HIGH TEMPERATURE ACCLIMATION IN BELL PEPPER LEAVES

By

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HIGH TEMPERATURE ACCLIMATION

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CHAPTER I

Introduction

Heat stress is a major limiting factor for the survival and development of plants and plays a significant role in determining crop productivity. Plant temperature may exceed surrounding air temperature during summer. This is due to heat absorption being greater than heat loss during the day (Treshow, 1970). Temperatures greater than the optimum growth temperature can cause injury to plants (Levitt, 1980a), resulting in physiological disorders (Reuther et al., 1979), reduced yield (El Ahmadi and Stevens, 1979), decreased product quality (Treshow, 1970) and increased product decay (Lee and Yang, 1984).

Heat stress is a function of exposure temperature and duration (Levitt, 1980a). High temperature can cause direct or indirect heat injury in plants (Levitt, 1980a). Exposure to an extremely high temperature for seconds or minutes may induce direct heat injury in plants. Direct injury occurred during a 20 min exposure to 52.5C in excised sour orange roots (Ingram and Buchanan, 1981). Indirect heat injury occurs during progressive exposure to moderately high temperatures. Pecan roots were killed by a 4 day exposure to 43C (Woodruff and Woodruff, 1934).

The temperatures causing direct or indirect injury to plants varied inversely with the exposure duration, with the critical temperature decreasing linearly as exposure time increased exponentially (Anderson et al., 1990; Ingram, 1985, 1986). Ingram (1985) presented a mathematical model to describe the interaction of exposure temperature and duration on plant thermotolerance using electrolyte leakage data. The model has been used to predict critical exposure temperatures and durations causing heat injury to plant tissues (Ingram, 1986; Anderson et al., 1990; Inaba and Crandall, 1988).

Two procedures have been developed to measure plant thermotolerance level. One approach is to expose intact plants in a growth chamber at a sublethal high temperature (Levitt, 1980a). After a defined time period, the plants are moved to a greenhouse to observe symptoms of heat injury. Another approach is to submerge small pieces of plants (contained in test tubes with distilled water) into a temperature-controlled water bath at a series of temperatures for a defined period (Julander, 1945; Sullivan, 1972; Ingram and Buchanan, 1981). Alternatively, samples can be exposed to one temperature for varying periods of time (Anderson et al., 1990). Heat exposure temperature and duration are two factors that appear to influence heat injury level of plants during heat stress. Compared to heat killing temperature, heat killing time is a more sensitive

criterion to evaluate plant heat tolerance (Chen et al., 1982).

Plant membrane properties are important for plants to survive and develop at high temperatures due to the membrane association of many important physiological activities. Electrolyte leakage has been used to estimate cell membrane stability in response to heat stress (Inaba and Crandall, 1988; Lester, 1985; Martineau et al., 1979). When plant tissues were exposed to heat stress, the cellular membranes were injured based on an increased membrane permeability. Measurement of electrolyte leakage is a quantitative procedure to evaluate the degree of heat injury in plant tıssues (Ingram and Buchanan, 1984). Sullivan and Ross (1979) used electrolyte leakage to identify genetic variation in heat tolerance ın grain sorghum.

Exposure to various environmental stresses, including freezing (Harber and Fuchigami, 1986), water stress (Kobayashi et al., 1981), high temperature (Field, 1981) and sulfur dioxide exposure (Peiser and Yang, 1979; Bressan et al., 1979), can increase ethylene production in plant tissues. Ben-Yehoshua and Aloni (1974) reported that an elevated ethylene level was observed in water-stressed orange leaves and was followed by a decreased amount of ethylene after the stress was relieved. According to Saltveit and Dilley (1978), ethylene production increased with increasıng temperature from 10C to 38C and then decreased sharply in etıolated pea seedlings. Measurement of

ethylene production was a reliable approach for determining the extent of tissue injury from environmental stresses (Tingey, 1980). Ethane evolution generally increased with continuous exposure to stress, indicating increased tissue injury (Kimmerer and Kozlowski, 1982). Ethane was produced from severely injured tissues (Peiser and Yang, 1979), but ethylene was produced in the living tissues (Kobayashi et al., 1981). Release of ethane from stressed tissue indicated lipid peroxidation (Peiser and Yang, 1979). Decreased ethylene at high temperature was related to the loss of membrane integrity (Lieberman, 1979).

Functions of ethylene in various physiological processes were studied by applying exogenous ethylene to plant tissues (Abeles et al., 1989; Taylor and Gunderson, 1986). Abeles et al. (1989) reported that exogenous ethylene stimulated the synthesis of three peroxidases in cucumber cotyledon tissue. Ethylene enhanced mRNA synthesis resulting in de novo protein synthesis in potato tubers (Theologis and Giudice, 1979). Induction of new mRNA following ethylene treatment was observed by Christoffersen and Laties (1982) in carrots. The results indicated that exogenous application of ethylene altered gene expression, triggering the production of new mRNA and subsequent synthesis of new proteins. The effect of ethylene on genetic regulation was confirmed by the observation that cycloheximide, a protein synthesis inhibitor, blocked abscission induced by ethylene (Abeles and Holm, 1966). Ethylene acting in a physiological

process must bind to an ethylene receptor (Yang, 1985). Ethylene activated a particular part of the genome which triggered a series of biochemical reactions leading to a variety of physiological responses such as fruit ripening (McGlasson, 1985), abscission (Reid, 1985) and senescence (Kader, 1985).

At least two approaches can be used to examine the effects of ethylene in plant physiological responses. One approach is to use ethylene action inhibitors such as carbon dioxide (Burg and Burg, 1967) or silver ions (Beyer, 1976, 1979) to block or delay physiological effects induced by ethylene. Inhibition of ethylene-induced sex expression in cotton flowers by silver ions and high CO_2 levels was observed by Beyer (1976). Understanding the action of ethylene also can be achieved by addition or removal of ethylene to regulate the ethylene level in the tissues (Yang, 1985).

Plants can acclimate to high temperatures as a result of pretreatments including high temperature exposure (Anderson et al., 1990; Wallner et al., 1982; Lester, 1986), growth regulator application [Engelbrecht and Mothes, 1960 (cited by Leopold, 1964)], and moderate water stress (Julander, 1945; Wehner and Watschke, 1981). As a result of pretreatment, plants survived at temperatures that would normally be lethal. Schroeder (1963) reported that avocado fruit tissues pretreated at 50C for 2-3 min showed markedly higher survival rates when subsequently heated to 55C for 10

min. According to Wallner et al.(1982), heat pretreatment (38C day/24C night for 24 h) administered to kentucky bluegrass can delay the killing time at 50C from 108 to 176 min. Anderson et al. (1990) reported that thermotolerance of pepper leaf disks was increased following exposure to 38C for 4 h.

The mechanism of acclimation to high temperature is complicated. Heat stress injury has been associated with alterations in membrane composition (Martineau et al., 1979; Lester, 1985), protein denaturation (Bernstam, 1978), and biochemical lesions (Levitt, 1980a). Raison et al. (1980) proposed the following hypothesis: a temperature shift can cause the membrane proteins to move toward the aqueous interface at low temperatures or toward the interior of the bilayer at high temperatures. The balance between the relative strengths of hydrophobic and hydrophilic interactions among proteins, lipids and the aqueous environment may explain why membranes lose physiological function at high temperature. Levitt (1980a) suggested that acclimation may result from an increase in protein thermostability by changes in protein three dimensional structure, such as increased hydrophobic bonds or the presence of stabilizing agents (cations or sugars). Increase in saturation of membrane fatty acids was also attributed to acclimation to high temperature in plants (Levitt, 1980a).

Synthesis of heat shock proteins is one of the responses by plants to elevated temperatures. Heat

pretreatments to plants or tissues can induce synthesis of new proteins, termed heat shock proteins (HSP) (Cooper and Ho, 1983; Key et al., 1981; Krishnan et al., 1989). Heat shock proteins were first found in the fruit fly, Drosophila melanogaster, following exposure to a sublethal high temperature and were accompanied by decreased levels of normal protein synthesis (Ashburner and Bonner, 1979). Increased HSP levels were also observed in roots of maize seedlings following a temperature shift from 25 to 40C (Cooper and Ho, 1983). Three size groups of HSP have been described (Necchi et al., 1987); high molecular mass from 70 to 103 kD, intermediate in the 32 to 62 kD range, and low mass proteins in the 16 to 17 kD range. Production of HSP is a transitory, induced response to high temperatures (Cooper and Ho, 1983). The induction of HSP is biphasic with an early group appearing immediately after heat shock exposure, and a late group of HSP which appear after a prolonged exposure period (Cooper and Ho, 1983). Various HSP have been found associated with several organelles including nuclear (Lin et al., 1984) and mitochondrial regions (Chou et al., 1989). When tissues were returned to normal growing temperatures, synthesis of HSP ceased with the restoration of normal protein synthesis and decreased HSP levels (Cooper and Ho, 1983). The number and types of high-temperature-induced HSP vary with genotype (Clarke and Critchley, 1990).

Synthesis of HSP resulted from the translation of HSP mRNAs at the higher temperatures (Key et al., 1981). According to Ho (1987), heat exposure activates the transcription of HSP genes, and represses the transcription of other genes. Activation of HSP genes results in elevated HSP mRNA levels leading to subsequent synthesis of HSP. In soybean seedlings, mRNAs coding for HSP became very abundant following a 2 h heat treatment (Kimpel et al., 1990). Heat shock gene expression was generally regulated at the transcription and translation levels (Burke et al., 1985).

High temperature treatments which induced HSP synthesis led to the development of thermotolerance (Kimpel and Key, 1985; Lindquist, 1986). A high correlation between the synthesis of HSP and thermotolerance level was observed in soybean seedlings (Lin et al., 1984). Kee and Nobel (1986) reported that shifting desert succulents from 30/20C (day/night) to 50/40C resulted in accumulation of two low molecular weight proteins and increased thermotolerance by 6 to 8C. The induction of HSP in response to high temperatures suggests that HSP provide a basis for plants to survive at a normally lethal temperature. However, germinating pollen (Herrero and Johnson, 1980; Xiao and Mascarenhas, 1985) and rat fibroblasts (Widlitz et al., 1986) were able to establish a thermotolerance response in the absence of HSP synthesis. Petko and Linquist (1986) reported that HSP 26, one of the major HSP of eukaryotic cells, was not required for thermotolerance in yeast. These observations indicated

that, in some plants, an increased thermotolerance level could be achieved without the synthesis of HSP.

Despite the asserted role of heat shock proteins in protecting plant tissues from injury induced by high temperature, there is little information about the molecular mechanism of action of HSP. The function of HSP in cellular thermal tolerance is still largely unknown.

