INTERACTION OF WAX, FUNGICIDE AND ETHYLENE TREATMENTS ON

STORAGE AND SHELF-LIFE OF SATSUMA MANDARINS

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INTERACTION OF WAX, FUNGICIDE AND ETHYLENE TREATMENTS ON STORAGE AND SHELF-LIFE OF SATSUMA MANDARINS

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VITA

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THESIS ABSTRACT

INTERACTION OF WAX, FUNGICIDE AND ETHYLENE TREATMENTS ON STORAGE AND SHELF-LIFE OF SATSUMA MANDARINS

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Satsuma mandarins (*Citrus unshiu* Marc.) were once the center of an important industry along the Gulf Coast of the United States. Several freezes in the early 1900's essentially wiped out the industry. In recent years there has been renewed interest in development of the industry. Currently little is known about the optimal postharvest practices for these small delicate fruit. Without improved knowledge of postharvest practices, the industry will not be able to expand into new markets, such as retail stores.

Retail stores require fruit to be of a specific external quality, namely, orange, blemish free fruit with a turgid appearance. Since Satsumas can reach maturity prior to obtaining the required orange peel color, fruit often need to be degreened with an exogenous application of ethylene. Fungicides can be used to prevent disease related blemishes, while the use of wax may allow fruit to keep their turgid appearance for longer periods.

In order to identify the most efficient means of providing fruit that meet retail store specifications, a postharvest study was initiated to determine the best combination of degreening, waxing and treating with fungicides for both storage and shelf-life. Fruit were assigned to one of twelve treatments with combinations of ethylene (ethylene or no ethylene), wax (waxed or no wax), and fungicide (Imazalil, 2-(4-thiazolyl) benzimidazole, or no fungicide). Fruit with an ethylene treatment were exposed to 5ppm of ethylene for 24 hours at room temperature.

Both external peel (color and amount of disease) and internal quality (Brix, titratable acidity, and Vitamin C) measures were taken for each treatment. Treating fruit at 5ppm of ethylene for 24 hours at room temperature had only a minor effect in promoting color development. Waxing the fruit had the greatest effect on preserving fruit quality, especially size and weight. Fungicide had a minor effect on preserving fruit quality, in part because disease incidence was low. In future studies, the impact of ethylene on internal quality needs to be monitored with respect to taste. Waxing also had a detrimental effect on Vitamin C in both years of the study.

Higher rates of ethylene, higher temperatures, or longer exposure time to ethylene are most likely needed to achieve acceptable degreening. In a pilot study, fruit were exposed to higher concentrations of ethylene in order to determine if a higher rate of ethylene provides better color development. Results of the pilot study indicates that higher levels of ethylene and storage at room temperature provide for better color development compared to storage in a cold environment or no ethylene. Future research needs to identify the proper application rate of ethylene that promotes color development while minimizing fruit damage from excessive amounts of ethylene.

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I. INTRODUCTION AND LITERATURE REVIEW

During the early 1900's, the Gulf Coast was the center of a young and prosperous citrus industry. The industry evolved around a small, delicate fruit called the Satsuma mandarin, *Citrus unshiu* Marc. (Rucker, 1996; Ebel et al., 2004). Satsuma mandarins originated in China and are a member of the mandarin group of citrus. There are four mandarin species including: 1) *Citrus reticulata* (Clementine), 2) *Citrus deliciosa* (Mediterranean), 3) *Citrus nobilis* (King), and 4) *Citrus unshiu* (Satsumas) (Saunt, 2000). Satsumas are, therefore, closely related to the more common tangerine and Clementine. Currently, there are several varieties of mandarins in commercial production. The varieties are placed into groups according to maturity date: 1) Goko Wase (very early group), 2) Wase (early group), 3) Nakate or Chusei (midseason group), and 4) Common Group or Bansei (late group) (Saunt, 2000). In Alabama 'Owari', a late variety, is the most commonly grown.

