or 16 days. Because of a loss of yield due to infestation by pepper maggot (*Zonosemata electra* Say), insufficient red 'Jalapeño' fruit were available to allow sampling after 4 days in storage. An additional group of 25 fruit of each type was also kept in storage at 2.5°C and 7°C and for those types which showed no injury after 16 days, samples from these groups were taken at 23 and 30 days to see if symptoms ultimately appeared.

2. Experiment 2:

Full color (red) Cherry 'Bomb' peppers were placed in storage rooms at temperatures of 2.5°C, 7°C, and ~15°C. Five experimental units were pulled randomly from the bags after durations of 0, 5, 10, or 15 days.

3. Experiment 3:

Yellow banana peppers were placed in storage rooms at temperatures of 2.5°C, 7°C, and ~15°C. Five experimental units were pulled randomly from the bags after durations of 0, 2, 4, 6, or 8 days.

4. Experiment 4:

'Serrano Chili' peppers were placed in storage rooms at temperatures of 2.5°C and 0°C, a lower temperature than those used in 1998. Five experimental units were randomly pulled from the bags after durations of 0, 15, 30, or 45 days. Peppers at 2.5°C were sampled only after 0 or 15 days due to disruption of the experiment.

C. Determination of Physiological Changes

1. Chilling-Injury Symptoms

Incidence of chilling-injury symptoms was observed. Symptoms were noted when the fruit were removed from storage and again after 12, 24, and 48 hours at room temperature. Symptoms observed included surface pitting, scald, and browning of the seeds. Fruit decay and the visible presence of pathogens also were recorded.

2. Ethylene Evolution

Peppers were placed in glass mason jars fitted with rubber septa. The largest types ('Cubanelle', 'Hungarian Wax', and Poblano 'Ancho San Luis') were placed in quart jars (0.95 L), and the Cherry 'Bomb' and 'Jalapeño' peppers were placed in pint jars (0.47 L). The 'Serrano' peppers, which are much smaller than the other types, were placed in eight-ounce (0.235 L) jars. The headspace volume was calculated by placing an experimental unit in the appropriate jar, filling the rest of the jar with water, and measuring the volume of the water. The headspace volume for each type was calculated

as the average of the volumes measured for five experimental units. Fruit that had not been subjected to a temperature treatment (0 days) were kept at 20°C overnight after harvest before being placed in jars to account for cultivar- and harvest-related differences in C_2H_4 production.

For measurement of C_2H_4 evolved, samples were removed from the headspace with a 10 ml syringe after both 12 and 24 hours out of storage. One ml was injected into a Shimadzu GC-8A gas chromatograph equipped with a 50 cm alumina column with an injection/detector temperature of 110°F and a column temperature of 40°F. After the twelve hour measurement was taken, the lids were removed for ~15 minutes to aerate the jars and prevent anaerobic respiration.

3. Internal Ethylene Concentrations

After the second headspace sample was taken, two, three, or four of the fruit were sampled for internal C_2H_4 production. Samples were taken with a 10 ml syringe from the locule midway down the length of the fruit. One ml was injected into a Shimadzu GC-8A gas chromatograph for determination of internal C_2H_4 concentration.

4. Ion Leakage

Twenty-four hours after removal from storage, groups of two fruit from experiment 3 and four fruit from experiment 4 were observed for ion leakage as an indication of CI, using a procedure based on that of Murata et al. (106). Five 0.5 cm disks from the pericarp of each fruit were excised with a #4 cork borer, avoiding placental tissue. These were placed in 50 ml beakers and washed three times with distilled, deionized water (ddH_2O). The disks were floated on 5 ml ddH_2O at room temperature for four hours to eliminate ions from cells disrupted by the cork borer. The solution was discarded and the disks were then floated on 10 ml ddH_2O in beakers covered with parafilm for 24 hours on an agitator at 125 rpm. The 10 ml of solution was transferred

from each beaker to a flat-bottomed test tube with a pipette. A probe was immersed in the solution to measure the resistance on a CSI Model 31 Conductivity Bridge. The solution was discarded. The beakers containing the disks were then placed in the freezer overnight. When the beakers containing the disks were removed from the freezer, 10 ml ddH_2O was added, and the disks were allowed to thaw for six hours at 20°C. The disks were then processed in a Virtis "45" tissue homogenizer (The Virtis Co., Inc., Gardiner, NY). The 10 ml of solution was transferred to a flat-bottomed test tube with a pipette. A probe was immersed in the solution to measure the total resistance on a CSI Model 31 Conductivity Bridge. Values are given as the percentage of the total ions, which was determined by adding the resistance with the total and then calculating the percentage.

D. Statistical Analysis

All data were analyzed using the Statistical Analysis System (SAS Institute, Cary, NC). The analyses of variance are in Appendix B. In the experiments where interaction terms were significant, 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. Those interactions which were found to be significant were then analyzed using Duncan's New Multiple Range Test ($P = 0.05$) or regression analysis. A logarithmic transformation to base 10 was done on all ethylene data prior to analysis.

CHAPTER IV

RESULTS

A. Comparison of Chile Pepper Types - Experiment 1

1. Development of Chilling-Injury Symptoms

In the first experiment, seven types of chile peppers were evaluated for susceptibilty to chilling injury (CI). Symptoms observed on peppers stored at 2.5°C and 7°C included surface pitting, scald, seed browning, and rot; no peppers exhibited these symptoms after storage at ~15°C, a temperature that was not expected to be chilling. Susceptibility to postharvest chilling and types of CI symptoms varied among pepper types. Pictures of symptoms exhibited by the types of chile peppers are found in Appendix A. For peppers stored at 2.5°C (Table 1) and peppers stored at 7°C (Table 2), there were differences among the pepper types in both the length of time in storage that led to chilling-injury symptom development and the length of time the peppers were at room temperature before symptoms were visible.

Table 1. Days in storage and subsequent hours at 20°C resulting in manifestation of first visible chilling-injury symptoms on chile peppers stored at 2.5°C (Experiment 1).

Cultivar	Days	Hours
Cherry	8	0
Cubanelle	16	0
Hungarian Wax	4	0
Jalapeño, mature green	8	24
Jalapeño, full color (red)	8	0
Poblano	8	24
Serrano	23	0

Table 2. Days in storage and subsequent hours at 20°C resulting in manifestation of first visible chilling-injury symptoms on chile peppers stored at 7°C (Experiment 1).

Cultivar	Days	Hours
Cherry	12	24
Cubanelle	23	0
Hungarian Wax	16	0
Jalapeño, mature green	12	0
Jalapeño, full color (red)	12	12
Poblano	8	48
Serrano	>30	>48

a. 'Hungarian Wax'

'Hungarian Wax' peppers were the most susceptible to chilling-injury symptom development at 2.5°C, being the first type in this study to manifest symptoms (Table 1). Scald, a surface browning, was observed on 'Hungarian Wax' fruit along with pitting (Picture 1). After storage at 2.5°C, approximately 50% of the peppers exhibited scald after 4 days. After 6 days, 80% of the peppers had injury symptoms, and 100% of the peppers were injured after 12 and 18 days (Table 1). Approximately 90% of the 'Hungarian Wax' peppers had scald and pits after 16 days at 7°C (Table 2). All symptoms developed while in storage, before fruit were removed to room temperature.

b. 'Serrano'

'Serrano' fruit were the most resistant to CI symptom development. Half of the fruit pitted in storage, but only after 23 days at 2.5°C, and 100 % of the peppers had injury after 30 days at 2.5°C (Table 1). They had no CI symptoms after storage at 7°C for 30 days plus 48 hours at room temperature (Table 2). Some 'Serrano' peppers had seed browning (Picture 2) but no external symptoms appeared during or after 16 days at 2.5°C.

c. Poblano 'Ancho San Luis'

Poblano 'Ancho San Luis' peppers were the most susceptible to chilling-injury symptom development at 7°C; approximately 20% of the peppers manifested small pits after 8 days in storage plus 48 hours at room temperature (Table 2). Approximately 40%

of the fruit also developed pits after 8 days at 2.5°C and manifested large, deep pits after 24 hours at room temperature (Table 1). Many fruit also exhibited seed browning after 8 days at 2.5°C (Picture 3).

d. Cherry 'Bomb'

After 8 days at 2.5°C, all of the Cherry 'Bomb' peppers developed small pits which were visible upon their removal from storage (Picture 4 and Table 1). At 7°C, approximately 40% of the Cherry peppers pitted after 12 days, but only after 24 hours at room temperature (Table 2).

e. 'Cubanelle'

Approximately 20 % of the 'Cubanelle' peppers had scald when they were removed from storage after 16 days at 2.5°C (Picture 5 and Table 1). At 7°C, both pits and scald were observed on 40% of the 'Cubanelle' fruit when they were removed from storage after 23 days (Table 2). During 48 hours at room temperature, no symptoms developed on fruit that were symptom-free upon removal from storage.

f. 'Jalapeño'

Approximately 40% of both the green and red 'Jalapeño' peppers pitted after 12 days at 2.5°C (Table 1). The red 'Jalapeño' fruit pitted in storage (Picture 6), and the green 'Jalapeño' fruit exhibited pits after 24 hours out of storage (Picture 7). At 7°C, approximately 20% of both the green and red 'Jalapeño' fruit were pitted after 16 days (Table 2). The green peppers pitted in storage, and symptoms were observed on the red fruit after 12 hours at room temperature.

2. Ethylene Evolution

The analyses of variance for C_2H_4 evolved after the first 12 hours, the second 12 hours, and the total after 24 hours at room temperature following removal from storage are found in Appendix B. In general, more C_2H_4 was produced in the first 12 hours out of storage (mean = 1.15 $\mu L \cdot kg^{-1} \cdot hr^{-1}$) than during the second 12 hour period (mean = 0.70

$\mu L \cdot kg^{-1} \cdot hr^{-1}$) for all types of peppers. For this reason, the data presented here represent C_2H_4 evolved during the first 12 hours out of storage.

The effects of temperature and duration of storage on C_2H_4 evolution were highly significant after the first 12 hours at room temperature (Appendix B.1). The interaction of the effects of temperature and duration of storage was highly significant (Appendix B.1). After storage for 0 or 4 days, the effects of temperature on C_2H_4 evolved were non-significant. The effects of temperature on C_2H_4 evolved from peppers stored for 8, 12, or 16 days were highly significant linearly and quadratically.

For all pepper types, more C_2H_4 was produced by peppers that had been stored at 2.5°C (mean = 2.69 $\mu L \cdot kg^{-1} \cdot hr^{-1}$) than at 7°C (mean = 0.79 $\mu L \cdot kg^{-1} \cdot hr^{-1}$) or ~15°C (mean = 0.24 $\mu L \cdot kg^{-1} \cdot hr^{-1}$). In addition, more C_2H_4 was evolved from peppers that had been stored at 7°C than at ~15°C. 'Serrano' peppers stored at 7°C and ~15°C produced significantly less C_2H_4 than the other types (Figure 8). For this reason, the vertical axis on the line graph representing this data (Figure 8) has a much lower minimum value than those of the graphs representing the data for the other six types of peppers. In general, C_2H_4 evolution from all pepper types increased with duration of storage at all temperatures (Figures 2, 3, 4, 5, 6, 7, and 8). No 'Jalapeño' peppers were sampled after 4 days in storage (Figures 5 and 6).

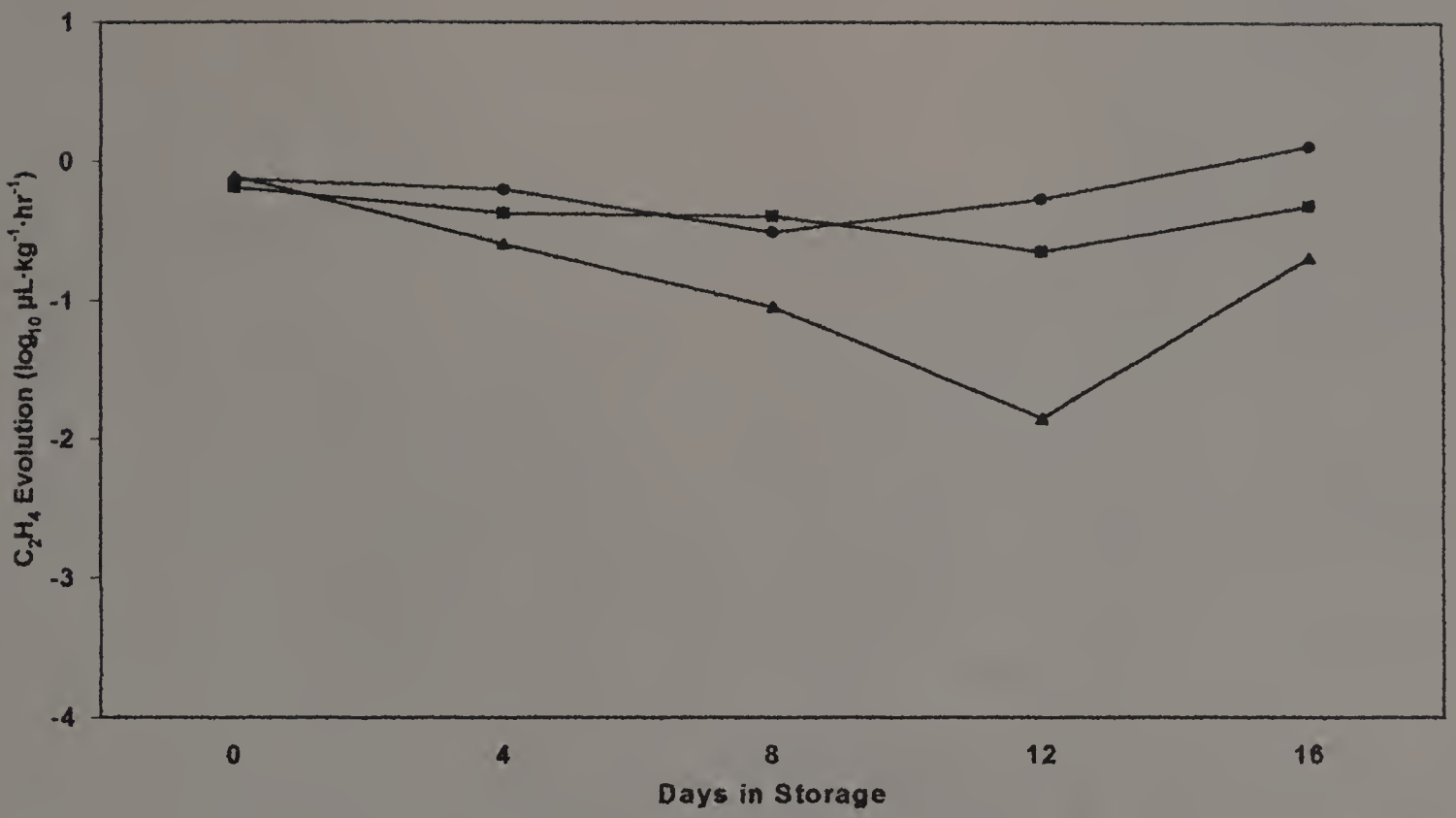


Figure 2. Ethylene evolved by Cherry 'Bomb' peppers after the first 12 hours at 20°C following removal from storage. ●, 2.5°C; ■, 7°C; ◆, ~15°C. (Experiment 1).

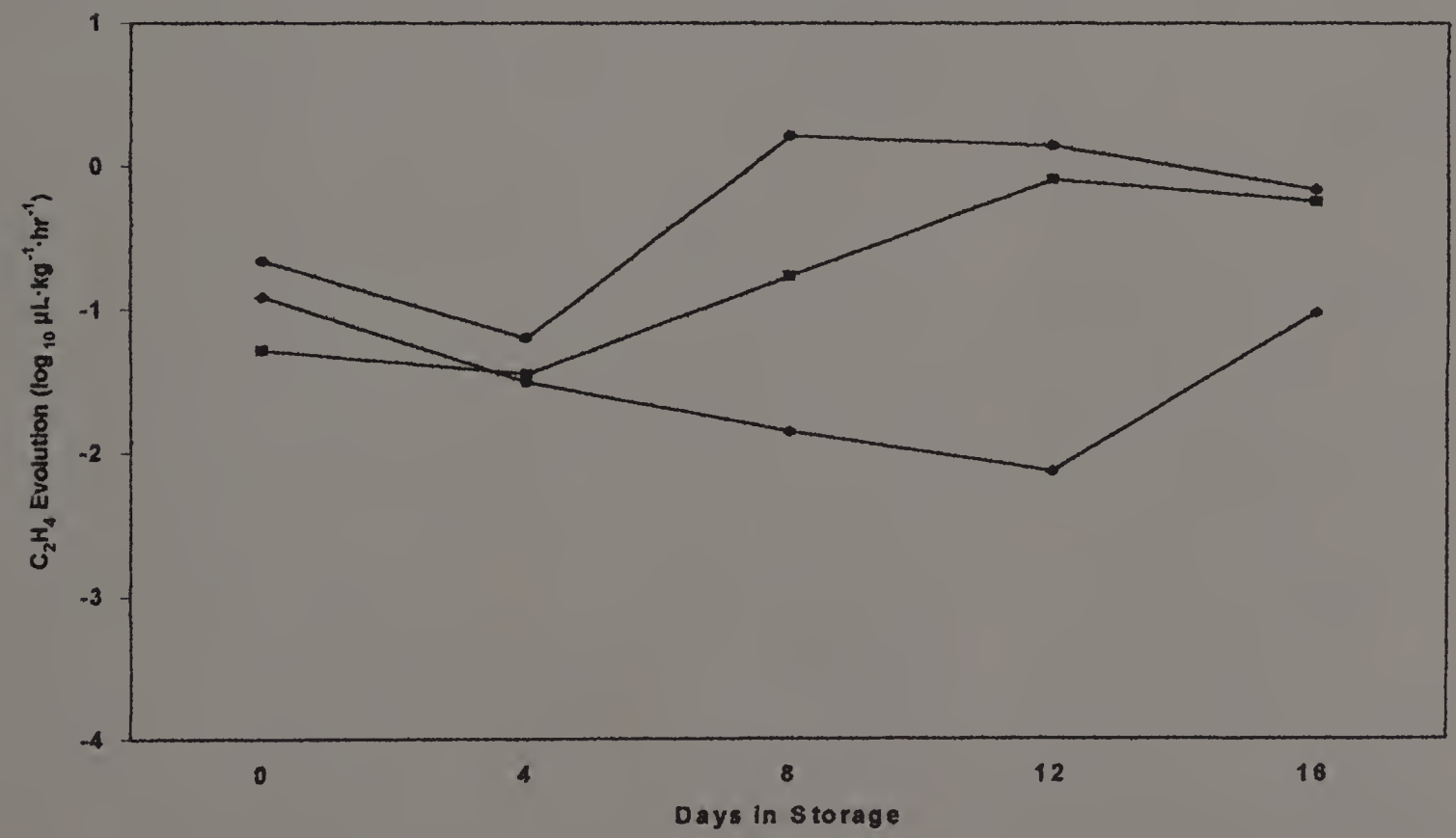


Figure 3. Ethylene evolved by 'Cubanelle' peppers after the first 12 hours at 20°C following removal from storage. ●, 2.5°C; ■, 7°C; ◆, ~15°C. (Experiment 1).

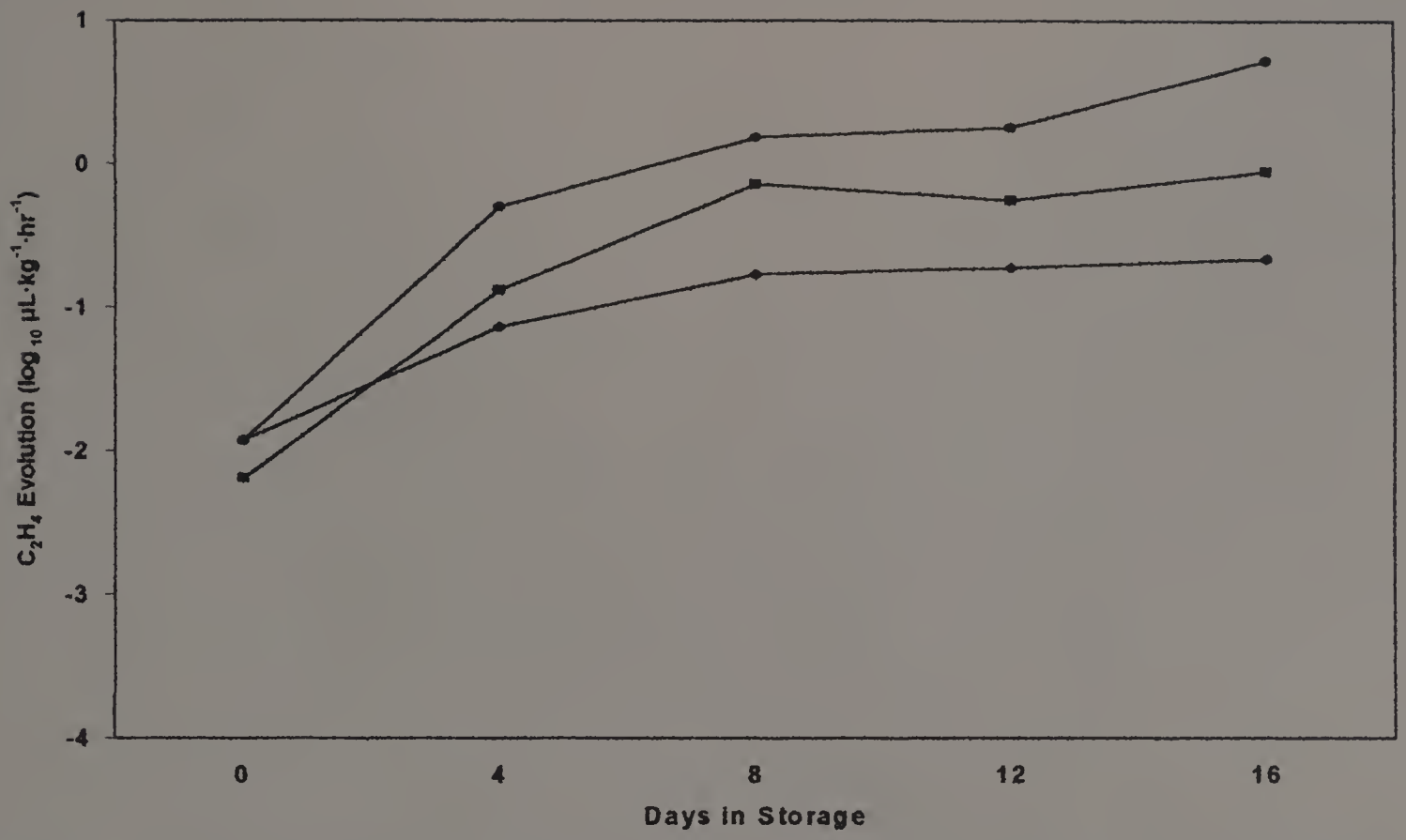


Figure 4. Ethylene evolved by 'Hungarian Wax' peppers after the first 12 hours at 20°C following removal from storage. ●, 2.5°C; ■, 7°C; ◆, ~15°C. (Experiment 1).

