

EFFECTS OF HEAT STRESS ON MEMBRANE
STABILITY AND REPRODUCTION
OF BELL PEPPER

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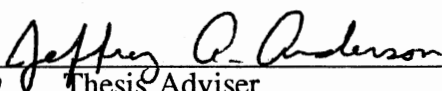
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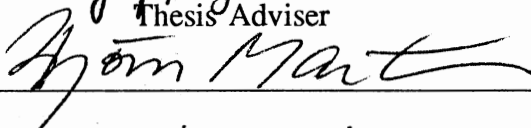
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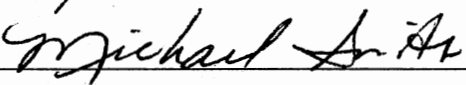
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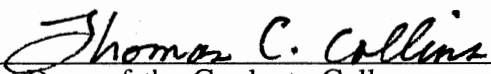
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CHAPTER I

INTRODUCTION

Land plants are injured by various environmental stresses resulting in reduced yield. Stresses include extremes of temperature (Reuther et al., 1979; Burke et al., 1976), water availability (Mahalakshmi et al., 1988) and gaseous pollutants (Tingey et al., 1988). Cellular level responses to stress include decline in enzymatic activity (Dann and Pell, 1989) protein denaturation (Bernstam, 1978), loss of membrane semipermeability (Levitt, 1980) and evolution of ethylene and ethane gases (Tingey, 1980).

Among the earliest symptoms of high temperature stress and cessation of cytoplasmic streaming are inactivation of photosynthesis (Alexandrov, 1964; Bjorkman, 1975). Loss of membrane semipermeability is one of the direct consequences of high temperature stress (Levitt, 1980), water stress (Becwar et al., 1986; Kobayashi, 1981) and freeze injury (Harber and Fuchigami, 1981). Electrolyte leakage occurs as a result of altered membrane permeability (Inaba and Crandall, 1988) and is proportional to the degree of injury (Martineau et al., 1979).

The production of volatile hydrocarbons such as ethane and ethylene by plant tissues occurs under a range of environmental stress conditions (Bressan et al., 1979; Peiser and Yang, 1979) or as a result of mechanical wounding (Konze and Kwiakowski, 1981). Ethane production was directly proportional to lesions formed

from sulfur dioxide exposure (Kimmerer and Kozlowski, 1982) and electrolyte leakage in water-stressed plum leaves (Kobayashi et al, 1981). Hence, ethane formation can be an index of injury in plant tissues.

Plant membranes are bilayers of lipids consisting of phospholipids, galactolipids and sterols (Singer and Nicholson, 1972). Linolenic acid, a major component of plant membranes (Galliard, 1978; Rochester et al., 1987), was reported to be the precursor of ethane (Lieberman and Mapson, 1962). Since fatty acids do not undergo peroxidation in situ, deesterification by phospholipase is required for oxidation. Lesham (1987) suggested that calcium may play a role in membrane deterioration through the following "phosphatidyl-linoleyl(-enyl) cascade". Influx of calcium into the cytoplasm increases activity of phospholipase via a calcium- calmodulin mechanism, leading to the release of free fatty acids. Free fatty acids with more than one double bond are susceptible to oxidation by lipoxygenase, leading to the formation of lipid peroxides and oxygen free radicals (Kacperska and Zebalska, 1989; Bilinski et al., 1989). Fatty acid peroxides increase membrane permeability which facilitates the entry of additional calcium and amplifies the cascade. Lipid peroxides are decomposed by cleavage enzymes to form volatile hydrocarbons including ethane and hexanal (Dumelin and Tappal, 1977; Galliard, 1978). The byproducts of membrane lipid peroxidation can result in disruption of membrane semipermeability, loss of metabolic functions and death of cells (Kimmerer and Kozlowski, 1982). Free radicals also mediate membrane disruption in plants under stress (Senaratna et al., 1987; Kendall and McKersie, 1989) through their involvement in lipid peroxidation (Mead, 1976) leading to the formation of hydrocarbons (Kacperska and Zebalska, 1989).

Intact membrane systems have free radical scavenging mechanisms involving antioxidants such as alpha-tocopherol (vitamin-E) (Riely et al., 1974) and enzymes, including superoxide dismutase (Bilinski et al., 1989). Studies using the free radical scavenger, propylgallate, showed that formation of ethylene could be inhibited in avocado and apple fruit discs (Apelbaum et al., 1980; Baker et al., 1978).

Extremes of environmental conditions can have a deteriorating effect at the whole plant level, resulting in reduced growth and reproduction. Temperature is one of the most important environmental factors influencing plant growth, development and yield (Bakker and Uffelen, 1988). Flowering and fruit set is affected by temperature, accounting in part for variation in fruit yield (Rylski, 1972 and 1973; Rylski and Spigelman, 1982). High temperature results in flower abortion and fruit drop (Rylski, 1973).

High temperature greatly influences the yield of bell pepper, primarily through reduced flowering and fruit set (Bakker, 1989). Supra-optimal temperatures are thought to affect reproduction through changes in assimilation demand (Walker and Ho, 1977) and endogenous hormone levels (El-Abd et al., 1986). Studies on physiological mechanisms responsible for high temperature inhibition of flowering and fruiting of tomatoes have shown that reduced levels of growth regulators are involved (Satti and Oebker, 1986). High temperature reduced proline content of anthers and pistils in tomato, resulting in lower pollen germination (Kuo et al., 1986). High temperature also stimulates ethylene production, which enhances boll drop in cotton (Guinn and Brummett, 1988).

Flowering and fruit set are phenomena that are influenced by hormonal factors. The involvement of cytokinins in the onset of flowering and inflorescence development has been demonstrated in many species (Ginzburg, 1974; Goh, 1977). Studies involving the application of growth retardants such as chloromequat chloride have reported improved fruit set (Nourai and Harris, 1983). Fruitone (3-chloro-phenoxy-propionic acid) has been used commercially to thin peaches (Buchanan et al., 1975).

Cytokinin application increases flower number and improves fruit set of certain plants. Cytokinins may have an indirect effect on photoperiodic induction by altering assimilate partitioning, hence floral stimulus translocation to the shoot apex (Ogawa and King, 1979). Application of cytokinin to flower clusters increased flower number (Skene and Antcliff, 1972) and promoted fruit set in grapes (Weaver et al., 1965). Treatment of tomato inflorescence with benzyl adenine (BA) increased soluble sugar content and led to continuous mobilization of sugar at fruit set and also increased the number of flowers and fruits (Satti and Oebker, 1986).

Chloromequat chloride (CCC) influences plant development by reducing growth of vegetative parts of the shoot system, leading to the diversion of metabolites and endogenous growth substances to the inflorescence. Application of CCC shortened veins, resulted in greater branching and increased leaf number in tomato (Mahmoud et al., 1986). Chloromequat chloride applied as a soil drench was effective in reducing flower bud abortion in greenhouse-grown tomatoes (Abdalla and Verkerk, 1970). Chloromequat chloride increased the dry weight of the first-formed inflorescence and reduced the dry weight of the remainder of the shoot system (Nourai and Harris,

1983). Chloromequat chloride retarded chlorophyll breakdown in leaf disks by stimulating protein synthesis (El-Antably, 1967) and increased concentrations of many amino acids (El-Fouly et al., 1970). Chloromequat chloride also increased carbohydrate content (Radwan et al., 1971). At high temperatures, tomato plants treated with CCC had reduced leaf yellowing and shedding until harvest. Also, CCC-treated plants had more tissue nitrogen due to a larger and more active root system (Abdalla and Verkerk, 1970).

Increased flower and fruit abortion in pepper is mediated by elevated ethylene levels in the plant tissues (Wein et al., 1989). Application of aminoethoxyvinylglycine (AVG), an inhibitor of ethylene biosynthesis, to apple flower clusters increased fruit set. Greene (1980) found that ethylene production also decreased from flowers treated with AVG .

High temperatures increase ethylene production and reduce auxin levels in the abscising organ by inhibiting polar auxin transport, and act directly at the pedicel base to separate the cells in the abscission zone (Sagee et al., 1990). Treatment of flowers with auxin improved fruit set of tomato (Mann and Minges, 1949) and pepper (Silveria et al., 1986) in cool weather. Auxin sprays were less effective in tomato and bell pepper at higher temperatures (Osborne and Went, 1953). Application of synthetic auxins, naphthalene acetic acid (NAA) and chlorophenoxy acetic acid (CPA), increased ethylene production and flower abscission (Wein and Zhang, 1991). Chlorophenoxy-propionamide (3-CPA) was used as an thinning agent in peach (Buchanan et al., 1975).

Objectives

1. To determine whether ethylene and ethane evolution is an accurate indicator of tissue response to temperature stress.
2. To determine how ethane production relates to membrane injury from high temperature stress.
3. To determine the effect of growth regulators on reproduction of bell pepper.

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CHAPTER II

HYDROCARBON EVOLUTION FROM TEMPERATURE STRESSED LEAVES

Introduction

Plant tissues are injured by a variety of stresses including chilling (Markhart, 1986), freezing (Burke et al., 1976), gaseous pollutants (Tingey et al., 1988) and high temperature (Reuther et al., 1979). Ethylene and ethane evolution has been used as an indicator of tissue injury (Tingey, 1980; Konze and Elstner, 1978). Studies in which plant tissues were exposed to subfreezing temperatures (Harber and Fuchigami, 1986), ozone (Tingey, 1980), sulfur dioxide or bisulfite (Bressan et al., 1979; Peiser and Young, 1979) and water deficits (Kobayashi et al., 1981) indicated that ethylene evolution accompanied stressed, yet viable tissues, and ethane was evolved from killed tissues.

Ethylene biosynthesis during the normal life cycle of plant growth and development occurs through the methionine pathway [methionine \rightarrow S-adenosyl-methionine (SAM) \rightarrow aminocyclopropane-1-carboxylic acid (ACC) \rightarrow ethylene] (Adams and Yang, 1980). Conversion of ACC to ethylene is mediated by ACC synthase (Boller et al., 1979), the rate controlling enzyme in the pathway of ethylene biosynthesis (Wang and Adams, 1982). Wounding induced the production of ACC

synthase (Yu and Yang, 1980). Based on in vitro studies, it was reported that free radicals were involved in the conversion of ACC to ethylene (Gardner and Newton, 1987). The ethylene-forming enzyme system is apparently membrane bound (Yang and Hoffman, 1984). Ethane evolution occurs from decompartmentalized or killed tissues (Elster and Konze, 1976). Ethane is produced from the decomposition of fatty acid peroxides (Riely et al., 1974; Dumelin and Tappal, 1977) in a reaction series involving free radicals (Peiser et al., 1982). Addition of linolenic acid stimulated ethane production from potato slices (John and Curtis, 1977; Konze and Elstner, 1978), suggesting that linolenic acid was the precursor of ethane. Increased electrolyte leakage was observed from tissues producing ethane, indicating that the plasma membrane had lost its selective permeability characteristics and the tissue was killed (Wallner et al., 1982).