Protein synthesis generally occurs through four major stages (Stryer, 1988). Protein synthesis can be repressed by inhibitors acting at various stages in the synthesis process. Treatment with protein synthesis inhibitors may be expected to modify HSP metabolism by blocking HSP synthesis. The role of HSP in thermotolerance has been examined in Dictyostelium (Loomis and Wheeler, 1980) and mammalian cells (Henle and Leeper, 1982). In these experiments, pretreatment with sublethal high temperature in the presence of cycloheximide blocked HSP synthesis and prevented the establishment of thermotolerance. However, Widelitz et al. (1986) reported that inhibition of HSP synthesis did not block the establishment of thermotolerance in rat fibroblasts in the presence of cycloheximide.

Water stress can damage plants through a variety of mechanisms. Protein synthesis (Dhindsa and Cleland, 1975), cell-wall extension (Hsiao, 1973) and enzyme activity (Hsiao, 1973) were greatly affected by secondary water stress. However, it is difficult to separate the effects of heat and water stress since high temperature stress is

usually accompanied by water stress in field-grown plants. High temperatures can increase evaporative demand and indirectly contribute to water deficiency. Even in the presence of adequate soil moisture, high temperature can reduce nutrient ion uptake (Davis and Lingle, 1961), photosynthesis (Bjorkman et al., 1980) and alter respiration and membrane permeability (Raison et al., 1980).

Mechanisms of heat and water stress injury to plants may be different, but tolerance to heat and water stress have been reported to be correlated (Levitt, 1980b; Sullivan, 1972). Heat stress has been used as a selection indicator of water stress resistance in grain sorghum (Sullivan and Ross, 1979), pasture grasses (Julander, 1945) and turfgrass (Wallner et al., 1982).

Exposure of plants to one stress may lead to the protection from another stress injury. Cloutier and Siminovitch (1982) reported that water stress conferred cold hardiness on several winter cereals. Water stress also has been associated with increased thermotolerance in plants (Wehner and Watschke, 1981; Julander, 1945). Levitt (1980a) proposed that most plants are less heat tolerant at high water contents. Bonham-Smith et al.(1987) reported that a 6 h progressive water stress from 0 to -1.25 MPa induced an elevated thermotolerance level in maize seedlings.

The mechanism of acclimation to high temperature induced by water stress is not clear. One of the possible mechanisms is the synthesis of HSP following water stress

treatment. Appearance and accumulation of heat shock proteins in water-stressed field cotton leaves were reported by Burke et al. (1985). In addition, increased heat tolerance following water stress was related to cell membrane stability (Blum and Ebercon, 1981) and accumulation of photosynthetic products (Julander, 1945). However, Becwar et al.(1983) reported that water stress pretreatment did not increase heat tolerance in turfgrass leaves.

Objectives

1. To determine the effects of acclimation temperature and duration on thermotolerance level of bell pepper leaves using excised pepper leaf disks.
2. To determine the effects of exogenous ethylene on thermotolerance of pepper leaf disks.
3. To determine the effects of protein synthesis inhibitors on acclimation to high temperature.
4. To determine the relationship between plant water potential and thermotolerance.

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CHAPTER II

HIGH TEMPERATURE ACCLIMATION IN BELL PEPPER LEAVES

Introduction

Plant growth and development are greatly affected by high temperature stress. Temperatures greater than the optimum growth temperature can cause injury to plants (Levitt, 1980), resulting in physiological disorders (Inaba and Crandall, 1988; Reuther et al., 1979), reduced yield (El Ahmadi and Stevens, 1979), decreased product quality (Treshow, 1970) and increased product decay (Lee and Yang, 1984).

Heat stress is a function of exposure temperature and duration (Levitt, 1980). High temperature can cause direct or indirect heat injury in plants (Levitt, 1980). Direct heat injury is induced by exposure to an extremely high temperature for seconds or minutes. Direct injury occurred during a 20 min exposure to 52.5C in excised sour orange roots (Ingram and Buchanan, 1981). Indirect heat injury occurs during prolonged exposure to moderately high temperatures. Pecan roots were killed by a 4 day exposure to 43C (Woodruff, 1934).

The temperatures causing direct or indirect injury to plants varied inversely with the exposure duration, with the critical temperature decreasing linearly as exposure time increased exponentially (Anderson et al., 1990; Ingram, 1985, 1986). Ingram (1985) developed a mathematical model to predict the critical exposure temperature for determining heat injury to plant tissues. The model has been used to describe the interaction of critical high temperatures and exposure times on membrane thermostability using electrolyte leakage data (Ingram, 1986; Anderson et al., 1990; Inaba and Crandall, 1988).

Several procedures to measure thermotolerance level have been developed. One method is to place plants in a growth chamber at an elevated temperature (Levitt, 1980). After a defined time period, the plants are moved to a greenhouse to observe symptoms of heat injury. Another method is to submerge small pieces or sections of plants (contained in test tubes with distilled water) into a temperature-controlled water bath at a series of temperatures for a defined period (Julander, 1945; Sullivan, 1972; Ingram and Buchanan, 1981). Alternatively, samples can be exposed to one temperature for varying periods of time (Anderson et al., 1990). Compared to heat killing temperature, heat killing time is a more sensitive criterion to evaluate plant heat tolerance (Chen et al., 1982).

Membrane properties are important for plants to survive and develop at high temperatures. Many important

physiological activities, including respiration and photosynthesis, take place in association with cell membranes. Electrolyte leakage has been used to estimate cell membrane thermostability in response to heat stress (Inaba and Crandall, 1988; Lester, 1985; Martineau et al., 1979). When plant tissues were exposed to heat stress, the plasmalemma was injured and permeability to solutes increased. The increased membrane injury was highly correlated with increased electrolyte leakage level in plant tissues (Martineau et al., 1978; Chen et al., 1982). Electrolyte leakage was a quantitative procedure to evaluate the degree of heat injury to plant tissues (Ingram and Buchanan, 1981). Sullivan (1972) used electrolyte leakage to identify genetic variation in heat tolerance in grain sorghum.

Plants are able to respond to high temperature with some degree of adaptation. Kramer (1980) defined acclimation of plants to heat stress as "the nonheritable modification of characters caused by exposure of plants to a mild climatic condition, such as warmer, or drier weather". Plants can acclimate as a result of pretreatments, including high temperature exposure (Wallner et al., 1982; Anderson et al., 1990; Schroeder, 1963), growth regulator application [Engelbrecht and Mothes, 1960 (cited from Leopold, 1964)], and moderate water stress (Julander, 1945; Wehner and Watschke, 1981). As a result of pretreatment, plants survived at temperatures that would normally be lethal. Pre-

treatment of 'Perlita' and 'Greenflesh' melon seedlings at 35C for 2 to 4 h ıncreased heat tolerance levels (Lester, 1986). According to Wallner et al.(1982), heat pretreatment (38C day/24C night for 24 h) administered to kentucky bluegrass delayed the killing time at 50C from 108 to 176 min. Anderson et al. (1990) reported that the thermotolerance level of pepper leaf disks was increased following exposure to 38C for 4 h.

The mechanism of acclimation to high temperature is complicated. Heat stress injury has been associated with alterations in membrane composition (Martineau et al., 1979; Lester, 1985), protein denaturation (Bernstam, 1978), and biochemical lesions, such as starvation (Levitt, 1980). Raison et al. (1980) proposed the following hypothesis: membrane proteins move toward the aqueous interface at low temperatures or toward the interior of the bilayer at high temperatures. The balance between the relative strengths of hydrophobic and hydrophilic interactions among proteins, lipids and the aqueous environment may explain why membranes lose physiological function at high temperature. Levitt (1980) suggested that acclimation may result from an increase in protein thermostability through changes in protein three dimensional structure, such as increased hydrophobıc bonds or the presence of stabilizıng agents (cations or sugars). Increased saturation of membrane fatty acids was also attributed to acclimation to high temperature in plants (Levitt, 1980).

Synthesis of heat shock proteins (HSP) may be involved in acclimation to high temperatures by plants. High temperature treatments which induced HSP synthesis led to the development of thermotolerance (Kimpel and Key, 1985; Lindquist, 1986). A high correlation between the synthesis of HSP and thermotolerance level was observed in soybean (Lin et al., 1984), maize (Cooper and Ho, 1983), desert succulents (Kee and Nobel, 1986) and wheat (Krishnan et al., 1989).

Our objectives were to evaluate the relationship between acclimation temperature and duration, and to determine the optimum acclimation treatment combination for bell pepper leaves.

Materials and Methods

'Early Calwonder' Pepper seeds (Capsicum annuum L.) were planted in 36-liter pots containing commercial potting soil (Fafard GP Mix, Springfield, Mass.) blended with 0.2 g/liter micro-nutrients (Micromax, Sierra Chemical, Milpitas, CA) in a growth chamber. Plants were monitored daily with watering based on soil color and pot weight. Irrigation water contained 1 g 20N-9P-17K soluble fertilizer/liter (Peters Professional, W.R. Grace & Co., Fogelsville, Pa.). The growth chamber was maintained at 23C (day)/20C (night) cycles with a 14 h photoperiod.

Disks (14 mm diameter) were punched from sixty- to eighty-day-old leaves with a cork borer and rinsed at least three times to remove the exogenous electrolytes. One disk was placed in each 25 mm x 150 mm test tube containing 0.5 ml distilled water.

The first series of experiments were conducted to evaluate the interaction of exposure temperature and duration during the acclimation process. Different exposure temperatures for each acclimation duration were chosen to compensate for the interaction between acclimation time and temperature. On each date, 120 leaf disks were randomly divided into 5 groups to receive one of the following acclimation treatments: 1) 1 h at 24, 34, 38, 42, or 46C; 2) 2 h at 24, 32, 36, 40, or 44C; or 3) 4 h at 24, 28, 32, 36, or 40C. Three subsamples with one disk per test tube were employed. Immediately following acclimation pretreatment, tubes containing leaf disks were placed in a water bath at 50.5C for 0, 1, 5, 10, 15, 30, 45 or 60 min. Tissue temperature was measured with a thermocouple threaded through an extra disk. Since tissue temperatures took nearly 3 min to equilibrate with bath temperature (± 0.3C), disks heat-treated for 1 min did not reach the target temperature of 50.5C. After high temperature treatments, 20 ml distilled water were added to each test tube. Tubes were held at 24C for 24 h on an orbital shaker in an incubator. Initial electrolyte leakage was measured with a conductance meter (Model 35, Yellow Springs Instrument Co., Yellow Springs,

Ohio). The tubes were then placed in an autoclave at 121C for 15 min. The final electrolyte leakage was measured after another 24 h incubation. Electrolyte leakage was calculated by dividing the initial leakage by the final leakage values.

The experiment was repeated on three dates for each acclimation duration. The experimental design was a 5 × 8 factorial arrangement with three replicates and three subsamples per treatment combination. The data were analyzed using the general linear models procedure (GLM) in PC-SAS (SAS Institute, 1985). The critical exposure time (Tmid) was calculated as the maximum electrolyte leakage plus the minimum electrolyte leakage divided by two.