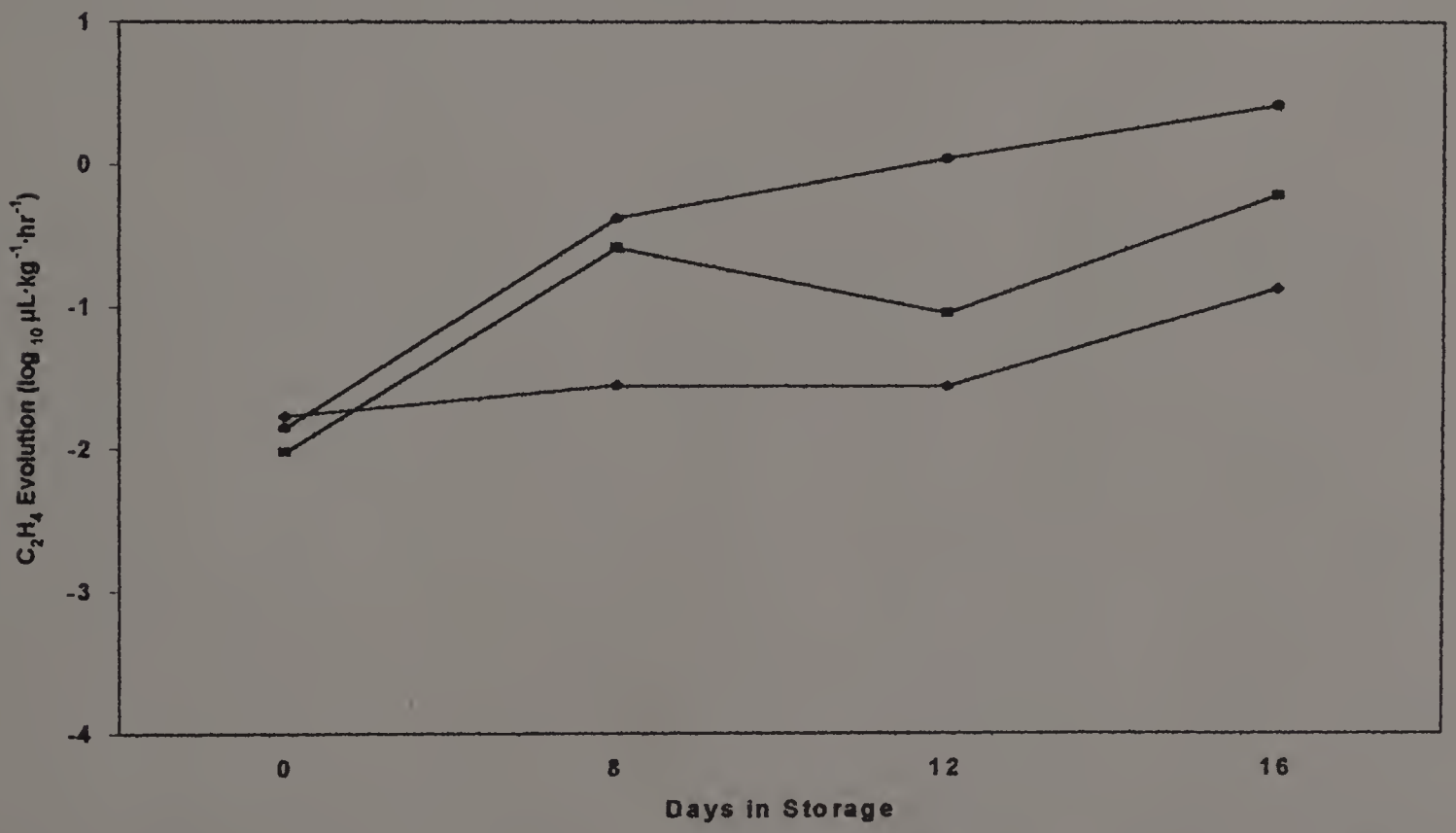


Figure 5. Ethylene evolved by mature green 'Jalapeño' peppers after the first 12 hours at 20°C following removal from storage. ●, 2.5°C; ■, 7°C; ◆, ~15°C. (Experiment 1).

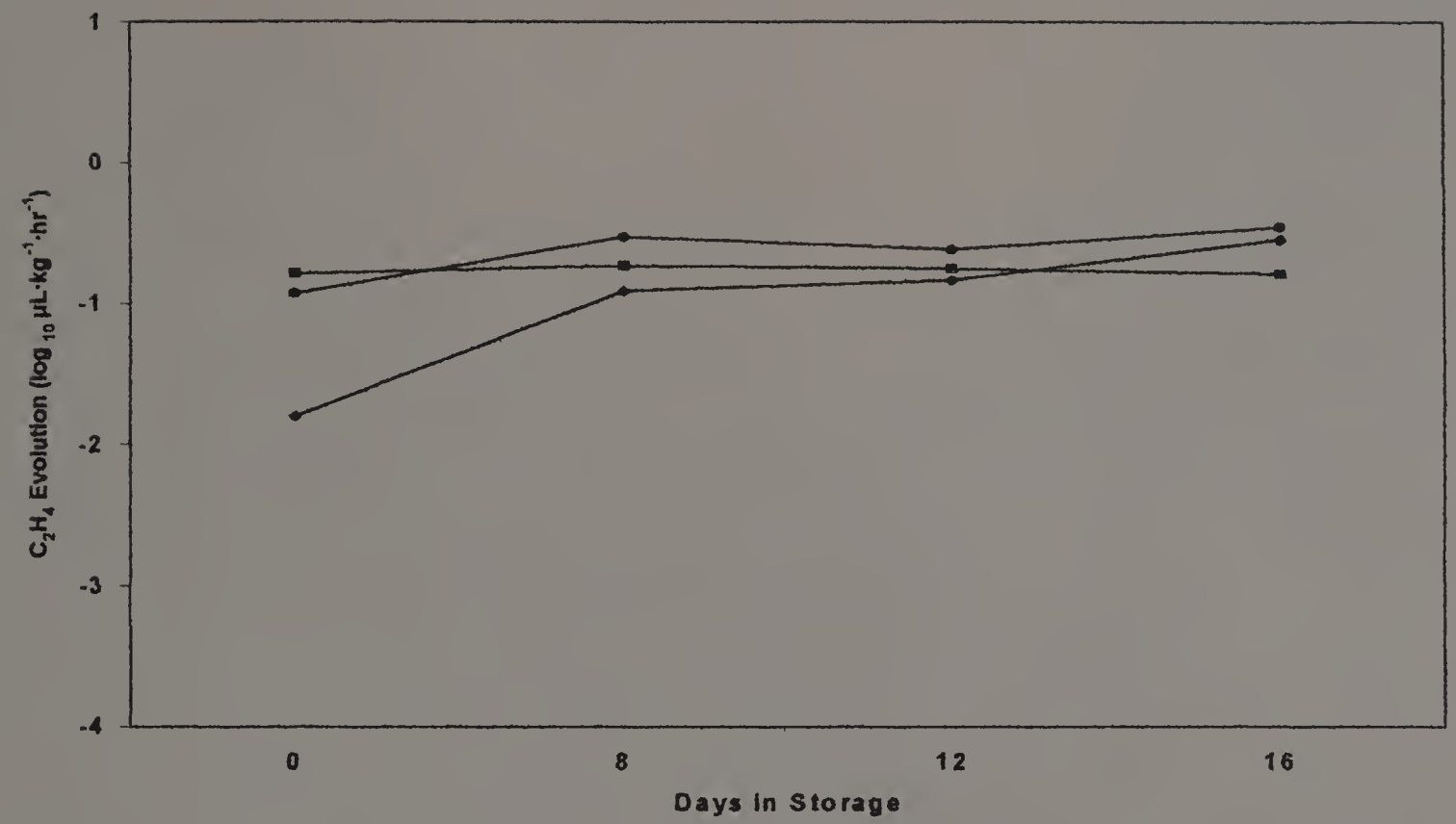


Figure 6. Ethylene evolved by full color (red) 'Jalapeño' peppers after the first 12 hours following removal from storage. ●, 2.5°C; ■, 7°C; ◆, ~15°C. (Experiment 1).

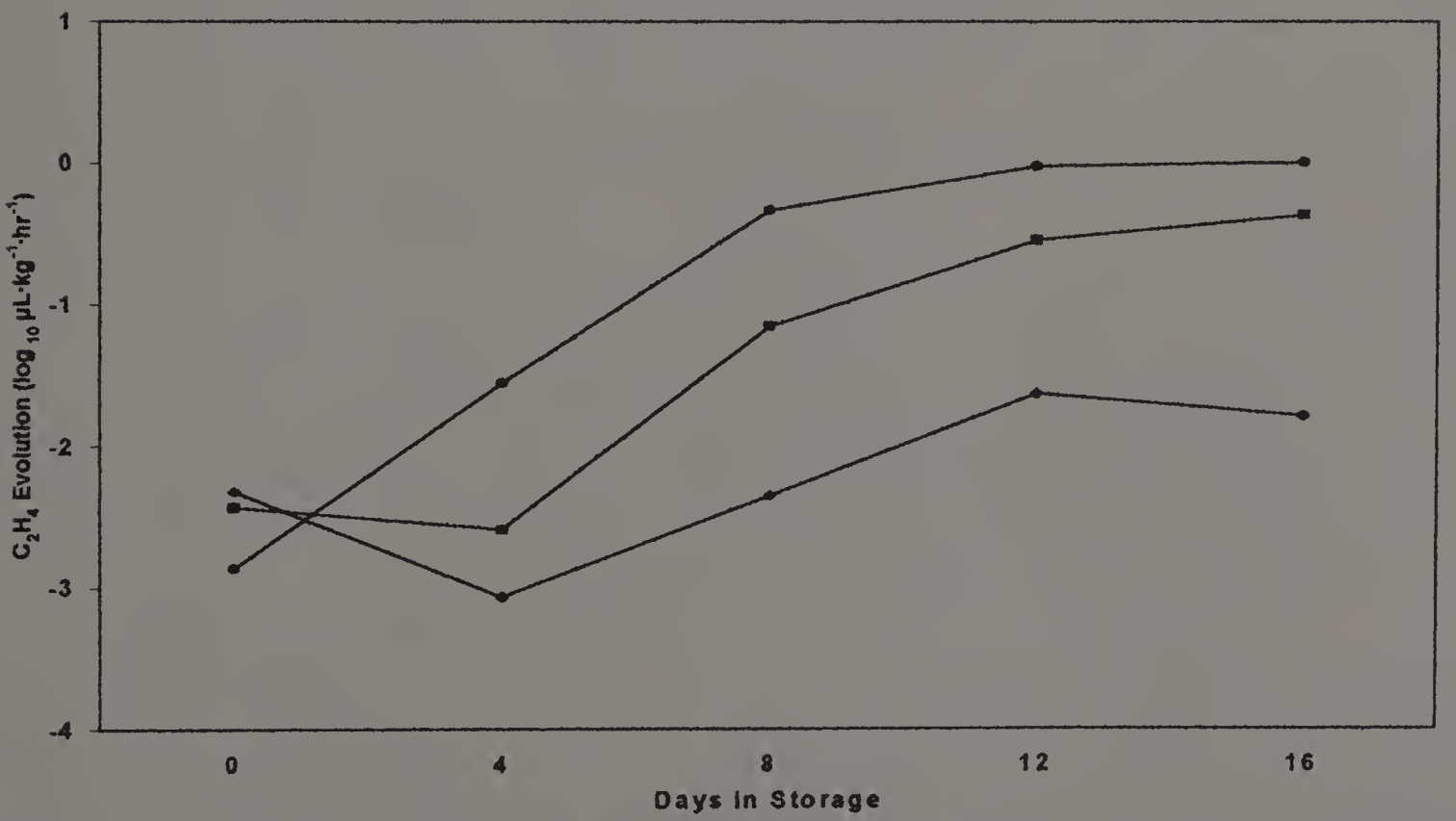


Figure 7. Ethylene evolved by Poblano 'Ancho San Luis' peppers after the first 12 hours at 20°C following removal from storage. ●, 2.5°C; ■, 7°C; ◆, ~15°C. (Experiment 1).

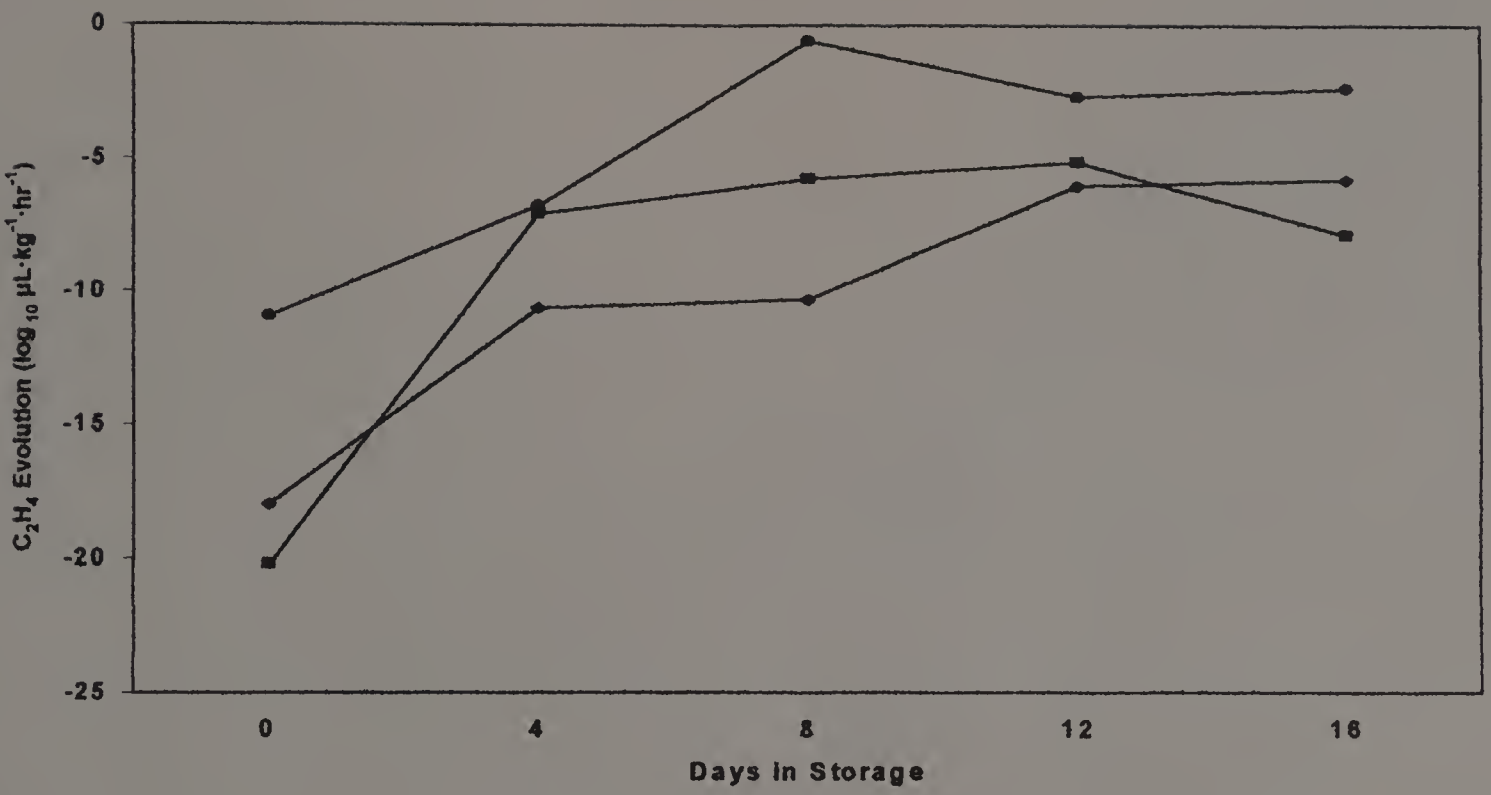


Figure 8. Ethylene evolved by 'Serrano' peppers after the first 12 hours at 20°C following removal from storage. ●, 2.5°C; ■, 7°C; ◆, ~15°C. (Experiment 1).

a. Ethylene Evolution after Storage at 2.5°C

Ethylene evolution from all pepper types generally increased with duration of storage at 2.5°C (Table 3). The effects of pepper type, duration of storage, and the interaction of pepper type and duration were highly significant for chile peppers stored at 2.5°C (Appendix B.2). The effects of pepper type on ethylene evolution for peppers stored at 2.5°C were highly significant for peppers stored for 8, 12, or 16 days and non-significant for peppers stored for 0 or 4 days (Appendix B.2). After storage at 2.5°C, there were differences in the amounts of C_2H_4 evolved by the seven types of peppers in this study (Table 3). After 8 days in storage at 2.5°C, 'Hungarian Wax' peppers produced significantly more C_2H_4 than the other types. After storage for 12 days, 'Hungarian Wax', Poblano 'Ancho San Luis', and 'Cubanelle' peppers produced significantly higher amounts of C_2H_4 than the other types. After 16 days in storage at this temperature, 'Hungarian Wax', 'Cubanelle', Poblano 'Ancho San Luis', and Cherry 'Bomb' peppers produced the most C_2H_4, followed by red and green 'Jalapeño' peppers.

After storage for 16 days at 2.5°C, 'Serrano' fruit produced the least amount of C_2H_4 of the types of peppers in this study (Table 3).

Table 3. Ethylene evolved ($\mu L \cdot kg^{-1} \cdot hr^{-1}$) by chile peppers after the first 12 hours at 20°C following storage at 2.5°C for 0 to 16 days (Experiment 1).

	Days in Storage				
Cultivar	0 Days	4 Days	8 Days	12 Days	16 Days
Cherry	0.5703	0.5547	0.7180 b[z]	0.4198 b	6.2526 abc
Cubanelle	0.2107	0.2609	1.6592 b	6.7282 a	8.8733 a
Hungarian Wax	0.0055	0.3274	9.9977 a	9.6854 a	9.9555 a
Jalapeño-MG [y]	0.0946		0.9881 b	1.9972 b	3.3625 cd
Jalapeño-FC [x]	0.1101		0.1975 b	1.9889 b	3.6681 bcd
Poblano	0.0316	0.1362	0.8400 b	7.6208 a	7.4489 ab
Serrano	0.0225	0.2341	0.1535 b	0.9057 b	1.8099 d

[z]Mean separation within columns by Duncan's New Multiple Range Test, P=0.05.
[y]Mature green
[x]Full color (red)

b. Ethylene Evolution after Storage at 7°C

Ethylene evolution from all pepper types generally increased with duration of storage at 7°C (Table 4). The effects of pepper type and duration of storage were highly significant, and the interaction of pepper type and duration was significant for chile peppers stored at 7°C (Appendix B.3). The effects of pepper type on C_2H_4 production for peppers stored at 7°C were highly significant for peppers stored for 8, 12, or 16 days and non-significant for peppers stored for 0 or 4 days (Appendix B.3). After storage at 7°C, there were differences in the amounts of C_2H_4 evolved by the seven types of peppers in this study (Table 4). After 8 days in storage at 2.5°C, 'Hungarian Wax' peppers produced the most C_2H_4, followed by Poblano 'Ancho San Luis', Cherry 'Bomb', and green and red 'Jalapeño' peppers. 'Cubanelle' and 'Serrano' peppers produced the least amounts of C_2H_4. After 12 days in storage at 7°C, 'Hungarian Wax' peppers produced the most C_2H_4, followed by Poblano, green and red 'Jalapeño', and 'Cubanelle' peppers. Cherry and 'Serrano' peppers produced the least amount of C_2H_4.

After 16 days in storage at this temperature, green 'Jalapeño', Poblano, 'Hungarian Wax', 'Cubanelle', Cherry, and red 'Jalapeño' peppers produced the most C_2H_4, and 'Serrano' peppers produced the least amount of C_2H_4 (Table 4).

Table 4. Ethylene evolved ($\mu L \cdot kg^{-1} \cdot hr^{-1}$) by chile peppers after the first 12 hours at 20°C following storage at 7°C for 0 to 16 days (Experiment 1).

	Days in Storage				
Cultivar	0 Days	4 Days	8 Days	12 Days	16 Days
Cherry	0.5751	0.4025	0.8393 a [z]	0.0222 b	1.4146 ab
Cubanelle	0.0986	0.0261	0.1754 ab	1.4015 ab	1.4934 ab
Hungarian Wax	0.0007	0.0596	0.9430 a	1.8977 a	1.6557 ab
Jalapeño-MG [y]	0.0931		0.7674 a	1.4917 ab	1.8113 a
Jalapeño-FC [x]	0.1315		0.6034 a	1.4229 ab	1.1200 ab
Poblano	0.0335	0.0507	0.8660 a	1.5785 ab	1.7402 a
Serrano	0.0658	0.7436	0.0416 b	0.6935 b	0.9084 b

[z]Mean separation within columns by Duncan's New Multiple Range Test, P=0.05.
[y]Mature green
[x]Full color (red)

c. Ethylene Evolution after Storage at ~15°C

Ethylene evolution from all pepper types generally increased with duration of storage at ~15°C (Table 5). The effects of pepper type were significant, and the effects of duration of storage and the interaction of pepper type and duration were highly significant for chile peppers stored at ~15°C (Appendix B.4). The effects of pepper type on C_2H_4 production for peppers stored at ~15°C were highly significant for peppers stored for 4, 8, 12, or 16 days and non-significant for peppers stored for 0 days (Appendix B.4). After storage at ~15°C, there were differences in the amounts of C_2H_4 evolved by the seven types of peppers in this study (Table 5). After 4 days in storage at ~15°C, Cherry 'Bomb' and 'Hungarian Wax' peppers produced the most C_2H_4, followed by Poblano 'Ancho San Luis', 'Serrano', and 'Cubanelle' peppers.

After 8 or 12 days in storage at ~15°C, Cherry peppers produced the most C_2H_4, followed by red and green 'Jalapeño' peppers. 'Hungarian Wax', Poblano, 'Cubanelle', and 'Serrano' peppers produced the least amounts C_2H_4. After 16 days in storage at this temperature, red 'Jalapeño', Cherry, and green 'Jalapeño' peppers produced significantly more C_2H_4 than 'Hungarian Wax', 'Cubanelle', and Poblano peppers. 'Serrano' peppers produced significantly less C_2H_4 than the other types of chile peppers after 16 days at ~15°C (Table 5).

Table 5. Ethylene evolved ($\mu L \cdot kg^{-1} \cdot hr^{-1}$) by chile peppers after the first 12 hours at 20°C following storage at ~15°C for 0 to 16 days (Experiment 1).

	Days in Storage				
Cultivar	0 Days	4 Days	8 Days	12 Days	16 Days
Cherry	0.5998	0.2096 a [z]	0.2847 a	0.2929 a	0.8418 a
Cubanelle	0.1309	0.0281 b	0.0386 b	0.1409 ab	0.4653 b
Hungarian Wax	0.0058	0.1122 ab	0.0527 b	0.1599 ab	0.4752 b
Jalapeño-MG [y]	0.1727		0.1850 ab	0.2819 a	0.8190 a
Jalapeño-FC [x]	0.0376		0.1980 ab	0.2928 a	0.8564 a
Poblano	0.0572	0.0369 b	0.0496 b	0.2385 ab	0.4640 b
Serrano	0.0274	0.0483 b	0.0251 b	0.0855 b	0.1403 c

[z]Mean separation within columns by Duncan's New Multiple Range Test, P=0.05.
[y]Mature green
[x]Full color (red)

B. Effects of Harvest Date on Red Cherry Peppers - Experiment 2

1. Development of Chilling-Injury Symptoms

Red Cherry 'Bomb' peppers were harvested on three dates: 4 September, 16 September, and 2 October, 1998. Development of chilling-injury symptoms varied for peppers harvested on different dates, with symptom manifestation increasing with later harvesting. No Cherry peppers exhibited chilling-injury symptoms after storage at ~15°C, a temperature not expected to cause injury. Peppers from the first harvest showed no CI symptoms after 15 days at 2.5°C or 7°C. Approximately 60% of the peppers harvested on

the second date pitted after 15 days at 2.5°C plus 48 hours at room temperature. They developed no symptoms after 15 days at 7°C. All of the peppers from the third harvest had pitted in storage after 10 days at 2.5°C, and approximately 80% of the peppers exhibited pits after 10 days at 7°C plus 24 hours at room temperature. Thus, with later harvesting the red Cherry peppers were more susceptible to chilling-injury symptoms, developing them at higher temperatures, and more rapidly at a given temperature.