The substrate specificity for hydrocarbon evolution in diatoms was reported to be 18- and 20-carbon polyunsaturated fatty acids (Bilinski et al., 1989). The ratio of saturated to unsaturated fatty acids changes during the growth and development of plants (Lester, 1990). Hence, tissue age could affect the amounts and types of hydrocarbons produced. Lipoxygenase, the enzyme that oxidizes linoleic and linolenic acids, has differential activity in various crop species (Siedow, 1991) and may also affect hydrocarbon evolution.

The amounts of hydrocarbons produced are proportional to the stress intensity (Field, 1981). Hyodo (1977) observed that the amount of ethylene produced from mandarin albedo tissue increased with greater incubation duration. Electrolyte leakage increased with increasing incubation temperatures in ryegrass (White et al., 1988).

Incubation duration and temperature may affect the amounts of hydrocarbons produced from pepper leaf disks. The objectives of this study were 1) to determine the effects of incubation duration and temperature on ethylene and ethane evolution from bell pepper leaf disks, 2) to determine the effect of tissue age on hydrocarbon evolution, 3) to compare the responses of broccoli, cucumber, squash and pepper and 4) to compare heat with freeze stress responses.

Materials and Methods

'Early Calwonder' pepper seeds (Capsicum annuum L.) were planted in 3-liter pots using commercial potting mix (Fafard GP mix, Springfield, Mass.) and were grown in a growth chamber at 23/20C day/night cycles. Photosynthetic photon flux density of about 500 $\mu\text{mol}/\text{sec}/\text{m}^2$ was supplied by fluorescent and incandescent bulbs. Relative humidity ranged from 45 to 65%. Plants were fertilized at each watering with 0.75 g/liter 20N-8.7P-16.6K soluble fertilizer (Peters Professional, W.R. Grace & Co., Fogelsville, Pa.). Watering was based on soil color and pot weight.

Leaf disks were excised with a cork borer and rinsed with at least 3 changes of distilled water to remove the exogenous electrolytes. Ten leaf disks were placed in each test tube containing 2 ml of distilled water to prevent secondary water stress. Temperature treatments were applied for 15 or 30 min in a circulating bath. Tissue temperature was measured with thermocouples threaded through an extra disk at each exposure temperature.

Ten leaf disks were placed in a test tube with 20 ml of distilled water for electrolyte leakage determination following stress treatments. Initial electrolyte leakage was measured with a conductance meter (Model 35, Yellow Springs

Instrument Co., Yellow Springs, OH) after 24 h at 24C on an orbital shaker. Then all samples were autoclaved and the final leakage was measured after an additional 24 hs at 24C. Data was expressed as initial/final leakage values.

Ten leaf disks were blotted and placed in a 14.5 ml serum bottle for ethylene and ethane measurements. Disks were incubated for 24 hs at 24C unless stated otherwise. One ml of headspace gas was removed with a syringe and injected into a gas chromatograph (model 540, Tracor Instruments Inc., Austin, TX) equipped with a flame ionization detector.

Data were analyzed using the general linear models procedure (GLM) in PC SAS and Duncan's multiple range test was used to separate means when appropriate. The midpoint temperature (Tmid) of sigmoidal response curves was calculated using the equation described by Ingram (1985).

Time course of gas evolution. Test tubes containing leaf disk samples were subjected to heat treatments of 21 (control), 44, 48 or 50C for 30 min. Gas samples were taken after 2, 4, 6, 22.5 and 46.5 h incubation. Ethane and ethylene evolution was measured from two subsamples on each of three dates (replications) using gas chromatography (GC). The experiment was a split plot design with treatment temperature as whole plot and time as subplot.

Incubation temperature. Leaf disks were pretreated at 21 (control), 46, 50 or 54C for 15 min. Samples were then incubated for 24 h at 21, 24, 27, 30 or 33C following heat stress exposure. Ethylene and ethane evolution and electrolyte leakage were measured after 24 h at 24C. Ethane, ethylene and electrolyte leakage was measured from two subsamples on each of three dates (replications) using gas

chromatography (GC). The experiment was a split plot design with pretreatment temperature as whole plot, and incubation temperature as subplot.

Tissue age. Leaves were tagged and dated as they emerged from the bud. Disks were sampled from 30, 60, 90 and 120 day-old leaves from the same plant. Parallel electrolyte leakage and gas evolution determinations were conducted. Ethane, ethylene and electrolyte leakage was measured from three replications. The experiment was a split plot design with treatment temperature as whole plot and age as subplot.

Crop species. Seeds of tomato (Lycopersicon esculentum Mill.), bell pepper (Capsicum annuum L.), cucumber (Cucumis sativus L.), squash (Cucurbita pepo L.) and broccoli (Brassica rapa L.) were sown such that all had a uniform emergence date. Leaf disks were taken from a 20-day-old leaf on a 45-day-old plant (days from emergence). Samples were subjected to heat treatments, and gas evolution and electrolyte leakage measurements were taken after 24 h at 24C. The experiment was a split plot design with treatment temperature as whole plot and crop as subplot. Ethane, ethylene and electrolyte leakage was measured from three replications.

Heat and freezing stress. Pepper leaf disks were exposed to temperatures from 0 to -8C for 30 min and another set was subjected to heat treatments from 40 to 55C with 21C as a control. Freezing stress samples were nucleated with ice chips at temperatures colder than -4C to avoid random supercooling. Thus disks exposed to temperatures from 0 to -5C and colder had frozen. Ethylene and ethane evolution and electrolyte leakage were measured after 24 h at 24C. The experiment was a completely randomized design with three replications. Tmid values were calculated

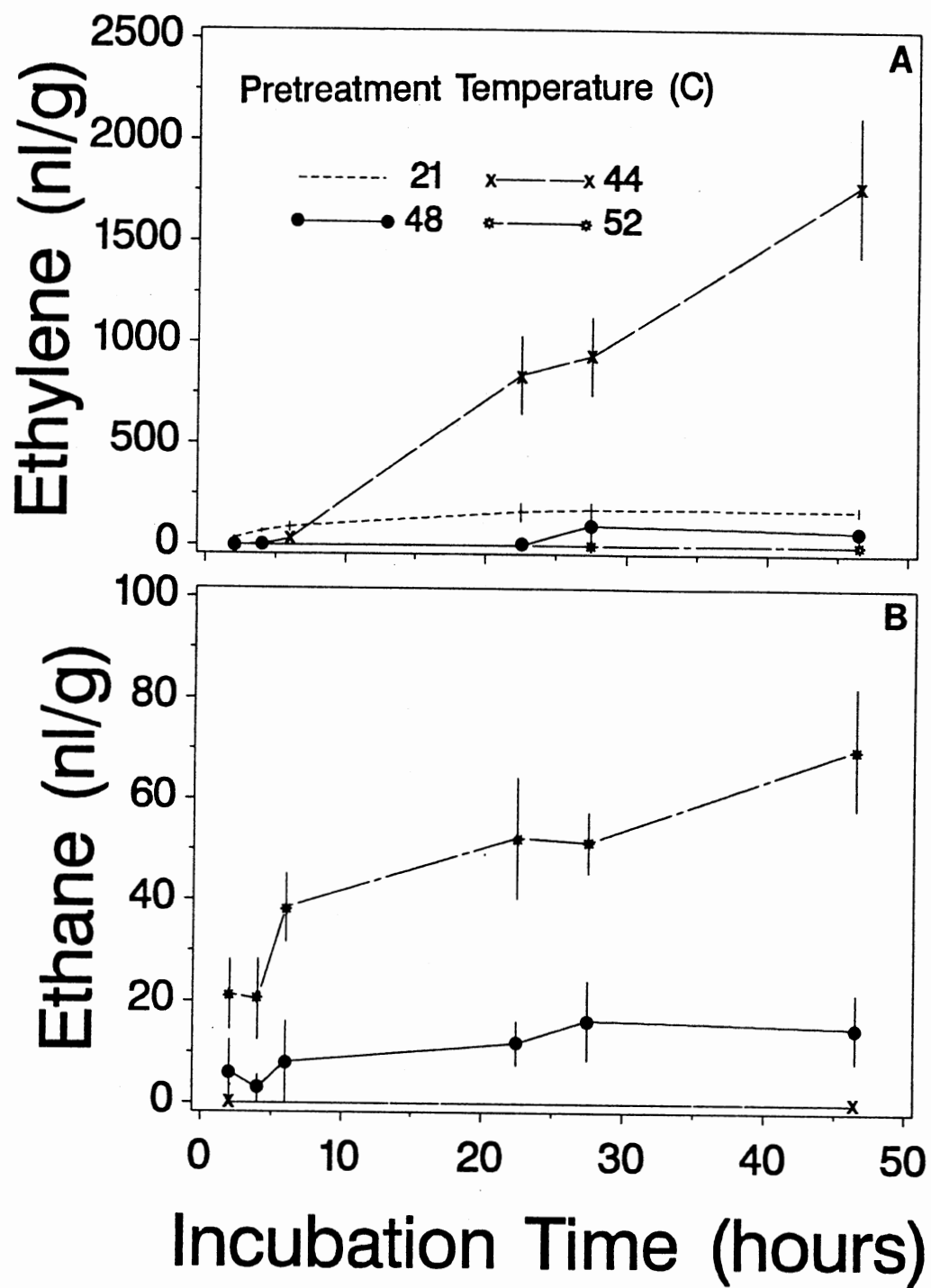
from Ingram's equation. Ethane:ethylene ratio was calculated from transformed values.

Results and Discussion

Time course of gas evolution. The time course for evolution of ethylene and ethane from pepper leaf disks was determined for tissues exposed to 21, 44, 48 or 52C for 30 min. Low levels of ethylene were produced from tissues treated at 21C, highest levels from 44C and undetectable amounts from 52C (Fig. 1A). Exposure to 48C yielded results intermediate to 21C and 52C treatments. The rate of ethylene production from disks exposed to 21C (control) was greatest for the first 4 h at $19 \text{ nl} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ then, declined to $3 \text{ nl} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ from 4 to 46.5 h. Tissues exposed to 44C exhibited a lag period of about 4 h followed by an evolution rate of $38 \text{ nl} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ from 4 to 46.5 h. A lag period was also observed in pea seedlings exposed to 36C (Saltveit and Dilley, 1978).

High levels of ethylene from disks pretreated at 44C, relative to 21C, were an indication of the production of stress ethylene (Field, 1980). The lack of ethylene production from tissues pretreated at temperatures above 44C indicated extensive tissue damage (Field, 1981), and reflected the requirement for intact membrane systems for ethylene production (Odawara et al., 1972). The highest rate of ethylene production from tissues pretreated at 21C was during the first 4 h, suggesting that wound ethylene may have been produced due to excision (Saltveit and Dilley, 1978). Control tissues returned to a basal rate 4 h after treatment.