In the second series of experiments, the optimum acclimation time for a 38C exposure was determined. Leaf disks were exposed to 38C for exposure durations of 0, 1, 2, 3 or 4 h. After 120 disks were punched and rinsed, one disk was placed in each of 120 test tubes. Test tubes were randomly divided into 5 groups: one group was placed in an incubator at 24C as control, the additional four groups were placed a in water bath at 38C for 1, 2, 3, or 4 h. Heat stress was conducted in a water bath at 50.5C for 0 to 60 min following acclimation treatments. After heat stress, electrolyte leakage was measured.

The experimental design was 5 × 8 factorial arrangement with 3 replicates and 3 subsamples per treatment combination. The experiment was repeated on three dates. The critical exposure time (Tmid) was calculated using a

modification of Ingram's equation (1986) with temperature replaced by time.

Results and Discussion

Interaction of acclimation temperature and duration. Thermotolerance of leaf disks was significantly affected by acclimation pretreatments. Leaf disks treated at 38C for 1 h exhibited a critical exposure time (Tmid) of 20 min at 50.5C (Fig. 1). The mean Tmid value for controls (24C) was 10 min. Acclimation temperatures of 42C and 46C reduced thermotolerance compared with controls. Critical exposure times of 27 and 28 min were observed in disks treated for 2 h at 36C and 40C, respectively (Fig. 2). Leaf disks treated at 32C also exhibited increased heat tolerance compared with controls. The data indicated that two-hour exposure at 36 or 40C induced a higher thermotolerance level than exposure to 32 and 44C. Leaf disks exhibited a Tmid of 25 min following 4 h exposure to 36C, which conferred a higher heat tolerance level compared with exposure to 24, 28, 32 and 40C for 4 h (Fig. 3). Thermostability of pepper leaves was increased by two-hour treatment durations at 36 or 40C. The increased thermotolerance level in pepper leaf disks was consistent with the report by Anderson et al. (1990) that excised pepper leaf disks were capable of acclimation to high temperature *in vitro* following exposure to 38C.

Figure 1. Critical exposure time (Tmid) for pepper leaf disks exposed to 50.5C for 0 to 60 min following acclimation pretreatments at 24, 34, 38, 42 and 46C for 1 h. Values are means ± standard error of nine measurements (three subsamples per treatment combination on each of three dates).

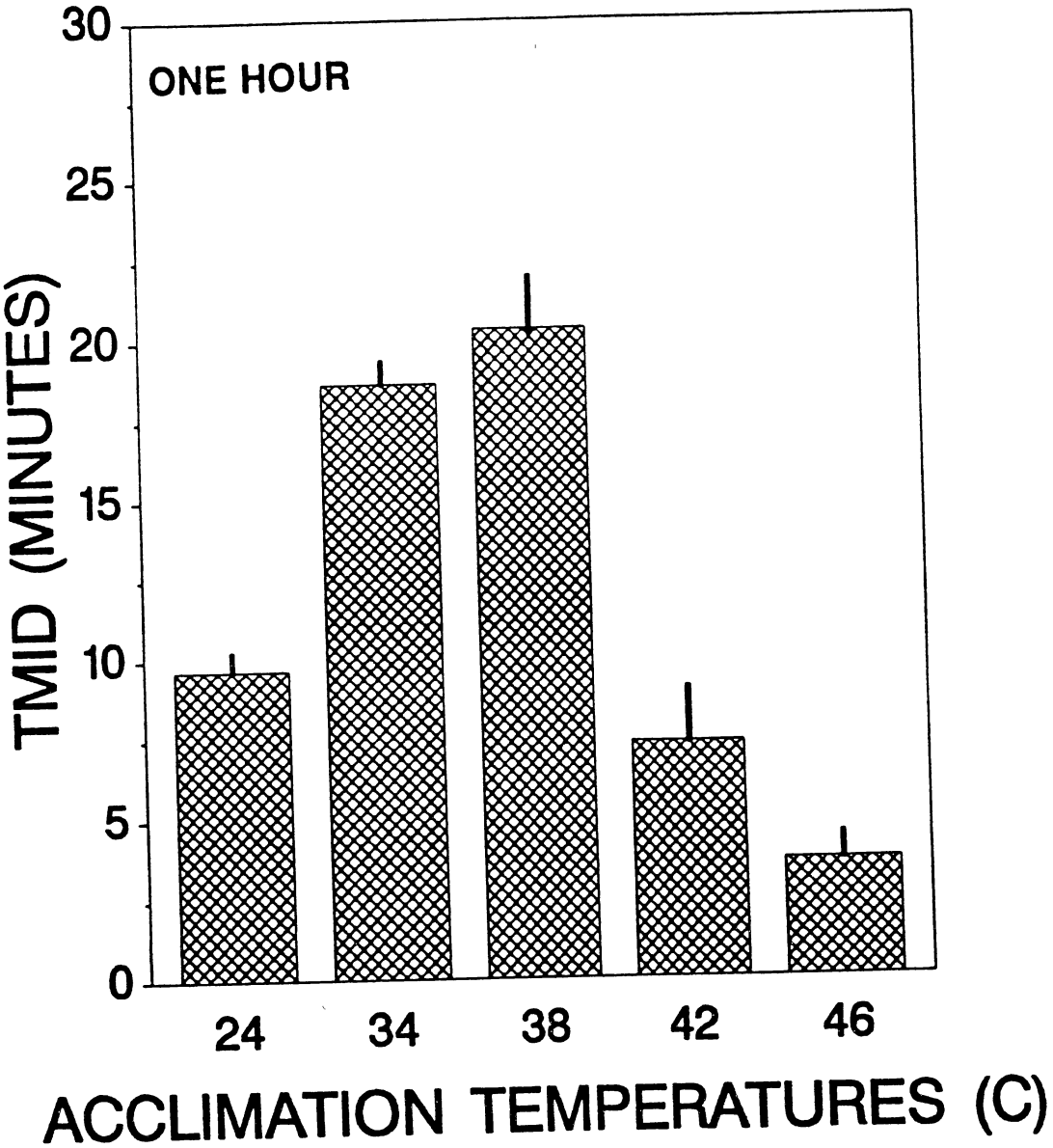

ONE HOUR
TMID (MINUTES)
30
25
20
15
10
5
0
24
34
38
42
46
ACCLIMATION TEMPERATURES (C)

Figure 2. Critical exposure time (Tmid) for pepper leaf disks exposed to 50.5C for 0 to 60 min following acclimation pretreatments at 24, 32, 36, 40 and 44C for 2 h. Values are means ± standard error of nine measurements (three subsamples per treatment combination on each of three dates).

