PHYSIOLOGICAL RESPONSE OF TOMATO FRUIT TO ETHYLENE AT HIGH TEMPERATURES

BY

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This work is dedicated to our children Tanaka Michael and Zvakanaka Gloria Masarirambi, my guardian angels, and to the loving memory of my late grandmother Cecilia Masarirambi, who passed away while I was in America.

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Lycopene biosynthesis during ripening is inhibited at temperatures of 30C or higher. Tomatoes are typically harvested at a mature but unripe stage of development ("mature green") and treated with C_2H_4 to initiate ripening. Tomato pulp temperatures may be 30C or greater during parts of the harvest season in Florida and other production areas. In this study, the response of tomatoes to C_2H_4 at temperatures of 30C and greater was investigated in terms of color development, respiration, C_2H_4 production, suberization and infection by *G. candidum*. Tomatoes held in air at 20 to 35C for 24, 48 or 72 hr, then transferred to 20C, ripened slowly and nonuniformily compared to fruit previously treated with C_2H_4 at the same temperatures and for the same periods of time. Prior exposure to 100 ppm C_2H_4 at 30C and, in some instances, 35C stimulated red color development in air at 20C. When the duration of C_2H_4 treatment at 40C was increased to 48 or 72 hr, subsequent red color development was inhibited after transfer to air at 20C. Further

increase in C_2H_4 treatment concentration to 1,000 ppm did not alter the fruit response to high temperature. There were few ripening behavior differences in terms of respiration, C_2H_4 production and 1-amino-cyclopropane-carboxylic acid oxidase activity in air at 20C in tomatoes previously exposed to C_2H_4 at high temperatures (≥ 30C) for 24 hr compared to those treated at lower temperatures (i.e. 20 and 25C). Ethylene production was inhibited after 48 or 72 hr at 40C but lower temperature exposure stimulated C_2H_4 production in the following order: 35 > 30 > 25C. During ripening in air at 20C, ACC conversion to C_2H_4 increased in fruit previously exposed to C_2H_4 at 20 or 25C but did not change in fruit from 30 or 35C, while exposure to 40C resulted in the lowest ACO acitivity. Fruit previously treated with C_2H_4 for 72 hr at 30C lost less weight than fruit held at 30C for 24 hr or 48 hr, which lost as much weight as fruit held at 20 or 25C. Fruit held at 35C lost the most weight. There was enough evidence to conclude that the optimum conditions for suberization are between 25 and 30C and near saturation humidity. Wounded mature green tomatoes were inoculated with *Geotrichum candidum*, placed at 20, 25, 30, 35 or 40C, and treated with air or C_2H_4 and near saturation RH. Wounded tomatoes previously treated with C_2H_4 at 35, 30 or 25C subsequently exhibited increased disease development after 9, 10, or 11 days in air at 20C, respectively, compared to fruit previously treated with air only. 'Sour rot' severity in air at 20C after at least 9 days increased as the temperature of previous exposure while treating with air or C_2H_4 increased from 20 to 25 and 30C and then declined in fruit that came from 35C. A thermophilic bacteria, which caused decay only in wounded tomatoes at temperatures ≥ 35C, was identified as probably being a *Bacillus* species after Gram testing.

CHAPTER 1
INTRODUCTION

The tomato (*Lycopersicon esculentum*. Mill) is one of the most important vegetables in the world, ranking second in importance only to potatoes (*Solanum tuberosum*) in many countries (Purseglove, 1984). On a worldwide scale, the tomato continues to increase in importance for consumption as a fresh crop, for inclusion as a major constituent in many primary foods (Hobson and Grierson, 1993), for research into fundamental principles of growth and development in plants (DellaPenna and Giovannoni, 1991; Hobson and Grierson,1993) and also for studying the molecular basis of ripening (Grierson et al., 1987; Gray et al., 1994; Stevens and Rick 1986). In Florida, tomato is the highest valued vegetable in terms of quantities produced and monetary value. Florida produces about 40% of the commercially grown fresh tomatoes in the US, California produces about 27% of the US supply and 24% is imported, mainly from Mexico (Gull,1992). Tomato is the second largest fruit crop in dollar value in the USA and other parts of the world, with the annual worldwide production of processing tomato fruits being more than 20 million tons, > 50% of which are produced in the US (Thakur et al., 1996). In Southern African Development Community (SADC) countries, tomato is on the top of the priority list of vegetable species for which research is needed, the criteria for determining priority being based on increased consumption and production by local people (Opena and Kyomo, 1990).

Tomato is important nutritionally in a world which is increasingly becoming nutritionally conscious. One medium sized tomato fruit provides 40% of the RDA of Vitamin C (ascorbic acid), 20% of the RDA of vitamin A, substantial amounts of potassium, dietary fiber, calcium, and lesser amounts of iron, magnesium, thiamine, riboflavin, and niacin, yet it contains only 35 calories and is low in sodium (Florida Tomato Committee, 1996). Vitamin A deficiency is prevalent in many parts of the SADC region (Opena and Kyomo, 1990), a problem which can be alleviated by increased consumption of tomato fruit or its products.

The magnitude of postharvest losses in fresh fruits and vegetables is an estimated 5 to 25 percent in developed countries like the US and 20 to 50 percent in developing countries like SADC (Zimbabwe), depending upon the commodity (Kader, 1992). This magnitude of loss is quite high and unacceptable because it reflects wasted resources used during production of the tomato crop. In 1975, the United Nations General Assembly, reflecting international concern with ways to increase the world's food supply, called for a 50-percent reduction in overall food losses by 1985 (National Academy of Sciences, 1978). This goal has probably not been realized. As we near the year 2000, the demand for food resulting from increased population growth continues to escalate while food production lags behind. Reducing the loss of food already produced will help alleviate the food shortage problem.

Temperature is the environmental factor that most influences the deterioration rate of harvested commodities. For example, for each increase of 10C above the optimum storage temperature, the rate of deterioration increases by two- to threefold (Kader, 1992) due to increased metabolism leading to senescence. Temperature affects the commodity's

response to ethylene (C_2H_4), atmospheric composition and pathogens in many different ways. Temperature management, therefore, becomes the most effective tool that can be manipulated for extending the shelf life of fresh horticultural commodities (Kader, 1992; Kasmire and Cantwell, 1992; Mitchell, 1992). Warm season crops or crops of tropical or subtropical origin such as tomato are subject to chilling injury (CI) (Kader, 1992; Kasmire and Cantwell, 1992; Cantwell, 1994) when exposed to non-freezing temperature below a critical threshold that is unique for each crop. CI has been defined as a physiological defect of plants and their products that results in reduced quality and loss of product utilization following exposure to low but non-freezing temperatures (Parkin et al., 1989). When mature green (MG) tomatoes are ripened at the optimum temperature using recommended concentrations of ethylene (the ripening hormone) and stored at an appropriate temperature, maximum storage life can be achieved. Ethylene is required in the initiation and maintenance of ripening in climacteric fruit such as tomato (Oeller et al., 1991). Ethylene biosynthesis and its regulation in higher plants is now well documented (Adams and Yang, 1979; Kende, 1993; Yang, 1985; Yang and Hoffman, 1984), while the nature of the ethylene receptor and how it perceives ethylene is only coming into view now (Ecker, 1995; Jian et al., 1996; Schaller and Bleecker, 1995; Theologis, 1995).

High temperatures (≥ 30C) can have detrimental effects on tomato ripening and thus subsequent quality (Inaba and Chachin, 1989; Maezawa et al., 1993; Mitcham and McDonald, 1992; Mohammed et al., 1996; Tomes, 1963; Yakir et al., 1984). Detrimental effects on tomato fruit ripening caused by previous exposure to high temperatures include poor color development, delayed softening, delayed climacteric ethylene production (in

instances where speedy ripening is desired, as at the packing houses), suppression of fruit respiration, and physiological disorders such as sunburn or sun scald inflicted in the field.

High temperatures can also have beneficial effects on horticultural products like tomato fruit. Fruit quality and shelf life can be improved by heat treatment (Klein and Lurie, 1991; Lurie and Klein, 1992). Heat treatments can be used to disinfest fresh fruits and vegetables of economically important quarantined insects (Couey and Hayes, 1986; Paull, 1994; Sharp, 1990), to achieve disease control (Couey, 1989; Klein and Lurie, 1992), to delay ripening (Biggs et al., 1988; Lurie and Klein, 1992), and to protect against CI (Kadyrzhanova et al., 1996; Klein and Lurie, 1991; Lurie and Klein, 1992; McDonald et al., 1996; Whitaker, 1994). Prestorage heating of fruit shows promise as a nonchemical method for managing both pathological (Couey, 1989; Klein and Lurie, 1992) and physiological (Klein and Lurie, 1992) disorders of fruits and vegetables. The development of nonchemical treatments to alleviate pathological and physiological disorders is desirable because consumers are concerned that many chemicals used to control postharvest disorders could potentially be harmful to them. High temperatures have been reported to inhibit postharvest fungal germination and growth (Couey, 1989) although the mechanism and interactions of heat stress and susceptibility to disease are not fully understood (Paull, 1994).

There is high likelihood in sub-tropical regions (such as SADC and Florida) that tomato fruit can be exposed to high temperatures $\geq$ 30C shortly before harvest or after harvest when the tomatoes await packing. Florida usually experiences temperatures greater than 30C during the early and late parts of the tomato harvest season.

Temperatures up to 49C have been measured in tomato fruit that were exposed to sunlight for 1 hour (Showalter unpublished).

Tomato fruit often get wounded during harvesting. The wounds, if unhealed, serve as entry points for disease causing organisms like sour rot (*Geotrichum candidum*), which is a wound pathogen. Suberization is a fundamental process involved in wound healing in tomato pericarp (Dean and Kollatukudy, 1976). Suberin is a principal constituent of the cell wall in suberized tissue and blocks entry of pathogens. There is high likelihood that the optimum temperature of suberization is close to 30C. In sweetpotato (*Ipomea batatas*) the optimum temperature for suberization has been reported to be 29C at around 90 to 95 % RH (Kushman, 1975; Ryall and Lipton, 1979).

In Zimbabwe, a SADC country, research has mainly been centered on postharvest needs of agronomic crops while little work has been done on postharvest requirements of Zimbabwean-grown horticultural crops (Masarirambi, 1991). With increased urbanization in the SADC region, a trend that is likely to continue, postharvest research concerning tomato fruit (one of the most important SADC vegetables) needs to be prioritized and causes of tomato postharvest losses urgently need to be investigated in order to alleviate those losses.

Ethylene, a simple hydrocarbon, is a natural plant hormone that is used to hasten tomato fruit ripening (Gull, 1981; Watada, 1986; Varga and Bruinsma, 1986). Ethylene has profound effects on many aspects of plant physiology and pathology (Abeles et al., 1992; Ecker, 1995; Lieberman ,1979; Pegg, 1976). Stress ethylene (Abeles et al., 1992) is induced by mechanical wounding, bruising, insect infestation, attack by pathogens and

temperature extremes. The relationship between ethylene (exogenous and endogenous) and tomato fruit ripening disruption at high temperatures needs to be clarified.

Geotrichum candidum, the fungal pathogen that causes sour rot, is a ubiquitous fungus that occurs in all tomato growing areas (Bartz, 1991). However, no work has been done to find how high temperatures affect sour rot disease development on tomato fruit.

This research is concerned with ways to harness the beneficial effects of ethylene treatment at high temperature while reducing the deteriorative effects and improve our understanding of what effects such treatments have on physiological and pathological responses of tomato fruit during postharvest handling.

The objectives of this study were

1. to investigate the influence of high temperature on the effectiveness of exogenous ethylene in induction of tomato fruit ripening and storability at 20C,.

2. to investigate the effect of high temperatures on suberization of green tomatoes to heal harvested-inflicted wounds, and

3. to determine the effect of high temperature and ethylene on bacterial and fungal diseases of tomato.

CHAPTER 2
LITERATURE REVIEW

General

The cultivated tomato (*Lycopersicon esculentum* Mill) is a member of the Solanaceae or nightshade family and is one of eight Lycopersicon species (Varga and Bruinsma, 1986). A natural concentration of the *Solanaceae* is found in South America, from where tribes and genera spread into other areas throughout the world (D'arcy, 1979). The tomato is native to the Peru-Ecuador region of South America, having evolved from the cherry form (*Lycopersicon esculentum var. cerasiforme* (Dun) (Pierce, 1987; Purseglove, 1984; Varga and Bruinsma, 1986). Most *Lycopersicon* species are unattractive in color and flavor, and this has led consumption to be limited to cultivars of *L. esculentum.* The tomato plant is a perennial grown as an annual and is adapted to a wide range of nutritional and environmental conditions. *Lycopersicon esculentum* is a self-pollinating diploid organism with rather a small genome size in comparison with most other agricultural crops (DellaPenna and Giovannoni, 1991). The tomato is important in that it is one of a few agricultural crops which is amenable to genetic engineering and has served as a model system for such applications (DellaPenna and Giovannoni, 1991; Hobson and Grierson, 1993). Over the last 25 years, tomato production has overtaken that of bananas, pome fruits and recently grapes to become second only to citrus in terms of weight of crop produced on an annual basis (FAO, 1991).

Plant Growth

Tomato is considered to be day neutral, although it is not productive in long days without a diurnal temperature variation of at least 6C. Maximum plant growth is achieved at a mean temperature of 24C day and 18 to 20C night (Pierce, 1987). The growth habits include determinate (bush), semi-determinate and indeterminate (vining) types. The tomato inflorescence is a dichasm (Vriesenga and Honna, 1974), which is relatively small and consists of a five-lobed corolla and calyx. The staminal cone represents a fusion of five anthers around the ovary style and stigma, giving a structure ensuring a high level of self pollination and thus homozygosity (Pierce, 1987).

Fruit Development

The tomato fruit is a berry consisting of a pericarp, placental tissue and seeds located in the locular tissue. Growth of many fruit, including tomato, is characterized by a single sigmoidal growth curve, is initially slow due to cell division, then rapid due to cell expansion until physiological maturity (Bohner and Bangerth, 1988; Coombe, 1976; Rhodes, 1980; Tieman and Handa, 1996). After fertilization, the number of cells in a tomato fruit increases over a period of 2-3 weeks, after which growth is almost exclusively by cell expansion, as the fruit pericarp and developing seeds accumulate carbohydrates from the plant (Hobson and Grierson, 1993). Towards the end of development, the tomato fruit shows a declining growth rate and becomes physiologically mature, followed by ripening in attached or detached fruit. Ethylene evolution and respiration begin to rise above a basal level at physiological maturity (Brecht, 1987) and chloroplasts start to turn into chromoplasts as ripening begins (Hobson and Grierson, 1993), initially in the locular jelly surrounding the seeds, then in the pericarp starting from the blossom end of the fruit

progressing to the stem end (Brecht, 1987). Ethylene production by developing tomato fruit cv. Sunny increased three- to fivefold at the time of gel formation in the locules, followed by a larger (20-fold) increase at the breaker stage (Brecht, 1987).

Tomato Quality

Quality is the degree of excellence or acceptability of an item. To the receiver, quality is primarily dependent on appearance such as good uniform red color (Tijskens and Evelo, 1994), freedom from defects and blemishes, and firmness, where firm fruit that would stand market handling are desirable (Gull, 1986; Hobson, 1988; Kasmire and Kader, 1978; Polderdijk et al., 1993; Schuch et al., 1991; Sun, 1987). Tomato quality from the consumer's standpoint consists of attributes such as appearance, flavor, shelf-life and nutritional composition (Gull et al., 1989; Kader et al., 1978). Fruit quality varies depending on such factors as genetic make up, and environmental and physiological effects. In Europe, consumers expect tomatoes to be reasonably sound and uniform in shape, weighing about 75 grams, slightly wider than deep and having an average of three locules (Hobson and Grierson, 1993). The U.S. standards define six categories that reflect the normal distribution of fruit size for large-fruited cultivars, while the categories for European standards reflect distribution of fruit size in the small-fruit cultivars that are common in Europe (Kader and Morris, 1976a). Tomato breeders have traditionally selected for round, large-fruited and smooth cultivars on the assumption that consumers prefer fresh tomatoes with such characteristics.

Color and Firmness

External quality characteristics, those that can be perceived by the senses of sight and touch without ingesting the product, are important in product differentiation,

particularly in the purchase decision and food preparation (Shewfelt, 1990). The quality of tomatoes can be assessed by their visual appearance (surface color) and firmness (Thorne and Alvarez, 1982). The term color is based on human perception of light and refers to one narrow band of the electromagnetic spectrum, called the visible spectrum (Hendry, 1993). Because color is frequently the most important factor a consumer uses to judge the quality of many products, including tomato, work was initiated to develop objective measurements of color using a photoelectric sorting machine whose major benefits were found to be a reduction in labor and an improvement in uniformity and consistency in the color sorting operation (Gaffney and Jahn, 1970). Color, a primary indicator of maturity or ripeness, is derived from the pigments found in a product, such as lycopene, the red pigment found in ripe tomatoes of some cultivars. The color of a ripe tomato is determined by the ratio of two pigments, lycopene and β-carotene. The color of a ripe tomato may range from yellow to red, depending on the cultivar.

Firmness is an important quality parameter of tomato fruit as perceived by the shipper, retailer or consumer, and softening is considered as loss of quality (Hall, 1987; Jordan et al., 1985; Kader and Morris, 1976b). Jordan et al. (1985) considered firmness to be one of the most important quality parameters for shippers and sellers. The textural quality of tomatoes is influenced by skin toughness, flesh firmness, and internal fruit structure, i.e., the ratio between locular tissue and pericarp (Ahrens and Huber, 1990; Kader and Morris, 1976b). The tomato fruit softens as it ripens, with softening beginning at the blossom end and proceeding towards the stem scar. Softening is genetically controlled, while environmental and cultural conditions play a major role in determining relative fruit firmness. Other factors that influence firmness at harvest include picking

stage, plant age and condition, cluster position on the plant, and incidence of disease (Kader and Morris, 1976b). It is desirable to the tomato industry and the consumer that tomatoes remain firm until they reach an acceptable ripe color.

Composition

The tomato fruit provides an ample amount of vitamin A and C (Kasmire and Kader, 1978), modest amounts of calories and fiber, and an insignificant amount of fat. Ripe tomatoes contain approxmately 94% water; 1% protein ; 0.1% fat; 4.3% carbohydrate; 0.6% fibre; 250 I. U.vitamin A; 25mg/100g ascorbic acid; the alkaloid tomatine and seeds which contain 24 percent of a semi-drying oil (Purseglove, 1984). The balance between water content and the various constituents of a ripe tomato fruit is dependent on the genotype, the environment in which the plant is grown, the nutritional treatment, and, to a minor extent, the nature of the postharvest treatment, so that precise figures can be misleading (Hobson and Grierson, 1993). The taste of the tomato fruit is largely determined by the ratio of sugars and acids and high values of these two components are preferred. The pericarp has higher sugar content than the locule gel, whereas the locule gel is more acid than the pericarp (Stevens et al., 1979). High acid and sugar content are important to good tomato flavor, which greatly influences repeat purchases by consumers (Kasmire and Kader, 1978).

Pigments

Chlorophyll

During development of the tomato fruit, chlorophyll, which is responsible for the green color, reaches a peak in concentration relatively early in the growth of the fruit. Chlorophyll, the predominant plant pigment on earth, is found in the thylakoid membranes

of the chloroplast and functions in photosynthesis by absorbing light energy. The chlorophylls have a characteristic absorption pattern; that of chlorophyll a shows two major absorption bands: one in the red, but with three minor peaks in the yellow part of the spectrum, together with at least one major absorption peak in the blue to purple end of the visible spectrum (Hendry, 1993). Commercially, fresh market tomatoes are usually harvested at the mature green (MG) stage and transported to the packinghouse, where they are ripened in ripening rooms using ethylene.

Carotenoids

The second most abundant group of plant pigments on the planet earth after chlorophyll are carotenoids (Hendry, 1993). They are responsible for a range of yellow, orange and red colors (Gutterson, 1995). Carotenoids are localized in the chromoplasts. With few exceptions, carotenoids are tetraterpenoids and are based on or derived from a skeleton of carbon atoms linked symmetrically with alternating unsaturated bonds (Hendry, 1993). Carotenoids are synthesized in green tissue, with β-carotene being one of the major products. Lycopene is the precursor of β-carotene. The control of lycopene synthesis is not yet known. In many fruits, such as in tomato, β-carotene and lycopene are synthesized during ripening (Tucker,1993).

Pigment Degradation and Synthesis

Pigment changes in tomato have been widely studied, and reviews have been published by Goldschmidt (1980), Goodwin (1980), Grierson and Kader (1986), and Khudairi (1972). Ethylene stimulates chlorophyll degradation as well as biosynthesis of carotenoids (Dostal and Leopold, 1967; Medina et al., 1981) in MG tomatoes with coincident ripening to red. Degradation of the green pigment chlorophyll unmasks

previously present pigments like β-carotene. In most fruits, this loss of chlorophyll is accompanied by the biosynthesis of one or more pigments, usually either anthocyanins or carotenoids (Tucker, 1993). Chlorophyll degradation is enzymic, but the precise mechanism of chlorophyll degradation is unclear. Investigators of pigment changes in senescing fruit and leaves diverted most of their efforts towards carotenoids and anthocyanins, leaving the *in vivo* degradation of chlorophylls least understood (Goldschmidt, 1980). The first step in chlorophyll degradation would seem to be the 'solubilization' of chlorophyll in the stroma, which may be brought about by enzymes capable of attacking the thylakoid membranes or chlorophyll directly, but the mechanism is unknown (Tucker, 1993). As chlorophyll decreases, it is replaced by carotenes and xanthophyll. Chloroplasts change into chromoplasts. The colorless phytofluene and the red pigment lycopene increase with ripening of tomato fruit, whereas β-carotene peaks a little before full color development (Hobson and Grierson, 1993). The situation may be regarded as relatively simple in tomato, which is normally dominated by carotenes, mainly lycopene and β-carotene (Goldschmidt, 1980). The metabolic pathways involved from acetyl CoA through mevalonic acid to colorless carotenoid compounds and then to colored pigments have been unraveled by use of various mutants affecting the color of fully developed tomato fruit (Stevens and Rick, 1986).

Ethylene

Ethylene is a simple hydrocarbon, a colorless gas with a sweet odor. It is flammable at concentrations between 3.1 and 32% in air. It has been known for many years that C_2H_4 has many variable effects on plant growth and development (Abeles et al., 1992). Neljubow, a Russian graduate student in the 1880s, first showed that C_2H_4 added

to the illuminating gas used in Europe then was the cause of various abnormalities in growth and development of plants growing close to leaky pipes carrying the gas (Reid, 1992). The abnormalities included premature leaf fall and death of flowers. In the 1930s it was discovered that C_2H_4 was produced by plants, and that therefore the responses to C_2H_4 are part of normal plant growth and development (Reid, 1992). The acceptance of C_2H_4 as a plant hormone was slow. Ethylene is unique among plant hormones in that it is a vapor at physiological temperatures so that its production, accumulation, transportation, and function involve special problems not encountered in other fields of plant hormone physiology (Burg, 1962). The gas plays a role in the postharvest life of many horticultural crops, often deleterious, speeding senescence and reducing shelf-life, and sometimes beneficial, improving the quality of the product, like in tomato, by promoting faster and more uniform ripening before retail distribution (Watada, 1986; Reid, 1992).

Ethylene Biosynthesis

It has long been a goal of plant biologists, in particular, to be able to prevent or delay fruit ripening in a reversible manner by controlling C_2H_4 action or production (Theologis et al., 1992). In order to achieve this goal, it is important to understand C_2H_4 biosynthesis and its regulation.

The C_2H_4 biosynthetic pathway in plants has been unraveled and is now well documented (Adams and Yang ,1979 ; Kende, 1993; Yang, 1985; Yang and Dong, 1993; Yang and Hoffman, 1984). The amino acid methionine and ATP are converted to S-adenosyl methionine (SAM), which is in turn converted to a cyclic amino acid, 1-amino cyclopropane-1-carboxylic acid (ACC), which is converted to C_2H_4, cyanide, water and carbon dioxide. Conversion of SAM to ACC (the precursor of C_2H_4) is the rate limiting

step and is catalyzed by ACC synthase (ACS) (EC 4. 1. 1. 14), which is the key enzyme in the biosynthetic pathway of the plant hormone C_2H_4 (Kende et al., 1986; Yang and Hoffman, 1984). ACC conversion to C_2H_4 is mediated by ACC oxidase (ACO) which is also known as C_2H_4-forming enzyme (EFE) (Kende et al., 1986). In most cases, C_2H_4 biosynthesis is regulated by endogenous levels of ACC via ACC synthase and the *de novo* synthesis or turnover of ACC synthase (Henstrand and Handa, 1989).

ACC and ACC Synthase

ACC is the immediate precursor of C_2H_4 and its availability is necessary for ethylene production. Changes in C_2H_4 production rates and ACS activity levels during the maturation and ripening of tomato fruit (cv. Castlemart) were studied by Su et al. (1984) and it was found that a linear relationship existed between internal C_2H_4 concentration and C_2H_4 production rate; both of which increased exponentially as tomato fruit reached more advanced maturity. It was also observed that there was a small increase in ACS activity at the early MG stages, which was followed by a marked increase at the breaker stage, and that the ACC level followed the same pattern as ACS activity (Su et al., 1984). Enhanced expression of genes for ACS occurs early in ripening and is partly responsible for elevated C_2H_4 production (Biggs et al., 1986; Bleecker, 1987; Hobson and Grierson, 1993; Yang and Dong, 1993).

Clones for ACS cDNAs were identified by partially purifying the enzyme, raising monoclonal antibodies, and using these to identify *Escherichia coli* cells transcribing cloned ACS synthase mRNA and translating it into a protein recognized by the antibody (Sato and Theologis 1989; Van der Straeten et al., 1990). ACC synthase antisense tomato plants were produced by Oeller et al. (1991), and these plants exhibited a reduced ethylene

phenotype. Fruit borne on these plants showed an extremely abnormal pattern of ripening, displaying reduced lycopene accumulation, a considerably delayed rate of softening and a greatly reduced respiratory climacteric. When fruit from ACS antisense tomato plants were treated with C_2H_4 they ripened normally (Oeller et al., 1991).

ACC Oxidase

ACC oxidase catalyzes the formation of ethylene from ACC, which is the final step of C_2H_4 biosynthesis (Kende et al., 1986). Application of exogenous C_2H_4 was reported to induce the synthesis of ACO enzyme before the synthesis of ACS (Yang, 1985). In fruits, ACO is induced during ripening and contributes to the regulation of C_2H_4 biosynthesis (Oetiker et al., 1995). Enhanced expression of genes for ACO occurs with ripening (Biggs et al., 1986; Yang and Dong, 1993). ACO is encoded by a small multigene family in tomato (Hobson and Grierson, 1993).

When the pathway of C_2H_4 biosynthesis had not yet been fully elucidated, it was widely believed that at least part of the C_2H_4-forming system was membrane associated (Kende, 1993). It is now known that ACO is found in the cytoplasm of the cell. It was shown in tomato fruit that ACO is a soluble enzyme by Ververidis and John (1991) and that there is no evidence for membrane localization in other plant tissues (Kende, 1993). ACC oxidase activity was reported to be enhanced by C_2H_4 in preclimacteric tomato fruits and in the non-ripening tomato mutants *nor* and *rin* (Liu et al., 1985). Now there is mounting evidence that the activity of ACO also increases in some plants in response to internal or external factors that induce C_2H_4 formation (Kende, 1993) such as stress.

Ethylene Action

Although the pathway of C_2H_4 biosynthesis is now well understood, by contrast biochemical approaches toward dissection of the mechanisms by which plants perceive and respond to C_2H_4 has not been as fruitful (Ecker, 1995). It has been proposed that ethylene binds to either a zinc (Zn) or copper (Cu) metalloprotein (Burg and Burg, 1967). The favored model is that C_2H_4 binds to a protein binding site, thus stimulating release of a so-called second message instructing the DNA to form mRNA molecules specific for the effects of C_2H_4 (Reid, 1992; Reid, 1995). Ethylene binding in plants has been studied and a model for C_2H_4 action has been suggested where reversible association of C_2H_4 with a membrane-bound receptor would result in release or activation of a second message (Sisler and Yang, 1984). A drawback to understanding the mechanism of C_2H_4 action is the failure to purify the putative metalloprotein receptor for C_2H_4 (Sisler, 1991). Application of molecular genetics, in combination with biochemical approaches, are beginning to illuminate the individual steps leading from the induction of C_2H_4 biosynthesis to the physiological responses elicited by this hormone (Kende, 1993). *Arabidopsis thaliana* has been used genetically to investigate C_2H_4 signal transduction. The constitutive triple response (ctr) phenotype displayed by one mutant (ctr1) is defective in C_2H_4 signal transduction (Roman et al., 1995). Several other C_2H_4-insensitive mutants have been reported that include etr1, ein2, ein3, ein5, ein6 and ein7 (Ecker, 1995).

Signal Transduction

Gray et al. (1994) put together a schematic representation of interactions between C_2H_4 biosynthesis and tomato fruit ripening depicting C_2H_4 perception by the C_2H_4 receptor followed by signal transduction, resulting in the following: housekeeping genes

remain unchanged, some genes are switched off, and ripening genes are switched on. This in turn affects the following: changes in cell wall metabolism, production of colored carotenoids, synthesis of new flavor and aroma volatiles, the respiratory climacteric and loss of chlorophyll and chloroplast thylakoids. The C_2H_4 receptor and transduction apparatus is just emerging into view (Theologis, 1995). Schaller and Bleecker's 1995 study on ethylene sensors has put the receptor into sharper focus and it is now believed that a protein kinase cascade is involved when plants sense C_2H_4. A hypothetical model based on Sisler's ligand hypothesis has been proposed for the two component regulation of C_2H_4 action. Binding of C_2H_4 to the receptor activates the kinase domain, which autophosphorylates, then transfers the phosphate to the response regulator protein, resulting in release of a ligand, or activation of a transcription factor (Reid, 1995).The ctr1 gene product is a central component in the C_2H_4 signaling pathway, it acts downstream of etr1 and ein4 and is a negative regulator of ein2, ein3, ein5, ein6, ein7, etr1, and hsl1 (Ecker, 1995; Jian et al., 1996; Koshland, 1993; Schaller and Bleecker, 1995; Theologis, 1995). The picture that emerges is that C_2H_4 sensors are encoded by multigene families with members that are differentially expressed during plant growth and development (Ecker, 1995).