Figure 1. Time course for accumulation of ethylene (A) and ethane (B) from heat stressed pepper leaf disks. Disks were pretreated at 21 (control) 44, 48 or 52C for 30 min. Values are means \pm SE of six measurements (two duplicate subsamples on each of three dates).



Patterns of ethane evolution contrasted ethylene production. Ethane was not detected from tissues pretreated at 21 or 44C (Fig. 1B). Tissues pretreated at 52C had the highest rate of ethane production at $7 \text{ nl}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ during the first 6 h, which then declined to a rate of $1 \text{ nl}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ for the remainder of the experiment. Similarly, tissues pretreated at 48C emitted ethane at $4.5 \text{ nl}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ during the first 6 h, then at $0.3 \text{ nl}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ for the following 42 h.

Studies of hydrocarbon evolution from plant tissues have employed incubation periods from 1 to 24 h (Bressan et al., 1979; Kobayashi et al., 1981). Hyodo (1977) incubated mandarin albedo tissues at 26C and observed greater amounts of ethylene with increasing incubation time. The appropriate incubation interval is a function of the type and amount of tissue, treatments and objectives of the study. Since the present study explores the use of ethylene and ethane production as an indicator of tissue response to heat stress, cumulative amounts appear to be satisfactory to distinguish between healthy, injured and killed tissue. Although 6 h was sufficient to distinguish between treatments based on ethane measurements, longer periods were required for ethylene determination under our experimental conditions. Since 24 h incubation was sufficient to accumulate wound ethylene following exposure to 44C, a 24 h duration was chosen for subsequent experiments. It is recognized that values are cumulative amounts over a period encompassing different rates of production.

Incubation temperature. The effects of incubation temperature on evolution of ethylene and ethane and loss of electrolytes were determined for pepper leaf disks pretreated at 21, 46, 50 or 54C for 15 min and then held at 21, 24, 27, 30 or 33C incubation temperatures. The rate of ethylene production from tissues pretreated at

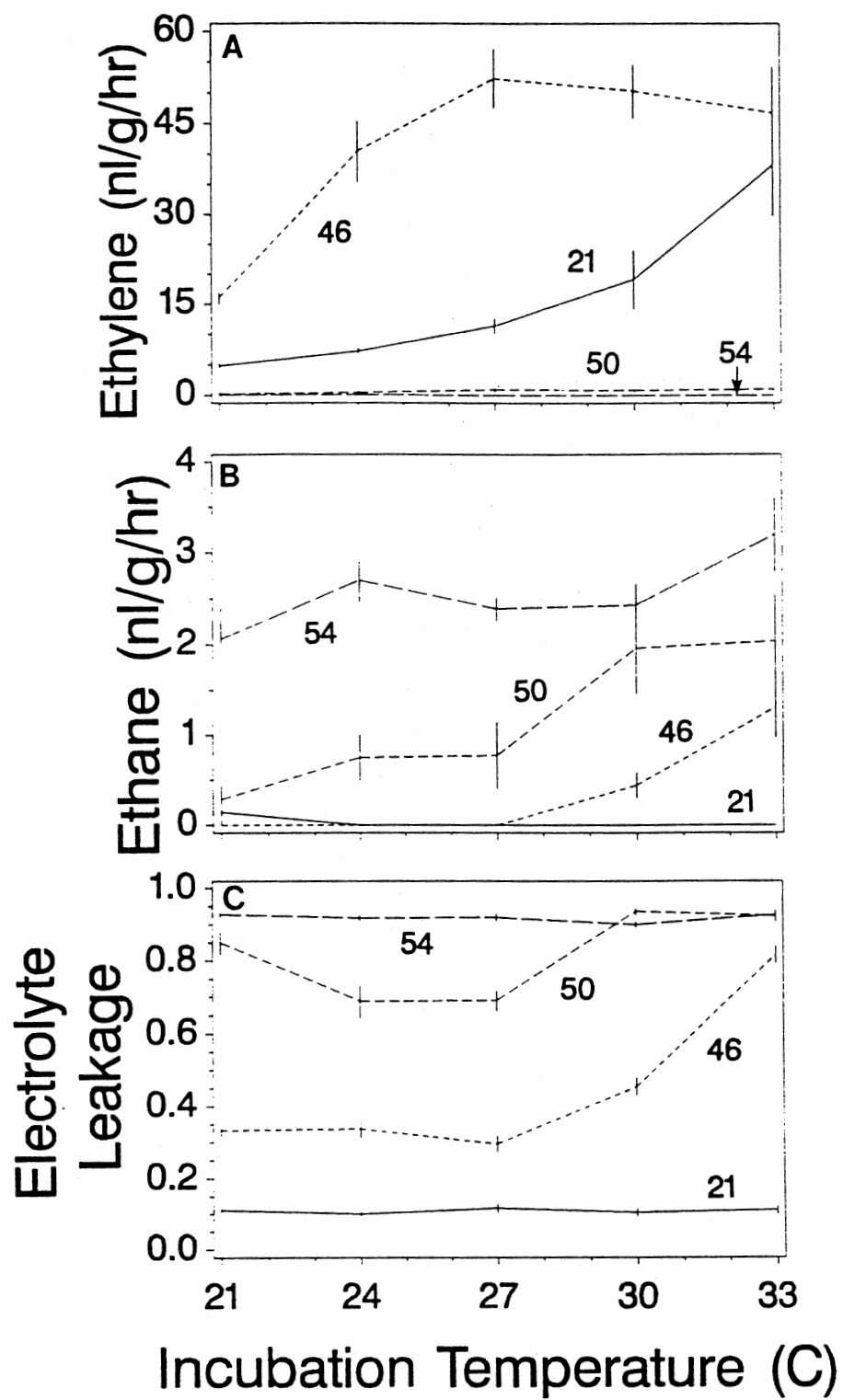
21C increased from 5 to 38 $\text{nl}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ as incubation temperature increased from 21 to 33C (Fig. 2A). Tissues pretreated at 46C produced the greatest amounts of ethylene at all incubation temperatures with mean rates greater than 40 $\text{nl}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ observed at temperatures greater than 21C. Ethylene was not detected following pretreatment at 50 or 54C.

Patterns of ethane production contrasted ethylene evolution. Tissues pretreated at 54C produced the greatest levels of ethane at all incubation temperatures (Fig. 2B). Ethane evolution increased with increasing incubation temperature following 50C exposure. Pretreatment at 46C yielded ethane only at incubation temperatures greater than 27C while 21C controls produced little or no ethane at any incubation temperature.

Electrolyte leakage values indicated that disks pretreated at 21C had no significant response to incubation temperature (Fig. 2C). Disks pretreated at 46C had increased leakage at incubation temperatures greater than 27C. Disks pretreated at 50 and 54C exhibited electrolyte leakage values greater than 0.6 at all incubation temperatures.

Electrolyte leakage data indicated that 50 and 54C pretreatments severely injured or killed the tissues based on previous work with plum leaves (Kobayashi et al., 1981). Similarly, the lack of ethylene production at these temperatures was an indication of membrane damage since intact membranes are required for ethylene production (Odawara et al., 1977). Production of ethane following 50 and 54C pretreatments also reflects severe damage since ethane is a breakdown product of membrane fatty acids (Konze and Elstner, 1978; Dumelin and Tappal, 1977).

Figure 2. Effect of incubation temperature on rate of ethylene (A) and ethane (B) production and electrolyte leakage (C) from pepper leaf disks. Disks were pretreated at 21 (control), 46, 50 or 54C for 15 min and then incubated at 21, 24, 27, 30 or 33C for 24 hs. Values are means \pm SE of six measurements (two duplicate subsamples on each of three dates).



The increased production of ethylene from tissues pretreated at 21C (control), as incubation temperature increased from 21 to 33C, indicated the stimulation of wound ethylene (Saltveit and Dilley, 1978). However, wounding was not severe enough to damage membrane integrity based on electrolyte leakage. Increased electrolyte leakage and ethane production from tissues pretreated at 46C and incubated at 30 or 33C indicated the compounding effect of elevated incubation temperature following pretreatment at an injurious, but sublethal temperature. Tissues pretreated at 54C were killed and not affected by subsequent incubation temperatures. Earlier work with ryegrass by White et al. (1988) also indicated that incubation temperatures had no significant effect on electrolyte leakage following a lethal 55C pretreatment. However, incubation temperature significantly affected efflux of cell solutes following sublethal 48 and 50C pretreatments. Studies with pea seedlings by Saltveit and Dilley (1978) indicated that higher incubation temperatures had a greater stimulation of ethylene. Subsequent studies will employ an incubation temperature of 24C to avoid compounding injury by higher incubation temperatures.

Tissue Age. The effects of tissue age on evolution of ethylene and ethane and loss of electrolytes was determined for disks from 30-, 60-, 90- and 120-day-old pepper leaves sampled from the same plant. Ethylene production from leaf disks exposed to high temperature stress was significantly affected by tissue age (Fig. 3A). Ethylene production increased markedly in all tissues, except 90-day-old disks, as exposure temperature increased from 21 to 42C (30-day-old disks) or 44C (60 and 120 day-old disks). Ninety-day-old disks exhibited a slight increase in ethylene production

