INFLUENCE OF ETHYLENE SUPPRESSION AND RIPENING ON TOMATO FRUIT COLOR, FIRMNESS, AND VOLATILE EMISSIONS

By

SONYA LEONORE STAHL

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In memory of Dr. Isaac Asimov, whose popular science essays inspired my interest in biochemistry, and in memory of Antonín Dvořák, whose music was my constant companion throughout my experiments and writing

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By

Sonya Leonore Stahl

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Ripening parameters of tomato (*Lycopersicon esculentum* 'BHN586', 'FL-47', and 'FL-91') were evaluated to determine the effects of ethylene suppression on color, firmness, and volatile emissions.

Tomato fruit 'BHN586' were obtained at turning stage and held at 20°C until ripe. Fruit color, firmness, and volatile emissions were measured over a period of 16 d following ripening. Hue angle did not change significantly in the 16 d post ripening, but firmness decreased from 5 to 3 N. Production of *cis*-3-hexenal decreased and ethyl vinyl ketone increased as the fruit progressed from ripe to overripe, and hexenal production fell and rose again. ß-ionone production remained low during the first week after attainment of ripe stage, but then rose sharply through day 11 and remained high through day 16.

Tomato fruit 'FL-47' were obtained at green, breaker, turning, and pink stages, stored at 20°C, and treated with air or 1-methylcyclopropene (1-MCP) at 250 ppb for 12 h at 20°C to determine the stage at which ethylene suppression would most delay color

change but not prevent ripening altogether. Breaker was determined to be the optimal stage for this treatment regimen, due to the difficulty of distinguishing between immature and mature green fruit, and the lack of response to 1-MCP exhibited by pink or turning fruit in this experiment. Production of C6 volatiles (*cis*-3-hexenal, *cis*-3-hexenol, *trans*-2-hexenal, and hexanal) was higher in ripe fruit derived from 1-MCP-treated breaker fruit than in ripe control fruit, but production of the carotenoid-derived volatiles (ß-ionone and 6-methyl-5-hepten-2-one) was unaffected by 1-MCP treatment at breaker stage. Amino acid derived volatile production was not affected consistently by 1-MCP: isovaleraldehyde and 3-methyl-1-butanol production decreased but 1-nitro-2-phenylethane production increased as a result of 1-MCP treatment.

Tomato fruit 'FL-91' were treated at breaker stage with air or 500 ppb 1-MCP for 12 h at 20°C and held until attainment of a hue angle below 50°, signifying full ripe. Red color developed more slowly and with less uniformity in 1-MCP-treated fruit, and softening was also delayed by treatment with 1-MCP. Upon reaching full ripe, both ripe control and ripe fruit derived from 1-MCP treated breaker fruit were sampled for volatile production. A second group of control fruit was held until overripe so that volatile production of untreated fruit could be quantified at the same chronological age as treated fruit. Where differences were found between treatments, they were often inconsistent for individual volatiles between experimental replications. *Cis*-3-hexenal production was observed to decrease in 1-MCP-treated fruit in some instances, but the C6 volatiles were largely unaffected by 1-MCP treatment. In most cases, carotenoid-derived volatiles were also unaffected by 1-MCP treatment, and the effect of 1-MCP treatment on amino-acid derived volatiles was inconsistent and varied.

CHAPTER 1
INTRODUCTION

Tomato Ripening

The tomato (*Lycopersicon esculentum*) is a solanaceous fruit that is important to the agricultural economy of the United States of America, and especially in Florida. Many developmental parameters are altered during tomato fruit ripening. Color change occurs as chlorophyll breaks down and lycopene production is initiated. Fruit firmness decreases to a softness acceptable for consumption. The development of red color and the softening of tomato fruit tissue can be delayed by 1-methylcyclopropene (1-MCP), a cyclic alkene that volatizes as a non-toxic, odorless gas. 1-MCP acts by suppressing the response to the hormone ethylene, thereby preventing or delaying processes dependent on ethylene. 1-MCP can therefore be used to determine which processes are directly regulated by that hormone.

Volatile Profile of Tomato

The flavor of tomatoes is derived from a combination of sugars, acids, and aroma volatiles. While sugars and acids are perceived by the gustatory receptors on the tongue, aroma volatiles are perceived by olfactory receptors and are therefore a 'smell' rather than a 'taste' contributor to tomato flavor. The volatile constituents most important to tomato flavor are the C6 compounds hexanal and *cis*-3-hexenal, which contribute a "green, grassy" odor that was also identified as "sweet", "ripe", and "tomato flavor". These, and other related compounds, are derived from fatty acids; however, other

important volatiles in tomato are derived from the metabolism of carotenoids and amino acids.

Volatile emissions in tomato are affected by a number of parameters throughout tomato development. Fertilizer regime, maturity at time of harvest, and postharvest handling such as bruising or storage at low or high temperatures can have profound effects on the volatile profile. Choice of cultivar can also influence volatile emissions, as some varieties are low-quality and produce less aroma.

Objectives

The primary objective of this study was to determine whether the volatile profile of tomato fruit would be altered by treatment with an inhibitor of ethylene action. The first objective was to evaluate the volatile emissions in ripe tomato fruit, continuing through overripening. Another objective was to determine the ripening stage at which to treat tomatoes with 1-MCP which would have the most pronounced effect on delaying development of ripe color, but would still allow fruit to continue to attain a full ripe stage. Then tomato volatile emissions in ripe, overripe, and ripe fruit derived from breaker fruit treated with an ethylene action inhibitor were evaluated and compared within treatments to determine if volatile production in treated fruit was more similar to ripe or to overripe untreated fruit. It was also of interest to employ 1-MCP treatment as a method of discovering which volatile pathways were directly dependent on ethylene.

CHAPTER 2
LITERATURE REVIEW

General Information

The tomato (*Lycopersicon esculentum*) is a member of the family Solanaceae. The genus name, *Lycopersicon*, is derived from a Greek word meaning "wolf peach" (Davies & Hobson, 1981). The fruit is a berry that is commonly eaten as a fresh or cooked vegetable and also has numerous applications in processed foods, such as ketchup, marinara sauce, and pre-prepared soups. It is a perennial plant, but it is grown as an annual. In the United States of America, it is second only to its relative the potato as the most abundantly produced of any vegetable. Nearly thirteen million metric tons of tomatoes were grown in the United States in 2005 (FAO 2006, http://faostat.fao.org/). The majority of the fresh-market cultivars are the familiar large round red varieties, but plums, clusters, round yellow, cherry, grape, and other specialty sizes are increasing in popularity (Sargent and Moretti, 2004).

The average American eats over nineteen pounds of fresh tomato every year (http://www.floridatomatoes.org/facts.html). It is third in popularity among vegetables, under the potato and lettuce. Tomatoes are a plentiful source of vitamins C and A, certain minerals, and antioxidants, especially lycopene, the pigment responsible for the color of red tomatoes. The tomato was legally declared a vegetable in 1893 in a Supreme case centering on tariffs (*Nix v. Hedden*, 149 U.S. 304, 1893).

Over four and a half million hectares of the world are devoted to tomato production (http://www.floridatomatoes.org/facts.html). Seventeen thousand hectares of this are in

the state of Florida, which is half of the fresh tomatoes produced in the United States. This represents a 660 million dollar value to Florida farmers each year and jobs for 33 thousand workers (http://www.floridatomatoes.org/facts.html).

The tomato fruit, a berry originating from a single, inferior, multi-locular ovary, undergoes experience a period of sigmoidal expansion and maturation prior to exhibiting the characteristic features of ripening. In this pre-ripening developmental period, the tomato fruit remain green. Upon completion of physical growth, the fruit attains a developmental state typically referred to as 'green' (Sargent and Moretti, 2004). For descriptive purposes, this green period is divided into four stages, M1 through M4. At the M1 stage, the locular tissue is firm and the seeds are immature (seeds are white and can be easily cut by passing a sharp blade through the fruit.) At the M2 stage, the seeds have matured and are tan, and gel is present in at least two locules. At the M3 stage, well developed gel is present in all of the locules, and the internal color is still green. The green maturation process concludes with 'mature green', which is the stage at which red color appears in the locule gel and internal, radial pericarp and columnella tissues (Sargent and Moretti, 2004, adapted from Kader and Morris 1976). Fruit harvested prior to attaining a physiologically mature, ‘green’ stage will continue to ripen, but will not attain the maximum potential quality (Hurr, Huber, and Lee, 2005).

The developmental period beyond the mature green stage through the full red-ripe stage has been divided for descriptive purposes into four stages based on the accumulation of surface lycopene distribution (USDA 1991: http://www.ams.usda.gov/standards/tomatfrh.pdf). Each stage indicates further breakdown of chlorophyll and accumulation of lycopene. Breaker fruit are mostly green

but show a blush of red or pink around the blossom end that constitutes less than ten percent of the fruit surface. Turning fruit have a larger area of red development, between 10% and one third of the surface. Pink fruit are between one and two thirds red, and light red are between two thirds and 90% red. When the tomato reaches the full red stage, it is table-ripe and ready to eat.

In commercial practice, tomatoes are usually hand-harvested unripe to prevent bruising and spoilage during storage and transportation. Care is taken to keep damaged, defective, immature, or fruit of an advanced stage of ripening—which will rot more quickly--out of postharvest channels to reduce the need for grading later on. Once out of the field and into the packinghouse, the fruit are washed in a chlorinated dump tank and then brushed, rinsed, and dried. Sometimes they are also waxed (Sargent, Brecht, and Olczyk, 2005 http://edis.ifas.ufl.edu/VH079). Tomato sorting occurs along a conveyor belt, as workers remove unsatisfactory fruit and classify the tomatoes by size and quality. In Florida, the quality grades are 'U.S. No. 1', '85% U.S. No. 1', 'U.S. Combination', and 'U.S. No. 2', and the sizes are '6x7' (between 2 9/32 and 2 19/32 inches in diameter), '6x6' (between 2 17/32 and 2 29/32 inches in diameter), and '5x6' (minimum diameter 2 25/32 inches) (http://www.floridatomatoes.org/domestic.html, USDA 1991: http://www.ams.usda.gov/standards/tomatfrh.pdf). The fruit are then packed into cartons and stacked into pallets. They are ripened by exposure to ethylene. This initiates ripening at a commercially desirable point in the supply chain and also assures uniform ripening of the harvest.

Flavor Chemistry

Flavor is an important component of a tomato's desirability, but with increasing focus from breeders on quality parameters including color, firmness, resistance to

disease, yield, size, and physical uniformity, many of today's tomato cultivars do not meet consumer standards for good taste (Hobson, 1988). Poor flavor components include sourness, bite (the stinging present after imbibing carbonated beverages), a 'green' taste reminiscent of unripe fruit, low pH, and most relevant to this discussion, low levels of many aromatic volatiles (Tandon, Baldwin, Scott, and Shewfelt, 2003). Stevens, Kader, Albright-Holton, and Algazi (1977) reported that since it is more fluid than the other tomato tissue, qualities of the locule gel are more readily perceived by taste receptors on the tongue and it is therefore a more significant contributor to tomato flavor than either pericarp or placental tissue.

The flavor of a ripe tomato is created by a complex combination of sugars, acids, and aroma volatiles (Krumbein and Auerswald, 1998). Tandon et al. (2003) report that fructose and glucose are the sugars most relevant to tomato flavor. These sugars comprise approximately three percent of the fresh weight of a ripe tomato, increasing as the fruit ripens (Tandon et al., 2003; Davies and Hobson, 1981). The sweetness of tomato fruit positively correlates to the amount of these sugars.

Organic acids are another important constituent of tomato flavor. Citric acid is the most abundant, at a concentration of 135-675 micrograms per gram fresh weight (mg/gfw) (Davies and Hobson, 1981). Citric acid is most plentiful in the locular gel, where it is present in concentrations between 634-1560 mg/100 gfw. It is followed by malic acid, which is present in concentrations between 21 and 225 mg/100 gfw in the whole fruit and 30-412 mg/100 gfw in just the locular gel (Davies and Hobson, 1981). As also reported by Davies and Hobson (1981), amino acids are also present, the most numerous in tomato fruit being glutamic acid, arginine, aspartic acid, and asparagine.

Aroma volatiles are present in very low concentrations--nanoliters per liter of blended fresh tomato fruit tissue--but are still very important to flavor. They are perceived by olfactory receptors in the nose, not by the taste receptors in the tongue (Acree, 1993). According to Buttery (1993), over four hundred volatiles have been identified in tomato fruit, but only around thirty of them comprise a significant portion of perceived flavor. These include various aldehydes, ketones, and alcohols. The volatiles that appear in the highest concentrations are *cis*-3-hexenal, hexanal, and 2-phenylethanol.

Volatiles can be classified according to their synthetic pathways and the compounds from which they originate. Some, like hexanal, cis-3-hexenal, trans-2-hexenal, cis-3-hexenol, and ethyl vinyl ketone, are derived from fatty acids (Buttery & Ling, 1992) including linolenic and linoleic acids (Goff and Klee, 2006). These lipid-derived volatiles are present in both leaves and fruit tissue. The maceration and blending with $CaCl_2$ of tomato leaves by Buttery and Ling (1992) and the consequent sampling of the resultant volatiles revealed high levels of *cis*-3-hexenal in the leaf tissue. This indicates that *cis*-3-hexenal is the volatile responsible for the green, grassy scent of tomato leaves. Buttery and Ling (1992) also sampled the *cis*-3-hexenal produced by tomato fruit tissue and found that it increased as the fruit ripened. The compound was found at a concentration of 0.1 parts per million (ppm) in mature green tomato, 0.3 ppm at breaker stage, and 15 ppm in ripe fruit.

Buttery and Ling (1992) also identify volatiles, including ß-ionone and 6-methyl-5-hepten-2-one, which are formed from carotenoids. ß-ionone is a cyclic compound, whereas 6-methyl-5-hepten-2-one is an open chain formed by oxidative cleavage of lycopene (Goff and Klee, 2006). Carotenoid-derived volatiles are found only in the fruit,

and not in leaf, flower, or stem tissue. There are also volatiles that are derived from amino acids. These are found exclusively in the fruit and are formed between the breaker and full-ripe stage (Buttery & Ling, 1992). For example, 1-nitro-2-phenylethane and 2-phenylethanol are formed from phenylalanine, isovaleraldehyde (3-methylbutanal) is formed from leucine, and 2-methylbutanal is formed from isoleucine. The pathways of some, like 2-isobutylthiazole, are unknown (Goff and Klee, 2006) although Schutte (1974) suggests that 2-isobutylthiazole may also originate from leucine via isovaleraldehyde.

Trained taste panelists have identified relationships between specific volatiles and certain facets of tomato flavor. Krumbein and Auerswald (1998) identified *cis*-3-hexenal as one of the most powerful components of flavor, producing a “fresh green” and “sweet” flavor. Hexanal was characterized as “green” and “grassy” by Krumbein and Auerswald (1998), and as “sweet”, “ripe”, and "tomato flavor" by Maul et al. (2000). 2-isobutylthiazole was described in Krumbein and Auerswald (1998) as producing both sweet and fruity flavors and causing a “bite” feeling in the mouth. It was also identified as “musty”. ß-ionone contributed to a sour taste, and *trans*-2-heptenal and 1-penten-3-one (ethyl vinyl ketone) produced a “green” aroma. 3-methylbutanal (isovaleraldehyde) was described as “unpleasant” (Krumbein and Auerswald, 1998; Baldwin, Scott, Shewmaker, and Schuch, 2000).

Ethylene

Ethylene (C_2H_4) is the structurally simplest of the plant hormones, as well as being the simplest alkene. It is derived from methionine via a pathway elucidated in detail by Yang and Hoffman (1984). The regulatory effects of ethylene on plant development are

numerous and varied. They include initiation of flower opening, seed germination, and leaf/flower senescence, and control of responses to both biotic and abiotic stresses (Yang and Hoffman, 1984). In climacteric fruit, those that experience an increase in respiration rate and ethylene production accompanying ripening, ethylene also regulates changes associated with fruit ripening (Yang and Hoffman, 1984). For thousands of years, humans have augmented the natural ethylene production of crops with their own artificially added doses in order to produce a uniform ripening response. For example, Theophrastus, who lived in the third century B.C.E., reports that agriculturists of his time damaged the skin of figs in order to initiate ripening through what is now understood to be a method of stimulating ethylene production via a wounding response (Galil, 1968). The process of wounding figs to promote ripening is still performed by present-day Egyptian farmers. In more modern times, citrus fruit such as oranges were placed in close proximity to burning kerosene in order to achieve degreening necessary for a brilliantly-colored orange or yellow product. Chace and Denny (1924) determined that the active factor in the burning kerosene was ethylene gas. In studies of apple fruit, Gane (1934) was the first to demonstrate that ethylene was a natural product of plant tissues.

The biosynthesis of ethylene begins with the amino acid methionine, which is converted to *S*-adenosylmethionine (SAM) by SAM synthetase (E.C. 2.5.1.6). SAM is converted to 1-aminocyclopropane-1-carboxylic acid, or ACC, by ACC synthase (E.C. 4.4.1.14) also called ACS (Yang and Hoffman, 1984). It then is oxidized by ACC oxidase (E.C. 1.14.17.4), also called ACO and once referred to as the ethylene forming enzyme (EFE), in order to produce ethylene (Alexander and Grierson, 2002).

Both the prevention of ethylene synthesis and the suppression of either ethylene perception or the response to ethylene interfere with ripening and other senescence processes. This was demonstrated by Brandt and Woodson (1992) by examining genotypic variation in carnation flowers. Cultivars with low amounts of ACC, indicating reduced activity of ACS, exhibited extended vase-life by an average of five days compared with a cultivar with normal levels of ethylene and its precursors. In addition, low activity of ACO, referred to in this study as EFE, the ethylene forming enzyme [as it was known before activity was obtained in vitro by Ververidis and John (1991)], resulted in even lower levels of ethylene in certain cultivars. One of these cultivars, 87-37G-2, responded to exogenously applied ethylene by finally producing its own ethylene and senescing earlier than untreated controls, indicating that its deficiency was in ethylene synthesis, not perception. Another synthesis-deficient cultivar, 799, failed to respond to external ethylene, indicating that is was both a perception and a synthesis mutant. It is clear that interfering with ethylene synthesis and/or perception will result in delayed, reduced, or halted senescence activities.

Although carnation served as an early model for exploration of ethylene effects, ripening fruits have become increasingly the system of choice. Carnations are still investigated as a floral system, but the bulk of the molecular work dealing with ethylene perception and action has been performed with fruit tissue. For example, the *Never-ripe* tomato is a mutant that is unable to perceive ethylene because of a mutation to its ethylene receptor. The altered receptor is unable to bind to ethylene, leaving it unengaged and able to repress ethylene responses even in the presence of the hormone (Hackett et al., 2000). Hackett et al. (2000) further demonstrated that inhibiting the *Nr* gene using

antisense constructs enabled the mutant tomatoes to recover ethylene perception and ripen normally.

Inhibitors of Ethylene Synthesis Action

One method of preventing the effects of ethylene on both cut flowers and edible crops is to employ an ethylene scrubber made of cement, silica gel, aluminum pellets, and more recently, potassium permanganate. The scrubbers absorb the ethylene from the surrounding atmosphere and prevent it from being absorbed by the product. Jayaraman and Raju (1992) found the potassium permanganate-based scrubber more effective than previously used materials in extending the shelf life of tomato, mango, and okra by a period of between three to eight days.

Silver thiosulfate (STS) is a compound that interferes with ethylene perception (Veen & Geijn, 1978; Bleecker & Kende, 2000). Ethylene is believed to interact with a copper cofactor in the receptor. It has been speculated that if silver is present, it takes the place of copper and interacts with the ethylene instead. While it has the same ability as copper to interact with ethylene, it lacks the ability to complete the receptor's function as copper does, disabling the receptor (Rodriguez et al., 1999). However, Veen and Geijn (1978) observed brown, necrotic spots on the leaves resulting from the toxic effects of high concentrations of silver. Silver is a toxic heavy metal that can causes injury even in small amounts because it binds to sulfhydryl groups non-discriminately. It may have effects of which science is still unaware. The toxicity of STS restricts it to use with flowers only, not for food crops, and in some countries its use or method of application have been restricted due to concerns about environmental pollution (Serek & Reid, 1993).

Sisler, Reid, and Yang (1986), Serek, Sisler, and Reid (1994), and Sisler and Blankenship (1993) examined a wide range of cyclic olefins in search of compounds that

might serve as antagonists or agonists of ethylene reception. 2,5-norbornadiene (NBD) and diazocyclopentadiene (DACP) were shown to have effects on the ethylene receptor, but they were deemed unsuitable for practical use. In the case of the former, the compound must be present continually in order to be effective (which is impractical for the supply chain of most produce), and in the case of the latter it was highly explosive. NBD also has a foul odor which further limits its desirability (Serek et al., 1994).

1-Methylcyclopropene, or 1-MCP, is a non-toxic, odorless gas that is effective in rendering ethylene receptor molecules inactive after short-term treatment. 1-MCP is a simple molecule, a cyclic alkene with a single methyl group attached to the olefinic carbons. 1-MCP apparently acts by non-competitively binding to the ethylene receptor because it has a lower binding constant, Kd=8 nl•liter^{-1} (Serek et al., 1994). It is presumed that by blocking ethylene binding, 1-MCP causes the receptor to remain engaged, maintaining the downstream cascade that suppresses ethylene-induced genes (Sisler and Serek, 1997).

The effectiveness of 1-MCP at extending the shelf life of many fruit and vegetable crops has been well documented. It has been approved for commercial use on horticultural crops since 2002, starting with apples (American Fruit Grower, 2002), and has now been approved for numerous other crops, including the tomato. Effects such as a delay in softening or in the development of ripe coloration have also been demonstrated on banana, avocado, melon, papaya, persimmon, and other crops, both climacteric and nonclimacteric (reviewed in detail in Blankenship & Dole, 2003). Commercially, it is available as *EthylBloc* for ornamentals and *SmartFresh* for edible crops. It is presently used as a gas that is volatized when a powder formulation is added to water, but a liquid

application is currently being investigated (Todd Eddington, Agrofresh, personal communication).

1-MCP inhibits many parameters of ripening, including ethylene synthesis, ethylene perception, loss in firmness, and changes in pigmentation. The effects can either be permanent or dissipate after a period of 1 to 2 weeks, depending on the concentration used and the period of exposure. The fact that 1-MCP interferes with perception of ethylene makes it a useful tool to study which ripening processes are ethylene dependent and which occur independent of ethylene. Most research with the effects of 1-MCP has focused on firmness and color. For example, tomato fruit were treated with 1 ppm 1-MCP for 24 h at 20°C. It was determined that the stage at which a tomato fruit is treated can determine the effectiveness of the 1-MCP treatment in delaying pigmentation changes associated with ripening. Tomatoes treated at mature green or breaker stage delay development of external red color for a week, and then ripen normally (Hurr et at., 2005).

1-MCP produces a delay in the loss of firmness associated with ripening. The amount of force required to compress the external tissue of a tomato a distance of 2 mm ranges from 20 Newtons (N) at the mature green stage to four or five N at full red ripe. 1-MCP causes this softening to be delayed from five to ten days later than usual (depending on the developmental stage at which the 1-MCP was applied). One possible explanation is that the enzymes polygalacturonase and cellulase, which are responsible for the breakdown of the pectin and cellulose that provide structural support for plant cells, are affected by ethylene suppression. The blocking of ethylene perception in avocado interferes with the activity of polygalacturonase and cellulase, but softening is inhibited only during the later stages of ripening with little effect during early to mid ripening

(Jeong, Huber, and Sargent, 2002; Jeong and Huber, 2004). This seems to indicate that the softening that occurs during early ripening may be largely ethylene independent, whereas the softening during overripening may be ethylene dependent since it can be stopped by the inhibition of ethylene recognition. A tomato transgenically altered with an antisense gene for polygalacturonase exhibited a definite decrease in postharvest softening compared with wild-type fruit (Kramer et al., 1992). There is a point at which ripening can be delayed excessively, however, where fruits do not soften sufficiently and become shriveled and decayed prior to ripening.

Effects of 1-MCP on Fruit Volatiles

Documented effects of 1-MCP on fruit volatile profile are variable and dependent on the species of fruit and specifics of treatment (concentration, duration of exposure, and temperature). Golding, Shearer, McGlasson, and Wyllie (1999) reported a decrease in total volatile production of bananas ripened at 20°C after treatment with 1-MCP (45 ppm, 1 h) at a mature green stage compared with ripe untreated bananas. This decrease was attributed mainly to a decrease in ester production. An increase in alcohol production in the treated fruit, compared with control fruit, was also observed. Golding et al. (1999) suggested that this increase in alcohols was due to a disruption of the final step in the formation of esters, in which alcohols are esterified by the enzyme alcohol acyl transferase, also known as AAT (E.C. 2.3.1.84) (Yahyaoui et al., 2002). Two esters, 3-methylbutyl isovalerate and pent-2-yl butanoate, increased in treated fruit compared with control fruit, implying that the enzyme remained functional and that availability of the substrate acyl CoA was the limiting factor in ester production (Golding et al., 1999).

Pelayo, Vilas-Boas, Benichou, and Kader (2003) reported that ester production was delayed, but not reduced, in bananas treated with 1-MCP (1 ppm, 24 h), and stored at

20°C, compared with untreated bananas. Aldehyde loss was also delayed in treated fruit compared with control fruit. The concentration of 1-MCP applied is critical. Bagnato, Barrett, Sedgley, and Klieber (2003) found that when bananas were treated at 300 ppb, ripening was delayed and the flavor was unaffected. Too low of a concentration (3 ppb) did not extend shelf life and too high of a concentration (30 ppm) prevented ripening entirely.

Apples have also been studied for the response of aroma volatiles to 1-MCP. During ripening of untreated apples, acetates and some esters increase and alcohols and aldehydes decrease (Lurie, Pre-Aymard, Ravid, Larkov, and Fallik, 2002). When 'Anna' apples were treated with 1-MCP (1 ppm, 20 h) at 20°C, the volatile profile associated with harvest time was retained. Taste panelists preferred the treated apples, which tasted freshly harvested, to the control apples, which displayed the characteristic aroma of very ripe apples.

In another study of apples using the cultivar 'Borkh', Defilippi, Dandekar, and Kader (2004) reported delayed and reduced esters when fruit were treated with 1-MCP (1 ppm, 20 h) at 20°C just after harvest, but aldehydes were unaffected. Data from adding ethylene back to transgenic fruit with the inability to synthesize it on their own implied that a continuous supply of ethylene is necessary for volatile synthesis (Defilippi et al., 2004).

Abdi, McGlasson, Holford, Williams, and Mizrahi (1998) used 1-MCP to determine whether parameters of plum ripening, such as the development of skin color and production of aroma volatiles, were ethylene dependent or independent. The cultivar 'Beauty' was treated with 1-MCP (26 and 39 ppm, 24 h) at 20°C and developed aroma

volatiles one week later than untreated plums, according to a taste panel. When the cultivar 'Shiro' received the same treatment, only the untreated fruit developed aroma volatiles at all.

Mitcham, Mattheis, Bower, Biasi, and Clayon (2001) found that European pears responded to various concentrations of 1-MCP with suppressed production of aroma volatiles, assessed subjectively but no quantities were recorded. Once the effects of the treatment wore off, volatiles began to form normally.

The processes regulated by ethylene are complex, and the response of a particular species or cultivar to 1-MCP, a powerful growth regulator, is not always predictable. This limits its commercial application and indicates that it is not suitable for every circumstance.

Factors Affecting Tomato Volatiles

The quantities and composition of volatiles in tomato fruit are highly dependent on genetic and environmental factors active throughout fruit development. Volatiles can vary between cultivars. For example, the variety 'FL-47' is known to be low-volatile, and consequently, low-quality (Tandon et al., 2003).

Preharvest conditions such as fertilizer regime can also affect the tomato's volatile production. The amount of aroma volatiles including hexenal, phenylacetaldehyde, benzaldehyde, ß-ionone, and 6-methyl-5-hepten-2-one was increased by elevated levels of nitrogen and potassium (Wright and Harris, 1985). This increase had a negative impact on tomato flavor (Wright and Harris, 1985).

Fruit maturity at harvest can influence the volatile profile. Maul et al. (1998) determined that tomato fruit that were harvested immature green, as indicated by the fruit taking 5 days to reach breaker stage in ethylene treatment, displayed lower levels of

many aroma volatiles, including *cis*-3-hexenal, 6-methyl-5-hepten-2-one, 2-isobutylthiazole, and ethyl vinyl ketone, compared with tomato fruit that were harvested mature green and only took one day to break in ethylene treatment.

After the tomato is harvested, ideally, care should be taken by the handlers and packers to ensure that an ideal volatile composition is preserved. Appropriate storage temperature is an important component of postharvest handling. The temperature during the final stages of ripening has the greatest effect on the fruit volatile profile once ripe. Maul et al. (2000) found that too low of a storage temperature will cause an increase in off-flavors and a decrease in aroma. Storage at a temperature below 12.5°C for even two days in the case of cv. 'BHN-189' or four days in the case of cv. 'Solimar' resulted in a loss of flavor quality, as evaluated by a trained taste panel. The quality was further reduced if the storage temperature was 5°C. The main loss in aroma volatiles consisted of a reduction in the lipid-derived volatiles (hexanal, *cis*-3-hexenal, ethyl vinyl ketone, *trans*-2-hexenal, *trans*-2-heptenal, and *cis*-3-hexenol) that accompanied the lower storage temperatures. The carotenoid-derived volatiles (ß-ionone and 6-methyl-5-hepten-2-one) were also reduced but only slightly. Tomatoes held at 20°C achieved higher ratings from the taste panel in aroma, flavor, and intensity of sweetness, even after twelve days of storage.