Fruit Ripening and C_2H_4

Ripening in tomato marks the end of maturation and the beginning of senescence, when the tomato fruit changes color, softens, and develops the flavor, texture, and aroma that constitutes optimum eating quality (Babbit et al., 1973; Maximova, 1996; Gull,1981; Rhodes, 1980). During development of a tomato fruit, its sensitivity to C_2H_4 increases (Trewavas, 1982; McGlasson, 1985) and treatment with C_2H_4 results in advancing the

onset of ripening (Gull, 1981; Varga and Bruinsma, 1986; Watada, 1986). The basic mechanism for C_2H_4 formation exists in tomato and appears to operate at a low level until activated when the fruit reaches a certain critical physiological age (Moore, 1989). The internal C_2H_4 concentration is less than 1 ul l^{-1} during development up to the start of the climacteric respiration rise. Such basal levels of C_2H_4 have been termed "system 1" production (McMurchie et al., 1972). The "system 1" C_2H_4 production is exhibited by nonclimacteric plant organs, non-ripening mutant tomatoes, and normal, unripe climacteric fruit. "System 1" is involved in regulation of the aging process and is responsible for the low rate of C_2H_4 production during growth, while "system 2" is responsible for the autocatalytic increase in C_2H_4 production called the climacteric that accompanies ripening (Abeles et al., 1992; McGlasson, 1985; MacMurchie et al., 1972).

Ethylene has been used to enhance ripening since ancient times. The earliest practices employed the emanations from ripe fruit or the smoke generated from burning combustible products (Sherman, 1985). The concentration of C_2H_4 required for the ripening of various fruits varies, but in most cases it is in the range of 0.1 to 1 ppm (Reid, 1992). Wells et al. (1978) showed differential responses to C_2H_4-treatment among tomatoes of different genotypes and recommended from their data that breeders pay attention to the response of breeding lines and potential cultivars to C_2H_4-induced ripening. Mature green 'Homestead' tomatoes and three advanced breeding lines: T3702, $0g^c$ and $hpog^c$ were treated with C_2H_4 and some compositional parameters of the treated fruit were compared with those of control fruit. There was no difference among the breeding lines in terms of the effect of C_2H_4 treatment on the levels of soluble solids, β-carotene, lycopene, dry matter and ascorbic acid (Wells et al., 1978).

The application of exogenous C_2H_4 to MG tomatoes at 100 ppm for 24 to 48 hr at 20 or 25C is sufficient to induce ripening, but less mature fruit need continuous exposure to the hormone (Gull, 1981; McGlasson et al., 1975). Cantwell (1994) recommended the use of 100 ppm C_2H_4 at 18 to 20C with high humidity (90% RH) and sufficient air flow to maintain a uniform temperature profile through the room to ensure uniform tomato ripening. The optimum temperature range for C_2H_4 to induce physiological responses is 20 to 25C, (Watada, 1986). Sawamura et al. (1978) concluded from their experiments that ripening of a tomato fruit attached to the plant is initiated by C_2H_4 that has to overcome the retarding influence exerted by ripening inhibitor(s) from the vegetative parts.

Sherman and Gull's (1987) research at the University of Florida resulted in the development of a flow-through system for the introduction of C_2H_4 into tomato ripening rooms. This system provides a safe, economical alternative to the catalytic generation of C_2H_4 from ethanol and "shot" methods for initiating tomato ripening. In principle, the flow-through system supplies a constant, ripening-effective, low-level blend of C_2H_4 and air, which passes over the tomatoes and escapes through a small exhaust port in the room (Sherman and Gull, 1987). Other methods of C_2H_4 application used are shot and catalytic generator. The shot method involves injection of C_2H_4 into the ripening room or a transport vehicle, which is then closed for a period of time. Catalytic generators convert ethyl alcohol to C_2H_4 and water in the presence of heat and a catalyst (Gull, 1981). Blankenship and Sisler (1991) compared gassing methods for tomatoes and found that comments from sensory panelists indicated no preference for one treatment over the other. Starting with MG fruit, it has been observed that there are no important biochemical, chemical or physiological differences between fruit ripened by naturally produced C_2H_4 or

externally applied C_2H_4 (Gull, 1981). Tomatoes exposed to externally applied C_2H_4 ripen more uniformly and in a shorter period of time than those which have not been exposed to C_2H_4. Therefore, there is less spoilage, and C_2H_4-treated fruit have a slightly higher vitamin content (DellaPenna and Giovannoni,1991; Gull, 1981; Kader et al., 1978; Watada, 1986).

Ripening Related Changes

The ripening of tomato has been well researched compared to other climacteric fruits. It is a fruit most extensively studied at the biochemical and genetic levels, leading to the conclusion that at least some ripening associated events are brought about by developmentally regulated changes in gene expression (Gray et al., 1994). There are physical, biochemical and physiological changes involving enzyme activity that take place with incipient tomato ripening. These alterations occur rapidly, are comprehensive, affect all cell compartments, and fundamentally alter the appearance, flavor, aroma, texture (Halsey and Jamison, 1957), disease resistance, and survivability or shelf life of the fruit (DellaPenna and Giovannoni, 1991; Hobson and Grierson, 1993).

The development of fruit color from green to red occurs due to the transition of chloroplasts to chromoplasts, a process in which chlorophyll is degraded and carotenoid pigments accumulate (Gray et al., 1994). Changes of tomato fruit from green to assorted shades of red and near- red depend on such factors as maturity, temperature, and duration of storage (Halsey and Jamison, 1957).

Fruit softening occurs during fruit ripening as a result of the action of cell wall degrading enzymes such as pectin methyl esterase (PME) and polygalacturonase (PG) (Ahrens and Huber, 1990; Baldwin and Pressey, 1988; Brady et al., 1982; Buescher and

Tigchelaar, 1975; DellaPenna et al., 1987; Hobson, 1965; Huber and Lee, 1988; Huber and O'Donoghue, 1993; Rushing and Huber, 1984; Sawamura et al., 1978). Tucker and Grierson (1982) reported that the cell wall degrading enzyme PG is not detectable in green tomatoes and that activity appears at the onset of ripening. PG is one of the major cell-wall-bound proteins in ripe fruit (Tucker and Grierson, 1982). Using radio-immunoassay techniques, Tucker and Grierson (1982) showed that PG was synthesized *de novo* and that the "Never ripe" mutant had low levels of PG. There is an inverse relationship between fruit firmness and PG activity within cultivars as the fruit soften with ripening. After the start of the production of C_2H_4, respiration increases, accompanying the ripening processes. Among these are the rise in water-soluble pectin (WSP), possibly also from *de novo* synthesis of WSP, or *de novo* synthesis of PG leading to solubilization of pectin, followed by the activity of PG that plays a role in the further solubilization of cell walls (Sawamura et al., 1978).

A study of the kinetics of C_2H_4 evolution and PG synthesis by individual tomato fruit in a ripening series, employing an immunological method and protein purification to identify and measure PG synthesis, showed that ethylene evolution preceded PG synthesis by 20 hours (Grierson and Tucker, 1983). Su et al. (1984) studied the changes in C_2H_4 production rate and development of ACS and PG activities of tomato fruit (cv. Castlemart) and found that the onset of the development of ACS activity precedes that of PG activity.

The composition of the fruit also changes dynamically with ripening, during which many changes are easily perceived, such as alterations in pigmentation, firmness, aroma, sweetness, and acidity (Biggs et al., 1986). Changes in the sugars and acids, solids, texture

and flavor occur, with the result that the quality also changes and the tomato becomes edible (Gross and Sams, 1984; Gross and Wagner, 1979; Halsey and Jamison, 1957).

Ripening of tomato is accompanied by a peak in respiration (referred to as the climacteric) and a concomitant burst of C_2H_4 synthesis (Burg and Burg, 1962, Gray et al., 1994; Grierson and Kader, 1986; Peacock, 1972; Pratt and Goeschl, 1968; Sawamura et al., 1978). There is much evidence to suggest that ripening of climacteric fruit is initiated by ethylene synthesized from SAM in the Yang cycle via ACS and ACO mediation (Gray et al., 1994). Since ripening involves alterations in many different biochemical pathways, recent research has focused on identification of genes that control these processes and understanding their function and regulation (Gray et al.,1994). Jeffery et al. (1984) investigated C_2H_4-dependent vs C_2H_4- independent biochemical changes in ripening tomatoes. To determine whether the changes in organic acid metabolism were affected by C_2H_4, fruit was kept at 22C in either a normal atmosphere or a normal atmosphere supplemented with 27 $\mu l l^{-1}$ C_2H_4, and it was shown that in both atmospheres similar quantitative changes occurred in the citric acid cycle enzymes. Changes in the specific activities of invertase and PG, together with pigment changes, occurred between 2 and 3 days earlier in fruit exposed to C_2H_4, compared to those kept in normal atmosphere (Jeffery et al., 1984).

Respiration

Goodwin and Mercer (1988) defined respiration as an oxidative process in which complex molecules such as carbohydrates and organic acids are metabolized into carbon dioxide (CO_2) and water, consequently liberating energy used to produce adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and orthophosphate.

After harvest, MG tomato fruit are alive and they carry out the processes associated with life, including respiration. Respiration is an important process in plant tissues where the energy generated is used to drive metabolic processes that sustain cellular activities. The loss of stored food reserves in the commodity during respiration means (1) the hastening of senescence as the reserves that provide energy to maintain the commodity's living status are exhausted, (2) reduced food value, (3) loss of flavor or quality, especially sweetness, acidity, and firmness, and (4) loss of salable weight (Kader, 1992). The respiration rate of tomato has been classified as moderate and the rate of deterioration (perishability) of harvested commodities in general is proportional to their respiration rates (Kader, 1992). Respiration is usually measured as net, whole organ CO_2 exchange.

Respiration and Ripening

A close examination of the respiration course of a climacteric fruit at a suitable ripening temperature of 20C shows a declining trend to the lowest value termed as the "pre-climacteric minimum" followed by a more or less sharp rise, depending on the species, to the "climacteric peak" and a subsequent "postclimacteric" decline in the rate of respiration (Biale and Young 1981). The red ripe, edible stage of tomato fruit is usually shortly after the respiratory peak (Ahrens and Huber, 1990).

Energy derived from respiration is required to drive the metabolic processes associated with ripening. Ripening requires the synthesis of novel proteins and mRNAs, as well as new pigments and flavor compounds (Tucker, 1993). The respiratory pathways used by the fruit are glycolysis, the oxidative pentose phosphate pathway (OPP) and the tricarboxylic acid (TCA) pathway.

Gene Expression During Tomato Fruit Ripening

A phenotype is a result of gene expression, and the expression of certain genes increases or decreases depending on developmental stage and prevailing environmental conditions. Environmental factors affecting plant gene expression include interactions with pathogens (biotic) and abiotic factors such as light, temperature, anoxia, drought, nutrient excess, nutrient deficiency, non-essential chemicals (Robinson et al., 1993) and hormones such as C_2H_4.

Work has been published on the role of C_2H_4 in the biochemical control of ripening in tomato fruit (Bleecker, 1987; Goodenough, 1986; Gray et al., 1994; Sawamura et al., 1978; Yang, 1987). Ethylene is thought to regulate fruit ripening by coordinating the expression of genes that are responsible for a variety of processes, including enhancement of a rise in the rate of respiration, autocatalytic C_2H_4 production, chlorophyll degradation, carotenoid synthesis, conversion of starch to sugars, and increased activity of cell wall degrading enzymes (Biggs et al., 1986; Davies and Grierson, 1989; Gray et al., 1994; Maunders et al., 1987). Changes in populations of protein and mRNA during normal tomato fruit development and the extent to which these changes differ in fruit from three abnormally ripening mutants, namely rin, nor and Nr have been investigated by Biggs et al. (1986). Their results show that the relative preponderance of many proteins change during development of tomato fruit, and that in fruit from the ripening mutants a number of proteins that normally accumulate in wild type fruit fail to do so, or do so at reduced levels. Analysis of mRNA populations based upon *in vitro* translation of poly (A)+RNAs from different stages of fruit development from normal and mutant phenotypes indicates even more extensive changes in gene expression during the ripening process (Biggs et al.,

1986). Maximal expression of the majority of mRNAs coincided with the time of greatest C_2H_4 production in tomato fruit (Davies and Grierson, 1989). Several genes induced during tomato fruit ripening and those involved in C_2H_4 biosynthesis have been cloned. By testing RNA from senescing leaves against a tomato fruit ripening-related cDNA library, seven cDNA clones were identified whose mRNA prevalence increased during both fruit ripening and leaf senescence (Davies and Grierson, 1989). ACC synthase isoforms are encoded by a multigene family and the members are differentially expressed in response to developmental, environmental, and hormonal factors. Two members of the family, LE-ACS2 and LE-ACS4, are induced during fruit ripening and upon treatment of MG fruit with exogenous C_2H_4 in a dose dependent manner (Lincoln et al., 1993). Both genes are superinduced by wounding tomato pericarp tissue during various stages of ripening. Jones et al. (1989) investigated molecular changes involved in the ripening of tomato (cv. Rutgers) fruit and found that *in vitro* translation of total RNA extracted from developing and wounded tomato fruit indicated that ripening-related changes in polypeptide populations are initiated in the gel and placental tissues before spreading to external tissues. Expression of ripening-related cDNA probes showed that PG activity was first activated in placental tissue and the data supported the view that ripening in tomato fruit is under genetic control, and are consistent with evidence that ripening is initiated in tomato gel tissue (Brecht, 1987). The appearance of PG mRNA in tomatoes was observed as one of a series of changes in gene expression occurring during development and ripening (Bennet and DellaPenna, 1987; Bird et al., 1988; DellaPenna et al., 1987; Grierson et al., 1984; Smith et al., 1986).

Postharvest Temperature

Temperature control is important to maintain postharvest quality of tomato fruit. The magnitude of both quality and product loss are maturity and time-temperature dependent (O'Brien et al., 1978). Preharvest field temperature can influence composition and quality of vegetables at harvest as well as their postharvest responses, and temperatures that injure or weaken the tissues prior to harvest also reduce storage life and increase susceptibility to decay (Kader et al., 1974). Tomatoes are living commodities subject to continuing changes, some of which are desirable and some that are not, and they respond to their environment, especially temperature, RH, and composition of the atmosphere (Morris and Kader, 1978). Control of environmental factors as critical as temperature helps in controlling deterioration. The recommended storage temperature for tomato differs with the maturity or ripeness stage of the fruit (Hardenburg, et al., 1986).

The tomato is a subtropical species and is adversely affected by exposure to low temperature and may suffer from CI if exposed to low but nonfreezing temperature. Symptoms of CI in tomatoes may include decreased flavor, lack of uniform ripening and possible failure to develop a deep red color, softness, mealiness when ripened, and, if stored long enough at low temperature, increased decay, especially *Alternaria*, and reduced table quality (Cantwell, 1994; Hardenburg et al., 1986; Morris and Kader, 1978). The optimum temperature for ripening MG tomatoes is around 20C. The optimum ripening and holding conditions for tomatoes are 20 to 22C, with high RH (85 to 95%). Above 29C, tomatoes develop more orange than red color and fruit will be more soft (Gull, 1981). Superior quality tomatoes result if the fruit are maintained in "cool" conditions (about 15C) rather than being held at lower temperatures and then later ripened

at temperatures above 20C (Cantwell, 1994). Light red tomatoes with 60 to 90% red color can be held up to a week at 10C, although if held longer, they will probably not have a normal shelf life during retailing (Hardenburg et al., 1986).

High Temperature Effects on Ripening

Temperature stresses affect ripening, and exposure of MG tomatoes to high temperatures attenuates some of the normal ripening processes (Hobson and Grierson, 1993). Thorne and Alvarez (1982) reported that CI occurred in tomato fruit below 12C, and that ripening was disrupted above 27C. High field temperature exposure can also result in physiological disorders, including increased deterioration and sunburn or sun scald; however, serious problems can also occur during handling and storage following injury that was not visible at harvest (Kader et al., 1974). High temperature exposure (30C) has been shown to delay ripening as indicated by delays in the change of color from green to red, softening, and C_2H_4 production (Inaba and Chachin, 1989; Cheng et al., 1988; Mitcham and McDonald, 1992; Mohammed et al., 1996).

Inaba and Chachin (1989) studied the effects of high-temperature stress on tomato respiration, metabolism and mitochondrial activities. In their study, MG tomatoes (cv. Sakata Tvr) were stored at 5, 10, 15, 20, 25, 30, 35 or 40C. It was observed that during storage, respiration rate and C_2H_4 production were suppressed at 5, 10 and 35C and that ripening and C_2H_4 production were suppressed and injury was obvious at 40C (Inaba and Chachin, 1989). The peak respiratory rate of tomato fruit was highest in those stored at 30C, even though the level of C_2H_4 production was lower than those stored at 25C. This may indicate that the respiration is not directly controlled by the level of C_2H_4 production. Cheng et al. (1988) investigated responses of detached tomato fruit (cv. Flora-Dade)

during high temperature storage (37C) and found that tissue softening, red color development, and climacteric production of C_2H_4 were delayed by high temperatures.

The effect of storage temperature on changes of PG and PME activities during tomato ripening were determined by Ogura et al. (1975). The activities of PG and PME were determined at intervals during tomato storage at 4C, room temperature, and 33C. The enzyme activities in fruit stored at 4C and 33C did not increase and it was inferred that one reason for extension of the storage life of tomatoes previously stored at 33C for several days may be repression of the development of PG and PME activities during storage (Ogura et al., 1975). The effect of high temperature on cell wall modifications associated with tomato fruit ripening was also studied by Mitcham and McDonald (1992). Tomato fruit (cv. Sunny) were harvested at the MG stage and kept for 4 days at either 21C or 40C, then subsequently stored at 21C. Red color development was inhibited in heated fruit, as was softening, such that, 14 days after treatment, heat-stressed fruit were twice as firm as non-heated fruit and contained only one-third the amount of soluble polyuronides that was present in the non-heated fruit (Mitcham and McDonald, 1992).

Tomato fruit respiration was reported to be suppressed by high temperature stress and tomato mitochondria developed the cyanide-insensitive pathway of respiration with increased storage temperature, especially at 30 and 35C and with extended storage time (Inaba and Chachin, 1989).

High Temperature Effects on Color Development

Tomatoes ripened at 30C and above have been reported to have poor color development (Atta-Aly, 1992; Goodwin, 1980; Maezawa et al., 1993; Morris and Kader, 1978; Tomes, 1963; Yakir et al., 1984). High temperatures in the range of 30 to 35C are

known to inhibit lycopene accumulation in tomato, but β-carotene is not affected by those high temperatures (Goodwin, 1980). According to Morris and Kader (1978), temperatures above 27C result in undesirable yellow color due to inhibition of lycopene formation. Cheng et al. (1988), Masarirambi et al. (1995), and Mitcham and MacDonald (1992), reported delayed red color development of tomato due to prior high temperature treatment. Tomatoes previously stored at 37C had a significant delay in red color development during postharvest ripening at 20C compared to that of the control samples (20C), and the delay in color development was thought to be a result of inhibition of lycopene biosynthesis or its precursors phytoene and phytofluene (Yakir et al., 1984). A temperature of 25C seems to be optimal for the biosynthesis of lycopene and temperatures above 30C suppress its biosynthesis (Varga and Bruinsma, 1986). Generally, the failure of fruit to ripen properly at temperatures beyond 30 to 35C may be because C_2H_4 synthesis is inhibited, although this inhibition is reversible (Rhodes, 1980). This is because C_2H_4 is required to initiate ripening of MG tomato fruit.

High Temperature Effects on C_2H_4 Biosynthesis and Action

Generally, C_2H_4 biosynthesis and hence its production declines as temperature increases from 30C (Atta-Aly, 1992; Atta-Aly and Brecht, 1995; Biggs et al., 1988; Cheng et al., 1988; Inaba and Chachin, 1989; Lurie and Klein, 1991; Yang et al., 1990). The effect of high temperature on tomato (cv. Rutgers) fruit was studied by Atta-Aly (1992), who harvested tomato fruit at the breaker stage, stored them at 15C until the pink stage and then transferred them to 20C at 6-hour intervals, followed by 25 to 35C and then returned back to 20C. Maximum C_2H_4 production occurred when fruit were held at 20C, but, as the temperature rose from 20 to 30C, both C_2H_4 production and ACC levels declined (Atta-

Aly, 1992). For fruit held at 35C, C_2H_4 production decreased while ACC levels sharply increased. The inhibition of C_2H_4 biosynthesis at temperatures between 20 and 30C is attributed only to the reduction in ACC synthesis, while at 35C both ACC synthesis and its conversion to C_2H_4 were inhibited, but the inhibition was sudden and more pronounced on ACC conversion to C_2H_4 (Atta-Aly, 1992).

The biochemical basis of high temperature inhibition of C_2H_4 biosynthesis in ripening tomato fruit was investigated by Biggs et al. (1988), who incubated tomatoes (cv. Rutgers) at 34C or above and observed a marked decrease in ripening-associated C_2H_4 production. Determination of pericarp enzyme activities involved in C_2H_4 biosynthesis following transfer of fruits from 25C to 30 or 40C revealed that ACS activity declined rapidly while ACO activity declined slowly (Biggs et al., 1988). Recovery of C_2H_4 production following transfer of pericarp discs from high to permissive temperature was inhibited in the presence of cycloheximide, indicating the necessity for protein synthesis (Biggs et al., 1988). Ethylene production was significantly inhibited during high temperature storage, but recovered and reached a maximum peak similar to that of the control upon removal of tomatoes stored at 37C for 3 days and transfer to 21C for 3 days (Cheng et al., 1988).

High Temperature Effects on Gene Expression

The effect of high temperature stress on ripening-related processes has been investigated at the molecular level (Kadyrzhanova et al., 1996; Kagan-Zur et al., 1995; Lurie and Klein, 1992; Lurie et al., 1996; Picton and Grierson, 1988). The effect of heat stress (36C) on ripening-related changes of harvested MG tomatoes at both the physiological and molecular levels was investigated by Lurie et al. (1996). Ethylene

production, color development, and softening were inhibited during heating and recovered afterwards at 20C. Levels of mRNA for ACO, phytoene synthase, and PG decreased dramatically during the heat treatment but recovered afterward, whereas the mRNA for HSP17 increased during the high temperature treatment and decreased when fruit were removed from heat (Lurie et al., 1996).

The effects of extended heat stress on PG and PME gene expression at the mRNA, protein and enzyme activity levels in ripening tomato fruit were investigated (Kagan-Zur et al., 1995). Steady state levels of PG mRNA declined at temperatures of 27C and above, a marked reduction in PG protein and activity was observed at temperatures of 32C and above. Accumulation of PG mRNA was partly restored when heat-stressed fruit were transferred to 20C. In fruit held at 34C, both PG and PME protein activity continued to accumulate for about 4 days, but thereafter PG protein and activity declined while little change was observed in PME protein and activity (Kagan-Zur et al., 1995). During recovery of heat-stressed fruit at 20C, increases in mRNA levels of both PG and PME were observed, while levels of PG protein and activity declined in fruit that were heat stressed for 4 or more days. PME protein and activity level were unchanged. Collectively, these data suggest that PG gene expression was being gradually and irreversibly shut off during heat stress, while PME gene expression was much less sensitive to heat stress (Kagan-Zur et al., 1995). On the other hand, increased activity of ACS begins within 30 minutes of a stress episode (Mitchell, 1996) such as exposure to high temperature.

Heat Shock Proteins

Plants and other organisms ranging from bacteria to humans respond to increased temperature by synthesizing a group of specific proteins referred to as heat shock proteins

(HSP) (Bressan and Handa, 1992; Czarnecka et al., 1984; Nover and Sharf, 1984; Nover et al., 1983; Nover et al., 1989; Sebehat et al., 1995). Plant tissues produce two major classes of HSP: high molecular-weight HSP from 70-95kDa, and low-molecular weight HSP from 15-30kDa (Neumann et al., 1987; Bressan and Handa, 1992). It is now known that exposure of plants to one stress can protect the plant from other stresses such as chemical toxins, chilling injury, high temperature injury and attack by pathogens. Sebehat et al. (1995) showed that tomato fruit previously stored at high temperature were protected from CI when exposed to low chilling temperature. Tomato fruit injected with radiolabelled methionine at harvest and subjected to 38C for 48 hr, accumulated high levels of specific radiolabeled proteins as visualized on 2-D gels, which were not present if the fruit were held at 20C. Heated fruit held at 20C for 4 days before being put at low temperature were sensitive to CI, while heated fruit transferred immediately to low temperature were not (Sebehat et al., 1995). The regulation of synthesis of many HSP involves controlling transcription of the HSP genes into mRNA, although control can also be at the translational level (Bressan and Handa, 1992).

Beneficial Effects of High Temperatures

Heat treatments have been used beneficially for insect disinfestation or to control diseases (Couey and Hayes, 1986; Jacobi and Wong, 1992; Klein and Lurie, 1992; McGuire, 1991; Paull, 1990; Paull, 1994). Commercial, ecological, and agrotechnical considerations have recently caused renewed interest in the use of physical rather than chemical means to maintain postharvest quality of horticultural crops (Klein and Lurie, 1992). This is because some chemicals used for control of postharvest pathogens and insect pests may be potentially harmful to humans and because many such chemicals have

lost their Environmental Protection Agency (EPA) registration and others will continue to be withdrawn. Prestorage heating shows promise as a nonchemical method for managing both pathological and physiological disorders of fruits and vegetables (Klein and Lurie, 1992). Exposing fruits to high temperatures attenuates some of the ripening processes while enhancing others and heated fruit become more advanced in some ripening characteristics than nonheated fruit while maintaining their quality longer at 20C. Klein and Lurie (1992) studied the effects of continuous storage at high temperatures on firmness of tomatoes and found that softening was slower if fruit were stored at temperatures between 30 and 40C than at 20C. When heated fruit were transferred to 20C, softening increased but was still slower compared to nonheated fruit. By the end of shelf life, however, titratable acidity was the same in heated and nonheated tomatoes, while soluble solids concentrations remained higher in heated fruit. The 10% to 30% increase in the sugar: acid ratio induced by heating is a characteristic that may make heat-treated fruit attractive to consumers (Klein and Lurie, 1992). Heated tomatoes were redder, but not softer, than nonheated fruit after storage at 12C (Lurie and Klein, 1992). In the case of heated tomatoes, a simultaneous increase in CO_2 production and decrease in C_2H_4 production occurred during exposure to high temperatures, and upon removal from heating, CO_2 production fell below that of controls, while ethylene production recovered and even exceeded that of nonheated fruit (Lurie and Klein, 1991). Although prestorage heat treatments are a promising alternative to chemical treatments, several aspects of the technique require continued research, and fine tuning the heating regime for specific commodities may be necessary (Klein and Lurie, 1992).

High temperature treatments have been reported to protect tomatoes exposed to low chilling temperatures from CI and other stress (Kadyrzhanova et al., 1996; Klein and Lurie, 1991; Lurie and Klein, 1992; McDonald et al., 1996; Whitaker, 1994). McDonald et al. (1996) determined the effects of prestorage heat treatments on chilling tolerance of tomatoes. MG tomato (cv. Agriset) fruit were either treated with C_2H_4 or not, immersed in 42C water for 60 min, and held at 38C for 48 hr, or not treated, then stored at either 2C (chilled) or 13C (nonchilled) for 14 days before ripening at 20C. Treatment of tomatoes in 42C water for 60 min or 38C air for 48 hr before storage at 2C for 14 days reduced CI and subsequent decay upon rewarming (McDonald et al., 1996). Prestorage short- and long-term heat treatments could allow for storage of MG tomatoes at lower temperatures with little loss of their ability to ripen normally (McDonald et al., 1996). MG tomatoes heat treated at 42C for 2 days became largely insensitive to CI when subsequently stored at an otherwise chilling temperature of 2C for at least 3 weeks and, furthermore, the heat-treated fruit ripened normally when transferred to 20C, while control fruit developed typical severe symptoms of CI (Kadyrzhanova et al., 1996).

Suberization

Suberin is found as an indistinct protective coating over underground plant parts. Suberin also covers the cork cells produced as scar tissue after wounding, such as upon leaf abscission and on potato tubers after cutting (Salisbury and Ross, 1979). Suberization is the production of suberin to cover wounded parts of plant organs. Most work on suberization has been done with sweetpotato roots (*Ipomea batatas*) or white potato tubers (*Solanum tuberosum)* which require exposure to favorable conditions to promote healing (periderm formation), in wounds sustained during harvesting before storage. The

favorable conditions to promote wound healing (curing) in sweetpotatoes by suberization have been reported to be warm temperature and high RH i.e., 7 days at 29C and 90 to 95% RH (Kushman, 1975; Ryall and Lipton, 1979). Rapid wound healing is essential in order to decrease water loss and pathogen invasion during storage. The curing process is primarily one of periderm formation to minimize the invasion of rotting organisms. Bacterial entry into potato tubers through wounds and lenticels was investigated by Fox et al. (1971), who found that entry was affected by the environment, especially the RH that prevailed. Light and electron microscope examination of tubers previously inoculated with *Erwinia carotovora* var. *atroseptica* showed that resistance to invasion was apparently due to the laying down of a barrier of suberin in the intercellular spaces and in two bands within the cell wall (Fox et al., 1971). The authors also reported that if the suberization process was complete, the barrier created prevented invasion. If incomplete, pectolysis spread through the gaps and, in such cases, it was possible for the suberin to be bypassed by the bacteria.

The effect of low concentrations of C_2H_4 during curing and storage was studied by Kitinoja (1987). Freshly harvested sweetpotatoes were cured in 2 ppm C_2H_4 or air at 30C, and it was observed, among other things, that C_2H_4-cured roots formed callus and their rate of water loss was somewhat higher than that of air-cured sweetpotatoes at the same temperature. In this case, C_2H_4 did not appear to promote suberization to heal sweetpotato harvest-inflicted wounds. However, no studies have been undertaken to investigate the factors affecting suberization in tomatoes to heal harvest-inflicted wounds, especially the effect of C_2H_4 when used to ripen tomatoes.