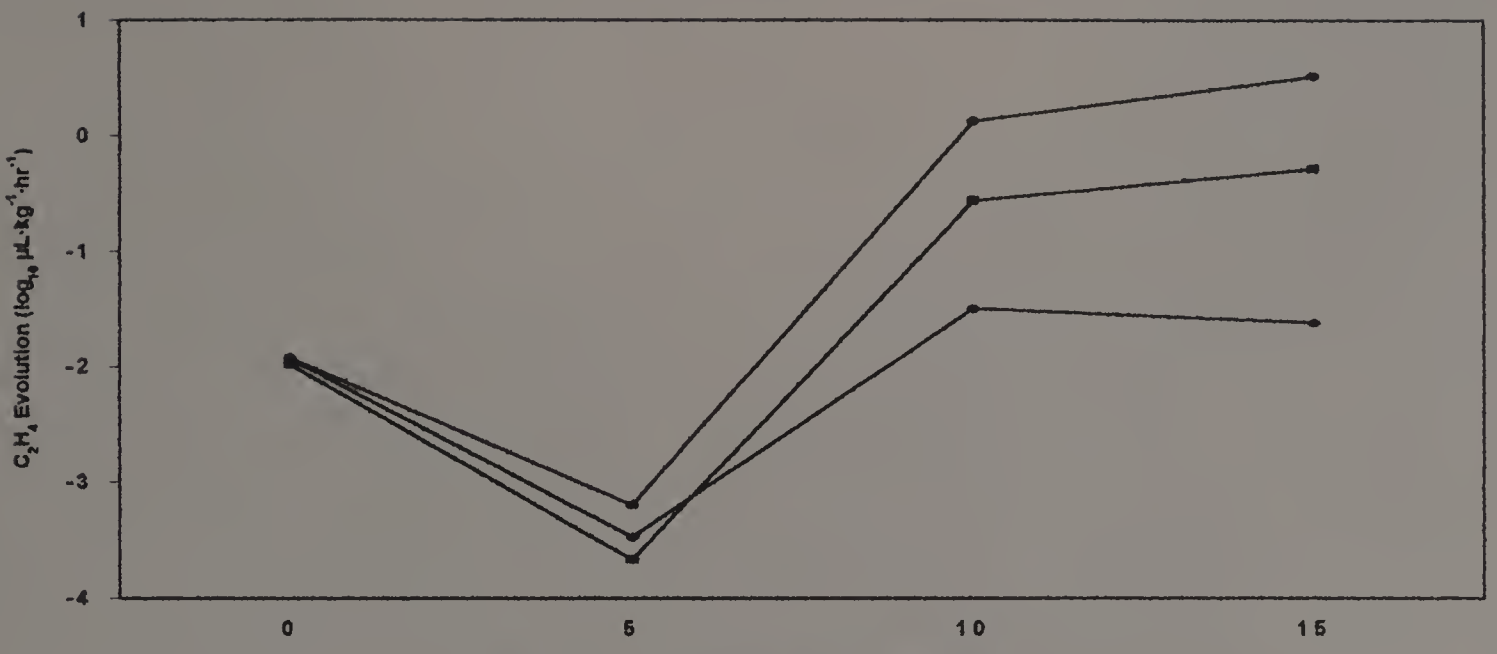
2. Ethylene Evolution

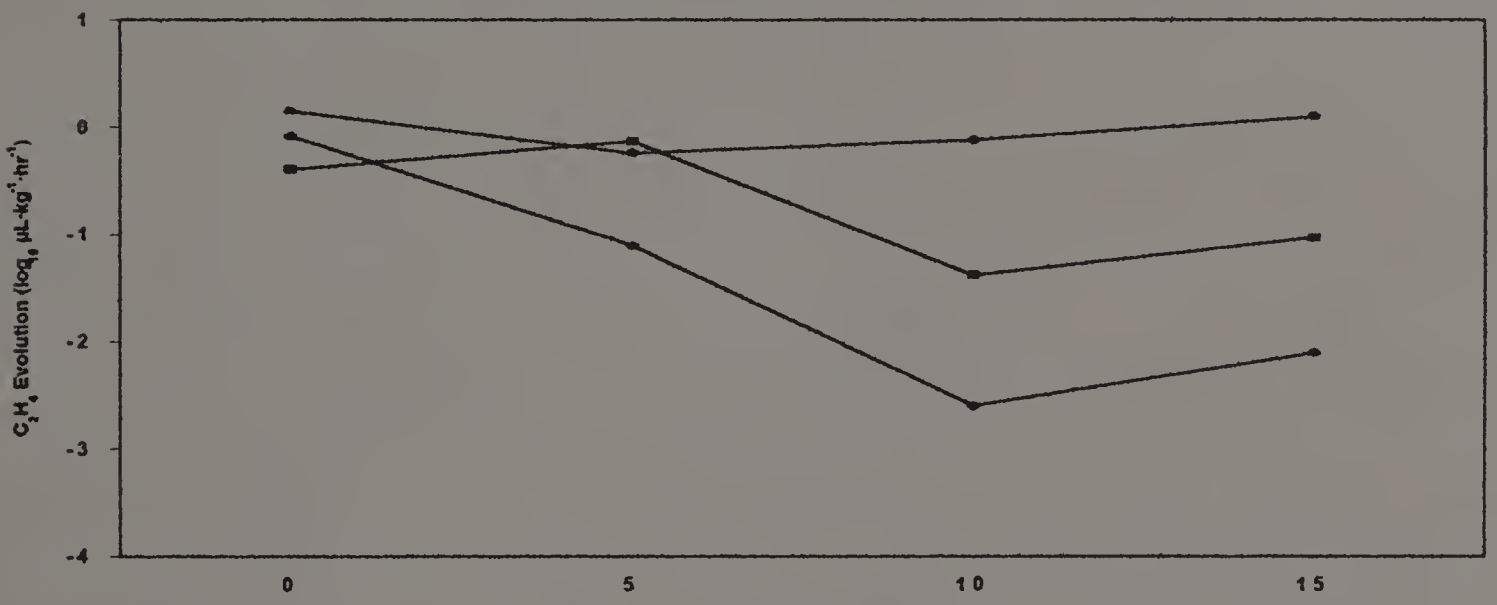
The analyses of variance for C_2H_4 evolved after the first 12 hours, the second 12 hours and the total after 24 hours at room temperature following storage are found in Appendix B. In general, more C_2H_4 was produced in the first 12 hours out of storage (mean = 1.58 $\mu L \cdot kg^{-1} \cdot hr^{-1}$) than during the second 12 hour period (mean = 0.41 $\mu L \cdot kg^{-1} \cdot hr^{-1}$) for peppers from all harvests. For this reason, the data presented here are from the first 12 hours out of storage.

The effects of harvest date on C_2H_4 evolution by red Cherry 'Bomb' peppers after the first 12 hours out of storage were non-significant (Appendix B.8). The effects of temperature on C_2H_4 evolution after the first 12 hours at room temperature were highly significant linearly and quadratically (Appendix B.8). The effects of duration of storage were significant, and the effects of the interaction of temperature and duration were highly significant (Appendix B.8). The effects of temperature on C_2H_4 evolution after the first 12 hours out of storage were non-significant for peppers in storage for 0 or 5 days and highly significant linearly and quadratically for peppers stored for 10 or 15 days (Appendix B.8).

For red Cherry peppers harvested on all three dates, more C_2H_4 was produced by peppers that had been stored at 2.5°C (mean = 2.51 $\mu L \cdot kg^{-1} \cdot hr^{-1}$) than at 7°C (mean = 1.40 $\mu L \cdot kg^{-1} \cdot hr^{-1}$) or ~15°C (mean = 0.81 $\mu L \cdot kg^{-1} \cdot hr^{-1}$). In addition, more C_2H_4 was evolved from peppers that had been stored at 7°C than at ~15°C. Peppers harvested on 4

September 1998, the first harvest date in this experiment, produced much less C_2H_4 than peppers from the other two harvest dates after storage at all temperatures for 0 or 5 days (Figure 9). However, the amounts of C_2H_4 evolved after 10 or 15 days in storage at all temperatures for peppers stored on all three harvests dates were similar (Figure 9). For fruit in storage for 10 or 15 days, more C_2H_4 was produced by peppers stored at 2.5°C than by fruit stored at 7°C or ~15°C. The effects of storage duration on C_2H_4 evolution after the first 12 hours were significant (Appendix B.8). In general, C_2H_4 evolution from peppers harvested on all three dates and stored at 2.5°C increased with duration of storage (Figure 9).





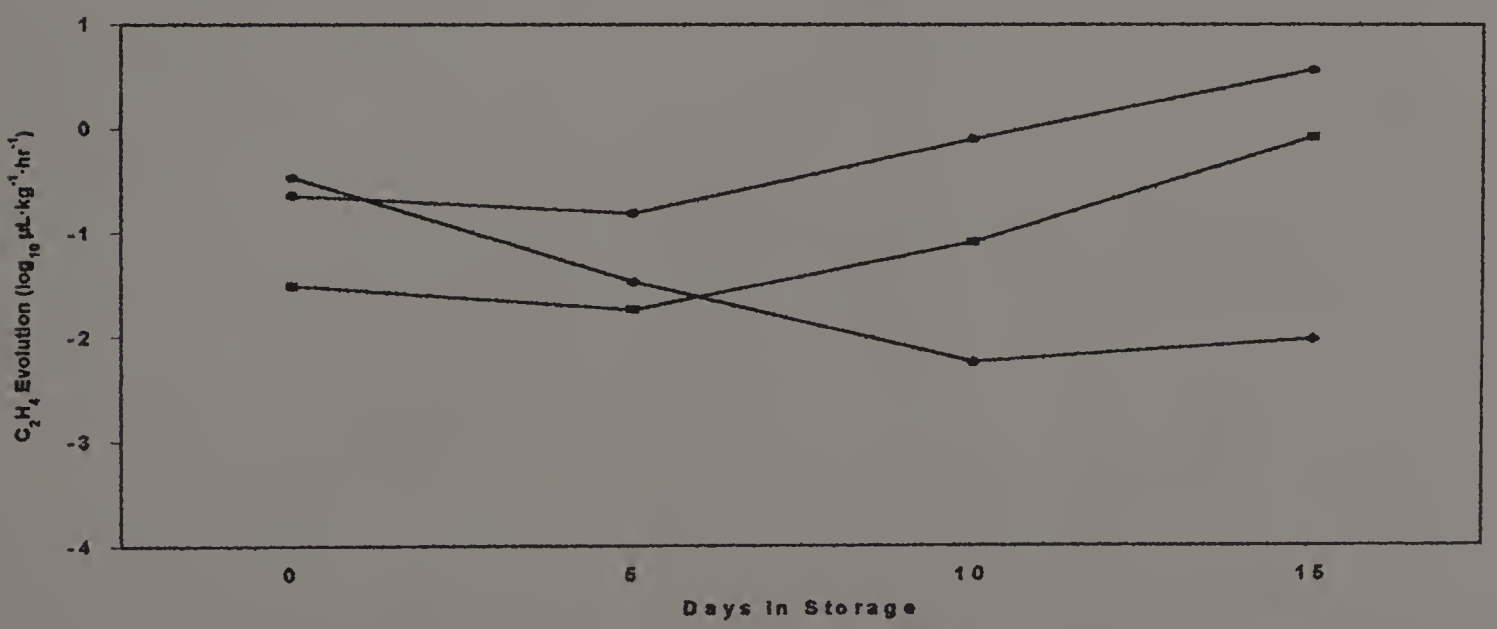


Figure 9. Ethylene evolved by red Cherry 'Bomb' peppers after the first 12 hours at 20°C following removal from storage. ●, 2.5°C; ■, 7°C; ◆, ~15°C (Experiment 2).

C. Comparison of Banana Types of Peppers - Experiment 3

1. Development of Chilling-Injury Symptoms

Three cultivars of banana-type peppers, 'Hungarian Wax', 'Hungarian Yellow Wax', and 'Sweet Banana', were evaluated for susceptibility to chilling-injury symptom development. Symptoms were exhibited on all three cultivars of banana-type peppers stored at 2.5°C and were visible when the fruit were removed from storage to room temperature. After 2 days at 2.5°C, approximately 25% of the 'Sweet Banana' peppers had many small, translucent pits. After 4 days, larger, irregular, translucent pits were observed on approximately 90% of the 'Sweet Banana' peppers, appearing as if small pits had coalesced. After 4 days at 2.5°C, approximately 50% of the 'Hungarian Wax' and 60% of the 'Hungarian Yellow Wax' peppers exhibited light brown, irregular pits, mostly on the stem end. A few 'Hungarian Yellow Wax' peppers had these pits from the stem end to the blossom end, but only on one side. Some 'Hungarian Wax' fruit also exhibited the small, translucent pits observed on the 'Sweet Banana' peppers.

After 6 days in storage at 2.5°C, all peppers had chilling injury. 'Hungarian Wax' and 'Hungarian Yellow Wax' peppers had brown, irregular pits that were larger than ones seen after 4 days. 'Sweet Banana' fruit had more severe CI symptoms, exhibiting brown and black pits, blackening of the stem and calyx, and rot. When the peppers were removed from storage after 8 days, most fruit of all cultivars had pits, scald, brown seeds, and black stems and calyx. Many fruit also had soft rot.

None of these cultivars had CI symptoms after 8 days at 7°C or developed symptoms during 48 hours at room temperature following storage. No chilling-injury symptoms appeared on fruit that were stored at ~15°C, a temperature not expected to cause injury.

2. Ethylene Evolution

The analyses of variance for C_2H_4 evolved after the first 12 hours and the total after 24 hours at room temperature following storage are found in Appendix B. In general, more C_2H_4 was produced in the first 12 hours out of storage (mean = 0.29 $\mu L \cdot kg^{-1} \cdot hr^{-1}$) than during the second 12 hour period (mean = 0.11 $\mu L \cdot kg^{-1} \cdot hr^{-1}$) for all cultivars of banana peppers. For this reason, the data presented here represent C_2H_4 evolved during the first 12 hours out of storage.

The effects of cultivar were non-significant (Appendix B.12). The effects of temperature, storage duration, and the interaction of temperature and duration were highly significant (Appendix B.12). The effects of temperature on C_2H_4 evolution were non-significant for banana peppers at harvest (0 days) or after 2 days in storage and highly significant linearly and quadratically for peppers stored for 4 or 6 days. The effects of temperature on C_2H_4 evolution were highly significant linearly and significant quadratically for peppers stored for 8 days (Appendix B.12).

More C_2H_4 was produced by banana peppers that had been stored at 2.5°C (mean = 0.55 $\mu L \cdot kg^{-1} \cdot hr^{-1}$) than at 7°C (mean = 0.17 $\mu L \cdot kg^{-1} \cdot hr^{-1}$) or ~15°C (mean = 0.14 $\mu L \cdot kg^{-1} \cdot hr^{-1}$). In addition, more C_2H_4 was evolved from peppers that had been stored at 7°C than at ~15°C. After 6 or 8 days in storage, more C_2H_4 was evolved from banana peppers stored at 2.5°C than the other two temperatures (Figure 10). 'Sweet Banana' peppers had higher levels of C_2H_4 evolved than 'Hungarian Wax' and 'Hungarian Yellow Wax' peppers after 0 or 2 days in storage at all temperatures (Figure 10). However, the amounts of C_2H_4 evolved after 4, 6, or 8 days in storage at all temperatures for all three cultivars of banana peppers were similar (Figure 10). In general, C_2H_4 evolution from all three cultivars of banana peppers stored at all three temperatures increased with duration of storage (Figure 10).

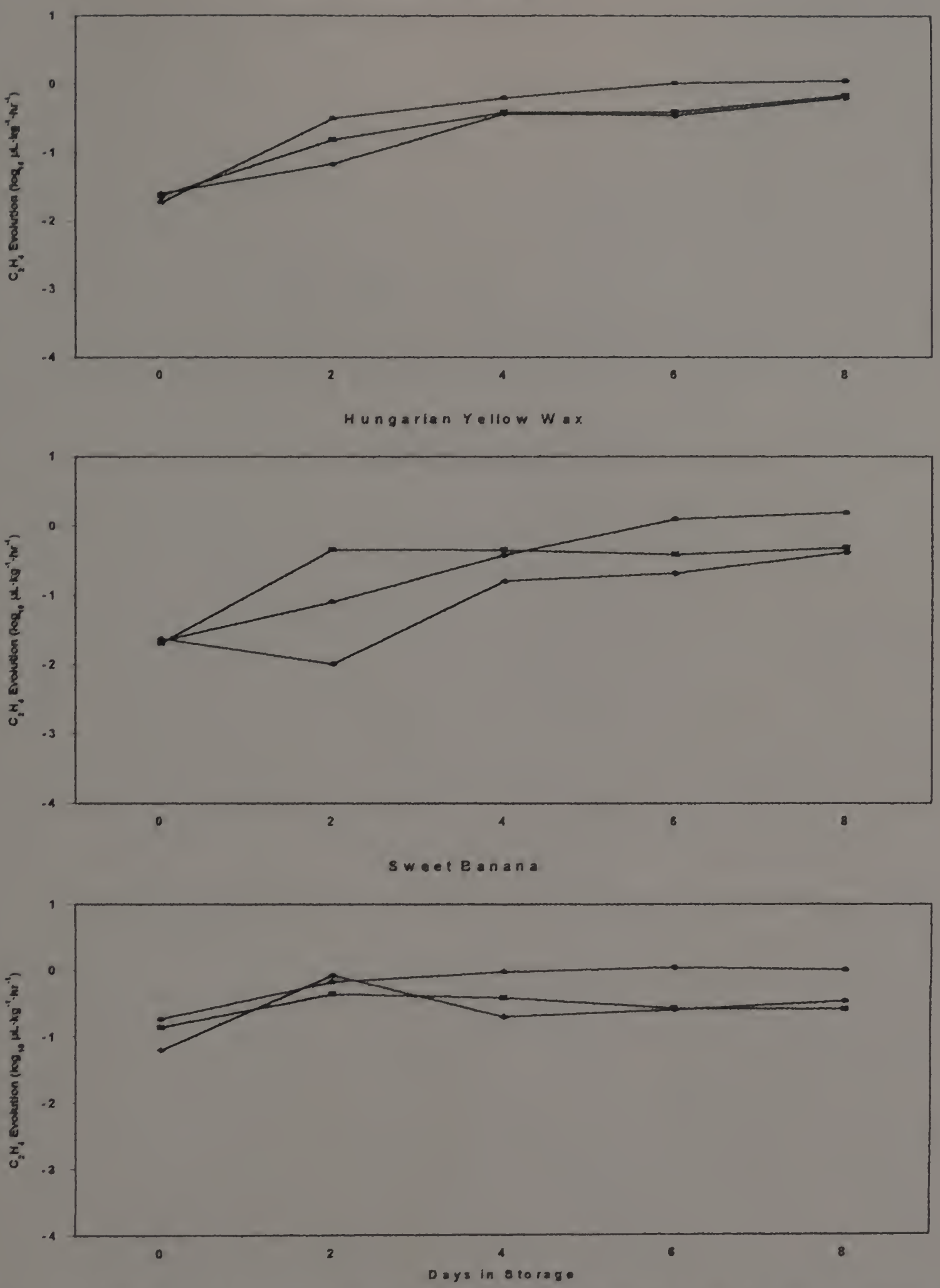


Figure 10. Ethylene evolved by banana peppers after the first 12 hours at 20°C following removal from storage. ●, 2.5°C; ■, 7°C; ◆, ~15°C. (Experiment 3).

a. Ethylene Evolution after Storage at 2.5°C

The effects of cultivar on ethylene evolution after storage at 2.5°C were non-significant for banana peppers (Appendix B.13). The effects of duration of storage on C_2H_4 production were highly significant (Appendix B.13).

b. Ethylene Evolution after Storage at 7°C

The effects on C_2H_4 evolution of cultivar and storage duration were highly significant for banana peppers stored at 7°C (Appendix B.14). The effects of the interaction of cultivar and duration were significant (Appendix B.14). The effects of cultivar after storage at 7°C were non-significant for banana peppers stored for 0, 2, or 6 days, significant for peppers stored for 4 days, and highly significant for peppers stored for 8 days (Appendix B.14). Ethylene evolution from all pepper types generally increased with duration of storage at 7°C. After storage at 7°C, there were differences among the three cultivars of peppers in this study in the amounts of C_2H_4 evolved (Table 6). After storage for 4 days at 7°C, 'Hungarian Wax' peppers produced the most C_2H_4, followed by 'Hungarian Yellow Wax' peppers, which, in turn, produced significantly more C_2H_4 than 'Sweet Banana' peppers. After 8 days at 7°C, 'Sweet Banana' peppers produced significantly less C_2H_4 than the other two cultivars of banana peppers (Table 6).

Table 6. Ethylene evolved ($\mu L \cdot kg^{-1} \cdot hr^{-1}$) by banana peppers after the first 12 hours at 20°C following storage at 7°C for 0 to 8 days (Experiment 3).

	Days in Storage				
Cultivar	0 Days	2 Days	4 Days	6 Days	8 Days
Hungarian Wax	0.0019	0.0642	0.3766 a [z]	0.1681	0.4467 a
Hungarian Yellow Wax	0.0019	0.2003	0.2900 ab	0.2448	0.3183 a
Sweet Banana	0.0316	0.1641	0.1027 b	0.0781	0.0988 b

[z]Mean separation within columns by Duncan's New Multiple Range Test, P=0.05.

c. Ethylene Evolution after Storage at ~15°C

The effects of cultivar on C_2H_4 evolution after storage at ~15°C were non-significant (Appendix B.15). The effects of storage duration and the interaction of cultivar and duration were highly significant (Appendix B.15). The effects of cultivar on ethylene evolution after storage at ~15°C were non-significant for banana peppers stored for 0, 2, 4, or 6 days and significant for peppers stored for 8 days (Appendix B.15). Ethylene evolution from all pepper types generally increased with duration of storage at ~15°C. After storage at ~15°C, there were differences in the amounts of C_2H_4 evolved by the three cultivars of peppers in this study (Table 7). After 8 days in storage, 'Hungarian Wax' peppers produced significantly more C_2H_4 than 'Hungarian Yellow Wax' or 'Sweet Banana' peppers (Table 7).

Table 7. Ethylene evolved ($\mu L \cdot kg^{-1} \cdot hr^{-1}$) by banana peppers after the first 12 hours at 20°C following storage at ~15°C for 0 to 8 days (Experiment 3).

	Days in Storage				
Cultivar	0 Days	2 Days	4 Days	6 Days	8 Days
Hungarian Wax	0.0019	0.0415	0.1282	0.1697	0.3613 a [z]
Hungarian Yellow Wax	0.0018	0.0007	0.1128	0.0754	0.2572 b
Sweet Banana	0.0229	0.5645	0.0347	0.0553	0.2358 b

[z]Mean separation within columns by Duncan's New Multiple Range Test, P=0.05.

3. Ion leakage

The effects of cultivar, temperature, and storage duration on ion leakage were highly significant (Appendix B.18). The effects of the interaction of temperature and duration were highly significant (Appendix B.18). The effects of temperature on ion leakage from the pericarps of banana peppers were non-significant for the peppers stored for 0 or 2 days (Appendix B.18). The effects of temperature on ion leakage from peppers stored for 4, 6, or 8 days were highly significant linearly and quadratically. However, comparisons among temperatures were inconsistent at different storage times. After 4

and 6 days, banana peppers stored at ~15°C had the highest percentage of ion leakage, followed by those stored at 7°C. After 8 days, banana peppers stored at 2.5°C had the highest percentage of ion leakage, and those stored at ~15°C had the lowest percentage. For all three cultivars stored at all three temperatures, ion leakage increased with duration of storage (Figure 11).