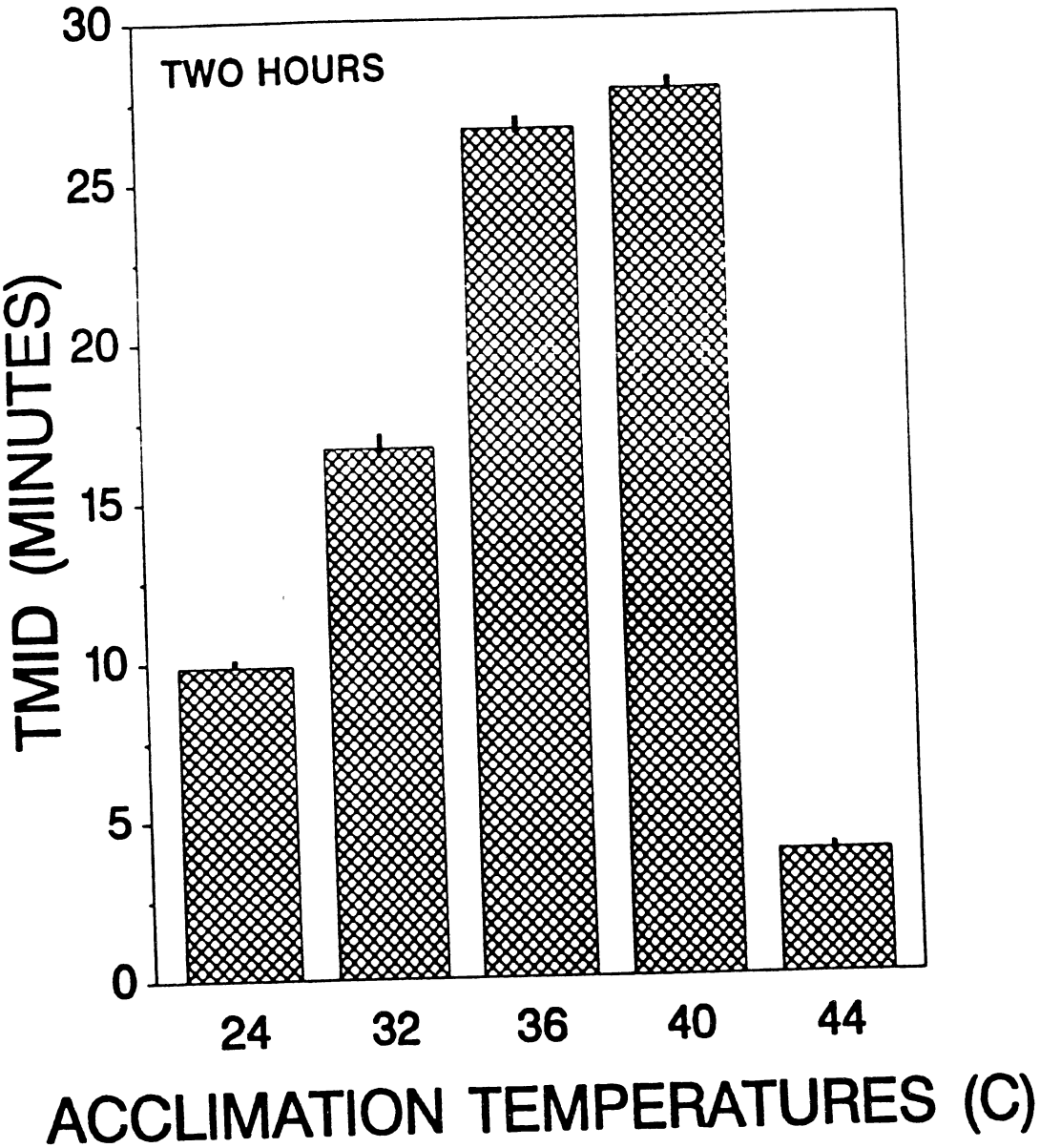
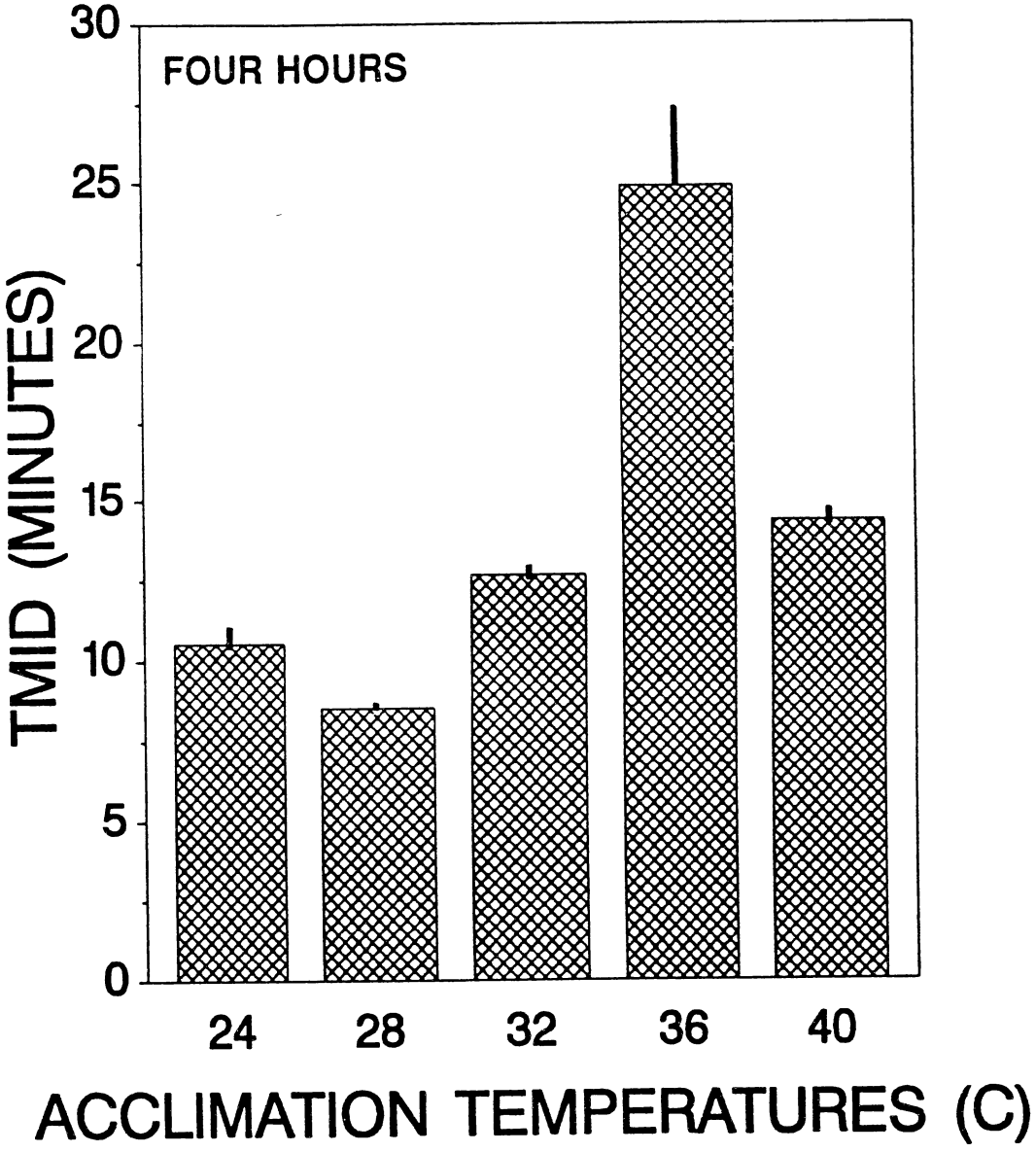
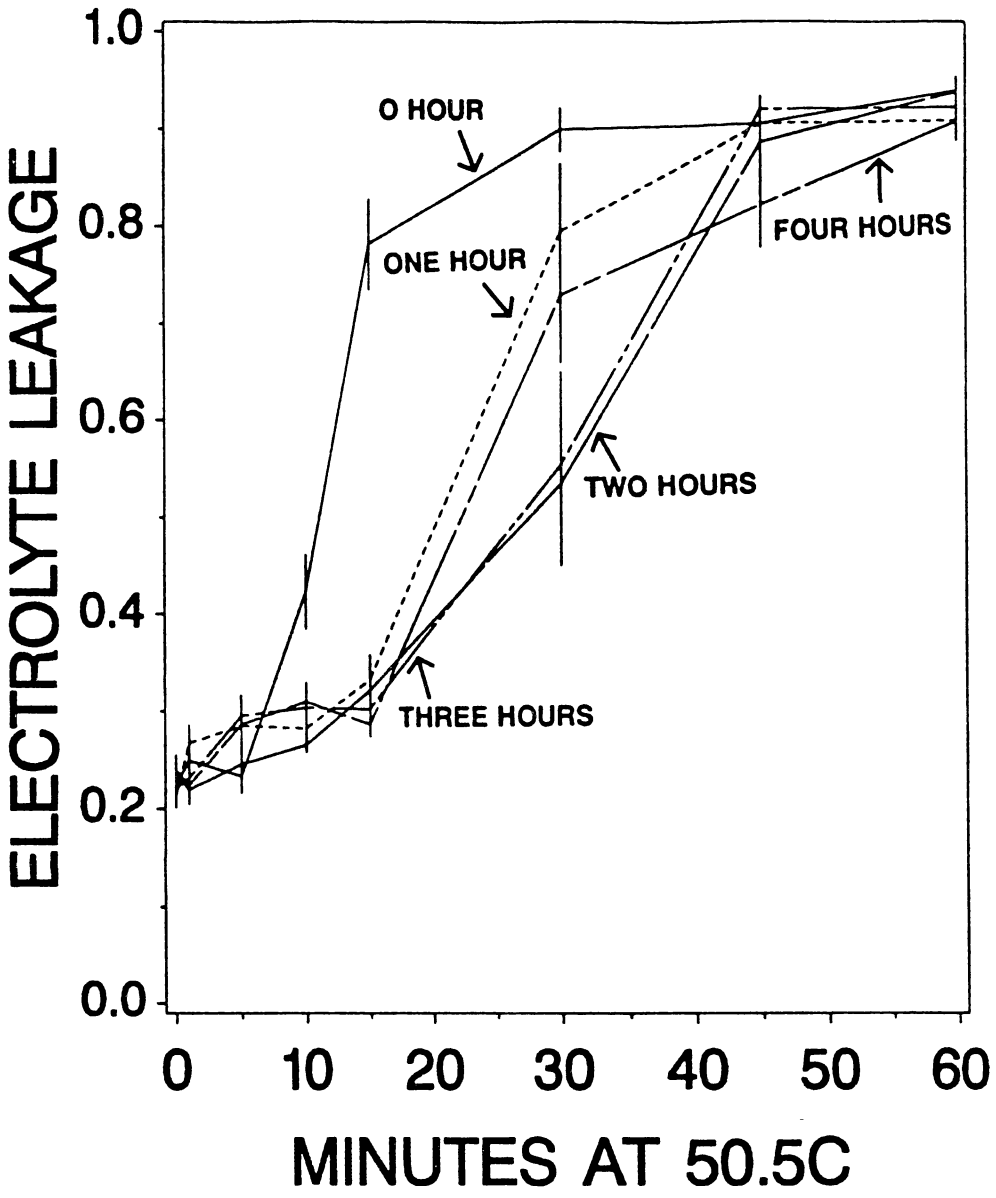

TWO HOURS
TMID (MINUTES)
30
25
20
15
10
5
0
24
32
36
40
44
ACCLIMATION TEMPERATURES (C)

Figure 3. Critical exposure time (Tmid) for pepper leaf disks exposed to 50.5C for 0 to 60 min following acclimation pretreatments at 24, 28, 32, 36 and 40C for 4 h. Values are means ± standard error of nine measurements (three subsamples per treatment combination on each of three dates).


FOUR HOURS
TMID (MINUTES)
30
25
20
15
10
5
0
24
28
32
36
40
ACCLIMATION TEMPERATURES (C)

Optimum acclimation duration at 38C. Acclimation treatments at 38C for 0, 1, 2, 3 or 4 h were chosen to determine the optimum exposure time to induce high temperature acclimation. Heat tolerance was influenced by all acclimation pretreatments at 38C from 1 to 4 h (Fig. 4). Control disks exhibited an abrupt increase in electrolyte leakage following 5 min heat stress at 50.5C. The disks pretreated at 38C for 1, 2, 3, or 4 h had longer critical exposure times compared with controls. The critical exposure time to cause heat injury in controls at 24C was 12 min. Leaf disks exposed to 38C for 2 and 3 h exhibited critical exposure times of 32 and 30 min, respectively. The optimum acclimation effect was induced by 2 h or 3 h pretreatment at 38C. The data indicated that following three hours acclimation treatment at 38C, continuous acclimation treatment did not increase the thermotolerance level in leaf disks.

Figure 4. Effect of acclimation pretreatment at 38C for 0, 1, 2, 3 or 4 h on electrolyte leakage from pepper leaves exposed to 50.5C for 0 to 60 min. Values are means ± standard error of nine measurements (three subsamples per treatment combination on each of three dates).


ELECTROLYTE LEAKAGE
1.0
0.8
0.6
0.4
0.2
0.0
0 HOUR
ONE HOUR
TWO HOURS
THREE HOURS
FOUR HOURS
0
10
20
30
40
50
60
MINUTES AT 50.5C

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CHAPTER III

EFFECT OF EXOGENOUS ETHYLENE ON THERMOTOLERANCE OF BELL PEPPER LEAF DISKS

Introduction

Ethylene is produced by all higher plants and regulates a wide variety of phenomenon in plant growth and development. Ethylene plays an important role in regulating fruit ripening (McGlasson, 1985), senescence (Kader, 1985) and abscission (Reid, 1985). Increased ethylene production is one of the responses of plants or detached plant organs to environmental stresses. Phaseolus vulgaris leaves exposed to 35 to 37.5C produced higher levels of ethylene than leaves held at 25C (Field, 1981). Ethylene production from water-stressed leaves increased with intensity of stress until the tissue was kılled (Kobayashi et al., 1981).

Accordıng to Lieberman et al. (1966), methionine is the initial substrate for ethylene synthesis. Ethylene production occurs via the following pathway: methionine to S-adenosylmethionine (SAM), to 1-aminocyclopropane-1-carboxylic acid (ACC), to ethylene (Adams and Yang, 1979). The step converting SAM to ACC is rate limiting in the biosynthetic pathway, with ACC synthase the main control

site of ethylene biosynthesis (Yang, 1980). Wang and Adams (1982) reported that application of cycloheximide, a protein synthesis inhibitor, blocked ethylene production in chilled cucumber by reducing the accumulation of ACC.

The production of stress ethylene was correlated with visible tissue injuries (Peiser and Yang, 1979; Kimmerer and Kozlowski, 1982). Stress-induced ethylene production has been used as an indicator of the extent of plant tissue injury from environmental stresses. Ben-Yehoshua and Aloni (1974) reported a three-fold increase in water-stress-induced ethylene production from detached leaves of 'Valencia' orange and a decreased amount of ethylene after the stress was relieved. Elevated ethylene production was also observed after exposure to sulfur dioxide (Peiser and Yang, 1979; Bressan et al., 1979), wounding (Saltveit and Dılley, 1978), chillıng (Wang and Adams, 1982) and freezing (Harber and Fuchigami, 1986).