In another study, Boukobza and Taylor (2002) compared low temperature (6°C), moderate temperature (21°C and 35°C), and high temperature (45°C) storage environments. The tomatoes were held at the trial temperature for three days (in the low temperature vs moderate temperature experiment) or six hours (in the high temperature vs moderate temperature experiment) before being transferred to 21°C for 4-6 hours to

mimic retail conditions. Low-temperature storage caused an eventual decrease in the amounts of isobutylthiazole, methylbutanal, and methylbutanol, possibly due to a reduction in enzyme activity or a decreased level of available amino acid precursors. High-temperature storage reduced the amounts of hexanal, hexenal, hexenol, isobutylthiazole, methylbutanal, methylbutanol, and 6-methyl-5-hepten-2-one, an effect believed to be due to the denaturing effect of the heat on the enzymes of the lipid oxidation pathway from which the volatiles would have formed. The reduction in aroma volatiles was aggravated by increasing the temperature further or increasing the duration of holding at 21°C.

Bruising can cause changes in a tomato's volatile profile, most notably a reduction in the amounts of *cis*-3-hexenal, 6-methyl-5-hepten-2-one, ethyl vinyl ketone, *cis*-3-hexenol, and 2-isobutylthiazole. These changes were identified by comparing bruised locular tissue to unbruised locular tissue. Placental tissue responded to bruising with increased levels of ethyl vinyl ketone and ß-ionone compared with unbruised placental tissue, and the amount of *trans*-2-hexenal in bruised pericarp tissue was higher than in unbruised pericarp tissue. These alterations are due possibly to the disruption of the enzymatic schedule by the damage resulting from the physical impacts. (Moretti, Baldwin, Sargent, and Huber, 2002).

A relationship between ethylene and the synthesis of many tomato volatiles is evident from a number of studies. Oeller, Wong, Taylor, and Pike (1991) investigated tomato plants which expressed an antisense construct for ACC synthase, suppressing the ethylene biosynthesis pathway. Ethylene production in the transformant was reduced nearly 200-fold, and the fruit failed to ripen beyond an orange color. Their aroma was

never developed. Antisense fruits ripened upon exposure to exogenous ethylene, and ripening was accompanied by the development of aroma volatiles. Baldwin et al. (2000) reported that ethylene is required for carotenoid synthesis, possibly increasing ethylene's effects on at least those volatiles that are derived from carotenoids. Kausch and Handa (1997) found that the ethylene-insensitive Never-ripe (*Nr)* mutant accumulated the mRNA for lipoxygenase, an enzyme important in the synthesis of volatiles from linolenic and linoleic acids, but did not accumulate the lipoxygenase protein. This implies that ethylene is not necessary for the accumulation of the mRNA for lipoxygenase, but that it is necessary for the translation or stability of the lipoxygenase protein and, consequently, for the formation of lipid-derived volatiles, which is supported by Ealing's (1994) work showing lipoxygenase activity increasing and then decreasing along with ethylene production in the early stages of tomato ripening (Ealing, 1994). Another important enzyme, alcohol dehydrogenase (ADH), catalyzes the conversion of hexanal to hexenol. ADH activity was reported to be ethylene-independent, which is supported by its elevated activity, possibly due to decreasing oxygen concentration, in ripening grape fruit, which are nonclimacteric (Zhu et al., 2005).

During normal tomato ripening, Baldwin, Nisperos-Carreido, and Moshonas (1991) found that the amounts of most aroma volatiles increased, including *cis*-3-hexenol, *cis*-3-hexenal, *trans*-2-hexenal, 6-methyl-5-hepten-2-one, and 2-isobutylthiazole. The increase in many of the volatiles followed the same trend as the climacteric increase in respiration and production of ethylene and lycopene during ripening. Ethyl vinyl ketone decreased during ripening, but in only one of the cultivars investigated, 'Sunny'. In the cultivar 'Solimar', a small increase in ethylene production during the breaker stage corresponded

with the appearance or significant increase in the amounts of *cis*-3-hexenol, *cis*-3-hexenal, *trans*-2-hexenal, 6-methyl-5-hepten-2-one, and 2-isobutylthiazole.

The production of 1-hexenal, *trans*-2-hexenal, and 6-methyl-5-hepten-2-one was reduced in table ripe tomato fruit ('308' and '311') treated at mature green stages with 1-MCP (2 ppm, 15 h) and stored at 21°C, compared with untreated fruit. Production of ethyl vinyl ketone and *cis*-3-hexenal was enhanced in 1-MCP-treated fruit compared with the controls (Mir, Canoles, and Beaudry, 2004). The amount of 1-hexanal and 2-isobutylthiazole was higher in turning-harvested fruit than in green-harvested fruit, but the amount of ethyl vinyl ketone and *cis*-3-hexenal was greater in green-harvested fruit than in turning-harvested fruit. The volatiles altered by treating tomatoes with 1-MCP were the same as the volatile altered by harvesting fruit at an earlier developmental stage. This implies that 1-MCP treatment may encourage flavors associated with harvesting tomatoes at an earlier stage of maturity (Mir et al., 2004), which often has a deleterious effect on flavor (Maul et al., 1998). However, the magnitude of the changes in aroma caused by 1-MCP in this study is small enough to indicate that it may not be a concern to the perception of consumers.

Tieman et al. (2006) monitored the production of 'M82' tomato volatiles as the fruit progressed from immature green to light red stage. Emissions of *cis*-3-hexenal, *trans*-2-hexenal, ß-ionone, and 6-methyl-5-hepten-2-one remained low during early ripening and increased dramatically between turning stage and light red stage. The largest increase in 2-phenylethanol was at breaker stage. Maximum emission of 3-methyl-1-butanol was at turning stage, and 2-isobutylthiazole decreased from immature green onwards. Mir et al. (2004) examined the volatile profile of '308' and '311' tomatoes

during the mature green, turning, and full ripe stages. However, neither work quantitatively addressed the changes occurring in tomato volatile emissions during the period following the attainment of full ripe stage, nor has the volatile profile of 1-MCP treated fruit been compared with fruit past this initial attainment of full ripe. The objective of the present study was to examine the development of overripe tomatoes and compare them with tomatoes of the same age which had undergone 1-MCP treatment to determine if 1-MCP continues to affect tomatoes past the initial point of ripening, or if changes in volatile emissions between 1-MCP ripened and untreated ripe tomatoes were due to the 1-MCP treatment directly or to the age of the fruit. It was also of interest to examine untreated tomatoes past full ripe stage because tomatoes are often purchased when full ripe or nearly full ripe, and subsequently held for a period of days prior to consumption, and it was of interest to discover for how many days is the volatile profile associated with the first day of "full ripe" (as defined by attaining a hue angle of 50°) maintained before being altered by overripening.

CHAPTER 3
MATERIALS AND METHODS

Plant Material

Tomato (*Lycopersicon esculentum*) fruit, cvs. Florida 47, Florida 91, and BHN586, employed for the studies performed were obtained from West Coast Tomato, Inc., in Palmetto, FL, North Florida Tomatoes in Quincy, FL, and University of Florida North Florida Research & Education Center Suwannee Valley extension stations in Live Oak, FL. Depending on the specific experiment, fruit were obtained at several developmental stages, including mature-green, breaker, turning, pink and full red, as defined on the basis of surface lycopene distribution (USDA 1991: http://www.ams.usda.gov/standards/tomatfrh.pdf). Each stage reflects further breakdown of chlorophyll and accumulation of lycopene. Breaker fruit are mostly green but show a blush of red or pink around the blossom end that constitutes less than ten percent of the fruit surface. Turning fruit have a larger area of red development, between 10% and one third of the surface. Pink fruit are between one and two thirds red, and light red are between two thirds and 90% red. When the tomato reaches the full red stage, it is generally considered by consumers to be ripe and ready to eat.

Fruit used in all experiments were obtained on the day of harvest, and transported immediately to Postharvest facilities at the University of Florida. Upon arrival, fruit were washed in 1.34 millimolar Na-hypochlorite prepared from household bleach, selected for uniformity in color and freedom from blemishes, and placed in storage facilities at 20°C until treated. No fruit used in any of these studies were treated with exogenous ethylene.

Specific conditions of 1-MCP treatment and storage duration are described in separate sections below.

Fruit Color Measurements

Fruit external color was measured daily using a Minolta Chroma Meter CR-400 (Minolta Camera Co Ltd, Japan) calibrated using a white tile as the standard. The chroma meter records lightness (on a scale of black to white, where black is numerically zero), chroma (color saturation), and hue angle, a spectral scale in which 45° represents red and 180° represents green. The measurements were taken at three equatorial locations and the three hue angle values were averaged.

Analysis of Whole Fruit Firmness

Fruit firmness was measured every other day nondestructively using an Instron Universal Testing Instrument (model 4411; Canton, Mass). The Instron was fitted with a 5 kg load cell and a blunt-ended, convex 7.5 mm probe. After establishing zero force contact with the surface of the tomato, the probe was driven with a cross-head speed of 50 mm/min, targeting an area over locular tissue and not internal walls. The force of compression was recorded in Newtons (N) at the point at which the tomato skin had been compressed a distance of 2 mm. The same fruit were measured for the duration of the experiment. Firmness was measured at three equatorial points for each tomato, and the values averaged.

Evaluation of Volatile Profile of Ripe to Overripe Fruit

This experiment was performed to determine the changes in volatile profile of tomatoes between the onset of full ripeness (as designated by an average hue angle of below 50°) and over the subsequent 16 d as the tomato becomes overripe.

Fruit Selection and Cleaning

Tomato (cv. BHN586) fruit ranging from breaker to pink stage were obtained from a packinghouse in February 2006. From these, 110 turning fruit were selected, dipped in 1.34 millimolar hypochlorite, rinsed, and arranged on paper towels to dry. Each fruit was individually marked with a ballpoint pen on a white adhesive label. Turning fruit were selected for this experiment so that the precise day of ripening (as designated by an average hue angle below 50°) could be identified through daily monitoring.

Identifying Onset of Ripe Stage Using Color Measurements

Fruit external color was measured daily using a Minolta Chroma Meter CR-400 (Minolta Camera Co Ltd, Japan) as described in the section 'Fruit Color Measurements'. The measurements were taken at three equatorial locations designated by the points 90°, 180°, and 270° in reference to the white label sticker. The three hue angle values were averaged, and those fruit that reached an average hue angle of below 50° were selected and divided into two identical samples. The day on which the hue angle reached this starting point was designated ‘Day 0’. On Day 1, 2, 6, 11, and 16 during storage at 20°C, five fruit were selected from each sample and removed for volatile analysis, with the Day 1 and 2 fruit representing ripe fruit and the Day 16 fruit representing significantly overripe fruit. Volatiles were analyzed using the method described above in the section 'Analysis of tomato volatiles'.

Determination of Fruit Developmental Stage for 1-MCP Treatment

This experiment was designed to determine the developmental stage at which 1-methylcyclopropene (1-MCP) would be most effective at delaying, but not preventing, the attainment of a full-ripe condition, as defined on the basis of fruit reaching a hue angle of 45°, representing full-red external pigmentation. Fruit (cv. FL-47) were obtained

from the University of Florida North Florida Research & Education Center in Quincy, FL. Upon return to Gainesville, fruit were cleaned and washed as described under 'Plant Material'. One experiment was performed using fruit that was obtained at the 'green' stage. Fruit for other experiments were obtained after color break.

Fruit at green, breaker, and pink stages were subsequently treated with 250 ppb 1-MCP for 12 h at 20°C in 174-L containers as described in detail under 'Exposure of Tomato Fruit to 1-MCP'. Following treatment, fruit were transferred to 20°C storage facilities for up to 14 d and evaluated daily for color using a Minolta Chroma Meter CR-400 (Minolta Camera Co Ltd, Japan) as described in detail under 'Fruit Color Measurements'. Fruit were also measured every other day for firmness using an Instron Universal Testing Instrument) model 4411; Canton, Mass) as described in detail under the section 'Analysis of whole fruit firmness'. Volatile analysis was performed on the breaker-stage treated fruit, once they had reached full ripe, using the method described in the section 'Analysis of tomato volatiles'.

Exposure of Tomato Fruit to 1-MCP

For all 1-MCP experiments in this study, 1-MCP gas was generated by weighing the appropriate quantity of *SmartFresh*® powder (1-Methylcyclopropene, 0.14% A.I.) (AgroFresh, a division of Rohm & Haas) to produce the desired atmosphere inside 174-L and 20-L treatment chambers. The chamber selected was based on the number of tomatoes being treated. The powder (26 mg for the 20-L chambers and 112 mg for the 174-L chambers) necessary to produce an atmosphere of 250 ppb (for the 174-L chambers) or 500 ppb (for the 20-L chambers) 1-MCP in the treatment chambers was added to a 125-mL Erlenmeyer flask containing 40 mL of tap water. After swirling to dissolve the 1-MCP powder, the flask was placed in the treatment chambers that were

immediately sealed. The chambers were sealed by clamping a clear Plexiglas cover and foam gasket to the open end of each treatment chamber (174-L chambers) or by inserting a threaded Gamma-Seal (B&A Products Ltd. Co., Oklahoma) (20-L containers). Control fruit were placed in a treatment chamber with a flask containing 40 mL of water alone. Treatment was performed at 20°C for twelve hours, after which time the containers were opened and the fruit transferred to 20°C normoxic storage facilities. At intervals during storage at 20°C, the fruit were removed and evaluated for color, firmness, and volatile production.

Analysis of the Effects of 1-MCP on Tomato Ripening and Volatile Profiles

Tomato fruit (cv. 'FL-91') were obtained in October 2005 from a packinghouse at the green stage and transported to Gainesville the same day. Fruit were dipped in 1.34 millimolar Na-hypochlorite, rinsed, and dried at room temperature. Fruit were held at 20°C until color break and then selected for color uniformity at breaker stage.

Two experiments were performed. For each experiment, fifteen identical breaker-stage fruit were selected and these fifteen fruit were subdivided into three groups of five fruit each. One group was treated with 500 ppb 1-MCP for 12 h at 20°C in 20-L treatment chambers as described earlier in the section 'Exposure of Tomato Fruit to 1-MCP'. The other two groups were designated as untreated controls. The extra control group was needed as described below.

Following treatment with 1-MCP, the fruit were transferred to 20°C storage facilities for up to 14 d and evaluated daily for color using a Minolta Chroma Meter CR-400 (Minolta Camera Co Ltd, Japan) as described in detail under 'Fruit Color Measurements'. Fruit were also measured every other day for firmness using an Instron

Universal Testing Instrument) model 4411; Canton, Mass) as described in detail under 'Analysis of Whole Fruit Firmness' above.

The untreated tomatoes reached a full red condition, as designated by the hue angle of each fruit averaging below 50°, after 7 to 10 d. Within 1 day of this, one of the two groups of untreated fruit was sampled to determine the aroma volatile constituency as described below. At this point, the 1-MCP treated fruit were still at a pink stage.

1-MCP treated fruit were held until they reached full red (average hue angle < 50°), approximately 4 to 11 d later than the control fruit, and then sampled to determine their volatile profile as described below. The other untreated control group, which had been full red (average hue angle < 50°) for 4 to 11 d, was analyzed at the same time. Thus for this experiment, the three treatments consisted of: 1. Fruit treated with 1-MCP at the breaker stage and ripened at 20°C, 2. Control fruit ripened at 20°C, and 3. Control fruit held at 20°C until the 1-MCP-treated fruit reached a full-ripe condition.

The experiment was repeated using fruit from another harvest from the same source three weeks later.

Analysis of Tomato Volatiles

In both the ripening evaluation and 1-MCP experiment described in these studies, fruit with an average hue angle of below 50° were used to determine the aroma volatile profile. To compose a single 100 g sample, a longitudinal wedge of approximately 60 degrees was removed from each of the five fruit and the tissue diced on a plastic tray using a sharp knife into cubes of approximately 1 cm^3. All tissues, including the pericarp, locular gel, juice, and seeds were retained in order to obtain a representative whole-fruit volatile profile. Each sample was held between 5 min to 1 h in a hexagonal

weigh-boat (Fisher, Canada) and then loaded into glass tubes measuring 2.54 cm x 61 cm (Volume: 309.1 mL). Both ends of the tubes were sealed with a rubber septum.

Volatiles were collected on Super-Q resin columns for 1 h using the method described by Schmelz, Alborn, and Tumlinson (2001). Filtered air was passed through the glass tubes and across the tomato samples at a flow rate of 618 mL/min. After 1 hour, a 5 µl nonyl acetate internal standard was injected into each resin column using a glass syringe. The contents of the columns were eluted with 150 µl methylene chloride, and stored in 300 µL glass inserts inside 1.8 mL glass jars at -80°C to preserve volatile integrity.

The volatiles within the glass jars were analyzed using an Agilent Technologies 6890N gas chromatograph with a DB-5 column (Agilent Technologies) and a flame ionization detector, using helium as the carrier gas. The injector temperature was 220°C, the detector temperature was 280°C, and the oven temperature was between 40 and 250°C. The carrier gas flow rate was 1.2 mL/min. The software package ChemStation (Agilent Technologies) was used to analyze the peaks produced by the volatiles, where retention times were compared with known standards and used to identify peaks. The peak areas were then exported into Microsoft Excel.

Statistical Analysis

Volatile production data was analyzed using the least squares method with a confidence interval of 95% in JMPIN, the student version of the JMP program made by SAS. Firmness and color data was analyzed using standard errors calculated by Microsoft Excel.

CHAPTER 4
RESULTS AND DISCUSSION

Evaluation of Volatile Profile of Ripe to Overripe Fruit

The changes in volatile emissions as tomato fruit (‘M82’) progress from immature green, through mature green, breaker, turning, and pink, to light red stage, have been observed and recorded in Tieman et al. (2006). The experiments in the present study were designed to determine the changes in tomato volatile emissions in the successive stages, starting at the day on which fruit reach a full ripe stage (as defined by an average hue angle below 50°) and continuing until 16 d following ripening.

Tomato fruit ('BHN586') were obtained at turning stage and ripened at 20°C. Color was monitored daily until individual fruit attained an individual average hue angle below 50°. Figure A-1 shows the hue angles of the fruit at the start of the experiment and each consecutive day during storage. The hue angle quickly decreased as the tomato ripened in 20°C storage.

The tomatoes upon reaching hue angle values below 50° (Figure A-1, day 4) were held for up to 17 days at 20°C (Figure A-2), during which time fruit were periodically measured for volatile emissions. According to these results, a tomato, once reaching red, does not experience further significant color change even as many as seventeen days after ripening.

Figure A-3 shows the average firmness of the tomatoes sampled. A ripe tomato is usually around 5 N, and for the first three days after ripening (using hue angle < 50° as an indicator of ripening) the tomatoes maintained a firmness comparable to the normal

firmness of full ripe tomatoes. However, firmness continued to decrease as the tomatoes progressed from ripe to overripe, declining to between 3 and 4 N by 11 d following ripening.

Changes in volatile emissions of the 'BHN586' tomato fruit during ripening and over-ripening are shown in Figures A-4 to A-8. Volatiles are discussed in order of their importance to tomato aroma, as noted by other researchers (Buttery and Ling, 1992; Buttery, 1993; Krumbein and Auerswald, 1998).

The "C6" compounds hexanal and *cis*-3-hexenal greatly impact the aroma of tomato leaves and fruit, producing a "green, grassy" scent (Buttery and Ling, 1992) or a sweet or "fresh green" flavor (Krumbein and Auerswald, 1998). The flavor of hexanal alone has also been referred to as "sweet", "ripe", and "tomato flavor" (Maul et al., 2000), identifying it as a key component of tomato aroma and taste. *Cis*-3-hexenal and hexanal are derived from the fatty acids linoleic and linolenic acids and increase in concentration as the tomato ripens (Buttery and Ling, 1992). Tieman et al. (2006) observed low levels of *cis*-3-hexenal in 'M82' tomato fruit up until the turning stage, at which point a dramatic increase in production occurred through the light red stage. According to Buttery and Ling (1992), *cis*-3-hexenal emission in tomato fruit increases slightly at color break, but experiences a fifty-fold increase at the full-ripe stage. In the present study, 'BNH586' tomato fruit experienced a decrease in *cis*-3-hexenal production as fruit progressed from full ripe to 16 d overripe (Figure A-4). Since *cis*-3-hexenal is a key component of ripe tomato aroma, it is not surprising that its production rises to the highest point 1 or 2 d after attaining a hue angle below 50° and then drops again. Interestingly, Mir et al. (2004) found that *cis*-3-hexenal production decreased in '308'

and ‘311’ tomatoes as they progressed from mature green to turning and then to full red, but this contrasting data may be explained by the difference in cultivar. Hexanal production in ‘BNH586’ tomatoes fruit fell between 1 and 2 d after attaining full red, but thereafter increased and remained high during the 16 d of observation, as shown in Figure A-5.

Ethyl vinyl ketone, or 1-penten-3-one, is another ‘C6’ volatile derived from fatty acids and found in both vegetative and reproductive tissues of the tomato plant. As with the other ‘C6’ volatiles, it imparts a ‘green’ aroma (Krumbein and Auerswald, 1998). The ‘308’ and ‘311’ tomatoes investigated in Mir et al. (2004) were found to produce less ethyl vinyl ketone at the turning stage than when mature green, after which production remained constant through the full ripe stage. As shown in Figure A-6, production of ethyl vinyl ketone in ‘BNH586’ tomato fruit remained constant within the first three days after attaining a full ripe stage, after which it rose over the course of the next week. Ethyl vinyl ketone emissions peaked on day 11 and remained statistically constant for the next 5 d. This pattern is consistent with the trend noted by Mir et al. (2004).

ß-ionone is a cyclic compound synthesized from the metabolism of carotenoids (Buttery and Ling, 1992). Carotenoid-derived volatiles are formed in the fruit but not in the vegetative tissues of tomato plants,. ß-ionone has been characterized as imparting a sour taste to taste panelists (Tandon et al., 2003). Production of ß–ionone increased dramatically as the 'BHN586' tomato fruit progressed from ripe to overripe (Figure A-7). ß–ionone-production was relatively constant within the first three days after fully ripening, and increased sharply at 11 d. At 11 and 16 d after ripening, ß-ionone production had increased approximately six-fold compared with values observed at the

full-ripe stage. Tieman et al. (2006) reported a dramatic increase in ß-ionone production between the turning and light red stages, at which time their observations ceased. The data in Figure A-7 illustrate that this volatile continues to increase through and after full ripening. There was no significant difference in ß-ionone production between the two identical groups sampled on any of the days measured.

Other tomato flavor volatiles are synthesized from amino acids. Isovaleraldehyde, also called 3-methylbutanal, is formed from the amino acid leucine. It has been associated with an unpleasant flavor (Krumbein and Auerswald, 1998). Tieman et al. (2006) observed a steady increase in isovaleraldehyde production in 'M82' tomato fruit in the time from immature green to turning stage, after which point emissions of isovaleraldehyde decreased again as the tomato progressed to light red. The 'BNH586' tomatoes used in the present experiment experienced steady isovaleraldehyde production for the first three days after attaining full ripe stage, after which production increased slightly. By day 11, however, it had fallen to well below the full-ripe rate of production, and emissions of isovaleraldehyde remained low over the next 5 d (Figure A-8).

Determination of Fruit Developmental Stage for 1-MCP Treatment

The purpose of these experiments was to determine the optimal stage for treating the tomato fruit with 1-MCP in order to be certain the 1-MCP would sufficiently delay ripening. Tomato fruit (*Lycopersicon esculentum* 'FL-47') at green, breaker, turning, and pink stages of development were treated with 1-MCP (250 ppb, 12 h, 20°C) or air and ripened at 20°C. Fruit surface color was recorded daily and firmness every other day.

Fruit at the green stage were found to be unsuitable for 1-MCP experiments, due to the unpredictable numbers of immature-green fruit in the samples. As noted by Hurr et al., (2005), 'green' tomato fruit treated with 1-MCP eventually diverge into 2 distinct

populations in terms of the degree of suppression of softening and surface red color development. Softening and color development of immature green fruit (an assignment made retrospectively based on the number of days to color break of the untreated controls) were acutely and irreversibly inhibited by the ethylene antagonist. Mature-green fruit, on the other hand, eventually softened and colored to a degree exhibited by control fruit, though both ripening parameters were significantly delayed. Aside from the difficulties in properly classifying green fruit, there is ample evidence that even mature-green fruit, once detached, never develop the characteristic volatiles associated with high quality. Definitive classification of green fruit as immature green or mature green requires either nuclear magnetic resonance technology (NMR) (Abbott, 1999) or X-ray technology (Brecht, Shewfelt, Garner, and Tollner, 1991) which are expensive and time-consuming, or cutting the fruit open. In view of the inability of distinguishing mature versus immature-green fruit, subsequent experiments were performed using fruit at the breaker stage and beyond.

Treating tomato fruit with 1-MCP at the pink or turning stage was also problematic because in the first five days following treatment, no difference in color or firmness was observed between the ripening of control fruit and 1-MCP treated fruit. This parallel ripening pattern is shown in Figures B-1 through B-4. Color of fruit treated at the pink stage did not appear to have been affected by the 1-MCP treatment, and the difference in average hue angle between 1-MCP treated and control fruit was less than one degree throughout the six days following ripening, indicating statistically similar values. No difference was observed between the firmness of treated and untreated pink fruit over the course of the observation. Fruit treated with 1-MCP at either the pink or turning stages

softened just as rapidly as air-treated control fruit. It is possible that differences may have emerged after the six days observed, but since at the time the experiment was terminated all fruit had already softened and reddened sufficiently to indicate full ripe within a similar period of time, there was nothing to indicate that 1-MCP had any effect on their development during ripening.

These results are in contrast with those of Hurr et al. (2005), who found that pink and turning 'FL-47' tomato fruit did respond to 1-MCP treatment (1 ppm, 24 h, 20°C), as did all other stages of tomato fruit development through full-ripe, with delayed softening and color development. This may be due to the fact that the concentration used was four times what was used in these experiments (1 ppm vs 250 ppb) and the duration of treatment was twice as long (24 h vs 12 h), and a treatment of that magnitude may be required to affect the development of pink or turning fruit. However, an initial concentration of only 150 ppb successfully produced a delay in ripening parameters such as softening and development of red color in 'Prisca' tomato fruit after 20 h of treatment (Hoeberichts, Van der Plas, and Woltering, 2002). It is possible that the difference in cultivar may be responsible for the difference in 1-MCP effectiveness. Blankenship and Dole (2003) stated that there is evidence of cultivar differences in response to 1-MCP for apple and banana fruits. This might be due to cultivar differences in the magnitude of ethylene production or in inherent sensitivity to ethylene. Another possible explanation for the deviation of the present results from previous work is that the fruit used in this experiment were stressed due to being transported in the trunk of a car for 1 h just after harvest, where high temperatures may have had a negative effect. This is unlikely,

however, since fruit color development, which is highly sensitive to high-temperature injury, was not impaired.

Due to the problems with green, pink, and turning fruit enumerated above, breaker fruit were employed for all further experiments. Fruit ('FL-47') treated with 1-MCP (250 ppb, 12 h, 20°C) at the breaker stage were monitored for changes in color and firmness until they attained a full ripe stage, the stage at which 90% or more of the fruit is covered by red pigmentation and the hue angle is below 50°. In this experiment, the length of time required for fruit to reach full red was significantly affected by 1-MCP. As shown in Figure B-5, control fruit reached an average hue angle below 45° eight days earlier than 1-MCP-treated fruit. Additionally, development of red pigmentation occurred with more uniformity over the surface in control fruit, although uniform red pigmentation did eventually develop in all fruit. Fruit treated at breaker stage with 1-MCP also exhibited a delay in loss of firmness compared with control fruit.

1-MCP-treated fruit experienced a delay in development of the ripening parameters of color and firmness compared with untreated fruit. It was of interest to discern whether this delay would affect volatile emissions. With that in mind, volatile analysis was performed as described earlier on ripe fruit derived from air- or 1-MCP-treated breaker fruit, and on breaker fruit taken before 1-MCP treatment.

Fatty-Acid Derived Volatiles

The amounts of *cis*-3-hexenal and *cis*-3-hexenol produced in ripe control fruit were higher than the amounts produced in breaker fruit (Figures B-6 and B-9). This increase in *cis*-3-hexenal during ripening in tomato fruit corroborates the data from Tieman et al. (2006), which indicated an increase in *cis*-3-hexenal between the turning and light red stages, but not Mir et al. (2004), who reported a decrease in *cis*-3-hexenal between

mature green, turning, and ripe fruit. The amount of both *cis*-3-hexenal and *cis*-3-hexenol produced in ripe fruit derived from 1-MCP-treated breaker fruit was higher than the amounts in ripe control fruit (Figs B-6 and B-9). In the case of *cis*-3-hexenal, this is consistent with the increase observed by Mir et al. (2004) in '308' and '311' tomatoes treated with 2 ppm 1-MCP for 15 h at 21°C. However, Mir et al. (2004) did not observe any change in the amount of *cis*-3-hexenol throughout their experiment. This difference might be attributed to cultivar differences, or difference in 1-MCP treatment or volatile sampling procedure. Mir measured volatiles in fruit homogenates whereas in the present study the volatiles were measured from a heterogenous mixture of skin, pericarp, locule gel, juice, and seeds diced into cubes of approximately 1 cm^3.

Hexanal and *trans*-2-hexenal were found in statistically similar amounts in breaker and ripe control fruit, and higher levels in ripe fruit derived from 1-MCP-treated breaker fruit (Figures B-7 and B-10). This contradicts the decrease observed in Mir et al. (2004) between ripe control and ripe fruit derived from 1-MCP-treated mature green fruit, and also the increase observed in that study in hexanal production between the mature green and turning stages. However, once the tomatoes in Mir et al. (2004) had reached turning stage, their hexanal emissions remained constant, and it is possible that the conclusion can be drawn from these combined results that the increase in hexanal associated with tomato ripening is initiated at the breaker stage, and not turning stage. Mir et al. (2004) did not measure tomatoes at breaker stage, and the tomatoes observed in that study retained constant hexanal emissions from the turning through full-ripe stage. An increase in the amount of *trans*-2-hexenal produced in ripe control fruit compared with breaker fruit does appear at the 90% confidence level, although not at a 95% confidence level.