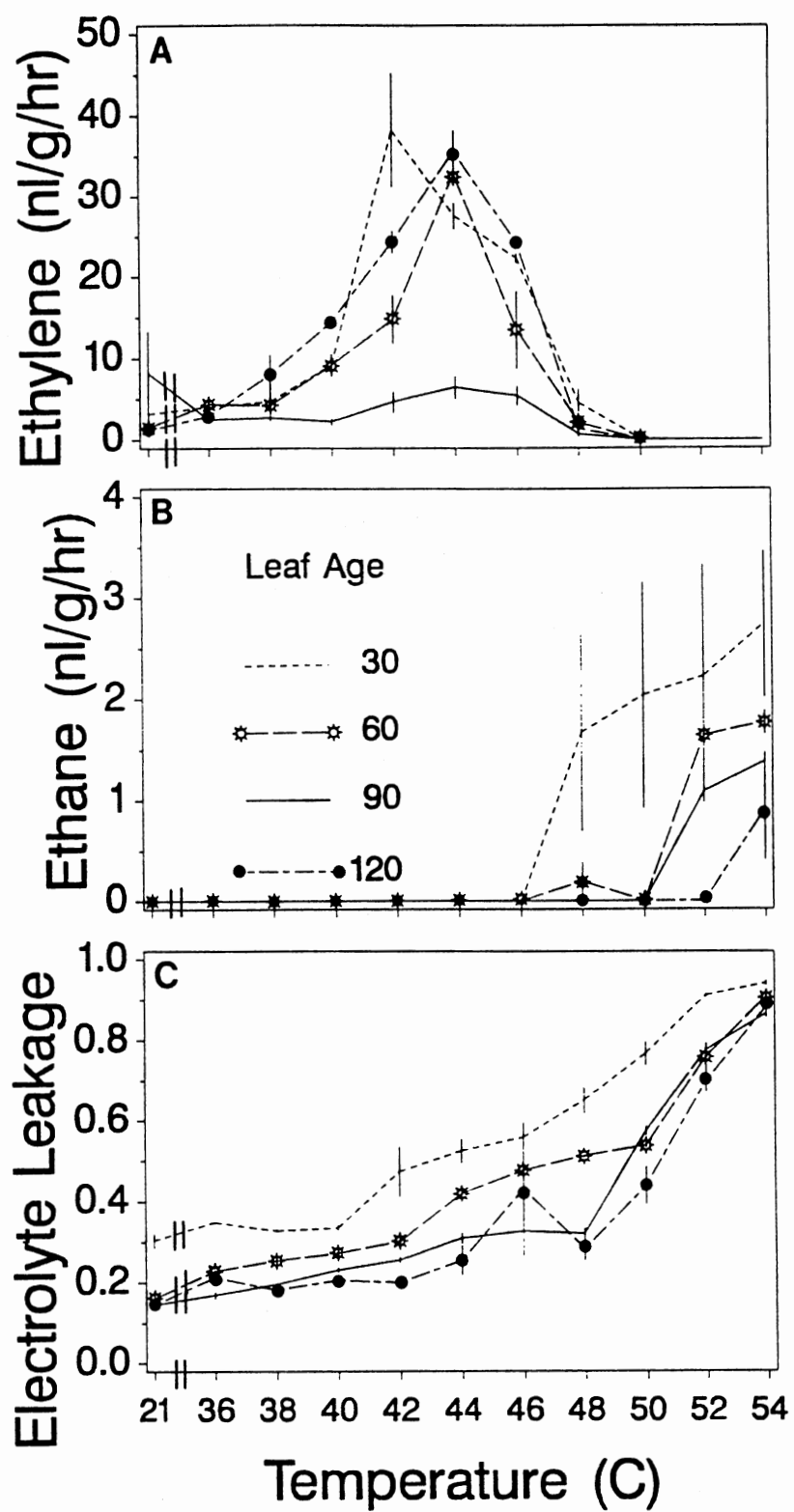
at 44C, but the rate was less than from tissues of other ages. No detectable ethylene was evolved from any disks at temperatures greater than 50C.

The temperature producing the greatest amount of ethylene was lower for 30-day-old leaf disks compared with older tissues. Since ethylene production is an indicator of stress, the peak temperature for ethylene production may have indicated damage to 30-day-old leaves at lower temperatures than older disks.

Ninety-day-old leaf disks had a smaller peak of ethylene at temperatures that produced a significant stimulation in 30-, 60- and 120-day-old tissues. Lester (1990) found that the ratio of saturated to unsaturated fatty acids increased as tissue age increased in muskmelon. Unsaturated fatty acids with more than one double bond undergo peroxidation which is an important biochemical process in ethylene production (Bilinski et al., 1988). Hence, the lower amount of ethylene produced from 90-day-old leaf disks may reflect limited substrate availability. One hundred twenty-day-old leaf tissues may have begun senescence resulting in an increase in phospholipase and lipoxygenase activity (Senaratna et al., 1987; Dhindsa et al., 1981).

Ethane production was greatest from thirty-day-old disks with measurable amounts detected following exposure at 48C and increasing to $2.7 \text{ nl}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ at 54C (Fig. 3B). Heat treatments at 54C yielded 1.8, 1.3, and $0.9 \text{ nl}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ from 60-, 90- and 120-day-old disks, respectively. Ethane production tended to decrease with age at 54C. John and Curtis (1977) also observed lesser amounts of ethane from mature bean leaves compared to young leaves.

Figure 3. Effect of tissue age on evolution of ethylene (A), ethane (B) and electrolyte leakage (C) from pepper leaf disks pretreated at 21 (control), and 36 to 54C. Leaf disks were from 30, 60, 90 or 120-day-old leaves from the same plant. Values are means \pm SE of three measurements.



Electrolyte leakage values also increased as treatment temperatures increased greater than about 40C (Fig. 3C). Electrolyte leakage values greater than 0.5 were observed from 30-, 60-, 90- and 120-day-old leaf disks at 44, 48, 50, and 52C, respectively. Loss of electrolytes from 30-day-old disks was greater than from older tissues at all temperatures, including 21C controls. Electrolyte leakage values from 30-day-old leaf disks exceeded 0.5 at a lower temperature than older tissues, indicating that younger tissues were less heat tolerant. A gradual physiological change was observed in membrane response to heat stress as leaf age increased from 30 to 120 days old, possibly reflecting changes in lipid composition of the membranes.

Crop species. Thermotolerance levels of cucumber, squash, pepper and broccoli were determined using disks from 20-day-old leaves from 45-day-old plants. Low levels of ethylene were produced from heat-stressed broccoli, cucumber and pepper leaf disks from 21 to 40C, then volumes increased with increasing temperature, reaching a maximum at about 44C (Fig. 4A). Little or no ethylene was detected following treatment at temperatures greater than 50C. In contrast, squash produced very little ethylene with no temperature-related peak.

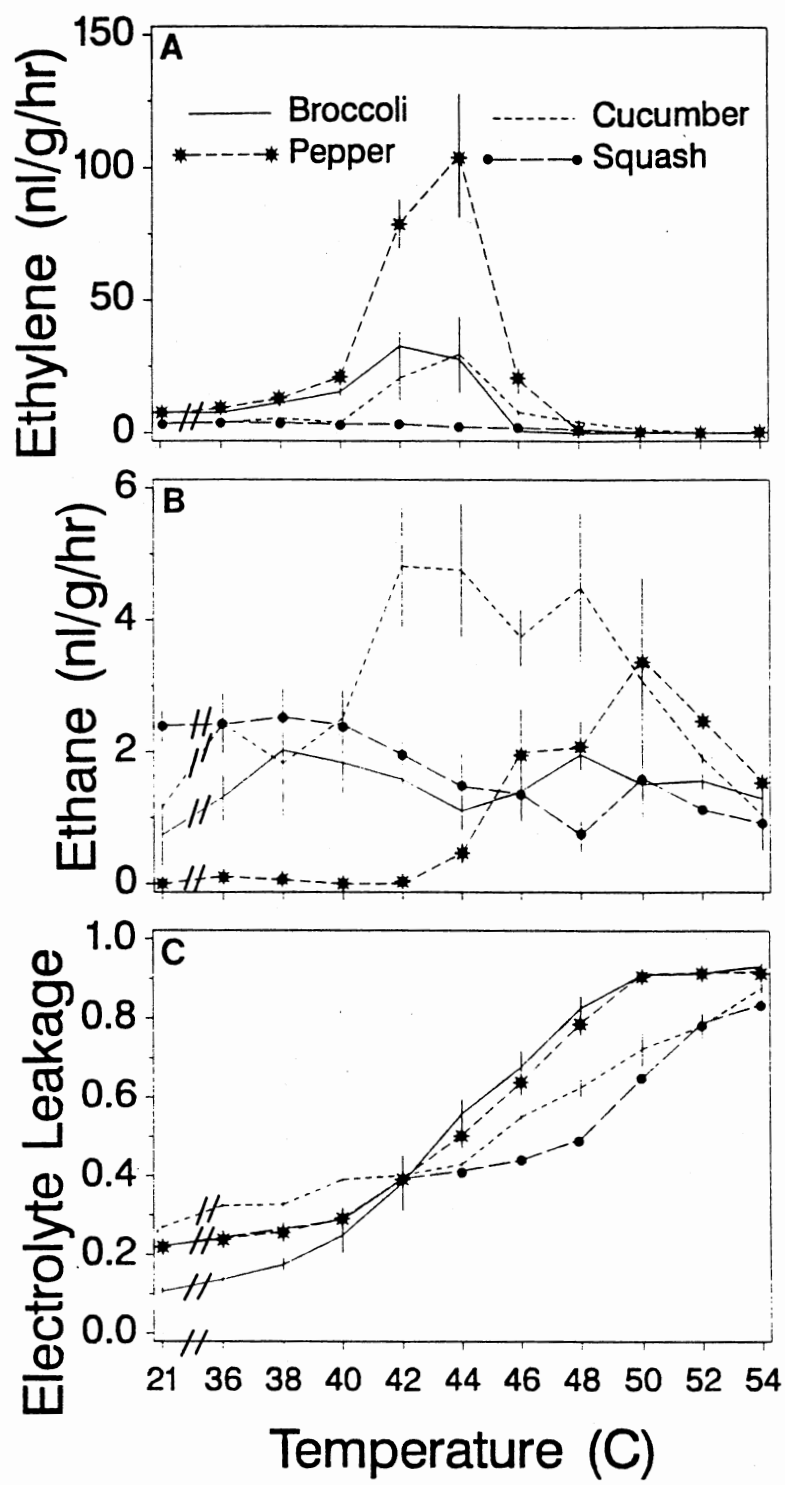
Ethane was produced from heat stressed pepper disks following exposure to temperatures greater than 42C (Fig 4B). In contrast, cucumber, squash and broccoli produced ethane at all treatment temperatures. Pepper had a distinct pattern of gas evolution with ethane production increasing at temperatures ethylene production declined. A similar response was observed in water-stressed plum leaves (Kobayashi et al., 1981) and frozen rhododendron leaf disks (Harber and Fuchigami, 1986). Squash, cucumber and broccoli had concomitant evolution of ethylene and ethane

following exposure to 21 to 46C. Differing ethane evolution patterns in the plant species examined may be related to differences in fatty acid saturation, varying amounts and activity of phospholipase, lipoxygenase and hydroperoxide cleavage enzymes, and antioxidant activity. Galliard (1978) suggested that cucumber and tomato had hydroperoxide cleavage enzymes with different substrate specificity, leading to differential amounts of carbonyl fragments.

Predicted midpoints of electrolyte leakage responses were 43.8 ± 0.5 , 44.7 ± 0.5 , 49.7 ± 1.6 and 51.7 ± 1.2 C for broccoli, pepper, cucumber and squash, respectively. Midpoints were close to the temperatures at which ethylene production declined to nondetectable levels in all crop species. Broccoli had the lowest inflection temperature indicating that, as a cool season crop, it is susceptible to heat stress. Greater midpoint temperatures for squash and cucumber were consistent with the dogma that summer crops are thermotolerant. The relatively low inflection temperature for pepper was probably a result of tissue age and slow growth habit. Pepper was at a much earlier stage of development at forty days than broccoli, squash and cucumber.