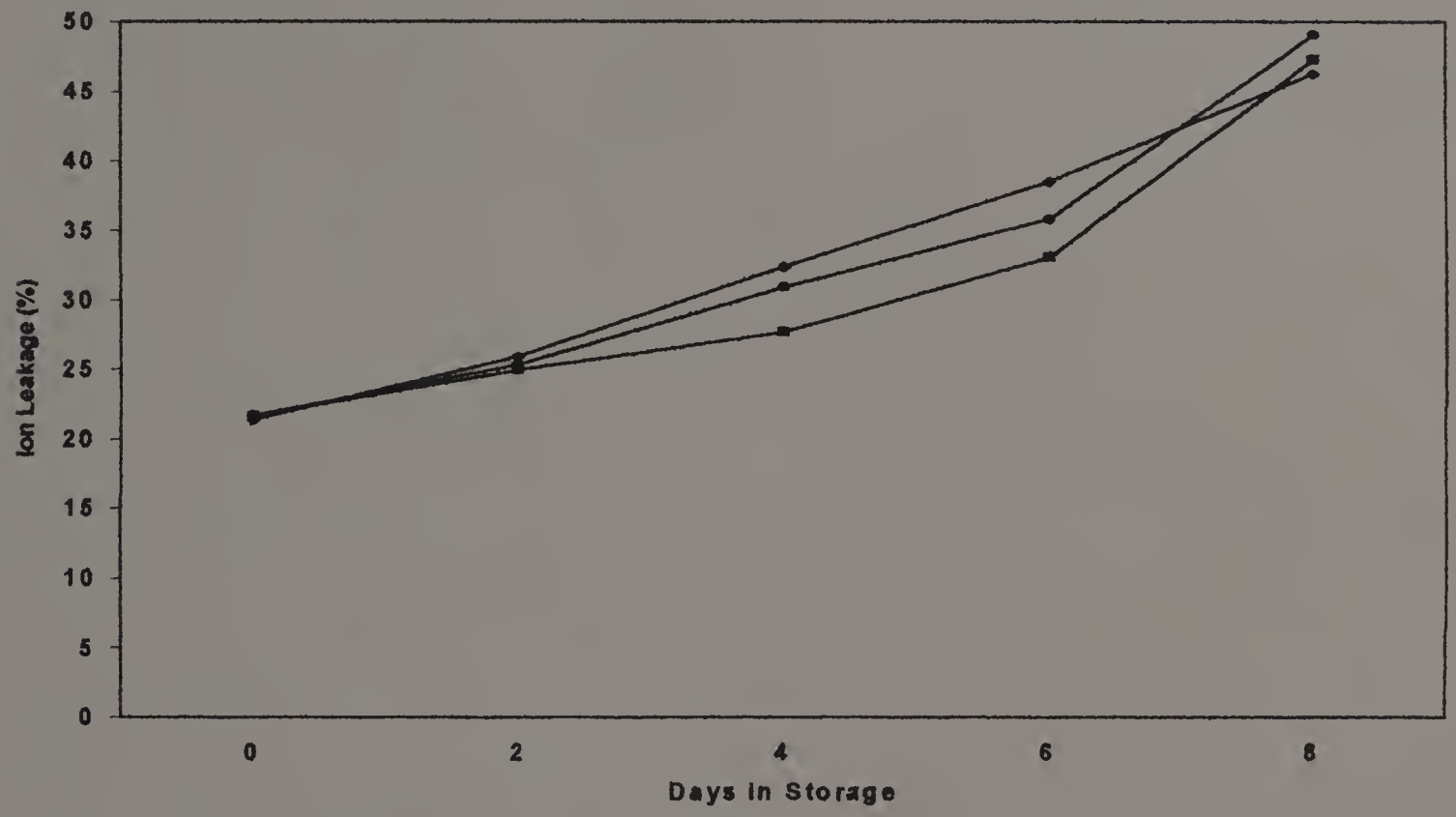


Figure 11. Ion leakage from the pericarps of banana peppers following storage at 2.5°C (●), 7°C (■), ~15°C (◆) (Experiment 3).

a. Ion Leakage after Storage at 2.5°C

The effects of cultivar, duration of storage, and the interaction of sultivar and duration were highly significant on ion leakage from banana peppers stored at 2.5°C (Appendix B.19). There were differences among the three types of banana peppers in the amounts of ion leakage from the pericarps. Following storage at 2.5°C, there were no significant differences in ion leakage from the peppers stored for 0 or 2 days, but the effects of duration of storage at this temperature were highly significant for banana peppers stored for 4, 6, or 8 days (Appendix B.19). 'Hungarian Yellow Wax' peppers had

significantly higher ion leakage than the other two cultivars after storage for 4 or 6 days (Table 8). After storage for 8 days at 2.5°C, 'Hungarian Wax' had the highest amount of ion leakage, followed by 'Hungarian Yellow Wax' and 'Sweet Banana' peppers.

Table 8. Ion leakage (%) from the pericarps of banana peppers following storage at 2.5°C for 0 to 8 days (Experiment 3).

	Days in Storage				
Cultivar	0 Days	2 Days	4 Days	6 Days	8 Days
Hungarian Wax	23.67	23.02	25.76 b [z]	33.54 b	52.24 a
Hungarian Yellow Wax	20.16	24.76	43.36 a	43.74 a	49.33 ab
Sweet Banana	21.19	24.53	25.59 b	30.16 b	45.65 b

[z]Mean separation within columns by Duncan's New Multiple Range Test, P=0.05.

b. Ion Leakage after Storage at 7°C

For banana peppers stored at 7°C, the effects of cultivar, duration of storage, and the interaction of cultivar and duration were highly significant on ion leakage from the pericarps (Appendix B.20). Following storage at 7°C, there were no significant differences in ion leakage from the peppers stored for 0 or 2 days, but the effects of duration of storage were highly significant for peppers stored for 4, 6, or 8 days (Appendix B.20). 'Hungarian Yellow Wax' peppers had significantly higher ion leakage than the other two cultivars after storage for 4 or 6 days (Table 9). After storage for 8 days at 7°C, 'Sweet Banana' peppers had significantly less ion leakage than 'Hungarian Wax' or 'Hungarian Yellow Wax' peppers.

Table 9. Ion leakage (%) from the pericarps of banana peppers following storage at 7°C for 0 to 8 days (Experiment 3).

	Days in Storage				
Cultivar	0 Days	2 Days	4 Days	6 Days	8 Days
Hungarian Wax	22.22	25.89	25.08 b [z]	27.17 b	48.72 a
Hungarian Yellow Wax	20.53	24.46	33.08 a	42.02 a	49.25 a
Sweet Banana	22.22	24.55	24.95 b	30.04 b	43.82 b

[z]Mean separation within columns by Duncan's New Multiple Range Test, P=0.05.

c. Ion Leakage after Storage at ~15°C

For banana peppers stored at ~15°C, the effects of cultivar, duration of storage, and the interaction of cultivar and duration on ion leakage from the pericarps were highly significant (Appendix B.21). Following storage at ~15°C, there were no significant differences in ion leakage from the peppers stored for 0, 2, or 8 days, but the effects of storage duration were highly significant for banana peppers stored for 4 or 6 days at this temperature (Appendix B.21). 'Hungarian Yellow Wax' peppers had significantly higher ion leakage than the other two cultivars after storage for 4 days (Table 10). After storage for 6 days at ~15°C, 'Hungarian Wax' peppers had significantly less ion leakage than 'Hungarian Yellow Wax' or 'Sweet Banana' peppers.

Table 10. Ion leakage (%) from the pericarps of banana peppers following storage at ~15°C for 0 to 8 days (Experiment 3).

	Days in Storage				
Cultivar	0 Days	2 Days	4 Days	6 Days	8 Days
Hungarian Wax	21.41	26.85	26.86 b [z]	31.38 b	46.38
Hungarian Yellow Wax	21.32	27.48	39.07 a	39.65 a	43.84
Sweet Banana	21.41	23.47	31.26 b	44.50 a	48.56

[z]Mean separation within columns by Duncan's New Multiple Range Test, P=0.05.

D. Storage Temperatures for Serrano Peppers - Experiment 4

1. Development of Chilling-Injury Symptoms

Chilling-injury symptoms developed on 'Serrano Chili' peppers after extended storage times at very low temperatures, and included pitting, dark splotches, and rot. Upon removal from storage after 30 days at 0°, approximately 80% of the peppers were soft and had black rot on the stems. After 24 hours at room temperature, some of these fruit had developed large, deep pits. After 45 days at 0°, all fruit were soft, splotchy, and had a foul smell, with rot extensively present.

'Serrano Chili' peppers manifested no chilling-injury symptoms after 15 days in storage at 2.5°C, which duplicated results recorded in 1998, even though different cultivars were used in the two years.

2. Ethylene Evolution

The effects of temperature on C_2H_4 production after the first 12 hours (Appendix B.22), the second 12 hours (Appendix B.23), and the total 24 hours (Appendix B.24) at room temperature were significant, highly significant, and non-significant, respectively. The effects of storage duration on C_2H_4 production were highly significant after the first 12 hours and the 24 hour total and significant after the second 12 hour period. The data presented here represent C_2H_4 evolved after the total 24 hours at room temperature after storage.

Although there was a difference in the amounts of C_2H_4 evolved between the two groups of 'Serrano Chili' peppers sampled after 0 days in storage, approximately 12 hours after harvest, those stored for 15 days at 0°C or 2.5°C had similar levels (Figure 12). In general, C_2H_4 evolution from Serrano peppers increased with duration of storage at 0°C or 2.5°C with the largest increase from 0 to 15 days (Figure 12).

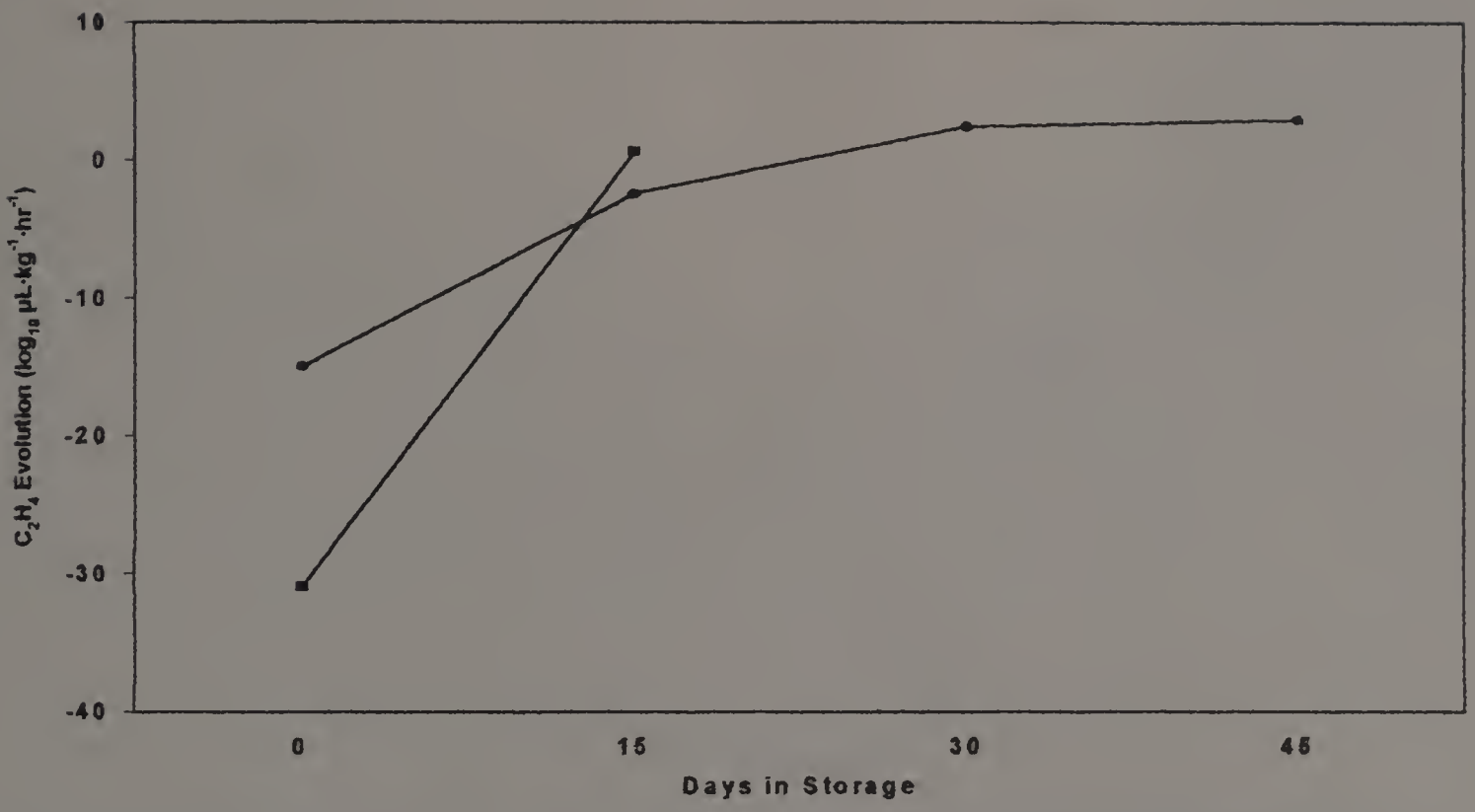


Figure 12. Ethylene evolved by 'Serrano Chili' peppers after the total 24 hours at 20°C following storage at 0°C (●) or 2.5°C (■) (Experiment 4).

3. Ion Leakage

The effects of temperature on ion leakage were non-significant, and the effects of storage duration on ion leakage were highly significant for 'Serrano Chili' peppers (Appendix B.26). Ion leakage from pericarp tissue of Serrano peppers increased with storage duration, but temperature had no significant effect on it (Figure 13). After storage at a chilling temperature of either 0° or 2.5°C, there was no difference in the levels of ion leakage from fruit stored for 0 or 15 days. In general, ion leakage from the pericarps of Serrano peppers increased with duration of storage at both 0°C and 2.5°C (Figure 13).

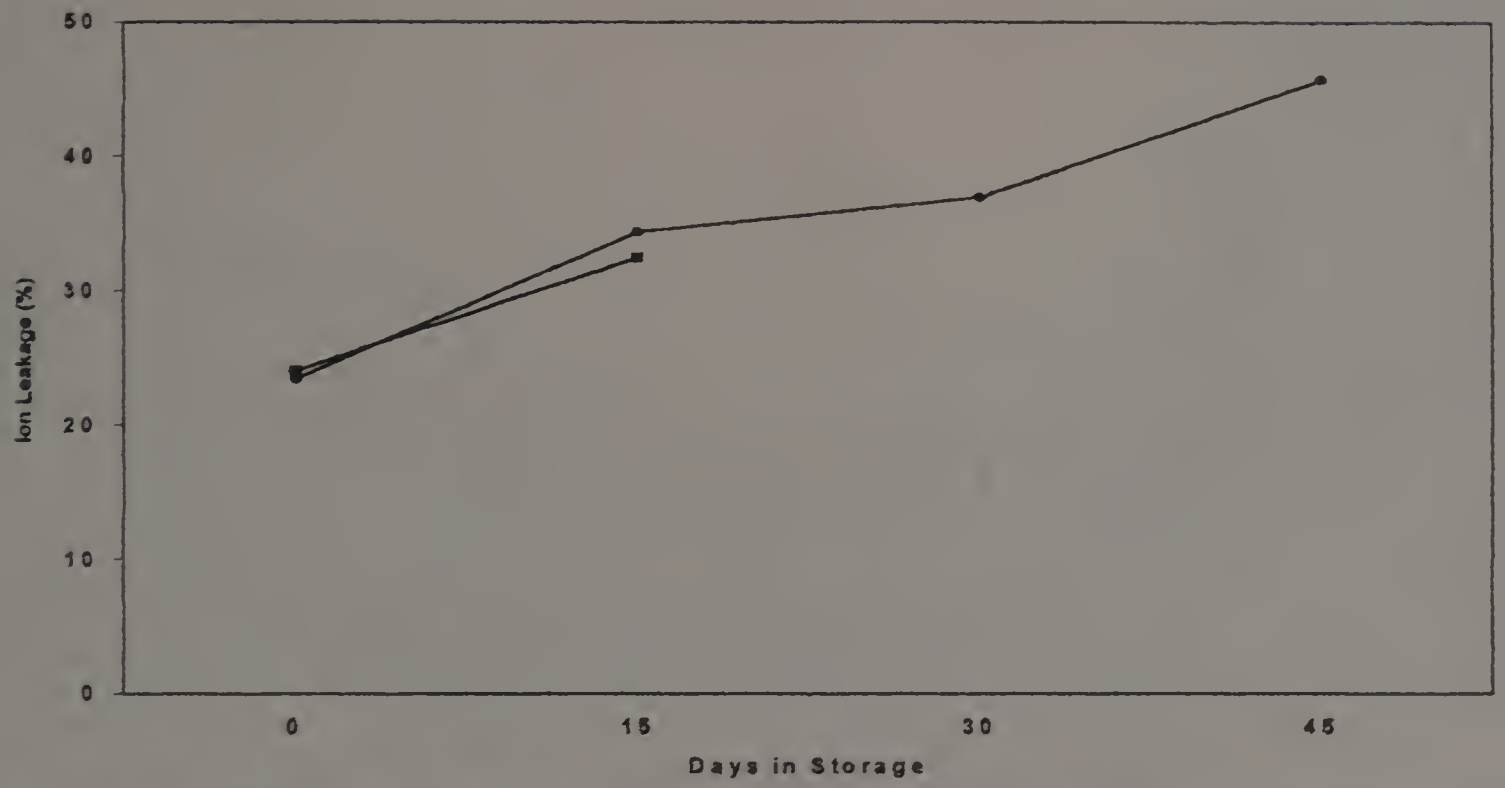


Figure 13. Ion leakage from the pericarps of 'Serrano Chili' peppers following storage at 0°C (●) or 2.5°C (■) (Experiment 4).

CHAPTER V

DISCUSSION

A. Introduction: Assessment of Postharvest Chilling Injury

The determination of chilling injury on harvested plant parts can be done directly or indirectly. Direct assessment of chilling injury is by observation of injury symptom development, while indirect assessment includes measurement of physiological changes associated with injury to the plant material, regardless of visible symptoms. Chilling-injury symptoms are the result of cellular breakdown and the accumulation of toxic compounds (87). In addition to visible physiological changes, rot caused by invading pathogens such as *Alternaria* and *Erwinia* species is an indication of postharvest chilling injury due to reduction of disease resistance of cells (92). In many cases, symptoms of chilling injury are not visible on fruit until after several hours or days at a non-chilling temperature. Differences in the time until manifestation of visible symptoms also indicate differences in susceptibility to chilling stress (86).

Indirect assessment of chilling injury can be done by measuring physiological responses to a suboptimal temperature. Ethylene production is often used as an indication of chilling stress, as its production increases in whole plants and plant parts under many adverse conditions (29, 148). Ethylene produced by fruits induces ripening (13), which produces many changes that could influence both responses to postharvest storage temperature and the measurements being used to characterize those responses (157). To reduce the effects of ripening on the peppers in this study, it was desired that the harvested fruit of each group be of similar physiological age. In the first year, 1998, efforts were made to insure that the fruit came from flowers fertilized at the same time so they would be of the same chronological age and would have experienced similar conditions. A consistent rate of physiological change as the fruit matured was assumed.

As pepper flowers are only open to pollination for a few days, flowers were tagged at anthesis. However, fruit bearing tags marked with the same date were observed to be of great variability in size and color development. This was particularly evident with Cherry 'Bomb' peppers developing from flowers pollinated at the same time, some of which were small and green while others even on the same plant were fully grown and red. Tagging at anthesis only served to identify fruit of similar chronological age and appeared to be physiologically meaningless. Similar results were recently reported by Villavicenzio et al. (149). For this reason, this process was not repeated in 1999, when uniform fruit were chosen according to size and visual appearance.

The extent of the effects of chilling can also be assessed indirectly by determining damage to plant cell membranes. Chilling has an adverse effect on membranes, as they are, to a large part, comprised of lipids which undergo a phase-change when exposed to suboptimal temperatures (87,123). This solidification of membrane lipids purportedly causes the membranes to "crack" at the critical temperature (87). As a consequence of this damage to membranes, ions leak from the organelle or cell. The percentage of total ions that leak from the plant material is an indication of the extent of chilling injury. Ion leakage was measured for experiments 1 and 2 (data not shown). However, the procedure was flawed and the data were meaningless. The disks excised from the pericarp were not placed on an agitator, and they were not homogenized for accurate total ion measurement. In the second year (experiments 3 and 4), the procedure was adjusted to achieve more accurate measurements of ion leakage.

B. Development of Chilling-Injury Symptoms

1. Cultivar Differences

Differences in response to postharvest chilling among cultivars of species other than peppers have been observed (3, 6, 114, 152). In addition, bell peppers harvested after turning red have been shown to be more resistant to chilling than those harvested at the mature green stage (76). The recommendations given in the current edition of United States Department of Agriculture (USDA) Handbook 66 (48) are to store all peppers at the same conditions recommended in the same volume for green bell peppers (48). Similar postharvest performance among types of peppers is assumed. This study confirmed the hypothesis that there is variability among different types of peppers in their susceptibility to postharvest chilling injury or development of injury symptoms.

Not only was there great variability among the types of peppers in this study in how quickly the fruit manifested chilling-injury symptoms (Tables 1 and 2), there was also variety in the types of symptoms exhibited. Some pepper types exhibited injury as scald, which was unexpected, while only Poblano 'Ancho San Luis' peppers had unusually large, deep pits which appeared water-soaked. These symptoms, observed on Poblano peppers stored at 2.5°C, resembled freezing injury, while the small pits on Poblanos stored at 7°C were consistent with chilling-injury symptoms observed on other pepper types. For peppers stored at 2.5°C, a temperature expected to cause injury, there were significant differences in the durations of storage until symptoms appeared (Table 1). In the first year, 'Hungarian Wax', a banana-type cultivar, had visible chilling-injury symptoms when the fruit were removed from storage after the first sampling interval of 4 days. Because of this, this experiment was repeated in 1999 with 'Hungarian Wax' and two other cultivars of the same type, 'Hungarian Yellow Wax' and 'Sweet Banana'. Because chilling-injury symptoms had been observed on fruit upon removal from storage at the first interval of time, 4 days, the sampling interval for the second year was reduced

to every 2 days. 'Sweet Banana' fruit had visible symptoms after only 2 days at 2.5°C; 'Hungarian Wax' and 'Hungarian Yellow Wax' manifested chilling-injury symptoms after 4 days in storage at 2.5°C.

Cherry and Poblano peppers were also more susceptible to development of chilling-injury symptoms than most other types in this study. Both of these types manifested symptoms after 8 days at 2.5°C (Table 1). 'Jalapeño' peppers, harvested both green and red, and 'Cubanelle' peppers were more resistant to postharvest chilling. The Jalapeños had visible symptoms after 12 days, and Cubanelles were injured after 16 days in storage. Although red bell peppers have been shown to be less susceptible to chilling than green bell peppers (76), the only difference between Jalapeños harvested green and those harvested red was in the duration of exposure to room temperature after removal from cold storage which resulted in symptom development. Red Jalapeños had visible symptoms when removed from storage after 12 days. Green Jalapeños had no symptoms upon removal from storage after the same amount of time, but they manifested chilling-injury symptoms after 24 hours at room temperature.

'Serrano' peppers were observed to be the most resistant to chilling injury of the types studied, not manifesting visible symptoms until after 23 days at 2.5°C (Table 1) and remaining free of injury after 30 days at 7°C (Table 2). This resistance was explored further the second year, 1999, with 'Serrano Chili' fruit kept again at 2.5°C and also at 0°C. Those stored at 2.5°C were sampled only after 0 and 15 days due to disruption of the experiment; those stored at 0°C were sampled after 0, 15, 30, or 45 days. As in the first year, Serrano peppers were free of injury after 15 days at 2.5°C, but manifested chilling-injury symptoms after 30 days at 0°C.

Not only were there significant differences in the susceptibility to chilling at the temperatures expected to cause injury, 0°C and 2.5°C, there were also surprising differences in susceptibility to chilling on fruit stored at 7°C, a temperature recommended as safe (48). Poblano peppers are susceptible to chilling injury at this

temperature and developed symptoms after only 8 days (Table 2). Cherry peppers manifested symptoms after 12 days. 'Hungarian Wax', which was the most susceptible to chilling injury at 2.5°C, was not very susceptible to chilling injury at 7°C. 'Hungarian Wax' and both green and red Jalapeños manifested symptoms after 16 days. 'Cubanelle' peppers, which, like 'Hungarian Wax', were susceptible to chilling at 2.5°C, first exhibited symptoms after 23 days in storage at 7°C.