Tieman et al. (2006) had found an increase in the amount of *trans*-2-hexenal produced as 'M82' tomato fruit ripen, but this increase was not observed in 'FL-47'. The increase in *trans*-2-hexenal may have occurred between the turning and light red stages, but remained undetected because the only stages at which volatiles were sampled were breaker and full red. A possible explanation is that the increase may have occurred transiently, only between the turning and light red stages, and this trend is corroborated by the data from Tieman et al. (2006). Additionally, the *trans*-2-hexenal production data presented in Figure B-10 is not consistent with the observations made by Mir et al. (2004), in which *trans*-2-hexenal was reported to have been produced at higher levels in untreated fruit, although in both experiments there was an increase in the amount produced in ripe fruit compared with fruit in the early stages of development.

Mir et al. (2004) had observed a decrease in ethyl vinyl ketone production from mature green to turning, after which production levels remained constant. Treatment with 1-MCP (2 ppm, 15 h, 21°C) reduced the levels of ethyl vinyl ketone in '308' and '311' tomatoes to the level they had exhibited when mature green. However, in this experiment, there was no difference in the amount of ethyl vinyl ketone produced by 'FL-47' tomatoes between any of the treatments/stages (Figure B-8).

Carotenoid Derived Volatiles

The amount of ß-ionone produced was statistically similar for all treatments (Figure B-11). The amount of 6-methyl-5-hepten-2-one produced was much higher in both ripe control and ripe fruit derived from 1-MCP-treated breaker fruit than in breaker fruit (Figure B-12). An increase in both of these volatiles was observed in Tieman et al. (2006) between the turning and light red stages and in Mir et al. (2004) between mature green, turning, and full ripe. In the case of 6-methyl-5-hepten-2-one, at least, this increase upon

ripening seems not to have been compromised by exposure to 1-MCP at the breaker stage. In contrast to the results reported here, Mir et al. (2004) reported decreased levels of both volatiles in response to 1-MCP. In this case it may be more practical to attribute the differences in observations to the difference between the 1-MCP concentrations in each experiment [2 ppm in Mir et al (2004) vs 250 ppb in the present study] rather than cultivar differences because the untreated 'FL-47' fruit in Figure R behaved consistently with the 'M82' fruit in Tieman et al. (2006) and the '308' and '311' fruit in Mir et al. (2004).

Amino Acid Derived Volatiles

The amounts of isovaleraldehyde and 3-methyl-1-butanol produced in ripe control fruit were higher than the amounts in both breaker fruit and ripe fruit derived from 1-MCP-treated breaker fruit, which were statistically similar at the 95% confidence level (Figures B-13 and B-15) but different at the 90% confidence level, where ripe fruit derived from 1-MCP-treated breaker fruit were found to produce higher emissions of 3-methyl-1-butanol than breaker fruit. Tieman et al. (2006) observed maximum emissions of 3-methyl-1-butanol at the turning stage, after which it decreased, which may seem to contradict these results. However, comparing only the breaker and light red stage data from Tieman et al. (2006), there was a small overall increase in 3-methyl-1-butanol. Whether or not the 1-MCP-treated fruit observed here experienced this transient increase is unknown because turning fruit were not measured for volatile content. In contrast with the results presented in Figure P, untreated '308' and '311' fruit observed in Mir et al. (2004) maintained a constant level of 3-methyl-1-butanol production throughout the mature green, turning, and full ripe stages, while 1-MCP treated fruit produced higher levels of 3-methyl-1-butanol.

2-methylbutyraldehyde was produced in higher amounts in ripe control fruit than in breaker fruit. Neither was statistically different from the amount produced in ripe fruit derived from 1-MCP-treated breaker fruit at the 95% confidence level (Figure B-14) but at the 90% confidence level ripe control fruit was observed to produce higher levels of 2-methylbutyraldehyde than ripe fruit derived from 1-MCP-treated breaker fruit.

1-nitro-2-phenylethane was produced in higher amounts in ripe fruit derived from 1-MCP-treated breaker fruit than in breaker or ripe control fruit (Figure B-17). There was no statistical difference observed in the amounts of 2-phenylethanol produced between any of the treatments/stages (Figure B-16). Since 1-nitro-2-phenylethane and 2-phenylethanol are both derived from phenylalanine, it is reasonable to assume that they would respond similarly to 1-MCP if their production trends were dependent on ethylene. This expectation was not fulfilled by the results in this study. However, as noted in Figures V and W, the volatiles exhibited different patterns of response to 1-MCP. Buttery and Ling (1992) stated that amino acid-derived volatiles such as 1-nitro-2-phenylethane and 2-phenylethanol are formed between the breaker and full ripe stages, indicating that an increase should have been detected at least between breaker and ripe control fruit. That increase was not observed in this study for either volatile.

Volatiles of Other Derivation

The derivation of 2-isobutylthiazole is unknown (Goff and Klee, 2006), although Schutte (1974) has suggested that 2-isobutylthiazole may originate from leucine using isovaleraldehyde as an intermediate. The flavor attributed to 2-isobutylthiazole is sweet and fruity and it was observed to cause a ‘bite’ feeling in the mouth (Krumbein and Auerswald, 1998). Both ripe control and ripe fruit derived from 1-MCP-treated breaker fruit produced higher levels of 2-isobutylthiazole than those produced by breaker fruits,

and there was no difference observed in the amounts produced between ripe control and ripe fruit derived from 1-MCP-treated breaker fruit (Figure B-18). In Tieman et al. (2006), the maximum emission of 2-isobutylthiazole was during the immature green stage, thereafter declining as tomato development proceeded. However, Tieman et al. (2006) only measured fruit volatiles up until light red stage, so it is possible that the increase observed in the present study, which appears not to have been impacted by exposure to 1-MCP, occurred entirely after the fruit attained a full red color. The increase observed by Tieman et al. (2006) is contradicted by the data in Mir et al. (2004), which shows an increase in 2-isobutylthiazole production between mature green and turning, after which the amount produced remains constant in untreated '308' and '311' tomato fruit. Mir et al. (2004) also shows a decrease in the amount of 2-isobutylthiazole in 1-MCP fruit compared with ripe control fruit, but this was not observed in the present study.

Response of Tomato Fruit Color and Firmness to 1-MCP

Tomato fruit (*Lycopersicon esculentum* 'FL-91') at the breaker stage were treated with 1-MCP (12 h, 20°C, 500 ppb) or air and ripened at 20°C. Firmness decreased more rapidly in air-treated fruit, as shown in Figures C-1 through C-4. This is consistent with previous studies involving the effect of 1-MCP in delaying 'FL-47' tomato softening, such as Hurr et al. (2005), in which a week to 10 d was required for 1-MCP treated fruit to soften to the same firmness as control fruit. Hoeberichts et al. (2002) also demonstrated that 1-MCP treatment causes tomato fruit ('Prisca') to attain a softness associated with ripening after a delay, compared with controls.

Development of red coloration occurred more slowly in 1-MCP -treated fruit than in control fruit, as shown in Figure C-5, and with less uniformity. Since color was

measured at three equatorial positions, it is not evident that for some 1-MCP treated fruit, patches of green or yellow remained at the stem end of the fruit for several days. These areas did eventually develop red color, resulting in a uniform full red fruit.

Tomato Volatile Production in Ripe, Overripe, and 1-MCP-treated 'FL-91' Tomato Fruit

These experiments were designed to determine whether or not emission of the thirteen volatiles considered most important to tomato aroma would be affected by ethylene suppression. In addition, these experiments sought to compare the volatile emissions of 1-MCP treated fruit not only with control fruit of a similar ripening stage, but with control fruit of similar age, and which were therefore overripe. The four trials performed within this experiment will be discussed together due to the fact that they involved tomatoes of the same cultivar ('FL-91'), obtained at the same stage (breaker) from the same packing house, treated at the same stage (breaker) with the same concentration of 1-MCP (500 ppb) for the same time period (12 h). Experiments 3 and 4 were performed on fruit obtained three weeks later than Experiments 1 and 2. Where no interaction is described, no significant differences were found between treatments at the 95% confidence level using the least squares method.

Fruit designated as 'ripe control' were sampled upon reaching a hue angle of 50° after treatment in air. Fruit designated as '1-MCP' were treated at breaker stage with 500 ppb 1-Methylcyclopropene (1-MCP), 12 h, 20°C) and then sampled upon reaching a hue angle of 50°. Since 1-MCP delays the change in pigmentation associated with tomato ripening by suppressing the ethylene response, this change in pigmentation occurred 4 to 11 d later than the 'ripe control' fruit. Fruit designated as 'overripe' were treated in air, but

sampled concurrently with the 1-MCP-treated fruit, between 4 to 11 days after the ‘ripe' reached a hue angle of 50°. The rationale behind the sampling of overripe air-treated fruit as well as ripe air-treated fruit was to rule out the possibility that differences in the tomato volatile profile between air-treated and 1-MCP-treated ripe fruit were due to fruit age rather than a response to delayed ripening. The 1-MCP-treated fruit were 4 to 11 d older than the air-treated fruit when they reached the 50° hue angle, and sampling the overripe fruit was necessary to distinguish between volatile differences due to fruit age and not to any direct effects of 1-MCP on ethylene-mediated processes.

Fatty-Acid Derived Volatiles

Cis-3-hexenal was found in higher amounts in ripe control fruit than in overripe fruit in Experiments 2 and 3, and higher amounts in ripe control fruit than in ripe fruit derived from 1-MCP-treated breaker fruit in experiment 2, which contradicts the results observed earlier in this study (Figures C-7 and C-8) and also the observations of Mir et al. (2004). There were no differences in cis-3-hexenal production between treatments in Experiments 1 or 4 (Figures C-6 and C-9).

Hexanal was found in higher amounts in overripe fruit than in ripe control fruit in Experiment 1, but no significant differences were found between ripe fruit derived from 1-MCP-treated breaker fruit and either ripe control or overripe fruit in this experiment (Figure C-10). There were no differences between treatments in Experiments 2 and 3 (Figures C-11 and C-12). In Experiment 4, higher amounts of hexanal were found in ripe fruit derived from 1-MCP-treated breaker fruit than in ripe control fruit, supporting the results observed earlier in this study (Figure C-13). There were no differences between ripe fruit derived from 1-MCP-treated breaker fruit and overripe fruit or between overripe fruit and ripe control fruit at the 95% confidence level, although at the 90% confidence

level, ripe fruit derived from 1-MCP-treated breaker fruit produced more hexanal than ripe control fruit in experiment 1, and overripe fruit produced more hexanal than ripe control fruit in experiment 4. These indications that 1-MCP treatment may cause some 'FL-91' tomato fruit to produce higher levels of hexanal contradicts data presented in Mir et al. (2004), in which '308' and '311' tomato fruit treated with1-MCP showed a 50% reduction in hexanal production, resulting in emission rates comparable to those of mature green fruit.

Ethyl vinyl ketone production was statistically similar for all treatments in Experiments 1, 2, and 4 (Figures C-14, C-15, and C-17). In Experiment 3, greater amounts of ethyl vinyl ketone were found in overripe fruit than in ripe fruit derived from 1-MCP-treated breaker fruit (Figure C-16). No differences were found between ripe control fruit and either overripe fruit or ripe fruit derived from 1-MCP-treated breaker fruit at the 95% confidence level, but overripe fruit produced more ethyl vinyl ketone than ripe control fruit at the 90% confidence level. This lack of statistical difference mirrors the results for the ethyl vinyl ketone measured earlier in this study.

There were no statistical differences in the production of *trans*-2-hexenal between any of the treatments at the 95% confidence level in all four experiments (Figures C-18 through C-21). However, at the 90% confidence level, overripe fruit produced more *trans*-2-hexenal than ripe control fruit in Experiments 3 and 4.

Cis-3-hexenol production was statistically similar for all treatments at the 95% confidence level in Experiments 1, 2, and 4 (Figures C-22, C-23, and C-25). However, at the 90% confidence level, overripe fruit produced higher levels of *cis*-3-hexenol than ripe control fruit in Experiments 1 and 4. Overripe fruit in experiment 3 produced higher

levels of cis-3-hexenol than ripe fruit or ripe fruit derived from 1-MCP-treated breaker fruit (Figure C-24). No differences were found between cis-3-hexenol production in ripe control fruit and ripe fruit derived from 1-MCP-treated breaker fruit. This similarity between treatments is fairly consistent with the observations of Mir et al. (2004), in which no differences were found in the amounts of *cis*-3-hexenol produced by mature green, turning, ripe control, and ripe fruit derived from 1-MCP-treated mature-green fruit.

Carotenoid Derived Volatiles

ß-ionone was found in higher amounts in both overripe and ripe fruit derived from 1-MCP-treated breaker fruit than in ripe control fruit in Experiment 3 (Figure C-28). There were no differences in ß-ionone production between treatments in Experiments 1, 2, or 4 (Figures C-26, C-27, and C-29), corroborating results presented earlier in this study. Both findings contradict the observations of Mir et al. (2004), in which ripe fruit derived from 1-MCP-treated mature green fruit produced much lower levels of ß-ionone than ripe control fruit.

6-methyl-5-hepten-2-one production was statistically similar for all treatments in Experiments 1 and 2 at the 95% confidence level (Figures C-30 and C-31), although at the 90% confidence level, ripe fruit derived from 1-MCP-treated breaker fruit produced more 6-methyl-5-hepten-2-one than ripe control fruit in Experiment 1. Higher levels of 6-methyl-5-hepten-2-one were identified in overripe fruit than in both control ripe and ripe fruit derived from 1-MCP-treated breaker fruit in Experiment 3 (Figure C-32). In Experiment 4. higher levels of 6-methyl-5-hepten-2-one were found in overripe fruit than in ripe fruit derived from 1-MCP-treated breaker fruit, and at the 95% confidence level, production of 6-methyl-5-hepten-2-one in ripe control fruit was statistically similar to both overripe fruit and ripe fruit derived from 1-MCP-treated breaker fruit. (Figure C-33).

At the 90% confidence level, however, overripe fruit produced more 6-methyl-5-hepten-2-one than ripe control fruit in Experiment 4. The statistical similarity between ripe fruit derived from 1-MCP-treated breaker fruit and ripe control fruit echoes the similarity observed earlier in this study and contradicts the findings of Mir et al. (2004), in which 1-MCP was observed to produce a decrease in 6-methyl-5-hepten-2-one emissions in '308' and '311' tomato fruit.

Amino Acid Derived Volatiles

Higher levels of isovaleraldehyde were observed for ripe control fruit than in both overripe fruit and from ripe fruit derived from 1-MCP-treated breaker fruit in Experiment 2 (Figure C-35). In Experiment 3, isovaleraldehyde was found in higher amounts in ripe fruit derived from 1-MCP-treated breaker fruit than in both overripe and ripe control fruit (Figure C-36). In Experiment 4, isovaleraldehyde was found in higher amounts in the overripe fruit compared with ripe fruit derived from 1-MCP-treated breaker fruit, both of which were statistically similar to ripe control fruit (Figure C-37). There were no differences in isovaleraldehyde production between treatments in Experiment 1. (Figure C-34)

3-methyl-1-butanol was found in higher amounts in ripe control fruit than in overripe fruit, both of which were statistically similar to the amount in ripe fruit derived from 1-MCP-treated breaker fruit at the 95% confidence level, in Experiment 1 (Figure C-38). At the 90% confidence level, however, ripe control fruit produced higher levels of 3-methyl-1-butanol than ripe fruit derived from 1-MCP-treated breaker fruit in Experiment 1. In Experiment 2, higher amounts of 3-methyl-1-butanol were found in ripe control fruit than in both overripe fruit and in ripe fruit derived from 1-MCP-treated breaker fruit (Figure C-39). Higher levels of 3-methyl-1-butanol were identified in ripe

fruit derived from 1-MCP-treated breaker fruit than in both overripe and ripe control fruit in experiment 3 (Figure C-40), corroborating results from Mir et al. (2004). There were no differences in 3-methyl-1-butanol production between treatments in Experiment 4 (Figure C-41).

1-nitro-2-phenylethane was found in higher amounts in both overripe fruit and in ripe fruit derived from 1-MCP-treated breaker fruit than in ripe control fruit in Experiments 1 and 3, corroborating observations described earlier (Figures C-42 and C-44). In Experiment 4, higher levels of 1-nitro-2-phenylethane were identified in overripe fruit than in both ripe fruit derived from 1-MCP-treated breaker fruit and in ripe control fruit (Figure C-45). There were no differences in production of 1-nitro-2-phenylethane between treatments in Experiment 2 (Figure C-43).

Higher levels of both 2-phenylethanol and 2-methylbutyraldehyde were identified in ripe control fruit than in both overripe fruit and in ripe fruit derived from 1-MCP-treated breaker fruit in Experiment 2 (Figures C-47 and C-51). No differences were observed in the production of either 2-phenylethanol or 2-methylbutyraldehyde in overripe fruit and in ripe fruit derived from 1-MCP-treated breaker fruit in experiment 2 at the 95% confidence level, although at the 90% confidence level, higher levels of 2-methylbutyraldehyde were observed in ripe fruit derived from 1-MCP-treated breaker fruit than in overripe fruit. There were no differences in 2-phenylethanol or 2-methylbutyraldehyde production between treatments in Experiments 1, 3, or 4 (Figures C-46, C-48, C-49, C-50, C-52, and C-53).

It is interesting to note that as with the earlier experiment in this study, the reaction of 1-nitro-2-phenylethane and 2-phenylethanol, both derived from phenylalanine, to ethylene suppression was not similar.

Volatiles of Other Derivation

2-isobutylthiazole was found in higher amounts in ripe fruit derived from 1-MCP-treated breaker fruit than in both overripe and ripe control fruit in Experiment 3 (Figure C-56), consistent with the report of Mir et al. (2004). There were no differences in 2-isobutylthiazole production between treatments in Experiments 1, 2, or 4 (Figures C-54, C-55, and C-57). There were no differences observed between ripe control and ripe fruit derived from 1-MCP-treated breaker fruit in the study discussed earlier, and the decrease observed in Mir et al. (2004) was minor, indicating that ethylene action may not be an important factor in the formation of 2-isobutylthiazole.

General Discussion

It is evident from the results of these studies that factors other than ethylene recognition affect volatile emissions. Numerous preharvest factors can influence volatile production in tomato fruit, such as fertilizer regime (Wright and Harris, 1985), choice of cultivar (Tandon et al., 2003), and climatic conditions during the growing season (cite). Harvesting conditions, such as fruit maturity at harvest (Maul et al., 2000) and storage temperature (Boukobza and Taylor, 2002; Maul et al., 2000), can also play a role in altering the volatile profile of tomato fruit. Bruising is also a factor (Moretti et al., 2001), which means that postharvest handling practices are likely influential. These natural variations were minimized by obtaining the fruit for the experiments in this study from the same packing house during the same season, and from the same cultivar. However, in the 'FL-91' experiment, the tomato fruit for experiments 3 and 4 were

obtained three weeks later than the fruit for experiments 1 and 2. A significant increase was found in the total volatile emissions (pooling data from all three treatments) from Experiments 1 and 2 compared with Experiments 3 and 4. Upon examination of the weather in October 2005 from Quincy, FL, the source of the tomatoes for this experiment, a possible explanation arises from the temperature data (Florida Automated Weather Network, http://fawn.ifas.ufl.edu). In the first three weeks of October, during which the fruit from the first harvest were obtained, the temperature averaged around 20°C. During the last week of October, approximately half a week before the start of experiments 3 and 4, temperatures dropped to between 1° and 4°C and remained there for 9-12 h per night for three consecutive days. Johnson, Gould, Badenhop, and Johnson (1968) observed tomatoes of the same varieties differing in the amount of *cis*-3-hexenol emitted in their juice depending in which week the tomatoes were harvested, showing that there is precedent in the literature for such variation.

Another source of volatile variation lies in the method of sample processing. In order to obtain a representative whole-fruit volatile profile, all tissues of the tomato fruit—including the pericarp, locular gel, and seeds—were retained after dicing and their volatile emissions collected and analyzed. Since each sample represents tissue taken from five fruit, precise amounts of locule gel, or any other fruit tissue, would not be consistent from sample to sample. In addition, sometimes as many as fourteen samples were prepared on one day before being loaded into the glass tubes and sealed with septa. The last sample diced was on the lab bench in an open weigh-boat for only five minutes, whereas the first sample diced might have remained in the open air for an hour, during

which time some volatiles may have escaped, or experienced changes in synthetic/degradation pathways.

There is evidence in the literature of volatile emissions being affected by the amount or method of tissue processing involved in the volatile sampling technique. Carbonell-Barrachina, Agustí, and Ruiz (2006) compared hexanal production in blended tomatoes with hexanal production observed in tomato paste by Buttery et al. (1990) and hexanal production observed by Maul et al. (1998) using a gas chromatograph headspace analysis technique. Carbonell-Barrachina et al. (2006), who used a dynamic headspace technique, found that hexanal, as well as the other compounds analyzed, was higher in their study than in tomato paste and lower than in the tissues analyzed by gas chromatograph headspace analysis. In an effort to develop a non-destructive method of volatile sampling, they also compared aroma volatile emissions from blended tomatoes with emissions from whole fruit. Blended tomatoes exhibited much higher aroma production than whole tomatoes, which supports the statement that tissue disruption can lead to additional production of some volatiles. Since the present study involved tomatoes that were never blended, only finely chopped, some differences between these results and previous studies may be attributed to divergent techniques.

Evidence in Zhu et al. (2005) would indicate that ethylene suppression would diminish production of fatty-acid derived volatiles and have no direct impact on production of volatiles derived from amino acids. While in one of the four ‘FL-91’ experiments *cis*-3-hexenal content was higher in untreated ripe fruit than in ripe fruit derived from 1-MCP-treated breaker fruit, the rest of the data from the fatty-acid derived volatiles in this study did not indicate that ethylene action inhibition had caused a

decrease in fatty-acid derived volatile production. In fact, in some experiments, it produced an increase in hexanal emissions, contradicting previous studies (Mir et al., 2004). As for amino acid derived volatiles, whose production was not expected to be directly affected by ethylene suppression, there were isolated cases of ripe control fruit exhibiting higher production of amino acid derived volatiles than ripe fruit derived from 1-MCP-treated breaker fruit, but the reverse was also observed, as well as an observation of no difference between treatments. Since the results were inconsistent between samples and between experiments, it is possible that taste differences existed between individual samples, but it is difficult to formulate a broad declaration about what kind of difference in taste would be present.

Although no taste panels were used in the present study, one worker in the laboratory could distinguish between treated and untreated ripe fruit by odor. In the future, it may be productive to augment quantitative volatile analysis, such as the gas chromatography employed within this study, with parallel taste tests performed by a trained taste panel. Such panels have already examined the volatile content of tomatoes, but have yet to explore potential differences in subjective taste due to ethylene inhibition.

APPENDIX A
'BNH586' HUE ANGLE, FIRMNESS, AND VOLATILE EMISSIONS

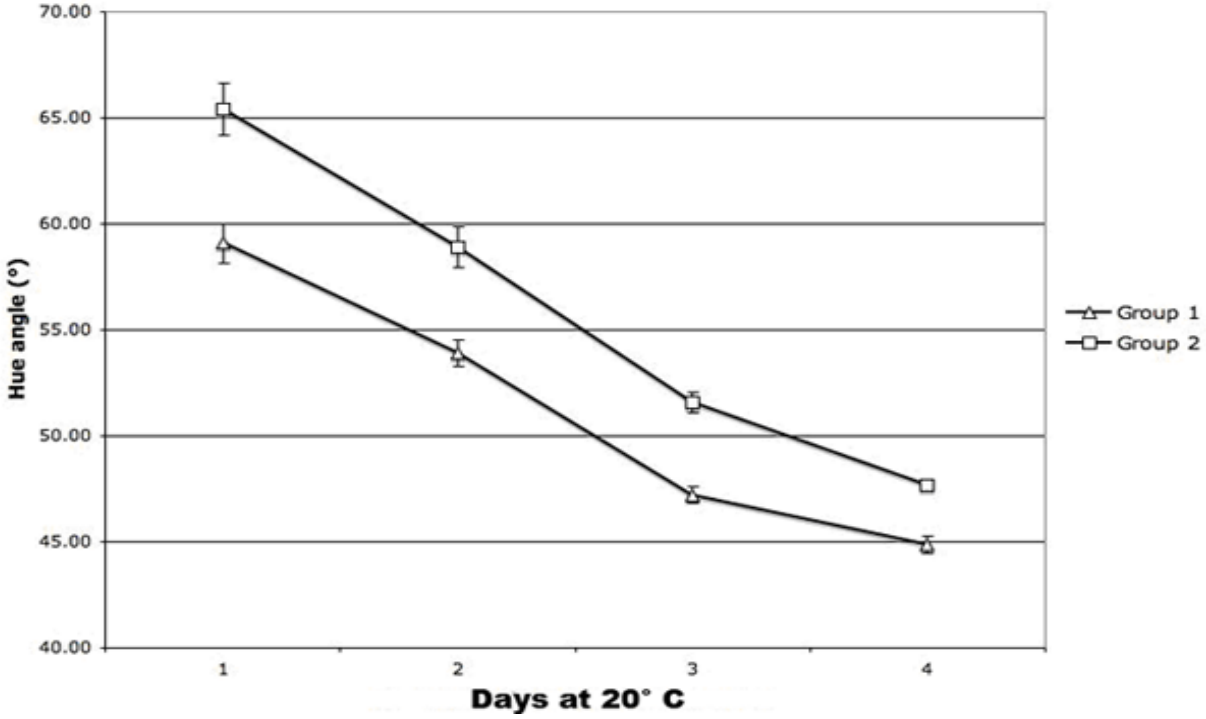

Figure A-1. Hue angle of 'BHN586' tomato fruit during storage at 20°C. Fruit were selected at the turning stage and placed directly in 20°C storage facilities. Hue angle was determined daily at the equatorial region of twenty-five individual fruit. Values represent the means (n= 75. 3 measurements each on each of 25 individual fruit), +/- standard error

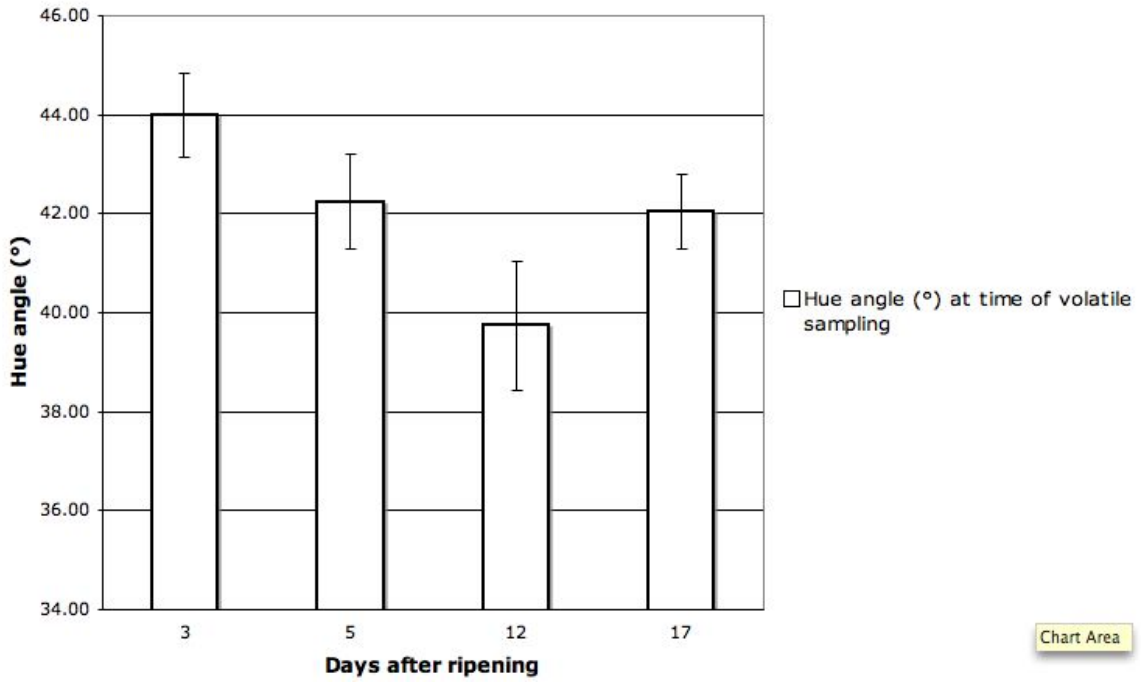

Figure A-2-a

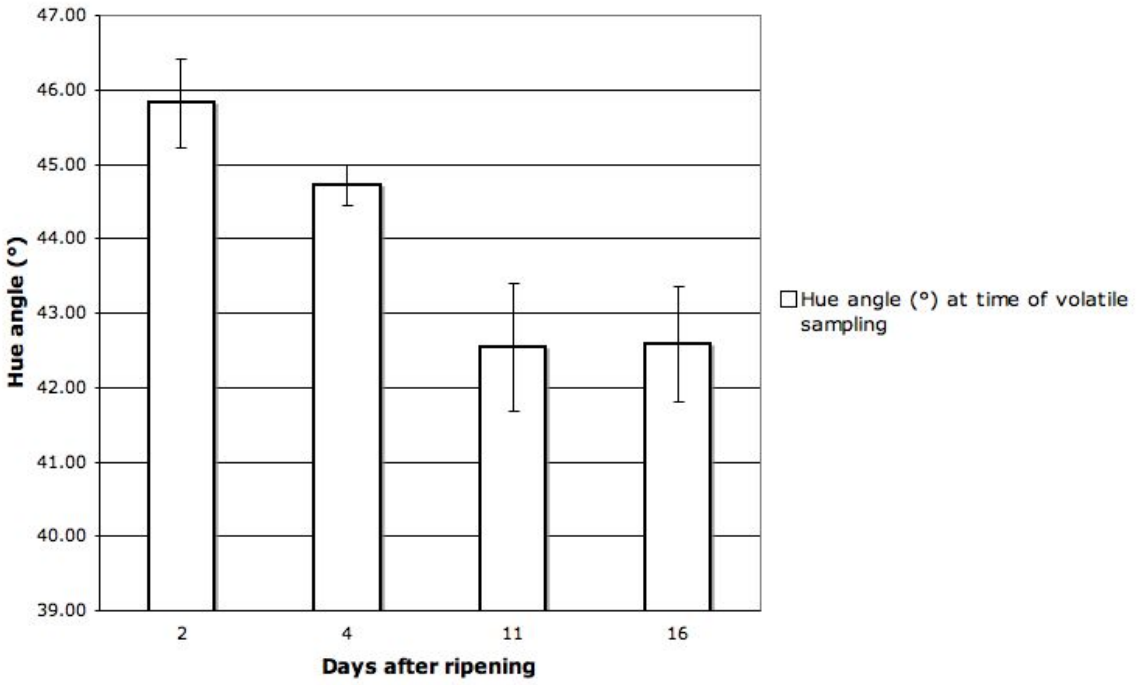

Figure A-2-b

Figure A-2. Hue angle of 'BHN586' tomato fruit during storage at 20°C after attaining a hue angle of 50°. Fruit were obtained at the turning stage and hue angle determined daily at the equatorial region of five individual fruit. On the days indicated, 5 fruit were removed and volatiles measured. ‘Days after ripening’ refers to days following the date on which a 50° hue angle was observed on all

five individual fruit measured. Figures A-2-a and A-2-b depict the two identical groups of fruit from the same source, selected and stored at the same time and at the same stage, and belonging to the same cultivar. Values represent the average of those five fruit, +/- standard error.