Heat stress injury has been associated with alterations in membrane composition (Martineau et al., 1979; Lester, 1985), protein denaturation (Bernstam, 1978) and metabolic imbalances (Levitt, 1980). Electrolyte leakage was a reliable measure of changes in membrane permeability, serving as an indicator of high temperature injury (Chen et al., 1982; Inaba and Crandall, 1988; Martineau et al., 1979). Increased membrane permeability and elevated ethylene production were also observed in heat-stressed Phaseolus vulgaris (Field, 1981). According to Saltveit and Dilley

(1978), ethylene production increased with increasing temperature from 10C to 38C and then decreased sharply with increasing temperature in etiolated pea seedlings.

Functions of ethylene in various physiological processes were studied by applying exogenous ethylene to plant tissue (Abeles et al., 1989; Taylor and Gunderson, 1986). Abeles et al. (1989) reported that exogenous ethylene stimulated the synthesis of three peroxidases in cucumber cotyledon tissue. Ethylene enhanced mRNA synthesis resulting in de novo protein synthesis in potato tubers (Theologis and Giudice, 1979). Induction of new mRNA following ethylene treatment was observed by Christoffersen and Laties (1982) in carrots. Abeles and Holm (1966) found that abscission in bean explants occurred following the synthesis of RNA and proteins induced by ethylene treatment. The effect of ethylene on genetic regulation was confirmed by the observation that protein synthesis inhibitors, such as cycloheximide and actinomycin D, blocked abscission induced by ethylene (Abeles and Holm, 1966). The results indicated that exogenous application of ethylene altered gene expression which resulted in the production of new mRNA and concomitant synthesis of new proteins. Ethylene activated a particular part of the genome which triggered a series of biochemical reactions leading to a variety of physiological responses such as abscission (Reid, 1985) and senescence (Kader, 1985).

Yang (1985) presumed that for ethylene to act it must bind to an ethylene receptor which has a high affinity and specificity for ethylene. At least two approaches can be used to examine the effects of ethylene in plant physiological responses. One approach is to use ethylene action inhibitors such as carbon dioxide (Burg and Burg, 1967) or silver ions (Beyer, 1976) to counteract ethylene action by interfering with the binding site, which can block or delay physiological effects of ethylene. Inhibition of ethylene-induced sex expression in cotton flowers by silver ions and high CO_2 levels was observed by Beyer (1976). Ethylene action also can be studied by addition or removal of ethylene to regulate the ethylene level in the tissues (Yang, 1985).

Ethylene production from heat-stressed plant tissues is an indicator of tissue injury. However, it is not clear whether the increased ethylene production is related to an adaptive response to high temperature stress. Thus, our objective was to determine the effects of exogenous ethylene on heat tolerance of pepper leaves.

Materials and Methods

'Early Calwonder' pepper seeds (Capsicum annuum L.) were planted in 36-liter pots containing commercial potting soil (Fafard GP Mix, Springfield, Mass.) blended with 0.2 g/liter micro-nutrients (Micromax, Sierra Chemical, Milpitas, CA) in a growth chamber. Plants were monitored

daily with watering based on soil color and pot weight. Irrigation water contained 1 g 20N-9P-17K soluble fertilizer/liter. The growth chamber was maintained at 23C (day)/20C (night) cycles with a 14 h photoperiod.

Disks (14 mm diameter) were punched with a cork borer from sixty- to eighty-day-old leaves and rinsed at least three times to remove the exogenous electrolytes. Ten disks were placed in each of twenty vials (14.5 ml). The vials were randomly divided into two groups. To the control group, ambient air was flushed into the vials three times using a 10 ml syringe. To the ethylene-treated group, 96 ppm ethylene (in air) was flushed into the vials three times. After 4 hours incubation at 24C, the mean ethylene level in control vials was 0.18 ± 0.02 ppm and 76.66 ± 1.58 ppm in ethylene-treated samples.

After gas measurements, the leaf disks were transferred to test tubes to receive heat stress treatments in a temperature-controlled water bath at 50.5C for 0, 1, 5, 10, 15, 30, 45 or 60 min. Twenty ml distilled water were added to each test tube after heat stress treatments. Tubes were held at 24C on an orbital shaker in an incubator. Electrolyte leakage was measured after 24 h with a conductance meter (Model 35, Yellow Springs Instrument Co., Yellow Springs, Ohio), recording the results as the initial leakage. Tubes were then placed in an autoclave at 121C for 15 min. After another 24 h incubation, the final electrolyte leakage was measured. Electrolyte leakage data were reported

as the initial leakage divided by the final leakage. Critical exposure time was defined as the duration at 50.5C which resulted in an electrolyte leakage value equal to the maximum electrolyte leakage plus the minimum electrolyte leakage divided by two.

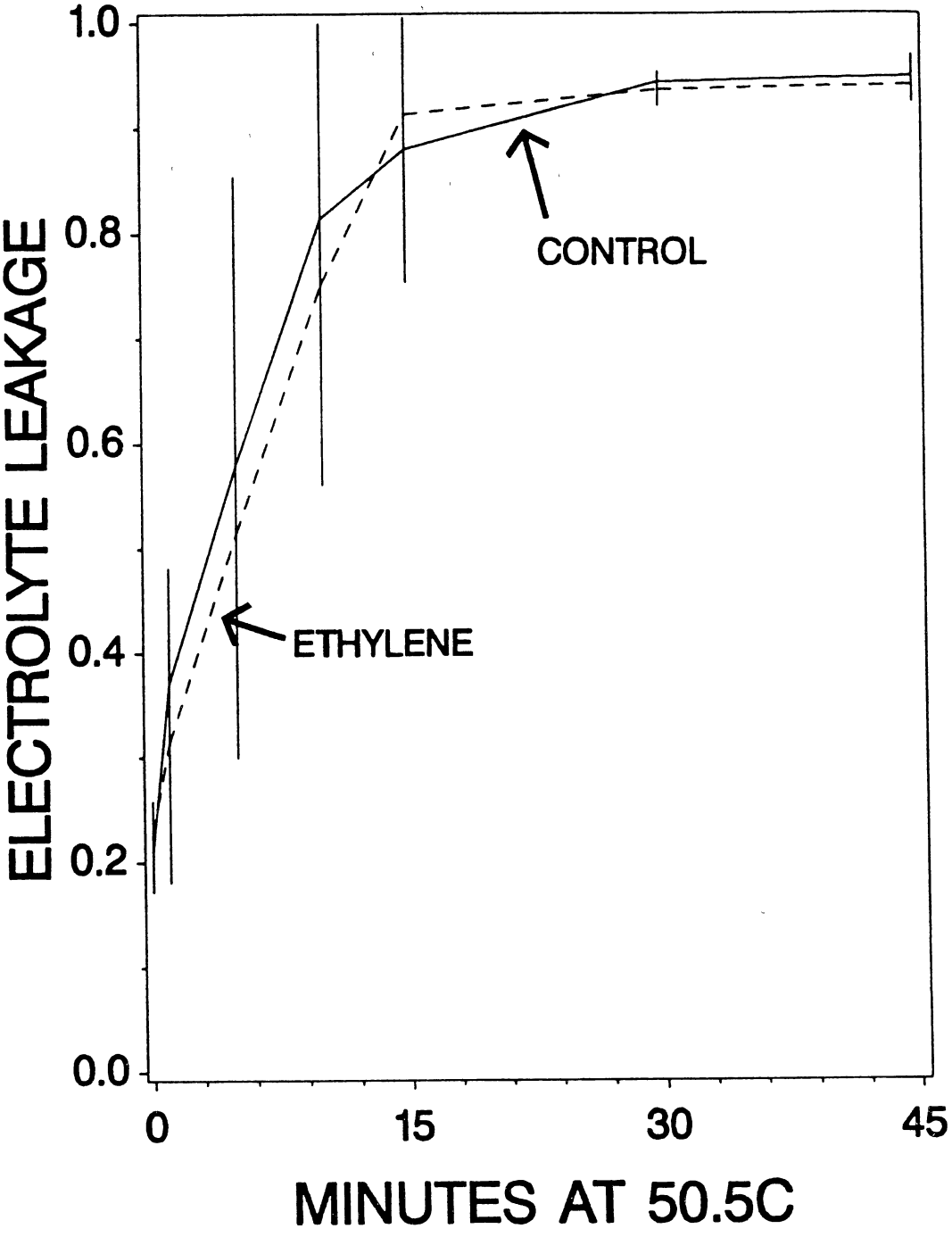
The experiment was repeated on 4 dates. The experimental design was a 2 × 8 factorial arrangement with four replicates and three subsamples per treatment combination. The data were analyzed using the general linear models procedure (GLM) in PC-SAS (SAS Institute, 1985).

Results and Discussion

The overall pattern of electrolyte leakage was similar in ethylene-treated disks and controls (Fig. 5). The critical exposure times at 50.5C for ethylene-treated disks and controls were 5 and 6 min, respectively. Since no significant differences in electrolyte leakage were observed between ethylene-treated and control leaf disks, it appears that exogenous ethylene did not affect the thermotolerance of pepper leaves.

Based on the observation that ethylene treatment failed to affect thermotolerance in pepper leaves, two interpretations could be made. First, elevated ethylene production from plant tissues exposed to temperatures that trigger acclimation is not related to heat stress resistance. Secondly, the lack of effect of ethylene on high

Figure 5. Electrolyte leakage from ethylene-pretreated (76.7 ± 1.6 ppm) and control pepper leaf disks following exposure to 50.5C. Values are means ± standard error of twelve measurements (three subsamples per treatment combination on each of four dates).


1.0
0.8
0.6
0.4
0.2
0.0
ELECTROLYTE LEAKAGE
CONTROL
ETHYLENE
0
15
30
45
MINUTES AT 50.5C

temperature acclimation could have resulted from difficulty in penetrating to the site of action.

In conclusion, the results of our experiment indicated that thermotolerance of pepper leaf disks was not affected by exogenous ethylene. The data suggested that at least some plant physiological processes are not responsive to exogenous ethylene.

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CHAPTER IV

THE EFFECTS OF PROTEIN SYNTHESIS INHIBITORS ON HIGH TEMPERATURE ACCLIMATION IN BELL PEPPER LEAVES

Introduction

Plants can acclimate to high temperature following pretreatments at sublethal temperatures (Anderson et al., 1990; Lester, 1986; Schroeder, 1963). Heat-pretreatments to plants or tissues can induce synthesis of new proteins, termed heat shock proteins (HSP) (Cooper and Ho, 1983; Key et al., 1981; Krishnan et al., 1989). Synthesis of HSP is considered an adaptive response by plants to tolerate heat stress.