The climate along the Alabama Gulf Coast is extremely well suited for Satsuma production. Cool nights promote excellent internal and external quality development during the ripening period (Ebel et al., 2004). In 1924, Alabama Satsuma acreage reached the height of production with approximately 4,900 bearing hectares (Dozier, 1924). Over the years, however, several weather phenomena, especially freezes and hurricanes, reduced yields and destroyed trees. Three devastating freezes occurred during the 1930's injuring trees and eliminating Satsuma fruit production during those years (Winberg, 1948). Another major freeze occurred in 1940 and destroyed what was left of this once promising industry (Winberg, 1948).

Since the early 1980's, there has been renewed interest in the production of Satsumas along the Gulf Coast. Without further research, however, the new industry is destined to be destroyed in the same manner as its predecessor (Ebel et al, in press). For the past several years, due in large part to federal and state grants, researchers have developed new, innovative techniques and strategies to ensure the Alabama Satsuma industry survives for many years to come.

There are currently two main research focuses of this large-scale project. The first involves marketing. Marketing research has focused primarily on consumer preferences for the Satsuma mandarin. Campbell et al. (2004) examined consumer preference for external attributes, such as price, size, and color. Their research verified the industry standards of large, orange, non-blemished fruit as being what consumers prefer. Market segments within the Satsuma market were also identified. More recent marketing research has focused on identification of optimal internal quality via sensory evaluation, comparison of preference for various mandarins, and packaging preference (Campbell, unpublished data).

The second focus of the research deals with production issues, including freeze prevention and postharvest handling. Ongoing freeze prevention research is being conducted with regards to cold tolerant selections (Zhang et al., 2002) and methods of freeze protection, such as micro-sprinkler irrigation (Nesbitt et al., 2000). The lack of a means to eliminate or reduce freeze injury was the main reason the industry suffered devastating losses in the past.

Without an understanding of the optimum postharvest storage methods, both marketing and freeze prevention are useless. This is due to the fact that, consumers will not purchase a product that is of inferior quality and growers will not produce a product that they are unable to sell. In order to address the postharvest issues confronting the growers, a research study was implemented to examine several postharvest handling techniques relating to fruit quality. Research into postharvest handling and storage will help producers maintain high fruit quality from tree to consumer.

Post Harvest Handling

Satsuma mandarins are a very delicate fruit and are prone to bruising and superficial damage to the peel. Proper handling with care is required throughout the harvest and storage process to ensure fruit remain acceptable to consumers. All fruit should be harvested and handled in a way to minimize internal and external damage to the fruit. Stems should be clipped flush to the fruit so as to decrease the chance of puncturing the fruit when it is washed and sorted later. Preventing puncture wounds to the peel and gentle washing and brushing minimizes the chance of water loss and disease infestation (Petracek, Kelsey, and Davis, 1998).

External Quality

Satsuma mandarins are small, usually two to three inches in diameter. The fruit are usually seedless and extremely easy to peel. Because of their ease of peeling, Satsumas have been given several nicknames, such as the “zipper-skin” and the “kid-glove” fruit (Saunt, 2000). High quality Satsumas have a turgid peel that is blemish-free. Consumers often confuse Satsumas with tangerines at first glance; however, the

Satsuma's ease of peeling and seedless nature quickly distinguishes them from other fruit.

Internal Quality

Citrus fruit, such as Satsumas, are unlike most other fruit and vegetable crops in that internal quality (ripeness) cannot be determined by external color alone. Some cultivars of Satsumas reach full color before they have met acceptable internal quality, while others meet quality standards after color development (Cohen, 1983; Ebel et al., 2004). Since peel color does not develop at the same rate as internal quality, internal tests must be preformed to determine maturity indices. Internal tests usually include the measurement of soluble solids (% Brix) and titratable acids (T.A.) in the fruit. These are two important components of flavor.