Heat and freezing stress. A comparison of heat and freezing stress responses was conducted by exposing one set of pepper leaf disks to temperatures from 40 to 55C with 21C as a control and another set to 0 to -8C. Electrolyte leakage and ethane:ethylene ratio exhibited large transitions as treatment temperatures decreased from -4 to -5C in freezing experiments and between 48 and 50C in heat-stressed disks (Figs. 5, 6). All disks exposed to temperatures lower than -4C were induced to freeze

Figure 4. Evolution of ethylene (A), ethane (B) and electrolyte leakage (C) from heat stressed leaf disks from broccoli, cucumber, squash or pepper. The values are means \pm SE of three replications.



by adding chips of ice to the tubes. Leaf disks exposed to temperatures from 0 to -4C supercooled based on a lack of water soaking (Lucas, 1956; Marcellos and Single, 1976). The increased amount of ethylene observed from exposure temperatures from -1 to -4C was an indication of stress-induced ethylene (Elstner and Konze, 1976). Nondetectable amounts of ethylene from tissues treated at -5C and lower temperatures reflected the requirement for an intact membrane system for ethylene biosynthesis (Field, 1981). Ethane production was observed from tissues following exposure to -4C and lower temperatures. Electrolyte leakage increased from 0.15 to 0.80 as treatment temperature decreased from -4C to -5C. There was a high correlation ($r=0.97$) between ethane:ethylene ratio and electrolyte leakage.

The transition from uninjured to damaged tissue was more abrupt in disks exposed to low temperatures. The damaged tissues exhibited water soaking upon thawing and increased electrolyte leakage and ethane:ethylene ratios. Thus, low temperature produced a disjunctive response in this frost-susceptible species with little or no apparent damage to disks that supercooled and severe injury to frozen tissues. This is in accord with observations of other frost-susceptible plant tissues that do not tolerate ice formation (Burke et al., 1976). Freezing stress in these tissues probably resulted in intracellular freezing with ice crystals leading to mechanical damage of the membrane systems (Burke et al., 1976).

Ethylene production from heat-stressed tissues increased following exposure to temperatures from 21 to 43C, then declined to nondetectable amounts at 50C. Ethane production was observed from tissues following exposure to temperatures greater than 48C. Electrolyte leakage increased from 0.13 to 0.75 as treatment temperature

Figure 5. Ratio of ethane and ethylene (EER), and electrolyte leakage (EL) from pepper leaf disks following low temperature exposure. Ethylene and ethane values were transformed $[(\text{nl} \cdot \text{g}^{-1} \cdot \text{h}^{-1} + 0.5)^{-2}]$ to accommodate zero values (nondetectable amounts). Disks exposed to temperatures colder than -4°C were induced to freeze by adding ice chips to the tubes. Values are means \pm SE of nine measurements (three duplicate subsamples on each of three dates).

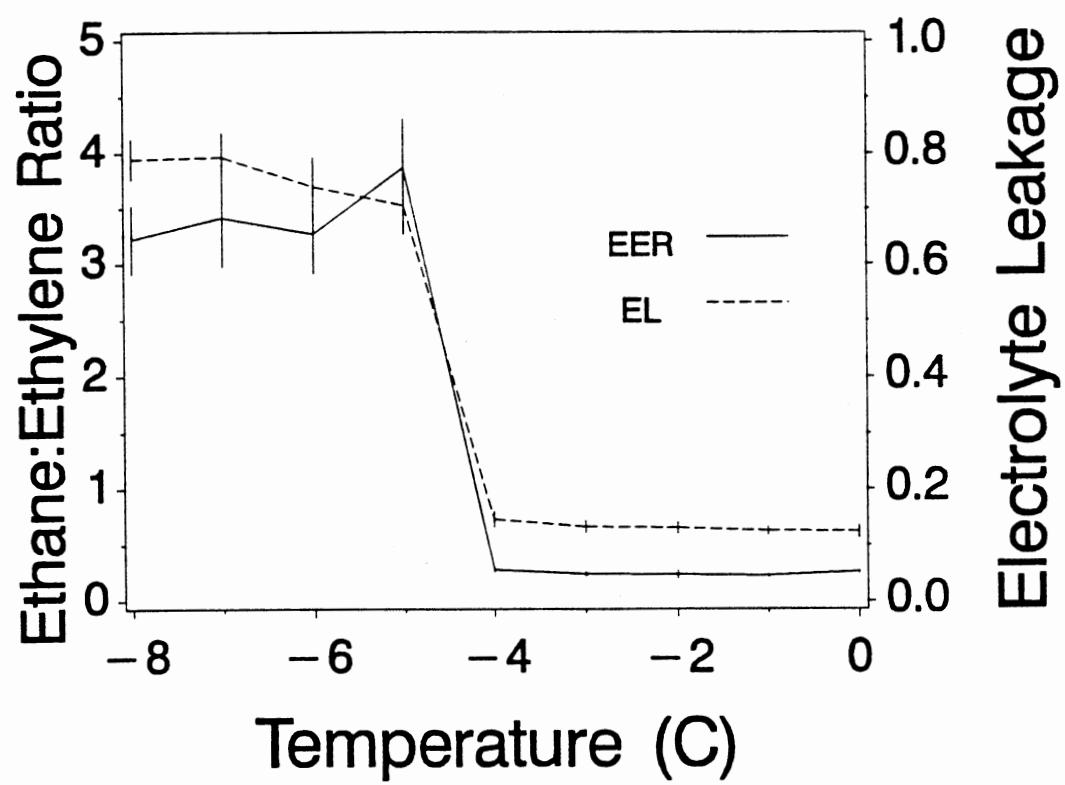
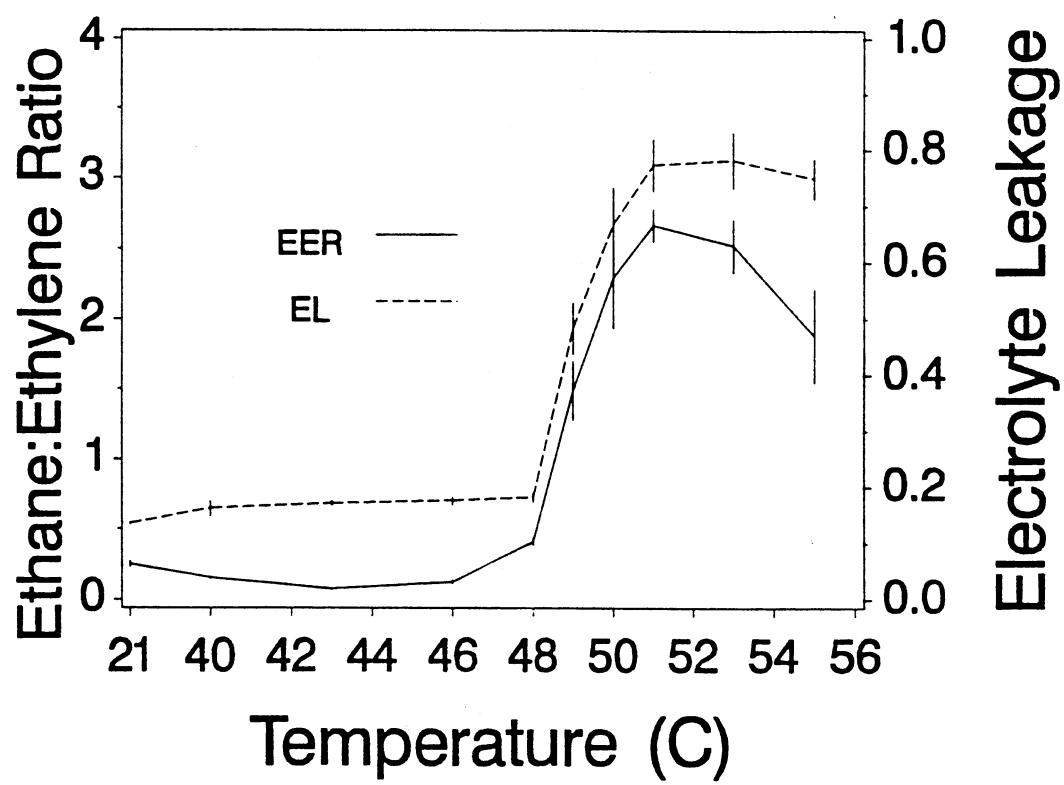


Figure 6. Ratio of ethane and ethylene (EER), and electrolyte leakage (EL) from pepper leaf disks following high temperature exposure. Ethylene and ethane values were transformed $[(\text{nl}\cdot\text{g}^{-1}\cdot\text{h}^{-1} + 0.5)^{-2}]$ to accommodate zero values (nondetectable amounts). Values are means \pm SE of nine measurements (three duplicate subsamples on each of three dates).



increased from 21(control) to 55C. There was a strong correlation ($r=0.84$) between ethane:ethylene ratio and electrolyte leakage.

Tissues exposed to high temperature stress exhibited a more gradual increase in electrolyte leakage and ethane:ethylene ratio than disks exposed to low temperatures. The range of electrolyte leakage values corresponding to uninjured to killed tissues spanned from 48 to 51C. Ethane:ethylene ratios decreased slightly from 21 to 46C, reflecting an increase in ethylene production at injurious, sublethal temperatures, then increased to a maximum at 51C followed by a decline at 55C. High temperature stress affects permeability via biochemical deterioration of membranes (Field, 1981; Lester, 1985) while intracellular ice formation can lead to mechanical damage of the membrane (Levitt, 1980). Even though freeze and heat stress affected membrane permeability and hydrocarbon evolution similarly in pepper leaf disks, the mechanisms of injury differ at the subcellular level.

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CHAPTER III

EFFECTS OF FATTY ACID INFILTRATION ON ETHANE PRODUCTION AND ELECTROLYTE LEAKAGE FROM PEPPER LEAF DISKS

Introduction

The production of volatile hydrocarbons such as ethane and ethylene by plant tissues occurs under a range of environmental stress conditions (Bressan et al., 1979; Peiser and Yang, 1979) or as a result of mechanical wounding (Konze and Kwiatkowski, 1981). Ethane production was directly proportional to lesions developing after sulfur dioxide exposure (Kimmerer and Kozlowski, 1982) and electrolyte leakage from water-stressed plum leaves (Kobayashi et al, 1981). Hence, ethane formation can be an index of injury in plant tissues.

Plant membranes are bilayers of lipids consisting of phospholipids, galactolipids and sterols (Singer and Nicholson, 1972). Linolenic acid, a major component of plant membranes (Galliard, 1978; Rochester et al., 1987), was reported to be the precursor of ethane (Lieberman and Mapson, 1962). Since fatty acids do not undergo peroxidation in situ, deesterification by phospholipase is required for oxidation. Lesham (1987) suggested that calcium may play a role in membrane deterioration through a "phosphatidyl-linoleyl(-enyl) cascade". Influx of calcium into the cytoplasm increases activity of phospholipase via a calcium-calmodulin mechanism, leading to

the release of free fatty acids. Free fatty acids with more than one double bond are susceptible to oxidation by lipoxygenase, leading to the formation of lipid peroxides and oxygen free radicals (Kacperska and Zebalska, 1989; Bilinski et al., 1989). Lipid peroxides are decomposed by cleavage enzymes to form volatile hydrocarbons including ethane and hexanal (Dumelin and Tappal, 1977; Galliard, 1978). The byproducts of membrane lipid peroxidation can result in disruption of membrane permeability, loss of metabolic functions and death of cells (Kimmerer and Kozlowski, 1982). Free radicals also mediate membrane disruption in plants under stress (Senaratna et al., 1987; Kendall and McKersie, 1989) through involvement in lipid peroxidation (Mead, 1976), leading to the formation of hydrocarbons (Kacperska and Zebalska, 1989). Intact membrane systems have free radical scavenging mechanisms involving antioxidants such as alpha-tocopherol (vitamin-E) (Riely et al., 1974) and enzymes, including superoxide dismutase (Bilinski et al., 1989). Propylgallate, a free radical scavenger, inhibited ethylene formation in avocado and apple fruit disks (Apelbaum et al., 1980; Baker et al., 1978).