Heat shock proteins were first found in the fruit fly, Drosophila melanogaster, following exposure to sublethal high temperature and were accompanied by decreased levels of normal protein synthesis (Ashburner and Bonner, 1979). Increased HSP levels were also observed in roots of maize seedlings following a temperature shift from 25 to 40C (Cooper and Ho, 1983). Three size groups of HSP have been described (Necchi et al., 1987); high molecular mass from 70 to 103 kD, intermediate in the 32 to 62 kD range, and low mass proteins in the 16 to 17 kD range. Production of HSP is a transient, induced response to heat stress at sublethal

high temperatures (Cooper and Ho, 1983). The induction of HSP is biphasic with an early group appearing immediately after heat shock exposure, and a late group of HSP appearing after a prolonged exposure period (Necchi et al., 1987). Various HSP have been found associated with several organelles including nuclei (Lin et al., 1984) and mitochondria (Chou et al., 1989). When tissues were returned to normal growing temperatures, synthesis of HSP ceased with the recovery of normal patterns of protein synthesis and the decline of HSP mRNAs (Cooper and Ho, 1983). The number and types of high temperature-induced HSP vary with genotype (Clarke and Critchley, 1990).

Synthesis of HSP resulted from the translation of HSP mRNAs at the higher temperatures (Key et al., 1981). Heat exposure activates the transcription of HSP genes, and represses the transcription of other genes (Ho, 1987). Activation of HSP genes results in elevated HSP mRNA levels leading to subsequent synthesis of HSP. In soybean seedlings, mRNAs encoding for HSP became very abundant following a 2 h heat treatment (Kimpel et al., 1990). Heat shock gene expression was generally regulated at the transcription and translation levels (Burke et al., 1985).

High temperature treatments which induce HSP synthesis generally lead to the development of thermotolerance. A high correlation between synthesis of HSP and thermotolerance was observed in soybean seedlings (Lin et al., 1984). Kee and Nobel (1986) reported that shifting desert succulents from

30/20C (day/night) to 50/40C resulted in accumulation of two low molecular weight proteins and increased heat tolerance by 6 to 8C. The induction of HSP in response to high temperatures suggests that HSP may provide a basis for plants to withstand a normally lethal temperature. However, germinating pollen (Herrero and Johnson, 1980; Xiao and Mascarenhas, 1985) and rat fibroblasts (Widlitz et al., 1986) were able to establish a thermotolerance response in the absence of HSP synthesis. Petko and Linquist (1986) reported that HSP 26, one of the major HSP of eukaryotic cells, was not required for thermotolerance in yeast. These observations indicated that, in some plants, an increased thermotolerance level could be achieved without the synthesis of HSP.

Pretreatments at non-lethal temperatures leading to increased thermotolerance have been associated with increased HSP synthesis (Lin et al., 1984; Lindquist, 1986). However, these correlative data did not demonstrate that accumulation of HSP was directly responsible for protection from heat injury. There is little information available concerning the molecular mechanism of action of HSP. The function of HSP in cellular thermotolerance is still largely unknown.

Protein synthesis generally occurs through four major stages (Stryer, 1988): 1) amino acid activation, 2) initiation of the polypeptide chain, 3) elongation, and 4) termination of translation. Protein synthesis can be

repressed by various inhibitors acting at various stages in the synthesis process.

Treatment with protein synthesis inhibitors may be expected to modify HSP metabolism by blocking HSP synthesis. The role of HSP in thermotolerance had been examined in experiments with Dictyostelium (Loomis and Wheeler, 1980) and mammalian cells (Henle and Leeper, 1982). In these experiments, pretreatment at sublethal high temperatures in the presence of cycloheximide blocked HSP synthesis and prevented the establishment of thermotolerance. However, Widelitz et al. (1986) reported that inhibition of HSP synthesis by cycloheximide did not inhibit the establishment of thermotolerance in rat fibroblasts. Our objective was to provide additional information on the role of protein synthesis in acclimation to heat stress. Several protein synthesis inhibitors, acting at various stages in protein synthesis, were administered to pepper leaf disks prior to acclimation treatments.

Materials and Methods

'Early Calwonder' pepper seeds (Capsicum annuum L.) were planted in 36-liter pots containing commercial potting soil (Fafard GP Mix, Springfield, Mass.) blended with 0.2 g/liter micro-nutrients (Micromax, Sierra Chemical, Milpitas, CA). Plants were monitored daily with watering based on soil color and pot weight. Irrigation water contained 1 g 20N-9P-17K soluble fertilizer/liter. The

growth chamber was maintained at 23C (day)/20C (night) cycles.

Disks (14 mm diameter) were punched with a cork borer from sixty- to eighty-day-old leaves and rinsed at least three times to remove the exogenous electrolytes. Three hundred and thirty-six leaf disks were randomly divided into seven groups to receive various protein synthesis inhibitor treatments. Each group containing 48 leaf disks was vacuum-infiltrated in one of seven protein synthesis inhibitor solutions [distilled water control, anisomicin (500 μM), aurintricarboxylic acid (100 μM), cycloheximide (1 μM), ethionine (500 μM), norvanine (1000 μM) and puromycin (10 μM)]. All inhibitors were dissolved in distilled water except aurintricarboxylic acid, which was first dissolved in ethanol (10% V/V) prior to dilution with distilled water. Effective concentrations of each inhibitor without toxic effects, based on electrolyte leakage, were determined in preliminary experiments (data not presented). Disks were held at 24C for 30 min in the inhibitor solutions after infiltration to ensure uptake. Disks were then transferred to test tubes contaıning one-half ml of distilled water. Tubes containing one leaf disk each were either placed in an incubator at 24C as a control, or in a water bath at 38C for 2 h to induce acclimation.

Disks then received heat stress treatments in a temperature-controlled water bath at 50.5C for 0, 1, 5, 10, 15, 25, 35 or 50 min. Thermotolerance of treated pepper leaf

disks was evaluated using electrolyte leakage. Twenty ml distilled water were added to each test tube following heat treatment. Tubes were held at 24C for 24 h on an orbital shaker in an incubator. Electrolyte leakage was measured with a conductance meter (Model 35, Yellow Springs Instrument Co., Yellow Springs, Ohio), recording the results as the initial leakage. The tubes were then placed in an autoclave at 121C for 15 min. After another 24 h incubation, the final electrolyte leakage was measured. Electrolyte leakage data were reported as initial leakage divided by the final leakage. Critical exposure time (Tmid) was defined as the duration at 50.5C which resulted in an electrolyte leakage value equal to the maximum electrolyte leakage plus the minimum leakage divided by two.

The experiment was repeated on four dates. The experimental design was a 2 × 7 × 8 factorial arrangement with four replications and three subsamples per treatment combination. The data were analyzed using the general linear models procedure (GLM) in PC-SAS (SAS Institute, 1985).

Results and Discussion

Thermotolerance levels of non-acclimated and acclimated leaf disks were significantly different in the absence of protein synthesis inhibitors. Non-acclimated disks were severely injured following an 8 min exposure to 50.5C (Table 1). In contrast, a critical exposure time of 28 min was observed in the acclimated disks. The data indicated that a

sublethal temperature treatment at 38C for 2 h conferred a higher thermotolerance level compared to 24C, in the absence of inhibitors.

Significant differences in critical exposure time among the disks treated with inhibitors and distilled water were observed following acclimation treatment at 38C (Table 1). Acclimation treatment at 38C did not increase the thermotolerance levels of the disks treated with protein synthesis inhibitors. In contrast, the disks infiltrated with distilled water exhibited an increased level of thermal tolerance following 2 h acclimation at 38C. The results suggested that protein synthesis inhibitors blocked high temperature acclimation in pepper leaf disks. Cycloheximide is a glutarimide antibiotic which can inhibit initiation, elongation and termination stages in the protein synthesis process (Vazquez, 1979). Puromycin acts by interrupting polypeptide chain elongation as a result of a structure similar to aminoacyl-tRNA (Stryer, 1988). Aurintricarboxylic acid has been widely used to block protein synthesis initiation by interacting with small ribosome subunits (Marcus et al., 1970). Anisomycin suppresses protein synthesis by specifically blocking peptide bond formation and acting on the termination step (Grollman, 1967). Both ethionine and norvanine act at the level of amino acid activation (Vazquez, 1979). Even though applied inhibitors acted on different protein synthesis stages, they resulted

Table 1. Critical exposure times (Tmid) for pepper leaf disks infiltrated with water or protein synthesis inhibitors prior to acclimation pretreatments at 24C or 38C for 2 h. Leaf disks were exposed to heat stress at 50.5C for 0 to 50 minutes following acclimation pretreatments. Means ± standard error of four replications with three subsamples per treatment combination on each date are presented.

	Tmid (min)[z]	
	Pretreatment temperature(C)	
	24	38
Distilled water	8	28
Anisomycin (500 µM)	6	8
Aurintricarboxylic acid (100 µM)	5	5
Cycloheximide (1 µM)	6	8
Ethionine (500 µM)	6	7
Norvanine (1000 µM)	8	5
Puromycin (10 µM)	5	7

[z]Tmid values were calculated as the duration at 50.5C which resulted in an electrolyte leakage value equal to the maximum electrolyte leakage plus the minimum leakage divided by two.

in the same effect that acclimation and, conceivably, HSP synthesis was blocked.

Electrolyte leakage values from controls and disks infiltrated with inhibitors, then held at 24C, were not significantly different. The results indicated that no measurable toxic effect was involved in the action of inhibitors on the acclimation process.

The effectiveness of protein synthesis inhibitors in preventing increased thermotolerance in leaf disks exposed to 38C provided additional support that protein synthesis is involved in the high temperature acclimation process. Either HSP synthesis or another process blocked by inhibitors appeared to have a fundamental role in high temperature acclimation in pepper leaves.

This study did not provide direct evidence that HSP were not produced in the disks treated with protein synthesis inhibitors. Further experiments must be carried out to obtain direct evidence that HSP are responsible for the acquisition of thermal tolerance in pepper leaves using tools such as two dimensional SDS polyacrylamide gel electrophoresis. Additional challenges include the elucidation of the mechanism(s) of HSP action.

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CHAPTER V

EFFECT OF WATER STRESS ON TOLERANCE TO HIGH TEMPERATURES IN BELL PEPPER LEAVES

Introduction

Plant growth and development are greatly affected by water deficit stress. Many metabolic processes, including protein synthesis (Bewley et al., 1983; Dhindsa and Cleland, 1975), cell-wall extension (Hsiao, 1973), photosynthesis (Hsiao, 1973) and enzyme activity (Hsiao, 1973), were inhibited by water stress. Cell extensibility was increased during water stress (Acevedo et al., 1971). However, it is difficult to separate the effects of heat and water stress under field conditions since high temperature stress is usually accompanied by a secondary water stress. High temperatures can increase evaporative demand and indirectly contribute to water deficiency. Even in the presence of adequate soil moisture, high temperature can reduce nutrient ion uptake (Davis and Lingle, 1961), photosynthesis (Bjorkman et al., 1980) and alter respiration and membrane permeability (Raison et al., 1980).

Mechanisms of heat and water stress injury to plants may be different, but tolerance to heat and water stress have been reported to be correlated (Levitt, 1980; Sullivan,

1972). Heat stress has been used as a selection indicator for water stress resistance in grain sorghum (Sullivan and Ross, 1979), pasture grasses (Julander, 1945) and turfgrass (Wallner et al., 1982). However, Blum and Ebercon (1981) reported that drought and heat tolerance were not correlated in wheat.