The industry standard for measuring internal quality is the Brix (soluble solids) to titratable acidity ratio (Spiegel-Roy and Goldschmidt, 1996). Certain states, such as Louisiana, have instituted a minimum ratio of 10:1 in order to eliminate inferior quality fruit from entering the market. Both variety and weather conditions determine when the ratio is met. Most Alabama growers utilize the 'Owari' variety, which generally reaches the 10:1 ratio in early November (Ebel et al., 2004).

Ethylene

Generally fruit are classified in one of two categories according to ethylene production: non-climacteric and climacteric. Satsumas are a non-climacteric fruit. As Satsumas ripen, ethylene production and respiration does not dramatically change as happens in climacteric fruit such as apples and tomatoes (Kader, 2002).

The non-climacteric classification does not mean that Satsumas fail to respond to ethylene. In areas where temperatures are constantly high, color break in the peel of Satsumas is prevented or delayed, which requires artificial means of degreening (Tuset et al., 1988). The most common means of degreening is by exposing the fruit to ethylene gas for less than 36 hours (Ritenour et al., 2003). Ethylene is applied to the fruit, causing chlorophyll degradation, which results in breakdown of the green color allowing the underlying yellow-orange colors to become visible caused by stimulation of yellow and orange carotenoid production, (Plaza, 2004).

Degreening with ethylene enables farmers to harvest fruit weeks earlier than if normal coloration were allowed to occur. This allows producers to better manage when their product is available for market, thereby, allowing them to obtain a higher price for their fruit (Poole and Gray, 2002).

Color is both subjective and objective. Subjective measurements take place when a grower or consumer visually evaluates a fruit and makes a determination of color. Objective measurements entail using a colorimeter that takes several color readings on the color scale. The two main color readings are the hue angle and chroma. Hue angle is a measure of color and decreases as the peel turns from green (160°) to yellow (90°) to orange (45°). Chroma is a measurement of the color's intensity from near white to pure color (McGuire, 1992; Voss, 1992).

Ethylene can also cause negative side effects such as an increased sensitivity to chilling injury, stem-end rot decay, and development of off-flavors (Porat et al., 1999). According to the University of Florida's recommendations for degreening citrus, fruit should not be exposed to concentrations of ethylene greater than 5ppm because higher ethylene concentrations have been known to promote decay (Ritenour et al., 2003).

Fungicides

Postharvest diseases such as stem-end rot (*Diplodia natalensis* (Pole Evans) and *Phomopsis citri* H. Fawc. non (Sacc.) Traverso & Spessa) are important problems for Satsuma growers. Fruit undergoing degreening are even more susceptible to these disease pressures. Satsumas develop stem-end rot on the button portion (where the stem is connected) of the fruit. Infection normally develops prior to harvest and progresses after harvest. The infection travels through the core of the fruit and develops unevenly throughout the fruit (Brown, 1984). Fruit infected with stem-end rot are unsuitable for fresh market purposes. Green (*Penicillium digitatum* Pers.:Fr.) and Blue Mold (*P. italicum* Wehmer) are initiated through open wounds made during harvest or during processing of the fruit. Proper handling and storage during postharvest handling, lessens the chance of pathogens being introduced into the fruit (Gross, 2002).

Fungicides such as Thiabendazole (TBZ) and Imazalil can be used to help control postharvest decay during storage (Tuset et al., 1988). Applying a postharvest dip of TBZ or Imazalil before Satsumas are packaged and shipped will decrease the incidence of *Diplodia sp., Phomopsis sp., Anthracnose sp.* and *Penicillium sp*. (Brown et al., 1983; Kader, 2002). Imazalil and TBZ can also be applied in combination with wax before storage and shipping (Brown, 1984). Gentle handling of the fruit and good sanitation of packinghouse equipment and storage areas will also help control the spread of postharvest pathogens (Gross, 2002).