2. Development of Scald

The visible symptoms of chilling injury vary with the commodity (86). Symptoms previously described on peppers include small, black pits on the pericarp, sheet pitting, blackening of the calyx and stem, seed browning, and increased susceptibility to alternaria rot (90). Chilling injury is manifested on apples as scald, a browning of the surface (154). In this study, scald was observed as a chilling-injury symptom on some types of chile peppers. In 1998, 'Hungarian Wax' and 'Cubanelle' peppers, which are morphologically similar, exhibited scald as a symptom of chilling injury. Some 'Cubanelle' peppers had minor scald, a light browning of a small area of the pericarp, after 16 days at 2.5°C. When stored at this temperature for 4 days, nearly all 'Hungarian Wax' peppers had severe scald, a darker brown coloring of much of the fruit, and manifested both scald and black pits after 16 days.

In the second year, 1999, 'Hungarian Wax' and two other cultivars of banana-type peppers, 'Hungarian Yellow Wax' and 'Sweet Banana', were evaluated for similarities in susceptibility to chilling and duplication of scald as a symptom of chilling injury on chile peppers. The incidence of scald similar to that observed on 'Hungarian Wax' in 1998 was observed on all three banana-type cultivars in 1999. Because the sampling interval was reduced to every 2 days in the second year, the development of scald was observed in stages. After 2 days at 2.5°C, numerous small, transparent pits were observed on 'Sweet Banana'. After 4 days, these were larger, had coalesced to form irregular pits, and had

darkened to a light brown. 'Hungarian Wax' and 'Hungarian Yellow Wax' peppers did not develop chilling-injury symptoms after two days at 2.5°C. They did, however, have numerous light brown, irregular pits when removed from storage after 4 days. These symptoms first appeared closest to the stem end and only on one side. After 6 days in storage, most peppers from all three cultivars had severe scald, usually concentrated on one side and running from stem to blossom end. Peppers after 8 days in cold storage were covered with dark brown scald on both sides from stem end to blossom end.

3. Effects of Harvest Date

Exposure to preharvest chilling has been shown to have conflicting effects on postharvest chilling injury. Bell pepper fruit harvested in the summer, without exposure to low field temperature, have been demonstrated to be more susceptible to postharvest chilling stress than peppers harvested in autumn of the same year (159). In contrast, bell peppers, tomatoes, and squash which experience some field chilling are more susceptible to postharvest chilling-injury symptom manifestation than those which did not experience preharvest chilling (63). The effect of harvest date on susceptibility to chilling injury was observed on red Cherry 'Bomb' peppers harvested on three dates in 1998. There were 12 days between the first and second harvests. The third harvest was 16 days after the second harvest. During the period of time between the last two harvest dates peppers experienced preharvest chilling. Field temperatures reached 2.8°C (37°F) on at least two nights during the week before the third harvest.

Fruit from the three harvests showed dramatic differences in their susceptibility to postharvest chilling injury. All injury symptoms noted appeared as pits, first small and translucent, then larger and black. Peppers harvested on the earliest date were resistant to postharvest chilling injury. No peppers from the first harvest developed pits, including those stored at 2.5°C for 15 days, the last sampling for this harvest. Fruit from the second harvest, 12 days after the first, developed chilling-injury symptoms. Pits were first

observed on peppers from the second harvest stored at 2.5°C for 15 days, the last sampling. The fruit were free of injury upon removal from storage, but pits were visible 48 hours after the fruit were removed to room temperature. Peppers from this harvest stored at 7°C did not manifest chilling-injury symptoms after 15 days. The peppers from the third harvest date, 16 days after the second harvest and 28 days after the first harvest, were much more susceptible to development of chilling-injury symptoms than the peppers harvested on either date earlier in the season. Pits were observed on peppers from the third harvest after 10 days in storage at 2.5°C. They were visible on these fruit when they were removed from cold storage, before being placed at room temperature. Peppers from this third harvest were the only ones that manifested chilling-injury symptoms after storage at 7°C, the recommended safe temperature. Fruit kept at this temperature for 10 days pitted within 24 hours of removal to room temperature. The incidence of visible chilling-injury symptoms was clearly greater with peppers that had experienced some field chilling. This may be a result of the cumulative effect of preharvest and postharvest chilling stress.

C. Physiological Changes

1. Ethylene Evolution

Ethylene (C_2H_4) production is often used as an indication of chilling stress, as its production increases in whole plants and plant parts under many adverse conditions (29, 148). There is typically a burst of postharvest C_2H_4 production when the fruit are removed from the chilling temperature, and this rate of production usually decreases to a steady level over time (157). For this reason, C_2H_4 measurements were taken from the headspace of jars containing the peppers 12 hours out of storage.

As C_2H_4 production is a response to chilling, it follows that with decreasing temperature and increased exposure time to a given temperature, the levels of C_2H_4

measured should increase. This was observed consistently in this study. The highest levels of C_2H_4 were produced by peppers which had been exposed to the lowest temperatures for the longest periods of time (Figures 2 and 4). Furthermore, similar data were recorded when internal C_2H_4 concentrations were measured 24 hours after removal from storage (Appendix C).

a. Cultivar Differences

Although the appearance of visible symptoms of chilling injury do not necessarily correspond with the physiological changes associated with CI, in this study the two observations closely paralleled one another. There were significant differences among the types of chile peppers in this study in both C_2H_4 evolution and the incidence of chilling-injury symptoms. 'Serrano' peppers, which did not exhibit pits until after 23 days in storage at 2.5°C (Table 1), produced significantly less C_2H_4 than all the other types in the first 12 hours at room temperature after storage (Figure 8). The initial measurements, taken ~12 hours after harvest, were much lower than those of the other six types of peppers. Although the levels of C_2H_4 increased with storage duration, they were consistently lower than those of the other pepper types (Figure 8).

In the first year, many headspace samples from the jars containing 'Serrano' peppers had levels of C_2H_4 undetectable by the gas chromatograph, even with these smaller fruit being placed in smaller jars. Because this was a possible cause of the significant difference between 'Serrano' and the other types, seven fruit instead of five were used as an experimental unit in the second year. The amounts of C_2H_4 evolved from these groups were comparable to those measured the first year. The appearance of chilling-injury symptoms on 'Serrano Chili' peppers after storage at 0°C paralleled the increase in C_2H_4 production after 30 or 45 days in storage.

'Hungarian Wax' peppers were the most susceptible to chilling-injury symptom manifestation, exhibiting CI symptoms in the form of scald after only 4 days at 2.5°C

(Table 1). This cultivar was also the one which produced the highest levels of C_2H_4 after storage at this temperature (Figure 4). 'Hungarian Wax' and two other cultivars of banana peppers, 'Hungarian Yellow Wax' and 'Sweet Banana' were evaluated for differences in susceptibility to chilling injury in the following year. Not only were all three of similar susceptibilities to chilling-injury symptom manifestation, exhibiting scald after 2 or 4 days at 2.5°C, they also had similar levels of C_2H_4 evolution (Figure 10). These three banana pepper cultivars produced levels of C_2H_4 similar to those produced by 'Hungarian Wax' peppers in the first year.

b. Effects of Harvest Date

In the experiment with Cherry 'Bomb' peppers harvested on three dates, the highest levels of C_2H_4 evolution were from peppers stored at the lowest temperatures for 10 or 15 days, regardless of harvest date. This difference in C_2H_4 production reflects a difference in temperature stress (Figure 9). Exposure to low temperature for longer periods of time may result in the production of C_2H_4 as a response to the stress. Peppers stored at ~15°C, a temperature not expected to cause chilling stress, produced much less C_2H_4 than those peppers stored at 2.5°C or 7°C. In addition, the levels of C_2H_4 production generally increased with duration of exposure to these lower temperatures (Figure 9).

Although there were striking differences among the three harvests of Cherry 'Bomb' peppers in the incidence of chilling-injury symptoms, there were few differences in the amounts of C_2H_4 evolved. The peppers from the first harvest produced much less C_2H_4 than peppers from the other two harvests after 5 days in storage at all temperatures, but after 10 or 15 days the amounts were similar (Figure 9). In contrast to this, peppers from the second harvest only exhibited pits after 15 days at 2.5°C, and peppers from the third harvest pitted after 10 days at this temperature. No pits were observed on peppers from the first harvest. Because this cultivar was harvested at full color (red), the C_2H_4

measured was a product of fruit ripening as well as chilling stress. Full color Cherry 'Bomb' peppers (143) and New Mexican chile peppers (16) have been shown to produce higher levels of C_2H_4 than green fruit (143). This could account for the similarities in C_2H_4 evolution among the three harvest dates.

2. Ion Leakage

The lipids which comprise plant cell membranes are adversely affected by exposure to suboptimal temperatures, which causes them to undergo a phase change and "crack" (87, 123). This causes solutes, including measurable ions, to leak from the cell or organelle. The measurement of solutes which have leaked out of plant cells can be an indication of cellular damage due to chilling or softening of the tissue due to ripening (87, 123).

In this study, the percentage of ions leaked from the pericarp tissue of banana peppers increased from ~23% at the time of harvest (0 days) to 45-50% after 8 days in storage (Figure 11). The highest percentages were with peppers that had been stored at 2.5°C or ~15°C. This is reflective of cellular damage or softening which results in a loss of cell integrity. Ion leakage generally increased with duration of storage, which reflects the irreversible processes of tissue softening and cell damage associated with senescence.

'Serrano Chili' peppers stored at 0°C or 2.5°C had similar levels of ion leakage after storage for wither 0 or 15 days (Figure 13). The percentage of ions leaking from the pericarp tissue increased with the duration of storage as a result of the effects of cellular damage from the chilling and/or senescence.

Although there were similarities among the three banana cultivars and 'Serrano Chili' in the levels of ion leakage from the initial measurements, ~12 hours after harvest, there were differences in the rates of increase over time. The percentage of ion leakage from the banana cultivars was ~30% after 4 days in storage at 2.5°C (Figure 11, Table 8). 'Serrano Chili' peppers had comparable levels of ion leakage after 15 days at this

temperature (Figure 13). This corresponds with their different susceptibilities to chilling-injury symptom manifestation.

D. Summary

In order for a food commodity to be acceptable to the consumer, it must meet certain visual criteria. Because of undesirable changes that take place when a commodity is stored at a non-optimal temperature, it is necessary to establish the optimum storage temperature for perishable commodities. If fresh harvested plant parts are kept at too high a temperature, there may be desiccation, unwelcome physiological changes, and incidence of pathogen growth (154). In this study, at the non-chilling control temperature of ~15°C, many 'Cubanelle', 'Hungarian Wax', 'Jalapeño', Poblano 'Ancho San Luis', and 'Serrano' peppers ripened, undergoing visible changes in color from green or yellow to orange or red, accompanied by an increase in ethylene over time. Some peppers of all types exhibited rot, most notably on the stem and calyx. After sixteen days at the control temperature, some 'Cubanelle' and 'Jalapeño' peppers were nearly liquefied with soft rot. Invasion by *Botrytis* was noted on many 'Cubanelle' peppers, and *Rhizopus* was evident on 'Hungarian Wax', Poblano, 'Serrano', and 'Jalapeño' peppers harvested both green and red (Mark Mazzola, University of Massachusetts, personal communication) (data not shown).

Although it is desirable to store food commodities at a lower temperature to decrease the respiration rate, retard ripening, and deter pathogen growth, when fresh food commodities are stored at too low a temperature other physiological and pathogenic problems may arise (154). Many perishable fruits and vegetables of tropical, sub-tropical, and temperate origin are at risk of a physiological disorder called chilling injury when stored at low but non-freezing temperatures (18, 86). Although chilling may not have

affected the taste or nutritional content of the commodity, the visible symptoms of chilling injury will deter the consumer from purchases.

Because the symptoms of chilling injury may not be visible until the commodity has been removed from cold storage and kept at room temperature for up to a few days, it is imperative to establish the lowest safe temperature. Current scientific research on the proper storage of all harvested plant parts, both edible and ornamental, is combined into USDA Handbook 66, where one can find comprehensive storage recommendations to optimize postharvest life. In the current edition of this handbook (48), recommendations for the postharvest handling of fresh bell peppers include storage at 7°C or above to avoid chilling injury. The current recommendation for the postharvest handling of other types of fresh peppers is simply to store them under the conditions recommended for bell peppers (48). There is no citation referring to research that gives scientific basis for this recommendation. The study described here was designed to explore differences among different types of peppers in their susceptibility to postharvest chilling injury.

There are dramatic differences among different types of chile peppers in their susceptibility to postharvest chilling, both in the storage duration which results in injury and the types of symptoms manifested. While all the cultivars in this study developed small, black pits, such as those previously described on bell peppers as chilling-injury symptoms, Poblano peppers exhibited large, deep pits similar to freezing injury. 'Cubanelle' and three cultivars of banana-type peppers, 'Sweet Banana', 'Hungarian Wax', and 'Hungarian Yellow Wax', exhibited scald as a symptom of chilling injury. Although no differences were observed among cultivars of the same type in the experiment which compared the three banana-type cultivars, it cannot be concluded that differences exist only among types and not among cultivars of the same type. The experiment may have been compromised by differences in the ripening stage at harvest among the cultivars. Although much effort was expended in harvesting banana peppers when fully yellow but before turning orange, many of the 'Sweet Banana' fruit retained a

green tinge at harvesting. This was observed by other local growers of 'Sweet Banana' cultivars in 1999 (John Howell, University of Massachusetts Extension, personal communication). In addition, no differences were observed between Jalapeños harvested green and those harvested red. All fresh peppers cannot be assumed to respond to cold stress in a similar way as sweet bell peppers, and 7°C cannot be considered a safe storage temperature for all pepper types.

Preharvest chilling may have a cumulative effect on chilling injury manifestation postharvest. Red Cherry 'Bomb' peppers harvested early in the season may withstand longer durations of time at low storage temperature than those harvested later in the season. Preharvest conditions should be considered in the postharvest storage of chile peppers.

The measurement of physiological changes associated with chilling injury is a good indicator of the differences among pepper cultivars in their susceptibility to the development of chilling-injury symptoms. Ethylene production and ion leakage are reliable indicators of physiological disruption due to chilling injury. However, it must be remembered that ethylene is also a product of the ripening process, and more ethylene may be evolved by full color peppers than those harvested green.

The current recommendations must be expanded to account for differences among pepper types. Specifically, they should include the following. Serrano peppers can be stored at temperatures as low as 2.5°C for three weeks and at temperatures as low as 0°C for two weeks. Banana-type peppers can be stored at 7°C for two weeks. Poblano peppers must be stored at a temperature higher than 7°C if they must be in storage more than four days. In addition, further research is warranted on other economically important peppers, including Habañeros, Cayenne, and New Mexican chiles.

APPENDIX A PICTURES

Picture 1. 'Hungarian Wax' peppers following storage at 2.5°C for 4 days.

2. ‘Serrano’ peppers following storage at 2.5°C for 8 days.

Picture 3. Poblano 'Ancho San Luis' peppers following storage at 2.5°C for 8 days.

Picture 4. Cherry 'Bomb' peppers following storage at 2.5°C for 8 days.

Picture 5. ‘Cubanelle’ peppers following storage at 2.5°C for 16 days.

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Picture 6. Full color 'Jalapeño' peppers following storage at 2.5°C for 12 days.

Picture 7. Mature green ‘Jalapeño’ peppers following storage at 2.5°C for 12 days.

APPENDIX B - ANALYSES OF VARIANCE

Appendix B.1. Analysis of Variance, ethylene evolution after the first 12 hours at 20°C following storage (Experiment 1).

Source	df	SS($x10^{-3}$)	MS($x10^{-3}$)	F
C	6	0.7559	0.1260	27.51**[z]
T	2	0.2985	0.1493	30.46**
D	4	0.2324	0.0581	18.51**
R	4	0.0264	0.0066	
CT	12	0.9736	0.0811	19.23**
CD	22	0.4964	0.0226	8.88**
CR	24	0.1100	0.0046	
TD	8	0.2469	0.0309	12.86**
T:D1 (0 Days)	2	0.0001	0.0000	0.01[ns y]
T:D2 (4 Days)	2	0.0014	0.0007	0.24[ns]
T:D2 (8 Days)	2	0.0816	0.0408	14.00**
Linear	1	0.0583	0.0583	19.98**
Quadratic	1	0.0234	0.0234	8.01**
T:D4 (12 Days)	2	0.2988	0.1494	51.23**
Linear	1	0.2302	0.2302	78.93**
Quadratic	1	0.0686	0.0686	23.54**
T:D4 (16 Days)	2	0.2018	01009	34.60**
Linear	1	0.1472	0.1472	50.49**
Quadratic	1	0.0545	0.0545	18.70**
TR	8	0.0399	0.0050	
DR	16	0.0503	0.0031	
CTD	44	0.6974	0.0159	6.66**
CTR	48	0.2026	0.0042	
CDR	88	0.2237	0.0025	
TDR	32	0.0767	0.0024	
CTDR	176	0.4188	0.0024	
nw = 1 N = 495				

[z]significant at $P = 0.01$
[y]non-significant

For Experiment 1:
C=Pepper Type
T=Temperature
D=Duration of Storage
R=Replication

Appendix B.2. Analysis of Variance, ethylene evolution after the first 12 hours at 20°C following storage at 2.5°C (Experiment 1).

Source	df	SS($x10^{-3}$)	MS($x10^{-3}$)	F
C	6	1.6989	0.2832	22.41** [z]
D	4	0.4601	0.1150	14.98**
R	4	0.6489	0.0162	
CD	22	1.1682	0.0153	7.64**
CR	24	0.3033	0.0126	
DR	16	0.1229	0.0077	
CDR	88	0.6117	0.0070	
C:D1 (0 Days)	6	0.0119	0.0002	0.02[ns y]
C:D2 (4 Days)	4	0.0541	0.0014	0.17[ns]
C:D3 (8 Days)	6	0.3384	0.0564	6.97**
C:D4 (12 Days)	6	1.4712	0.2452	30.32**
C:D5 (16 Days)	6	1.0511	0.1752	21.66**
nw = 1	N = 165			

[z]significant at $P = 0.01$
[y]non-significant

Appendix B.3. Analysis of Variance, ethylene evolution after the first 12 hours at 20°C following storage at 7°C (Experiment 1).

Source	df	SS($x10^{-3}$)	MS($x10^{-3}$)	F
C	6	0.0256	0.0043	12.97** [z]
D	4	0.0180	0.0045	18.75**
R	4	0.0013	0.0003	
CD	22	0.2130	0.0010	3.13* [y]
C:D1 (0 Days)	6	0.0012	0.0002	0.61[ns x]
C:D2 (4 Days)	4	0.0020	0.0005	1.56[ns]
C:D3 (8 Days)	6	0.0091	0.0015	4.81**
C:D4 (12 Days)	6	0.0099	0.0016	5.22**
C:D5 (16 Days)	6	0.0249	0.0042	13.22**
CR	24	0.0079	0.0003	
DR	16	0.0040	0.0002	
CDR	88	0.0273	0.0003	
nw = 1	N = 165			

[z]significant at $P = 0.01$
[y]significant at $P = 0.05$
[x]non-significant

Appendix B.4. Analysis of Variance, ethylene evolution after the first 12 hours at 20°C following storage at ~15°C (Experiment 1).

Source	df	SS($x10^{-3}$)	MS($x10^{-3}$)	F
C	6	0.00480	0.00080	13.33* [z]
D	4	0.00120	0.00030	30.00** [y]
R	4	0.00010	0.00004	
CD	22	0.00440	0.00020	5.00**
C:D1 (0 Days)	6	0.00010	0.00002	1.33[ns x]
C:D2 (4 Days)	4	0.00030	0.00008	5.33**
C:D3 (8 Days)	6	0.00070	0.00010	7.33**
C:D4 (12 Days)	6	0.00190	0.00030	21.33**
C:D5 (16 Days)	6	0.00490	0.00080	54.00**
CR	24	0.00140	0.00006	
DR	16	0.00020	0.00001	
CDR	88	0.00350	0.00004	
nw = 1	N = 165			

[z]significant at $P = 0.05$
[y]significant at $P = 0.01$
[x]non-significant

Appendix B.5. Analysis of Variance, ethylene evolution after the second 12 hours at 20°C following storage (Experiment 1).

Source	df	SS($x10^{-3}$)	MS($x10^{-3}$)	F
C	6	0.9990	0.1665	59.45** [z]
T	2	0.4562	0.2281	87.39**
D	4	0.4491	0.1112	48.79**
R	4	0.0170	0.0043	
CT	12	1.6647	0.1387	54.19**
CD	23	1.3307	0.0579	38.32**
CR	24	0.0671	0.0028	
TD	8	0.6143	0.0768	56.46**
T:D1 (0 Days)	2	0.0000	0.0000	0.06[ns y]
T:D2 (4 Days)	2	0.0004	0.0002	1.18[ns]
T:D3 (8 Days)	2	0.0073	0.0036	22.61**
Linear	1	0.0043	0.0043	26.65**
Quadratic	1	0.0030	0.0098	18.51**
T:D4 (12 Days)	2	0.5449	0.2725	1692.36**
Linear	1	0.4566	0.4566	2836.15**
Quadratic	1	0.8832	0.8832	548.57**
T:D5 (16 Days)	2	0.5420	0.2710	1683.29**
Linear	1	0.4582	0.4582	2846.21**
Quadratic	1	0.0838	0.0838	520.43**
TR	8	0.0209	0.0026	
DR	16	0.0365	0.0023	
CTD	46	2.3327	0.0507	36.48**
CTR	48	0.1228	0.0026	
CDR	92	0.1393	0.0015	
TDR	32	0.0437	0.0014	
CTDR	184	0.2551	0.0014	
nw = 1	N = 495			

[z]significant at $P = 0.01$
[y]non-significant

Appendix B.6. Analysis of Variance, ethylene evolution after the total 24 hours at 20°C following storage (Experiment 1).