Tomato fruit often get wounded during harvesting operations. The intact fruit is covered with wax layers to protect the inner cells, from desiccation and microbial invasion. Wounding exposes these inner cells, and their normal response is to produce and deposit protective materials in order to seal off the wound (Imaseki, 1985). The structure and composition of the aliphatic monomers of the polymeric material deposited during wound healing of tomato fruit, bean pods, and jade leaves were examined. Suberization is a fundamental process involved in wound healing in tomato pericarp and all plant tissues (Dean and Kolattukudy, 1976). New protective layers were reported to be produced in wounded tomato fruit, bean pods and jade plant (*Crassula*) leaves (Dean and Kolattukudy, 1976). The major aliphatic components of the polymeric material deposited during wound-healing of tomato fruit, bean pods, and jade leaves were clearly those characteristic of suberin (Dean and Kolattukudy, 1976) . So suberization in tomato fruit appears to follow a similar mechanism as in other plant tissues.

Suberization of the wound surface of potato tubers occurred after a 4-day lag period but was inhibited if the wound surface was washed with large amounts of water for 3 hours immediately after wounding (Soliday et al., 1978), but washing after 4 days did not inhibit suberization. Soliday et al. (1978) deduced that a factor removed by washing for 3 hours was responsible for the induction of suberization, that the factor was generated near the wound, and that it was required until induction of the enzymes involved in suberization occurred. Injured cells produce phenylalanine ammonia lyase (PAL), which is an important enzyme required for the formation of phenolic compounds thought to be involved in wound healing (Dean and Kollatukudy, 1976).

Postharvest Diseases of Tomato Fruit

Tomatoes are subject to pathological breakdown, the extent of which depends upon the vitality of the fruit, the environment that exists, the presence of physical injury, and the pathogenic organism (Morris and Kader, 1978). The occurrence of tomato diseases after harvest is often traceable to growing conditions and packinghouse operations (Bartz, 1991; Morris and Kader, 1978).

Disease Incidence

Although it is difficult to estimate the full extent of postharvest losses due to diseases, conservative estimates place losses of fruits and vegetables from spoilage at around 24% of harvested crops in the United States (USDA, 1965). Coursey and Booth (1972) estimated that losses in developing countries were about 50% of the harvested produce.

Disease in plants can be defined as any disturbance brought about by a pathogen or an environmental factor that interferes with manufacture, translocation, or utilization of food, mineral nutrients, and water in such a way that the affected plant changes in appearance and or yields less than a normal healthy plant of the same variety (Agrios, 1978). In general, plant diseases are caused by either living (biotic) or non-living (abiotic) agents. The living agents that cause plant diseases are fungi, bacteria, a few higher plants, nematodes, algae, viruses, mycoplasmas and viroids (Kucharek, 1994).

In surveys from 1974-1977 and 1980, more than 23% of the tomato fruit marketed through the New York (NY) terminal market were lost to biotic and abiotic disorders (Bartz, 1991). In this study, where virtually all the losses were at the consumer level, biotic diseases or postharvest decays accounted for 80% of the losses in the entire system.

Ceponis et al. (1986) reported on the condition of fresh tomato shipments on arrival at the NY market as a result of inspections made during 1972-1984 on about 11% of all tomato shipments. Sour/watery rot was found in 35.3% of all inspected shipments, of which Florida accounted for 42-48% of the total volume, gray mold rot in 27%, bacterial soft rot in 25.2%, and unidentified decays (lacking characteristic symptoms) in 24.3% (Ceponis et al., 1986). In a previous 3-year study, Ceponis and Butterfield (1979) reported that fresh tomato losses at retail and consumer levels in metropolitan NY were estimated at 9,000 MT annually; parasitic diseases, principally *Alternaria* rot, gray mold rot, *Rhizopus* rot and bacterial soft rot, caused two-thirds of these losses. Average tomato losses worldwide probably exceed these values, because modern handling technology (the use of biocides and refrigeration) and firm-fleshed tomato cultivars are not available in many countries (Bartz, 1991). Green tomato fruit, called 'mature greens' in the marketing system, are relatively resistant to most plant pathogenic organisms, but, as the fruit ripen, they become increasingly susceptible to a number of facultative parasites, many of which are only weakly pathogenic (Bartz, 1991). In Florida, postharvest tomato diseases rank in decreasing importance in the following order: bacterial soft rot (*Erwinia carotovora* (Jones) Sub sp. *carotovora*) sour rot (*Geotrichum candidum* Link) , rhizopus rot (*Rhizopus stolonifer*), and gray mold (*Botrytis cinerea).*

Influence of Temperature on Bacterial Diseases

Pathogen growth and probably development is favored by warm temperatures, with the optimum for many pathogenic organisms being about 30C (Perombelon and Kelman, 1980). Bartz et al. (1991) reported effects of temperature on the progress of bacterial soft rot in tomatoes. It was observed that the onset of bacterial soft rot in

tomatoes may be delayed up to 3 days or longer among fruit stored at normal ripening temperature, 20-22C, as compared with temperatures optimum for the disease, 30-35C. Temperatures above 25C reduced the period between inoculation and disease onset, which may increase the risk of spread of the disease among boxed fruit, while temperatures below 15C delayed disease onset. However, if temperature and exposure time are sufficient to induce CI, this may predispose the fruit to increased decay as they are being ripened and marketed (Bartz et al., 1991).

The differential between fruit temperature and dump tank water temperature will affect the amount of water containing possible bacterial pathogens that will infiltrate into the tomato fruit via the stem scar. Bartz and Showalter (1981) showed that tomato fruit immersed in bacterial suspensions with negative suspension/fruit temperature differentials (fruit warmer than suspensions) became infiltrated by water as evidenced by increased weight of the fruit. Isolation of *Serratia marcesens* (an easily recognizable red bacterium) from internal tissues of fruit previously immersed in suspensions of that organism or development of bacterial decay in fruit previously immersed in suspensions of *E. carotovora* subsp. *carotovora*, *Pseudomonas marginalis*, or *P. aeruginosa*, were evidence for corresponding bacterial infiltration (Bartz and Showalter, 1981). Pulp temperatures of up to 49C have been measured in Florida tomato fruit after exposure to sunlight for 1 hour (Showalter, unpublished). Such high temperatures will result in large differentials between fruit temperature and dump tank water temperature and predispose tomatoes to disease due to bacterial pathogens that will infiltrate them via the stem scar.

High Temperature Effects on Fungal Diseases

Heat treatments of fruits and vegetables applied as hot dry air, vapor heat or hot water dips have been reported to inhibit development of postharvest diseases (Couey, 1989; Fallik et al., 1993; Spalding and Reeder, 1986). Postharvest treatments with heat, irradiation, and systemic fungicides have proven effective in some instances, but the principle approach to the control of postharvest infections is by the application of protectant or eradicant fungicides to the crop in the field (Eckert, 1977). Heat has been reported to exert sublethal or lethal effects to inhibit fungal germination and growth. (Couey, 1989; Fallik et al., 1993 and Spalding and Reeder, 1986). Holding inoculated MG and pink tomato fruit for 3 days at 38C completely inhibited decay caused by *Botrytis cinerea*, one of the main postharvest pathogens of tomatoes in Israel (Fallik et al., 1993). Quality changes in tomatoes and resistance to *Alternaria* infection as influenced by varying storage temperature was investigated by Efiuvwevwere and Hobson (1989). Tomato fruit were stored at 27C continuously for 9 days or at 35, 18, then 27C for 3, 2, then 4 days, respectively, before inoculation with *Alternaria solani*. After inoculation, fruit were ripened for 6 additional days at 27C and evaluated for response to infection and quality changes. Larger lesions (mean of 9.1 mm) occurred in fruit stored at 35, 18 then 27C, while continuous storage at 27C resulted in smaller lesions (mean of 3.9 mm) (Efiuvwevwere and Hobson, 1989). These results indicate that fluctuating (nonuniform) temperature storage may be detrimental to tomato fruit quality and its resistance to fungal deterioration.

Ethylene Effects on Disease Development

Ethylene functions as a powerful hormone in many aspects of plant growth, development, and senescence (Lieberman, 1979). Ethylene biosynthesis is promoted by many stresses, including wounding and pathogen infection (Abeles et al., 1992; Qadir et al., 1996), and correlates with the induction of mRNAs for a diverse array of pathogen-related genes (Ecker, 1995). However, the specific role of C_2H_4 in postharvest pathogenesis is poorly understood (Kader, 1985; Pegg, 1976). Since C_2H_4 is intimately involved in the metabolism of other growth substances, both in terms of stimulation and repression, and is produced in most tissues as a result of physical or physiological damage, it follows that it is involved in most, if not all, toxin-induced symptoms (Pegg, 1981). Ethylene lowers the resistance threshold of tissue to toxins and pectolytic enzymes, (Pegg, 1981).

Enhancement of the growth of *Botrytis cinerea* Pers, exFr on strawberries by C_2H_4 was reported by El-Kazzaz et al. (1983b). In their study of C_2H_4 effects on *in vitro* and *in vivo* growth of certain postharvest fruit infecting fungi, El-Kazzaz et al. (1983b) observed stimulation of germ tube elongation of *Botrytis cinerea* by exposure to 1, 10, 100, and 1000 ppm C_2H_4. The role of exogenous C_2H_4 in development of *Botrytis cinerea*, *Alternaria alternata*, and *Alternaria consortiale*, all important fungal pathogens, was examined (Kipczynska, 1996). Exogenous C_2H_4 and ethephon markedly increased the spore germination and slightly increased the growth of mycelia of the tested fungi; by contrast, 2,5 norbornadiene, a competitive inhibitor of action, inhibited development of the fungi.

On the other hand, C_2H_4 has also been shown to induce resistance to certain pathogens in harvested plant organs (Kader, 1985). Ethylene treatment was found to inhibit apple rot development caused by *Gloeosporium album* (Lockhart et al., 1968). Ethylene treatment of Florida 'Robinson' tangerines for 3 days resulted in the tangerines being resistant to *Colletotrichum gloeosporioides* (Brown, 1978).

Both the direct stimulatory effects of C_2H_4 on postharvest fruit fungi and the indirect inhibitory effects via possible modifications of the host metabolism have practical implications in the postharvest biology of fresh horticultural crops (Kader, 1985). The mechanism of C_2H_4-induced resistance to infection have been postulated. Two aspects of possible C_2H_4 involvement in disease resistance that have received much attention in recent years are the effect of the hormone on peroxidases, polyphenol oxidase and PAL activities and its role in the synthesis of specific anti-fungal compounds (Pegg, 1976). It is assumed that certain oxidases in diseased tissue, like wall-bound amino acid oxidases and glucose oxidase, release hydrogen peroxide from their substrates, leading to cellular damage and necrosis. In many host-pathogen combinations, the pathogen may penetrate the cell wall, but as soon as it has established contact with the protoplast of the cell, the nucleus moves toward the intruding pathogen and soon disintegrates, and brown, resin-like granules form in the cytoplasm, first around the pathogen and then throughout the cytoplasm (Agrios, 1978). The necrotic tissue isolates the pathogen from the living substance, starving it to death because it depends on living tissue for its nutrition, growth and multiplication. The faster the host cell dies after invasion, the more resistant to infection the plant or plant part seems to be (Agrios, 1978). Furthermore, the peroxide is then used in oxidative reactions to protect the cell from damage. Polyphenol oxidases, on

the other hand, are thought to play a role in resistance *via* the oxidation of phenolic compounds to phenolic acids, which are further oxidized to toxic antifungal quinones (Pegg, 1976).

Ethylene does not appear to play a role in bacterial growth and development, although a few soil-borne species such as *Mycobacterium parafenicum* can grow on ethylene and convert it into C_2H_4 oxide (Abeles et al., 1992). Bacteria that produce C_2H_4 may enhance disease induction compared to non-C_2H_4 producing bacteria. Ethylene promotes senescence in plant tissues, thereby predisposing weakened plant tissues to attack by disease-causing microorganisms.

Ethylene does not appear to have an effect on the growth and development of certain fungi (Abeles et al., 1992) although some fungi have been reported to produce C_2H_4, i.e.(*Penicillium citrinum* (Honma et al., 1996), *Botrytis cinerea* (Qadir et al., 1996), and *Penicillium digitatum* (Fuji et al., 1985). Fergus (1954) showed that C_2H_4 production rate by *P. digitatum* followed the growth curve of the fungus. Although oxygen is required for C_2H_4 production by both fungi and higher plants, the two pathways are different (Abeles et al., 1992).

Additional research is needed to evaluate the potential benefits of avoiding exposure to C_2H_4 on the rate of rot development vs. possible use of C_2H_4 treatment to induce disease resistance in the commodity and the biochemical basis of this response (Kader, 1985).

CHAPTER 3
COLOR DEVELOPMENT OF TOMATO FRUIT AFTER C_2H_4 OR AIR TREATMENT AT HIGH TEMPERATURES

Introduction

Poor color development has been reported when tomatoes are ripened at temperatures ≥ 30C (Atta-Aly, 1992; Goodwin, 1980; Maezawa et al.,1993; Tomes, 1963; Yakir et al., 1984). However the inhibition of red color development by high temperatures, i.e. ≥ 30C, is reversible (Atta-Aly, 1992; Atta-Aly and Brecht, 1995; Cheng et al., 1988; Masarirambi et al., 1995). When 'Sunny' tomato fruit at the breaker stage were held at 30, 35 or 40C and then transferred to 20C for ripening, increasing delays in red color development were noticed that corresponded to the temperature and duration of exposure to high temperatures (Atta-Aly and Brecht, 1995). Previous storage of tomatoes at 37C led to a significant delay in red color development during postharvest ripening at 20C compared to that of control samples held continuously at 20C, and the delay in color development was postulated to be a result of inhibition of the synthesis of lycopene or its precursors, phytofluene and phytoene (Yakir et al., 1984). When MG tomato fruit were commercially treated with 80 ppm C_2H_4 for one night at 21C, then stored at 37C for 3 or 7 days, and finally ripened at 21C, red color development was delayed by the high temperature treatment, but color development was rapid when the tomatoes were transferred to 21C (Cheng et al., 1988). However, in this study, the tomatoes were not treated with C_2H_4 while they were at the high temperature. Picton and

Grierson (1988) used fruit at the breaker stage and found that ripening tomato fruit incubated at 35C failed to achieve normal pigmentation, softened little and showed a marked decline in C_2H_4 evolution.

Typically, tomatoes are held in ripening rooms for 1 to 3 days, when daily inspections are made to ascertain when nearly all of the fruit have begun to develop red color. Because tomatoes are not cooled prior to being placed in ripening rooms, the fruit pulp temperatures may well be over 30C for a substantial amount of time while the fruit are treated with C_2H_4, although the ripening rooms are maintained/set at 20 to 21C (Brecht and Sargent, unpublished). Sherman and Talbolt (1986) reported that ripening rooms are generally not designed for efficient cooling of tomatoes and therefore there is high likelihood of tomato pulp temperatures remaining high for some time while in the ripening room.

In a unique experiment, Yang et al. (1990) attempted to distinguish between an effect of high temperature ($\geq$ 30C) on C_2H_4 biosynthesis and sensitivity of the tissue to C_2H_4 by exposing MG 'Sunny' tomato fruit to saturating levels of C_2H_4 and monitoring changes in indicators of ripening. Exogenous C_2H_4 did not overcome inhibition of autocatalytic C_2H_4 formation, ACC synthesis, color development or softening during storage at 30 or 37C. Therefore, Yang et al. (1990) concluded that ripening inhibition at high temperature is not merely the result of decreased production of ACC and C_2H_4, but also a reduced sensitivity to C_2H_4.

Since high temperature exposure of MG tomatoes can have both negative and beneficial effects, we felt compelled to elaborate on what happens when MG tomatoes are treated with C_2H_4 at high temperature, then transferred to 20C, a temperature commonly

used to ripen tomatoes. We bore in mind the fact that ripening rooms are not designed for efficient cooling and that tomatoes often remain at high temperatures for considerable time while being treated with C_2H_4. Furthermore, Yang et al. (1990) reported that exogenous C_2H_4 supplied to tomatoes at 30 or 37C did not restore autocatalytic production of C_2H_4, ACS activity, color development, or softening. There is no information of tomato fruit response in terms of color development in air at 20C following C_2H_4 treatment or air at high temperatures and yet color is the most important visual criterion of maturity in tomatoes. The color of vegetables, including that of tomatoes, is a highly significant factor in the salability and commercial value of the product (Ryall and Lipton, 1979). Therefore, the objective of these studies was to investigate color development of tomatoes at 20C after previously treating MG tomatoes with C_2H_4 or air at high temperatures.

Materials and Methods

Plant Material

Large (USDA, 1976) MG 'Agriset 761', 'Sunbeam', and 'Sunny' tomato fruit were obtained from a commercial packinghouse in the Ruskin area on the day of harvest, picked from plots at the IFAS NFREC, Quincy, or picked from plots at commercial farms in Quincy. The tomato fruit were transported to Gainsville and either treated immediately ('Sunny' and 'Sunbeam') or left at 8C overnight ('Agriset 761'). Fruit that were transferred from the field directly to the laboratory were washed in water with 100 ppm chlorine, pH 7.0, rinsed, dried, and graded. Fruit from all lots that were showing red color development, or were misshapen or wounded (unsuitable fruit) were discarded.

Temperature and C_2H_4 Treatment

In the first experiment to determine whether tomato fruit are able to respond to C_2H_4 at high temperature, six buckets, each containing 30 'Sunbeam' fruit, were placed at 20 or 35C and tomatoes in three of the six buckets at each temperature were treated with either air or about 100 ppm C_2H_4 for 24, 48 or 72 hr. In the experiments to determine the response of tomatoes to C_2H_4 at a range of temperatures, three buckets, each containing 30 'Agriset 761' or 'Sunny' fruit, were placed at each of the following five temperatures: 20, 25, 30, 35 or 40C and around 95% RH and treated with about 100 ppm C_2H_4. Ethylene and air were mixed to obtain 100 ppm C_2H_4, which was applied at constant pressure in a flow-through system via a gas mixing board equipped with needle valve flow meters. High RH was maintained around the tomatoes by passing the air and C_2H_4 mixture or air alone through water before entering the buckets. In all treatments, the fruit were transferred to air at 20C and 95% RH for ripening after the initial 24, 48 or 72 hr.

Color Measurements

'Agriset 761' and 'Sunny' fruit color was monitored daily during ripening at 20C for three, 10-fruit replicates per time-temperature combination daily for up to 11 days. The color of 'Sunbeam' fruit was also measured at 20C for three, 10-fruit replicates per time-temperature-atmosphere combination for up to 9 days. The surface color of the tomatoes was measured at random spots near the blossom end of each fruit (Hobson et al., 1983). For 'Agriset 761', color was measured using a Hunterlab Color Quest colorimeter (Hunter Assoc., Reston, Virginia. USA) fitted with a 2.5-cm viewing port aided by a Zenith 286 computer, while color of 'Sunbeam' and 'Sunny' tomato fruit was measured using a Minolta Chromameter, CR200 (Minolta Co., Ltd. Japan) with an 8-mm port. Both

instruments were calibrated using a standard white tile before the color of the tomato samples was measured. The color of the tomatoes seen visually is due to the light that is reflected. Light reflected by the specimen is picked up by a three-element optical sensor in the colorimeter or Chromameter, the output from which is converted into color specifications that are presented in alphanumeric form as three parameters: L*, a measure of lightness on a scale of 0 (black) to 100 (white); a*, which denotes greenness when negative and redness when positive; and b*, which denotes blueness when negative and yellowness when positive (Hobson et al, 1983). Chroma was calculated using the formula that follows: chroma = $(a^{*2}+b^{*2})^{1/2}$. Chroma represents the purity of color of a specific hue. The hue angle values, which represent the shade of color, were calculated as follows: hue angle = $\tan^{-1}b^*/a^*$. The experiments described here were designed as completely randomized designs (CRD) with duration of treatment, atmosphere of treatment and temperature as factorial combinations. Analysis of Variance (ANOVA) was done and Least Significant Difference (LSD) values were calculated at $P \leq 0.05$ using the Statistical Analysis System (SAS, 1986).

Results

L* Value

Generally, the L* values of 'Sunbeam' tomato fruit previously exposed to air or C_2H_4 at 20C or 30C decreased with time as the fruit ripened during the 9 days of the experiment (Figure 3-1 A, B and C). There were no significant differences in the L* values of 'Sunbeam' fruit after treatment with air or C_2H_4 at 20 or 35C for 24, 48 or 72 hr. Significant L* value differences ($P \leq 0.05$) were evident by the 4th, 5th, and 6th day at 20C for fruit that were treated with air or C_2H_4 for 24, 48, or 72 hr, respectively (Figure 3-1 A,

B, and C). By the 4th day at 20C, 'Sunbeam' fruit treated with C_2H_4 at 20C had the lowest L* values compared to fruit treated with air or C_2H_4 at 35C or air at 20C, which were similar. Two days later, 'Sunbeam' fruit treated with C_2H_4 at 35C had the lowest L* value followed in increasing order by 20C/ C_2H_4,35 C/ air, and 20C/air. After 8 days at 20C, 'Sunbeam' fruit exhibited similar L* values for all treatments.

Ripening of 'Agriset 761' and 'Sunny' fruit at 20C in air after C_2H_4 treatment at 20, 25, 30, 35, or 40C for 24, 48, or 72 hr varied depending on previous treatments of temperatures and duration of C_2H_4 exposure. The L* values of 'Sunny' fruit measured over 7 days and the L* values of 'Agriset 761' measured over 11 days in air at 20C generally decreased with time as the fruit ripened. There were no significant ($P < 0.05$) L* value differences for 'Sunny' and 'Agriset 761' fruit previously treated with C_2H_4 at 20, 25, 30, 35, or 40C for 24 hr (Figure 3-2 A and 3.3 A). However, differences were observed for 'Sunny' and 'Agriset 761' fruit similarly treated for 48 or 72 hr duration (Figure 3-2 B, C and 3-3 B, C). The L* value differences became larger as the duration of previous C_2H_4 treatment at 20, 25, 30, 35 or 40C increased from 48 to 72 hr. Tomato fruit that were exposed to C_2H_4 at 40C had the highest L* values, followed in decreasing order by 20, 30, 25, and 35C for 'Sunny' fruit measured over 7 days (Figure 3-2 C), while 'Agriset 761' tomato fruit previously exposed to C_2H_4 at 40C had the highest L* values followed by 35, 20, 30 and 25C in decreasing order during the 11 days in air at 20C (Figure 3-3).

a *Value

'Agriset 761', 'Sunbeam' and 'Sunny' fruit changed from green to red with time at 20C, as indicated by increases in a* values changing from negative to positive.

Generally, 'Sunbeam' a* values increased with time in air at 20C for fruit from all treatments. 'Sunbeam' tomatoes previously treated with C_2H_4 at 20C for 24 hr had a* values that rose fastest, but were then overtaken by a* values of fruit previously treated with C_2H_4 at 35C for 24 hr. Tomatoes previously treated with C_2H_4 or air for 48 hr had a* values that all rose similarly, although fruit previously treated with air had a* values that lagged behind C_2H_4 treated fruit. Tomatoes treated with C_2H_4 at 20C for 72 hr had a* values that rose fastest then were equaled, first by those treated with C_2H_4 at 35C, then those treated with air at 35C, while fruit previously treated wth air at 20C had a* values that were slowest to increase. Overall, fruit treated with C_2H_4 at either 20 or 35C for 24, 48, or 72 hr had higher a* values, which increased faster compared to fruit treated with air. The a* values of 'Sunbeam' fruit treated with C_2H_4 at 20 or 35C for 24, 48, or 72 hr changed from negative to positive values by at least 1 day earlier compared to fruit treated with air (Figure 3-4 A, B, and C). At the end of this experiment, after 6 to 9 days, 'Sunbeam' fruit from all treatments had similar a* values. It took about 3 days and about 4 days for 'Sunbeam' a* values to change from negative to positive for fruit treated with C_2H_4 at 20C and 35C, respectively, for 24 hr, while about 5 days were needed for fruit treated with air to reach positive a* values (Figure 3-4 A).

There were little significant differences ($P \leq 0.05$) in a* values of 'Agriset 761' and 'Sunny' fruit treated with C_2H_4 at 20, 25, 30, 35, or 40C for 24 hr (Figure 3-5 A, 3-6 A). However, significant ($P \leq 0.05$) differences in a* values were observed when 'Agriset 761' and 'Sunny' fruit were ripened at 20C after previous C_2H_4 treatment for 48 hr or 72 at 20, 25, 30, or 40C (Figure 3-5 B, C and 3-6 B, C). The highest a* values were measured for 'Sunny' fruit previously treated with C_2H_4 at 25 > 30 > 35C for 48 hr, while

among 'Sunny' fruit previously treated with C_2H_4 for 72 hr, fruit that came from 25C initially exhibited the highest a* values, then fruit that came from 35C, which exhibited higher or equal a* values. The a* values of 'Agriset' fruit were highest in fruit previously treated with C_2H_4 for either 48 or 72hr at 30>25>20>35>40C (Figure 3-6 B and C). The a* value of 'Sunny' fruit treated with C_2H_4 at 25C for 48 hr changed from negative to positive during the first day after transfer to air at 20C, while fruit treated with C_2H_4 at 20, 30 or 35C achieved positive a* values during the second day in air at 20C, (Figure 3-5 B). It took between 3 and 4 days for 'Sunny' fruit a* values to change from negative to positive for fruit treated with C_2H_4 at 40C for 48 hr. The a* value changes for 'Sunny' fruit treated with C_2H_4 for 72 hr were different from those of fruit treated with C_2H_4 for 48 hr before transfer to air at 20C. The a* values for 'Sunny' fruit treated with C_2H_4 for 72 hr at 20, 25, 30, or 35C were already positive when the fruit were transferred to air at 20C, when initial color measurements were done (Figure 3-5 C), while fruit previously treated with C_2H_4 at 40C showed negative a* values for 5 days. 'Sunny' fruit treated with C_2H_4 at 40C for 72 hr had significantly lower a* values at the end of the experiment than fruit treated with C_2H_4 for the same duration at 20, 25, 30 or 35C (Figure 3-5 C).

It took 'Agriset 761' about 1, 2, 3, or 4 days for the a* values of fruit treated with C_2H_4 for 48 hr at 30, 25, 20, or 35C, respectively, to change from negative to positive. About 5 days elapsed before a* values changed from negative to positive for 'Agriset 761' fruit treated with C_2H_4 for 48 hr at 40C. 'Agriset 761' fruit from all treatments had negative a* values after C_2H_4 treatment for 72 hr (Figure 3-5 C). The a* values changed from negative to positive within the next 24 hr after transfer to air at 20C for fruit previously treated with C_2H_4 for 72 hr at 30, 25 or 20C, while fruit treated with C_2H_4 at

35C for 72 hr took 2 days for a* values to change from negative to positive. After 5 days in air at 20C, 'Agriset 761' fruit treated with C_2H_4 for 72 hr at 40C had an average a* value of zero, which was significantly lower ($P \leq 0.05$) than that of fruit treated with C_2H_4 at other temperatures (Figure 3-6 C).

b* Value

'Sunbeam' fruit treated with air at 20C had higher b* values for the first 3 days after 24 hr treatment (Figure 3-7 A). No clear treatment differences in b* values were observed after 48 hr treatment (Figure 3-7 B). 'Sunbeam' fruit treated with air for 72 hr at 35C had lower b* values than fruit from other treatments.

'Sunny' b* values generally remained the same over a 6 or 7 day period at 20C for fruit treated with C_2H_4 at 20, 25, 30, 35 or 40C for 24 or 48 hr, with up and down fluctuations (Figure 3-8 A and B). Significantly ($P \leq 0.05$) higher b* values were recorded on fruit previously treated with C_2H_4 at 30 or 35C than fruit treated with C_2H_4 at 25C, which had higher b* values than C_2H_4-treated fruit from 20 or 40C . 'Sunny' fruit previously treated with C_2H_4 at 30 or 35C for 72 hr had significantly the higher b* values compared to fruit treated with C_2H_4 at 20, 25, 30, or 40C for 72 hr. Higher b* values of fruit previously treated with C_2H_4 at 30 or 35C were maintained for about 3 days, until decreasing to values similar to fruit from 20, 25 and 40C after 6 days in air at 20C.

The b* values of 'Agriset 761' fruit were not significantly different for fruit treated with C_2H_4 for 24 or 48 hr at 20, 25, 30, 35 or 40C (Figure 3-9 A and B). However, increasing the duration of C_2H_4 treatment of 'Agriset 761' fruit to 72 hr resulted in significantly ($P < 0.05$) different b* values due to previous temperature of exposure (Figure 3-9C). The highest b* values of 'Agriset 761' fruit in increasing order were for fruit

treated with C_2H_4 at 25, 30 or 35C. 'Agriset 761' fruit treated with C_2H_4 at 40C had significantly lower b* values, while fruit treated with C_2H_4 at 20C for 72 hr had intermediate b* values, similar to fruit treated with C_2H_4 at 25 and 30C (Figure 3-9 C).

Hue. The hue of 'Sunbeam' fruit treated with air or C_2H_4 at 20 or 35C for 24, 48, or 72 hr decreased with time in air at 20C. The hue in air at 20C of 'Agriset 761' fruit treated with C_2H_4 for 24, 48, or 72 hr at 20 or 35C decreased faster than fruit held in air at 20 or 35C for the same durations (Figure 3-10 A, B and C).

The hue of 'Sunny' fruit decreased with time at 20C in all treatments. The hue of 'Sunny' fruit treated with C_2H_4 at 20, 25, 30, 35, or 40C for 24 hr was similar for the 7 days of measurement in air at 20C (Figure 3-11 A). Hue was similar for 'Sunny' fruit treated with C_2H_4 at 35, 25, or 30C, but significantly ($P \leq 0.05$) lower than fruit previously treated with C_2H_4 at 40C for 48 hr or 20C for 72 hr (Figure 3-11 B and C). Fruit of 'Sunny' treated with C_2H_4 at 20C for 48 or 72 hr had significantly lower hue than fruit treated with C_2H_4 at 40C for a similar period of time. The largest hue values were maintained by 'Sunny' tomato fruit previously exposed to C_2H_4 at 40C for 72 hr. All time/temperature combination treatments except 72 hr/40C reached similar final hues. Generally, the hue angle of 'Agriset 761' decreased with time in air at 20C in all treatments. There were no hue angle differences for 'Agriset 761' fruit treated with C_2H_4 at 20, 25, 30, 35, or 40C for 24 hr (Figure 3-12 A). The hue of 'Agriset 761' fruit treated with C_2H_4 for 48 hr at 30C showed the most rapid decline, while fruit from 20 and 25C changed more slowly. On the other hand, the hue of 'Agriset 761' tomatoes previously treated with C_2H_4 at 35 or 40C changed little for the first 3 days, then the hue of fruit from 35C declined faster to equal the final hues of 20 and 25C fruit. The hue of 'Agriset 761'

changed little after 40C treatment with C_2H_4 for 72 hr. In other treatments, the hue declined in the order: 35 < 20 < 25 <30C.