Wax

Citrus along with a variety of other fruits and vegetables are commonly waxed. Waxes improve the appearance of fruit by providing an enhanced shine on the outer surface. They can also enhance the overall appearance and quality of the fruit to the consumer by reducing water loss and preventing shrinkage (Petracek, Dou, and Pao, 1998). Water loss prevention is not only beneficial to consumers, but also to growers and retail stores due to the fact most citrus is sold on a weight basis. Waxes also provide a modified atmosphere within the fruit which decreases respiration and delays ripening (Bayindirli et al., 1995). In addition to improving the façade of the fruit, waxes can be used as fungicide carriers (Bayindirli et al., 1995). Fungicides can be mixed directly in the waxes and applied during one process.

Waxes used on citrus are most often water or petroleum based, although petroleum based waxes are being used less often because they are more expensive and harder to use (Brown et al., 1983). Water based waxes also provide more air exchange within the fruit causing less of a chance of off-flavors than with petroleum based waxes.

Vitamin C

Consumers are becoming increasingly aware of the nutritional value of the food they eat. It has long been known that citrus fruit, such as Satsumas, contain a large amount of vitamin C. Vitamin C is one of the most important essential vitamins for human consumption found in Satsuma mandarins. According to Lee and Kader (2000) more than 90% of the Vitamin C humans consumed comes from fruit and vegetables. Vitamin C includes L-ascorbic acid (AA) and dehydroascorbic acid (DHA). AA is the most biologically active form, however, DHA can be readily converted into a usable form, so both forms need to be measured to get an accurate representation of Vitamin C content in a fruit or vegetable (Lee and Kader, 2000).

There are many factors that affect Vitamin C content in fruit and vegetables. Vitamin C can be affected by the intensity of light during the growing season and by postharvest bruising or physical injuries to the fruit (Lee and Kader, 2000). By studying how Vitamin C changes after the fruit is harvested, growers will be better able to provide a high quality product to their consumers.

Until recently, the extent to which vitamins play in preventative health has been unknown. Most notably, a diet high in citrus helps with a reduced risk of esophageal cancers (Chen et al., 2002), bladder cancer (Jacobs et al., 2002) and other types of cancers (Van Duyn and Pivonka, 2000). There is also some evidence that a diet high in deep yellow-orange fruit may help with the prevention of coronary heart disease, stroke and hypertension (Van Duyn and Pivonka, 2000; Anonymous, 1997). Other benefits of a diet high in citrus are decreased birth defects due to the high foliate content (Anonymous, 1997). It is important for women to have a diet high in foliate immediately before and during pregnancy. A study by Hemila (1996) also linked vitamin C to the prevention of the common cold as well as the lessening of the symptoms associated with a cold.

II. INTERACTION OF WAX, FUNGICIDE, AND ETHYLENE TREATMENTS ON STORAGE AND SHELF-LIFE OF SATSUMA MANDARINS: EFFECT ON FRUIT QUALITY AND DISEASE INCIDENCE

Introduction

During the early 1900's, the Alabama Gulf Coast was the center of a young and prosperous citrus industry. The industry evolved around a small, delicate fruit called the Satsuma mandarin. Satsumas are a member of the species *Citrus unshiu*, Marc., which is closely related to the tangerine and Clementine (Rucker, 1996; Ebel et al., 2004). At the height of production, Alabama Satsuma acreage peaked at approximately 4,900 bearing hectares (Dozier, 1924).

The climate in Alabama is extremely well suited for Satsuma production. Cool nights promote both internal and external quality development (Ebel et al., 2004). However, over the years several weather phenomena, namely freezes and hurricanes, reduced yields and damaged trees. Three devastating freezes occurred during the 1930's, which severely injured trees and eliminated Satsuma production along the Gulf Coast. Another major freeze occurred in 1940 and destroyed what was remaining of this once promising industry (Winberg, 1948).