The objective of this study was to determine the effects of infiltration with fatty acids and propylgallate on membrane permeability and ethane evolution.

Materials and Methods

Pepper (Capsicum annuum L. 'Early calwonder') leaf disks were infiltrated with stearic (18:0), oleic (18:1), linoleic (18:2) or linolenic acid (18:3). Free fatty acids (Sigma Chemical Co., St. Louis, Mo) were dissolved in 3 ml reagent-grade ethanol and diluted to 5 mM with 0.01% (v/v) Tween 60 (polyoxyethylene sorbitan). Leaf disks vacuum infiltrated with only 0.01% Tween 60 and ethanol served as controls. In

a second fatty acid experiment, pepper leaf disks were vacuum infiltrated with alpha-linolenic acid (18:3 n-3), gamma-linolenic acid (18:3 n-6), cis-11,14,17 eicosatrienoic acid (20:3 n-3) and cis-8,11,14-eicosatrienoic acid (20:3 n-6). Fatty acids were dissolved in 1 ml reagent-grade ethanol and diluted to 5 mM with distilled water. Leaf disks infiltrated with 0.3% ethanol served as controls.

The effects of propylgallate on hydrocarbon evolution, electrolyte leakage and efflux of potassium ions were evaluated by infiltrating pepper leaf disks with 5 mM alpha-linolenic acid and 0.1 or 1.0 mM propylgallate. Treatments with pepper leaf disks infiltrated with only propylgallate, linolenic acid or ethanol were the controls.

Gas measurements. Ten leaf disks for each treatment replication were blotted after infiltration and placed in a 14.5 ml vial and incubated for 4 h at 24C. Ethane was measured by thoroughly mixing the vial atmosphere, then removing 1 ml of headspace gas with a syringe and injecting into a gas chromatograph (model 540, Tracor Instruments Inc., Austin, TX) equipped with a flame ionization detector and an alumina-stainless steel column.

Electrolyte leakage. Twenty ml of distilled water were added to a test tube containing three infiltrated disks. Initial electrolyte leakage was measured with a conductance meter (Model 35, Yellow Springs Instrument Co., Yellow Springs, OH) after 4 h incubation at 24C on an orbital shaker. Then all of the samples were autoclaved and the final leakage was measured after an additional 4 h at 24C.

K⁺ leakage. Potassium leakage was determined from a 1:1 dilution of the solution used for initial electrolyte leakage measurements with an atomic absorption

spectrophotometer (model 2380, Perkin-Elmer Corporation, Norwalk, CT.)

Concentrations were calculated from standard curves.

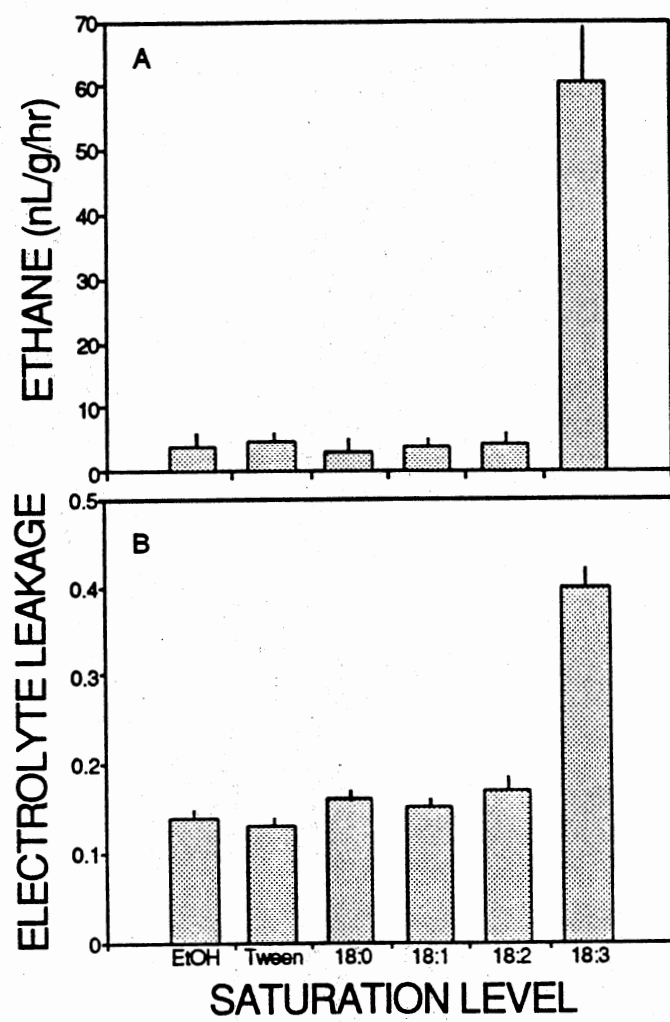
Data analysis. The first two fatty acid experiments were randomized block designs with three replications and three subsamples. The third experiment was a split plot design with three replications and three subsamples. Fatty acids were whole plots and propylgallate was subplot. Analysis of variance was conducted with orthogonal contrasts or Duncan's multiple range test carried out as appropriate.

Results and Discussion

The first fatty acid experiment involved a saturation series of 18-carbon fatty acids. Leaf disks infiltrated with alpha-linolenic acid (18:3) generated significantly greater amounts of ethane and electrolyte leakage compared to the other treatments (Fig. 7). Similarly, Konze and Elstner (1978) reported enhanced production of ethane from potato tuber slices incubated in alpha-linolenic acid.

Fatty acids are released during membrane perturbation by phospholipase. Free fatty acids stimulate oxidative enzymes like lipoxygenase (Shridhara and Lesham, 1986), leading to formation of lipid peroxides (Galliard, 1978). Increased membrane permeability was correlated with increased lipid peroxide levels in tobacco leaves undergoing senescence (Dhindsa et al., 1981), suggesting that lipid peroxides may be a common metabolic intermediate in the membrane deterioration pathway. Bilinski et al. (1989) reported that fatty acids with more than one double bond were susceptible to peroxidation in yeast cells. Lipoxygenase oxidizes linoleic and linolenic acids to form lipid peroxides. However, a number of lipoxygenase isozymes with varying substrate specificity have been reported (Siedow, 1991). Lipid peroxides decompose in the

Figure 7. Evolution of ethane (A) and electrolyte leakage (B) from pepper leaf disks infiltrated with 5 mM 18-carbon fatty acids containing from 0 to 3 double bonds, distilled water and ethanol (EtOH), or with distilled water, ethanol and Tween 60 (Tween). Values are means \pm SE of nine measurements (three duplicate subsamples on each of three dates).



presence of cleavage enzymes to produce volatile hydrocarbons, including ethane and hexanal. The substrate for the cleavage enzymes differs among crop species (Dumelin and Tappal, 1977; Galliard, 1978). Hence, the differential decomposition of linoleic and linolenic acid in our experiment could have reflected specificity by lipoxygenase and/or the cleavage enzyme(s) present in pepper leaf tissues. The lack of ethane from oleic acid was an indication of greater stability of less unsaturated fatty acids (Bilinski et al., 1989).

The effects of double bond location in trienoic fatty acids on electrolyte leakage and ethane production were determined by infiltration of pepper leaf disks with alpha-linolenic acid (18:n-3), gamma-linolenic acid (18:3 n-6), cis-11,14,17 eicosatrienoic acid (20:3 n-3) and cis-8,11,14-eicosatrienoic acid (20:3 n-6). Eicosapentaenoic acid (20:5 n-3) was also included to determine the effect of five vs. three double bonds. Pepper leaf disks infiltrated with alpha linolenic acid produced the greatest amount of ethylene compared to the other fatty acids with three double bonds (Fig. 8). However, eicosapentaenoic acid (20:5 n-3) had the highest ethane production ($147 \pm 20 \text{ nl}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) and electrolyte leakage (0.52 ± 0.05) of the fatty acids examined. Similar results were observed in diatom suspensions incubated in eicosapentaenoic acid (20:5 n-3) and alpha-linolenic acid (18:3 n-3). The lack of effect on ethane production by cis-8,11,14-eicosatrienoic acid (20:3 n-6) and reduced stimulation by gamma-linolenic acid (18:3 n-6) and cis-11,14,17 eicosatrienoic acid (20:3 n-3) compared with alpha-linolenic acid, may reflect enzymatic specificity.

Propylgallate significantly reduced ethane evolution from pepper leaf disks infiltrated with alpha-linolenic acid. However leakage of potassium ions and electrolytes was not affected (Table 1). Similar responses were observed in algal

Figure 8. Evolution of ethane (A) and electrolyte leakage (B) from pepper leaf disks infiltrated with 5 mM 18- and 20-carbon fatty acids containing a double bond 3 (n-3) or 6 (n-6) carbons from the nonpolar end of the molecule, or with distilled water, ethanol and Tween 60. Values are means \pm SE of nine measurements (three duplicate subsamples on each of three dates). Treatments with the same letter are not significantly different at $P=0.05$.

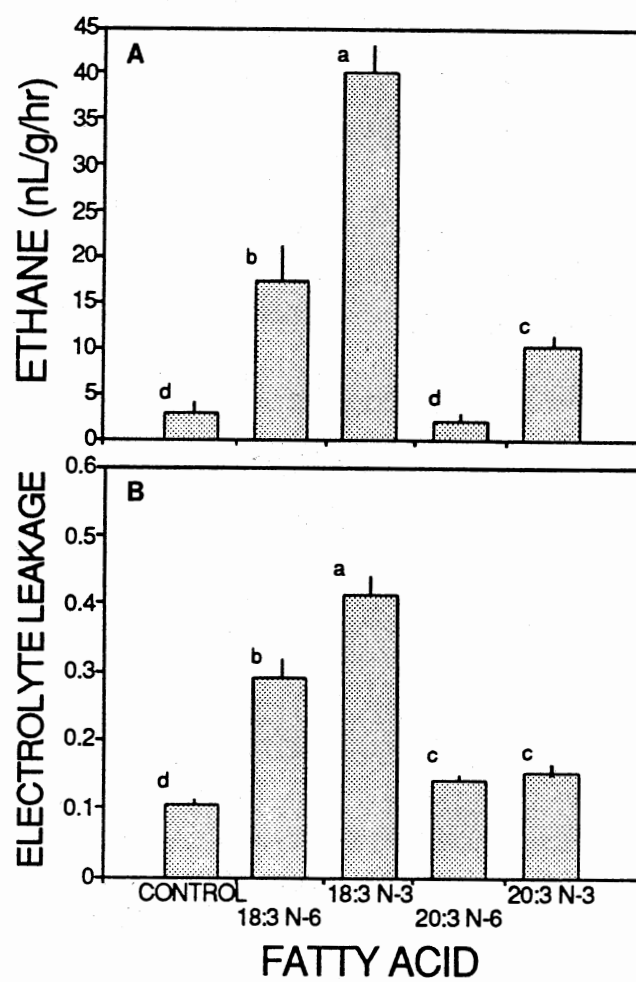


Table 1. Effects of propyl gallate and linolenic acid on ethane evolution and loss of K^+ and total electrolytes from pepper leaf disks. Values are means \pm SE of nine measurements (three duplicate samples on each of three dates).