Chroma

The chroma of 'Sunbeam' fruit generally increased at 20C after a lag of 3 or 4 days for fruit previously treated with C_2H_4 or air at 20 or 35C. There were no significant chroma differences for 'Sunbeam' fruit over the first 5, 4 or 3 days at 20C for fruit treated with air or C_2H_4 for 24, 48, or 72 hr, respectively, but significant ($P \leq 0.05$) differences were observed by day 6, 5, or 4 for fruit treated with air or C_2H_4 at 20C for 24, 48, or 72 hr, respectively (Figure 3-13 A). Fruit treated with C_2H_4 at 20 or 35C had significantly higher chroma than fruit treated with air at 20 or 35C, and in increasing order: 20C/air, 35C/air, 20C/ C_2H_4, and 35 C/ C_2H_4.

The chroma of 'Sunny' fruit increased moderately from about 25 to 35 or 40C, sometimes showing upward and downward shifts for all treatments. No chroma differences were observed for 'Sunny' fruit treated with C_2H_4 for 24 hr at 20, 25, 30, 35 or 40C. Chroma values were significantly ($P \leq 0.05$) higher in fruit treated with C_2H_4 for 48 or 72 hr at 30 or 35C compared to fruit previously treated with C_2H_4 at 25, 20, or 40C in decreasing order for a similar period. (Figure 3-14 B and C). The chroma of fruit previously treated with C_2H_4 at 40C was the lowest compared to fruit treated with C_2H_4 at 20, 25, 30 or 35C, and maintained that way for 6 days at 20C. However, chroma did not change after 72 hr at 40C. The chroma of 'Agriset 761' fruit increased with time for all treatments. No chroma differences were measured for 'Agriset 761' fruit treated with C_2H_4 for 24 or 48 hr (Figure 3-15 A and B). However, for 'Agriset 761' fruit treated with C_2H_4 for 72 hr, fruit previously exposed to C_2H_4 at 40C changed chroma more slowly and

maintained the lowest chroma values compared to fruit from the other temperatures (Figure 3-15 C).

Discussion

L* Value

Tomato fruit from all treatments ripened at 20C, although at different rates depending on previous treatment because 20C is a conducive temperature for tomato ripening. The L* values of tomato fruit from all treatments decreased over time at 20C because as tomato fruit ripen they become darker due to carotenoids synthesized as fruit enter their last stages of development. In 'Sunbeam' fruit, significant L* value differences were only evident by the 4th, 5th, and 6th day of ripening at 20C for fruit previously treated with C_2H_4 or air for 24, 48, or 72 hr, respectively (Figure 3-1 A). These results for 'Sunbeam fruit indicate a delayed response as a result of treatment. 'Sunbeam' fruit treated with C_2H_4 at 20 or 35C had lower L* values than fruit treated with air at the same temperatures because C_2H_4 promotes tomato fruit ripening.

No significant differences in L* values were observed for 'Sunny' or 'Agriset 761' fruit treated with C_2H_4 for 24 hr at 20, 25, 30, 35, or 40C, probably because 24 hr is not long enough for high temperatures ($\geq$ 30 C) to have an impact on tomato ripening. We previously observed similar results (Masarirambi et al., 1995). The L* values are important because consumers are likely to be attracted to deep red tomatoes whose L* values are relatively low. 'Sunny' and 'Agriset 761' tomato fruit treated with C_2H_4 for 48 or 72 hr at 40C had the highest L* values. For 'Sunny' fruit, the L* values for temperatures <40C were in decreasing order: 20C > 30C >25C >35C, while in 'Agriset 761' they were in the following decreasing order: 35C >20C >30C >25C. It appears that

the highest temperature, 40C, and the lowest temperature, 20C, both lead to slower decrease in L* value over time and this may be attributable to decreased metabolism at both temperatures. Fruit treated at 25C developed the lowest L* values, probably due to high metabolism that led to faster ripening. The interesting thing is that even at high temperature (35C), C_2H_4 was capable of promoting ripening upon transfer to the recommended ripening temperature of 20 C.

a* Value

When tomato fruit a* values change from negative to positive it visually means the tomato fruit are changing from green to red, typical of changes that take place when a tomato fruit ripens. In the case of 'Sunbeam', fruit treated with C_2H_4 at 20 or 35C for 24, 48, or 72 hr had higher a* values that changed faster compared to fruit treated with air. What is interesting here is that 'Sunbeam' tomato fruit treated with C_2H_4 at 35C were able to perceive C_2H_4 and this enabled the fruit to ripen faster and more uniformly at 20C compared to control fruit that were held in air. At the end of the experiment after (6 to 9 days), 'Sunbeam' fruit from all treatments had similar red color development most likely because of C_2H_4 production by some ripening fruit in the container stimulating ripening of the other fruit. The formation of lycopene by tomatoes requires C_2H_4 (Jeffery et al., 1984).

Little a* value differences were observed for 'Agriset 761' and 'Sunny' fruit treated with C_2H_4 at all temperatures for 24 hr. It is likely that 24 hr was not long enough to influence the fruit response to C_2H_4 at the different temperatures, whereas increasing the duration of C_2H_4 treatment to 48 or 72 hr at the various temperatures resulted in significant differences in a* values. The highest a* values, implying most ripe fruit in terms

of red color development, were measured for 'Agriset 761' and 'Sunny' fruit treated with C_2H_4 at 25 or 30C. However, there were differences between the two cultivars. Cultivar differences in terms of red color development after exposure to high temperature have previously been reported (Hall, 1963; Yakir et al., 1984). Fruit treated with C_2H_4 at 40C ripened slowly, more so as the time at 40C increased from 48 to 72 hr in both 'Agriset 761' and 'Sunny' fruit. However, the fruit from the 48 hr 40C treatment ended up ripening satisfactorily and, from a practical point of view, such a treatment could be used to not only delay ripening but also to control tomato postharvest diseases. Tomato fruit ripening in terms of red color development has been reported to be reversibly inhibited at temperatures above 30C (Atta-Aly, 1992; Cheng et al., 1988). Atta-Aly and Brecht (1995) held breaker 'Sunny' tomatoes at 30, 35 or 40C and then transfered them to air at 20C. Increasing delays in red color development of fruit were observed in air at 20C that corresponded to the temperature and time of exposure to high temperatures (Atta-Aly and Brecht, 1995). When MG tomato fruit were treated with C_2H_4, stored at 37C for 3 to 7 days, then ripened at 21C, it was observed that red color developmeent was delyed by the high temperature treatment, but developed rapidly when the tomatoes were transferred to 21C (Cheng et al., 1988). Yakir et al. (1984) proposed that the delay in red color development was caused by inhibition of lycopene biosynthesis. In terms of controlling tomato postharvest diseases using high temperatures, Fallik et al. (1993) held inoculated MG and pink tomatoes for 3 days at 38C and completely inhibited decay caused by *Botrytis cinerea*, one of the main postharvest pathogens of tomatoes in Israel. Increasing the duration of C_2H_4 treatment at 40C for more than 72 hr resulted in fruit showing signs of heat injury, which resulted in failure to ripen satisfactory and led to subsequent rots

(data not shown). Heat injury as a result of prolonged exposure to high temperature has been previously reported (Atta-Aly and Brecht, 1995; Biggs et al., 1988).

b* Value

The fruit of 'Sunbeam' did not exhibit any meaningful b* value trends. Generally, the b* values do not seem to be a useful color component in studying the ripening of tomatoes in this particular case. The b* value denotes blueness when negative and yellowness when positive (Hobson et., 1983). In the case of 'Sunny' fruit, significantly higher b* values were recorded upon transfer to 20C for fruit that were treated with C_2H_4 at 30 or 35C compared to fruit treated with C_2H_4 at the other temperatures. This is probably because, at 30 or 35C, chlorophyll degradation occurs as it would with the ripening process, revealing the underlying yellow carotenoids. Lower b* values for fruit treated with C_2H_4 at 20 or 25C probably are due to a masking effect of C_2H_4-promoted synthesis of the red pigment, lycopene. Delay in red color development, as evidenced by prevalence of yellowness, was observed by Yakir et al. (1984) after tomatoes were previously stored at 37C. They speculated that high temperatures inhibit lycopene development or that of its precursors phytoene and phytofluene. In 'Agriset 761' fruit, there were no significant differences in the b* values for fruit treated with C_2H_4 at 24 or 48 hr at the five temperatures although treatment differences were apparent with increased duration of C_2H_4 to 72 hr. High b* values can mean that fruit are at an advanced stage of ripening since tomato fruit of red-colored cvs first turn pink before they turn red. This explains why both 'Sunny' and 'Agriset 761' fruit treated with C_2H_4 at 40C for at least 48 hr to 72 hr had lower b* values compared to fruit from other temperatures, partly because 40C inhibited ripening.

Hue

The hue of the fruit is the actual color or shade of color expressed as an angle from 0 to 360°, which decreased with time at 20C in all treatments and cvs. As the hue angle of the tomato fruit decreases the color of the fruit changes from green through pink to red. The hue changes are an inverse of the a* value changes and the likely causes are similar to explanations for a* changes in the different treatments.

'Sunbeam' fruit treated with C_2H_4 at 20 or 35C for 24, 48, or 72 hr had degreened more and changed to red faster compared to fruit previously treated with air only, when transferred to air at 20C and around 95% RH. Tomato fruit ripening and uniformity of ripeness are enhanced by C_2H_4 treatment at 20 to 24C (Hardenburg et al., 1986). However, this is the first report where C_2H_4 treatment at 35C caused faster and more uniform ripening after transfer to air at 20C. In this case, C_2H_4 was perceived at 35C, but some step(s) in signal transduction must have been blocked, preventing color development while at that temperature. 'Agriset 761' and 'Sunny' fruit treated with C_2H_4 at 25, 30, or 35C degreened more after treatment and developed red color faster than fruit treated with C_2H_4 at 20 or 40C. Red color development in 'Agriset 761' and 'Sunny' tomatoes was slowest in fruit previously treated with C_2H_4 at 40C. Inhibition of red color development in tomatoes while at high temperatures has previously been reported (Cheng et al., 1988; Yakir et al., 1984; Maezawa et al., 1993).

Chroma

Generally, there were no chroma differences for 5, 4, or 3 days in 'Sunbeam' fruit treated with air or C_2H_4 at 20 or 35C for 24, 48, or 72 hr, respectively, because it was during these days when the tomato color was a mixture of green, red and yellow, resulting

in generally less pure color. Later loss of chlorophyll led to increased intensity of the developing red color. When fruit chroma started to increase with ripening of 'Sunbeam' fruit, it became clear that fruit previously treated with C_2H_4 at either 20 or 35C ripened faster than fruit treated with air at either temperature.

The chroma of 'Agriset 761', 'Sunbeam' and 'Sunny' fruit remained about the same for about 3 days in air at 20C for all treatments. Thereafter, chroma started to increase, indicating that as the fruit changed color from green to red they passed through a mixed color due to the simultaneous presence of green and red pigments, after which the green color was replaced with a more intense or pure red color as chlorophyll completely disappeared and more lycopene accumulated with ripening. Rhodes (1980) reported that the transition of chloroplasts rich in the green pigment, chlorophyll, into chromoplasts rich in the red and yellow carotenoid pigments was responsible for the color changes of fruit during ripening. The fact that there were no chroma differences in 'Sunny' and 'Agriset 761' fruit treated with C_2H_4 for 24 hr at 20, 25, 30, 35 or 40C implies that 24 hr was not long enough for the high temperatures ($\geq$ 30C) to affect C_2H_4 perception and signal transduction. In 'Agriset 761', increasing the duration of treatment to 48 hr did not alter the fruit response in terms of chroma development in air at 20C and the differential response from 'Sunny' may be due to cv physiological differences. Furthermore, 'Agriset 761' was stored overnight at 8C and thus it probably took a longer time for the tomato pulp temperatures to rise to the treatment temperatures, unlike in 'Sunny' fruit, which were placed at the treatment temperatures on the day of harvest and promptly treated with C_2H_4. It is not known how long it took tomato pulp temperatures to equilibrate to that of the temperature of treatment, but this time is a function of initial pulp temperature. Fruit

chroma differences were observed when 'Sunny' fruit were treated with C_2H_4 for 48 or 72 hr at the different temperatures and for 72 hr in 'Agriset 761' because increasing the duration of C_2H_4 treatment affected the intensity of color development. In all cases, 40C had the least pure color and the fruit ripened more slowly compared to fruit from other treatments. Treating tomatoes with C_2H_4 for 48 or 72 hr at 25, 30 or 35C stimulated color development following transfer to air at 20C and this is why more red color was attained faster by tomatoes that came from those treatments. It is well known that ripening can be initiated and accelerated by C_2H_4 application at temperatures around 20C (Gull, 1981; McGlasson et al., 1975; Sherman, 1985), but this is the first demonstration that C_2H_4 can still be applied at high temperatures of up to 35C and is still perceived, and that signal transduction to achieve ripening in tomato is completed in air at 20C. Of particular interest is that prior exposure of tomatoes to high temperatures between 30 and 35C while treating with C_2H_4 for 48 or 72 hr actually stimulated red color development after transfer to air at 20C. This observation raises a question concerning the necessity of reducing tomato fruit temperatures in ripening rooms in order to treat them with C_2H_4, since our results suggest that treating tomatoes with C_2H_4 at temperatures between 30 and 35C for 48 to 72 hr will actually stimulate subsequent ripening in air at 20C. In any case, ripening rooms have been reported to not be designed for efficient cooling of tomatoes (Sherman and Talbot, 1986).

Summary

In the experiments described, there were little ripening behavior differences in terms of color development of tomato fruits treated with C_2H_4 at high temperatures ($\geq$30 C) for 24 hr compared to those treated at lower temperatures. On the other hand, fruit

handled without exposure to exogenous C_2H_4 ripened slowly and nonuniformly compared to fruit previously treated with 100 ppm C_2H_4. When the duration of C_2H_4 treatment at 40C of 'Agriset 761' and 'Sunny' fruit was increased to 48 or 72 hr, subsequent red color development was inhibited while in air at 20C, whereas prior exposure to C_2H_4 at 30C, and in some instances 35C, stimulated red color development at 20C. From our results, it can be inferred that tomatoes can perceive C_2H_4 at moderately high temperatures (30 to 35C) that inhibit ripening, but the response to C_2H_4 is apparent only after transfer to 20C. Red color development was promoted by C_2H_4 treatment at 35C compared to air treatment at the same temperature. The observation that the C_2H_4 response occurs after removal of exogenous C_2H_4 and transfer to 20C indicates that inhibition of the C_2H_4 response mechanism at high temperatures involves reactions downstream from the initial protein binding reaction.

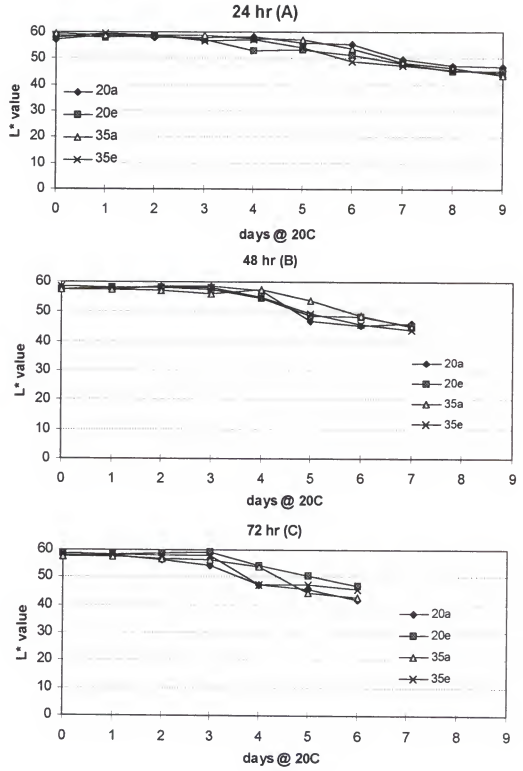


Figure 3-1. The L* values of 'Sunbeam' tomatoes during ripening in air at 20C following treatment with air (a) or 100 ppm C_2H_4 (e) at 20 or 35C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5% =1.56.

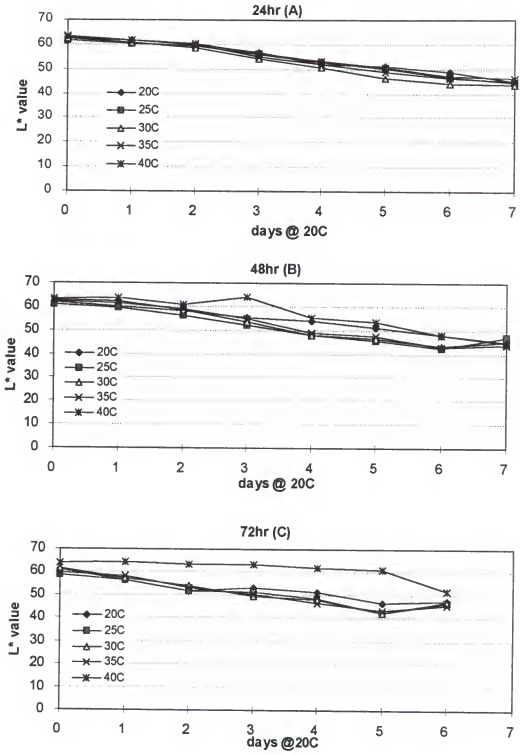


Figure 3-2. The L* values of 'Sunny' tomatoes during ripening in air at 20C following treatment with 100 ppm C_2H_4 at 20, 25, 30, 35 or 40C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5% =0.70.

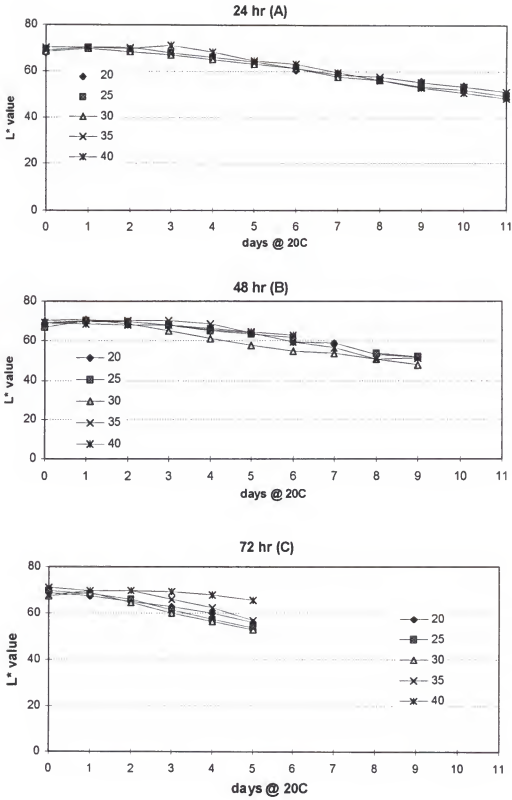


Figure 3-3. The L* values of 'Agriset 761' tomatoes during ripening in air at 20C following treatment with 100 ppm C_2H_4 at 20, 25, 30, 35 or 40C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5% =3.34.

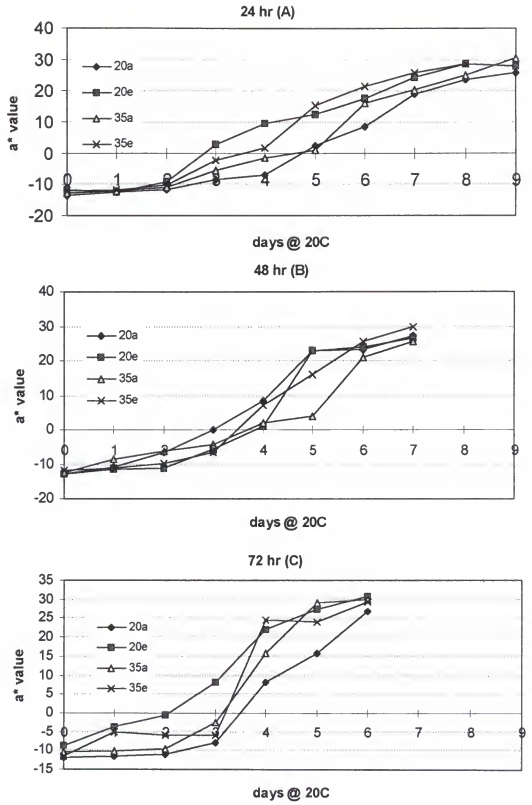


Figure 3-4. The a* values of 'Sunbeam' tomatoes during ripening in air at 20C following treatment with air (a) or 100 ppm C_2H_4 (e) at 20 or 35C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5% =4.19.

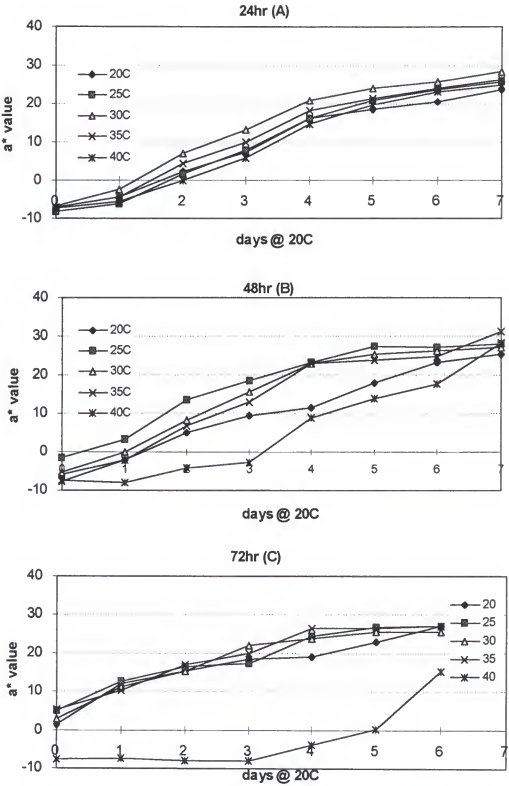


Figure 3-5. The a* values of 'Sunny' tomatoes during ripening in air at 20C following treatment with 100 ppm C_2H_4 at 20, 25, 30, 35 or 40C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5% =1.29.

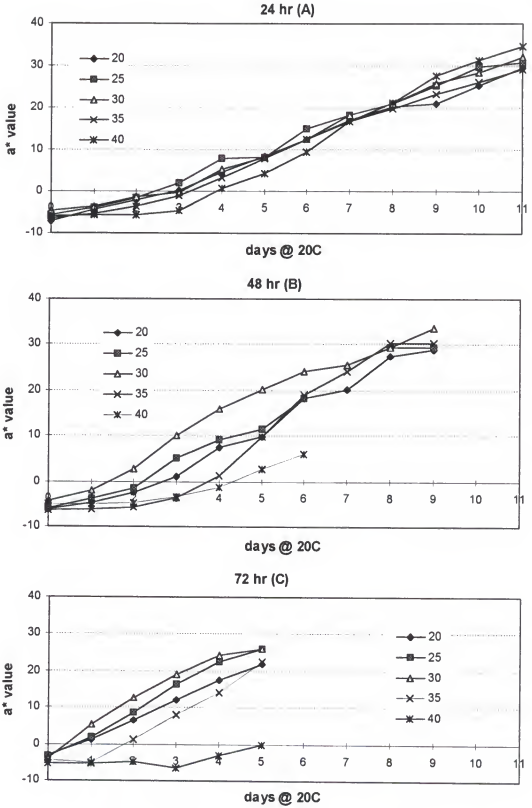


Figure 3-6. The a* values of 'Agriset 761' tomatoes during ripening in air at 20C following treatment 100 ppm C_2H_4 at 20, 25, 30, 35 or 40C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5% =6.48.

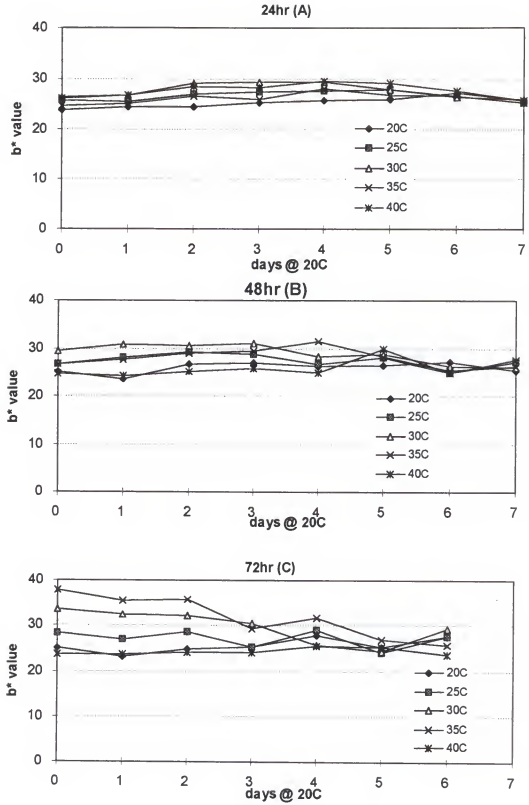


Figure 3-7. The b* values of 'Sunbeam' tomatoes during ripening in air at 20C following treatment with air (a) or 100 ppm C_2H_4 (e) at 20 or 35C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5% =0.39.

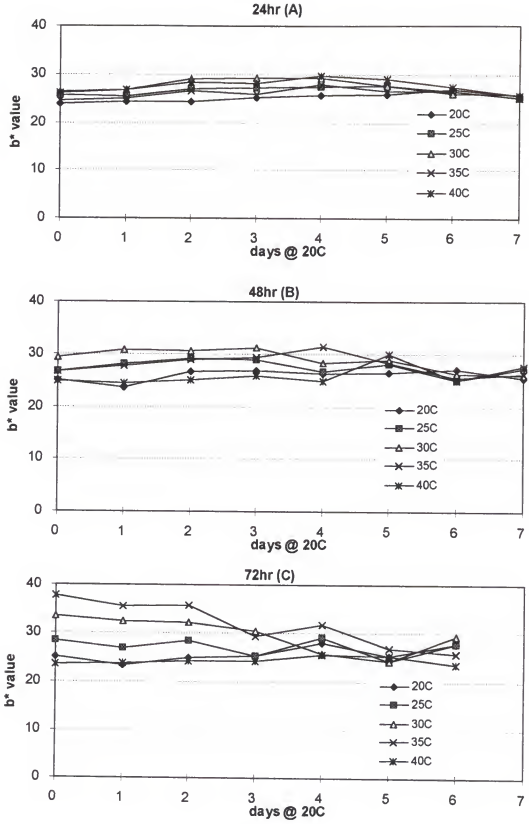


Figure 3-8. The b* values of 'Sunny' tomatoes during ripening in air at 20C following treatment with 100 ppm C_2H_4 at 20, 25, 30, 35 or 40C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5% =0.54

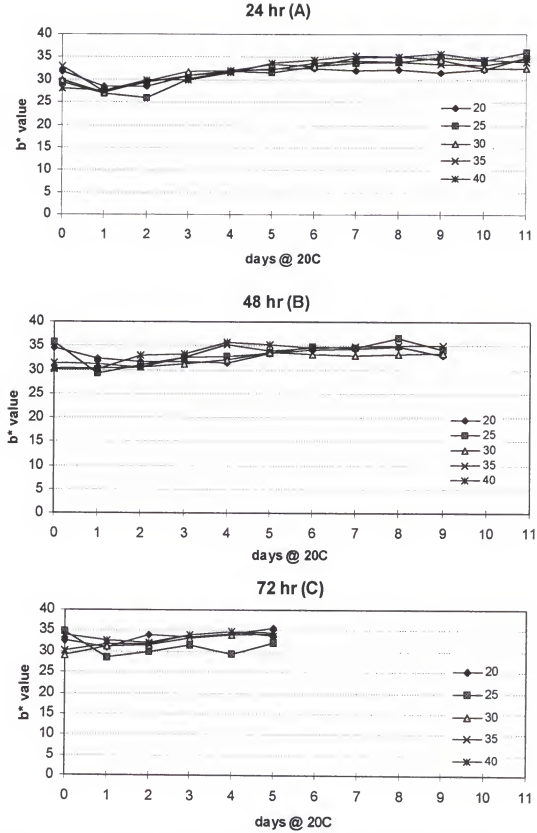


Figure 3-9. The b* values of 'Agriset 761' tomatoes during ripening in air at 20C following treatment 100 ppm C_2H_4 at 20, 25, 30, 35 or 40C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5% =1.28.