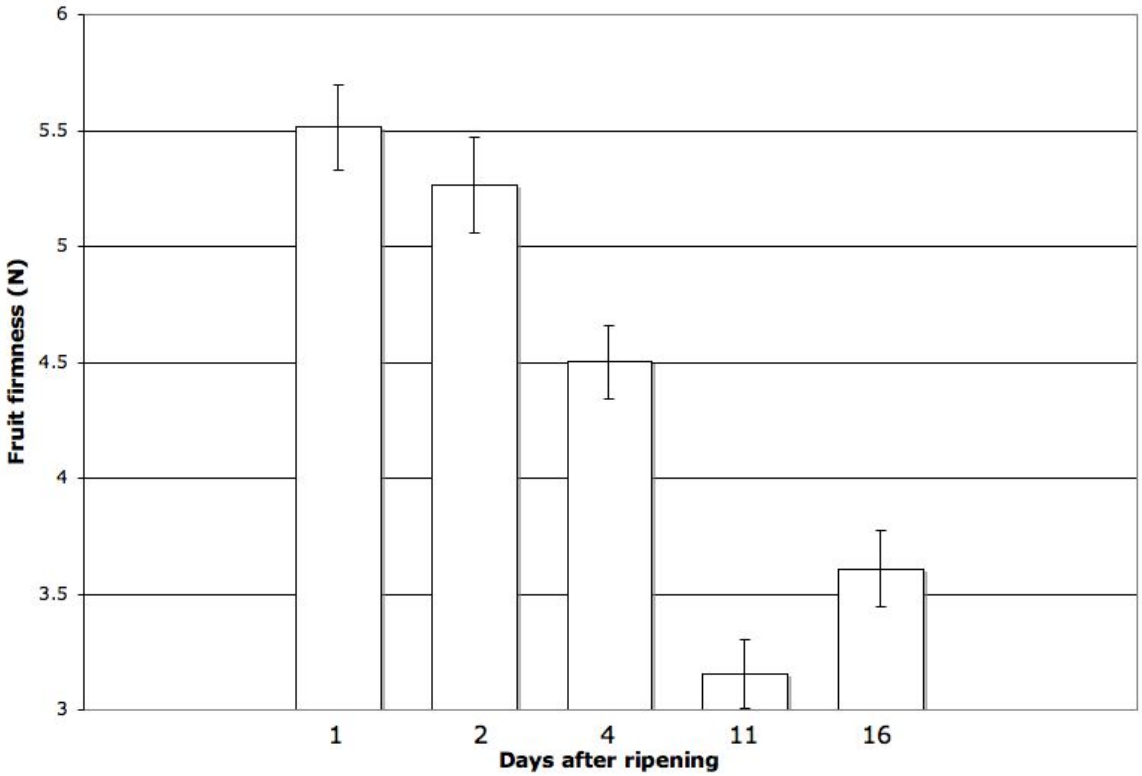

Figure A-3. Fruit firmness (N) of 'BHN586' tomato fruit during storage at 20°C after attaining a hue angle of 50°. Fruit were obtained at the turning stage and hue angle determined daily at the equatorial region of five individual fruit. On the days indicated, 5 fruit were removed and volatiles measured. ‘Days after ripening’ refers to days following the time at which a 50° hue angle was observed on all five individual fruit measured. Values represent the average of those five fruit, +/- standard error.

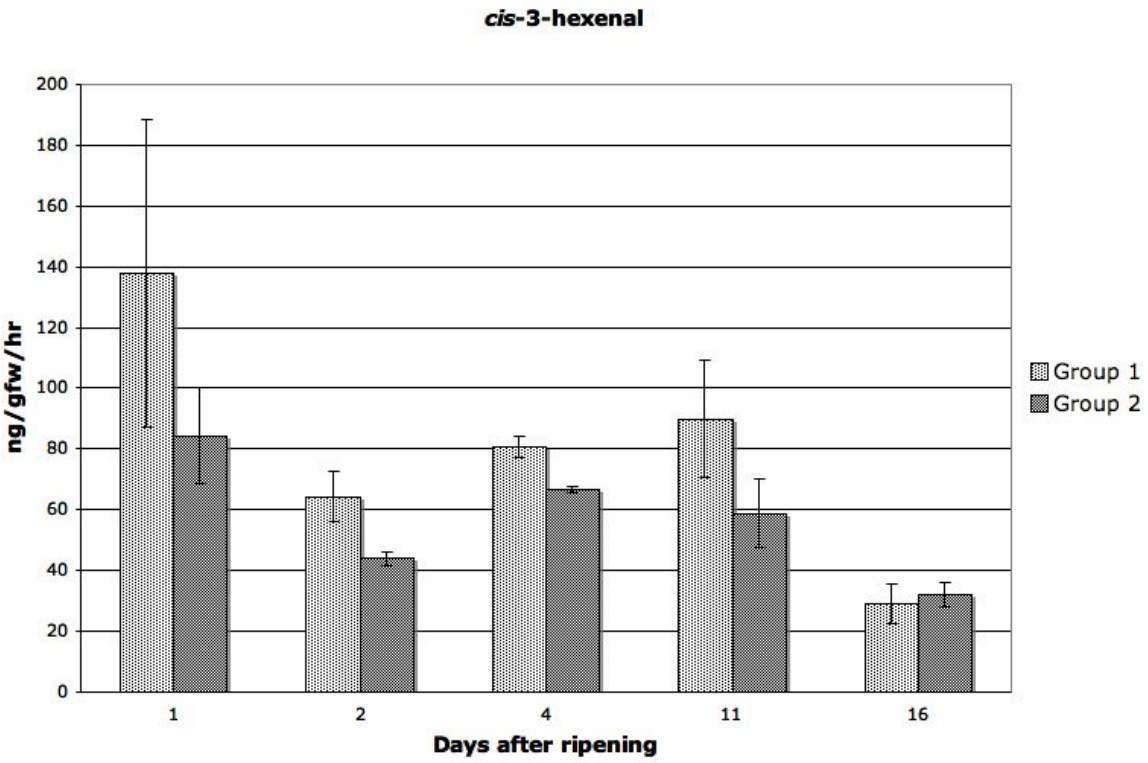

Figure A-4. *Cis*-3-hexenal production in ripe ‘BNH586' tomato fruit. Fruit were ripened at 20°C, and volatile production was measured on 1, 2, 4, 11, and 16 days after fruit reached an average hue angle of 50°. Group 1 and Group 2 are identical samples taken from fruit obtained at the same time from the same source and stored for the same duration. Values represent the average of five fruit, +/- standard error.

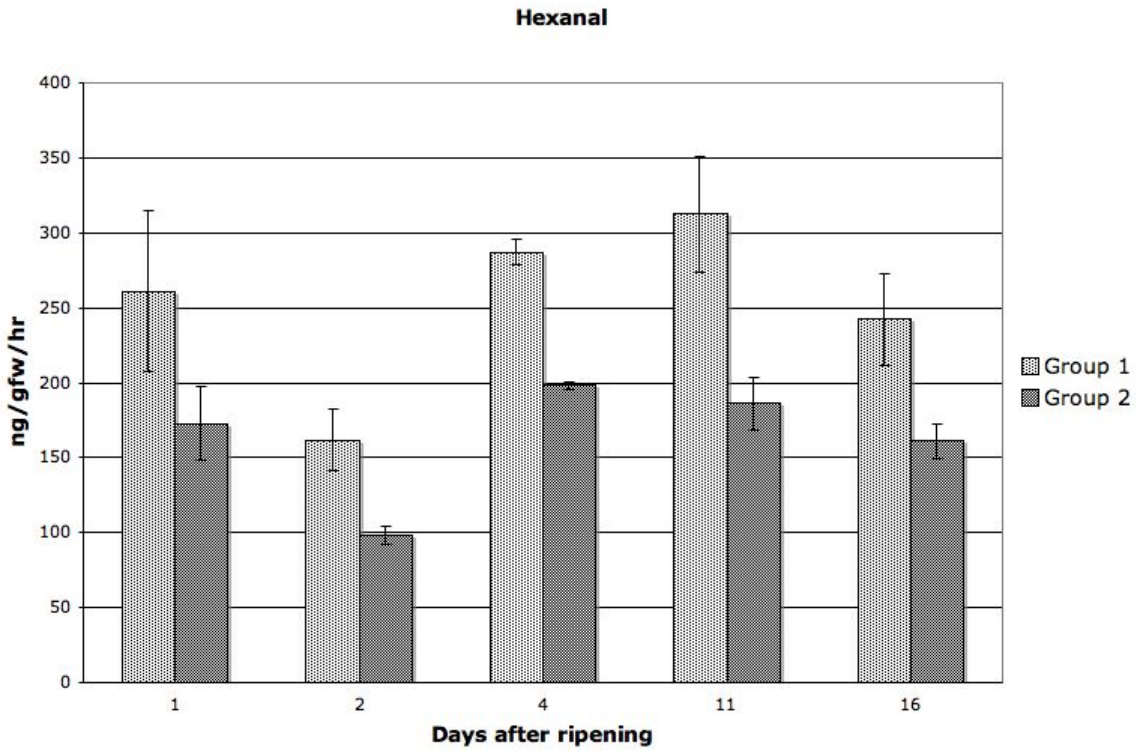

Figure A-5. Hexanal production in ripe ‘BNH586' tomato fruit. Fruit were ripened at 20°C, and volatile production was measured on 1, 2, 4, 11, and 16 days after fruit reached an average hue angle of 50°. Group 1 and Group 2 are identical samples taken from fruit obtained at the same time from the same source and stored for the same duration. Values represent the average of five fruit, +/- standard error.

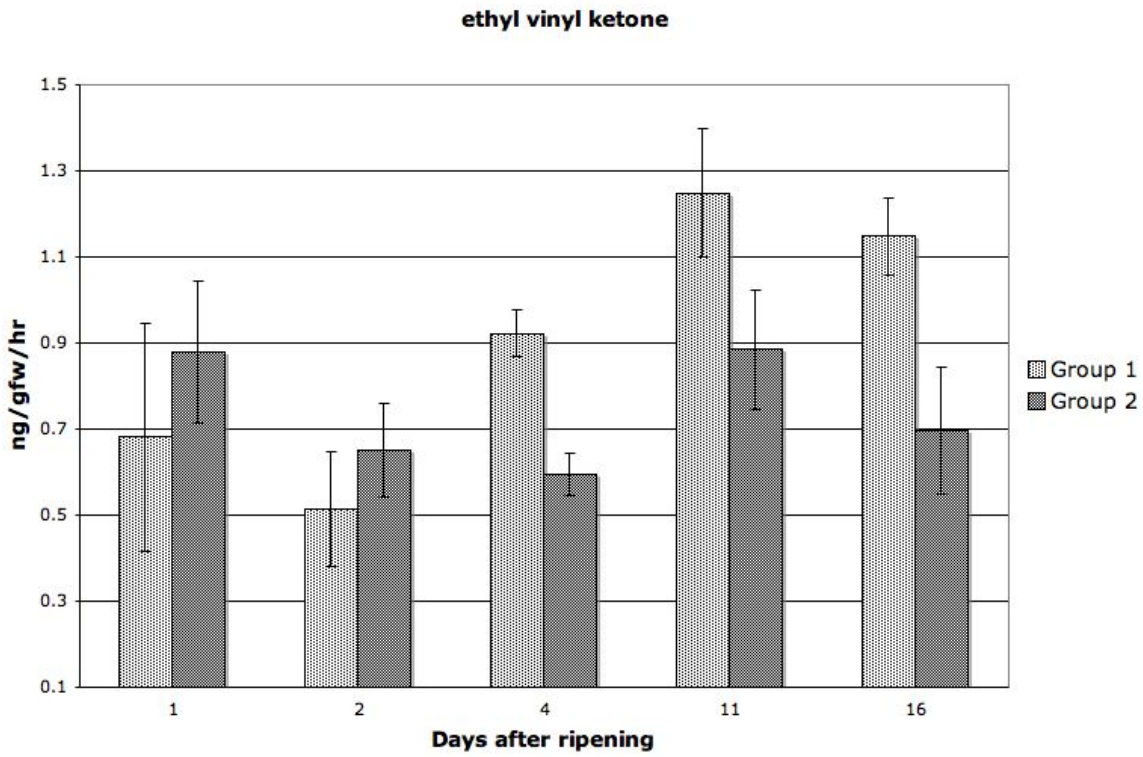

Figure A-6. Ethyl vinyl ketone production in ripe ‘BNH586' tomato fruit. Fruit were ripened at 20°C, and volatile production was measured on 1, 2, 4, 11, and 16 days after fruit reached an average hue angle of 50°. Group 1 and Group 2 are identical samples taken from fruit obtained at the same time from the same source and stored for the same duration. Values represent the average of five fruit, +/- standard error.

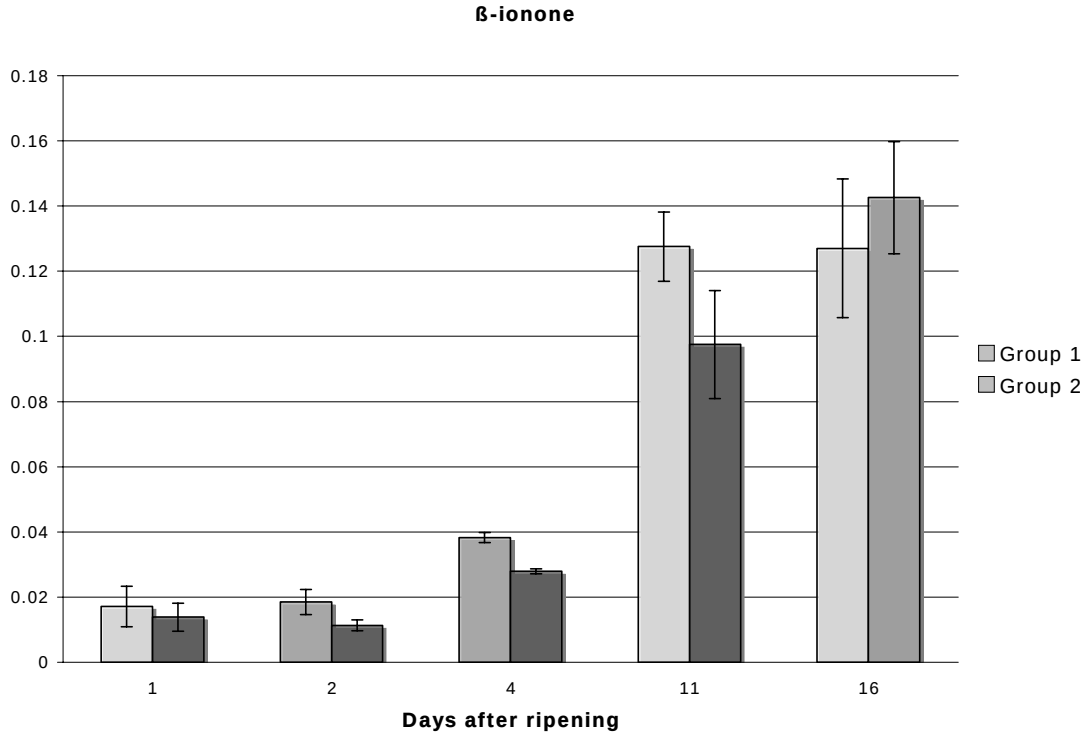

Figure A-7. ß-ionone production in ripe ‘BNH586' tomato fruit. Fruit were ripened at 20°C, and volatile production was measured on 1, 2, 4, 11, and 16 days after fruit reached an average hue angle of 50°. Group 1 and Group 2 are identical samples taken from fruit obtained at the same time from the same source and stored for the same duration. Values represent the average of five fruit, +/- standard error.

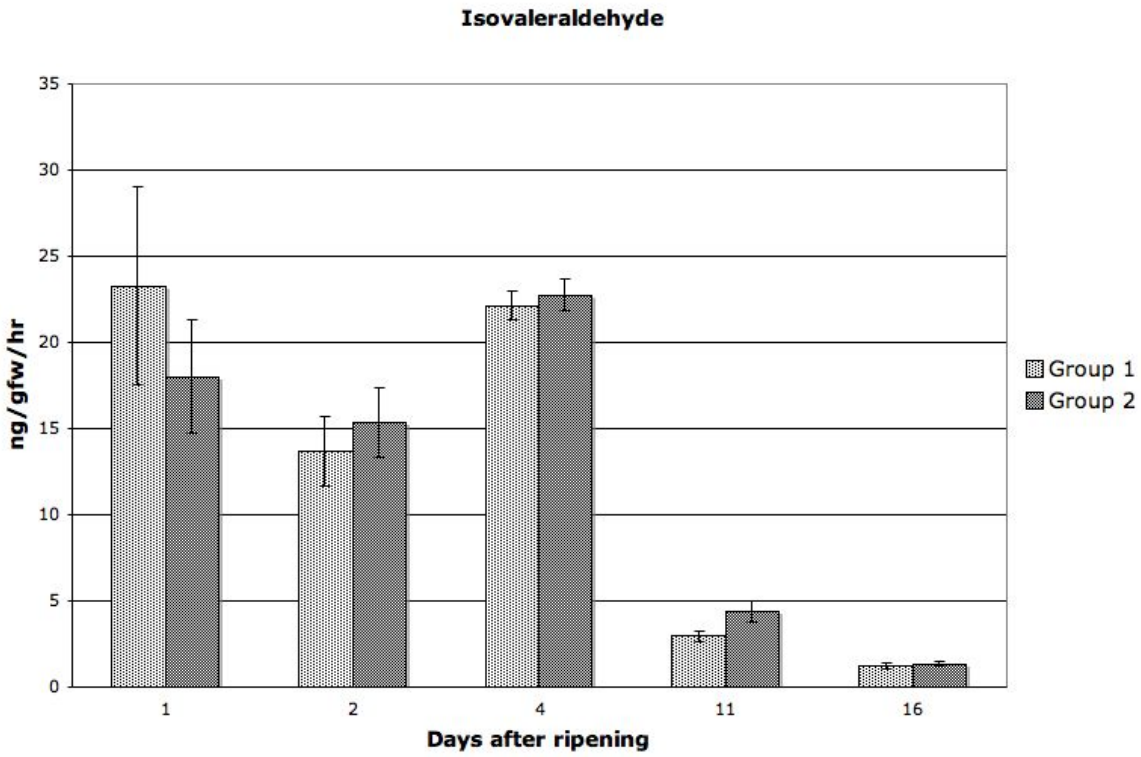

Figure A-8. Isovaleraldehyde production in ripe ‘BNH586' tomato fruit. Fruit were ripened at 20°C, and volatile production was measured on 1, 2, 4, 11, and 16 days after fruit reached an average hue angle of 50°. Group 1 and Group 2 are identical samples taken from fruit obtained at the same time from the same source and stored for the same duration. Values represent the average of five fruit, +/- standard error.

APPENDIX B
'FL-47' HUE ANGLE, FIRMNESS, AND VOLATILE EMISSIONS

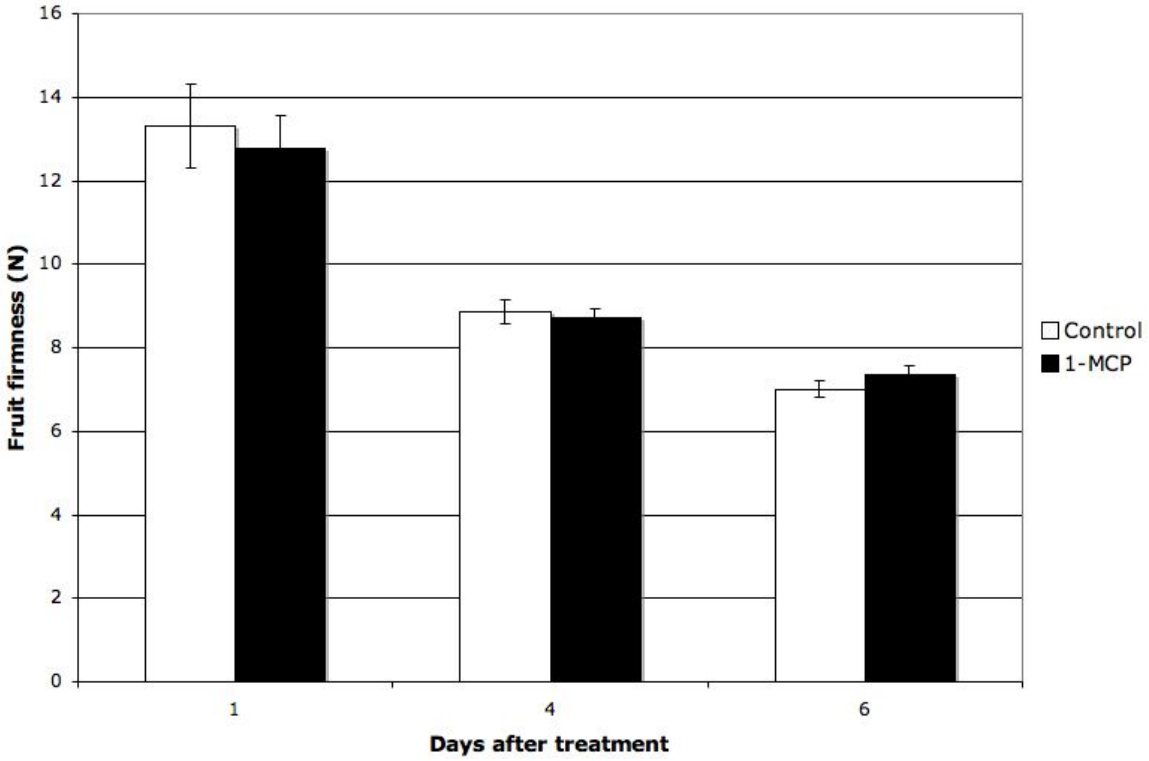

Figure B-1. Firmness (N) of 'FL-47' tomato fruit after treatment with air or 1-MCP (250 ppb, 12 h, 20°C) at the pink stage and ripened at 20°C. Days represent time since treatment. Values represent the average of fifteen fruit, +/- standard error.

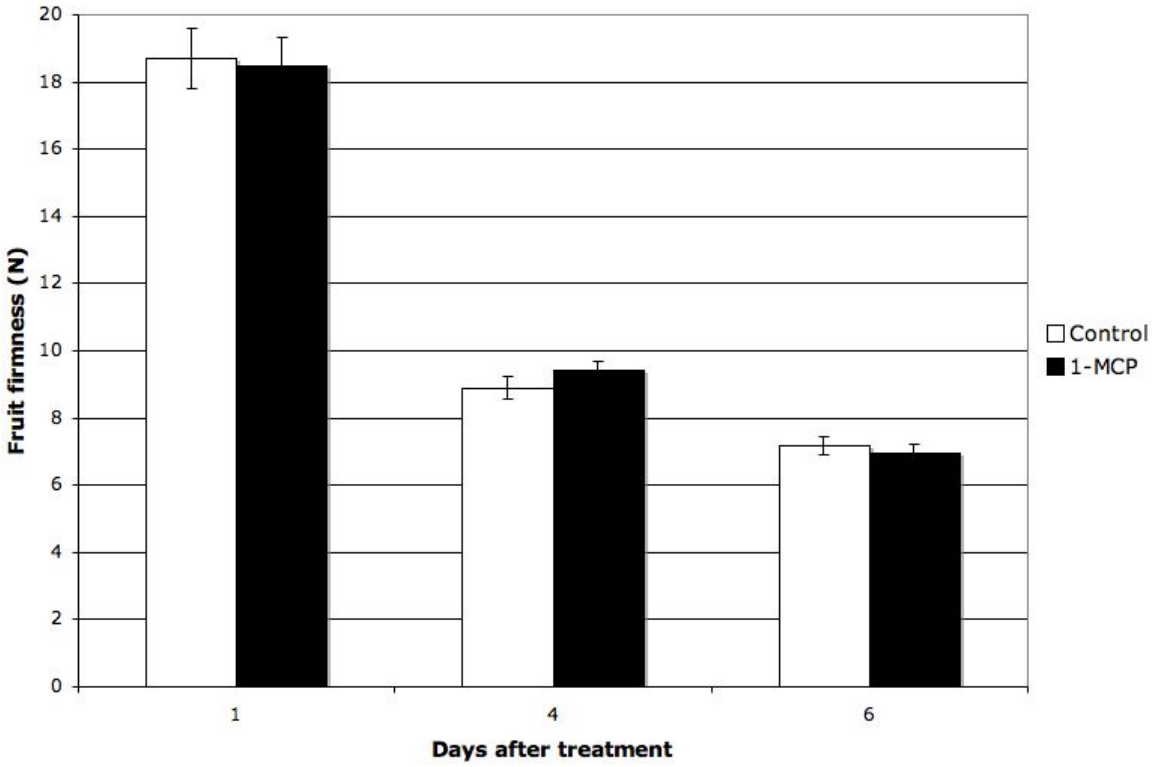

Figure B-2. Firmness (N) of 'FL-47' tomato fruit after treatment with air or 1-MCP (250 ppb, 12 h, 20°C) at the turning stage and ripened at 20°C. Days represent time since treatment. Values represent the average of fifteen fruit, +/- standard error.

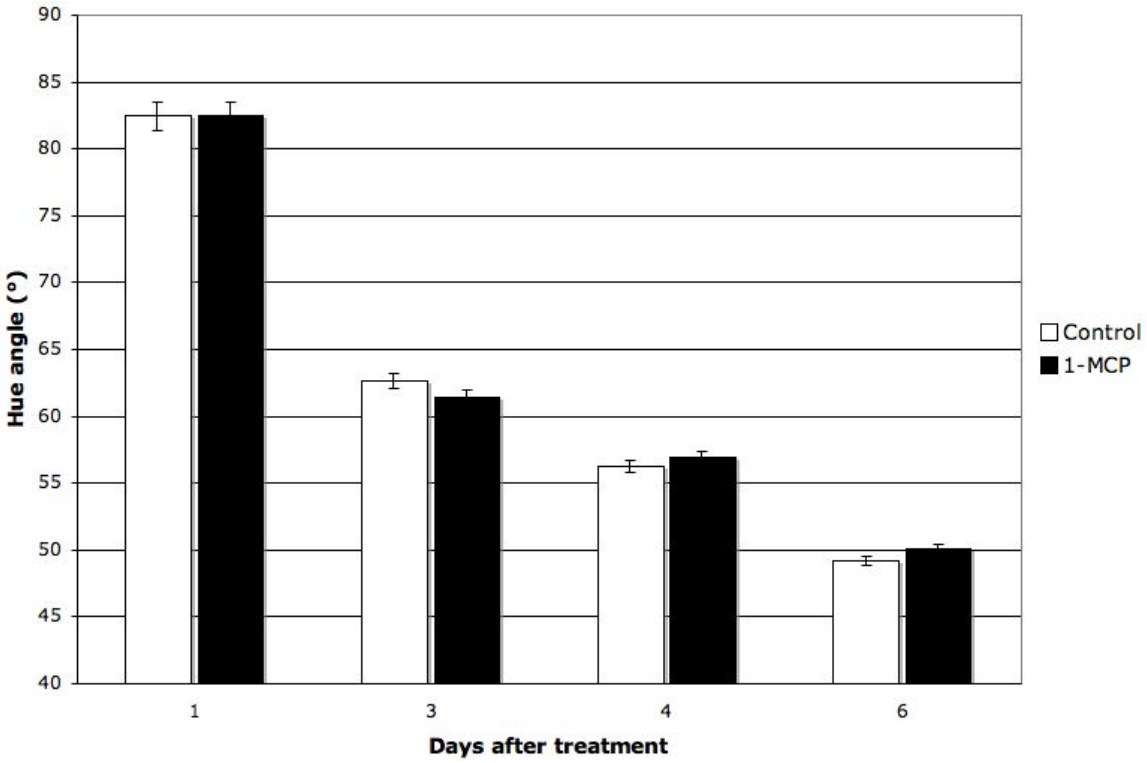

Figure B-3. Hue angle (°) of 'FL-47' tomato fruit after treatment with air or 1-MCP (250 ppb, 12 h, 20°C) at the pink stage and ripened at 20°C. Days represent time since treatment. Values represent the average of fifteen fruit, +/- standard error.