Since the early 1980's, there has been renewed interest in redeveloping a Satsuma mandarin industry along the Gulf Coast. Several farmers along the coast of Alabama have planted trees in an effort to revitalize Satsuma production. Without further research, however, the new industry will succumb to freezes just as its predecessor. For the past several years, due in large part to federal and state initiatives, researchers have begun developing new, innovative techniques and strategies to encourage redevelopment of the Satsuma industry in Alabama.

There are currently two main research focuses of this large-scale project. The first involves marketing. Marketing research has focused primarily on consumer preferences for the Satsuma. Campbell et al. (2004) examined consumer preference for external attributes, such as price, size, and color. Their research verified the industry standards of orange color, larger size, and non-blemished fruit, while identifying three market segments within the Satsuma market. New marketing research is focusing on consumer preference based on sensory evaluation, comparison of preference for various mandarins, and new value-added products (Campbell, unpublished data).

The second emphasis concerns production issues. Such production issues include freeze prevention and postharvest handling. Freeze prevention research is being conducted with regards to cold tolerant selections (Zhang et al., 2002) and methods of freeze protection, such as micro-sprinkler irrigation (Nesbitt et al., 2000). The lack of a means to eliminate or reduce the effect of freeze damage is the main reason the industry sustained devastating losses in the past.

Without an understanding of the optimum postharvest storage methods, both marketing and freeze prevention are useless. Consumers will not purchase a product of inferior quality and growers will not produce a product they are unable to sell. Consumers prefer a uniformly orange, blemish-free fruit (Campbell et al., 2004). This study examined several postharvest treatments relating to fruit quality. Research into postharvest handling and storage will help maintain a high quality fruit from tree to consumer. This project was designed to take the next step in the production process by providing information on the best methods for postharvest handling and storage of Satsumas.

The study was conducted over two growing seasons, 2003/2004 and 2004/2005. The objectives of this study were: (1) to determine efficacy of degreening with ethylene and (2) to determine how wax and fungicides interact with ethylene to affect storage and shelf-life. Results from this study will assist growers, retailers, and researchers in maximizing the storage and shelf-life of Satsuma mandarins.

Materials and Methods

Fruit utilized within this study were from mature Satsuma mandarin trees (*Citrus unshiu*, Marc. 'Owari') budded onto *Poncirus trifoliata* (L.) Raf. 'Rubidoux' rootstock. Satsumas from the Gulf Coast Research and Extension Center in Fairhope, AL were monitored weekly, starting in October, in order to determine optimum harvest date. Satsumas were harvested after the fruit reached a 10:1 soluble solids to titratable acidity ratio. The optimum (10:1) ratio was reached in mid-November and harvest followed, which is the typical harvest period for this cultivar in this region (Ebel et al. 2004). During harvest, care was taken to prevent puncturing or causing damage to the fruit. After harvest, the fruit were transported to a grading house where they were washed and graded for size. Fruit with large blemishes and deformities were culled. Fruit from the various trees were randomly mixed in order to minimize tree effect among treatments. Fruit were boxed and transported to Auburn University where they were placed in cold storage ($4^{o}C$) overnight.

Approximately 800 fruit were placed in 9 kg corrugated ventilated boxes and placed in the center of an atmospheric controlled chamber where 5 ppm of ethylene gas was supplied by a compressed gas cylinder (Airgas Gas; Radnor, PA) at a rate of one room volume exchange each hour for 24 hours at room temperature. Ethylene application varied between years. In 2003, ethylene was applied directly into the headspace at one end of the chamber and exited an 8cm port at the other end of the lid. Air was circulated in the chamber and around the boxes of fruit by a fan (Model: HANF75, the Holmes Group, Inc., Millford, MA). In 2004, ethylene was applied through the bottom of the chamber via a manifold of 2 cm PVC pipe with exit holes drilled 10 cm apart to release the ethylene gas uniformly throughout the chamber. A fan was used to circulate air and a ventilation hole cut in the top of the chamber. The concentration of ethylene in the headspace was measured every 6-10 hours to ensure it was maintained near 5ppm (Table 1). A 1cm^3 gas volume was withdrawn for determination of ethylene concentration by gas chromatography (Perkins Elmer Sigma 3B Model Gas Chromatograph) fitted with a flame ionization detector. Ethylene was separated on a Porepak-Q column (76cm x 1.6 cm) at 70ºC with helium as the carrier gas. Fruit not treated with ethylene remained in cold storage.