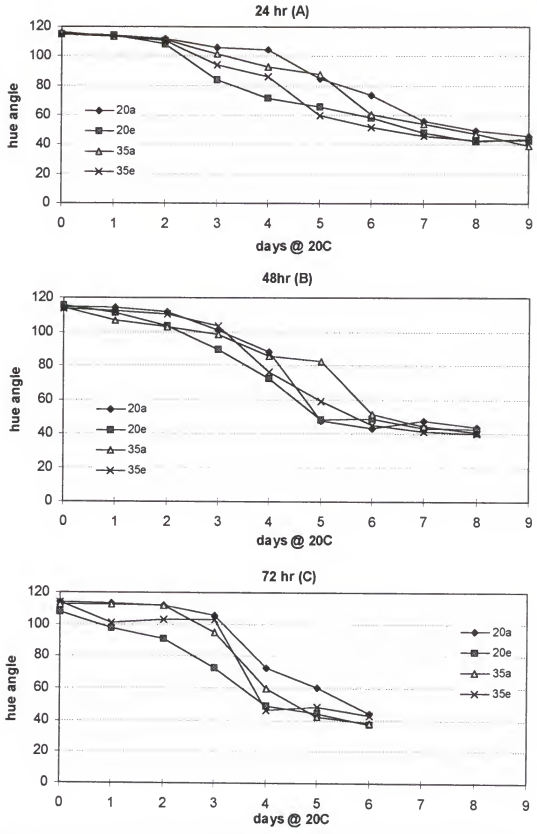


Figure 3-10. Hue angle values of 'Sunbeam' tomatoes during ripening in air at 20C following treatment with air (a) or 100 ppm C_2H_4 (e) at 20 or 35C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5% =7.45

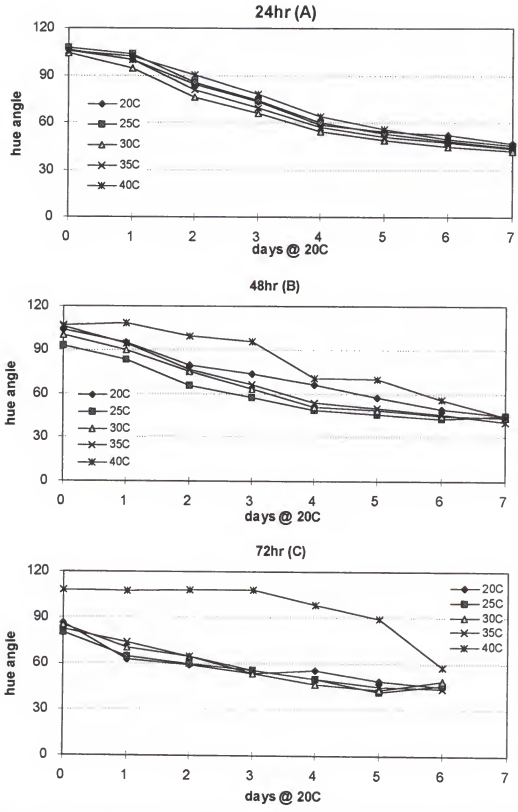


Figure 3-11. Hue angle values of 'Sunny' tomatoes during ripening in air at 20C following treatment with 100 ppm C_2H_4 at 20, 25, 30, 35 or 40C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5% =2.18.

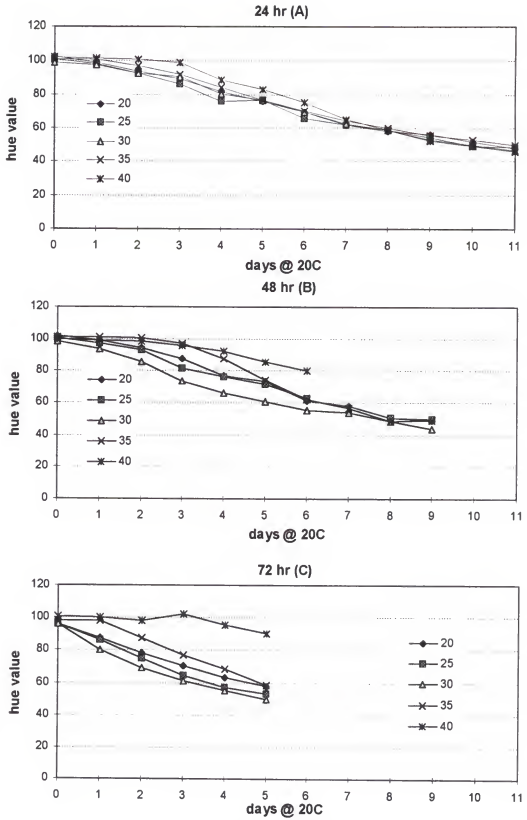


Figure 3-12. Hue angle values of 'Agriset 761' tomatoes during ripening in air at 20C following treatment 100 ppm C_2H_4 at 20, 25, 30, 35 or 40C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5% =8.99.

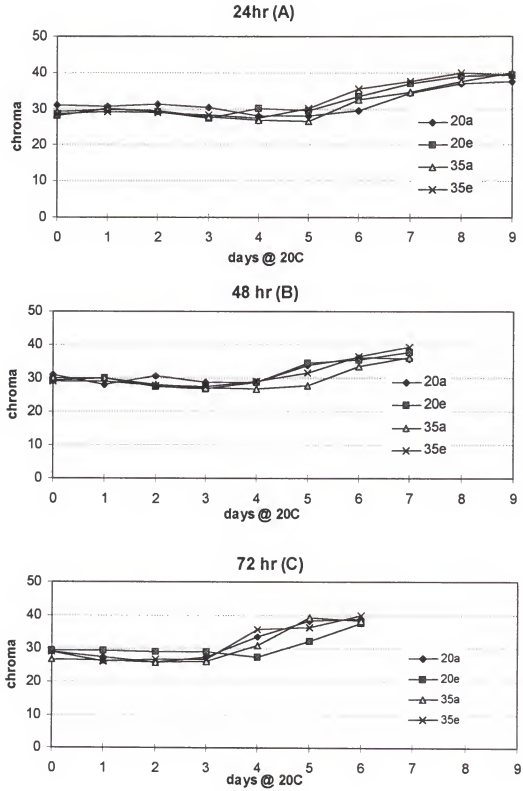


Figure 3-13. Chroma values of 'Sunbeam' tomatoes during ripening in air at 20C following treatment with air (a) or 100 ppm C_2H_4 (e) at 20 or 35C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5%=1.16.

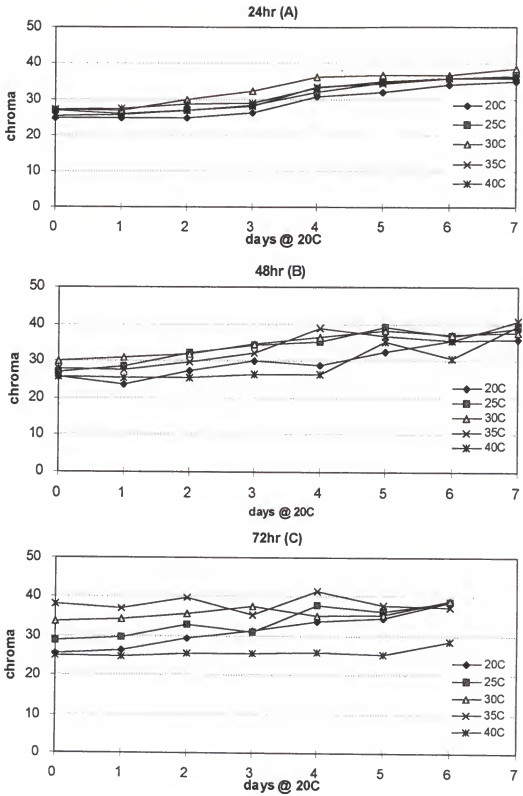


Figure 3-14. Chroma values of 'Sunny' tomatoes during ripening in air at 20C following treatment with 100 ppm C_2H_4 at 20, 25, 30, 35 or 40C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5% =0.80

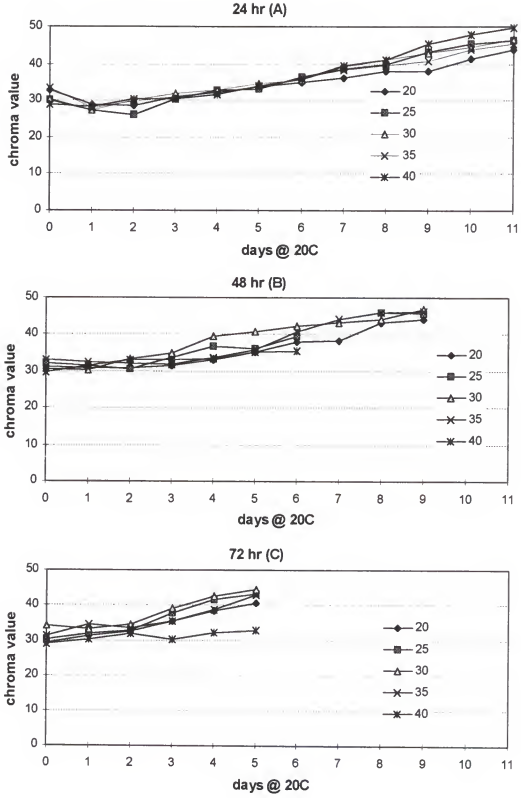


Figure 3-15. Chroma values of 'Agriset 761' tomatoes during ripening in air at 20C following treatment with 100 ppm C_2H_4 at 20, 25, 30, 35 or 40C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5% =2.98.

CHAPTER 4
PHYSIOLOGICAL RESPONSE OF TOMATO FRUIT TO C_2H_4 AT HIGH TEMPERATURE

Introduction

The ripening process involves a complex and coordinated series of changes in pigmentation, flavor, texture, and aroma resulting from physiological and biological activity (Babbit et al., 1973; Maximova, 1996; Gull, 1981; Lurie et. al., 1996; Rhodes, 1980). Ripening in tomato is characterized by a rise in C_2H_4 production and concomitant increase in respiration called the climacteric. (Biale and Young, 1981; McGlasson, 1985). The ripening process is irreversible once endogenous (autocatalytic) C_2H_4 production increases to a certain level (McGlasson, 1985). Treatment with C_2H_4 will advance the onset of ripening in climacteric fruit such as tomato (Gull, 1981; Varga and Bruinsma, 1986; Watada, 1986).

Exposure of MG tomatoes to temperatures $\geq$ 30C attenuates many aspects of ripening. High temperature exposure of MG tomatoes has been reported to disrupt ripening as evidenced by poor color development (Goodwin, 1986; Maezawa et al., 1983; Morris and Kader, 1978; Tomes, 1963; Yakir et al., 1984), delayed softening (Cheng et al., 1988; Inaba and Chachin, 1989; Mitcham and McDonald, 1992; Mohammed et al., 1996) perturbed respiration rate (Cheng et al., 1988; Inaba and Chachin, 1989) and suppressed C_2H_4 production (Atta-Aly, 1992; Biggs, 1988; Cheng et al., 1988; Inaba and Chachin, 1989). Fruit exposed to 40C for 4 days (Atta-Aly and Brecht, 1995) or 9 days

(Inaba and Chachin, 1989) failed to ripen normally and showed symptoms of high temperature injury even when transferred to 20C (Atta-Aly and Brecht, 1995). Cheng et al. (1988) investigated the responses of detached tomato (cv. Flora-Dade) fruit during high temperature (37C) storage and subsequent ripening at 21C. Ethylene-treated MG fruit were stored at 37C and 55% RH for 3 or 7 days, then ripened at 21C and 55% RH, while a control group was kept at 21C and 55% RH continuously. What is peculiar with this experiment is that the tomatoes were put at the treatment temperatures after C_2H_4 treatment. Effects were monitored for 17 days and it was observed that tissue softening, red color development, and climacteric production of C_2H_4 were delayed in fruit previously exposed to high temperature (Cheng et al., 1988). It was also noted that the rate of respiration and respiratory control were higher in mitochondria isolated from the stressed fruit than from the control. Inaba and Chachin (1989) presented detailed information on the effect of high temperature stress on tomato respiration, metabolism and mitochondria activity. When MG tomatoes (cv. Sakata TVR) were stored for 9 days at 5, 10, 15, 20, 25, 30, 35 or 40C, the respiration rate and ethylene production were found to be suppressed at 5, 10, and 35C, while at 40C fruit injury was seen. Mitochondrial oxidation of malate, succinate, α-ketoglutarate and citrate were suppressed at 30 and 35C, while the respiratory control and ADP:O ratios at 35C were consistently lower than those at 25C (Inaba and Chachin, 1989).

The effect of high temperature on C_2H_4 biosynthesis by tomato (cv. Rutgers) fruit at the pink stage was studied by Atta-Aly (1992), who transferred fruit at 6 hr intervals from 15C to 20C, followed by 25C and 30C, and then returned to 20C. As the temperature increased from 15C to 30C, CO_2 production by the fruit increased, while

maximum C_2H_4 production occurred when fruit were held at 20C. Ethylene production and ACC concentration declined as the temperature rose from 20 to 30C. Although this study provides information on the effect of high temperature exposure on pink tomatoes, in Florida it is MG tomatoes that are likely to be exposed to high temperatures at harvest and after harvest before the tomatoes are loaded into ripening rooms.

Biggs et al. (1988) investigated the basis of high temperature inhibition of C_2H_4 biosynthesis in ripening tomato (cv. Rutgers) fruit. A marked decrease in ripening-associated C_2H_4 production was observed when tomato fruit were incubated at 34C or above compared to fruit at 21C. C_2H_4 production resumed after transfer of fruit to a permissive temperature (21C), indicating that high temperature inhibition of C_2H_4 biosynthesis was not associated with permanent tissue damage. Determination of pericarp enzyme activities involved in C_2H_4 biosynthesis following transfer of fruit from 25C to 35 or 40C revealed that ACS activity declined rapidly while ACO activity declined slowly (Biggs et al., 1988). Recovery of ACS activity was more rapid compared to that of ACO when high temperature stress was removed. However, in this study, Biggs et al. (1988) did not treat tomato fruit with C_2H_4 while at the high temperature. The effect of high temperature and C_2H_4 treatment on the ripening of tomatoes was investigated by Yang et al. (1990) who attempted to distinguish between an effect of high temperature on C_2H_4 biosynthesis and sensitivity of the tissue to C_2H_4, by exposing detached MG 'Sunny' fruit to saturating levels of C_2H_4 at 30 and 37C and monitoring changes in indicators of ripening.

The influence of high temperature stress on postharvest quality of processing and non-processing tomato cultivars was studied by Mohammed et al. (1996). A processing

tomato cv ('Dorado'), and a non-processing cv. ('Star Pak') were exposed to one of three prestorage heat treatments over a 5 hr period under unshaded (34 +/-2C), shaded (29 +/-2C) and pre-cooled (20 +/-2C) conditions followed by storage for 18 and 36 days at 20C. At the end, evaluation was done for heat injury symptoms and disease infection in relation to changes in physical, chemical and physiological characteristics of the fruit during storage at 20C. Unshaded fruit showed effects of heat injury, which resulted in reduced bioelectrical resistance, increased electrolyte leakage, increased total acidity and decreased total soluble solids during storage at 20C, while precooling and shading proved to be effective methods to remove or prevent accumulation of field-heat and minimize heat injury (Mohammed et al., 1996). As in previous studies, Mohammed et al. (1996) did not investigate how high temperatures would affect subsequent development of MG fruit after high temperature and C_2H_4 exposure, considering that in Florida tomatoes are harvested at the MG stage.

Although tomato has the status of being the second most frequently purchased grocery produce item in the USA (Resurreccion, 1986) reports of poor quality are common. There are a number of causes of poor quality in tomatoes, including immaturity, CI, physical damage, failure to develop full red color, and insect and disease damage. Although tomato fruit are harvested at the MG stage, usually there is a considerable amount of immature fruit (Kader et al., 1977). In most cases, the immature fruit fail to ripen satisfactory after C_2H_4 treatment in ripening rooms.

The high temperatures prevalent during parts of the tomato harvesting season in regions like Florida are cause for concern because they may aggravate or contribute to tomato quality problems. While there have been studies on the effect of high temperatures

on tomato fruit ripening, there is a dearth of information on the physiological effects of exposing tomato fruit to C_2H_4 at high temperature. We felt that it was necessary, in light of the limited information available, to investigate the effects of tomato high temperature exposure while treating with C_2H_4 on subsequent tomato characteristics. The objective of this study was therefore to investigate ripening changes of tomato fruit after C_2H_4 treatment at high temperatures.

Materials and Methods

Plant Material

Large (USDA, 1976), MG 'Colonial', 'Sunbeam', and 'Sunny' tomatoes were obtained from packinghouses in the Ruskin area or harvested from plots in the Quincy area and transported to Gainesville. Fruit that were picked from plots were first washed in 100 ppm chlorine at a pH of around 7.0 and air dried at the grading table. The tomatoes were regraded, and defective, nonuniform fruit, or fruit showing red color were discarded.

Effect of Treating Tomatoes With Air or C_2H_4 at High Temperature on Subsequent Respiration and C_2H_4 Production

The first experiment was conducted to determine the effect of exposing tomatoes to C_2H_4 at high temperatures on subsequent C_2H_4 production and respiration rate (CO_2 production) at 20C. Thirty 'Sunbeam' tomatoes were put in each bucket , and 6 buckets were placed at 20 or 35C, then treated with air or 100 ppm C_2H_4. In another experiment, the same number of 'Sunbeam' tomatoes were treated with 100 or 1000 ppm C_2H_4. Air and C_2H_4 were mixed at constant pressure in a flow- through system via a gas mixing board utilizing needle valve flowmeters. The fruit were transferred in three, 10-fruit

replicates per time-temperature combination for ripening at 20C in air at 95% RH after 24, 48, or 72 hr at 20 or 35C.

The second experiment, almost a repeat of the first except that more temperatures were considered, was conducted to determine the effect of exposing tomatoes to C_2H_4 at high temperatures on subsequent C_2H_4 production at 20C. Three buckets, each containing 30 'Sunny' fruit, were exposed to 100 ppm C_2H_4 at 20, 25, 30, 35 or 40C. Ethylene and air were mixed and passed through water to achieve near saturation humidity and then the 'Sunny' tomatoes were treated with the resultant 100 ppm C_2H_4 as previously described. After 24, 48, or 72 hr, the fruit were transferred to air at 20C for ripening. While at the ripening temperature (20C), the fruit were evaluated daily for respiration and C_2H_4 production.

Respiration

Respiration was measured as CO_2 produced by three samples of 10 tomatoes enclosed in 8-liter jars for 1 hr. A 1-ml sample of gas was taken through a serum cap from the jars containing the tomatoes and 0.5 ml of the sample was injected into a Gow-Mac GC Series 580 GC (Gow-Mac Instrument Co, Bridgewater, NJ., USA). The GC was equipped with a thermal conductivity detector, with a 102 cm x 3.18 mm, 80/100 mesh Poropak Q column at 40C, and the injector and detector temperatures set at 90C with helium as the carrier gas. The respiration rates are presented as ml CO_2 kg fresh wt^{-1} hr^{-1}.

Ethylene Measurement

Three samples of 10 tomatoes per treatment were weighed and enclosed in 8-liter jars for 1 hr in a static system. Gas samples were taken via a sampling port using a 1 ml syringe, of which 0.5 ml was injected into a Photovac 10A10 (Photovac International

Inc., Deer Park, NY. USA) portable photoionization GC at ambient temperature. The GC was equipped with a 76 cm x 3.18 mm, 80/100 mesh activated alumina column with nitrogen at 15 ml min^{-1} as the carrier gas. The amount of C_2H_4 produced by the 10 fruit over the 1 hr period was recorded in ppm by a Hewlett Packard Integrator, HP 3390A, and expressed as μl C_2H_4 kg fresh wt^{-1} hr^{-1}.

Effect of Treating Tomatoes With C_2H_4 at High Temperate on Subsequent ACC Oxidase (ACO) Activity

The third experiment was conducted to determine the effect of exposing tomatoes to C_2H_4 at high temperatures on subsequent ACO activity at 20C. Three buckets, each containing 30 'Colonial' fruit, were treated with 100 ppm C_2H_4 at 20, 25, 30, 35, or 40C. After 24, 48, or 72 hr, The fruit were transferred to air at 20C, where subsequent ACO activity was measured daily as described below.

ACO Activity

ACO activity was measured *in vivo* in 'Sunny' fruit using the method of Hoffman and Yang (1982), in which tomato pericarp tissue plugs were removed with a # 6 (12 mm diameter) cork borer inserted radially into a fruit over a locule. Four plugs from four fruit were put into each of 10 tared, 18x150 mm test tubes containing 2 ml of a 2% KCl, 1 mM ACC, 1mM aminooxyacetic acid (AOA) solution and the tissue weight was recorded. The test tubes with tissue plugs were attached to a vacuum evaporator/shaker ("Evapomix" Buchler Instruments Inc., Fort Lee, NJ. USA), and a vacuum of 15 mm Hg was applied for 1 min. After releasing the vacuum, the 4 plugs per tube were weighed and each put into 50-ml Erlenmeyer flasks, the head space of which was flushed with C_2H_4-free air and then closed with a rubber serum cap for 1 hr. After 1 hr, a 1 ml gas sample was

withdrawn by inserting a syringe needle through the serum cap and pumping the syringe plunger 10 to 15 times before withdrawing a sample for injection onto the GC column. From each flask, 0.5 ml of gas was injected into the Potovac GC and the amount of C_2H_4 produced was recorded as previously described. ACO activity assessed as C_2H_4 production was calculated using the formula below:

C_2H_4 Production (nl $g^{-1}hr^{-1}$) = [C_2H_4] (nl/L) x void space (L)/[tissue wt (g) x time (hr)]

Effect of Treating Tomatoes With C_2H_4 at High Temperature on Subsequent Flesh Firmness and Internal Composition

The fourth experiment was carried out to investigate the effect of exposing tomatoes to C_2H_4 at high temperature on fruit flesh firmness and internal composition. Three buckets, each containing 30 'Sunny' fruit, were placed at each of the following temperatures: 20, 30, 35, or 40C, and then treated with 100 ppm C_2H_4.

Flesh Firmness

Flesh firmness of 'Sunny' tomatoes was measured using a Cornell Firmness Tester (Hamson, 1952) as modified by Gull (1987). A single tomato fruit was placed on a stand under a 1 kg weight and the handle of the tester was lowered until contact with either of two, opposite equatorial sides of the fruit was achieved. The instrument was zeroed by manipulating the sliding inclined plane under the depth gauge. The handle was then released completely and compression in mm was noted after 5 seconds.

Fruit Composition

Longitudinal wedges from 10-fruit (cv. Sunny) replicates were taken from the median of each fruit, bagged and stored at -20C for later use. After 1 week, the wedges were removed from -20C and placed at ambient temperature where, upon thawing, they

were blended in a Waring commercial blender (New Hartford, Connecticut, USA). The blended tomato puree was centrifuged for 45 min at 650 x g in a swinging bucket rotor (Damon/IEC Division, International Equipment Co., Needham Heights, Mass., USA). After centrifugation, the (supernatant) juice was removed from the centrifuge tubes by decanting into small vials for later use to measure total soluble solids (TSS), pH, and titratable acidity (TA).

Total Soluble Solids (TSS)

The amount of TSS of juice extracted from tomato fruit was measured by a Reichhert-Jung, Abbe Mark II, digital refractometer (Cambridge Instruments Inc. Buffalo, NY, USA). A few drops were transferred to the prism using a transfer pipette and the temperature-adjusted reading of TSS was recorded.

pH

The pH of the tomato juice was measured using a Corning pH meter 140 (Corning Medical and Scientific Instruments, Halsted Essex, England , UK). A probe was inserted into a beaker containing a sample of clear juice derived from the tomato fruits, and the pH was recorded.

Titratable Acidity (TA)

Samples were prepared by weighing 6.00g of clear tomato juice into a 100-ml beaker, 50 ml distilled water was added, and a magnetic stirrer was placed into the solution. Titration was done using the Fisher Burette/dispenser, model 381, connected to a Fisher automatic titrimeter, model 381, which was connected to a Fisher stirrer, model 385 (Fisher Scientific Co., Pittsburgh, PA, USA), on which the beaker was placed to receive 0.1N NaOH The samples were titrated to an end point of pH 8.2. The volume of

NaOH in ml was converted to % citric acid equivalents in the tomato juice as follows: % citric acid = (ml NaOH used x N NaOH x 6.4 meq/mole)/ 6.00 g of tomato juice sample used x 100.

These experiments were conducted using a completely randomized design (CRD) with factorial combinations of treatments. The results were analyzed by Analysis of Variance (ANOVA) using SAS for Personal Computers (1988) by SAS Institute Inc., Cary, NC, USA. Mean separation was by least significant difference (LSD) at $P \leq 0.05$.

Results

Effect of Treating Tomatoes With Air or C_2H_4 at High Temperature on Subsequent Respiration and C_2H_4 Production

Respiration. Respiration of 'Sunbeam' fruit was generally higher in fruit previously treated with C_2H_4 at 20C compared to those treated at 35C, while fruit treated with air at similar temperatures had lower respiration rates than fruit exposed to C_2H_4. However, there were fluctuations/ perturbations in the respiration rates over time in air at 20C with no particular trend in some cases (Figure 4-1 A, B, and C).

Ethylene production. Ethylene production by 'Sunbeam' fruit from all treatments generally increased with time at 20C up to a peak and then began to decline. In most cases, C_2H_4 production was higher in fruit previously treated with C_2H_4 at 20C or 35C compared to fruit treated with air at the same temperature for the three periods of treatment. (Figure 4-2 A, B, and C). For the first 2 or 3 days, C_2H_4 production was always lowest in fruit held in air only at 35C. Thereafter, fruit treated with air at 20C produced the least amounts of C_2H_4. The largest amount of C_2H_4 was produced by tomatoes exposed to C_2H_4 at 35C, with the exception of fruit treated with C_2H_4 at 20C for

24 hr (Figure 4-2 A, B, and C). Ethylene production by 'Sunbeam' fruit usually reached a peak between the 5th and 7th days after transfer to air at 20C.

Ethylene production by 'Sunny' fruit in air at 20C following treatment with 100 ppm C_2H_4 at 20, 25, 30, 35, or 40C for 24, 48, or 72 hr generally increased to a peak, and in some instances, subsequently declined during the duration of the experiment (Figure 4-3 A, B, and C). There were no significant differences in C_2H_4 production over time at 20C for 'Sunny' tomatoes treated with 100 ppm C_2H_4 at 20, 25, 30, 35, or 40C for 24 hr (Figure 4-3 A). Significant differences ($P \leq 0.05$) in C_2H_4 production after transfer of the fruit to air at 20C were observed in fruit previously treated with 100 ppm C_2H_4 for 48 or 72 hr (Figure 4-3 B and C). Ethylene production in air at 20C was higher in 'Sunny' fruit previously exposed to 100 ppm C_2H_4 at 30 or 35C for 48 hr or 72 hr compared to fruit exposed to C_2H_4 at 20 or 40C for the same period of time, while prior exposure of fruit to C_2H_4 at 25C resulted in an intermediate C_2H_4 production rate (Figure 4-3 B and C). Ethylene production in air at 20C was highest in fruit previously exposed to 100 ppm C_2H_4 at 35C and decreased in the following order: 30 > 25 > 20 > 40C. Ethylene production was inhibited after 48 or 72 hr at 40C, but was stimulated by exposure to lower temperatures in the order shown: 35 > 30 > 25.

Ethylene production by 'Sunny' fruit treated with C_2H_4 at 20, 25, 30, 35, or 40C for 24 hr reached a peak on the 6th or 7th day, i.e. the last 2 days of the experiment (Figure 4-3 A), and, by the 7th day, fruit previously treated with C_2H_4 at 40C for 24 hr produced the highest amounts of C_2H_4 (> 8.0 $\mu l\ kg^{-1}hr^{-1}$). Fruit treated with C_2H_4 at the five temperatures for 48 hr had reached the C_2H_4 production peak between the 3rd and 5th days in air at 20C, with the fruit treated with C_2H_4 for 48 hr at 35C producing the largest

amounts of C_2H_4 (8.0µl kg^{-1} hr^{-1}). Fruit treated with C_2H_4 for 72 hr had maximum C_2H_4 production on the 2nd or 3rd day in air at 20C, except for fruit from 40C, which peaked on the 4th day. Fruit previously exposed to C_2H_4 at 35C produced the largest amounts of C_2H_4 (11.0 µl $kg^{-1}hr^{-1}$) while at the ripening temperature.

Effect of Treating Tomatoes With C_2H_4 at High Temperate on Subsequent ACC Oxidase (ACO) Activity

During ripening, conversion of ACC to C_2H_4 increased in 'Sunny' fruit exposed to C_2H_4 at 20 or 25C but did not change in fruit previously treated at 30 or 35C (Figure 4-4, A, B, C). ACO activity was lowest in air at 20C after exposure to 40C. Untreated fruit ripened slowly and nonuniformly compared to fruit previously treated with 100 ppm C_2H_4. Increasing the C_2H_4 treatment concentration to 1000 ppm did not alter the responses to high temperatures described above (data not shown).

Effect of Treating Tomatoes With C_2H_4 at High Temperature on Subsequent Flesh Firmness and Internal Composition

Flesh firmness. Flesh firmness of whole 'Sunny' fruit was significantly ($P \leq 0.05$) affected by previous temperature of exposure while treating with C_2H_4, but there were no significant differences caused by duration of treatment (Table 4-1). Fruit softening after 9, 10, or 11 days in air was more in fruit previously treated with C_2H_4 at 30C, less in fruit that were treated with C_2H_4 at 35 or 40C, and intermediate in fruit previously treated with C_2H_4 at 20C for all durations of treatment (Table 4-1). Flesh firmness was highest in fruit that were treated with C_2H_4 at 40 > 35 > 20 > 30C (Table 4-1).

Fruit composition. There were minor compositional differences caused by treatment with C_2H_4 at 20, 30, 35, or 40C. Duration of treatment at the different temperatures did not affect compositional changes in 'Sunny' tomato fruit. Fruit

Table 4-1. Quality attributes of 'Sunny' tomato fruit after 11, 10, and 9 days in air at 20C following C_2H_4 treatment at 20, 30, 35 or 40C for 24, 48, or 72 hr respectively.

Temperature (C)	Duration (hr)	Deformation (mm)	pH	TA (%)	TSS (%)
20	24	7.13	3.95	1.09	4.36
	48	7.67	4.16	1.08	4.53
	72	8.13	4.28	1.03	4.13
30	24	9.47	3.93	0.99	4.70
	48	8.93	3.93	1.36	4.73
	72	10.27	4.28	1.10	4.30
35	24	6.46	3.83	1.06	4.67
	48	8.13	4.10	1.03	4.13
	72	5.73	4.00	1.00	4.57
40	24	8.53	3.80	1.07	4.70
	48	5.07	4.07	1.114	4.23
	72	5.13	4.10	1.09	4.10
Overal LSD @ 5%	1.07	0.11	0.08		0.16

ANOVA		Pr >F		
	Deformation	pH	TA	TSS
Duration (D)	0.4116	0.0002	0.0130	0.0001
Temp.(T)	0.0001	0.0195	0.0361	0.0004
D X T	0.0026	0.2147	0.0085	0.0047

previously treated with C_2H_4 at 20C had the highest pH of 4.28 compared to fruit treated with C_2H_4 at 30, 35 or 40C, which had similar pH of around 3.98 (Table 4-1). Little differences were observed in % TA and TSS due to C_2H_4 treatment of tomatoes at the various temperatures.