Linolenic acid (mM)	Propyl gallate (mM)	Ethane (nl·g ⁻¹ ·h ⁻¹)	Electrolyte leakage	K ⁺ (μg·g ⁻¹)
0	0	3.4 ± 1.3	0.12 ± 0.01	0.2 ± 0.5
0	0.1	4.2 ± 1.2	0.11 ± 0.01	0.2 ± 0.3
0	1.0	1.4 ± 0.8	0.13 ± 0.01	1.2 ± 0.5
5	0	53.9 ± 11.5	0.30 ± 0.01	7.1 ± 0.7
5	0.1	20.6 ± 2.8	0.31 ± 0.03	5.0 ± 1.0
5	1.0	10.3 ± 1.0	0.37 ± 0.02	6.4 ± 0.6
Source ^z		Significance ^y		
LA*PG				
Linear		*	ns	ns
Quadratic		*	ns	ns

^z LA = linolenic acid, PG = propyl gallate

^y ns, * = not significant, significant at P = 0.05

suspensions (Schobert and Elstner, 1980) and tomato fruit disks (Baker et al, 1978) incubated in propylgallate. The formation of ethane from alpha-linolenic acid is thought to occur in several steps involving free radicals, including lipid peroxidation and the decomposition of the fatty acid peroxides. Propylgallate may have inhibited the latter step leading to the decline in ethane formation, whereas electrolyte leakage may have been a consequence of an earlier step in the reaction pathway. These studies suggest the requirement for free radical scavenging activity prior to the peroxidation stage for reduction in membrane damage.

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CHAPTER IV

EFFECTS OF GROWTH REGULATORS ON BELL PEPPER REPRODUCTION AT HIGH TEMPERATURE

Introduction

Temperature is one of the most important environmental factors influencing plant growth, development and yield (Bakker and Uffelen, 1988). High temperature results in flower drop and fruit abortion accounting in part for variation in fruit yield (Rylski, 1972 and 1973; Rylski and Spigelman, 1982).

Loss of flower buds and flowers is a major problem in the production of bell peppers in regions of variable summer temperatures (Wein, 1990). High temperatures greatly influence the yield of bell pepper, primarily through reduced flowering and fruit set (Bakker, 1989). Supra-optimal temperatures affect reproduction through changes in assimilation demand (Walker and Ho, 1977) and endogenous hormone levels (El-Abd et al., 1986). Studies on physiological mechanisms responsible for high temperature inhibition of flowering and fruiting of tomatoes have shown that reduced levels of growth regulators are involved (Satti and Oebker, 1986). Abortion of fruits is related to assimilation rate and distribution of photosynthates (Schapendonk and Brouwer, 1984). High temperatures favor increased flower formation (Bakker, 1989) and create high assimilation demand since fruits are the main sink for

assimilates (Walker and Ho, 1977). The inability to meet the increased assimilation demand can result in abortion of newly formed fruits. Unfavorable distribution of carbohydrates at high temperature was responsible for flower and fruit drop in tomatoes (Satti and Oebker, 1986).

Flowering and fruit set are phenomena that are influenced by hormonal factors. The involvement of cytokinins in the onset of flowering and inflorescence development has been demonstrated in many species (Ginzburg, 1974; Goh, 1977). Studies involving the application of growth retardants such as chloromequat chloride have reported improved fruit set (Nourai and Harris, 1983). Fruitone (3-chloro-phenoxy-propionic acid) has been used commercially to thin peaches (Buchanan et al., 1975).

Cytokinin application increases flower number and improves fruit set. Cytokinins may have an indirect effect on photoperiodic induction by altering assimilate partitioning, hence floral stimulus translocation to the shoot apex (Ogawa and King, 1979). Application of cytokinin to flower clusters increased flower number (Skene and Antcliff, 1972) and promoted fruit set in grapes (Weaver et al., 1965). Treatment of tomato inflorescence with BA increased soluble sugar content and led to continuous mobilization of sugar at fruit set and increased the number of flowers and fruits (Satti and Oebker, 1986).

Chloromequat chloride (CCC) shortens veins, results in greater branching and increases leaf number (Mahmoud et al., 1986). Flower bud abortion was reduced in greenhouse-grown tomatoes by soil drench of CCC. Chloromequat chloride increased the dry weight of the first-formed inflorescence and reduced the dry weight of the

remainder of the shoot system (Nourai and Harris, 1983). The reduction in growth of vegetative parts of the shoot system leads to the diversion of metabolites or endogenous growth substances to the inflorescence. Chloromequat chloride retards chlorophyll breakdown in leaf disks by stimulating protein synthesis (El-Antably, 1967). Chloromequat chloride increases carbohydrate content (Radwan et al., 1971) and increases concentrations of many amino acids (El-Fouly et al., 1970).

Increased flower and fruit abortion are mediated by elevated ethylene levels in the plant tissues (Wein et al., 1989). High temperature stimulated ethylene production and boll drop in cotton (Guinn and Brummett, 1988). Application of aminoethoxyvinylglycine (AVG), an inhibitor of ethylene biosynthesis, to apple flower clusters decreased ethylene evolution and increased fruit set (Greene, 1980).

High temperatures increase ethylene production and reduce auxin levels in the abscising organ, inhibit polar auxin transport, and act directly at the pedicel base to separate the cells in the abscission zone (Sagee et al., 1990). Treatment of flowers with auxin improved fruit set of tomato (Mann and Minges, 1949) and pepper (Silveria et al., 1986) in cool weather. Auxin sprays were less successful in tomato and bell pepper at higher temperatures (Osborne and Went, 1953). Application of synthetic auxins, naphthalene acetic acid (NAA) and chlorophenoxy acetic acid (CPA), increased ethylene production and flower abscission (Wein and Zhang, 1991). Chlorophenoxy-propionamide (3-CPA) was used as a thinning agent in peach (Buchanan et al., 1975). The objective of this study was to determine the effect of growth regulators on reproduction of bell pepper.

Materials and Methods

1990 experiment. Seeds were sown in flats containing Terralite Metro Mix 200 (W.R. Grace & Co., Fogelsville, PA) medium. Seedlings were grown in a greenhouse at ambient temperature (34.6C day/22.6C night) and transplanted to 3-liter pots filled with Fafard GP Mix (Springfield, Mass) after about two months. Plants were fertilized at each watering with 300 mg.liter⁻¹ of 20N-8.7P-16.6K soluble fertilizer (Peter's Professional, W.R. Grace & Co., Fogelsville, PA). Watering was based on soil color and pot weight. Seedlings were treated in the flats with CCC as a soil drench at 4000 mg.liter⁻¹ @ 2 liter / flat on the day prior to transplanting. Fruitone (3-chlorophenoxy propionic acid) was applied at 0, 250 and 500 mg.liter⁻¹ as a spray at full bloom. Benzyl adenine was applied at 0 and 100 mg.liter⁻¹ at flowering. Since 10% ethanol was used to dissolve BA, it was used in all treatments, including control.

Plant height was measured at 5, 12, 19, 26 and 33 days after transplanting. Flowers were tagged at anthesis. Flower and fruit abortion and fruit and seed weight were recorded. Treatments were combined into a completely randomized design with a factorial combination of 12 treatments and 8 single plant replicates (Table 2). Data were analyzed in single degree of freedom contrasts versus controls using chi-square.

1991 experiment. Seedlings were divided into two groups, one set for destructive measurement of ethylene from flowers after treatment and another set for flower and fruit abortion and fruit weight measurements. Three flowers for ethylene measurements and ten flowers for flower and fruit abortion per plant were tagged a day prior to treatment. All flowers were pollinated at full bloom using an artist's brush. Seedlings were sprayed with 1000 mg.liter⁻¹ Ivory detergent as controls, 100 mg.liter⁻¹

Table 2. Factorial combination of growth regulators in 1990 greenhouse experiment. CCC was applied at 2 liter/flat on the day prior to transplant. CPA and BA were sprayed at full bloom.

Treatment	CCC (mg liter ⁻¹)	CPA (mg liter ⁻¹)	BA (mg liter ⁻¹)
1	0	0	0
2	0	250	0
3	0	500	0
4	0	0	100
5	0	250	100
6	0	500	100
7	4000	0	0
8	4000	250	0
9	4000	500	0
10	4000	0	100
11	4000	250	100
12	4000	500	100

BA, and 2 mg.liter⁻¹ cytokin at weekly intervals starting one week after transplanting, for a total of four weeks. Flowers received a localized spray of 200 mg.liter⁻¹ AVG, 18.6 mg.liter⁻¹ CPA or 18.6 mg.liter⁻¹ NAA at bloom. Three flowers were incubated in a 14.5 ml vial for four hours at 24C for ethylene measurements. One ml of headspace gas was removed with a syringe and injected into a gas chromatograph (model 540, Tracor Instruments Inc., Austin, TX) equipped with a flame ionization detector. Flower and fruit abortion and fruit weight were recorded for the ten tagged flowers. The experimental design was a randomized complete block design with six treatments and eight replications.

Results and Discussion

1990 experiment. Plants treated with CCC averaged 5 cm less in height increase (height at five weeks minus height at transplanting) than controls. A similar response was observed in tomato plants grown at 35C day/25C night cycles and treated with CCC (Abdalla and Verkerk, 1970). Total flower abortion (open and closed during spray) was not affected by CPA, but was reduced by BA and CCC (Table 3). A similar reduction in abortion of CCC-treated plants was obtained when only flowers blooming prior to the application of BA and CPA were analyzed (data not presented). Nourai and Harris (1983) and Abdalla and Verkerk (1970) observed reduced flower abortion in tomato following CCC application and suggested that abortion of flower buds occurred when vegetative parts of the shoot system were in competition with the inflorescence for metabolites or growth substances. Growth retardants like CCC acted to reduce or remove this competition. Plants treated with BA and/or CCC had a significantly lower percentage of flower abortion compared to

Table 3. Effect of growth regulators on greenhouse-grown bell pepper reproduction during summer 1990.