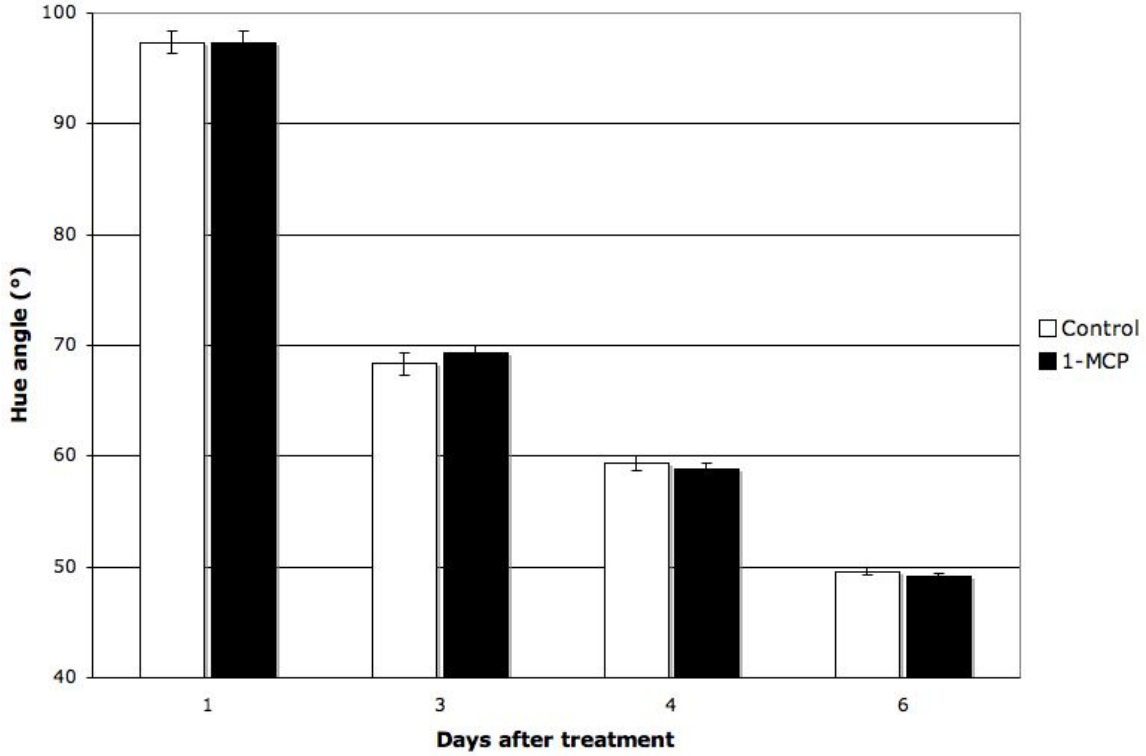

Figure B-4. Hue angle (°) of 'FL-47' tomato fruit after treatment with air or 1-MCP (250 ppb, 12 h, 20°C) at the turning stage and ripened at 20°C. Days represent time since treatment. Values represent the average of fifteen fruit, +/- standard error.

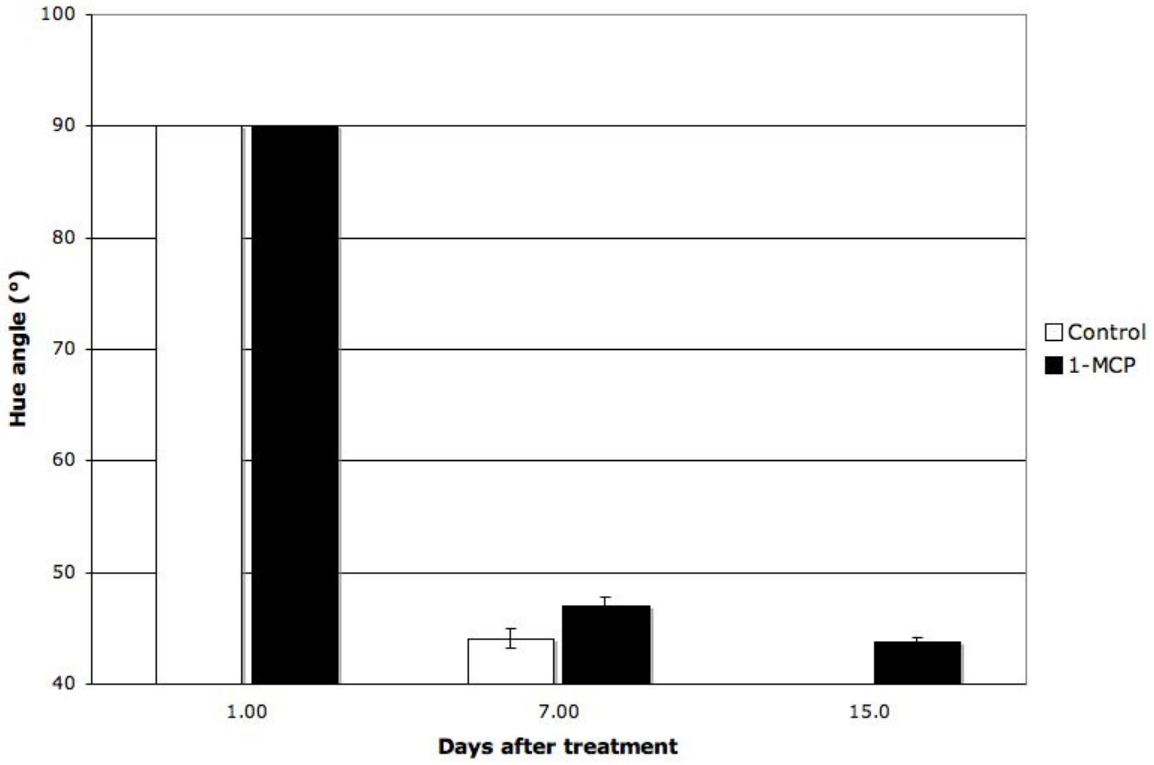

Figure B-5. Hue angle (°) of 'FL-47' tomato fruit after treatment with air or 1-MCP (250 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Days represent time since treatment. Values represent the average of fifteen fruit, +/- standard error.

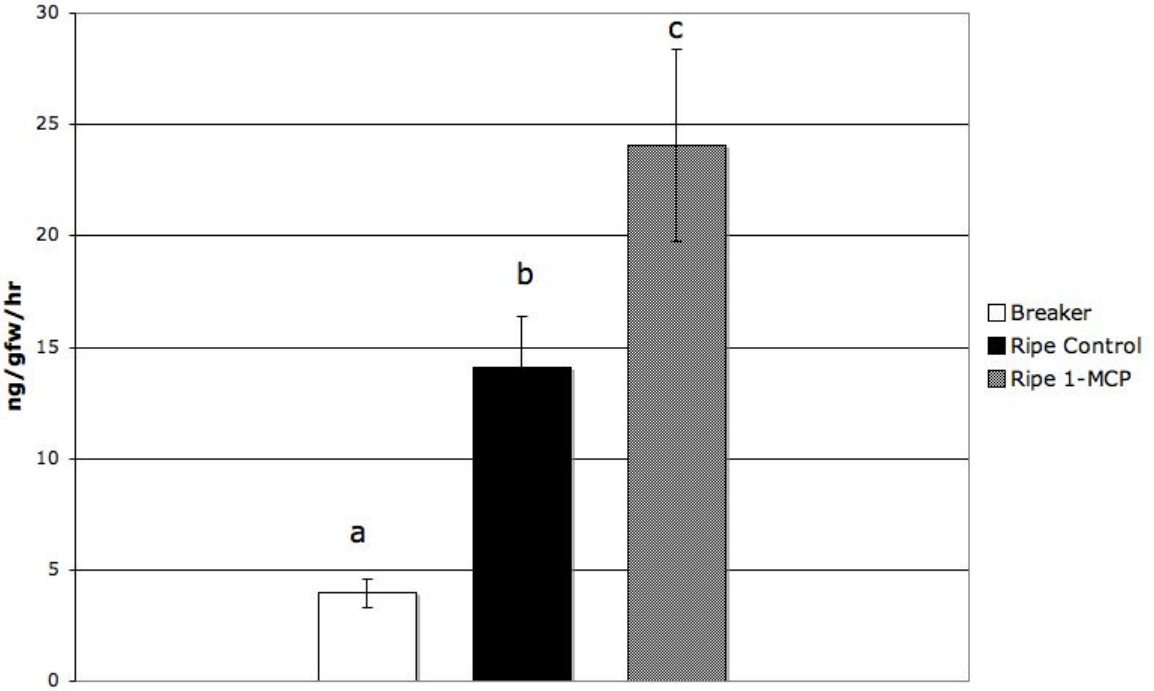

Figure B-6. *Cis*-3-hexenal production in 'FL-47' tomato fruit. Fruit were treated with air or 1-MCP (250 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 45°. 1-MCP treated fruit required an additional 8 days to reach this hue value. Breaker fruit were sampled without treatment. Values represent the average of fifteen fruit, +/- standard error.

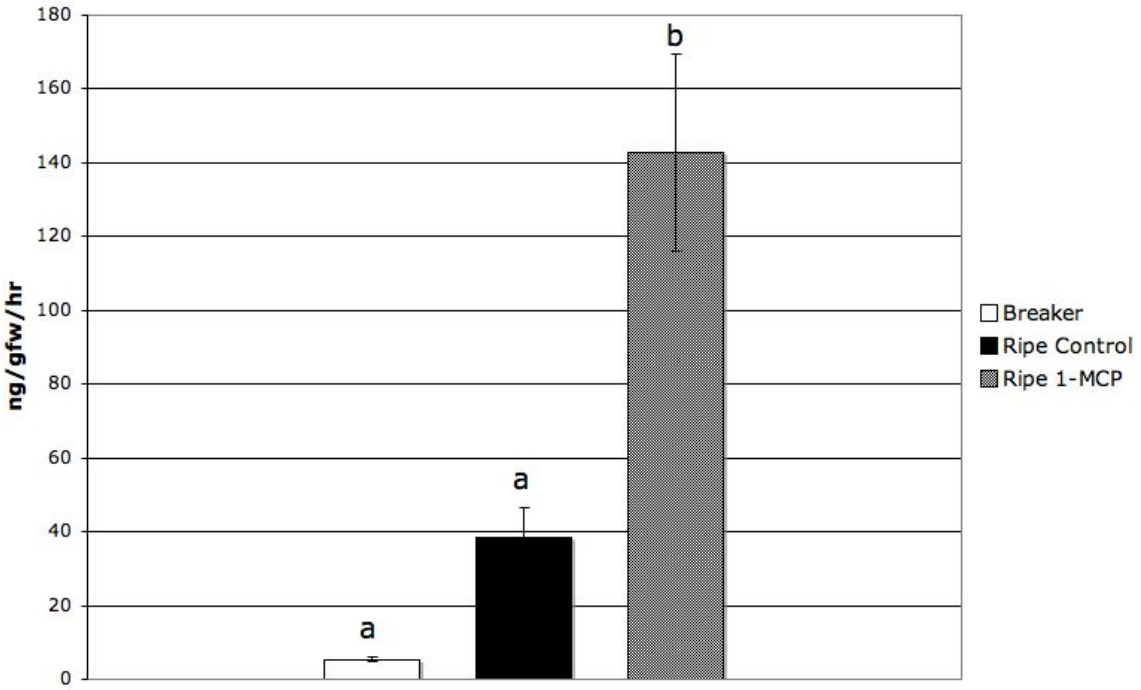

Figure B-7. Hexanal production in 'FL-47' tomato fruit. Fruit were treated with air or 1-MCP (250 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 45°. 1-MCP treated fruit required an additional 8 days to reach this hue value. Breaker fruit were sampled without treatment. Values represent the average of fifteen fruit, +/- standard error.

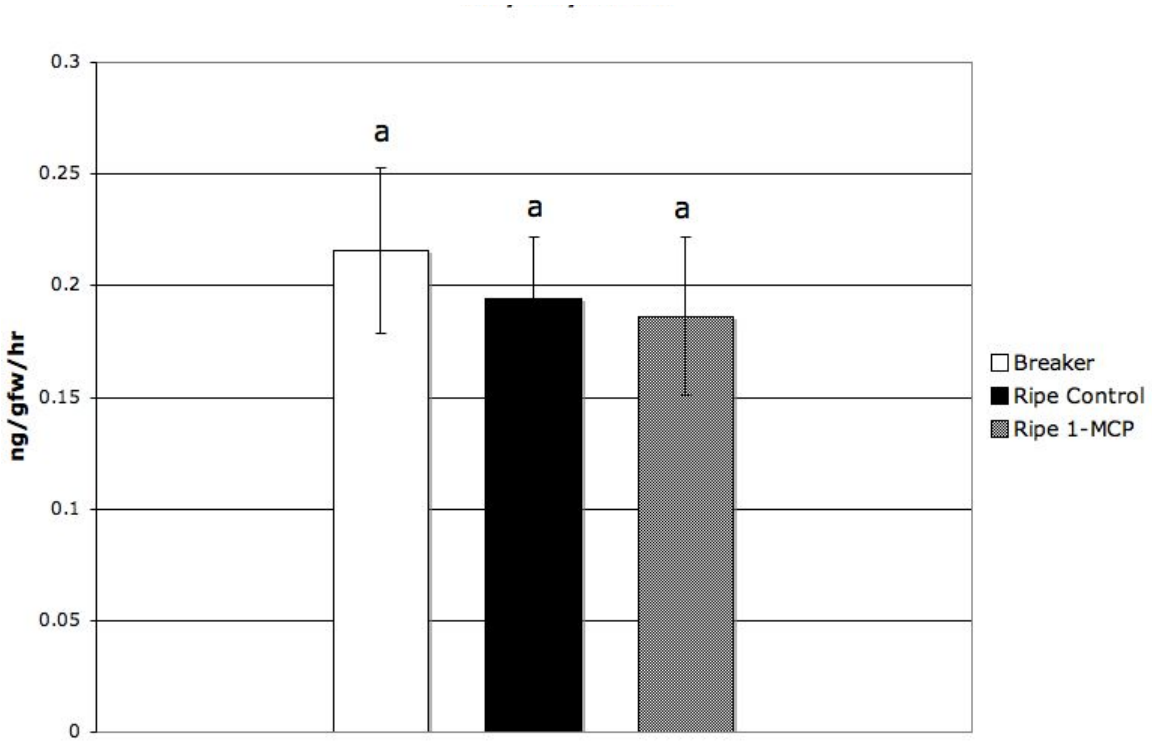

Figure B-8. Ethyl vinyl ketone production in 'FL-47' tomato fruit. Fruit were treated with air or 1-MCP (250 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 45°. 1-MCP treated fruit required an additional 8 days to reach this hue value. Breaker fruit were sampled without treatment. Values represent the average of fifteen fruit, +/- standard error.

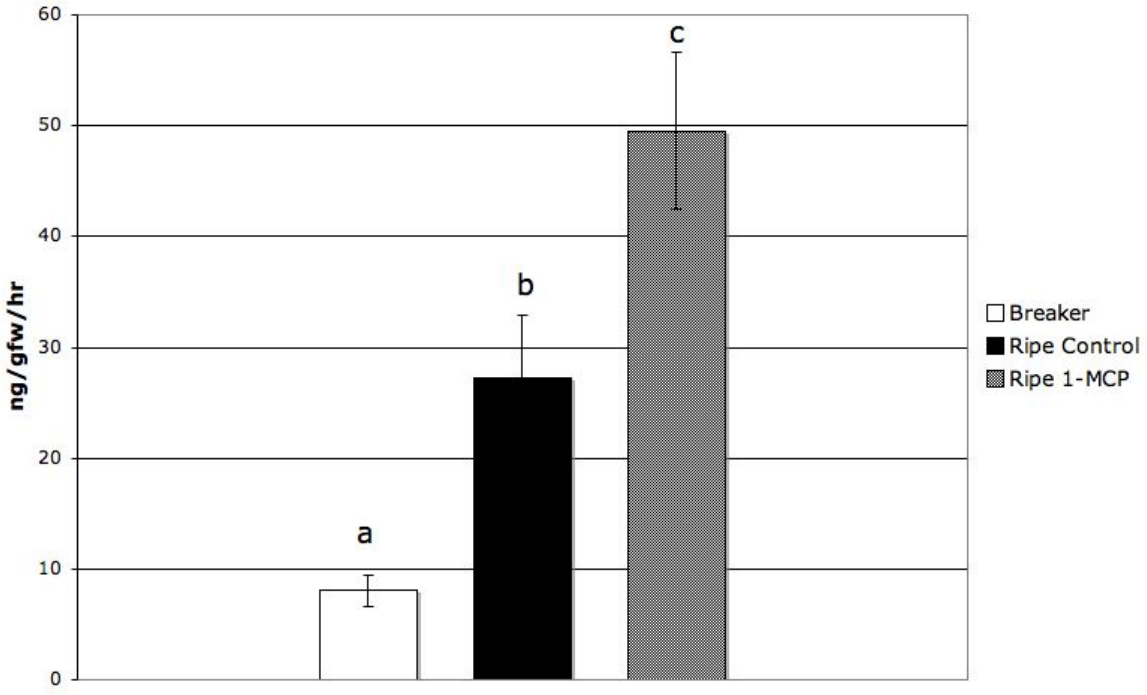

Figure B-9. *Cis*-3-hexenol production in 'FL-47' tomato fruit. Fruit were treated with air or 1-MCP (250 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 45°. 1-MCP treated fruit required an additional 8 days to reach this hue value. Breaker fruit were sampled without treatment. Values represent the average of fifteen fruit, +/- standard error.

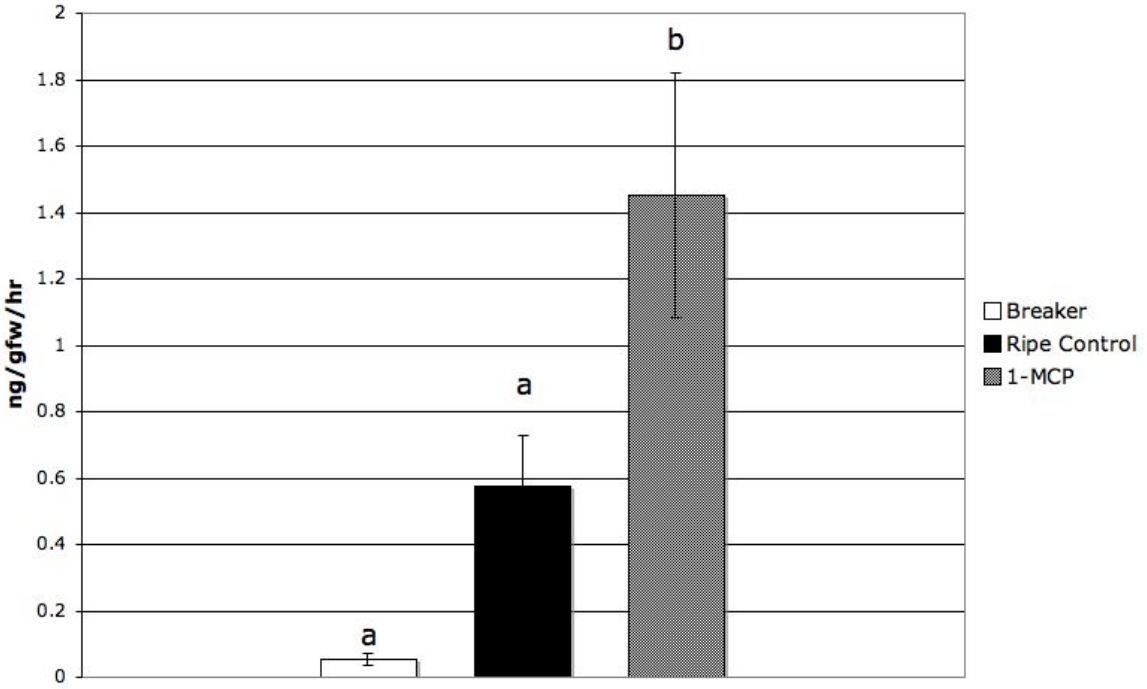

Figure B-10. *Trans*-2-hexenal production in 'FL-47' tomato fruit. Fruit were treated with air or 1-MCP (250 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 45°. 1-MCP treated fruit required an additional 8 days to reach this hue value. Breaker fruit were sampled without treatment. Values represent the average of fifteen fruit, +/- standard error.

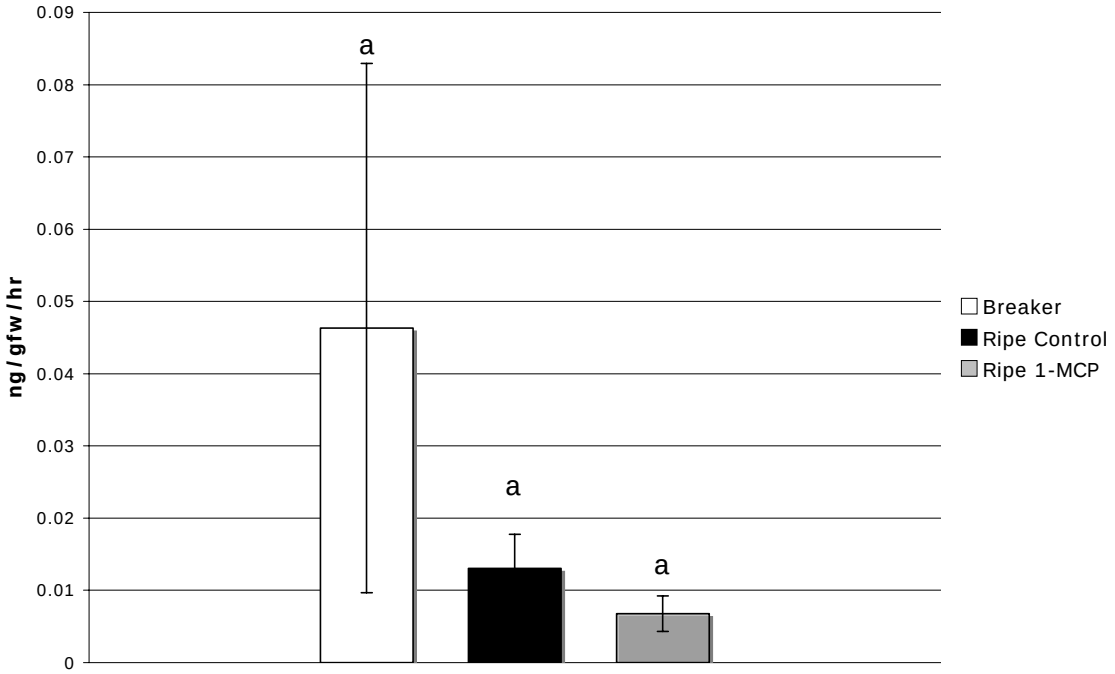

Figure B-11. ß-ionone production in 'FL-47' tomato fruit. Fruit were treated with air or 1-MCP (250 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 45°. 1-MCP treated fruit required an additional 8 days to reach this hue value. Breaker fruit were sampled without treatment. Values represent the average of fifteen fruit, +/- standard error.

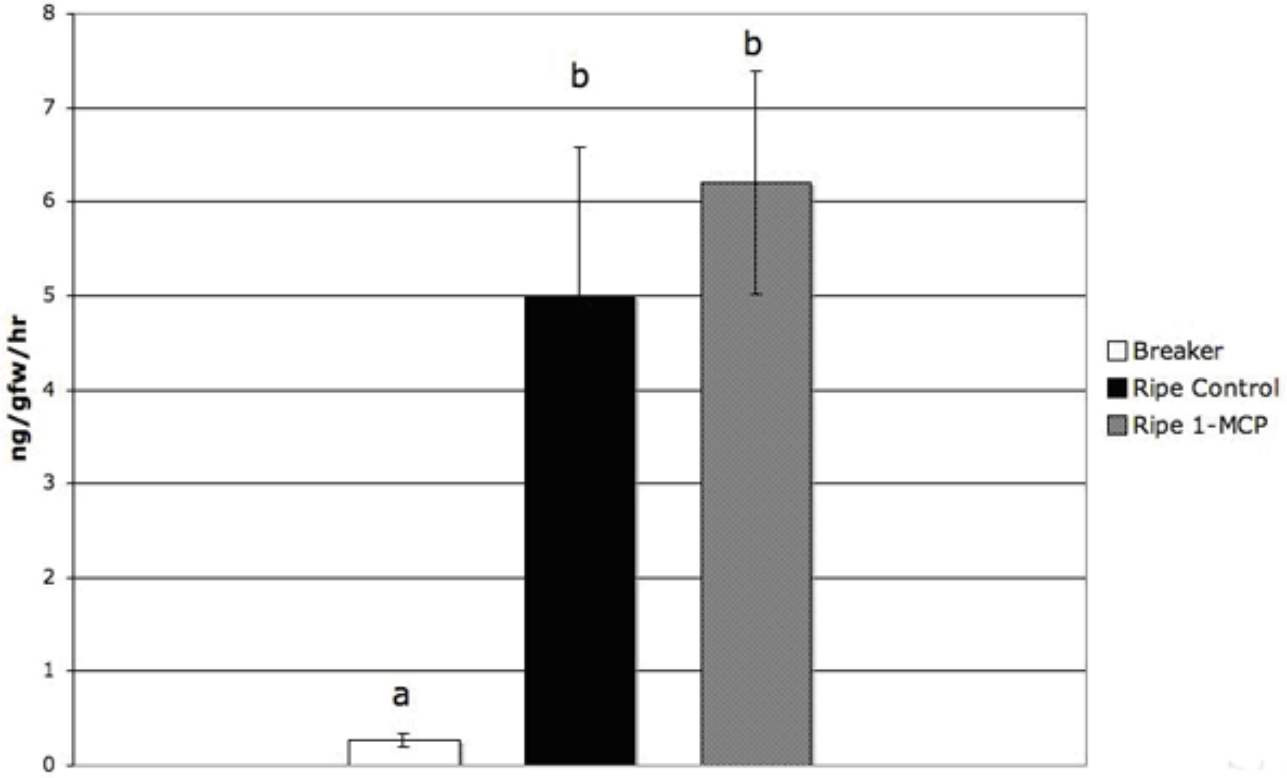

Figure B-12. 6-methyl-5-hepten-2-one production in 'FL-47' tomato fruit. Fruit were treated with air or 1-MCP (250 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 45°. 1-MCP treated fruit required an additional 8 days to reach this hue value. Breaker fruit were sampled without treatment. Values represent the average of fifteen fruit, +/- standard error.

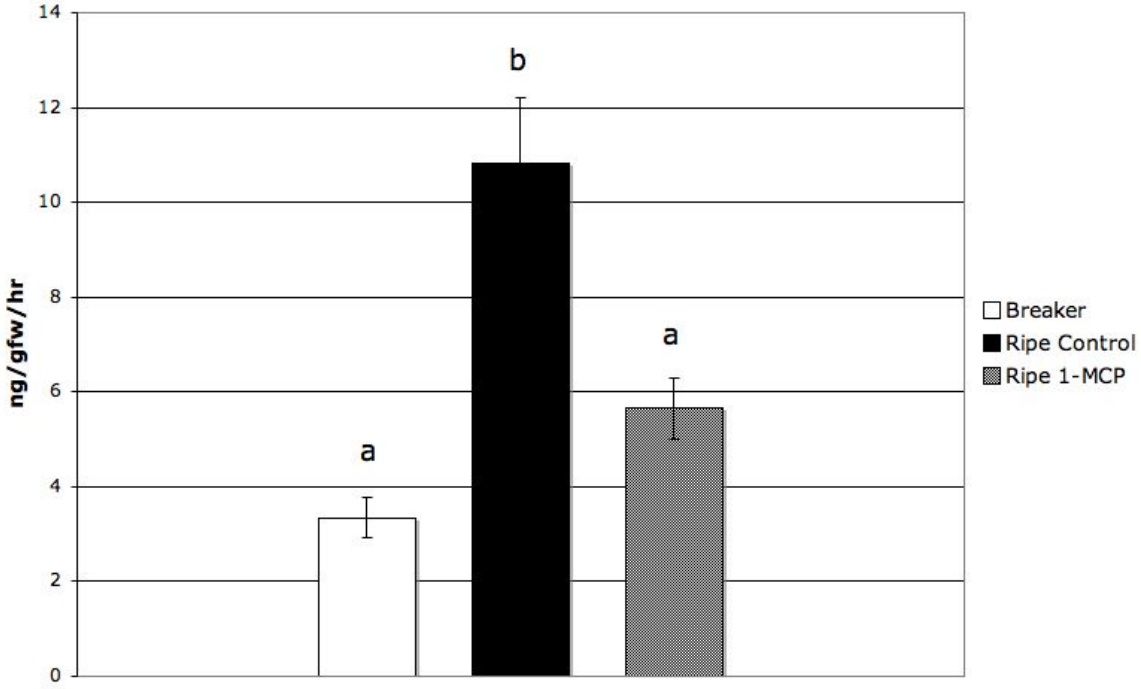

Figure B-13. Isovaleraldehyde production in 'FL-47' tomato fruit. Fruit were treated with air or 1-MCP (250 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 45°. 1-MCP treated fruit required an additional 8 days to reach this hue value. Breaker fruit were sampled without treatment. Values represent the average of fifteen fruit, +/- standard error.