Discussion

Effect of Treating Tomatoes With Air or C_2H_4 at High Temperature on Subsequent Respiration and C_2H_4 Production

Respiration. Heat treatments can alter senescence of fruit by affecting the rates of softening, chlorophyll loss, C_2H_4 synthesis, respiration and protein synthesis (Paull, 1990). The respiration rate of 'Sunbeam' fruit was higher in fruit previously treated with 100 ppm C_2H_4 at 20 > 30C compared to fruit treated with air only at those temperatures. It is well known that C_2H_4 treatment stimulates respiration rate in plant tissues (Abeles et al., 1992). The fluctuations in the respiration rate of tomatoes in air at 20C is consistent with the findings of Inaba and Chachin (1989) and Cheng et al. (1988), who reported that high temperature exposure of MG tomatoes perturbed respiration rates. It is also possible that the respiration rate of tomatoes in air at 20C fluctuated so much because of individual tomatoes being at different ripening stages, especially for the tomatoes that were not treated with C_2H_4.

Few ripening differences were observed for fruit treated with C_2H_4 for 24 hr at the various temperatures, probably because 24 hr was not long enough for the high temperatures (≥ 30C) to cause impairment in tomato physiology. It probably took a number of hours before the tomatoes heated up to the higher treatment temperatures, thus lessening the chances of high temperature impact with only 24 hr exposure. The general

increase in C_2H_4 production by 'Sunny' and 'Sunbeam' fruit from all treatments at 20C is expected because 20C is conducive to tomato ripening and, as ripening progresses, C_2H_4 production generally increases. In fact, under commercial conditions, tomatoes are ripened at this temperature (Gull, 1981; Hardenburg et al., 1986; McGlasson et al., 1975). It has also been reported that C_2H_4 treatment advances the onset of ripening in climacteric fruit like tomato (Gull, 1980; Watada, 1986). Increasing the duration of C_2H_4 treatment to 48 and 72 hr resulted in significant differences in C_2H_4 production in air at 20C (Figure 4-3 B and C), because 48 and 72 hr were apparently long enough for the tomatoes to be affected by the high temperatures. Fruit previously treated with C_2H_4 at 30 or 35C for 48 or 72 hr produced the largest amounts of C_2H_4 in air at 20C, possibly because those temperatures are stressful and lead to additional C_2H_4 production due to stress. Alternatively, C_2H_4 perception was maximum at 30 to 35C, and thus fruit from these temperatures had the maximum capacity for producing C_2H_4 in air at 20C, as evidenced by the large amounts of C_2H_4 produced at the ripening temperature. The fact that stress stimulates C_2H_4 production in plant tissues has been reported before (Abeles et al., 1992). However, 35C has been reported to suppress C_2H_4 production (Inaba and Chachin 1989; Atta-Aly, 1992). Those authors did not treat the tomatoes with C_2H_4 while at the high temperature, nor were the tomatoes transferred to 20C. In a rare experiment, Yang et al. (1990) added exogenous C_2H_4 (100 ppm) to the storage atmosphere of MG tomatoes to determine if inhibition of ripening at high temperatures was due to reduced C_2H_4 production or reduced sensitivity. The authors found that exogenous C_2H_4 did not overcome inhibition of autocatalytic C_2H_4 formation, color development or softening during storage at 30 or 37C. Our present findings seem to suggest that tomatoes perceived

C_2H_4 most at 30-35C, but that completion of the C_2H_4-induced changes was only apparent in air at 20C. In the present study, exposure of tomatoes to air at 20C enabled them to recover from whatever damage may have been caused by the high temperature stress.

Ethylene production. The least amount of C_2H_4 was produced by fruit previously treated with C_2H_4 at 40C for 48 or 72 hr because 40C is too extreme a high temperature, and exposure to that temperature for 48 or 72 hr led to damage of the fruit tissues. This is consistent with the findings of Cheng et al. (1988) who did a similar experiment where they exposed tomatoes to 37C (without C_2H_4) for 3 to 7 days and then ripened them at 21 C, where C_2H_4 production was found to be suppressed by prior exposure of tomatoes to 37C. Increasing the duration of C_2H_4 treatment beyond 72 hr at 40C resulted in injury to the tomato fruit that was characterized by failure to ripen and necrotic waterlogged, spots. Those fruit quickly rotted, probably because the injuries weakened the tomatoes disease defenses. Under commercial conditions, this scenario presents problems because, in Florida for example, tomato pulp temperatures rising to as high as 49C after exposure to sunlight for 1 hour have been observed (Showalter unpublished). Ethylene production was stimulated in air at 20C by prior C_2H_4 exposure in the following temperature order: 35 > 30 > 25C in 'Sunny'. The lag in reaching a peak in C_2H_4 production was associated with the duration of C_2H_4 treatment, with the number of days decreasing as the duration of C_2H_4 treatment increased from 24 to 48 to 72 hr (Figure, 4-3 A, B, and C). This implies that the longer the duration of C_2H_4 treatment under the conditions of the present study, the shorter the time to ripening, and thus the sooner the C_2H_4 climacteric occurred for temperatures <35C.

Ethylene production by 'Sunbeam' fruit was higher in fruit that had been previously treated with 100 ppm C_2H_4 at 20 or 35C compared to fruit treated with air at those temperatures. These results indicate that the tomato fruit were able to sense C_2H_4 at both 20 and 35C. In the case of 35C, the signal is perceived but is only processed in air at 20C, indicating that some downstream events in C_2H_4 signal transduction are affected by high temperature in a reversible manner. The reversible inhibition of ripening in tomato, as evidenced by delayed C_2H_4 production, has been reported by Biggs et al. (1988), who studied the biochemical basis of high-temperature inhibition of C_2H_4 biosynthesis in ripening tomato fruits. The untreated tomatoes ended up ripening satisfactorily, although more slowly than C_2H_4-treated fruit, because they were in a container such that those that had started ripening produced C_2H_4, which stimulated autocatalytic C_2H_4 production in the other tomatoes that had not yet started ripening, thereby causing them to ripen.

Effect of treating tomatoes with C_2H_4 at high temperate on subsequent ACC oxidase (ACO) activity. The fact that conversion of ACC to C_2H_4 increased in 'Colonial' fruit previously exposed to C_2H_4 at 20 or 25C but not 30 or 35C implies that the former temperatures are optimum for ACO activity while the latter are rather high and extreme temperatures. Temperatures > 35C may cause enzyme denaturation and loss of or reduced catalytic function (Whitaker, 1972) and this explains why previous exposure of tomatoes to 40C resulted in the lowest ACO activity and hence C_2H_4 production. Fruit treated with C_2H_4 ripened faster and more uniformly than fruit treated with air only. Ethylene is known to speed up ripening in climacteric fruits such as tomato and is considered the ripening hormone (Gull, 1981; Watada, 1986). Increasing the C_2H_4 concentration from 100 to

1,000 ppm did not alter the ripening response, indicating that the response to C_2H_4 at all temperatures was saturated at 100 ppm C_2H_4. Thus, the differences observed between treatments were due to temperature effects only, and not as a result of differential response to C_2H_4 at the different temperatures.

Effect of Treating Tomatoes With C_2H_4 at High Temperature on Subsequent Flesh Firmness and Internal Composition

Flesh firmness. Flesh firmness was affected by high temperature treatment of MG tomatoes and was highest in fruit treated with C_2H_4 at 40 > 35 > 20 > 30C. In this case, high temperatures greater than 35C slowed down degradation of the cell wall, probably partially inhibiting the activity of PG (Kagan-Zur et al., 1995; Lurie et al., 1996; Picton and Grierson, 1988). These findings are similar to those of Mitcham and McDonald (1992), who found that softening was inhibited in heated tomato fruit (fruit exposed to 40C for 4 days), such that 14 days after treatment, heat-stressed fruit were twice as firm as non-heated fruit. The difference with the present study is that in their experiment they did not treat fruit with C_2H_4 while at the high temperature.

Fruit composition. There were minor differences in composition of tomato fruit caused by heat treatment at 20, 30, 35, or 40C for 24, 48, or 72 hr. This is interesting because it means that heat treatments could possibly be used where desired without adverse effects on internal fruit quality attributes. Physical treatments like heat can be used beneficially for insect disinfestation and disease control (Couey and Hayes, 1986; Klein and Lurie, 1992).

Summary

This is the first study of the effect of high temperature and C_2H_4 treatment on subsequent ripening-associated processes of MG tomatoes. There were few ripening behavior differences in terms of C_2H_4 production and ACO activity in air at 20C in tomatoes previously exposed to C_2H_4 at high temperatures (≥ 30C) for 24 hr compared to those treated at lower temperatures (i.e. 20 and 25C). Ethylene production was inhibited after 48 or 72 hr at 40C, but lower temperature exposure stimulated C_2H_4 production in the following order: 35 > 30 > 25C. During ripening in air at 20C, ACC conversion to C_2H_4 increased in fruit previously exposed to C_2H_4 at 20 or 25C but did not change in fruit from 30 or 35C, while exposure to 40C resulted in the lowest ACO activity. Tomato fruit that were not treated with C_2H_4 ripened slowly and nonuniformly compared to those previously treated with C_2H_4.

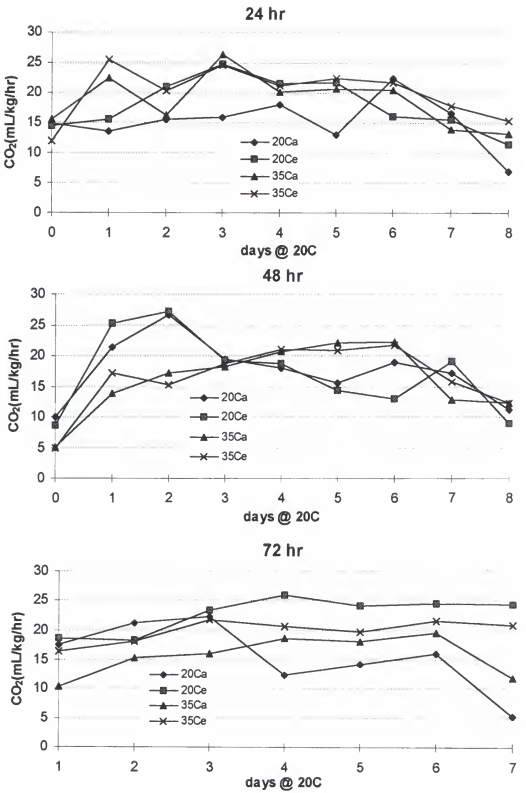


Figure 4-1. Respiration of 'Sunbeam' tomatoes during ripening in air at 20C following treatment with air (a) or 100 ppm C_2H_4 (e) at 20 or 35C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5%=10.5.

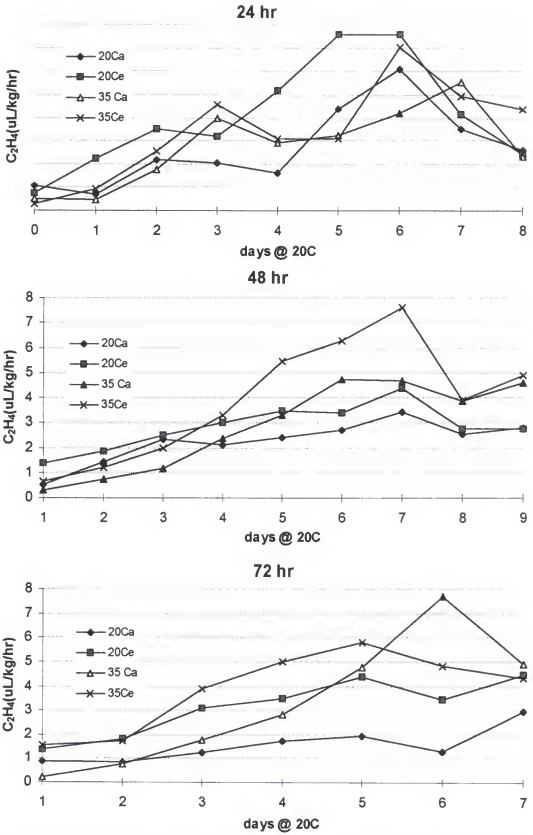


Figure 4-2. Ethylene production of 'Sunbeam' tomatoes during ripening in air at 20C following treatment with air (a) or 100 ppm C_2H_4 (e) at 20 or 35C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5%=0.35.

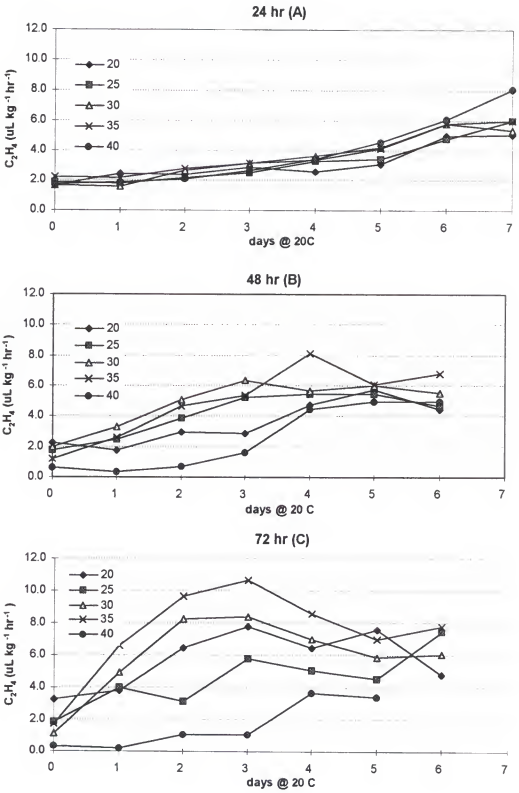
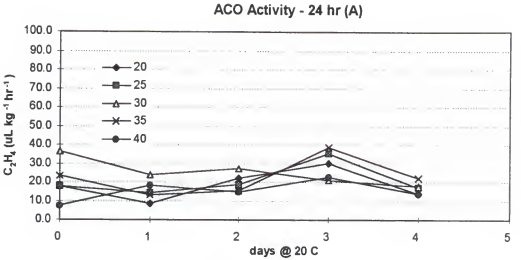
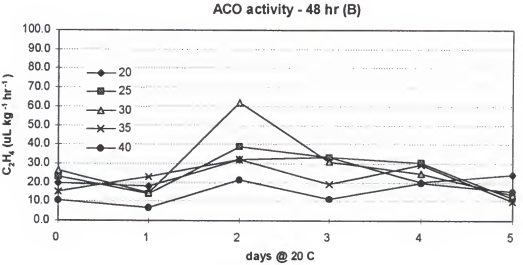


Figure 4-3. Ethylene production of 'Sunny' tomatoes during ripening in air at 20C following treatment with 100 ppm at 20, 25, 30, 35 or 40C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5% =2.8.





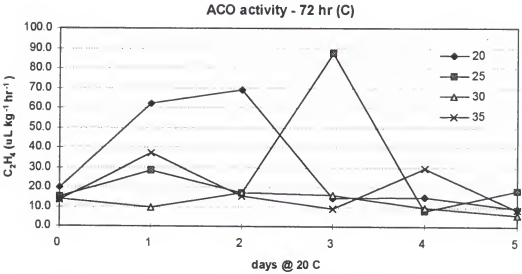


Figure 4-4. ACO activity of 'Sunny' tomatoes during ripening in air at 20C following treatment with 100 ppm at 20, 25, 30, 35 or 40C for 24 (A), 48 (B) or 72 hr (C). LSD @ 5% =6.98.

CHAPTER 5
SUBERIZATION RESPONSE DURING WOUND-HEALING IN TOMATO FRUIT FOLLOWING EXPOSURE TO C_2H_4 AT HIGH TEMPERATURE

Introduction

Living organisms (including tomato fruit) are packaged in envelopes that consist of polymeric structural components; in terrestrial plants, a waterproofing mixture of lipids, collectively called waxes, is associated with this outer layer (Kolattukudy, 1980). The polymeric structural components in higher plants are biopolyesters, with cutin found in the aerial parts and suberin at wound surfaces (Kolattukudy, 1980) These compounds prevent excessive water loss from the plant or plant part. Suberin and cutin function primarily as the structural components of barrier layers (Kolattukudy, 1980). Suberin can serve as a nearly impermeable chemical and physical barrier to fungal attack (Stelzig, 1987), thus preventing rots caused by some opportunistic fungi. Suberin-associated soluble compounds, such as phenolics or wax components, may also act as antifungal agents (Stelzig, 1987). It has been proposed that suberization might be a general response to fungal infection. In response to injury, plant tissue may form protective barriers of tightly packed cells, provided that the tissues are still capable of cell division, or the cells surrounding the injury can deposit lignin and suberin in their walls to protect against the action of macerating enzymes of pathogenic microorganisms (Eckert, 1978).

There have been a few previous studies on the synthesis of suberin in tomato fruit tissue (Dean and Kolattukudy, 1976; Rao et al., 1984; Sherf and Kolattukudy, 1993;

Wilson and Cocking, 1972). Rao et al.(1984) showed that isolated tomato fruit protoplasts behaved as if wounded and synthesized suberin. The multilamellar wall secreted by protoplasts isolated from tomato locule tissue that had been incubated at 20 to 25C was purified and an extract was obtained after depolymerization with BF_3-methanol. The extract was analyzed by thin layer chromatography and the presence of fatty acid methyl esters, fatty alcohols, dicarboxylic acid dimethyl esters, and w-hydroxyl acid methyl esters was detected, indicating the presence of suberin (Rao et al., 1984). Synthesis of suberin during wound-healing in jade leaves, tomato fruit, and bean pods was studied by Dean and Kolattukudy (1976). 'Bonny Best' tomato fruit were harvested at an immature green stage of growth and the fruit were wounded by removing the cuticle with a razor blade. The wounds were left to heal for 14 days under ambient laboratory conditions (temperature and RH not specified), after which the resulting surface layer was excised, lyophilized, solvent-extracted, and depolymerized by hydrogenolysis with $LiAlH_4$ or transesterified with BF_3. The components of the extract were analyzed and it was found that the major aliphatic monomers of the polymeric material deposited during the wound-healing of bean pods, jade leaves and tomato fruit were similar to those of suberin. From these results, it was concluded that suberization was a fundamental process involved in wound-healing in plants, irrespective of the chemical nature of the natural protective polymer of the tissue (Dean and Kolattukudy, 1976).

Wounded plant organs such as fruits produce C_2H_4 in response to wounding stress (Abeles et al., 1992; Riov and Yang, 1982; Yang, 1985). Stress seems to affect suberization (Kollatukudy, 1980), promoting it in many cases. Indirect evidence suggested that vascular coatings formed by plants in response to stress consist of suberin-

like substances containing lipid and phenolic compounds (Robb et al., 1991). The authors provided more direct chemical evidence that the coatings are suberin by using a natural pathogen, *Verticillium albo-atrum*, or a stress-responsive hormone, abscisic acid, to induce coating in two isolines of 'Craigella' tomato, one resistant and the other susceptible to the pathogen. The authors concluded that stress-induced coatings were mainly suberin. Several defense mechanisms, including hypersensitivity, suberization, wound periderm formation, and phytoalexin production in tubers of *Solanum tuberosum* were investigated (Vaughan and Lalai, 1991). It was found that phytotoxin produced by the pathogen *Verticillium dahliae* induced suberization in *S. tuberosum*. The effect of C_2H_4 on suberin formation during healing of harvest-inflicted wounds of tomatoes during ripening remains unclear. It was observed that exposure to 10 ppm C_2H_4 caused a rise in PAL activity at all temperatures and increased development of russet spotting in Iceberg lettuce (*Lactuca sativa* L)(Ritenour, et al., 1995). The deamination of L-phenylalanine to trans-cinnamic acid, which is catalyzed by PAL, is the first committed step leading to the formation of secondary products in the phenylpropanoid pathway in plant tissues (Camm and Towers, 1973; Ritenour et al., 1995). Suberin is one of the many secondary products produced in plants and plant parts and it can be speculated that C_2H_4 may play a role in the formation of suberin, but the details of C_2H_4's probable role in tomato wound healing are not yet known. Kitinoja (1987) found that when sweetpotatoes (*Ipomea batatus* (L) Lam) were cured at 30C in air containing 2 ppm C_2H_4, the roots formed callus and their rate of water loss was somewhat higher than that of air-cured sweetpotatoes, indicating that C_2H_4 exposure may interfere with suberization in sweetpotatoes.

Florida research has shown that poor dump tank water and wash water management practices can be major contributors to tomato fruit decay problems (Sherman et al., 1981). Chlorine (Cl_2) is the major biocide used to sanitize dump tank water and wash water, and can be obtained from aqueous solutions of sodium hypochlorite or calcium hypochlorite, or elemental Cl_2 in liquid form can be used. Sherman et al. (1981) recommended chlorinating dump tank and wash water to maintain a free Cl_2 concentration of 100 to 150 ppm. Grigg and Chase (1967) studied the effect of water or Cl solutions used to wash potatoes (*S. tuberosum*) prior to storage on shrinkage evaluation/weight loss after 1, 5, 6 and 7 months in storage. Using a 10% sodium hypochlorite (NaOCl) solution as a chlorine source, tubers of 'Sebago', 'Arenac' and 'Russet Burbank' were washed before storage in water or Cl solutions of 100, 500 and 1,000 ppm. No significant differences in weight loss were observed among treatments, although a trend of decreased weight loss with increased Cl solution concentration was apparent.

Since high temperature exposure of fruits and vegetables has potential beneficial and detrimental effects, we questioned how high temperatures before harvest, after harvest, and continued high tomato pulp temperatures in the ripening rooms would affect suberization of wounded MG tomatoes when exposed to C_2H_4 to initiate ripening. The suberization response of tomatoes during wound healing during and following exposure to C_2H_4 at high temperature has not previously been studied at all. And yet suberization is important in that it not only reduces water loss but seals off wounds, thereby keeping out potential opportunistic decay causing organisms. The objective of this study, therefore was to investigate the effect of C_2H_4 treatment at high temperatures and prior Cl

concentration in wash water (sanitization) on suberization of MG tomatoes to heal harvest-inflicted wounds.

Materials and Methods

Plant Material

Large (USDA, 1976) 'Sunny' and 'Solar Set' tomato fruit at the MG stage were obtained from packinghouses or harvested from plots in the Ruskin and Imokalee areas and transported to Gainesville in an air-conditioned van. Upon arrival in Gainesville, the fruit from the field were washed with water containing about 100 ppm Cl at around pH 7, air dried, then regraded, with wounded, deformed, and malformed fruit or fruit showing color discarded, while fruit from the packinghouse were just regraded.

Weight Loss in Wounded and Unwounded Tomatoes

In this experiment, 'Sunny' tomatoes were divided into two groups, with one group to be wounded and other used as an unwounded control. Wounding was done on two opposite surfaces of each tomato by abrasion, using sandpaper to create a circular wound about 10 mm wide and 2 mm deep. The weight of wounded and unwounded fruit was measured. Thirty fruit of each group (wounded or unwounded) were put in one bucket, and three buckets of each group were placed at 20, 25, 30, 35, or 40C and treated with 100 ppm C_2H_4 for 24, 48, or 72 hr. The fruit were treated with C_2H_4 by mixing C_2H_4 and air via a gas mixing board equipped with needle valve flowmeters. RH was maintained at near saturation by bubbling the air/ C_2H_4 mixture through water before entering the buckets. After 24, 48, or 72 hr at the designated temperatures, the fruit were transferred to air at 20C and around 95% RH

The fruit remained in air at 20C for 7 days, after which they were weighed to assess weight loss. The time required for completion of the suberization process has been reported to be between 5 to 7 days in sweetpotatoes (*I. batatas*) at around 29C in the presence of a high RH of about 85 to 90% (Kushman, 1975; Ryall and Lipton, 1979; Walter and Schadel, 1982).

In a second experiment (almost a repeat of the first one), 'Sunny' tomatoes were all wounded as previously described and weighed. Three buckets, each containing 30 fruit, where placed at each of the following four temperatures: 20, 30, 35, or 40C at near saturation humidity and treated with 100 ppm C_2H_4 as previously described. After 24, 48, or 72 hr of C_2H_4 treatment at the designated temperatures, 30 tomatoes from each treatment temperature were moved to air at 20C and around 95% RH to complete wound healing. The same tomatoes were weighed after 7 days in air at 20C to assess the amount of weight lost. In a third experiment, wounded (as previously described) 'Solar Set' tomatoes were placed in buckets, each containing 30 fruit. Three buckets were placed at each of the following temperatures: 20, 25, 30, or 35C, where the tomatoes where treated with about 100 ppm C_2H_4 for 24, 48, or 72 hr at near saturation humidity as previously described. The tomatoes were transferred to air at 20C and around 95% RH after 24, 48, or 72 hr as described previously. After 7 days in air at 20C and around 95% RH, shriveling of the wounded surfaces of the tomatoes was scored as follows: 1= 25% ; 2= 40%; 3= 60%; 4= 80%; 5= 100% shriveling based on severity of shrinkage of the area affected.

In part, water loss served as one indicator of the extent of suberization at the wound surface after C_2H_4 or air treatment for the various temperatures and durations of

treatment, the rationale being that less water loss in fruit implies that the wounds in those fruit suberized or healed faster than those that lost more water. The mechanism of the prevention of water loss in plant tissues by suberin is due to the wax, which provides the major barrier to moisture diffusion (Kolattukudy, 1980). Suberin, which functions primarily as the structural components of barrier layers, always has wax associated with it.

Effect of Temperature and C_2H_4 Treatment on Deformation at Wounds

After 'Sunny' fruit that had been treated with C_2H_4 for 72 hr were evaluated for shrivelling, five fruit per treatment temperature were set aside for use to measure deformation at wounded areas. Firmness was measured using a Cornell Firmness Tester (Hamson, 1952) as modified by Gull (1987). A single tomato fruit was placed on a stand under a 1 kg weight and the tester's handle was lowered until contact with the wounded area was achieved. The firmness tester was fitted with a 1-cm diameter convex tip probe, which was centered on the abraided area of the fruit. Deformation was measured as previously described. In this experiment, deformation was measured on two wounded areas and average deformation (mm/fruit) was calculated.

Effect of Temperature and C_2H_4 Concentration on Suberization

'Sunny' tomatoes were wounded as previously described. Thirty wounded fruit were put in each bucket and three buckets were placed at each of the following five temperatures: 20, 25, 30, 35 or 40C, at near saturation humidity and treated with 100 or 1000 ppm C_2H_4 as described before. After 24, 48, or 72 hr, the fruit were moved to air at 20C and 95% RH. After 7 days in air at 20C, the wound coverings of the tomatoes from the various temperatures were removed by separating the wounded tissue from the underlying healthy tissue using a razor blade and placing the tissue samples in small plastic

boats for staining. To perform the color test, 5 drops of a saturated solution of phloroglucinol in 18% hydrochloric acid (HCl) were placed on the detached wound surface, which was previously adjacent to the healthy tissue (Walter and Schadel, 1982). The tissue was blotted dry after 10 minutes and its color was visually scored on a scale of 1 to 4. The scale was as follows: 1= no color to faint pink around the edges; 2= pink, 3= red; 4= deep reddish purple. This method of wound periderm staining was originally developed in sweetpotato (Walter and Schadel, 1982). The phloroglucinol reacts with suberin, with more suberin resulting in higher scores.

Effect of Chlorine Treatment on Weight Loss and Decay

'Solarset' tomatoes were wounded as previously described and washed in Cl solutions of 100, 500 or 1,000 ppm NaOCl at a pH of around 7. The weight of the wounded tomatoes was recorded prior to them being placed at designated temperatures for C_2H_4 treatment. Three buckets containing 30 fruit each were placed at 30 or 35C and treated with 100 ppm C_2H_4 for 24, 48, or 72 hr as previously described. After 24, 48 or 72 hr, the tomatoes were transferred to air at 20C and 95% RH to complete the wound healing process. After 7 days, three, 10-fruit replicates were re-weighed to assess weight loss. The fruit were subsequently left in air at 20C for use to assess disease development.

After 20 days in air at 20C and 95% RH, incidence of disease was assessed by counting the number of diseased fruit and expressing the number as a percentage of the total number of fruit that were in a replicate.

Inoculum Preparation

G. candidum link was taken using a sterile wire loop from a stock spore culture that is maintained in the Postharvest Pathology Laboratory of Dr. J. A. Bartz at the

University of Florida and was inoculated onto potato dextrose agar (PDA) in petri dishies. *G. candidum* was grown on PDA in petri dishes at 20C under continous fluorescent light. After 7 days of growth on PDA at 24C, when spores had formed, the plates were flooded with sterile, distilled water and the fungal spores were suspended by rubbing a bent sterile glass rod on top of the culture to mix the spores. The resultant mixture of fungal spores and sterile water was poured into an Erlenmeyer flask. The spore suspension concentration of *G. candidum* was calculated from a previously calculated regression of optical density at 600 nm using a Spectronic 20 spectrophotometer (Bausch and Lomb, USA) to be 1 x 10^7 conidia-forming units (cfu) ml^{-1}.

Inoculation with *G. candidum* at 30 and 35C.

The 1 x 10^7 cfu ml^{-1} spore suspension was diluted to 1 x 10^6 cfu ml^{-1} by serial dilution, and the diluted spore suspension was later used to inoculate wounded tomatoes. Fruit were wounded by abrasion as previously described on four spots around the equatorial region. Three buckets, each containing 15 wounded 'Solar Set' tomatoes, were placed at each of the following temperatures: 20, 25, 30, or 35C for 24, 48, or 72 hr and treated with about 100 ppm C_2H_4 at near saturation RH as previously described. The wounded fruit were inoculated after C_2H_4 treatment by dipping the wounded spots into the spore suspension of *G. candidum* contained in a petri dish. The inoculated fruits were immediately placed in black plastic bags and stored at 20C with high RH maintained by occasionally misting the fruit with distilled water, to allow for disease development. The disease incidence was expressed as a percentage of the total number of fruit that were affected by *G. candidum*. Disease severity was scored on affected fruit on the following scale: 1=25% , 2=50%, 3= 75% and 4= 100% of the whole tomato fruit affected.