Source	df	SS($x10^{-3}$)	MS($x10^{-3}$)	F
C	6	0.87504	0.14584	43.02** [z]
T	2	0.34900	0.17450	51.93**
D	4	0.32427	0.08107	33.78**
R	4	0.01963	0.00491	
CD	22	0.82463	0.03758	21.92**
CR	24	0.08125	0.00339	
TD	8	0.40280	0.05035	32.28**
T:D1 (0 Days)	2	0.00003	0.00002	0.01[ns y]
T:D2 (4 Days)	2	0.00065	0.00032	0.17[ns]
T:D3 (8 Days)	2	0.03442	0.01721	8.96[ns]
T:D4 (12 Days)	2	0.41131	0.20566	107.11**
Linear	1	0.33289	0.33289	173.38**
Quadratic	1	0.07842	0.07842	40.84**
T:D5 (16 Days)	2	0.34960	0.17480	91.04**
Linear	1	0.28139	0.28139	146.56**
Quadratic	1	0.06821	0.06821	35.53**
TR	8	0.02690	0.00336	
DR	16	0.03832	0.00240	
CTD	44	1.32241	0.03005	19.26**
CTR	48	0.14654	0.00305	
CDR	88	0.15054	0.00171	
TDR	32	0.04991	0.00156	
CTDR	176	0.27469	0.00156	
$n_w = 1$	N = 495			

[z]significant at $P = 0.01$
[y]non-significant

Appendix B.7. Analysis of Variance, internal ethylene concentrations (Experiment 1)

Source	df	SS	MS	F
C	6	15.73	2.62	14.09** [z]
T	2	11.00	5.50	31.42**
D	4	26.02	6.51	23.40**
R	4	0.43	0.11	
CT	12	11.33	0.94	4.00**
CD	23	37.32	1.62	7.51**
CR	24	4.46	0.19	
TD	8	7.20	0.90	6.12**
T:D1 (0 Days)	2	0.01	0.00	0.03[ns y]
T:D2 (4 Days)	2	0.15	0.07	0.48[ns]
T:D3 (8 Days)	2	3.66	1.83	11.99**
Linear	1	3.46	3.46	22.70**
Quadratic	1	0.19	0.19	1.27[ns]
T:D4 (12 Days)	2	7.21	3.61	23.63**
Linear	1	6.77	6.77	44.36**
Quadratic	1	0.44	0.44	2.91[ns]
T:D5 (16 Days)	2	7.76	3.88	25.42**
Linear	1	4.57	4.57	29.92**
Quadratic	1	3.19	3.19	20.92**
TR	8	1.40	0.18	
DR	16	4.45	0.28	
CTD	46	37.87	0.82	2.86**
CTR	48	11.31	0.24	
CDR	92	19.92	0.22	
TDR	32	4.70	0.15	
CTDR	184	53.06	0.29	

$n_w = 3$ N = 1530

[z]significant at $P = 0.01$
[y]non-significant

Appendix B.8. Analysis of Variance, ethylene evolution after the first 12 hours at 20°C following storage (Experiment 2)

Source	df	SS(x10^{-3})	MS(x10^{-3})	F
H	2	0.00192	0.00096	0.81$^{ns\ z}$
T	2	0.08936	0.04468	85.92$^{**\ y}$
Linear	1	0.05110	0.05110	98.27**
Quadratic	1	0.03826	0.03826	73.58**
D	3	0.02782	0.00927	4.61$^{*\ x}$
R	4	0.00733	0.00183	
HT	4	0.00007	0.00002	0.03^{ns}
HD	6	0.05007	0.00835	7.14**
HR	8	0.00942	0.00118	
TD	6	0.04558	0.00760	6.85**
T:D1 (0 Days)	2	0.00254	0.00127	1.32^{ns}
T:D2 (5 Days)	2	0.00476	0.00238	2.47^{ns}
T:D3 (10 Days)	2	0.04087	0.02044	21.23**
Linear	1	0.02565	0.02650	26.64**
Quadratic	1	0.01523	0.01523	15.82**
T:D4 (15 Days)	2	0.08677	0.04338	45.05**
Linear	1	0.04246	0.04246	44.09**
Quadratic	1	0.04431	0.04431	46.01**
TR	8	0.00412	0.00052	
DR	12	0.02416	0.00201	
HTD	12	0.00733	0.00061	0.90^{ns}
HTR	16	0.00967	0.00060	
HDR	24	0.02799	0.00117	
TDR	24	0.02653	0.00111	
HTDR	48	0.03276	0.00068	
$n_w = 1$	N = 180			

^{z}non-significant
^{y}significant at $P = 0.01$
^{x}significant at $P = 0.05$

For Experiment 2:
H=Harvest
T=Temperature
D=Duration of Storage
R=Replication

Appendix B.9. Analysis of Variance, ethylene evolution after the second 12 hours at 20°C following storage (Experiment 2)

Source	df	SS($x10^{-3}$)	MS($x10^{-3}$)	F
H	2	0.00550	0.00275	$2.08^{ns\ z}$
T	2	0.07567	0.03784	$67.57^{**\ y}$
D	3	0.02964	0.00988	$5.26^{*\ x}$
R	4	0.00819	0.00205	
HT	4	0.01092	0.00273	2.08^{ns}
HD	6	0.04225	0.00845	7.28**
HR	8	0.01056	0.00132	
TD	6	0.04923	0.00821	9.02**
T:D1 (0 Days)	2	0.00140	0.00070	0.85^{ns}
T:D2 (5 Days)	2	0.00389	0.00195	2.37^{ns}
T:D3 (10 Days)	2	0.01346	0.00673	8.18**
Linear	1	0.00490	0.00490	5.94*
Quadratic	1	0.00860	0.00860	10.41**
T:D4 (15 Days)	2	0.10983	0.05492	66.73**
Linear	1	0.05250	0.05250	63.79**
Quadratic	1	0.05730	0.05730	69.66**
TR	8	0.00447	0.00056	
DR	12	0.02253	0.00188	
HTD	10	0.03412	0.00341	3.07*
HTR	16	0.02099	0.00131	
HDR	20	0.02321	0.00116	
TDR	24	0.02179	0.00091	
HTDR	40	0.04433	0.00111	
nw = 1	N = 165			

[z]non-significant
[y]significant at $P = 0.01$
[x]significant at $P = 0.05$

Appendix B.10. Analysis of Variance, ethylene evolution after the total 24 hours at 20°C following storage (Experiment 2)

Source	df	SS(x10^{-3})	MS(x10^{-3})	F
H	2	0.00080	0.00040	0.40$^{ns\ z}$
T	2	0.08869	0.04434	113.69$^{**\ y}$
D	3	0.02412	0.00804	8.20**
R	4	0.00759	0.00190	
HT	4	0.00203	0.00051	0.72^{ns}
HD	6	0.03851	0.00770	8.75**
HR	8	0.00791	0.00099	
TD	6	0.04162	0.00694	10.06**
T:D1 (0 Days)	2	0.00130	0.00065	1.06^{ns}
T:D2 (5 Days)	2	0.00756	0.00368	5.98$^{*\ x}$
Linear	1	0.00310	0.00310	4.99*
Quadratic	1	0.00420	0.00420	6.85**
T:D3 (10 Days)	2	0.02936	0.01468	23.87**
Linear	1	0.00240	0.00240	3.90*
Quadratic	1	0.02700	0.02700	43.84**
T:D4 (15 Days)	2	0.09796	0.04898	79.64**
Linear	1	0.00740	0.00740	12.03**
Quadratic	1	0.09060	0.09060	147.25**
TR	8	0.00310	0.00039	
DR	12	0.01182	0.00098	
HTD	10	0.01430	0.00143	2.51*
HTR	16	0.01140	0.00071	
HDR	20	0.01752	0.00088	
TDR	24	0.01664	0.00069	
HTDR	60	0.02287	0.00057	
nw = 1	N = 165			

^{z}non-significant
^{y}significant at $P = 0.01$
^{x}significant at $P = 0.05$

Appendix B.11. Analysis of Variance, internal ethylene concentrations (Experiment 2)

Source		df	SS	MS	F
H		2	3.92	1.96	40.00** [z]
	Linear	1	1.17	1.17	23.37**
	Quadratic	1	2.75	2.75	55.04**
T		2	4.37	2.19	43.72**
	Linear	1	2.11	2.11	42.16**
	Quadratic	1	2.26	2.26	45.24**
D		3	0.80	0.27	4.93* [y]
R		4	0.18	0.04	
HT		4	1.35	0.34	11.62**
HD		6	11.54	1.92	34.98**
HR		8	0.39	0.05	
TD		6	2.74	0.46	7.14**
T:D1 (0 Days)		2	0.12	0.06	0.96[ns x]
T:D2 (5 Days)		2	0.63	0.31	5.20*
	Linear	1	0.06	0.06	1.00[ns]
	Quadratic	1	0.57	0.57	9.39**
T:D3 (10 Days)		2	0.76	0.38	6.31*
	Linear	1	0.45	0.45	7.41*
	Quadratic	1	0.32	0.32	5.22*
T:D4 (15 Days)		2	5.60	2.8	46.30**
	Linear	1	3.76	3.76	62.23**
	Quadratic	1	1.84	1.84	30.37**
TR		8	0.40	0.05	
DR		12	0.65	0.05	
HTD		12	5.69	0.47	8.17**
HTR		16	0.46	0.03	
HDR		24	1.33	0.06	
TDR		24	1.54	0.06	
HTDR		48	2.81	0.06	
nw = 2	N = 360				

[z]significant at $P = 0.01$
[y]significant at $P = 0.05$
[x]non-significant

Appendix B.12. Analysis of Variance, ethylene evolution after the first 12 hours at 20°C following storage (Experiment 3)

Source		df	SS(x10^{-3})	MS(x10^{-3})	F
C		2	0.00016	0.00008	1.33$^{ns\ z}$
T		2	0.00785	0.00392	65.33$^{**\ y}$
	Linear	1	0.00642	0.00642	107.00**
	Quadratic	1	0.00140	0.00140	23.33**
D		4	0.00667	0.00167	23.86**
R		4	0.00012	0.00003	
CT		4	0.00064	0.00016	4.00$^{*\ x}$
CD		8	0.00185	0.00023	5.75**
CR		8	0.00047	0.00006	
TD		8	0.00527	0.00066	8.25**
TR		8	0.00049	0.00006	
DR		16	0.00108	0.00007	
CTD		16	0.00092	0.00006	1.50^{ns}
CTR		16	0.00069	0.00004	
CDR		32	0.00135	0.00004	
TDR		32	0.00241	0.00008	
CTDR		64	0.00272	0.00004	
T:D1 (0 Days)		2	0.00001	0.00001	0.00^{ns}
T:D2 (2 Days)		2	0.00003	0.00001	0.13^{ns}
T:D3 (4 Days)		2	0.00326	0.00163	21.45**
	Linear	1	0.00220	0.00220	28.55**
	Quadratic	1	0.00110	0.00110	14.34**
T:D4 (6 Days)		2	0.00551	0.00276	36.32**
	Linear	1	0.00450	0.00450	59.47**
	Quadratic	1	0.00099	0.00099	13.03**
T:D5 (8 Days)		2	0.00432	0.00216	28.42**
	Linear	1	0.00380	0.00380	49.47**
	Quadratic	1	0.00060	0.00060	7.37*
nw = 1	N = 225				

^{z}non-significant
^{y}significant at $P = 0.01$
^{x}significant at $P = 0.05$

For Experiment 3:
C=Pepper Cultivar
T=Temperature
D=Duration of Storage
R=Replication

Apendix B.13. Analysis of Variance, ethylene evolution after the first 12 hours at 20°C following storage at 2.5°C (Experiment 3)

Source	df	SS($x10^{-3}$)	MS($x10^{-3}$)	F
C	2	0.00046	0.00020	$2.56^{ns\ z}$
D	4	0.01054	0.00260	$13.89^{**\ y}$
R	4	0.00050	0.00010	
CD	8	0.00140	0.00020	1.89^{ns}
CR	8	0.00070	0.00010	
DR	16	0.00300	0.00020	
CDR	32	0.00300	0.00010	
nw = 1	N = 75			

[z]non-significant
[y]significant at P=0.01

Apendix B.14. Analysis of Variance, ethylene evolution after the first 12 hours at 20°C following storage at 7°C (Experiment 3)

Source	df	SS($x10^{-3}$)	MS($x10^{-3}$)	F
C	2	0.00020	0.00010	11.00** [z]
D	4	0.00070	0.00020	18.00**
R	4	0.00010	0.00002	
CD	8	0.00040	0.00010	2.50* [y]
C:D1 (0 Days)	2	0.00001	0.00001	0.00[ns x]
C:D2 (2 Days)	2	0.00010	0.00030	1.39[ns]
C:D3 (4 Days)	2	0.00020	0.00010	5.56*
C:D4 (6 Days)	2	0.00010	0.00040	1.94[ns]
C:D5 (8 Days)	2	0.00030	0.00020	8.33**
CR	8	0.00010	0.00001	
DR	16	0.00010	0.00001	
CDR	32	0.00050	0.00002	
nw = 1	N = 75			

[z]significant at $P = 0.01$
[y]significant at $P = 0.05$
[x]non-significant

Apendix B.15. Analysis of Variance, ethylene evolution after the first 12 hours at 20°C following storage at ~15°C (Experiment 3)

Source	df	SS($x10^{-3}$)	MS($x10^{-3}$)	F
C	2	0.00010	0.00010	$1.25^{ns\ z}$
D	4	0.00070	0.00010	$6.00^{**\ y}$
R	4	0.00003	0.00001	
CD	8	0.00100	0.00010	6.00^{**}
C:D1 (0 Days)	2	0.00001	0.00001	0.00^{ns}
C:D2 (2 Days)	2	0.00010	0.00050	2.04^{ns}
C:D3 (4 Days)	2	0.00002	0.00001	0.42^{ns}
C:D4 (6 Days)	2	0.00004	0.00002	0.83^{ns}
C:D4 (8 Days)	2	0.00010	0.00005	$1.04^{*\ x}$
CR	8	0.00030	0.00004	
DR	16	0.00040	0.00002	
CDR	32	0.00060	0.00002	
nw = 1		N = 75		

^{z}non-significant
^{y}significant at $P = 0.01$
^{x}significant at $P = 0.05$

Appendix B.16. Analysis of Variance, ethylene evolution after the total 24 hours at 20°C following storage (Experiment 3)

Source	df	SS(x10^{-3})	MS(x10^{-3})	F
C	2	0.00004	0.00002	1.00$^{ns\ z}$
T	2	0.00297	0.00149	37.25$^{**\ y}$
Linear	1	0.00260	0.00260	64.50**
Quadratic	1	0.00040	0.00040	9.75**
D	4	0.00419	0.00105	35.00**
R	4	0.00005	0.00001	
CT	4	0.00035	0.00009	4.50$^{*\ x}$
CD	8	0.00074	0.00009	4.50**
CR	8	0.00017	0.00002	
TD	8	0.00238	0.00030	10.00**
T:D1 (0 Days)	2	0.00002	0.00001	0.00^{ns}
T:D2 (2 Days)	2	0.00008	0.00004	1.25^{ns}
T:D3 (4 Days)	2	0.00136	0.00068	21.25**
Linear	1	0.00090	0.00090	27.19**
Quadratic	1	0.00050	0.00050	15.31**
T:D4 (6 Days)	2	0.00171	0.00086	26.88**
Linear	1	0.00090	0.00090	27.19**
Quadratic	1	0.00050	0.00050	15.31**
T:D5 (8 Days)	2	0.00220	0.00110	34.38**
Linear	1	0.00090	0.00090	27.19**
Quadratic	1	0.00050	0.00050	15.31**
TR	8	0.00033	0.00004	
DR	16	0.00042	0.00003	
CTD	16	0.00068	0.00004	2.00*
CTR	16	0.00028	0.00002	
CDR	32	0.00068	0.00002	
TDR	32	0.00101	0.00003	
CTDR	64	0.00134	0.00002	
$n_w = 1$	N = 225			

^{z}non-significant
^{y}significant at $P = 0.01$
^{x}significant at $P = 0.05$

Appendix B.17. Analysis of Variance, internal ethylene concentrations (Experiment 3)

Source	df	SS	MS	F
C	2	5.76	2.88	3.40$^{ns\ z}$
T	2	2.21	1.10	2.77^{ns}
D	4	11.66	2.92	22.78$^{**\ y}$
R	4	2.48	0.62	
CT	4	2.04	0.51	0.95^{ns}
CD	8	4.85	0.61	1.80^{ns}
CR	8	6.77	0.85	
TD	8	9.12	1.14	2.76$^{*\ x}$
T:D1 (0 Days)	2	0.00	0.00	0.001^{ns}
T:D2 (2 Days)	2	0.71	0.36	0.87^{ns}
T:D3 (4 Days)	2	1.83	0.91	2.23^{ns}
T:D4 (6 Days)	2	8.78	4.39	10.71**
Linear	1	0.49	0.49	1.20^{ns}
Quadratic	1	8.29	8.29	20.22**
T:D5 (8 Days)	2	0.00	0.00	0.00^{ns}
TR	8	3.19	0.40	
DR	16	2.06	0.13	
CTD	16	7.77	0.49	0.92^{ns}
CTR	16	8.59	0.54	
CDR	32	10.78	0.34	
TDR	32	13.22	0.41	
CTDR	64	33.66	0.53	

nw = 1 N = 675

^{z}non-significant
^{y}significant at $P = 0.01$
^{x}significant at $P = 0.05$

Appendix B.18. Analysis of Variance, ion leakage (Experiment 3)

Source		df	SS	MS	F
C		2	2324.09	1162.04	106.60** [z]
T		2	456.65	228.32	20.94**
	Linear	1	142.62	142.62	13.08**
	Quadratic	1	313.72	313.72	28.78**
D		4	56181.38	14045.35	1288.42**
R		4	51.97	12.99	1.19[ns] [y]
CT		4	884.00	221.00	20.27**
C:T1 (2.5°C)		2	1597.20	798.60	55.61**
C:T2 (7°C)		2	989.18	494.59	34.44**
C:T3 (~15°C)		2	613.14	306.57	21.35**
CD		8	4677.23	584.65	53.63**
C:D1 (0 Days)		2	69.37	34.68	2.47[ns]
C:D2 (2 Days)		2	47.41	23.70	1.69[ns]
C:D3 (4 Days)		2	3828.26	1914.13	136.47**
C:D4 (6 Days)		2	2830.54	1415.27	100.91**
C:D5 (8 Days)		2	216.92	108.46	7.73**
CR		8	64.41	8.05	0.74[ns]
TD		8	988.26	123.53	11.33**
T:D1 (0 Days)		2	2.56	1.28	0.09[ns]
T:D2 (2 Days)		2	75.50	37.75	2.76[ns]
T:D3 (4 Days)		2	518.04	259.02	18.91**
	Linear	1	149.45	149.45	10.91**
	Quadratic	1	366.86	366.86	26.79**
T:D4 (6 Days)		2	665.27	332.63	24.29**
	Linear	1	85.77	852.77	6.26**
	Quadratic	1	579.50	579.50	42.32**
T:D5 (8 Days)		2	183.43	91.71	6.70**
	Linear	1	102.98	102.98	7.52**
	Quadratic	1	80.45	80.45	5.87* [x]
TR		8	120.22	15.03	1.38[ns]
DR		16	272.87	17.05	1.56[ns]
CTD		16	2121.31	132.58	12.16**
CTR		16	280.27	17.52	1.61[ns]
CDR		32	5454.40	17.01	1.56[ns]
TDR		32	427.55	13.36	1.23[ns]
CTDR		64	1129.76	17.65	1.62[ns]
$n_w = 1$	N = 675				

[z]significant at $P = 0.01$
[y]non-significant
[x]significant at $P = 0.05$

Appendix B.19. Analysis of Variance, ion leakage following storage at 2.5°C (Experiment 3)

Source	df	SS	MS	F
C	2	1602.72	801.36	39.71** [z]
D	4	21408.17	5352.04	191.42**
R	4	56.02	14.01	
CD	8	2797.27	349.66	16.49**
C:D1 (0 Days)	2	97.77	48.88	2.33[ns y]
C:D2 (2 Days)	2	26.90	13.45	0.44[ns]
C:D3 (4 Days)	2	2439.05	1219.53	58.06**
C:D4 (6 Days)	2	1499.15	749.58	35.69**
C:D5 (8 Days)	2	329.12	163.56	7.79**
CR	8	161.47	20.18	
DR	16	447.34	27.96	
CDR	32	678.86	21.21	
$n_w = 1$		N = 325		

[z]significant at $P = 0.01$
[y]non-significant

Appendix B.20. Analysis of Variance, ion leakage following storage at 7°C (Experiment 3)

Source	df	SS	MS	F
C	2	989.18	494.59	64.82** [z]
D	4	18146.94	4536.74	1216.28**
R	4	30.12	7.53	
CD	8	1838.60	229.83	18.55**
C:D1 (0 Days)	2	27.84	13.92	1.22[ns y]
C:D2 (2 Days)	2	19.15	9.57	0.84[ns]
C:D3 (4 Days)	2	650.31	325.16	28.43**
C:D4 (6 Days)	2	1861.87	930.94	81.39**
C:D5 (8 Days)	2	268.60	134.30	11.74**
CR	8	61.05	7.63	
DR	16	59.64	3.73	
CDR	32	396.53	12.39	
$n_w = 1$	N = 325			

[z]significant at $P = 0.01$
[y]non-significant

Appendix B.21. Analysis of Variance, ion leakage following storage at ~15°C (Experiment 3)

Source	df	SS	MS	F
C	2	613.15	306.57	20.17** [z]
D	4	17615.32	4403.83	372.57**
R	4	85.24	21.31	
CD	8	2161.09	270.14	14.36**
C:D1 (0 Days)	2	0.07	0.04	0.002[ns y]
C:D2 (2 Days)	2	139.13	69.57	3.85[ns]
C:D3 (4 Days)	2	1148.22	574.11	31.74**
C:D4 (6 Days)	2	1319.94	659.97	36.49**
C:D5 (8 Days)	2	166.88	83.44	4.61[ns]
CR	8	121.57	15.20	
DR	16	189.15	11.82	
CDR	32	601.95	18.81	
$n_w = 1$	N = 325			

[z]significant at $P = 0.01$
[y]non-significant

Appendix B.22. Analysis of Variance, ethylene evolution after the first 12 hours at 20°C following storage (Experiment 4)

Source	df	SS($x10^{-3}$)	MS($x10^{-3}$)	F
T	1	0.04511	0.04511	8.07* [z]
D	3	1.38759	0.46253	27.00** [y]
R	4	0.13905	0.03476	
TD	1	0.05808	0.05808	11.48**
TR	4	0.02236	0.00559	
DR	12	0.20551	0.01713	
TDR	12	0.02024	0.00506	
T:D1 (0 Days)	1	0.00041	0.00041	0.08[ns x]
T:D2 (15 Days)	1	0.10277	0.10277	19.30**
nw = 1 N = 30				

[z]significant at $P = 0.05$
[y]significant at $P = 0.01$
[x]non-significant

For Experiment 4:
T=Temperature
D=Duration of Storage
R=Replication

Appendix B.23. Analysis of Variance, ethylene evolution after the second 12 hours at 20°C following storage (Experiment 4)

Source	df	SS($x10^{-3}$)	MS($x10^{-3}$)	F
T	1	0.02060	0.02060	10.05** [z]
D	3	0.07076	0.02359	3.72* [y]
R	4	0.03082	0.00771	
TD	1	0.01959	0.01959	10.05**
T:D1 (0 Days)	1	0.00001	0.00001	0.01 [ns x]
T:D2 (15 Days)	1	0.04018	0.04018	20.09*
TR	4	0.00820	0.00205	
DR	12	0.07609	0.00634	
TDR	12	0.00778	0.00195	
nw = 1 N = 30				

[z]significant at $P = 0.01$
[y]significant at $P = 0.05$
[x]non-significant

Appendix B.24. Analysis of Variance, ethylene evolution after the total 24 hours at 20°C following storage (Experiment 4)

Source	df	SS($x10^{-3}$)	MS($x10^{-3}$)	F
T	1	0.00117	0.00117	$0.72^{ns\ z}$
D	3	0.51504	0.17168	$19.94^{**\ y}$
R	4	0.07235	0.01809	
TD	1	0.00257	0.00257	1.77^{ns}
TR	4	0.00646	0.00162	
DR	12	0.10328	0.00861	
TDR	12	0.00579	0.00145	
nw = 1	N = 30			

[z]non-significant
[y]significant at $P = 0.01$

Appendix B.25. Analysis of Variance, internal ethylene concentrations (Experiment 4)

Source	df	SS	MS	F
T	1	0.00	0.00	$0.00^{ns\ z}$
D	3	0.50	0.17	$11.93^{**\ y}$
R	4	0.04	0.01	
TD	1	0.00	0.00	0.00^{ns}
TR	4	0.00	0.00	
DR	12	0.17	0.01	
TDR	12	0.00	0.00	
$n_w = 1$	$N = 90$			