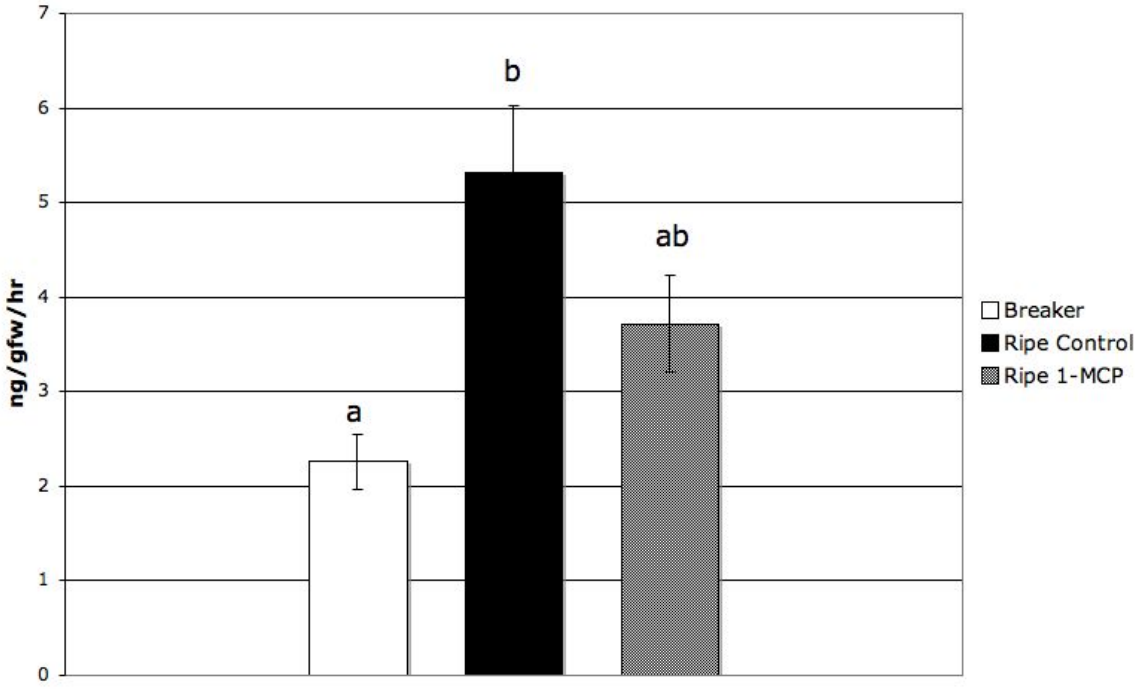

Figure B-14. 2-methylbutyraldehyde production in 'FL-47' tomato fruit. Fruit were treated with air or 1-MCP (250 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 45°. 1-MCP treated fruit required an additional 8 days to reach this hue value. Breaker fruit were sampled without treatment. Values represent the average of fifteen fruit, +/- standard error.

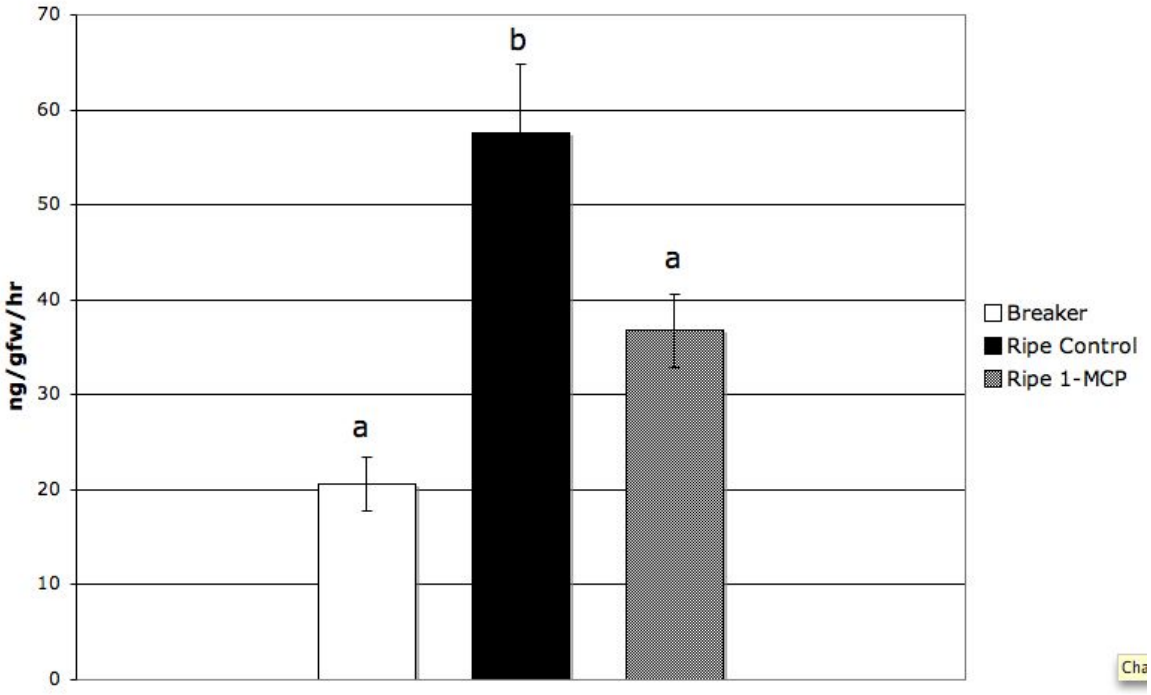

Figure B-15. 3-methyl-1-butanol production in 'FL-47' tomato fruit. Fruit were treated with air or 1-MCP (250 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 45°. 1-MCP treated fruit required an additional 8 days to reach this hue value. Breaker fruit were sampled without treatment. Values represent the average of fifteen fruit, +/- standard error.

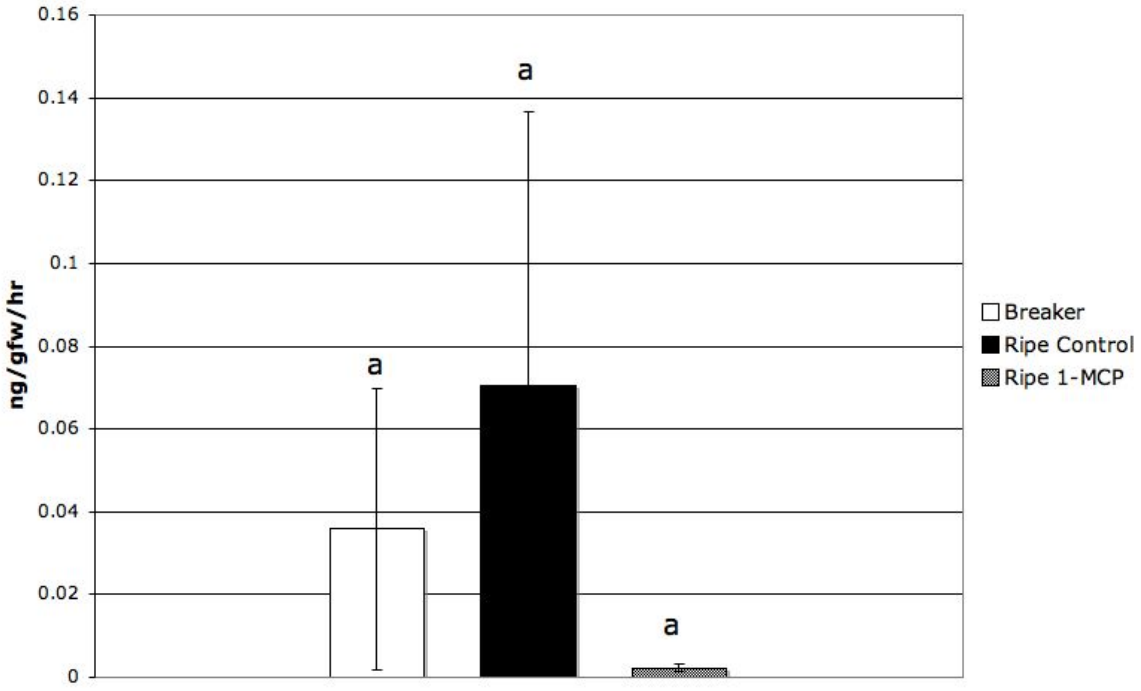

Figure B-16. 2-phenylethanol production in 'FL-47' tomato fruit. Fruit were treated with air or 1-MCP (250 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 45°. 1-MCP treated fruit required an additional 8 days to reach this hue value. Breaker fruit were sampled without treatment. Values represent the average of fifteen fruit, +/- standard error.

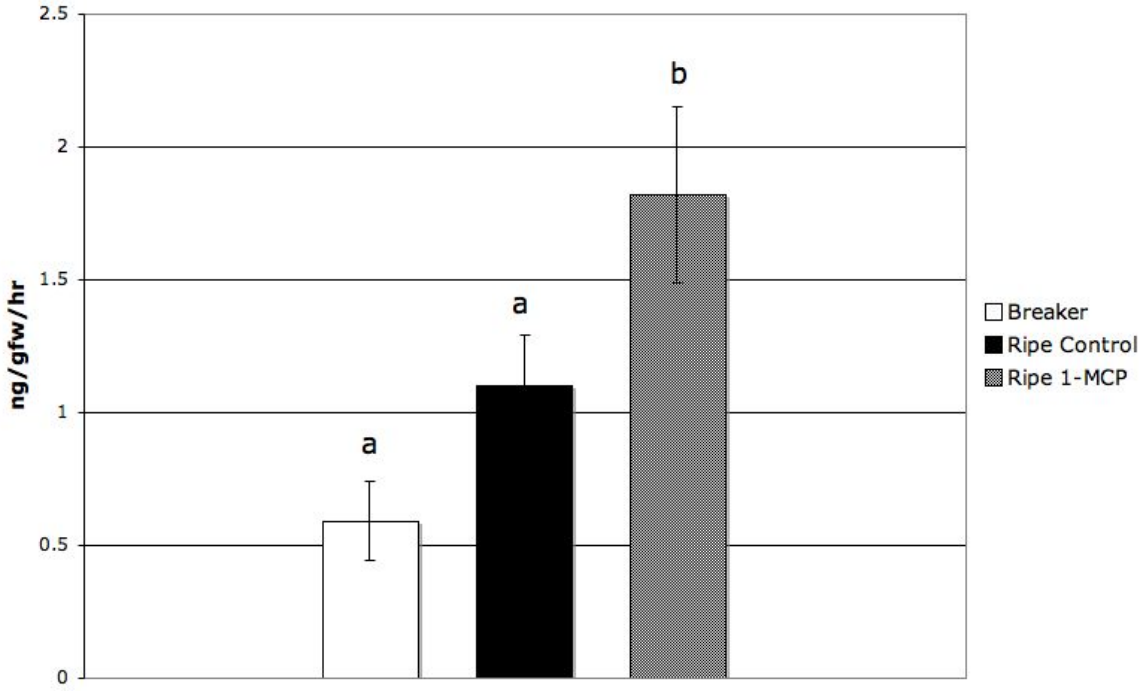

Figure B-17. 1-nitro-2-phenylethane production in 'FL-47' tomato fruit. Fruit were treated with air or 1-MCP (250 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 45°. 1-MCP treated fruit required an additional 8 days to reach this hue value. Breaker fruit were sampled without treatment. Values represent the average of fifteen fruit, +/- standard error.

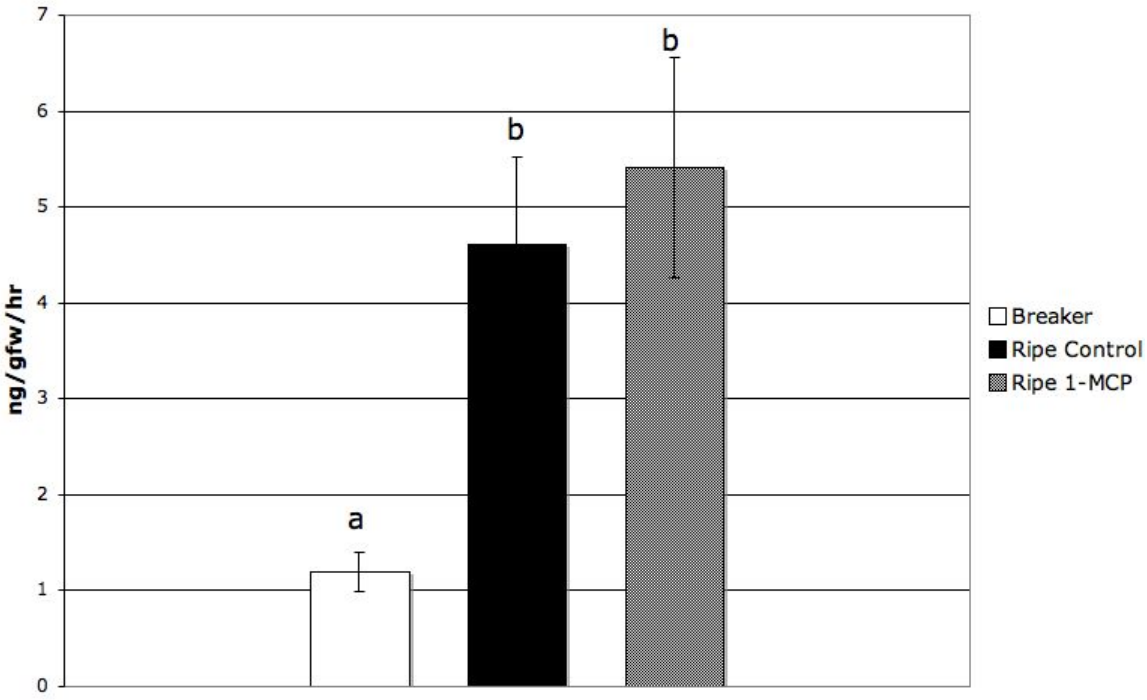

Figure B-18. 2-isobutylthiazole production in 'FL-47' tomato fruit. Fruit were treated with air or 1-MCP (250 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 45°. 1-MCP treated fruit required an additional 8 days to reach this hue value. Breaker fruit were sampled without treatment. Values represent the average of fifteen fruit, +/- standard error.

APPENDIX C
'FL-91' HUE ANGLE, FIRMNESS, AND VOLATILE EMISSIONS

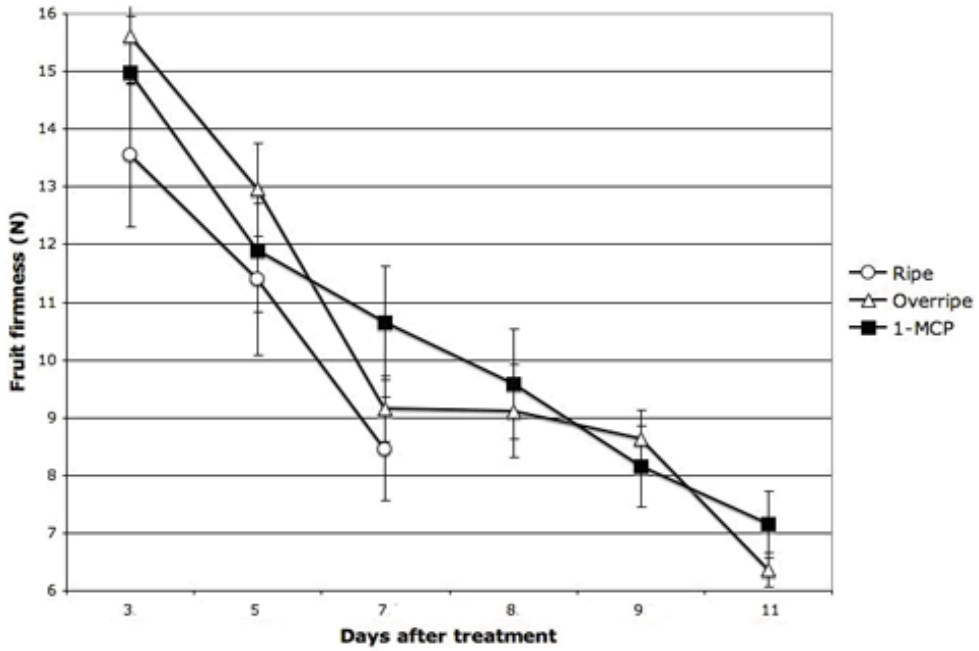

Figure C-1. Firmness (N) of 'FL-91' tomato fruit after treatment with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Days represent time since treatment. Values represent the average of five fruit, +/- standard error.

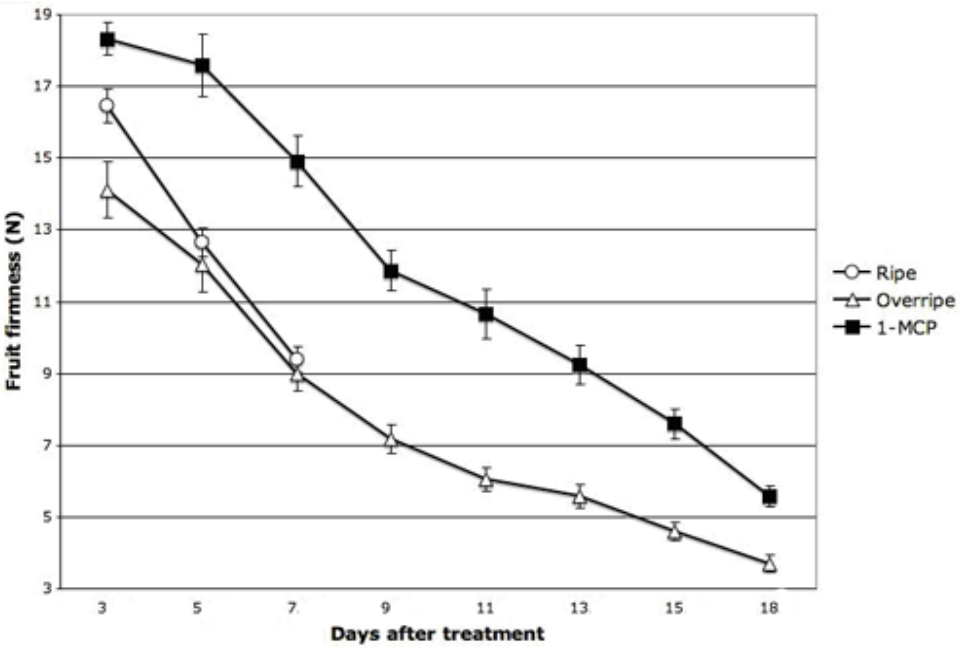

Figure C-2. Firmness (N) of 'FL-91' tomato fruit after treatment with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Days represent time since treatment. Values represent the average of five fruit, +/- standard error.

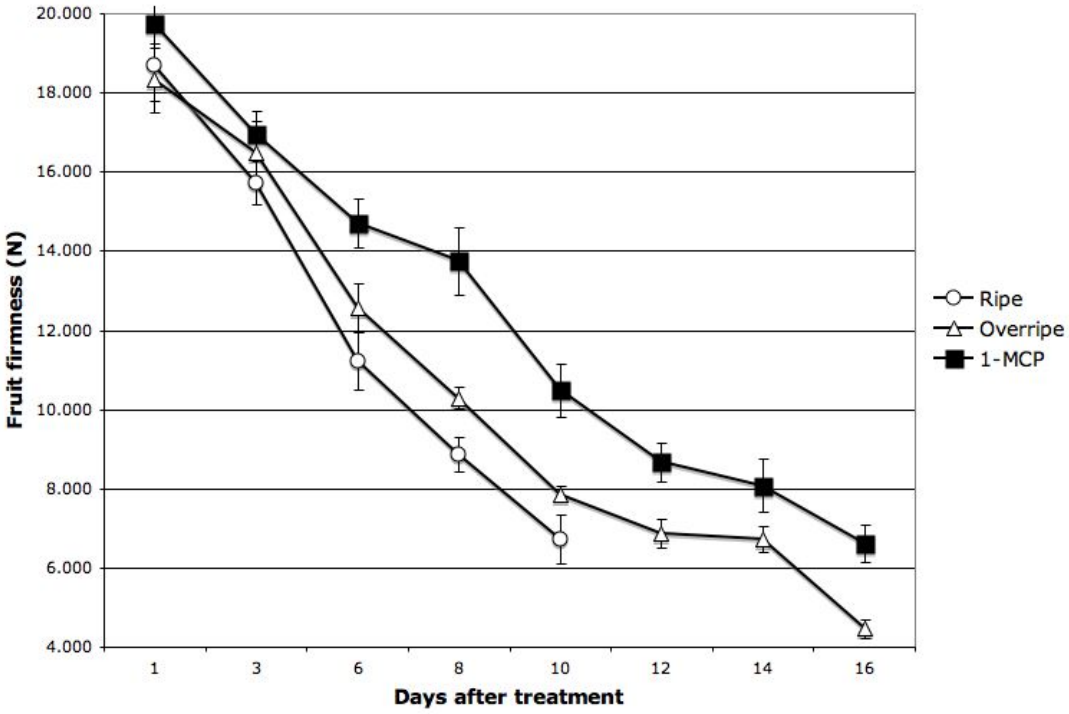

Figure C-3. Firmness (N) of 'FL-91' tomato fruit after treatment with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Days represent time since treatment. Values represent the average of five fruit, +/- standard error.

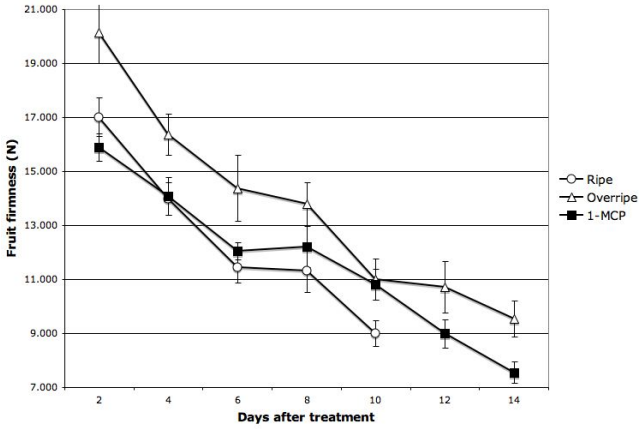

Figure C-4. Firmness (N) of 'FL-91' tomato fruit after treatment with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Days represent time since treatment. Values represent the average of five fruit, +/- standard error.

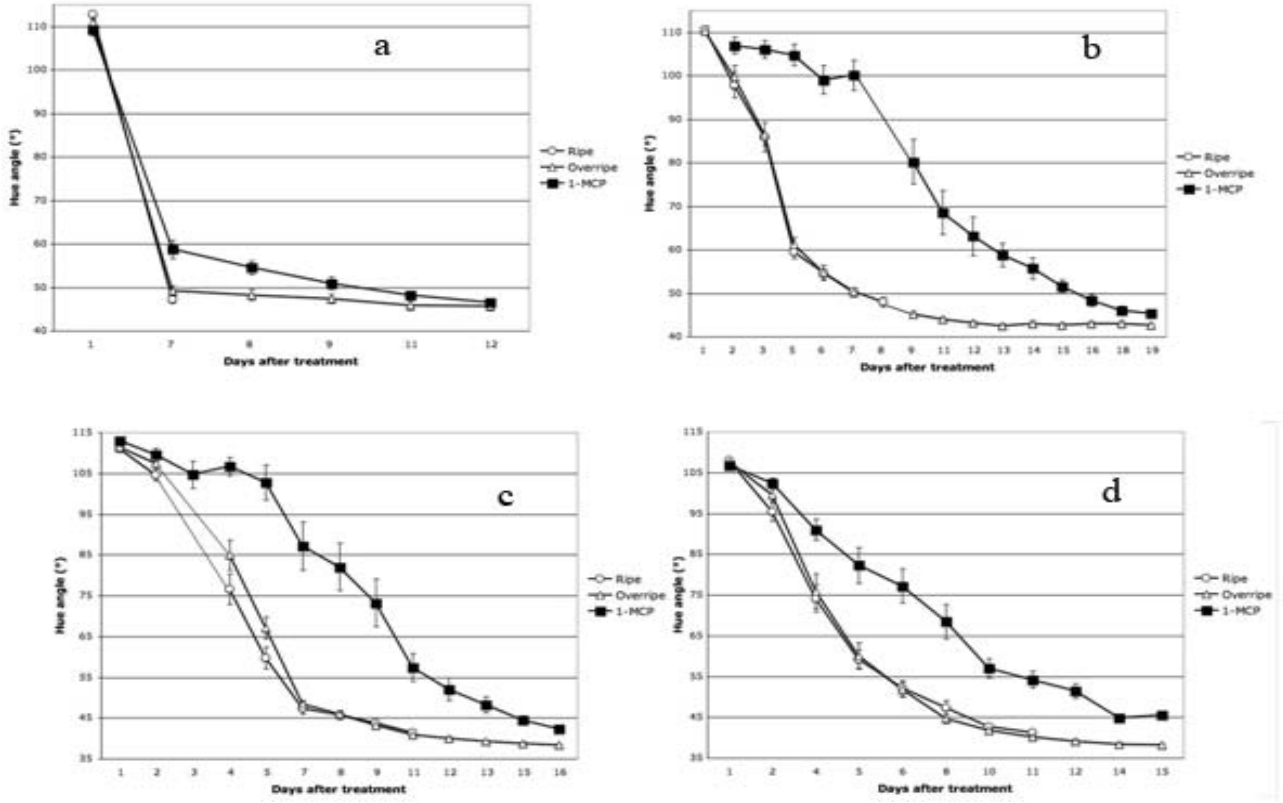

Figure C-5. Hue angle (°) of 'FL-91' tomato fruit after treatment with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Days represent time since treatment. Values represent the average of five fruit, +/- standard error. C-5-a) Experiment 1. C-5-b) Experiment 2. C-5-c) Experiment 3. C-5-d) Experiment 4.

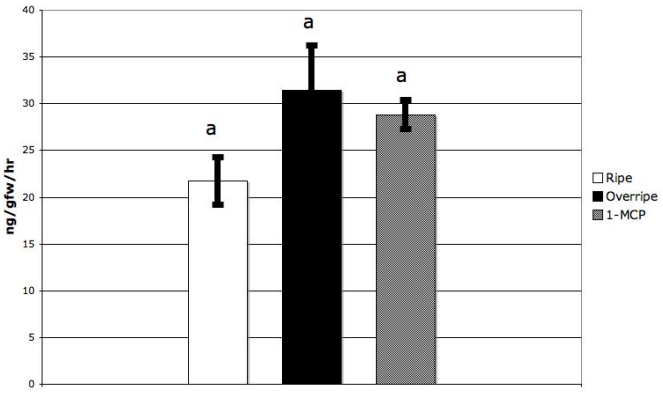

Figure C-6. *Cis*-3-hexenal production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 5 days to reach this hue value, at which time an additional control (hue angle at 50° + 5 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

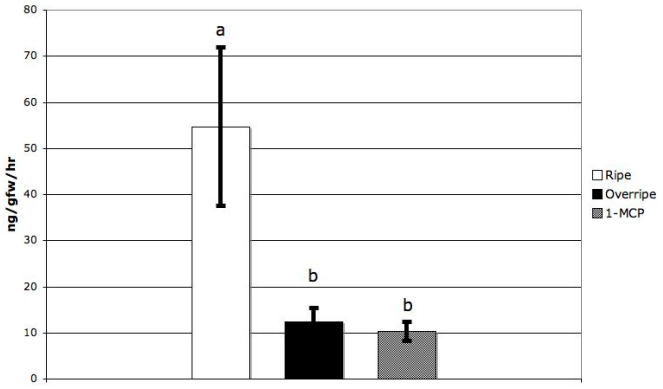

Figure C-7. *Cis*-3-hexenal production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 11 days to reach this hue value, at which time an additional control (hue angle at 50° + 11 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

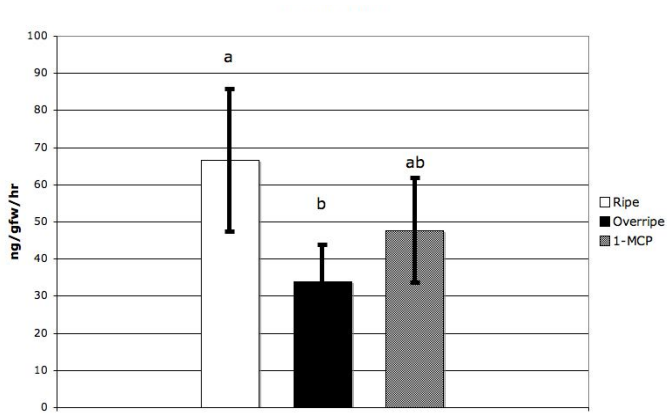

Figure C-8. *Cis*-3-hexenal production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 6 days to reach this hue value, at which time an additional control (hue angle at 50° + 6 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

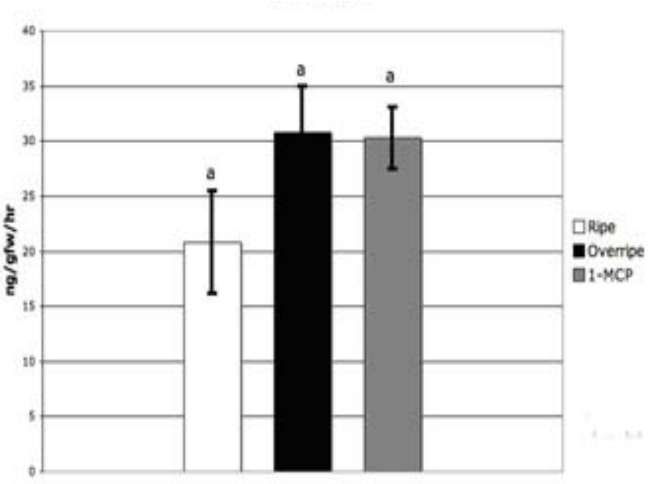

Figure C-9. *Cis*-3-hexenal production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 4 days to reach this hue value, at which time an additional control (hue angle at 50° + 4 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

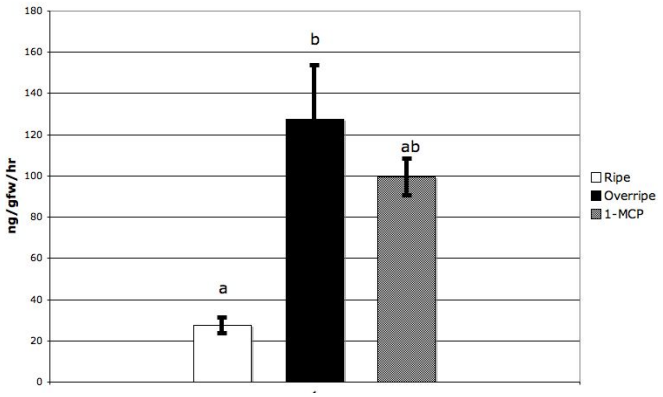

Figure C-10. Hexanal production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 5 days to reach this hue value, at which time an additional control (hue angle at 50° + 5 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