Exposure of plants to one stress may lead to the protection from another stress. Cloutier and Siminovitch (1982) reported that water stress conferred cold hardiness on several winter cereals. Water stress also has been associated with increased thermotolerance in plants (Wehner and Watschke, 1981; Julander, 1945). Levitt (1980) proposed that most plants are less heat tolerant at high water contents. Bonham-Smith et al.(1987) reported that a 6 h progressive water stress from 0 to -1.25 MPa induced an elevated thermotolerance level in maize seedlings.

The mechanism of acclimation to high temperature is complicated. One of the possible mechanisms involves the synthesis of heat shock proteins (HSP) following sublethal stress. HSP can protect cells and tissues from death that might otherwise be caused by high temperatures. Synthesis and accumulation of heat shock proteins in water-stressed, field-grown cotton leaves were reported by Burke et al. (1987). Increased heat tolerance following water stress was related to cell membrane stability (Blum and Ebercon, 1981) and accumulation of photosynthetic products (Julander, 1945). However, Becwar et al.(1983) reported that water

stress pretreatment did not increase heat tolerance in turfgrass leaves.

Many important physiological activities, such as respiration and photosynthesis, take place in association with plant membranes. Membrane properties are important for plants to survive and develop at high temperatures. Electrolyte leakage has been used as an indicator of cell membrane stability in response to heat stress (Chen et al., 1982; Inaba and Crandall, 1988; Martineau et al., 1979). Sullivan and Ross (1979) used electrolyte leakage to identify genetic variation in heat tolerance in grain sorghum. Stress-induced ethylene production has also been used as an indicator of plant tissue injury (Harber and Fuchigami, 1986; Kobayashi et al., 1981; Peiser and Yang, 1979). Ben-Yehoshua and Aloni (1974) reported that an elevated ethylene level was induced in water-stressed orange leaves and was followed by a decreased amount of ethylene after the stress was relieved. According to Saltveit and Dilley (1978), ethylene production increased with increasing temperature from 10C to 38C and then decreased sharply in etiolated pea seedlings. Ethylene production was judged a reliable indicator for determining the extent of tissue injury from environmental stresses (Tingey, 1980). Ethylene was stimulated by high temperatures, possibly due to an altered three dimensional structure of membrane-bound enzymes (Field, 1981). Relative ethylene and ethane production rates were related to the extent of membrane injury induced by stresses (Kobayashi et al., 1981).

The degree of stress-induced tissue injury has been estimated from the ratio of ethane and ethylene (Harber and Fuchigami, 1986; Bressan et al., 1979). A decreased ethylene:ethane ratio was observed in cucumber leaf tissues (Bressan et al., 1979) and sugar beet leaf tissues (Elstner and Konze, 1976) following exposure to sulfur dioxide and freezing, respectively.

Our objective was to determine the effect of water stress on thermotolerance of pepper leaves by measuring electrolyte leakage, ethylene and ethane production following water and heat stress.

Materials and Methods

'Early Calwonder' pepper seeds (Capsicum annuum L.) were planted in 36-liter pots containing commercial potting soil (Fafard GP Mix, Springfield, Mass.) blended with 0.2 g/liter micro-nutrients (Micromax, Sierra Chemical, Milpitas, CA) in a growth chamber. Plants were monitored daily with watering based on soil color and pot weight. Irrigation water contained 1 g 20N-9P-17K soluble fertilizer /liter. The growth chamber was maintained at 23C (day)/20C (night) cycles with a 14 h photoperiod.

Water stress. Seven hundred eighty-four leaf disks (14 mm diameter) were punched with a cork borer from sixty- to eighty-day-old leaves. Pre-weighed leaf disks were subjected to water stress treatments at 24C in a lighted incubator (approximately 85 $\mu E \cdot m^{-2} \cdot s^{-1}$). Disks were air dried for 0,

30, 60, 90, 120, 150, or 180 min. Disks were removed from the incubator at each time interval and weighed in order to calculate water loss. Disk water potential from each drying exposure interval was also determined at 30C using a Wescor HP-115 water potential data system (Wescor, Logan, Utah) and leaf cutter psychrometer (J.R.D. Merrill, Logan, Utah).

Heat stress. Following removal of leaf disks from the incubator, disks were transferred to test tubes containing one-half ml distilled water and placed in a temperature-controlled water bath at 50.5C for 0, 1, 5, 10, 15, 30, 45 or 60 min. Each test tube contained 13 leaf disks.

Ten disks were blotted and transferred to a 14.5 ml vial for ethylene and ethane measurements. After 24 h incubation at 24C in an incubator, 1 ml gas was drawn from each vial for injection into a Tracor 540 gas chromatograph (Model 540, Tracor Inc., Austin, TX) equipped with a flame ionization detector.

Twenty ml distilled water was added to each test tube for electrolyte leakage determination. Three subsamples were measured with one disk per test tube. Tubes were held in an incubator at 24C. Electrolyte leakage was measured after 24 h with a conductance meter (Model 35, Yellow Springs Instrument Co., Yellow Springs, Ohio), recording the results as the initial leakage. Tubes were then placed in an autoclave at 121C for 15 min. Final electrolyte leakage was measured after another 24 h incubation. Electrolyte leakage data were reported as initial leakage divided by the final

leakage. Critical exposure time was defined as the duration at 50.5C which resulted in an electrolyte leakage value equal to the maximum electrolyte leakage plus the minimum leakage divided by two.

Since tissues were severely injured following 150 and 180 min drying treatments, ethylene, ethane and electrolyte leakage data were not presented. In addition, only the data for 0 to 45 min heat exposure times at 50.5C were presented.

A factorially arranged, completely randomized design was used with drying treatments at seven levels (including control) and heat exposure time at eight levels. Each treatment combination contained three observations for electrolyte leakage and one observation for gas evolution. The experiment was repeated on three dates. Data were analyzed using the general linear models procedure of PC-SAS (SAS Institute, 1985).

Results and Discussion

Water potentials of leaf disks were consistent with leaf weight loss as air drying durations increased (Fig. 6). The effects of water stress on thermotolerance of pepper leaf disks were determined following exposure to 50.5C for 0 to 60 min.

Leaf disks exhibited a greater increase in electrolyte leakage (Fig. 7) and ethane:ethylene ratio (Fig. 8) following exposure to water stress compared with control disks. Electrolyte leakage from control disks increased with

Figure 6. Water potential and weight loss from pepper leaf disks following air drying for 0 to 180 min. Values are means ± standard error of three replications.

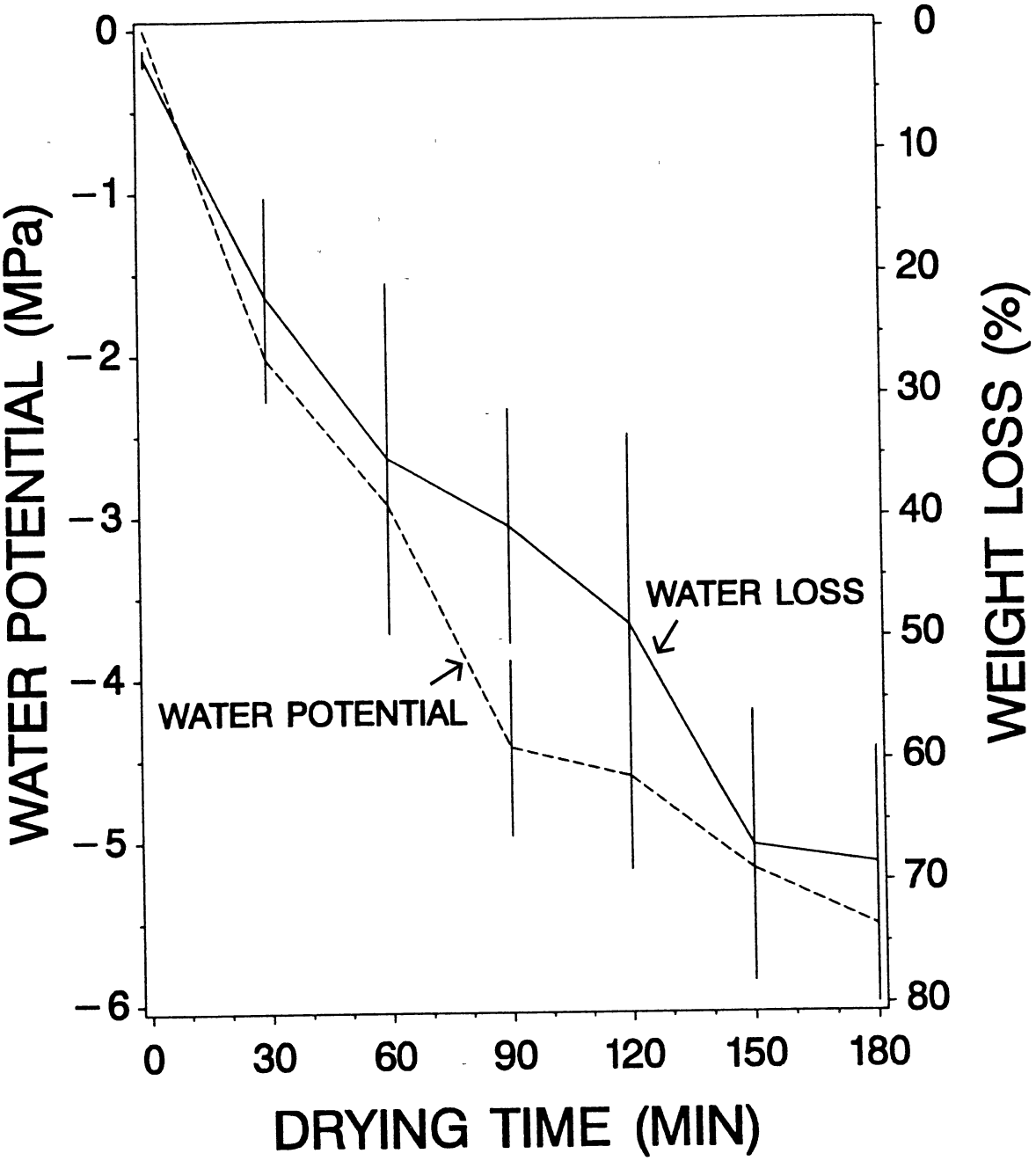

WATER POTENTIAL (MPa)
0
−1
−2
−3
−4
−5
−6
WEIGHT LOSS (%)
0
10
20
30
40
50
60
70
80
WATER LOSS
WATER POTENTIAL
0
30
60
90
120
150
180
DRYING TIME (MIN)

Figure 7. Electrolyte leakage from heat stress treated pepper leaf disks following air drying at 24C for 0 (-0.17 MPa), 30 (-1.64 MPa), 60 (-2.64 MPa), 90 (-3.06 MPa), or 120 min (-3.65 MPa). Values are means ± standard error of nine measurements (three subsamples per treatment combination on each of three dates).