[z]non-significant
[y]significant at $P = 0.01$

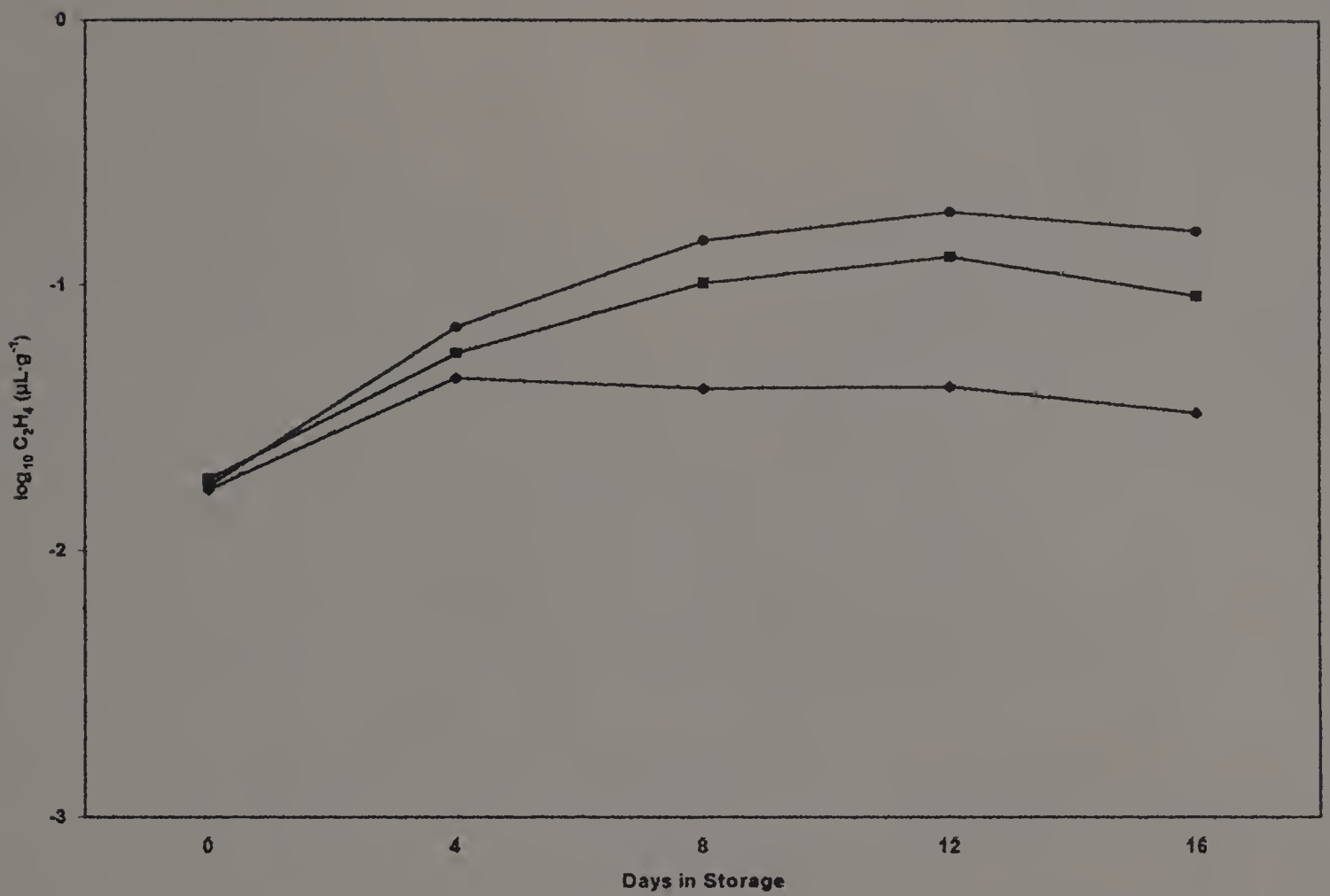
Appendix B.26. Analysis of Variance, ion leakage (Experiment 4)

Source	df	SS	MS	F
T	1	8.12	8.12	0.88^{ns} [z]
D	3	6261.66	2087.22	241.27^{**} [y]
R	4	19.84	4.96	
TD	1	9.83	9.83	1.27^{ns}
TR	4	37.01	9.25	
DR	12	103.81	8.66	
TDR	12	31.01	7.75	

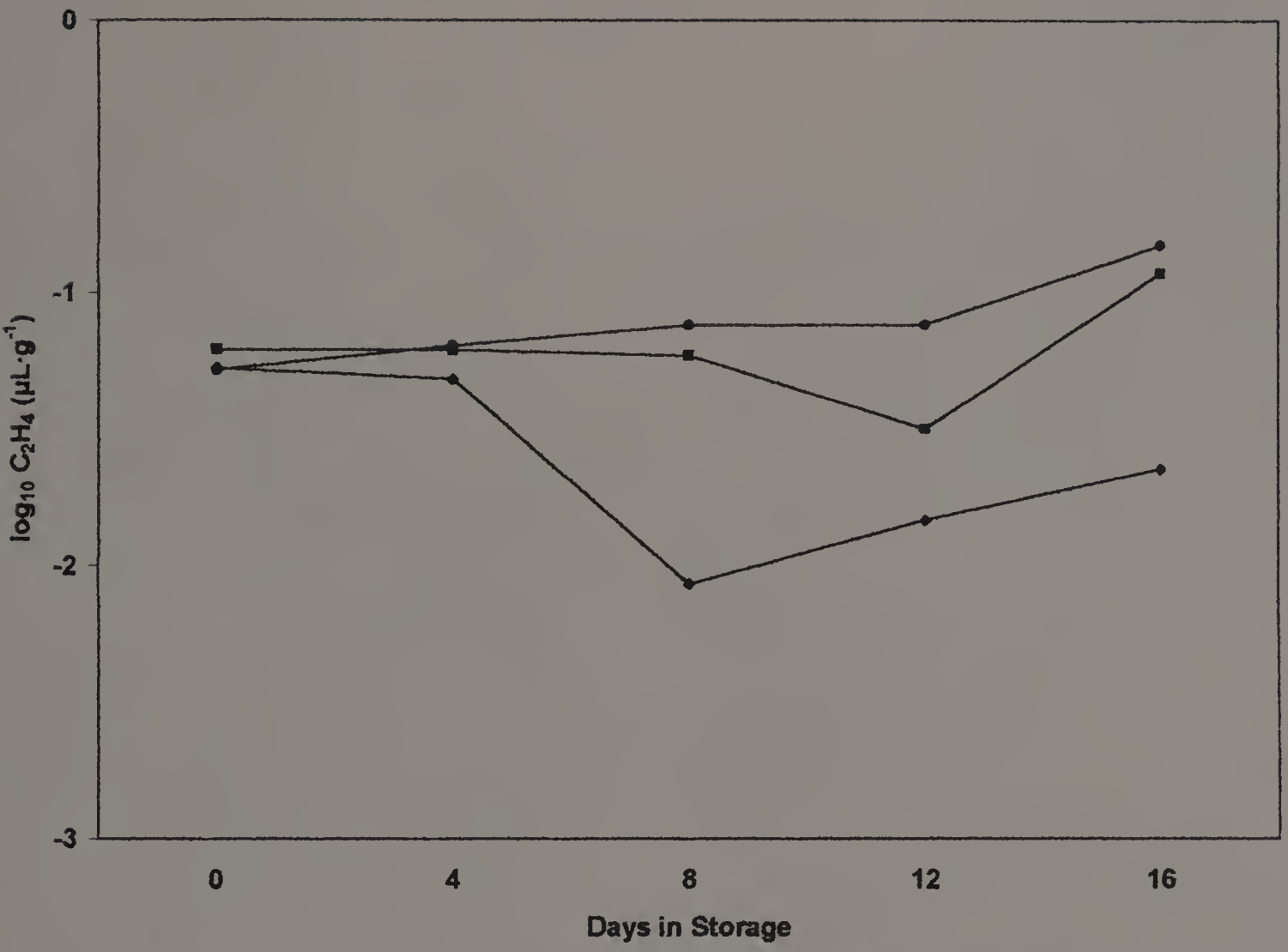
$n_w = 1$ N = 90

[z]non-significant
[y]significant at $P = 0.01$

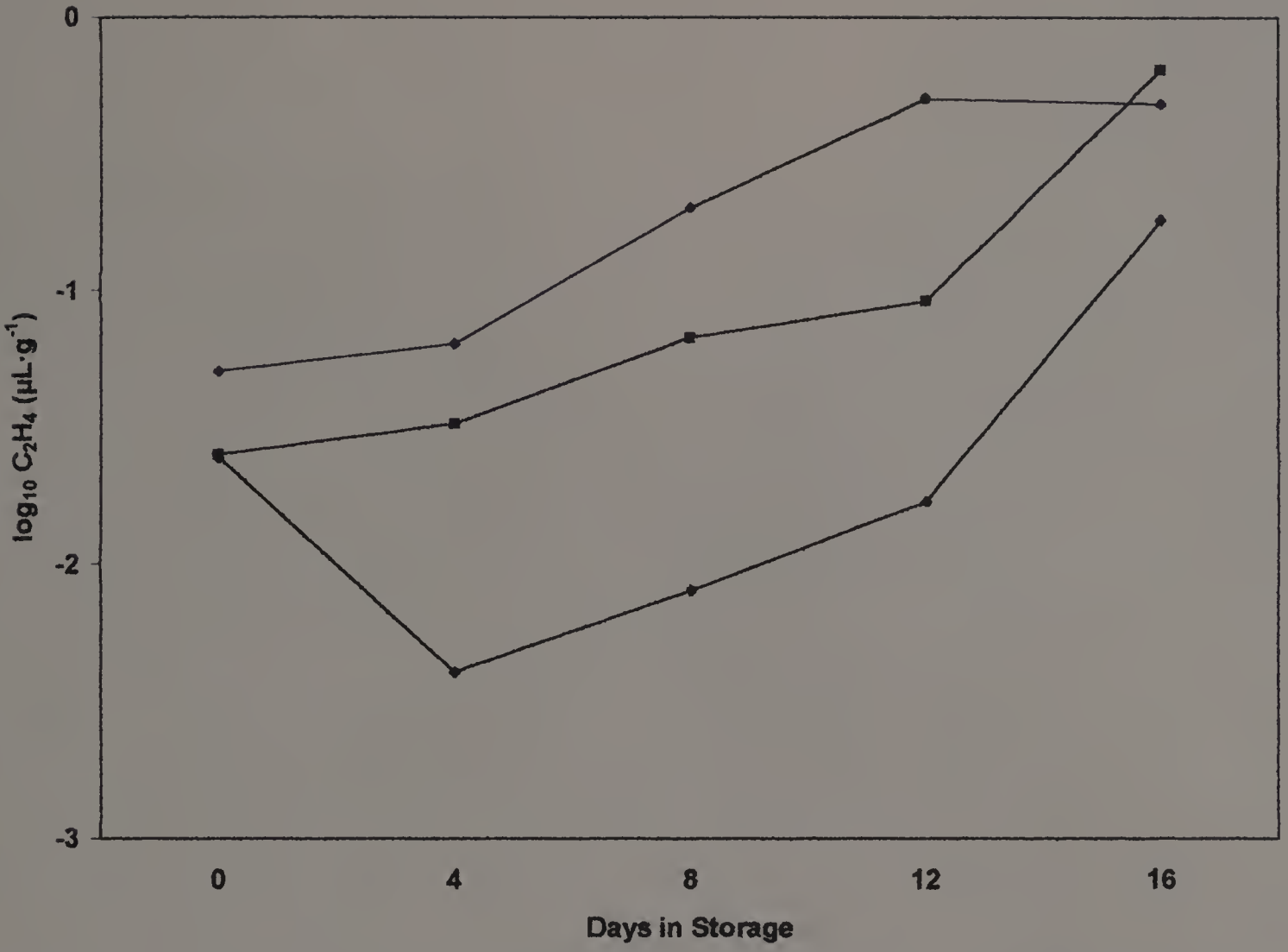
APPENDIX C - INTERNAL ETHYLENE DATA



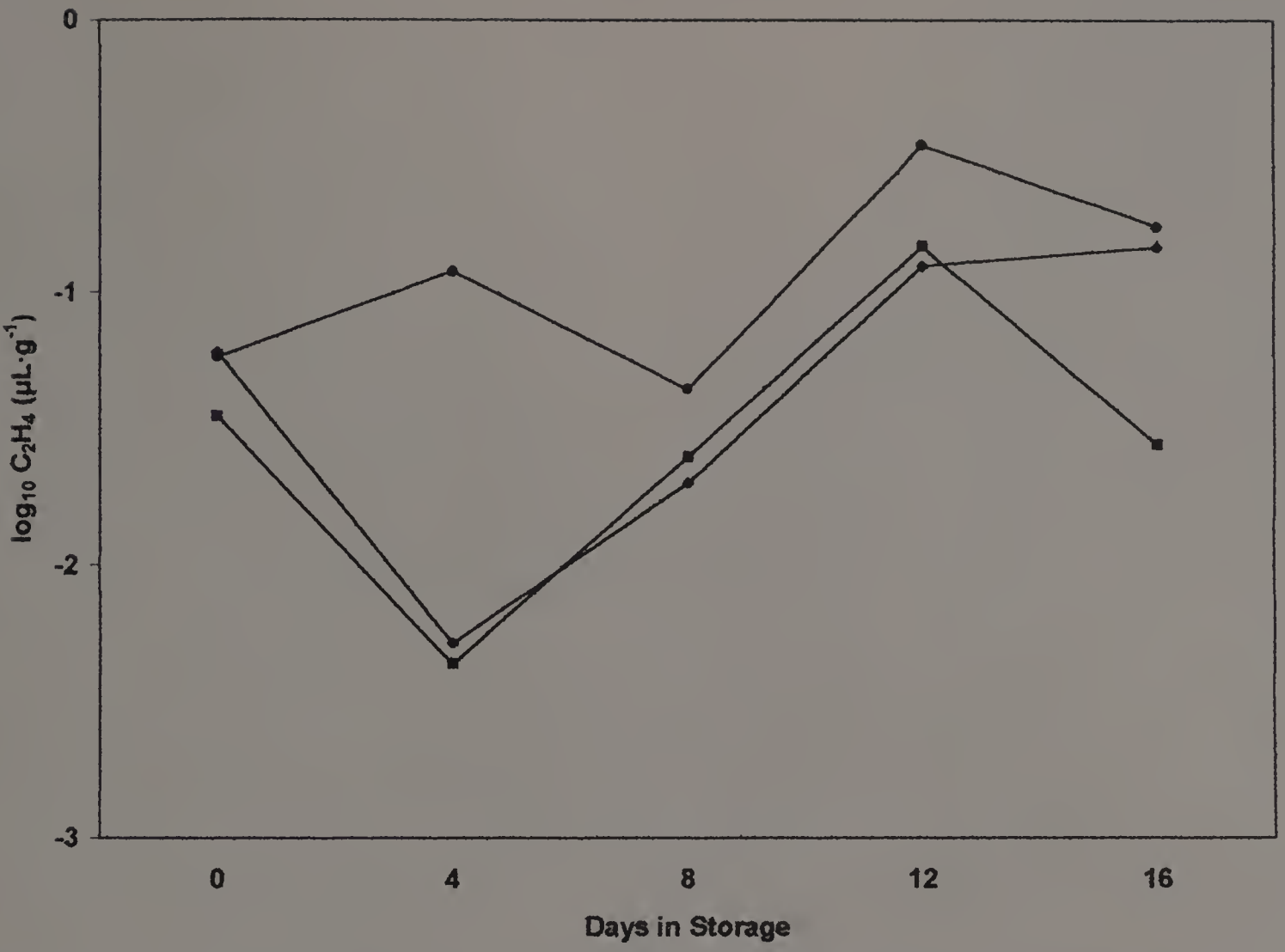
Appendix C.1. Internal ethylene concentrations of Cherry 'Bomb' peppers 24 hours after removal from storage. ●, 2.5°C; ■, 7°C; ◆, ~15°C. (Experiment 1).



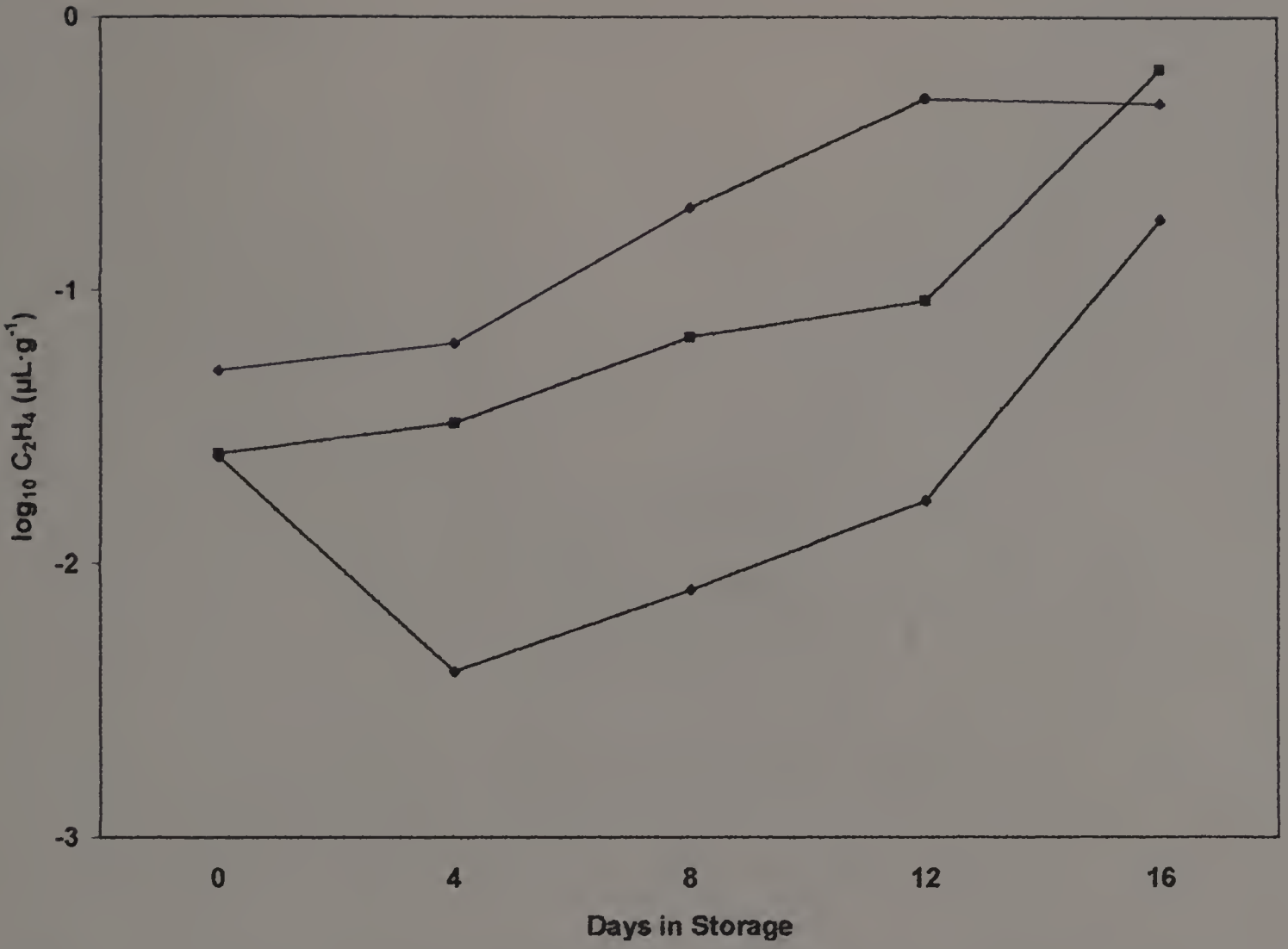
Appendix C.2. Internal ethylene concentrations of 'Cubanelle' peppers 24 hours after removal from storage.●, 2.5°C; ■, 7°C; ◆, ~15°C. (Experiment 1).



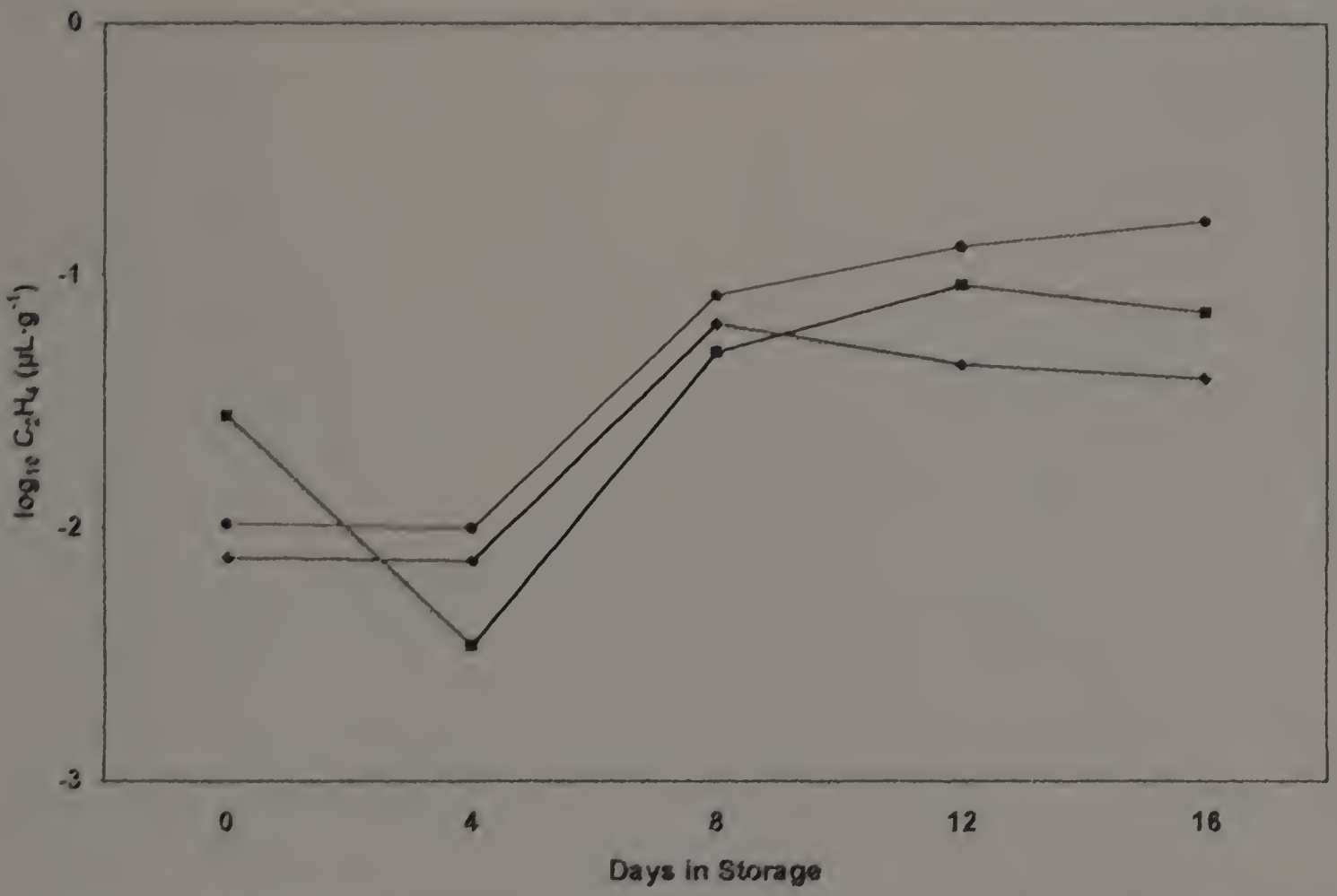
Appendix C.3. Internal ethylene concentrations of 'Hungarian Wax' peppers 24 hours after removal from storage.●, 2.5°C; ■, 7°C; ◆, ~15°C. (Experiment 1).