CCC ^x (mg liter ⁻¹)	BA ^x (mg liter ⁻¹)	CPA ^x (mg liter ⁻¹)	Percentage abortion			
			Flowers ^y			Fruit
			Open	Closed	Total	Total
0	0	0	58	76	67	77
0	0	250	45 [*]	91	66	17 [*]
0	0	500	35 [*]	90	63	60
0	100	0	0 [*]	32 [*]	15 [*]	22 [*]
0	100	250	15 [*]	43 [*]	26 [*]	56
0	100	500	34 [*]	43 [*]	36 [*]	66
4000	0	0	29 [*]	66	48 [*]	57
4000	0	250	31 [*]	58	41 [*]	75
4000	0	500	36 [*]	88	52 [*]	32 [*]
4000	100	0	5 [*]	21 [*]	18 [*]	35 [*]
4000	100	250	8 [*]	34 [*]	20 [*]	58
4000	100	500	3 [*]	41 [*]	14 [*]	55

^z Values with asterisks are significantly different than controls at P = 0.05

based on single degree of freedom contrasts.

^y Flowers were grouped as open or closed on the date of BA and CPA application.

^x CCC=chloromequat chloride (soil drench at transplanting), BA = benzyl-amino purine (spray at full bloom), CPA =chlorophenoxy propionic acid (spray at full bloom).

controls. Application of BA in an earlier study conducted by Skene and Antcliff (1972) increased flower numbers and fruit set in grapes. It was suggested that cytokinins alter assimilate partitioning, resulting in floral stimulus translocation to the shoot apex (Ogawa and King, 1979). Total flower abortion following application of CPA alone was not significantly different from controls in our study. Earlier work with bell pepper by Wein et al. (1989), where he used a combination of open and closed flowers found that application of chlorophenoxy acetic acid (4-CPA) resulted in increased flower abortion which was attributed to stimulation of ethylene production. Open flowers had significantly lower flower abortion compared to closed flowers. All treatments decreased flower abortion in open flowers, but closed flowers responded only to treatments with BA. Differences between open and closed flowers responses may reflect differential uptake or physiological differences between the two stages. Fruit abortion was significantly lower than the controls in plants treated with CPA ($250 \text{ mg liter}^{-1}$), BA ($100 \text{ mg liter}^{-1}$), CCC ($4000 \text{ mg liter}^{-1}$) and CPA ($500 \text{ mg liter}^{-1}$), and BA ($100 \text{ mg liter}^{-1}$) and CCC ($4000 \text{ mg liter}^{-1}$).

1991 experiment. Plants treated with AVG had a significant decrease in ethylene production (Table 4). AVG is an inhibitor of ethylene biosynthesis, blocking its conversion from methionine (Boller et al., 1979). Flower abortion was not significantly different than the controls for the flowers sprayed with AVG, however fruit abortion was (Table 4) significantly decreased. Greene (1980) observed a similar response in apples sprayed with $200 \text{ mg liter}^{-1}$ AVG.

Plants treated with NAA and CPA had elevated ethylene production. Studies by Wein and Zhang (1991) and Wein and Turner (1989) indicated that auxin

Table 4. Effect of growth regulators on greenhouse grown bell pepper reproduction conditions during summer 1991.

Treatment ^z	Ethylene	Percentage abortion			
		Flowers			Fruit
		Open	Closed	Total	
Control	3.2 ± 0.4 ^b	58	84	68	42
AVG	1.3 ± 0.4 ^a	66	63*	64	21*
NAA	6.2 ± 1.3 ^c	22*	42*	31*	13*
BA	3.5 ± 0.7 ^b	52	81	69	44
Cytokin	3.0 ± 0.3 ^b	39*	77	56	37
CPA	7.4 ± 0.7 ^c	10*	23*	14*	17*

^z Control = 0.1% Ivory Detergent, AVG = aminovinyglycine

at 200mg.liter⁻¹, NAA = Naphthalene acetic acid at 18.6

mg.liter⁻¹, BA = 100 mg.liter⁻¹, Cytokin = 2 mg.liter⁻¹,

CPA = 18.6 mg.liter⁻¹. * significant at P = 0.05 determined by

single degrees of freedom contrast with control.

application as a foliar spray increased ethylene production. However, flower and fruit abortion decreased significantly in CPA- and NAA-treated plants. These apparently contrasting results with decreased flower abortion following increased ethylene production can be explained by 1) the threshold ethylene level needed for initiation of abscission may not have been reached or 2) the localized auxin application may have prevented abscission (Wein et al., 1989).

Flower abortion from cytokin-treated plants was significantly lower than controls for only open controls whereas BA-treated plants had flower abortion similar to controls. Cytokinin, CPA and NAA reduced flower abortion in open flowers, whereas AVG, NAA and CPA reduced flower abortion in closed flowers. Total flower abortion was reduced by NAA and CPA. Fruit abortion was significantly lower than controls in flowers treated with CPA, NAA and AVG. Fruit weight was not significantly different from controls in any treatments (data not presented). High temperatures during early stages of fruit development resulted in scalding and lower fruit weight. Daily maximum/minimum temperatures for 1990 and 1991 are presented in figures 9 and 10. Based on our study, CCC as a soil drench or BA at anthesis but not before flowers opened, had a significant effect on plant growth and reproduction by decreasing flower and fruit abortion.

Figure 9. Maximum and minimum air temperatures recorded in the greenhouse during 1990.

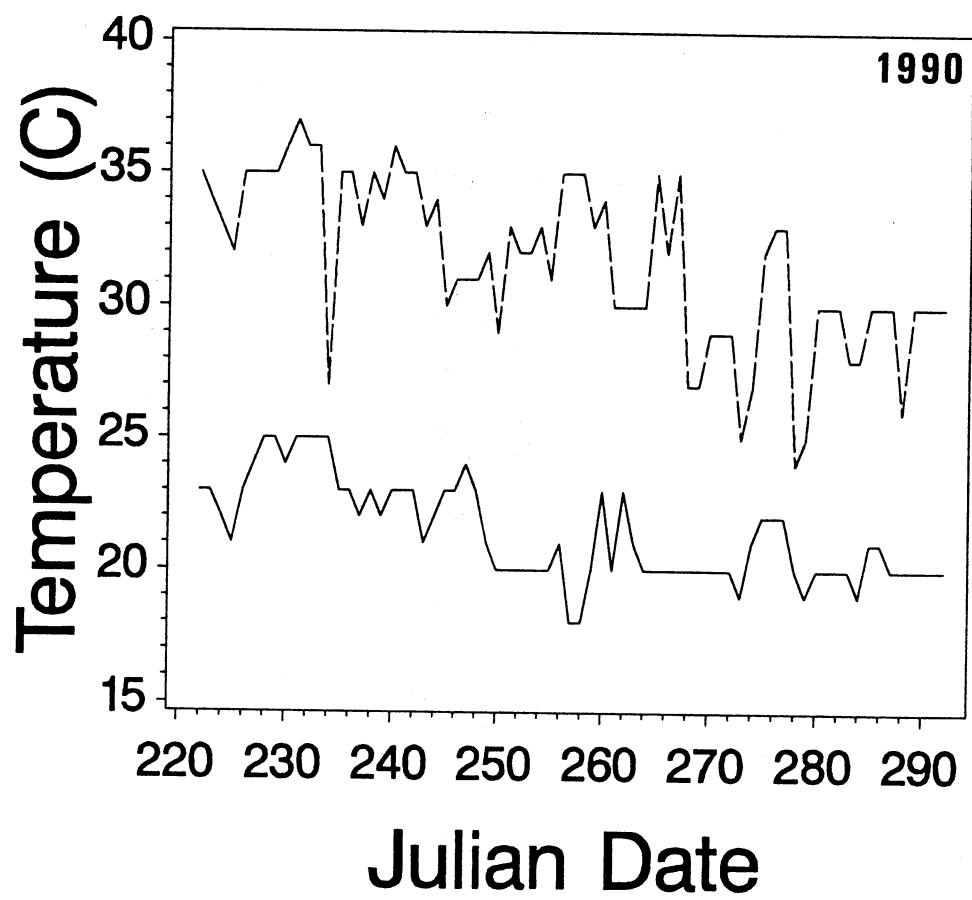
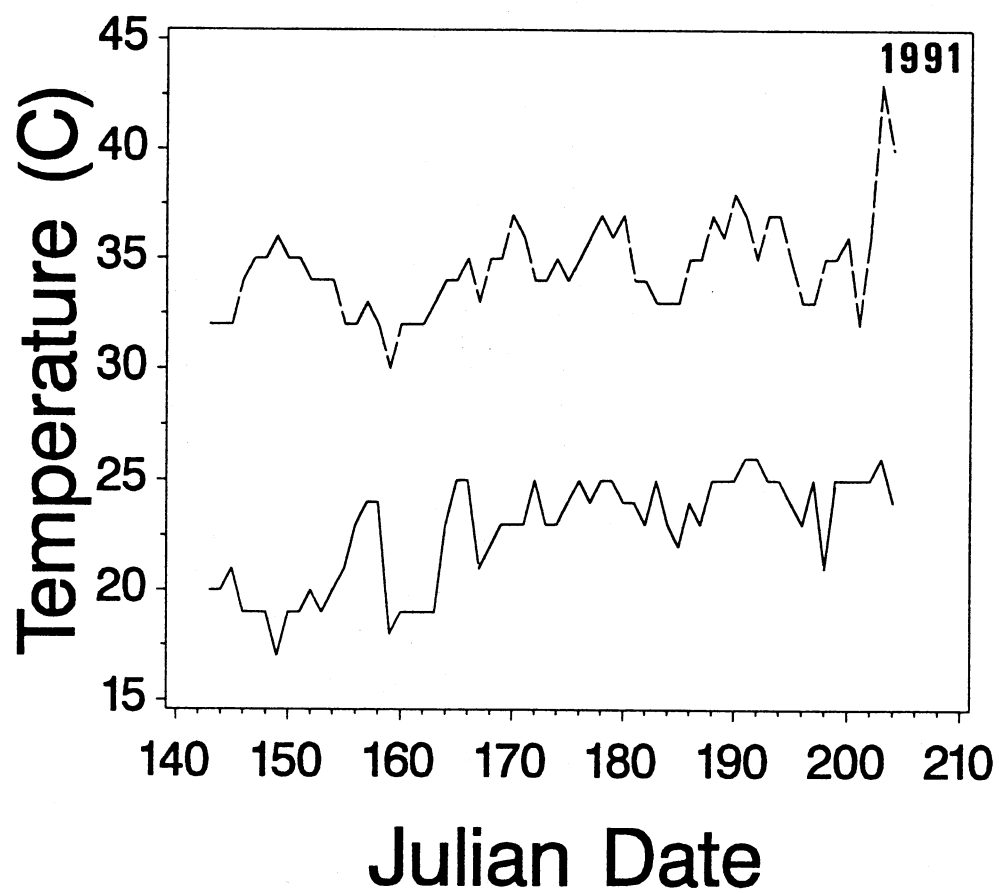


Figure 10. Maximum and minimum air temperatures recorded in the greenhouse during 1991.



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