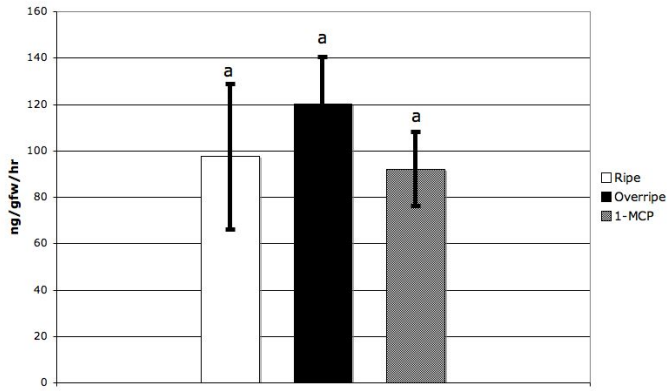

Figure C-11. Hexanal production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 11 days to reach this hue value, at which time an additional control (hue angle at 50° + 11 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

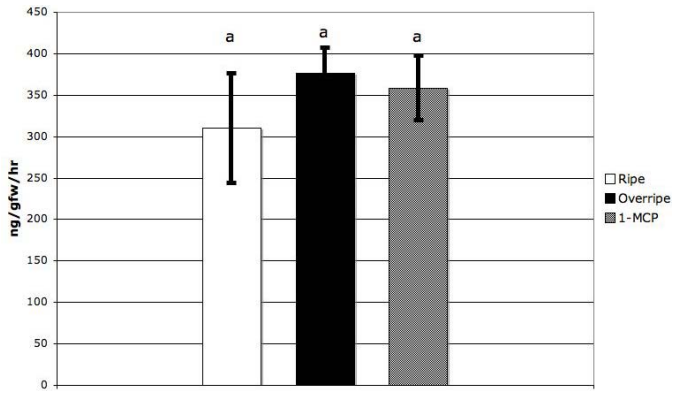

Figure C-12. Hexanal production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 6 days to reach this hue value, at which time an additional control (hue angle at 50° + 6 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

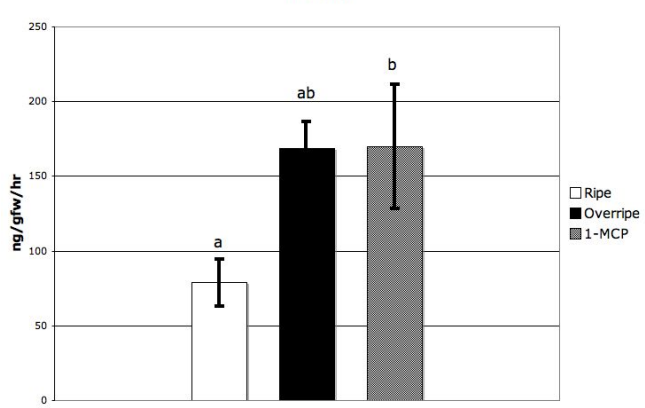

Figure C-13. Hexanal production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 4 days to reach this hue value, at which time an additional control (hue angle at 50° + 4 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

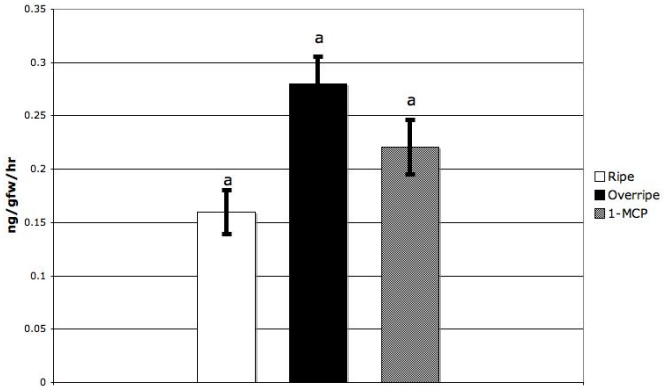

Figure C-14. Ethyl vinyl ketone production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 5 days to reach this hue value, at which time an additional control (hue angle at 50° + 5 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

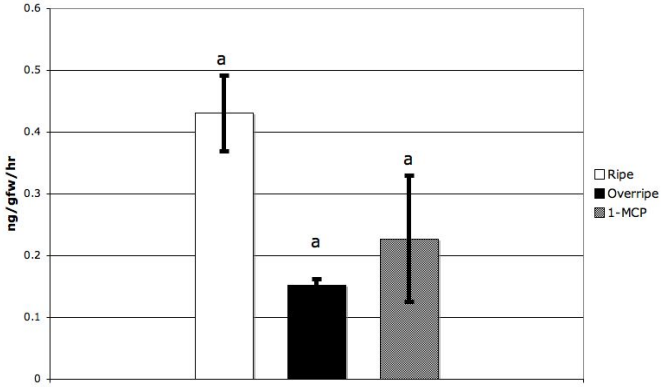

Figure C-15. Ethyl vinyl ketone production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 11 days to reach this hue value, at which time an additional control (hue angle at 50° + 11 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

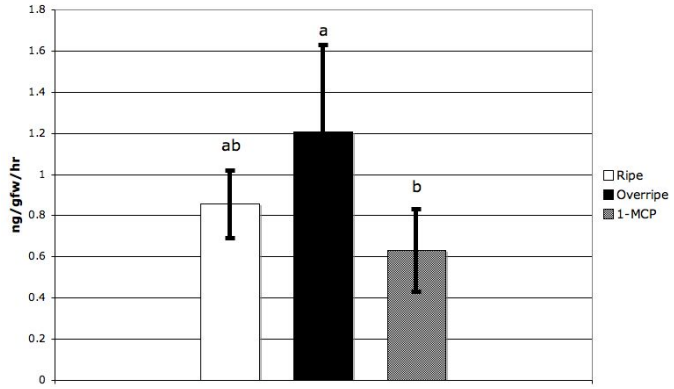

Figure C-16. Ethyl vinyl ketone production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 6 days to reach this hue value, at which time an additional control (hue angle at 50° + 6 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

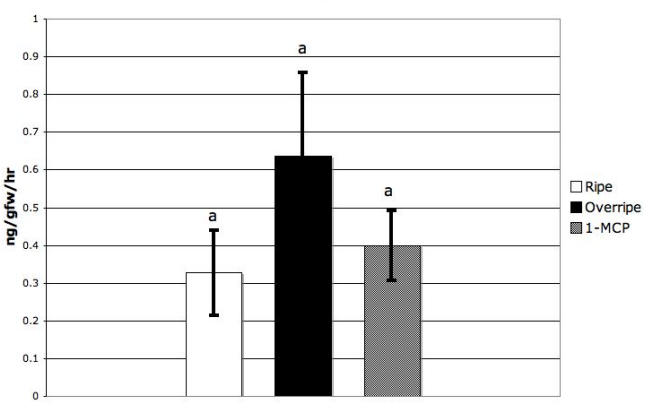

Figure C-17. Ethyl vinyl ketone production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 4 days to reach this hue value, at which time an additional control (hue angle at 50° + 4 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

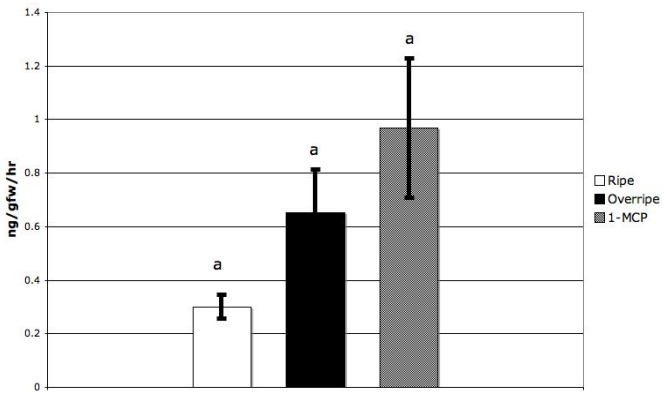

Figure C-18. *Trans*-2-hexenal production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 5 days to reach this hue value, at which time an additional control (hue angle at 50° + 5 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

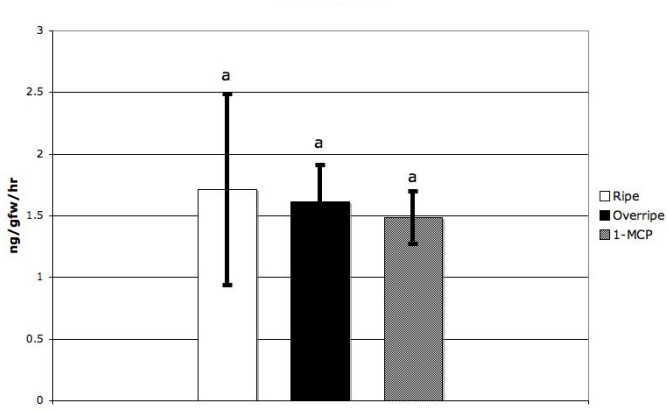

Figure C-19. *Trans*-2-hexenal production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 11 days to reach this hue value, at which time an additional control (hue angle at 50° + 11 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

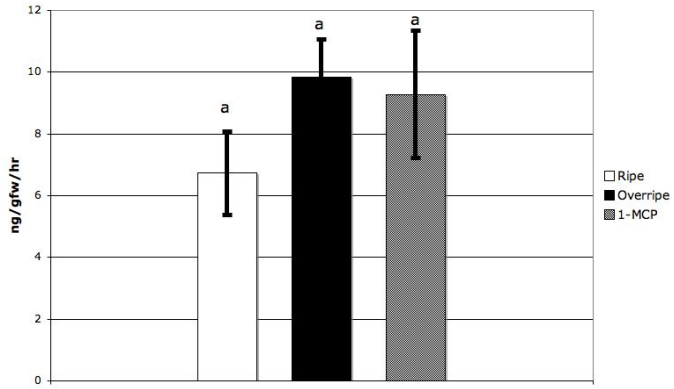

Figure C-20. *Trans*-2-hexenal production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 6 days to reach this hue value, at which time an additional control (hue angle at 50° + 6 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

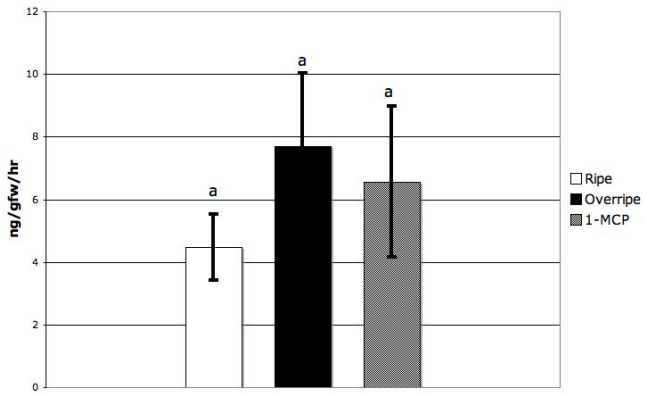

Figure C-21. *Trans*-2-hexenal production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 4 days to reach this hue value, at which time an additional control (hue angle at 50° + 4 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

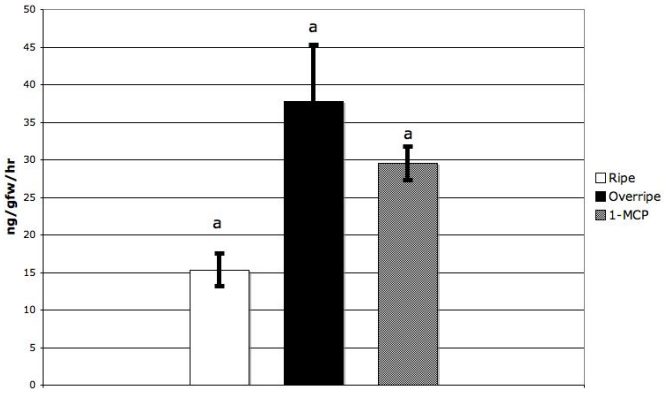

Figure C-22. *Cis*-3-hexenol production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 5 days to reach this hue value, at which time an additional control (hue angle at 50° + 5 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

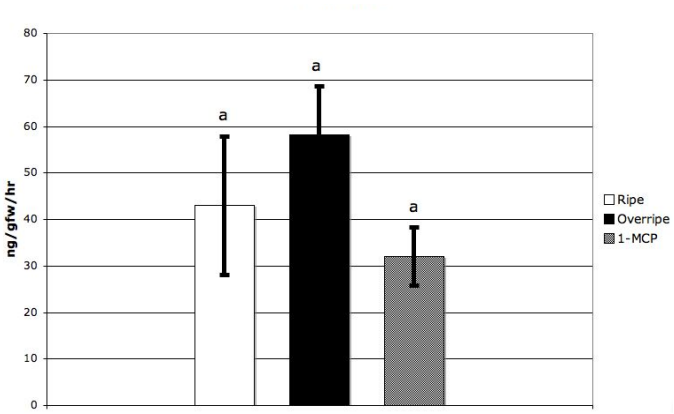

Figure C-23. *Cis*-3-hexenol production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 11 days to reach this hue value, at which time an additional control (hue angle at 50° + 11 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

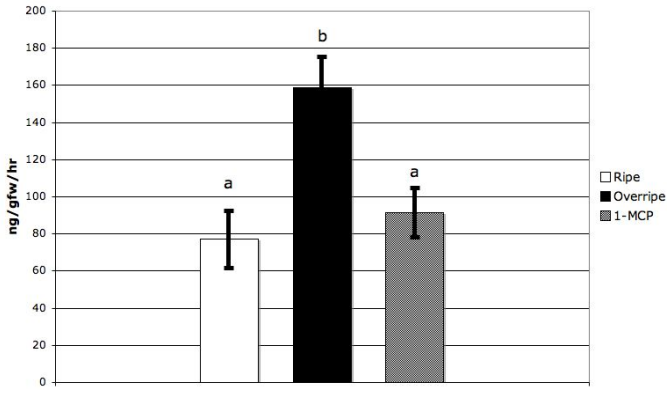

Figure C-24. *Cis*-3-hexenol production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 6 days to reach this hue value, at which time an additional control (hue angle at 50° + 6 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

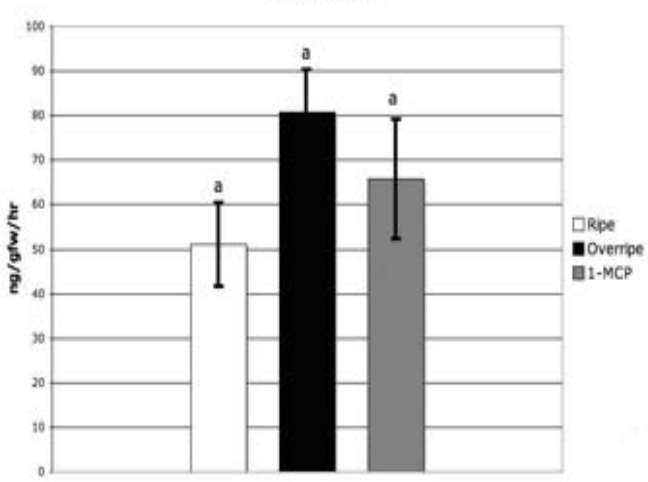

Figure C-25. *Cis*-3-hexenol production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 4 days to reach this hue value, at which time an additional control (hue angle at 50° + 4 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

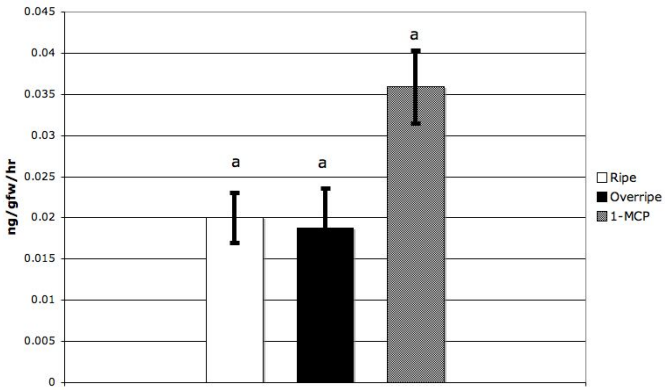

Figure C-26. ß-ionone production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 5 days to reach this hue value, at which time an additional control (hue angle at 50° + 5 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

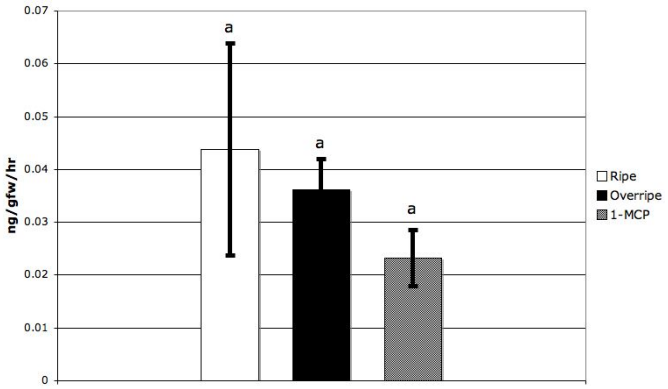

Figure C-27. ß-ionone production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 11 days to reach this hue value, at which time an additional control (hue angle at 50° + 11 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

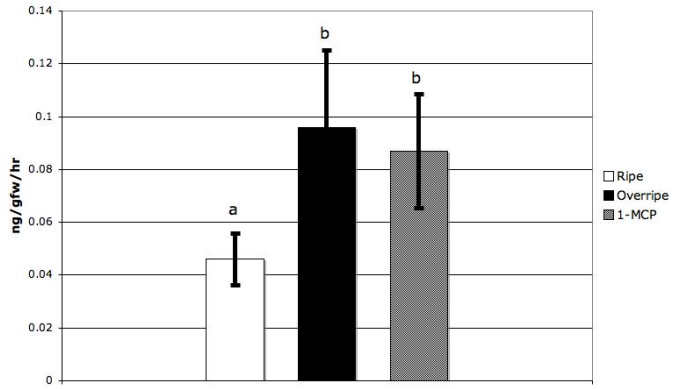

Figure C-28. ß-ionone production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 6 days to reach this hue value, at which time an additional control (hue angle at 50° + 6 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

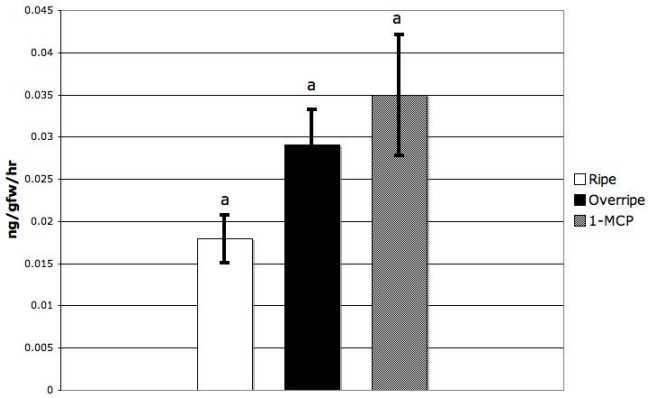

Figure C-29. ß-ionone production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 4 days to reach this hue value, at which time an additional control (hue angle at 50° + 4 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

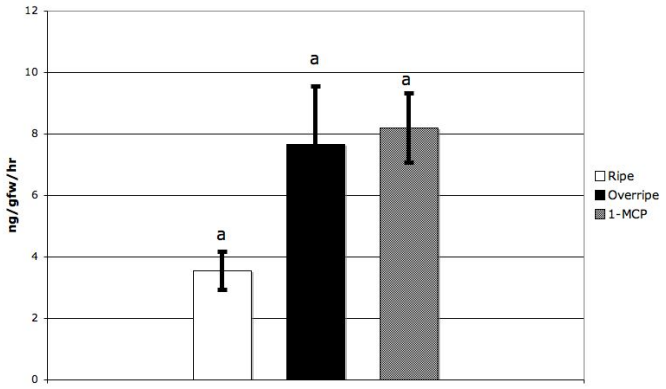

Figure C-30. 6-methyl-5-hepten-2-one production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 5 days to reach this hue value, at which time an additional control (hue angle at 50° + 5 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

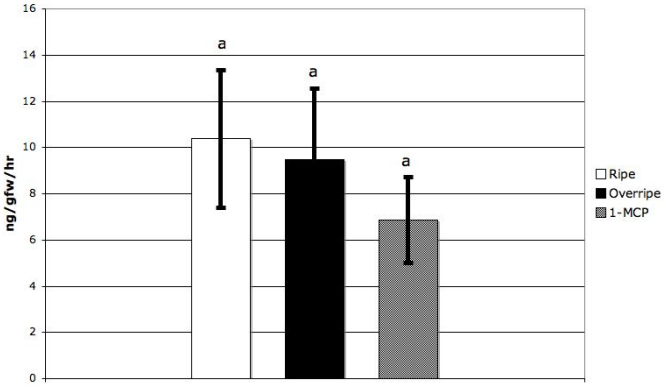

Figure C-31. 6-methyl-5-hepten-2-one production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 11 days to reach this hue value, at which time an additional control (hue angle at 50° + 11 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

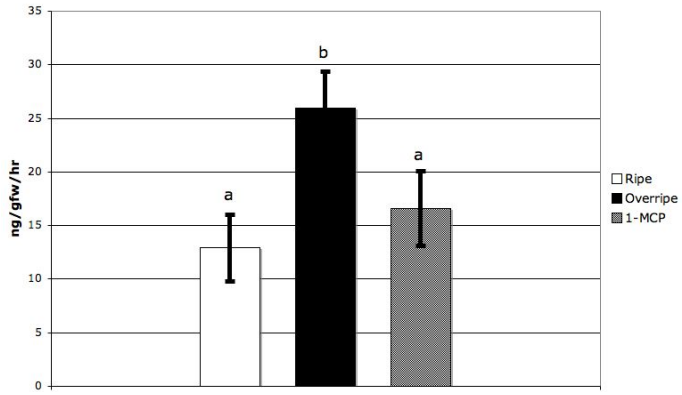

Figure C-32. 6-methyl-5-hepten-2-one production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 6 days to reach this hue value, at which time an additional control (hue angle at 50° + 6 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

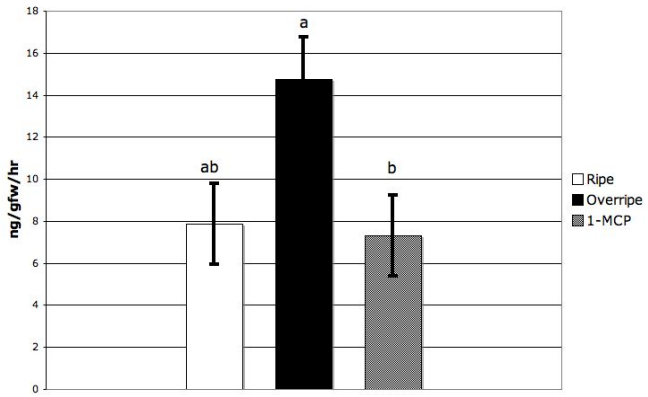

Figure C-33. 6-methyl-5-hepten-2-one production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 4 days to reach this hue value, at which time an additional control (hue angle at 50° + 4 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

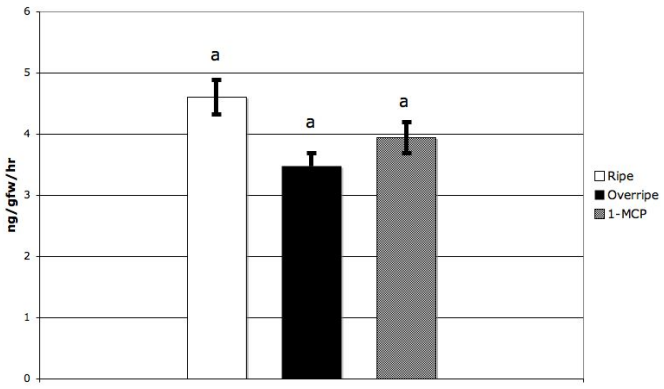

Figure C-34. Isovaleraldehyde production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 5 days to reach this hue value, at which time an additional control (hue angle at 50° + 5 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

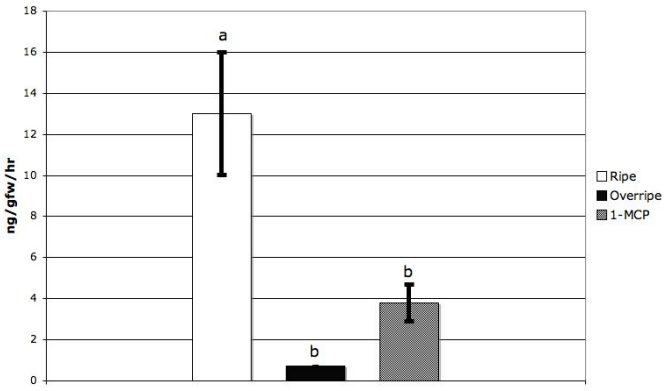

Figure C-35. Isovaleraldehyde production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 11 days to reach this hue value, at which time an additional control (hue angle at 50° + 11 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

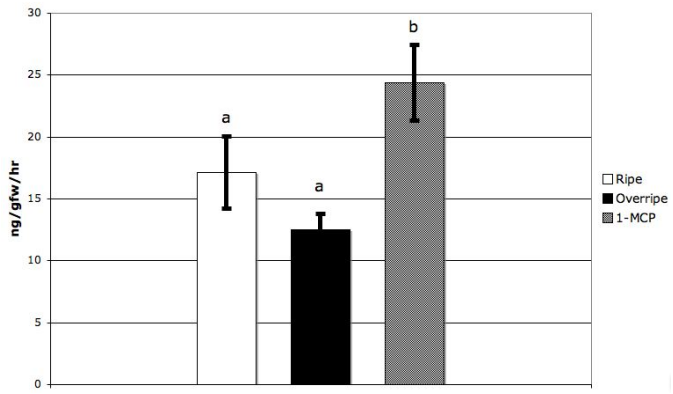

Figure C-36. Isovaleraldehyde production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 6 days to reach this hue value, at which time an additional control (hue angle at 50° + 6 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

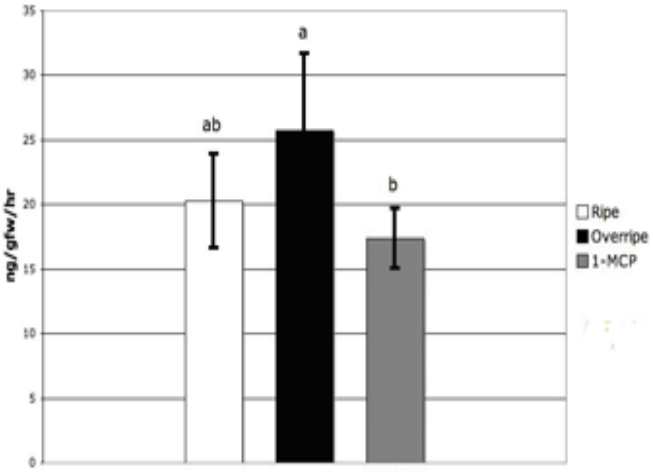

Figure C-37. Isovaleraldehyde production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 4 days to reach this hue value, at which time an additional control (hue angle at 50° + 4 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

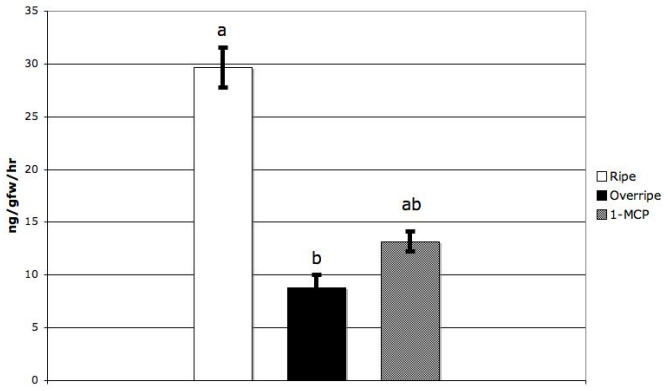

Figure C-38. 3-methyl-1-butanol production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 5 days to reach this hue value, at which time an additional control (hue angle at 50° + 5 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

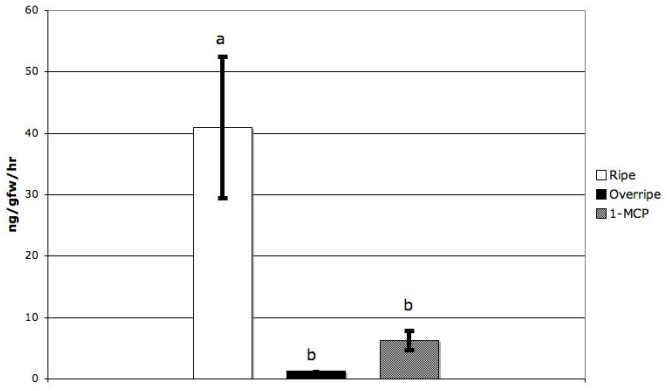

Figure C-39. 3-methyl-1-butanol production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 11 days to reach this hue value, at which time an additional control (hue angle at 50° + 11 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

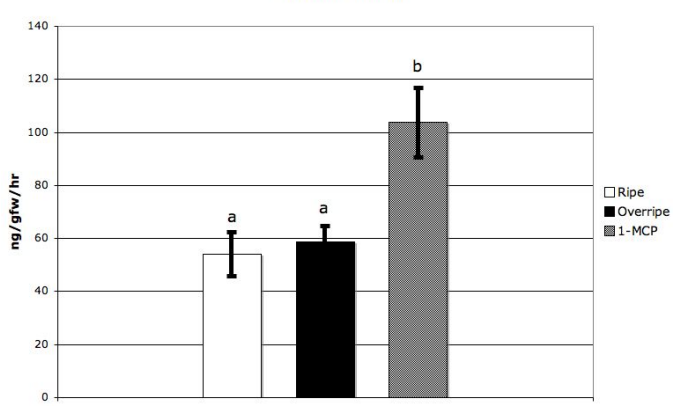

Figure C-40. 3-methyl-1-butanol production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 6 days to reach this hue value, at which time an additional control (hue angle at 50° + 6 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