After ethylene treatment, ethylene and non-ethylene treated fruit were treated with wax (Sta-Fresh 4201, FMC Technologies; Riverside, California) and fungicides (Table 2). Imazalil (Freshgard 700, FMC Technologies; Riverside, California) and 2-(4-thiazolyl) benzimidazole (TBZ) (Freshgard 598, FMC Technologies; Riverside, California), the fungicides used in this experiment, were applied at the labeled rates for citrus. For the non-waxed treated fruit, fungicides were applied at a rate of 1000ppm a.i.

in water. Groups requiring wax were treated with a 1:3 dilution wax/water solution. For treatments requiring a wax and fungicide combination, the fungicides were mixed directly in the wax/water solution. Imazalil was mixed with wax at a rate of 2000ppm a.i., while TBZ was mixed at a rate of 1000ppm a.i. The treatments were applied to the peel of the fruit using a rough cloth to simulate a brush application typical of commercial grading machines.

The day after treatments were applied (Day 0), five fruit from each treatment group were evaluated for fruit quality measurements, including: weight (g), size (cm), color (hue and chroma), Brix (%), Titratable acid (%), and disease incidence. Weight (g), length (cm) and width (cm) were measured collectively for all ten fruit in each treatment. Peel color was assessed by using a Minolta colorimeter (model CM-2002; Minolta Camera Co., Japan) and data were expressed as hue angle and chroma. Hue angle measures color change from green (160°) to yellow (90°) to orange (45°), while chroma is the intensity of hue angle color from near white to pure color (McGuier, 1992; Voss, 1992). Color measurements were taken on opposite sides of each fruit and averaged together in order to determine hue angle and chroma. Fruit were then visually evaluated for disease incidence. Disease incidence was measured by visual appearance using a 1-10 rating scale. A rating of one was determined as fruit having an infected area smaller than 5mm. A fruit with a rating of 10 was determined to have 50% or more of the fruit infected. Diseased fruit were then taken to the Auburn University Plant Diagnosis Laboratory for identification. The fruit were isolated on nutrient agar and identified according to disease clinic guidelines.

The fruit from each treatment were halved and juiced using an electric hand juicer (model HJ28; Black and Decker, Townsend, Md.). Composite juice samples were combined for all five fruit and poured through cheese-cloth to remove pulp from the sample. A 1mL aliquot was placed on a hand-held, temperature compensated refractometer (model 10494; Leica Microsystems Inc., Buffalo, N.Y.) to determine percent soluble solids.

A 10mL aliquot of juice was taken from each treatment group and titrated to a pH of 8.1 with 0.1 N KOH using an automated titrimeter (Metrohm Titrino model 751 GPD and Metrohm Sample Changer; Metrohm Corp., Herisau, Switzerland) interfaced with a personal computer utilizing a Titrino Worcell 4.4 software package (Brinkmann Corp., Westbury, N.Y.). Results were expressed as percent citric acid equivalents.

Storage and Shelf-life Treatments

For this study, storage was defined as the amount of time a fruit was held in cold storage at 4^oC, which is the industry standard for storing citrus fruit (Plaza et al., 2003). Fruit were sampled once every two weeks. Ten fruit from each treatment were randomly selected from cold storage and processed in the same manner as the Day 0 fruit.