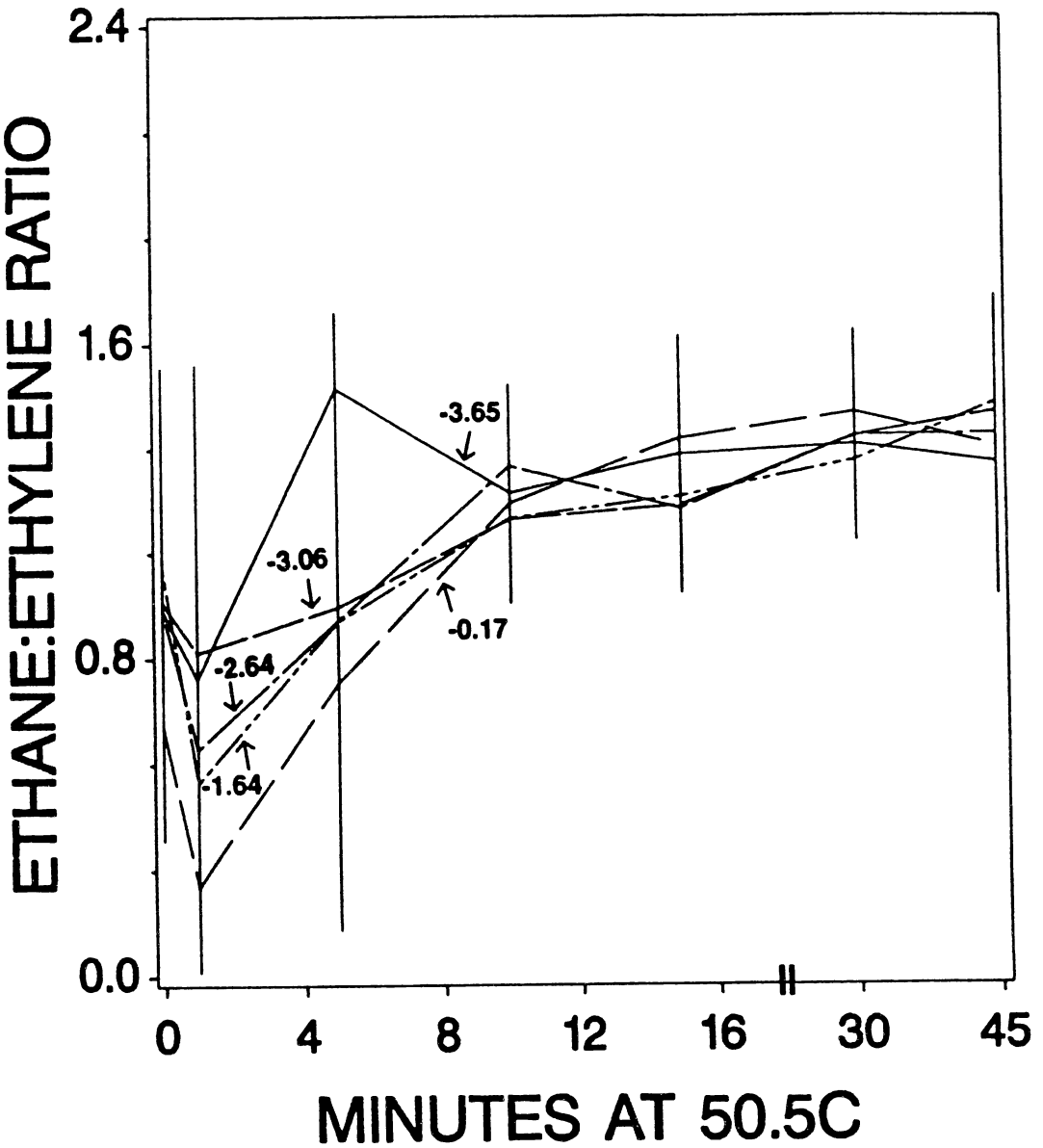
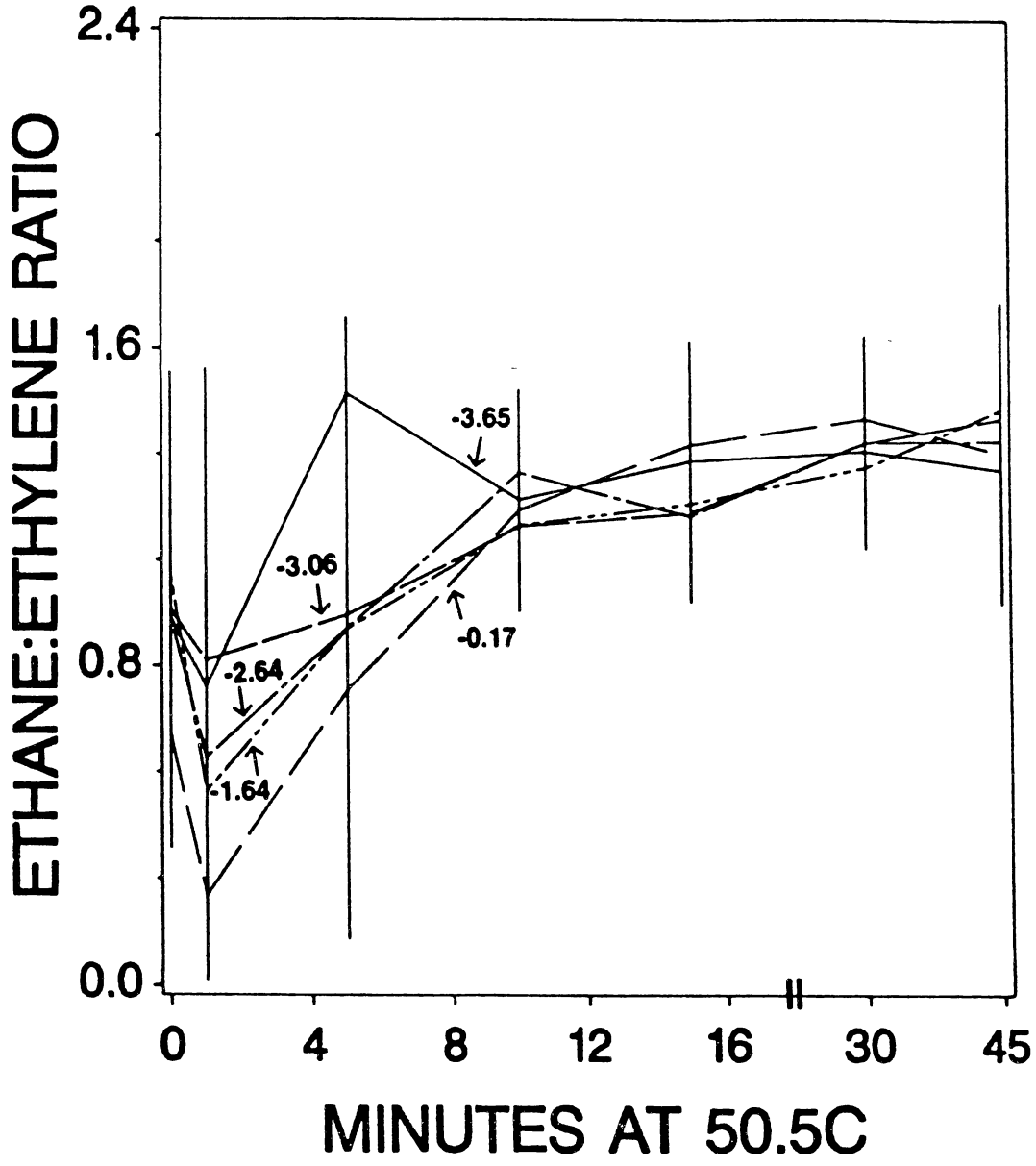

2.4
1.6
0.8
0.0
ETHANE:ETHYLENE RATIO
-3.65
-3.06
-2.64
-1.64
-0.17
0
4
8
12
16
30
45
MINUTES AT 50.5C

Figure 8. Ethane:ethylene ratio from heat-stress-treated pepper leaf disks following air drying at 24C for 0 (-0.17 MPa), 30 (-1.64 MPa), 60 (-2.64 MPa), 90 (-3.06 MPa), or 120 min (-3.65 MPa). Ethylene and ethane values were transformed to $(nl \cdot g^{-1}h^{-1} + 0.5)^{-2}$ to compensate for zero values (nondetectable amounts). The values represent means ± standard error of three replications.


2.4
1.6
0.8
0.0
ETHANE:ETHYLENE RATIO
-3.65
-3.06
-2.64
-1.64
-0.17
0
4
8
12
16
30
45
MINUTES AT 50.5C

exposure time from 0 to 30 min. The critical exposure time (Tmid) for control disks was 12 min. Shorter critical exposure times for water-stress-treated disks indicated that water stress had disturbed cell membrane structure prior to heat stress, compared with controls.

A large increase in ethylene evolution from control disks was observed with increasing exposure time from 0 to 1 min at 50.5C. Ethylene production in the controls reached a peak of 14 $nl \cdot g^{-1} \cdot h^{-1}$ after 1 min at 50.5C and then declined as exposure time increased. Little ethylene production was detected after 10 min. The rate of ethylene production from all water-stress-treated disks increased less abruptly than controls. Ethane evolution from water-stressed disks increased more rapidly than control disks. The rate of ethane production in control disks from 0 to 5 min was 0.17 $nl \cdot g^{-1} \cdot h^{-1}$, while water-stressed disks had sharply increasing ethane evolution rates after 0 to 5 min at 50.5C.

Disks with a more negative water potential had a higher ethane:ethylene ratio, indicating a decrease in ethylene production and an increase in ethane production. Ethane evolution increased following the decrease of ethylene, which indicated an increased tissue injury level with continuous exposure to stresses (Kimmerer and Kozlowski, 1982). Decreased ethylene evolution at high temperatures could be interpreted as a loss of membrane integrity (Lieberman, 1979). Release of ethane indicated that tissues had been severely injured (Peiser and Yang, 1979). Ethylene

and ethane were not produced by the same mechanism. Methionine is the precursor for ethylene synthesis (Lieberman et al., 1966). Ethane is produced from linolenic acid (John and Curtis, 1977).

In this study, water-stress-treated leaf disks were killed in less than 4 min when exposed to 50.5C, while the critical exposure time for leaf disks not water-stressed was 12 min. The data indicated that water stress did not confer thermotolerance to pepper leaves. On the contrary, heat injury in leaf disks was intensified following water stress. The result was consistent with the finding by Becwar et al. (1983) that decreased thermotolerance in turfgrass leaves was induced by water stress treatment (-1.5 MPa). Wallner et al. (1980) also failed to find significant differences in thermotolerance of drought-stressed and non-stressed tissues. The effect of water stress on heat tolerance in this study could have resulted from using too severe treatments. Further experiments could be carried out under milder water deficit stress.

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