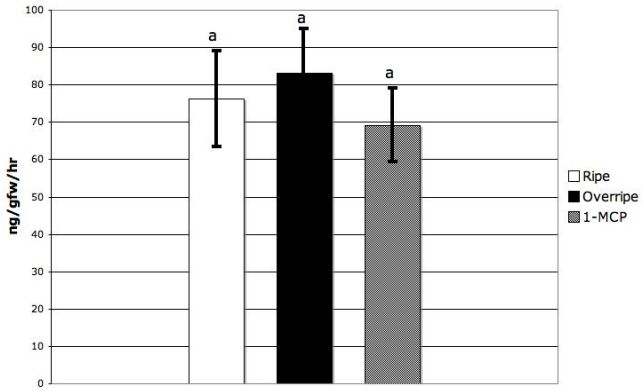

Figure C-41. 3-methyl-1-butanol production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 4 days to reach this hue value, at which time an additional control (hue angle at 50° + 4 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

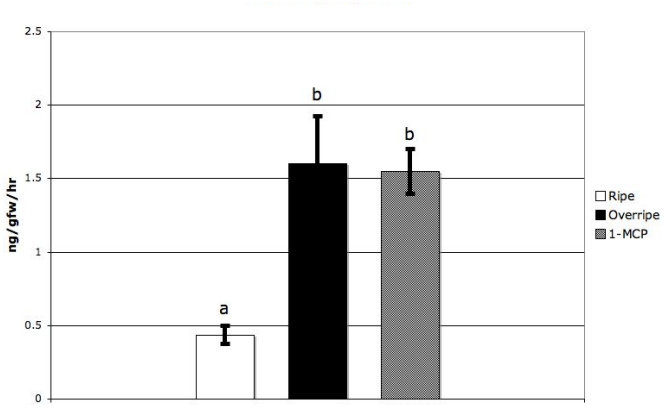

Figure C-42. 1-nitro-2-phenylethane production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 5 days to reach this hue value, at which time an additional control (hue angle at 50° + 5 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

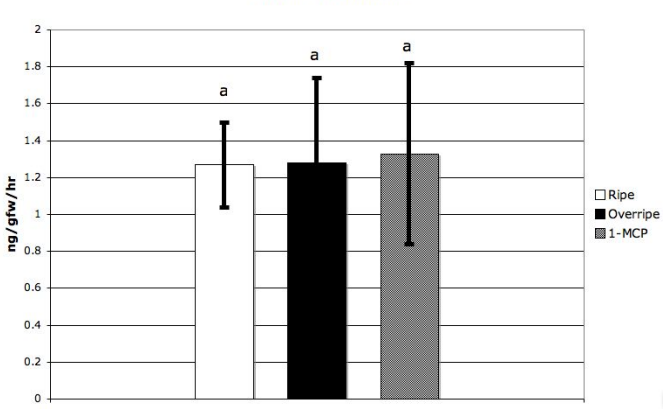

Figure C-43. 1-nitro-2-phenylethane production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 11 days to reach this hue value, at which time an additional control (hue angle at 50° + 11 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

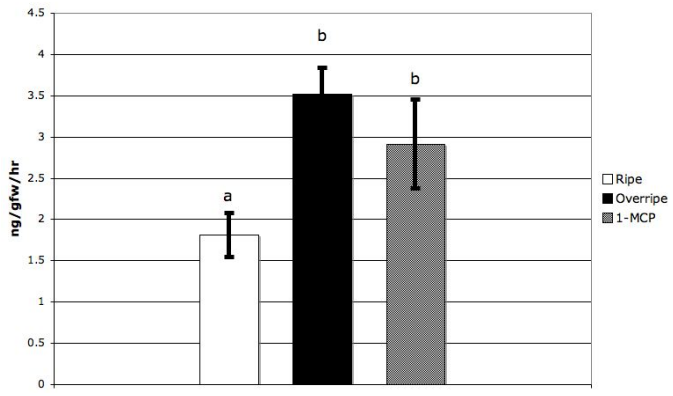

Figure C-44. 1-nitro-2-phenylethane production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 6 days to reach this hue value, at which time an additional control (hue angle at 50° + 6 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

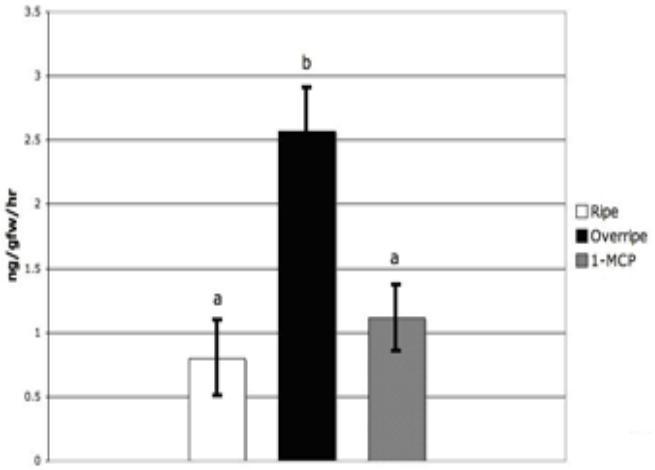

Figure C-45. 1-nitro-2-phenylethane production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 4 days to reach this hue value, at which time an additional control (hue angle at 50° + 4 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

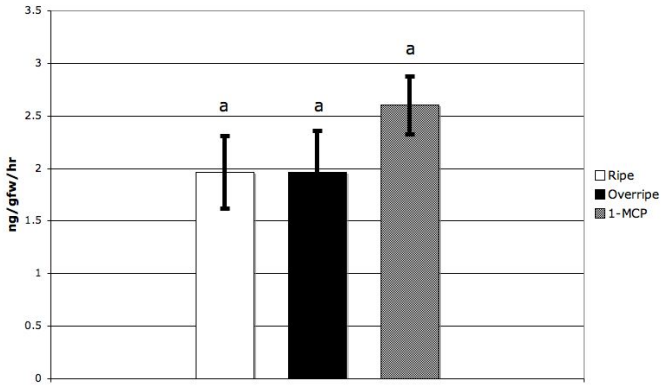

Figure C-46. 2-phenylethanol production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 5 days to reach this hue value, at which time an additional control (hue angle at 50° + 5 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

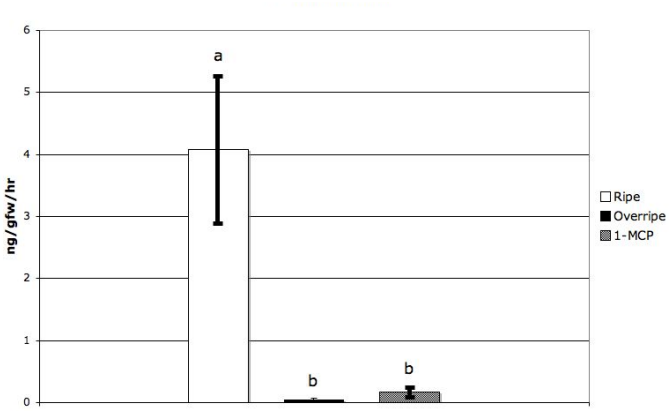

Figure C-47. 2-phenylethanol production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 11 days to reach this hue value, at which time an additional control (hue angle at 50° + 11 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

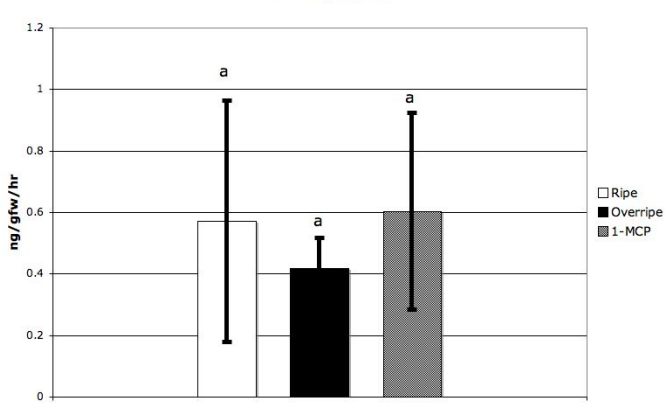

Figure C-48. 2-phenylethanol production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 6 days to reach this hue value, at which time an additional control (hue angle at 50° + 6 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

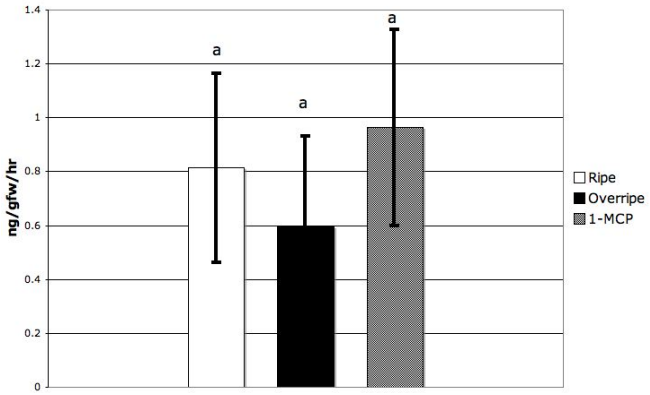

Figure C-49. 2-phenylethanol production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 4 days to reach this hue value, at which time an additional control (hue angle at 50° + 4 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

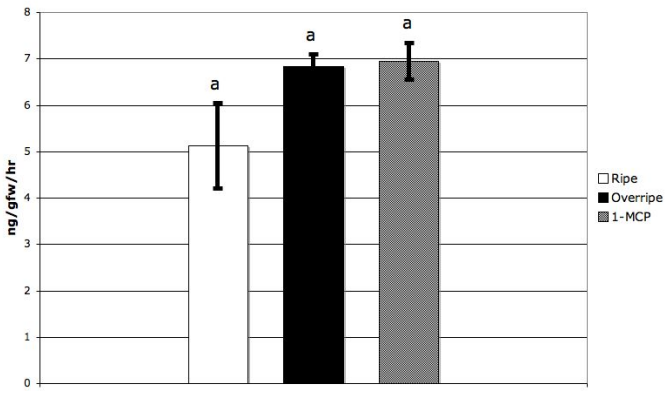

Figure C-50. 2-methylbutyraldehyde production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 5 days to reach this hue value, at which time an additional control (hue angle at 50° + 5 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

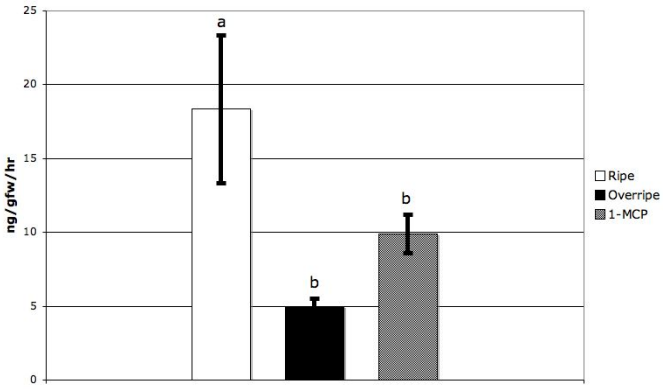

Figure C-51. 2-methylbutyraldehyde production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 11 days to reach this hue value, at which time an additional control (hue angle at 50° + 11 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

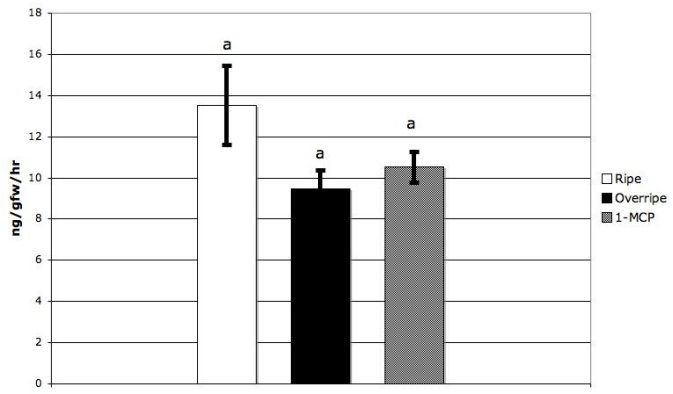

Figure C-52. 2-methylbutyraldehyde production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 6 days to reach this hue value, at which time an additional control (hue angle at 50° + 6 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

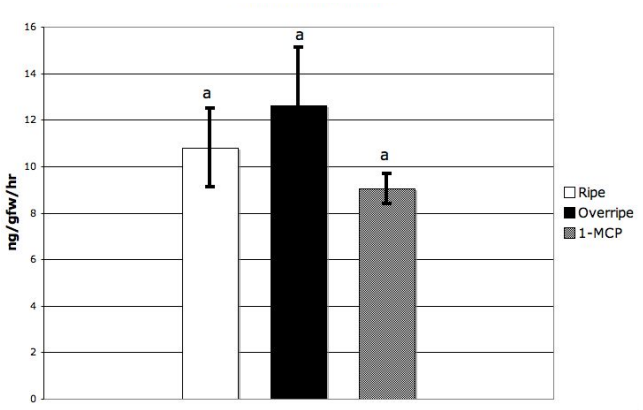

Figure C-53. 2-methylbutyraldehyde production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 4 days to reach this hue value, at which time an additional control (hue angle at 50° + 4 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

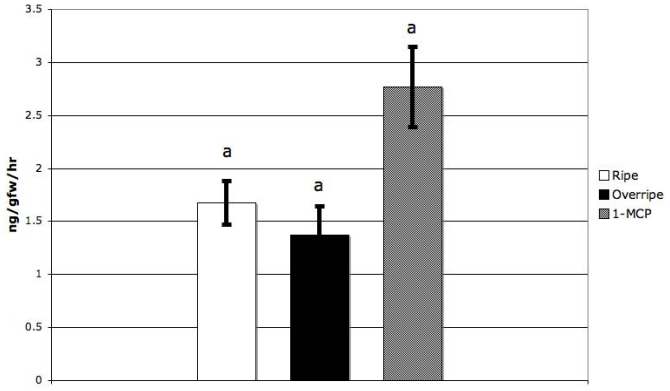

Figure C-54. 2-isobutylthiazole production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 5 days to reach this hue value, at which time an additional control (hue angle at 50° + 5 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

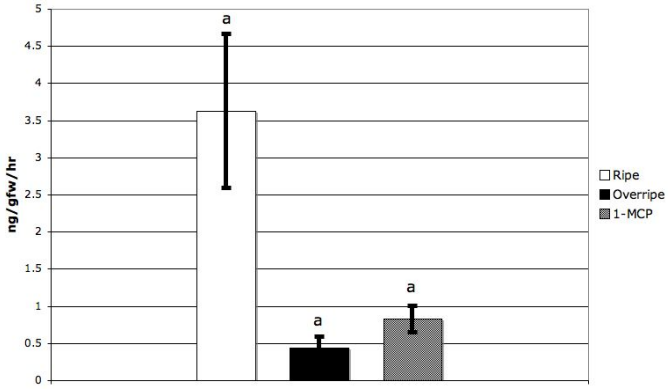

Figure C-55. 2-isobutylthiazole production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 11 days to reach this hue value, at which time an additional control (hue angle at 50° + 11 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

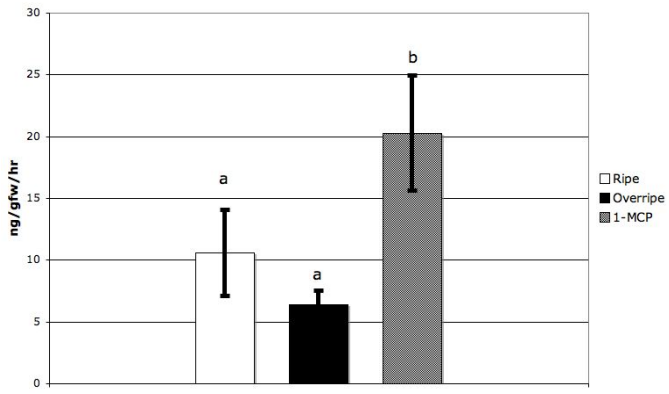

Figure C-56. 2-isobutylthiazole production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 6 days to reach this hue value, at which time an additional control (hue angle at 50° + 6 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

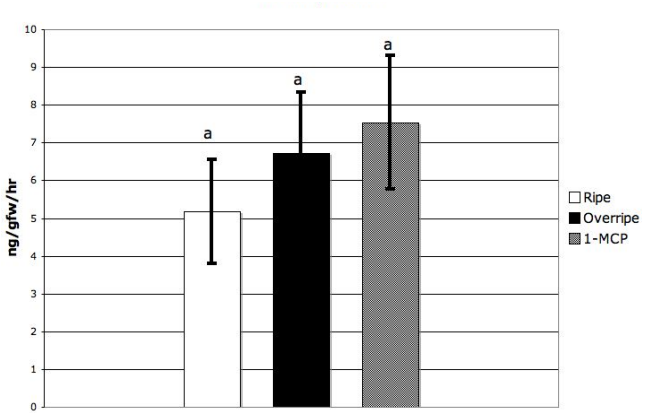

Figure C-57. 2-isobutylthiazole production in ripe 'FL-91' tomato fruit. Fruit were treated with air or 1-MCP (500 ppb, 12 h, 20°C) at the breaker stage and ripened at 20°C. Volatile production was measured when fruit reached an average hue angle of 50°. 1-MCP treated fruit required an additional 4 days to reach this hue value, at which time an additional control (hue angle at 50° + 4 days) sample was analyzed. Values represent the average of five fruit, +/- standard error.

LIST OF REFERENCES

Abbott JA, 1999. Quality measurement of fruits and vegetables. Postharvest Biol and Technol 15:207-225.

Abdi N, McGlasson WB, Holford P, Williams M, Mizrahi Y, 1998. Responses of climacteric and suppressed-climacteric plums to treatment with propylene and 1-methylcyclopropene. Postharvest Biol and Technol 14:29-39

Acree, 1993 Bioassays for flavor. In: Acree T., Teranishi R. (ed.) Flavor Science: Sensible Principles and Techniques. Am Chem Soc, Washington D.C., 1-20

Alexander L, Grierson D, 2002. Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. J of Exp Bot 53:2039-2055.

Bagnato N, Barrett R, Sedgley M, Klieber A, 2003. The effects on the quality of Cavendish bananas, which have been treated with ethylene, of exposure to 1-methylcyclopropene. Int J of Food Sci and Technol 38:745-750.

Baldwin EA, Nisperos-Carriedo MO, Moshonas MG, 1991. Quantitative analysis of flavor and other volatiles and for certain constituents of two tomato cultivars during ripening. J. Amer. Soc. Hort. Sci. 116(2):265-269.

Baldwin EA, Scott JW, Shewmaker CK, Schuch W, 2000. Flavor trivia and tomato aroma: Biochemistry and possible mechanisms for control of important aroma components. HortScience 35(6):1013-1022.

Blankenship SM, Dole JM, 2003. 1-Methylcyclopropene: a review. Postharvest Biol. Technol. 28:1-25

Bleecker AB, Kende H, 2000. Ethylene: a gaseous signal molecule in plants. Annu. Rev. Cell Dev. Biol. 16:1-18.

Boukobza F, Taylor AJ, 2002. Effect of postharvest treatment on flavour volatiles of tomatoes. Postharvest Biol and Technol 25:321-331.

Brandt AS, Woodson WR, 1992. Variation in flower senescence and ethylene biosynthesis among carnations. HortScience 27(10):1100-1102.

Brecht JK, Shewfelt RL, Garner JC, Tollner, EW, 1991. Using x-ray computer tomography to nondestructively determine tomato maturity. HortScience 26(1):45-47.

Buttery RG, Ling LC, 1992. Volatile components of tomato fruit and plant parts. In: Teranishi R, Buttery RG, Sugisawa H. (ed.) Bioactive Volatile Compounds from Plants. Am Chem Soc, Washington D.C., 24-34.

Buttery, RG, 1993. Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits. In: Acree T., Teranishi R. (ed.) Flavor Science: Sensible Principles and Techniques. Am Chem Soc, Washington D.C., 259-286

Carbonell-Barrachina AA, Agustí A, Ruiz JJ, 2006. Analysis of flavor volatile compounds by dynamic headspace in traditional and hybrid cultivars of Spanish tomatoes. Eur. Food Res Technol. 222:536-542

Chace EM, Denny FE, 1924. Use of ethylene in the coloring of citrus fruit. Ind and Eng Chem 16:339-340

Defilippi BG, Dandekar AM, Kader AA, 2004. Impact of suppression of ethylene action or biosynthesis on flavor metabolites in apple (*Malus domestica* Borkh) fruits. J. Agric. Food Chem. 52:5694-5701.

Davies JN, Hobson GE, 1981. The constituents of tomato fruit—the influence of environment, nutrition, and genotype. CRC Crit Rev Food Sci Nutr 15:205-280

Ealing, 1994. Lipoxygenase activity in ripening tomato fruit pericarp tissue. Phytochemistry. 36:547-552

Eddington, Todd, Agrofresh, personal communication

Florida Automated Weather Network, University of Florida IFAS, Gainesville, FL http://fawn.ifas.ufl.edu accessed July 2006.

Florida Tomato Committee Regulatory Bulletin No. 1 2005-2006. http://www.floridatomatoes.org/domestic.html accessed July 2006.

Florida Tomato Facts and Sizes, Florida Tomato Committee, Maitland, FL. http://www.floridatomatoes.org/facts.html accessed July 2006.

Food and Agriculture Organization (FAO) of the United Nations Statistical Databases http://faostat.fao.org accessed July 2006.

Galil, J. 1968. An ancient technique for ripening sycomore fruit in East-Meditteranean countries. Economic Bot 22:178-190.

Gane R, 1934. Production of ethylene by some fruits. Nature 134:1008.

Goff SA, Klee HJ, 2006. Plant volatile compounds: Sensory cues for health and nutritional value. Science 311:815-819.

Golding JB, Shearer D, McGlasson WB, Wyllie SG, 1999. Relationships between respiration, ethylene, and aroma production in ripening banana. J. Agric. Food Chem 47:1646-1651.

Hackett RM, Ho CW, Lin Z, Foote HCC, Fray RG, Grierson D, 2000. Antisense inhibition of the *Nr* gene restores normal ripening to the tomato *Never-ripe* mutant, consistent with the ethylene receptor-inhibition model. Plant Physiol 124:1079-1085.

Hobson G, September 1988. How the tomato lost its taste. New Scientist 29:46-50.

Hoeberichts, FA, Van der Plas LHW, Woltering EJ, 2002. Ethylene perception is required for the expression of tomato ripening-related genes and associated physiological changes evene at advanced stages of ripening. Postharvest Biol. And Technol. 26:125-133.

Hurr BM, Huber DJ, Lee JH, 2005. Differential responses in color changes and softening of 'Florida-47' tomato fruit treated at green and advanced ripening stages with the ethylene antagonist 1-Methylcyclopropene. HortTechnol 15(3):617-622

Jayaraman KS, Raju PS, 1992. Development and evaluation of a permanganate-based ethylene scrubber for extending the shelf life of fresh fruits and vegetables. J. Fd. Sci. Technol. 29(2):77-83.

Jeong J, Huber DJ, 2004. Suppression of avocado (*Persea americana* Mill.) fruit softening and changes in cell wall matrix polysaccharides and enzyme activities: Differential responses to 1-MCP and delayed ethylene application. J. Amer. Soc. Hort. Sci. 129(5):752-759.

Jeong J, Huber DJ, Sargent SA, 2002. Delay of avocado (*Persea americana*) fruit ripening by 1-methylcyclopropene and wax treatments. Postharvest Biol and Technol 28:247-257.

Johnson JH, Gould WA, Badenhop AF, Johnson RM, 1968. Quantitative comparison of isoamylol, pentanol, and 3-hexenol-1 in tomato juice: Varietal and harvest differences and processing effects. J. Agric. Food Chem. 16:255-258.

Kausch KD, Handa AK, 1997. Molecular cloning of a ripening-specific lipoxygenase and its expression during wild-type and mutant tomato fruit development. Plant Physiol. 113:1041-1050.

Kramer M, Sanders R, Bolkan H, Waters C, Sheehy RE, Hiatt WR, 1992. Postharvest evaluation of transgenic tomatoes with reduced levels of polygalacturonase: Processing, firmness, and disease resistance. Postharvest Biol. and Technol. 1:241–255.

Krumbein A, Auerswald H, 1998. Characterization of aroma volatiles in tomatoes by sensory analysis. Nahrung/Food 42(6):395-399.

Lurie S, Pre-Aymard C, Ravid U, Larkov O, Fallik E, 2002. Effect of 1-methylcyclopropene on volatile emission and aroma in Cv. Anna apples. J. Agric. Food Chem. 50:4251-4256.

Maul F, Sargent SA, Balaban MO, Baldwin EA, Huber DJ, Sims CA. 1998. Aroma volatile profiles from ripe tomato fruit are influenced by physiological maturity at harvest: an application for electronic nose technology. J. Amer. Soc. Hort. Sci.123(6):1094-1101.

Maul F, Sargent SA, Sims CA, Baldwin EA, Balaban MO, Huber DJ 2000. Tomato flavor and aroma quality as affected by storage temperature. J. Food Sci. 65:1228-1237.

Mir N, Canoles M, Beaudry R, 2004. Inhibiting tomato ripening with 1-methylcyclopropene. J. Am. Soc. Hort. Sci. 129(1):112-120.

Mitcham B, Mattheis J, Bower J, Biasi B, Clayon M, 2001. Response of european pears to 1-MCP. Perishables Handling Quarterly 108:16-19.

Moretti CL, Baldwin EA, Sargent SA, Huber DJ, 2002. Internal bruising alters aroma volatile profiles in tomato fruit tissues. HortScience 37(2):378-382.

Nix v. Hedden, 149 U.S. 304, 1893

Oeller PW, Wong LM, Taylor LP, Pike DA, Theologis A, 1991. Reversible inhibition of tomato fruit senescence by antisense RNA. Science 254:437-439

Pelayo C, Vilas-Boas EVdeB, Benichou M, Kader AA 2003. Variability in responses of partially ripe bananas to 1-methylcyclopropene. Postharvest Biol and Technol 28:75-85.

Rodriguez FI, Esch JJ, Hall AE, Binder BM, Schaller GE, Bleecker AB, 1999. A copper cofactor for the ethylene receptor ETR1 from Arabidopsis. Science 283: 996-998

Sargent and Moretti, 2004. Tomato. In: USDA Handbook 66. http://usna.usda.gov/hb66/138tomato.pdf accessed: July 2006.

Sargent SA, Brecht JK, Olczyk T, 2005. Handling Florida Vegetables Series—Round and Roma Tomato Types. SS-VEC-928 Gainesville, FL: University of Florida IFAS, http://edis.ifas.ufl.edu/VH079 accessed: July 2006.

Schmelz EA, Alborn HT, Tumlinson JH, 2001. The influence of intact-plant and excised-leaf bioassay designs on volicitin- and jasmonic acid-induced sesquiterpene volatile release in *Zea mays*. Planta 214:171-179.

Schutte L, March 1974. Precursors of sulfur-containing flavor compounds. Crit Rev in Food Technol 4:457-505.

Serek M, Reid MS 1993. Anti-ethylene treatments for potted Christmas cactus--Efficacy of inhibitors of ethylene action and biosynthesis. HortScience 28(12):1180-1181.

Sisler EC, Serek M, 1997. Inhibitors of ethylene responses in plants at the receptor level: Recent developments. Physiologia Plantarum 100:577-582.

Sisler EC, Reid MS, Yang SF, 1986. Effect of antagonists of ethylene action on binding of ethylene in cut carnations. Plant Growth Reg 4(3):213-218.

Sisler EC, Blankenship SM, 1993. Effect of diazocyclopentadiene on tomato ripening. Plant Growth Reg. 12:155-160.

Serek M, Sisler EC, Reid MS, 1994. Novel gaseous ethylene binding inhibitor prevents ethylene effects in potted flowering plants. J. Am. Soc. Hortic. Sci. 119, 1230–1233.

Stevens MA, Kader AA, Albright-Holton M, Algazi, M. Genotypic variation for flavor and composition in fresh-market tomatoes. J. Am. Soc. Hortic. Sci. 102:680-689.

Tandon KS, Baldwin EA, Scott JW, Shewfelt RL, 2003. Linking sensory descriptors to volatile and nonvolatile components of fresh tomato flavor. J of Food Sci 68(7):2366-2371.

Tieman D, Zeigler M, Schmelz M, Taylor M, Bliss P, Kirst M, Klee H, 2006. Identification of loci affecting flavor volatile emissions in tomato fruits. J Exp Bot 57(4):887-896.

USDA Agricultural Marketing Service, Fruit and Vegetable Division, Fresh Products Branch. 1991. United States standards for grades of fresh tomatoes. http://www.ams.usda.gov/standards/tomatfrh.pdf accessed: July 2006.

Veen H, van de Geijn SC 1978. Mobility and ionic form of silver as related to longevity of cut carnations. Planta 140:93-96.

Ververidis P, John P, 1991. Complete recovery *in vitro* of ethylene-forming enzyme activity. Phytochemistry 30:725-727.

Wright DH, Harris ND, 1985. Effect of nitrogen and potassium fertilization on tomato flavor. J. Agric. Food Chem. 33:355-358.

Yahyaoui FEL, Chalermchai WA, Latché A, Hackett R, Grierson D, Pech JC, 2002. Molecular and biochemical characteristics of a gene encoding an alcohol acyl-transferase involved in the generation of aroma volatile esters during melon ripening. Eur. J. Biochem 269:2359-2366.

Yang SF, Hoffman NE 1984. Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Physiol. 35, 155.

Zhu HL, Zhu BZ, Fu DQ, Xie YH, Hao YL, Luo YB 2005. Role of ethylene in the biosynthetic pathways of aroma volatiles in ripening fruit. Russian J of Plant Phys 52(5):691-695.

BIOGRAPHICAL SKETCH

Sonya Leonore Stahl was born on October 22, 1981, in New York City, NY, and grew up in Coral Springs, FL. She graduated from J. P. Taravella High School and entered college in 1999. In 2004, she graduated from the University of Florida with a Bachelor of Science degree in Natural Resources and Conservation and a Bachelor of Arts degree in Violin Performance. That summer, she returned to the same university to pursue a Master of Science degree in Horticultural Sciences, which she obtained in December 2006.

Ms. Stahl works at East Coast Fruit, continuing her lifelong ambition of working with fruits and vegetables--an interest that was born in the hydroponic greenhouse of Disney's EPCOT Center when she was six years old.