Statistical Analysis

The experiments were carried out using a completely randomized design (CRD) with duration, atmosphere, Cl concentration, and temperature of treatment as factorial combinations. In the weight loss experiments with wounded and unwounded tomato fruit, weight loss after 7 days in air at 20C was expressed as a % of the original fruit weight before C_2H_4 treatment at the designated temperatures and durations. Data for percent disease incidence and severity was transformed using the arc sine / angular transformation (Little and Hills, 1978). The results were analyzed by Analysis of Variance (ANOVA) using the Statistical Analysis System (SAS, 1988) and Least Significant Difference (LSD) values were calculated at $P \leq 0.05$.

Results

Weight Loss Assessment in Wounded and Unwounded Tomatoes

Tomatoes from all treatments lost weight while in air at 20C, with unwounded fruit losing less weight than their wounded counterparts (Table 5-1). In this first experiment, more weight loss occurred in tomatoes from 35C, while there were no significant differences in weight loss in tomatoes that came from 20 or 25C (Table 5-1). Wounded 'Sunny' tomatoes ($P \leq 0.05$) lost significantly more weight, about two times more than unwounded fruit, after 7 days in air at 20C. The duration of C_2H_4 treatment of wounded and unwounded 'Sunny' tomatoes at 20 and 25C did not affect weight loss significantly after 7 days in air at 20C (Table 5-1). However, wounded 'Sunny' tomatoes previously treated with C_2H_4 for 72 hr at 30C lost less weight than tomatoes previously treated with C_2H_4 for 24 or 48 hr and the weight loss was equal to tomatoes that were treated at 20 or 25C. Wounded 'Sunny' tomatoes previously treated with C_2H_4 at 35C lost

Table 5-1. Weight loss of wounded or non-wounded 'Sunny' tomatoes after 7 days in air at 20C following treatment with 100 ppm C_2H_4 at 20, 25, 30 or 35C for 24, 48 or 72 hr.

Temperature (C)	Duration (hr)	Wound Status (W or NW)	Wt loss (%) (%)
20	24	W	4.63
	48	W	4.67
	72	W	4.42
	24	NW	2.72
	48	NW	2.44
	72	NW	2.47
25	24	W	4.16
	48	W	4.35
	72	W	4.21
	24	NW	3.09
	48	NW	3.20
	72	NW	2.63
30	24	W	5.61
	48	W	5.98
	72	W	4.29
	24	NW	2.58
	48	NW	3.04
	72	NW	3.01
35	24	W	5.25
	48	W	6.01
	72	W	8.15
	24	NW	2.55
	48	NW	2.72
	72	NW	3.06
Overall LSD @ 5%	-	-	0.73
ANOVA			Pr > F
Duration (D)			0.6936
Wound (W)			0.0001
Temp. (T)			0.3330
D X W			0.3965
D X T			0.0920
W X T			0.0224

increasing weight as exposure time increased. Tomatoes wounded prior to C_2H_4 treatment at 20, 25, 30, or 35C lost significantly more weight than unwounded tomatoes after 7 days in air at 20C. Wounded 'Sunny' fruit treated with C_2H_4 at 40C for 24, 48, or 72 hr were affected on the wounded parts by an unidentified organism that appeared to be a bacteria. All fruit from 40C were discarded and therefore not used for weight loss measurements.

In the second experiment, duration of C_2H_4 treatment of 'Sunny' tomatoes at either 20, 30, or 35C had no effect on subsequent weight loss of tomatoes after 7 days in air at 20C (Table 5-2). The trend, however, was that prior C_2H_4 treatment of wounded tomatoes for 24 hr led to the highest percent weight loss, while treatment for 72 hr resulted in tomato fruit losing intermediate weight, and the least weight loss was recorded in tomatoes treated with C_2H_4 for 48 hr at 30 or 35C (Table 5- 2). In this experiment, wounded tomatoes previously treated with C_2H_4 at 20C had the highest percent weight loss, while fruit from 35C had similar percent weight loss to fruit from 30C (Table 5-2). There was no significant interaction between duration of C_2H_4 treatment and temperature of exposure.

Wound Healing Assessed by Visual Appearance and Deformation

Shriveling scores on wounded areas of 'Solarset' tomatoes after 7 days in air at 20C were significantly ($P \leq 0.05$) highest in tomatoes that were previously treated with C_2H_4 at 35C and least in tomatoes that were previously treated with C_2H_4 at 30C, while fruit treated with C_2H_4 at 20 and 25C had intermediate shriveling scores (Table 5-3). Duration of C_2H_4 treatment did not affect shriveling scores and did not influence the temperature effect on shriveling of tomato wounds as evidenced by the non-significant interaction (Table 5-3). As the temperature of previous exposure while treating tomatoes

Table 5-2. Weight loss of wounded 'Sunny' tomatoes after 7 days in air at 20C following treatment with 100ppm C_2H_4 at 20, 30 or 35C for 24, 48, or 72 hr.

Temperature (C)	Duration (hr)	Wt loss (%)
20	24	6.82
	48	6.97
	72	6.51
30	24	6.17
	48	5.12
	72	5.66
35	24	6.57
	48	5.58
	72	5.67
Overall LSD @ 5%	-	0.58
ANOVA		Pr > F
Duration (D)		0.063
Temp. (T)		0.002
D X T		0.353

Table 5-3. Shriveling scores of wounded 'Solar Set' tomatoes after 7 days in air at 20C following treatment with 100 ppm C_2H_4 at 20, 25, 30, or 35C for 24, 48 or 72 hr.

Temperature (C)	Duration (hr)	Shriveling score (1-5)	Deformation (mm)
20	24	2.67	
	48	2.67	
	72	3.00	11.67
25	24	3.00	
	48	3.00	
	72	3.00	5.5
30	24	2.33	
	48	2.00	
	72	2.33	4.83
35	24	4.33	
	48	5.00	
	72	5.00	13.33
Overall LSD @ 5%			2.44
		Pr > F	Pr > F
ANOVA			
Duration (D)		0.2545	
Temp. (T)		0.0001	0.0001
DXT		0.4158	-

with C_2H_4 for 72 hr increased from 20 to 30C, deformation at wounded spots decreased significantly ($P \leq 0.05$) and then increased drastically at 35C (Table 5-3). The least amount of deformation was recorded in fruit previously treated with C_2H_4 for 72 hr at 30C.

Effect of Temperature and C_2H_4 Concentration on Weight Loss and Suberization

Wounded 'Sunny' tomatoes previously treated with C_2H_4 at 30 or 35C for 48 hr lost significantly ($P \leq 0.05$) less weight after 7 days in air at 20C than tomatoes previously treated with C_2H_4 for 24 or 72 hr (Table 5-4). In this experiment, 'Sunny' fruit previously treated with 100 or 1,000 ppm C_2H_4 for 24 hr at 30C had a significantly ($P \leq 0.05$) higher percent weight loss after 7 days in air at 20C compared to fruit treated with C_2H_4 at 30C for 72 hr (Table 5-4). However, with C_2H_4 treatment at 35C, weight loss increased significantly with an increase in duration of exposure from 24 to 72 hr (Table 5-4). Increasing the C_2H_4 concentration at 35 or 30C from 100 ppm to 1,000 ppm did not significantly alter the weight loss of tomatoes.

Suberization color scores of excised wounded tissue after 7 days in air at 20C were significantly ($P \leq 0.05$) higher in fruit previously treated with C_2H_4 at 30C than fruit treated with C_2H_4 at 35C (Table 5-4). Suberization color scores increased as the duration of C_2H_4 treatment at 30C increased from 24 to 48 and 72 hr , while suberization color scores decreased as prior duration of C_2H_4 treatment at 35C increased from 24 to 48 and 72 hr (Table 5-4). In general, the concentration of C_2H_4 did not significantly ($P \leq 0.05$) affect suberization color scores (Table 5-4), although wounded fruit previously treated with 1,000 ppm C_2H_4 for 24 hr at 30C had a higher suberization color score than fruit previously treated with 100 ppm C_2H_4 for 24 hr at 30C.

Table 5-4. Weight loss and suberization of wounded 'Sunny' tomatoes after 7 days in air at 20C following treatment with 100 or 1,000 ppm C_2H_4 at 30 or 35C for 24, 48 or 72 hr.

Temperature (C)	[C2H4] (ppm)	Duration (hr)	Wt loss (%)	Suberization Color score (1-4)
30	100	24	4.52	2.67
		48	3.04	4.00
		72	3.86	4.00
	1 000	24	4.90	3.67
		48	2.85	3.67
		72	3.76	4.00
35	100	24	4.36	3.33
		48	3.96	2.33
		72	5.93	2.00
	1 000	24	4.04	3.33
		48	3.89	2.33
		72	5.09	2.33
Overall LSD @ 5%		-	0.34	0.41
ANOVA			Pr > F	Pr > F
Duration (D)			0.0001	0.6337
Temp (T)			0.0001	0.0001
C_2H_4 (E)			0.0632	0.3162
D X T			0.0001	0.0001
D X E			0.5292	0.2663
T X E			0.2650	0.7361

Effect of Chlorine Treatment on Weight Loss and Decay

There appeared to be no effect of the concentration of Cl used to wash wounded 'Solarset' tomatoes on weight loss after 7 days in air at 20C. However, in this experiment, the trend was that wounded tomatoes previously treated with C_2H_4 at 30C lost less weight than tomatoes treated with C_2H_4 at 35C (Table 5-5).

Disease incidence occurring naturally on wounded tomatoes was measured after 20 days in air at 20C (Table 5-5). Fruit that were treated with C_2H_4 at 30C had a higher incidence of disease than fruit that were treated with C_2H_4 at 35C. On the other hand, increasing the Cl concentration of the tomato washing solution resulted in reduced disease incidence. It was observed that 1,000 and 500 ppm Cl and 72 hr exposure at 30C reduced disease incidence compared to 100 ppm Cl, and shorter exposures at 30C while treating with C_2H_4. On the other hand, 100 and 500 ppm Cl plus 48 or 72 hr exposure, and 1,000 ppm Cl with all durations at 35C, reduced disease incidence (Table 5-5).

Inoculation with *G. candidum* at 30 and 35C

The incidence and severity of sour rot in 'Solar Set' fruit after 10 days in air at 20C increased as the temperature of C_2H_4 treatment increased up to 30C (Table 5-6). Increasing the duration of C_2H_4 treatment led to increased disease incidence and severity in 'Solar Set' tomatoes. Most diseased tomatoes were those that were previously treated with C_2H_4 at 30C prior to inoculation with *G. candidum* (Table 5-6). Disease incidence and severity after treatment with C_2H_4 at 35C was sharply reduced compared to tomatoes treated with C_2H_4 at 30C. However, as the duration of C_2H_4 treatment at 35C increased from 24 to 72 hr, incidence and severity of *G. candidum* increased (Table 5-6).

Table 5-5. Weight loss and disease incidence of wounded 'Solar Set' tomatoes after 7 and 20 days, respectively, in air at 20C following washing in 100, 500 or 1, 000 ppm Cl then treatment with 100 ppm C_2H_4 at 20, 25, 30 or 35C for 24, 48 or 72 hr.

Temperature (C)	Cl concentration (ppm)	Duration (hr)	Weight loss (%)	Disease incidence (%)
30	100	24	2.46	55.55
		48	3.79	88.89
		72	2.71	77.78
	500	24	3.41	55.55
		48	2.63	66.67
		72	2.64	33.33
	1, 000	24	2.87	33.33
		48	2.32	33.33
		72	2.45	44.44
35	100	24	2.72	77.77
		48	3.07	0.00
		72	2.98	33.33
	500	24	3.09	83.33
		48	3.83	0.00
		72	2.67	22.22
	1, 000	24	3.36	11.11
		48	3.11	33.33
		72	3.38	0.00
Overall LSD @ 5%		-	0.48	23.40
ANOVA			Pr > F	Pr > F
Duration (D)			0.414	0.9338
Clorine (Cl)			0.8595	0.0063
Temp. (T)			0.1027	0.0015
D X Cl			0.2097	0.8645
D X T			0.8098	0.2031
Cl X T			0.2573	0.3644

Table 5-6. Sour rot disease severity of *G. candidum*-inoculated 'Solar Set tomatoes after 10 days in air at 20C following treatment with C2H4 for 24, 48 or 72 hr at 20, 25, 30 or 35C.

Temperature (C)	Duration) (hr)	Disease Severity (%)
20	24	20.00
	48	20.00
	72	20.00
25	24	60.00
	48	60.00
	72	73.00
30	24	83.33
	48	80.00
	72	80.00
35	24	20.00
	48	20.00
	72	20.00
Overall LSD @ 5%		13.47
ANOVA		Pr > F
Duration (D)		0.87
Temp. (T)		0.0001
D X T		0.9738

Discussion

Weight Loss Assessment in Wounded and Nonwounded Tomatoes

Tomatoes from all treatments generally lost weight over time while in air at 20C and around 95% RH because of water loss through the stem scar or the wounded parts. The tomato fruit is mostly composed of water. Wounded fruit lost more water than non-wounded fruit because, apart from losing water through the stem scar, wounded parts had the protective barriers removed, thereby facilitating water loss through the wounded parts at times when the wound periderm was not yet fully formed. In the first experiment with 'Sunny' tomatoes, relatively more weight loss occurred in fruit that were treated with C_2H_4 at 35C than fruit that came from 20, 25 or 30C. In this case, 35C led to creation of higher vapor pressures deficits (vpd) and thus more water was lost from tomatoes while at that temperature via the stem scar and the wounds. The fact that more water was lost at 35C implies that treating tomatoes with C_2H_4 at 35C did not promote or inhibit synthesis of barriers at the wound sites, while C_2H_4 treatment at 30C resulted in slow healing of the wounds. Ryall and Lipton (1979) stated that very little wound healing occurred in sweetpotatoes at 35C or above. In our experiment, the most conducive conditions for wound healing were between 20 to 30C. In other words, suberization occurred least in tomatoes that were previously treated with C_2H_4 at 35C compared to 20, 25 or 30C.

Even though the stem scars of MG-harvested tomatoes still undergo suberization after harvest, this was not apparent in our study. The interesting result here is the observation that there was no differences in weight loss and thus water loss in tomatoes previously treated with C_2H_4 at 20 or 25C while 30C led led to a higher but small percent weight loss after 7 days in air at 20C and around 95% RH. Under normal circumstances at

a constant RH, as the temperature increases so will the vpd and the potential amount of water loss, and hence percent weight loss of tomatoes. This then implies that something happened with the effect of reducing water loss to a greater extent from wounded parts of tomatoes while being treated with C_2H_4 at 25 and 30C compared to 20 or 35C. If suberization provides a seal that acts as a protective barrier against water loss, then to some extent treating tomatoes with C_2H_4 at 25 and 30C was more conducive in promoting suberization, which provided a barrier to water loss. Dean and Kolattukudy (1976) investigated the synthesis of suberin during wound-healing in jade leaves, tomato fruit, and bean pods and concluded that suberization is the fundamental process involved in wound-healing, irrespective of the nature of the natural protective polymer of the tissue.

Wounded tomatoes generally lost about two times the weight lost by non-wounded fruit because wounding removed the protective barrier and resulted in water being lost on two fronts i.e. the stem scar and the wounded portions. Duration of C_2H_4 treatment and wounding influenced the temperature response of tomato fruit in terms of weight loss because the longer the wounded fruit were at the high temperature, the greater was the chance of water loss and thus weight loss.

Wounded 'Sunny' tomatoes treated with C_2H_4 at 20C had the highest percent weight loss, while fruit from 35C had intermediate percent weight loss, and 30C resulted in the least percent weight loss. These results indicate that relatively more effective layers of protective barriers on wounded parts were probably deposited in tomatoes that were treated with C_2H_4 while at 30C. This implies that treating tomatoes with C_2H_4 at 30C was the most effective way of promoting suberization under the conditions of this experiment. Actually, the current recommendations for curing sweet potatoes are to hold the roots at

29.4C and 85-90% RH for 4 to 7 days (Walter and Schadel, 1982). The fact that the duration of treatment did not affect the tomato suberization response may imply that 24 hr has the same effect as 48 or 72 hr in terms of inducing or inhibiting suberization on tomato stem scars and wounds. It is highly likely that suberization was completed in air at 20C. It takes about 5 to 7 days for suberization to be complete in sweetpotatoes depending on the environment (Kushman, 1975; Walter and Schadel, 1982). The time frame required for suberization to be completed in tomatoes under various conditions has not been determined yet. Water loss from tomatoes is an economic loss because tomatoes are sold by weight basis. The conditions that best curb water loss in tomatoes via wounds or stem scars need further investigation from an economic point of view.

Wound Healing Assessed by Visual Appearance and Deformation

Wounded tomatoes previously treated with C_2H_4 at 30C shriveled the least, while shriveling rating scores of tomatoes from 20 and 25C were intermediate, and tomatoes previously treated with C_2H_4 at 35C shriveled the most. Shriveling in plant tissues is usually the result of water loss, leading to the affected cells losing turgor and undergoing plasmolysis to a certain extent, thus producing the shriveled appearance. In this experiment, water was lost from the wounded parts of the tomato fruit and from the stem scar. The fact that tomatoes from 30C were shriveled the least implies that less water was lost, probably due to a more effective barrier of suberin, synthesized due to stimuli received at that temperature while treating wounded tomatoes with C_2H_4. The suberin would have then acted as a barrier that blocked excessive water loss. The high temperature of 35C not only increased the vpd while at that temperature, leading to more water loss and shriveling, but was probably not very effective in promoting synthesis of

suberin (Ryall and Lipton, 1979). Although tomatoes from the lower temperature of 20C were expected to lose the least amount of water, as indicated by shriveling scores, it was probably not the best temperature to promote wound healing and the fundamental process of suberization, while 25C had intermediate effects as far as promotion of wound healing was concerned, that is if the amount of water loss can be related to suberization.

Deformation of wounded areas decreased as the previous temperature of exposure while treating with C_2H_4 increased from 20 to 25 and 30C, then increased in fruit previously held at 35C. These results may imply that, as temperature increased up to 30C, the wounded areas increased, hardening after 7 days in air at 20C. Increased hardening associated with previous temperature of exposure may be associated with increased suberization or increased deposition of suberin. Suberin is a secondary product associated with increased strength of plant tissue. A sudden increase in deformation on wounded areas in fruit previously treated with C_2H_4 at 35C may imply that previous holding of wounded tomatoes at that temperature inhibited suberization. If suberization is not complete, there are gaps left in wounded tissue (Fox et al., 1971), and it is these gaps that lead to increased deformation, with the extent depending on the size of the gaps and their geometrical arrangement. Although suberization is reported to take about 7 days at 29.5C and around 85 to 95% RH in sweetpotatoes (Ryall and Lipton, 1979), it is possible that other factors were involved in delaying suberization after 7 days in air at 20C in fruit once held for 72 hr at 35C.

In the experiment where tomato fruit were treated with C_2H_4 at 30C or 35C, it was observed that there was less weight loss after 7 days in air at 20C following 30C treatment compared to fruit treated with C_2H_4 at 35C (Table 5-4). This is consistent with our

previous findings and further suggests that 30C is probably the most conducive temperature for suberin synthesis. The duration of C_2H_4 treatment did not affect weight loss, most likely because 24 hr is enough time just like 48 or 72 hr in affecting suberization of wounded tomatoes at both 30 and 35C. Higher color scores were observed on wounds of fruit previously treated with C_2H_4 at 30C compared to 35C, because the more intense the color the greater the amount of suberin deposited in those tissues. This method was developed in sweetpotato (Walter and Schadel, 1982). This further confirms that treating wounded tomatoes with C_2H_4 at around 30C provided the most conducive wound healing conditions, characterized by increased suberin deposition in the wounded areas. Again, the duration of C_2H_4 treatment was not a factor in affecting the color scores, just as was the case in the water loss experiment, further showing that 24 hr C_2H_4 treatment of wounded tomatoes has the same effects as 48 or 72 hr at either 30 or 35C.

Effect of Chlorine Treatment on Weight Loss and Decay

The concentration of the Cl_2 solutions used to wash wounded tomatoes did not influence the amount of weight loss in tomatoes and hence the amount of water lost. As prior concentration of Cl used to wash tomatoes increased from 100 to 500 and 1,000 ppm, disease incidence decreased, more so at both 30 and 35C, most likely because higher concentration of the biocide killed more inert potential decay organisms on tomatoes. On the other hand, as the duration of previous treatment with C_2H_4 at 35C increased, subsequent incidence of decay decreased because the longer the tomatoes were exposed to 35C, the more potential pathogens were killed. Holding inoculated MG and pink tomatoes for 3 days at 38C completely inhibited decay caused by *B. cinerea*, and inhibition was observed to increase with duration of treatment (Fallik et al., 1993). Our

results showed that Cl did not affect wound healing/suberization but affected the pathogens directly as evidenced by the absence of interaction between the two variables.

Chlorine solutions of a concentration of 100 ppm are normally used to wash tomatoes, where Cl_2 is the active ingredient for sanitizing the tomatoes in the dump tanks of commercial packinghouses. The results presented here are different from those of Grigg and Chase (1967), who found that increasing the concentration of Cl_2 solutions from 100 to 1,000 ppm resulted in reduced percent weight loss in potatoes, at least as far as the trend of water loss was concerned. The IFAS recommended levels of free Cl_2 are 100 to 150 ppm at pH 7 and water temperature that is 5C higher than the tomato pulp temperature (Sherman et al., 1981). However, recently, Sargent et al. (1992) reported that it appears that adequate dump tank sanitation can be achieved under packinghouse conditions by constantly maintaining the water at about 50 ppm free Cl, pH 7 and a temperature of 40C. The effect of these conditions on suberization to heal harvest-inflicted wounds in tomatoes warrants further investigation.

Natural Decay Development

It took about 20 days in air at 20C for wounded tomato fruit to develop disease symptoms from pathogens naturally occurring on fruit or in the storage room. This is because tomato fruit become more susceptible to disease as they senesce due to decreased natural defense systems/mechanisms. These include breakdown of barriers to diseases such as cell walls and the onset of the genetically programmed cell death. Fruit that were previously treated with C_2H_4 at 30C had a higher incidence of disease than fruit that were treated with C_2H_4 at 35C, probably because 30C was more conducive for infection of tomato tissues by its several naturally occurring postharvest pathogens and the inoculated

wound pathogen *G. candidum*. It is also quite likely that, at 30C, C_2H_4 influences tomato tissue in such a way that the fruit become prone to infection by postharvest pathogens. It is surprising that 30C seemed to be the best temperature for wound healing (suberization) based on water loss, shrivel, deformation and staining, yet 35C resulted in less decay. It is possible that 35C conferred disease resistance to tomatoes. Increasing the concentration of Cl in the washing solutions led to reduced disease incidence and severity, most likely because Cl killed potential pathogens on the fruit surface that were acquired in the field or during handling more effectively as the concentration increased. Bartz (1991) previously reported that most tomato diseases can be traced to previous conditions in the field or the handling system. However, under practical conditions, 100 ppm Cl concentration is used as a standard in the dump tank wash solutions. As mentioned above, lower concentrations of about 50 ppm have even been recently recommended (Sargent et al., 1992). Further research on the effect of lower Cl concentrations in wash solutions on subsequent long-term postharvest disease development of tomato fruit may only shed more light.

Inoculation with *G. candidum*

The incidence and severity of sour rot caused by inoculation with *G. candidum* after 10 days in air increased as the prior temperature at which wounded tomatoes were treated with C_2H_4 increased from 20C up to 30C. The maximum incidence and severity of sour rot occurred in fruit from 30C probably because fruit from that temperature ripened faster and thus become more suceptible to *G. candidum*. Prior treatment of fruit with C_2H_4 at 35C inhibited subsequent development of sour rot in wounded tomatoes because 35C probably made the fruit more resitant to *G. candidum* somehow.

Summary

This is the first study carried out to investigate the effect of C_2H_4 treatment at high temperature and Cl concentration in wash water used to sanitize tomatoes on suberization of tomatoes to heal harvest-inflicted wounds. Wounded tomatoes lost more weight than their nonwounded counterparts. Fruit previously treated with C_2H_4 for 72 hr at 30C lost less weight than fruit held at 30C for 24 hr or 48 hr, which lost as much weight as fruit held at 20 or 25C. Tomatoes previously treated with C_2H_4 at 35C lost the most weight, and weight loss increased as exposure time increased. Shriveling of wounded tomatoes was highest in tomatoes that were previously treated with C_2H_4 at 35C and least in tomatoes that were treated with C_2H_4 at 30C, while fruit from 20 and 25C showed similar, intermediate shriveling. As the temperature of previous exposure while treating tomatoes with C_2H_4 for 72 hr increased from 20 to 25 and 30C, deformation on wounded areas decreased; deformation increased in tomatoes that came from 35C. Suberization color scores of excised wounded tissue were higher in fruit previously treated with C_2H_4 at 30C than fruit treated at 35C. Disease incidence decreased as the concentration of Cl used in the washwater increased. Holding tomatoes at 35C for 24 to 72 hr resulted in the lowest incidence and severity of natural disease as well as sour rot from inoculation with *G. candidum* following the temperature treatments.

CHAPTER 6
EFFECTS OF TEMPERATURE AND ETHYLENE ON TOMATO FRUIT INFECTION BY *GEOTRICHUM CANDIDUM*

Introduction

Tomato postharvest diseases are influenced by many factors, including temperature and RH. Pathogen growth and probably development is favored by warm temperatures, with the optimum for many pathogenic organisms being about 30C (Perombelon and Kelman, 1980). The onset of bacterial soft rot in tomatoes may be delayed up to 3 days or longer in fruit stored at the optimum ripening temperature, ie. 20-22C, as compared to temperatures optimum for the disease, ie. 30 to 35C (Bartz et al., 1991). Temperatures close to 40C have been reported to possibly induce metabolic disorders and facilitate bacterial and fungal invasion (Rick and Coursey, 1979). However, high temperatures ≥ 30C have also been reported to inhibit fungal germination and growth (Couey, 1989; Fallik et al., 1993).

The important postharvest diseases of tomato fruit in Florida rank in increasing importance in the following order: gray mold (*Botrytis cinerea*), rhizopus rot (*Rhizopus stolonifer*), sour rot (*Geotrichum candidum* Link), and bacterial soft rot (*Erwinia carotovora* (Jones) Sub sp. *carotovora*) (Bartz, 1991). The occurrence of tomato disease after harvest is often traceable to growing conditions and packinghouse operations (Bartz, 1991; Morris and Kader, 1978).

Ethylene functions as a powerful regulator in most aspects of plant growth, development, senescence, and disease (Lieberman, 1979). A major problem in the study of C_2H_4 in pathogenesis is that it may be produced by the pathogen but it is also produced in response to physical wounding in the absence or presence of a pathogen (Saltveit and Dilley, 1978). Although most plant pathogens are capable of producing C_2H_4, there is little evidence in the majority of diseases to indicate that the pathogen contributes measurably to the C_2H_4 pool (Pegg, 1981). Ethylene can have phytotoxic effects on plants or plant tissues. Plants exposed to an atmosphere contaminated with coal gas containing C_2H_4 and carbon monoxide as impurities showed many of the symptoms associated with disease, e.g. in the short term, epinasty and foliar abscission and, over long periods, formation of adventitious root initials, stunting, chlorosis, necrotic lesions and death (Pegg, 1981). On the other hand, C_2H_4 has been implicated to play a role in disease resistance of plant tissues. A role for C_2H_4 in disease resistance is based on evidence from (1) plants treated with C_2H_4 or ethrel that subsequently show diminished infection or symptoms, (2) C_2H_4-induced changes in enzymes that are presumed to be associated with resistance, and (3) C_2H_4-stimulated biosynthesis of known antifungal compounds (Pegg, 1981). There is, however, no information on the effect of C_2H_4 in tomato fruit postharvest pathogenesis and the influence of high temperature on disease development, despite the fact that wounded tomatoes are treated with C_2H_4 at high temperatures for considerable time in the ripening rooms.

The influence of initial temperature while treating with C_2H_4 on development of bacterial soft rot in tomato fruit was investigated by Bartz et al. (1991). The authors found that the disease incidence among tomatoes that had been treated with C_2H_4 at 25C was

higher than among fruit treated at 20C, whereas the incidence among those treated at 20C was higher than among those treated at 15C.

Heat treatments to fruits and vegetables applied as hot water dips, vapor heat or hot, dry air have been reported to inhibit postharvest disease development (Couey, 1989; Fallik et al., 1993; Spalding et al., 1987). Fallik et al. (1993) examined the effect of exposure to 38C on decay caused by *Botrytis cinerea*, one of the major postharvest pathogens of tomatoes in Israel, in MG and pink tomatoes, and on ripening indices of previously heated tomatoes. Holding inoculated MG and pink tomato fruit for 3 days at 38C completely inhibited decay caused by *B. cinerea*, with conidial germination found to be more sensitive than mycelial growth to 38C, while inhibition of both processes increased with duration of treatment (Fallik et al., 1993). It was found that pink fruit held at 38C for 24 or 48 hr had reduced disease index, while inoculated pink fruit held at 38C for 72 hr had no decay development. Although nonheated MG fruit were much more resistant to disease than pink fruit, they still benefited from the heat treatment as far as decay development was concerned (Fallik et al., 1993). The authors further reported that the prophylactic effect of heating tomatoes on decay caused by *B. cinerea* was due to heat affecting the fungus rather than by an inhibitory effect on fruit ripening. However, in their experiment, they did not treat MG tomatoes with C_2H_4 while at the high temperature, considering that there is a practical possibility of MG tomatoes being treated with C_2H_4 at high temperatures.