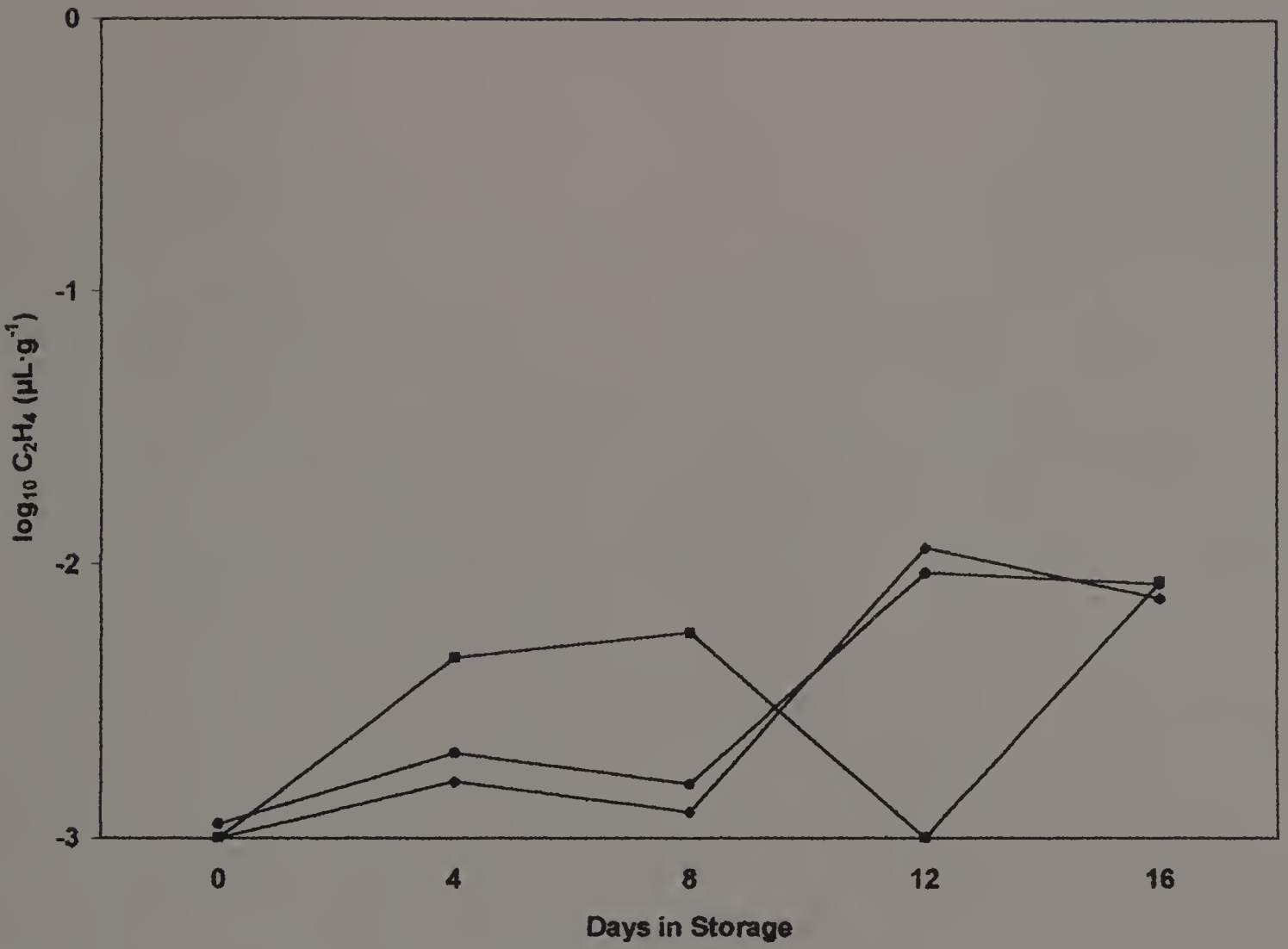
Appendix C.4. Internal ethylene concentrations of mature green 'Jalapeño' peppers 24 hours after removal from storage.●, 2.5°C; ■, 7°C; ◆, ~15°C. (Experiment 1).



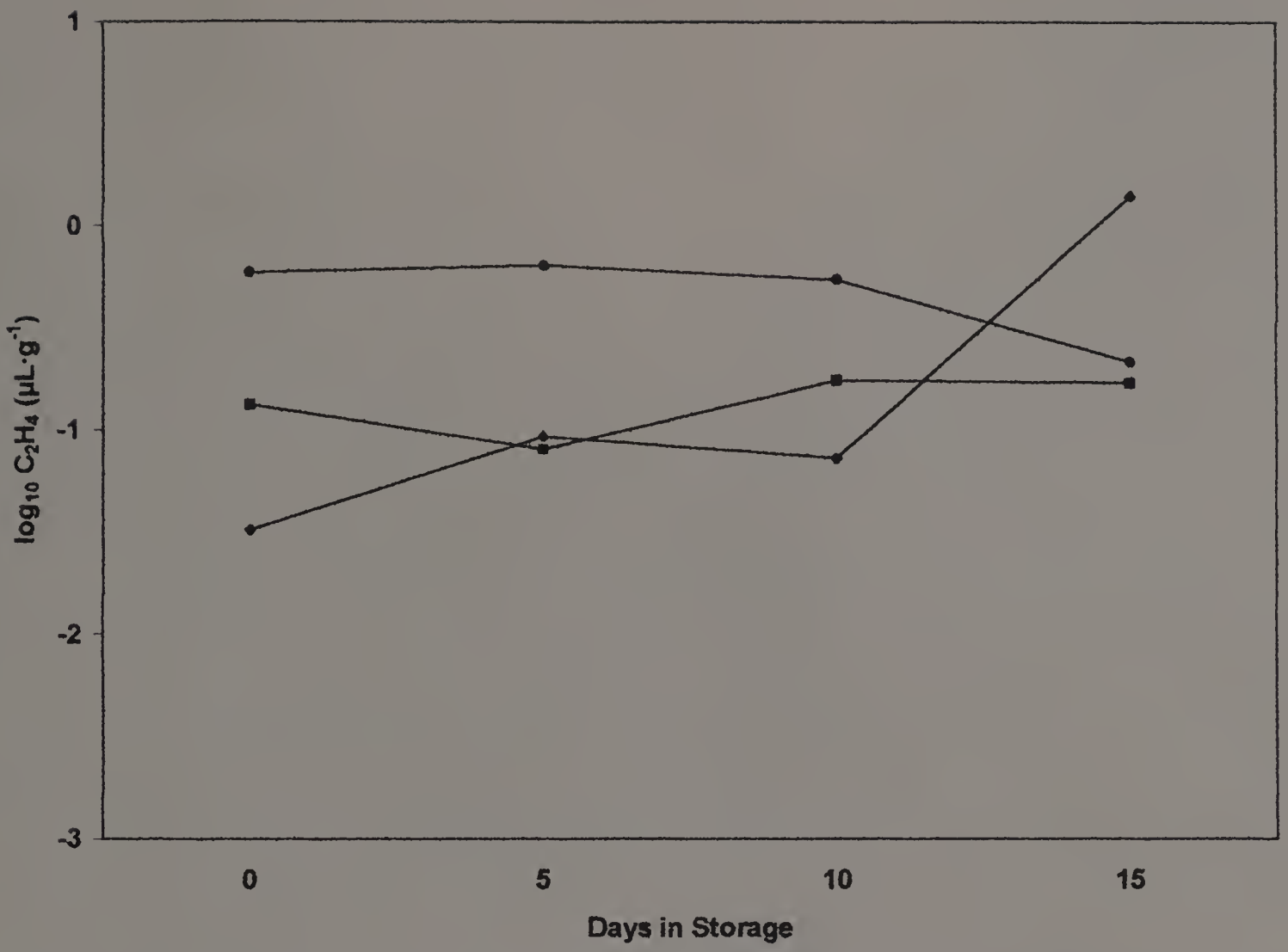
Appendix C.5. Internal ethylene concentrations of full color (red) 'Jalapeño' peppers 24 hours after removal from storage.●, 2.5°C; ■, 7°C; ◆, ~15°C. (Experiment 1).



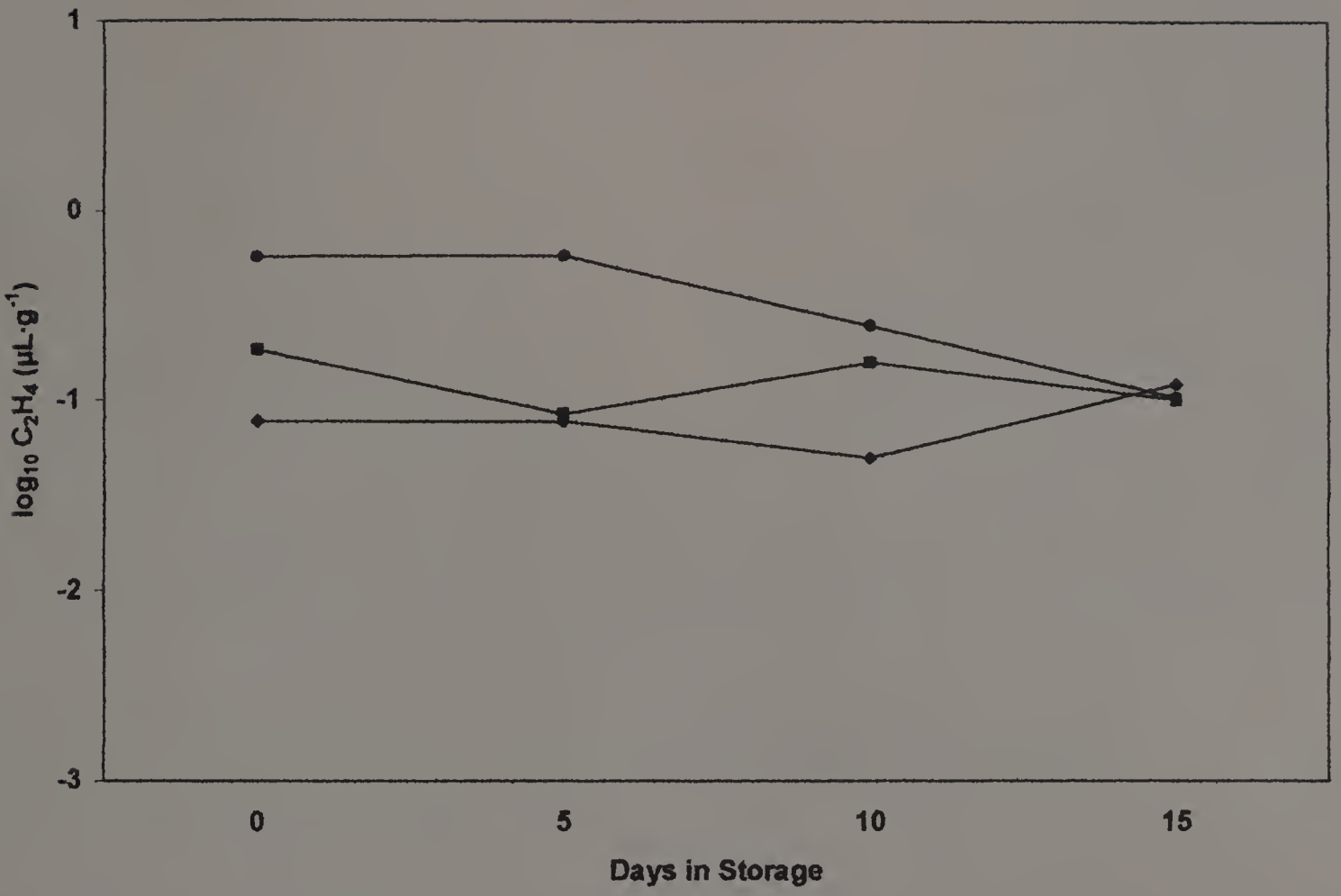
Appendix C.6. Internal ethylene concentrations of Poblano 'Ancho San Luis' peppers 24 hours after removal from storage. ●, 2.5°C; ■, 7°C; ◆, ~15°C. (Experiment 1).



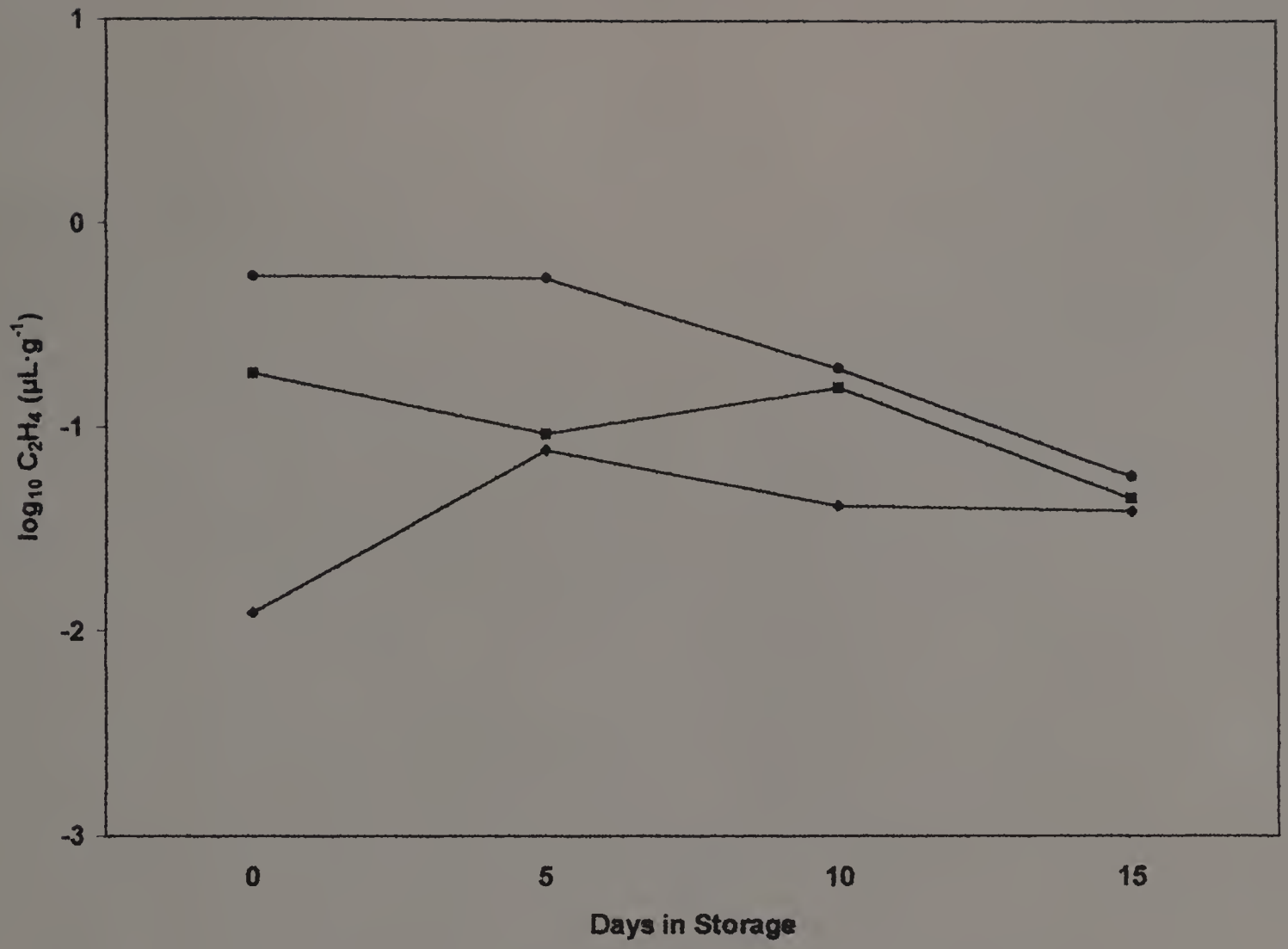
Appendix C.7. Internal ethylene concentrations of 'Serrano' peppers 24 hours after removal from storage. ●, 2.5°C; ■, 7°C; ◆, ~15°C. (Experiment 1).



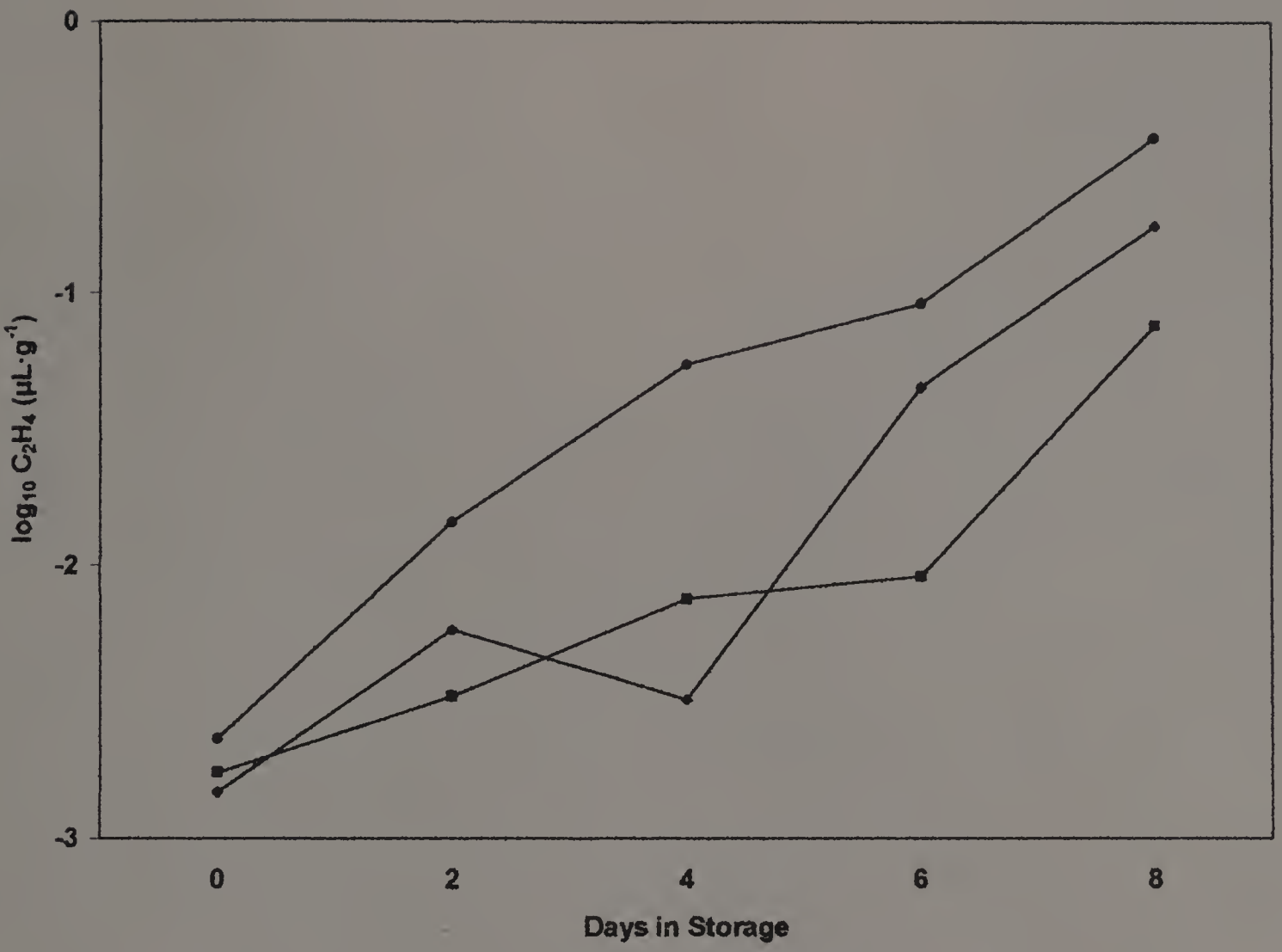
Appendix C.8. Internal ethylene concentrations of red Cherry 'Bomb' peppers after the first 12 hours following removal from storage at 2.5°C. ●, Harvest 1; ■, Harvest 2; ◆, Harvest 3. (Experiment 2).



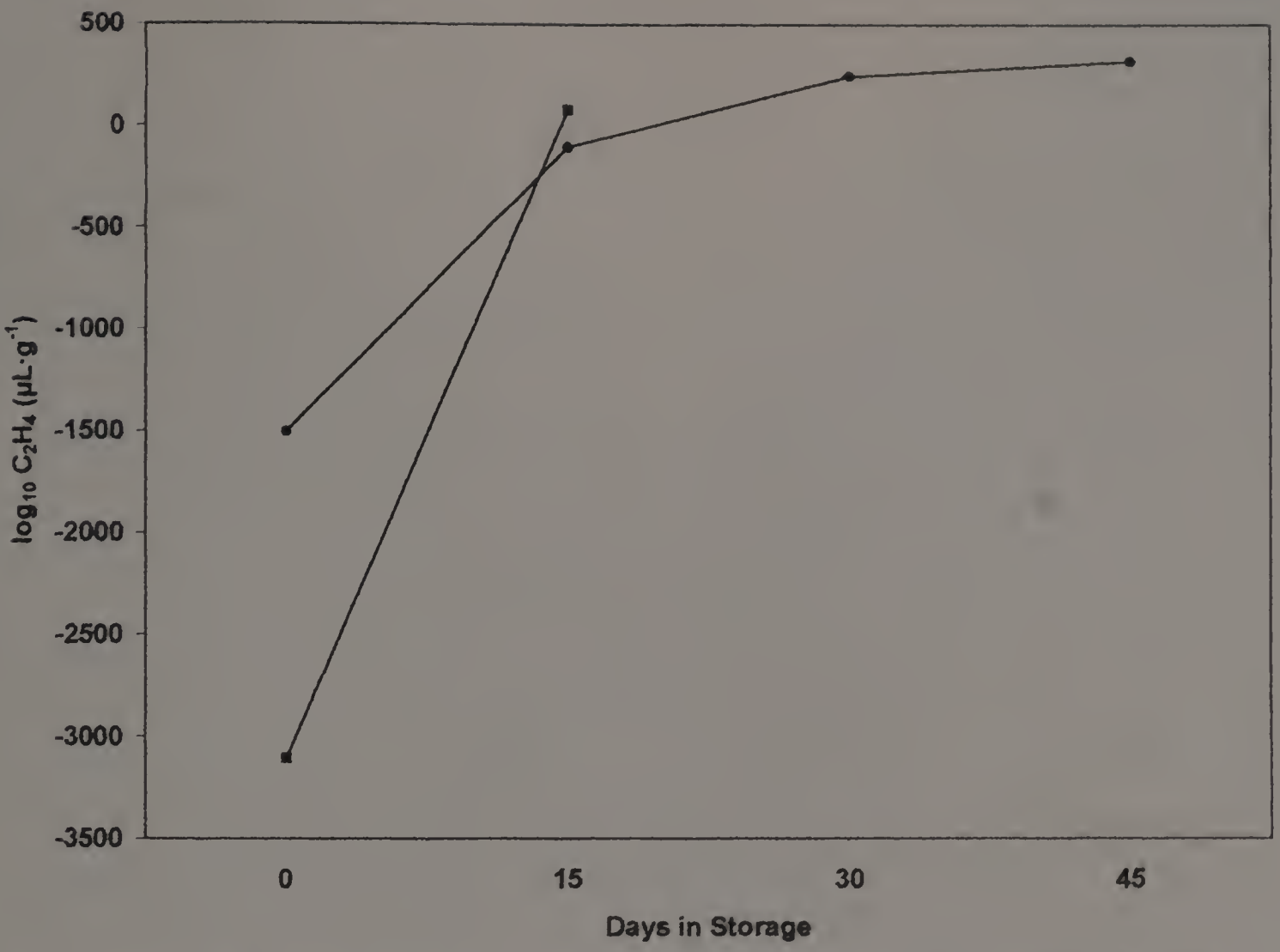
Appendix C.9. Internal ethylene concentrations of red Cherry 'Bomb' peppers after the first 12 hours following removal from storage at 7°C. ●, Harvest 1; ■, Harvest 2; ◆, Harvest 3. (Experiment 2).



Appendix C.10. Internal ethylene concentrations of red Cherry 'Bomb' peppers after the first 12 hours following removal from storage at ~15°C. ●, Harvest 1; ■, Harvest 2; ◆, Harvest 3. (Experiment 2).



Appendix C.11. Internal ethylene concentrations of banana peppers 24 hours after removal from storage. ●, 2.5°; ■, 7°C; ◆, ~15°C. (Experiment 3).



Appendix C.12. Internal ethylene concentrations of 'Serrano Chili' peppers 24 hours after removal from storage. ●, 0°C; ■, 2.5°C. (Experiment 4).

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