Sour rot of tomato is caused by *G. candidum* Link: Pers., a wound pathogen that requires an injury for entry. The pathogen is a saprophyte commonly found in stagnant water and wet soil (Brown, 1979; Butler, 1960; Gutter, 1978; Morris, 1982). Symptoms

of infection by *G. candidum* include brown, water-soaked lesions that contain white mycelium and, as the decay progresses, fruit become soft and unmarketable (Moline, 1984a). Sour rot disease is characterized by a sour odor caused by production of lactic acid by the pathogen (Bartz, 1991). 'Sour rot' is an important postharvest disease, not only in Florida, but in the whole of the USA, and other parts of the world. In the course of this project, in which the physiological response of tomato fruit to C_2H_4 at high temperatures was investigated, *G. candidum* was the most common fungal pathogen causing decay in the fruit. However, no similar work of potential benefit as that of Fallik et al. (1993), where heat treatment inhibited decay caused by *B. cinerea* has been done with *G. candidum*. The optimum temperature for the development of *G. candidum* was determined to be 30C and, additionally, it was determined that there was a drop in growth at 35 and 40C (Moline, 1984a). However in Moline's (1984a) experiment, the tomatoes were not treated with C_2H_4 while at the different temperatures. There is evidence that MG tomato pulp temperature may remain well over 30C for some time while in the ripening room while being treated with C_2H_4 (Brecht and Sargent, unpublished) and yet there is no information documented on pathological responses of tomato fruit following exposure to C_2H_4 at high temperature. It was previously observed in this study that MG tomatoes decayed at 35 or 40C while being treated with C_2H_4 due to infection caused by an unidentified organism that appeared to be a bacteria. Predominantly wounded tomatoes were affected. Therefore, the objectives of this study were to examine the effect of C_2H_4 treatment of MG tomatoes at high temperature on subsequent decay caused by *G. candidum* and to characterize the unidentified organism that caused decay primarily on wounds at 35 and 40C.

Materials and Methods

Plant Material

Extra large (USDA, 1976) 'Agriset 761' and 'Mountain Spring' tomatoes were harvested at the MG stage in Imokalee and the Quincy area, respectively, and transported to Gainesville in an air conditioned van. The fruit were washed in chlorinated (100 ppm NaOCl) water at pH around 7. The tomatoes were left to dry in air. The tomatoes were then graded, with malformed and wounded fruit, or fruit showing color, discarded. Large (USDA, 1976) 'Sunny' fruit used in experiments with the unidentified organism causing decay at 35 and 40C, came from packinghouses from various locations in Florida. Fruit from packinghouses were only regraded since they were previously washed and waxed at the packinghouse.

Inoculation of Tomatoes with *G. candidum*

The *G. candidum* preparation used in to inoculate MG tomatoes was prepared as previously described in Chapter 5 and serially diluted with sterile water into one of a 10-fold dilution series of 10^6, 10^5, 10^4, 10^3, 10^2 cfu ml^{-1}. The fruit were wounded using a thin sterile wire spike by poking. Each fruit was wounded three times around the stem scar/shoulder region. The tomato fruit were inoculated by adding 100µl of a spore suspension onto each wound using a pipette. Three fruit were used per *G. candidum* spore suspension concentration. Three buckets containing 75 fruit inoculated with the previously described concentrations of *G. candidum* spore suspension were placed at each of the following temperatures: 20, 25, 30, 35, or 40C at near saturation humidity. At each temperature, tomatoes were either treated with 100 ppm C_2H_4 or air. Near saturation

humidity was achieved by bubbling air or air plus C_2H_4 through water prior to passing over the tomatoes in each bucket.

After 24, 48, or 72 hr, the 75 fruit per duration were transferred to air at 20C, and placed on trays, which were stacked and covered with black plastic film in order to maintain high humidity. Observations on incidence and severity of sour rot development on the tomatoes were made each day for 11 days and recorded. Disease incidence was measured by counting the number of infected fruit and converting the number of diseased fruit to a % of the total number of fruits in a sample. Disease severity was assessed for an individual fruit using the following scale: 1=20%; 2=40%, 3=60%; 4=80%, and 5=100% diseased. The results shown are for 'sour rot' disease severity measured after 9, 10 or 11 days in air at 20C in fruit previously treated with air or C_2H_4 at the designated temperatures for 72, 48, or 24 hr, respectively. At the time of disease severity assessment, 100% of the tomatoes were infected with *G. candidum*.

Identification and Pathogenicity of a Thermophilic Bacterium

While at high temperatures of 35 and 40C, an unidentified pathogenic organism, which appeared to be a bacteria, was observed (as previously described) growing very vigorously on wounds of tomatoes, causing the tomato fruit to collapse within less than a week after transfer to air at 20C. The affected wound areas showed whitish, yeast-like scum and the tomato tissue appeared to have been eaten away by this unidentified organism. The pathogen was not identified by reference to standard manuals and so diagnosis of the disease remained incomplete. In such a circumstance, Agrios (1978) recommends use of Koch's postulates to verify the hypothesis that the isolated pathogen is the cause of the disease.

Isolation and Production/Multiplication of Pure Culture

The following steps of Koch's postulates were followed:

1. The pathogen must be found associated with the disease in all the diseased fruit examined.

2. The pathogen must be isolated and grown in pure culture on nutrient media, and its characteristics described (nonobligate parasites), or on a susceptible host plant (obligate parasite), and its appearance and effects recorded.

3. The pathogen from pure culture must be inoculated on healthy plants of the same species or variety in which the disease appears, and it must produce the same disease in the inoculated plants.

4. The pathogen must be isolated in pure culture again and its characteristics must be exactly like those observed in step 2.

Inoculation of Tomatoes with a Thermophilic Bacterium

Tomatoes were divided into two groups, one of which was used for inoculation of wounded fruit with the unidentified organism and the other group for inoculating uninjured fruit with a suspension of the unidentified organism. Fruit were wounded by abrading them with sandpaper as previously described. Fruit were inoculated with the unidentified organism taken from nutrient agar (NA) contained in a petri dish using a sterile wire loop and spreading the suspension on wounds in one group and on the surfaces of tomato fruit in another group. Three buckets, each containing 20 fruit, of which ten were wound inoculated and the other ten surface inoculated, were placed at each of the following temperatures: 20, 25, 30, 35, or 40C at near saturation humidity. After 24, 48, or 72 hr, the tomatoes were removed from the designated temperatures and

disease incidence and severity were assessed as previously described in studies with *G. candidum*. The fruit were then placed in trays at 20C, with high humidity maintained by occasionally misting them with sterile water, and covering them with black plastic so as to monitor the disease further.

Re-isolation and Characterization of the Thermophilic Bacterium

It is necessary to determine whether the Gram-stain is negative or positive in the classification and identification of plant pathogenic bacteria. In this reaction bacteria are treated with a crystal violet solution for 30 seconds, rinsed gently, treated with iodine solution, and rinsed again with water and then alcohol (Agrios, 1978). Gram-positive bacteria retain the violet iodine stain while Gram-negative bacteria have no affinity for the stain and remain nearly invisible.

A rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining as described by Suslow et al. (1982) was also used. The thermophilic organism was isolated many times and grown on nutrient agar. A dispensing pipette was used to put a few drops of 3% potassium hydroxide (KOH) solution on a clean glass slide. A sterile wooden toothpick was used to scrape off a sample of the unidentified bacterial organism from the NA. The bacterial sample was then placed on the slide and thoroughly mixed with the KOH solution using the sterile tooth pick. After 5 to 8 seconds, the toothpick was alternately raised and lowered just off the slide surface to detect a stringing effect. The KOH test was considered positive for Gram-negative bacteria if drop viscosity increased and stringing occurred within 15 seconds (Suslow et al., 1982).

Results

Inoculation of Tomatoes with *G. candidum*

Lesions on tomatoes were first observed about 2 days after inoculation with *G. candidum* when fruit were held at the designated temperatures. However, it took at least about 9, 10 and 11 days in air at 20C for all tomatoes previously treated with air or C_2H_4 at the designated temperatures for 72, 48, or 24 hr, respectively, to show obvious symptoms of 'sour rot' disease caused by *G. candidum*. Water-soaked, greasy lesions began at the wounds used to inoculate the fruit with *G. candidum* spore suspensions. Fruit at the advanced stage of the 'sour rot' disease emitted a characteristic lactic acid odor and showed white mycelia.

Tomatoes previously treated with air or C_2H_4 for 24, 48, or 72 hr at 40C were infected with an unidentified organism, which appeared to be a bacterium. The symptoms appeared yeast-like, primarily on the regions of inoculation with *G. candidum*. Infected tomatoes collapsed within 7 days after transfer to air at 20C and, for that reason, fruit previously treated with air or C_2H_4 for 24, 48, or 72 hr at 40C were not used in 'sour rot' disease severity assessment. Fruit that showed injury caused by the unidentified organism were discarded.

Generally, both 'Agriset 761' and 'Mountain Spring' tomatoes that were treated with C_2H_4 while at the designated temperatures had significantly ($P<0.05$) increased subsequent 'sour rot' severity after storage in air at 20C (Tables 6-1, 6-2, 6-3 and 6-4).

C_2H_4 appeared to have the greatest effect on 'sour rot' disease development in air at 20C compared to other factors such as duration of air or C_2H_4 treatment or the concentration of *G. candidum* spore suspensions used to inoculate the tomatoes (Table 6-4). As the duration of C_2H_4 treatment at 20, 25, 30, or 35C increased from 24 to 48 or

Table 6-1. Sour rot disease severity of *G. candidum*-inoculated 'Agriset 761' and 'Mountain Spring' tomatoes after 11 days in air at 20C following treatment with air or C_2H_4 for 24 hr at 20, 25, 30 or 35C.

Temperature (C)	Population (cfu)	$[C_2H_4]$ (ppm)	Disease Severity (%)[z]	
			'Agriset 761'	'Mountain Spring'
20	10^2	0	26.67	20.00
	10^3		26.67	20.00
	10^4		26.67	20.00
	10^5		26.67	40.00
	10^6		53.33	40.00
	10^2	100	26.67	40.00
	10^3		26.67	40.00
	10^4		33.33	40.00
	10^5		40.00	40.00
	10^6		40.00	40.00
25	10^2	0	26.67	40.00
	10^3		26.67	40.00
	10^4		26.67	40.00
	10^5		26.67	40.00
	10^6		33.33	46.67
	10^2	100	53.33	40.00
	10^3		60.00	46.67
	10^4		66.67	46.67
	10^5		66.67	46.67
	10^6		66.67	46.67
30	10^2	0	26.67	40.00
	10^3		26.67	46.67
	10^4		33.33	46.67
	10^5		60.00	46.67
	10^6		66.67	53.00
	10^2	100	60.00	46.67
	10^3		60.00	46.67
	10^4		60.00	53.33
	10^5		60.00	53.33
	10^6		66.67	60.00

Table 6-1--continued.

Temperature (C)	Population (cfu)	$[C_2H_4]$ (ppm)	Disease	Severity (%)[z]
			'Agriset 761'	'Mountain Spring'
35	10^2	0	20.00	40.00
	10^3		26.67	40.00
	10^4		26.67	46.67
	10^5		26.67	46.67
	10^6		33.33	53.33
	10^2	100	26.67	46.67
	10^3		26.67	46.67
	10^4		26.67	56.67
	10^5		33.33	53.33
	10^6		40.00	60.00
LSD @ 5%[Y]			4.34	3.45

[z]Sour rot disease incidence was 100% after 24 hr treatment plus 11 days at 20C
[Y]LSD calculated for combined 24, 48 and 72 hr C_2H_4 or air treatment durations.

Table 6-2. Sour rot disease severity of *G. candidum*-inoculated 'Agriset 761' and 'Mountain Spring' tomatoes after 10 days in air at 20C following treatment with air or C_2H_4 for 48 hr at 20, 25, 30 or 35C.

Temperature(C)	Population (cfu)	[C_2H_4] (ppm)	Disease Severity (%)	
			'Agriset 761'	'Mountain Spring'
20	10^2	0	26.67	40.00
	10^3		26.67	40.00
	10^4		26.67	40.00
	10^5		26.67	40.00
	10^6		33.33	46.67
	10^2	100	33.33	46.67
	10^3		33.33	46.67
	10^4		40.00	53.33
	10^5		46.67	53.33
	10^6		53.33	46.67
25	10^2	0	33.33	40.00
	10^3		33.33	40.00
	10^4		33.33	40.00
	10^5		33.33	40.00
	10^6		33.33	40.00
	10^2	100	40.00	46.67
	10^3		40.00	46.67
	10^4		60.00	46.67
	10^5		60.00	46.67
	10^6		60.00	46.67
30	10^2	0	40.00	46.67
	10^3		40.00	46.67
	10^4		40.00	46.67
	10^5		40.00	46.67
	10^6		46.67	53.33
	10^2	100	46.67	46.67
	10^3		46.67	53.33
	10^4		53.33	66.67
	10^5		73.33	60.00
	10^6		80.00	60.00

Table 6-2--continued.

Temperature(C)	Population (cfu)	$[C_2H_4]$ (ppm)	Disease	Severity (%)
			'Agriset 761'	'Mountain Spring'
35	10^2	0	40.00	40.00
	10^3		40.00	40.00
	10^4		40.00	40.00
	10^5		40.00	40.00
	10^6		53.33	40.00
	10^2	100	40.00	40.00
	10^3		46.67	40.00
	10^4		60.00	46.67
	10^5		60.00	53.33
	10^6		66.67	53.33

[z]Sour rot disease incidence was 100% after 48 hr treatment plus 10 days at 20C
[y]LSD calculated for combined 24, 48 and 72 hr C_2H_4 or air treatment durations.

Table 6-3. Disease severity of *G. candidum*-inoculated 'Agriset 761' and 'Mountain Spring' tomatoes after 9 days in air at 20C following treatment with air or C_2H_4 for 72 hr at 20, 25, 30 or 35C.

Temperature(C)	Population (cfu)	$[C_2H_4]$ (ppm)	Disease Severity (%)	
			'Agriset 761'	'Mountain Spring'
20	10^2	0	40.00	40.00
	10^3		40.00	46.67
	10^4		40.00	46.67
	10^5		46.67	53.33
	10^6		46.67	46.67
	10^2	100	40.00	53.33
	10^3		46.67	53.33
	10^4		73.33	53.33
	10^5		73.33	73.33
	10^6		86.67	73.33
25	10^2	0	40.00	46.67
	10^3		46.67	46.67
	10^4		40.00	53.33
	10^5		46.67	46.67
	10^6		46.67	46.67
	10^2	100	40.00	53.33
	10^3		73.33	53.33
	10^4		73.33	60.00
	10^5		73.33	60.00
	10^6		80.00	66.67
30	10^2	0	46.67	60.00
	10^3		46.67	60.00
	10^4		46.67	60.00
	10^5		46.67	60.00
	10^6		53.33	60.00
	10^2	100	60.00	60.00
	10^3		60.00	60.00
	10^4		60.00	66.67
	10^5		60.00	66.67
	10^6		80.00	86.67

Table 6-3--continued.

Temperature(C)	Population (cfu)	$[C_2H_4]$ (ppm)	Disease Severity (%)	
			'Agriset 761'	'Mountain Spring'
35	10^2	0	40.00	40.00
	10^3		46.67	40.00
	10^4		46.67	40.00
	10^5		53.33	40.00
	10^6		53.33	40.00
	10^2	100	40.00	40.00
	10^3		46.67	53.33
	10^4		46.67	46.67
	10^5		46.67	46.67
	10^6		46.67	60.00

[z]Sour rot disease incidence was 100% after 72 hr treatment plus 9 days at 20C
[y]LSD calculated for combined 24, 48 and 72 hr C_2H_4 or air treatment durations.

Table 6-4. Analysis of variance (ANOVA) of disease severity of *G. candidum*- inoculated 'Agriset 761' and 'Mountain Spring' tomatoes after 9, 10 and 11 days in air at 20C following treatment with air or C_2H_4 for 24, 48 or 72 hr at 20, 25, 30 or 35C.

ANOVA	Pr > F	Pr > F
	'Agriset 761'	'Mountain Spring'
Duration(D)	0.0001	0.0001
C_2H_4 (E)	0.0001	0.0001
Temp.(T)	0.0001	0.0001
Inoculum conc.(C)	0.0001	0.0001
D X E	0.7899	0.0033
D X T	0.0001	0.0001
D X C	0.4721	0.1443
E X T	0.0001	0.7566
E X C	0.0400	0.0062
T X P	0.0087	0.3815

72 hr, the disease severity increased significantly ($P \leq 0.05$) (Tables 6-1, 2, 3 and 4). Tomatoes previously treated with C_2H_4 at 20, 25, 30, or 35C for 24, 48, or 72 hr showed significantly ($P \leq 0.05$) higher 'sour rot' disease severity than fruit treated with air at the same temperatures and for the same durations, at least after 9 more days in air at 20C, when disease incidence was 100% (Tables 6-1, 2, 3 and 4).

As the temperature at which the tomatoes were treated with C_2H_4 increased from 20 to 25 to 30C, 'sour rot' disease severity generally increased significantly ($P \leq 0.05$) in both cultivars with a few exceptional cases (Tables 6-1, 2, 3 and 4), reaching a peak at 25 and 30C. The greatest 'sour rot' disease severity caused by *G. candidum* occurred in tomatoes previously treated with C_2H_4 at 30C, then declined in fruit previously treated with air or C_2H_4 at 35C.

Generally, an increase in the concentration of spore suspensions of *G. candidum* used to inoculate the tomatoes prior to C_2H_4 treatment at the designated temperatures led to significant ($P \leq 0.05$) increases (sometimes there was no change) in subsequent 'sour rot' disease severity (Tables 6-1, 2, 3 and 4). As the duration of C_2H_4 treatment increased from 24 to 48 or 72 hr at 20, 25, 30, or 35C, subsequent disease severity increased and peaked in fruit of both cvs. previously treated at 25 or 30C (Table 6-4).

There was a significant duration and ethylene treatment interaction in 'Mountain Spring', such that, as the duration of treatment increased, there was an increase in 'sour rot' disease severity (Table 6-4). There was no duration x ethylene interaction in 'Agriset 761'. There were significant ($P \leq 0.05$) duration of C_2H_4 treatment and temperature of treatment effects in both 'Agriset 761' and 'Mountain Spring,' such that, as both temperature and the duration of C_2H_4 treatment increased, there was a corresponding

subsequent increase in disease severity in both cultivars. There were no significant effects of duration of C_2H_4 treatment or concentration of *G. candidum* spore suspensions in both cultivars. Ethylene treatment and temperature of treatment interacted significantly in 'Agriset 761', such that C_2H_4 treatment led to increased subsequent disease severity as the temperature of treatment increased up to 30C. There was no interaction of ethylene treatment and temperature in 'Mountain Spring' tomatoes. Ethylene treatment (vs. air) and concentration of spore suspension of *G. candidum* interacted significantly ($P \leq 0.05$) in both cultivars, such that treating tomatoes with C_2H_4 together with increased concentration of spore suspensions led to increased disease severity. On the other hand, there was a significant interaction between temperature and concentration of *G. candidum* spore suspensions in 'Agriset 761' (and not in 'Mountain Spring'), which resulted in increased disease severity as both the temperature of treatment and concentration of spore suspensions increased.

Identification and pathogenicity of a thermophilic bacterium

The unidentified organism was established to be a bacteria whose growth was favored by high temperature. The unidentified organism was found to be a bacteria by growth characteristics after repeated isolation and growing of pure cultures on NA. Colonies of bacteria appeared spherically shaped on NA and were cream in color. This is the first time that this bacteria has been observed on wounded tomato fruit in Florida. Because the organism was not identified with the aid of reference manuals, diagnosis of the disease was accomplished using Koch's postulates as recommended by Agrios (1978).

Isolation and Production/Multiplication of Pure Culture

The pure culture bacteria was multiplied and grown on NA medium contained in petri dishes as previously described (Agrios, 1978). The growth of the pure culture of bacteria was similar on NA in all petri dishes.

Inoculation of Tomatoes with a Thermophillic Bacterium

No infection of wound-inoculated tomatoes was observed when tomatoes were incubated at 20, 25 or 30C and near saturation humidity while being treated with air or C_2H_4 for 24, 48, or 72 hr. Infection was observed on wounds of tomato fruit after 3 days in air or C_2H_4 at 35C and after 1 day in air or C_2H_4 at 40C. Inoculated, uninjured fruit did not develop the disease caused by the unidentified pathogen at any of the temperatures tested.

When the Gram test was conducted, no strings were formed after thoroughly mixing the bacterial sample and KOH solution, indicating that the bacteria was Gram positive. Gram positive bacteria have thicker cell walls than Gram negative species and so are not lysed in this test like Gram negative cells (thereby releasing DNA, characterized by high molecular weight, which forms the strings). Gram positive bacteria are probably *Bacillus* species.

Discussion

Inoculation of Tomatoes with *G. candidum*

It took about 9 days in air at 20C for all tomatoes to show obvious symptoms of disease caused by *G. candidum* because tomatoes become more susceptible to 'sour rot' as they ripen. The results for 'sour rot' disease severity shown here are for fruit treated with air or C_2H_4 at the designated temperatures for 24, 48, or 72 hr, then held in air at 20C

for 11, 10, or 9 days, respectively. MG fruit appeared to be resistant to infection by *G. candidum* but, as the fruit ripened, the disease progressed quickly. Moline (1984a) reported comparative research studies with two *Geotrichum* species inciting postharvest decays of tomato fruit and found that growth of *G. candidum* and *G. penicillatum* was significantly inhibited on MG fruit compared to red fruit. In ripe fruit, the disease progresses rapidly, particularly under warm conditions, and the epidermis covering the lesions usually cracks, allowing the watery contents to spill out (Bartz, 1991). Diseased fruit emitted a lactic acid or sour odor as previously reported (McColloch et al., 1968).

As the duration of C_2H_4 treatment at 20, 25, 30, or 35C increased from 24 to 48 or 72 hr, subsequent 'sour rot' severity also increased. This was probably because C_2H_4 stimulated ripening more as the duration of C_2H_4 exposure was increased from 24 to 48 or 72 hr. Under commercial conditions, periods of up to 8 days are sometimes used for C_2H_4 treatment of green-harvested tomatoes. This is done to ensure that the immature green fruit that are inevitably harvested with MG fruit (Kader at al., 1977) ripen. The fact that longer durations of C_2H_4 treatment predispose tomatoes to disease is generally because, as fruit ripen or senesce, the defense mechanisms weaken. Bartz (1991) reported that 'sour rot' disease advanced rapidly under warm conditions. In this study, it was observed that, as the temperature at which the tomatoes were previously exposed to air or C_2H_4 increased, so did subsequent disease severity (reaching a peak in fruit previously held at 25 or 30C and declining in fruit previously held at 35C. These results are consistent with the findings of Moline (1984a), who reported that maximal growth of *G. candidum* was observed at 30C and minimal growth occurred at 5C on PDA. However, in

Moline's experiment, tomatoes were not treated with C_2H_4 and were not exposed to temperatures above 30C.

As the concentration of spore suspensions of *G. candidum* used to inoculate tomatoes increased, there was a general subsequent increase in severity of 'sour rot' disease in most cases, with a few exceptions where no increase was observed (Table 6-1, 6-2, and 6-3). These results are not surprising because higher spore concentrations in suspensions of *G. candidum* are likely to increase chances of fruit infection.

Inoculation of Tomatoes with a Thermophilic Bacterium

The fact that there was no infection of wounded tomatoes observed at 20, 25 or 30C after inoculation with the unidentified organism implied that those temperatures were not conducive to growth of this bacterium and progression of the disease it causes. However, the disease that was observed at 35C after 3 days and at 40C after 1 day implied that the unidentified organism was thermophilic. Uninjured tomato fruit inoculated with this organism did not develop disease when incubated at any of the temperatures previously mentioned. This meant that the bacterium is a wound pathogen, i.e. requiring wounds for ingress into tomato tissue. This is the first observation in Florida of a pathogenic thermophilic bacterium. In Israel, rapid development of soft rot was observed in tomato fruit that had been severely affected by late blight, after a change from cool to warm weather (Volcani and Wahl, 1954). The fungus *Phytophtora infestans* (Mont.) Dby. and a bacterial organism were isolated by the authors from the necrotic tissue of those tomato fruit. Bacteria were isolated from the soft tissue bordering necrotic areas. The cultural and physiological characteristics of the bacterium were those of *Bacillus subtilis* Cohn emend Prazmowski, which in Israel causes diseases of fruits of *Capsicum annum* L

and *Solanum melongena* L. (Volcani and Wahl, 1954). In preliminary infection experiments, the authors reported that soft rot was readily induced in disease-free tomato fruit by depositing a concentrated bacterial suspension on epidermal slits made with a sterile needle and then incubating the fruit under high relative humidity at 30 to 35C for 3 to 4 days. In results similar to our findings, no infection occurred if inoculated fruit were incubated at 20 to 23C and, furthermore, inoculated uninjured fruit failed to develop disease at either the low or high temperatures (Volcani and Wahl, 1954).

The use of the KOH test as an effective Gram staining has been useful for rapid and accurate differentiation of a large number of bacteria originally isolated from plant tissue (Suslow et al., 1982). The test is based on the fact that the cell walls of Gram-positive bacteria do not lyse in 3% KOH, while the cell walls of Gram-negative bacteria are rapidly disrupted in the alkaline solution, releasing DNA, which results in the viscous threading.

Summary

This is the first study on the effect of high temperature while treating with C_2H_4 on subsequent disease development of *G. candidum* wound-inoculated MG tomatoes. Wounded tomatoes previously treated with C_2H_4 at the designated temperatures for 24, 48, or 72 hr, subsequently exhibited increased disease after 11, 10 or 9 days in air at 20C, respectvely, compared to fruit previously treated with air. As the duration of C_2H_4 treatment increased from 24 to 48 and 72 hr, so did subsequent 'sour rot' development in air at 20C. Subsequent 'sour rot' severity in air after at least 9 days increased as the temperature of previous exposure while treating with air or C_2H_4 increased from 20C to 25 and 30C, then declined in fruit that came from 35C. As the population (cfu) of

G. candidum used to inoculate fruit increased, it lead to increased subsequent disease severity. In the course of this experiment, an unidentified organism, which appeared to be a bacterium was observed on wounds of tomatoes that were exposed to 35 or 40C. This thermophilic organism was not identified using standard manuals, and Koch's postulates were done to assist in identification. The bacteria was identified as probably being a *Bacillus* species after Gram testing. The bacteria did not cause disease in nonwounded tomatoes or in fruit incubated at ≤ 30C.

CHAPTER 7
SUMMARY AND CONCLUSIONS

The tomato is one of the most important vegetables in the world, and is consumed as a fresh crop or included as a major constituent in many primary foods. The tomato is a model plant for studying the principles of growth and development, in particular fruit ripening. Temperature affects the commodity's response to C_2H_4, atmospheric composition and pathogens in many ways. High temperature ($\geq$ 30C) exposure of tomatoes can have detrimental effects, which include poor color development (lycopene biosynthesis is inhibited by high temperature), delayed softening, delayed climacteric C_2H_4 production, suppression of fruit respiration, and physiological disorders such as sunburn. In Florida, tomatoes are mostly harvested at the MG stage and ripened by treating with C_2H_4. However, during some parts of the harvest season tomato pulp temperatures may be $\geq$ 30C for some time while being treated with C_2H_4. This study was initiated to investigate the effect of treating MG tomatoes with C_2H_4 at temperatures of $\geq$ 30C on subsequent color development, respiration, C_2H_4 production, ACO enzyme activity, suberization to heal harvest-inflicted wounds, and infection by *G. candidum*, a wound pathogen.

Tomatoes held in air at 20 or 35C for 24, 48 or 72 hr, then transferred to 20C, ripened slowly and nonnuniformly compared to fruit previously treated with C_2H_4 at the same temperatures and for the same periods of time. It was found that prior exposure to C_2H_4 at 30C, and in some instances 35C, stimulated subsequent red color development in

air at 20C. This finding is new and interesting, and implies that tomatoes are able to perceive C_2H_4 at temperatures of 30 to 35C, but signal transduction was not completed while at the high temperature. In light of these new findings one wonders if there is a real need to cool tomatoes while treating with C_2H_4. Alternatively, the fruit can be treated with C_2H_4 while at the high temperature with a guarantee that fruit will ripen normally or even achieve stimulated ripening in air at 20C. The commercial applicability of this information remains to be tested and seen. However, signal transduction leading to ripening initiation was completed in air at 20C, indicating that inhibition of the C_2H_4 response mechanism at high temperatures involves reactions downstream from the initial protein binding reaction. When tomatoes were exposed to C_2H_4 at 40C for 48 or 72 hr, subsequent red color development was inhibited after transfer to air at 20C. It was likely that 40C impaired the normal tomato function and could have caused irreversible damage, which was not overcome in air at 20C. The fact that increasing the C_2H_4 concentration from 100 to 1,000 ppm did not alter the fruit response clearly shows that the inhibition of ripening at 40C and after subsequent transfer to 20C is not a result of an altered threshold for response to C_2H_4.

Few ripening behavior differences were observed in terms of respiration, C_2H_4 production and ACO activity in air at 20C in tomatoes previously treated with C_2H_4 at high temperature ($\geq$ 30C) for 24 hr compared to those treated at lower temperatures (i.e. 20 and 25C). This observation may imply that 24 hr was not long enough for the high temperatures to have impact, as evidenced by ripening differences in fruit exposed to C_2H_4 for 48 or 72 hr. It is also not known how long it took for the temperature of the fruit to equilibrate when placed at the designated temperatures. Ethylene production was

inhibited after 48 or 72 hr at 40C probably because the ability of the tomato tissues to perceive C_2H_4 was impaired at that temperature. Exposure to 40C also resulted in the lowest ACO activity, which could have been due to possible enzyme denaturation to some extent.

Wounded fruit previously treated with C_2H_4 for 72 hr at 30C lost less weight than fruit held at 30C for 24 or 48 hr, which lost as much weight as fruit held at 20 or 25C. Most of the weight loss in tomatoes is due to water loss. If suberization seals off wounds to reduce water loss, then 30C appears to be the optimum temperature for wound healing. The optimum conditions for wound healing in sweetpotatoes are a temperature of 30C and around 95% RH for 5 to 7 days. On the other hand, wounded MG tomatoes inoculated with *G. candidum* and treated with C_2H_4 at 35, 30 or 25C subsequently exhibited increased 'sour rot' disease development. Here we have a conflict in that, although 29C proved to be the optimum temperature to heal harvest-inflicted wounds in tomatoes, it is also the optimum temperature for the growth and development of *G. candidum*, which causes sour rot disease in wounded tomatoes. A thermophilic bacterium, which caused decay in wounded tomatoes only at temperatures ≥ 35C was identified as probably being a *Bacillus* species after gram testing.

Further work is recommended to investigate the effect of C_2H_4 treatment at high temperature on suberization at the microscopic level and its effect on ripening at the molecular level. Applied research is needed to determine the advisability of treating tomatoes with C_2H_4 at high temperatures than are currently employed by commercial tomato handlers. There is also need for more work to characterize the bacterial species that caused decay in wounded tomatoes at high temperature.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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