Shelf-life was defined as fruit held in ambient room conditions on a lab bench for one week in addition to the storage time held at 4^oC. Fruit from each treatment were randomly selected from cold storage during the same week as the storage fruit and held at room temperature and ambient relative humidity for one additional week in order to simulate the retail consumer environment.

Statistical Analysis

Storage and shelf life data were evaluated separately. Data were analyzed as a 2 (ethylene treatment) by 2 (wax treatment) by 3 (fungicide treatment) factorial, split-plot (over time) using the general linear model (GLM) procedure of the Statistical Analysis System (SAS Institute, 1985). Due to the difference in ethylene application procedure, data were analyzed separately for each year. Only potential interactions with ethylene were tested. Means for fruit weight (g), chroma and hue angle were determined using the SAS means procedure before applying the GLM procedure. Where all interactions were not significant, means were separated using Duncan's multiple range test ($P \leq 0.05$).

Results

Storage life

Ethylene treatment did not stimulate degreening of the fruit in 2003 and there was a mild effect in 2004 ($P=0.0445$) that diminished during storage (Table 3, Figure 1). Hue angle averaged 67.5 degrees in 2003 and 67.0 degrees in 2004 at harvest for untreated fruit indicating that the fruit were mostly yellow with some patches of green. Satsuma fruit continued to degreen in storage with hue angle decreasing to 58.5 degrees in 2003 and 61.7 degrees in 2004 for untreated fruit. There was no significant effect on chroma for either year. Any retention of green in the peel is detrimental to consumer preferences (Campbell et al., 2004). Ethylene applications did not achieve sufficient levels of degreening that would justify its use at these rates.

The major concern with degreening with ethylene is the development of fungal diseases, especially stem end rots (Porat et al., 1999). It is possible that these diseases would affect the rate of water loss from the fruit. Development of fungal pathogens was very low in this study and there was no effect of ethylene on weight loss (Table 3). Minor disease development occurred on fruit, but there was no difference among any of treatments. Postharvest diseases found included: *Penicillium notatum* (blue-green mold), a *Colletotrichum sp*. (gray and orange mold), a *Fusarium sp*.(white and orange mold), a *Botryosphaeria sp*. (dark gray mold), and an *Aspergillus sp.* (yellow-green mold). There was a significant interaction of ethylene and fungicide during storage on fruit width ($P=0.0429$), which also impacted the height to width ratio ($P=0.0416$), but the influence was small (data not shown).

Wax treatment was the most effective in maintaining fruit weight, with waxed fruit losing weight much slower than unwaxed fruit in 2003 (Figure 2), as indicated by a wax by week interaction ($P=0.0692$). The difference in weight loss was mainly due to shrinkage of fruit height ($P=0.0174$) rather than width ($P=0.0875$). There was no effect of wax on weight loss in 2004.

In 2003, wax and fungicide treatments did not affect Brix but there was a significant week * ethylene * wax interaction on titratable acidity ($P=0.0001$) and the Brix to titratable acidity ratio ($P<0.0001$). In general, titratable acidity of waxed fruit decreased more rapidly than unwaxed fruit (Figure 3). The significant decrease of titratable acidity for ethylene fruit was also reflected in faster increases in the Brix/titratable acidity ratio.

In 2003, there was also a week * ethylene * fungicide interaction of titratable acidity ($P=0.0042$) and a Brix/titratable acidity ratio ($P=0.0012$). Fungicide application interacted with ethylene to alter the rate titratable acidity decreased and the Brix/titratable acidity ratio increased (Figure 4). Ethylene without fungicides promoted a slight increase in titratable acidity whereas all other treatments had titratable acidity decrease.

In 2004, ethylene treated fruit influenced the rate of change of Brix (*P=0.0085*) and titratable acidity (*P=0.0080*), but not their ratio (*P=0.1949*). Brix increased more rapidly and titratable acidity did not decrease compared to untreated fruit (Figure 5). The net effect was a similar increase in Brix/titratable acidity ratio for both treatments. Fungicide treatments did not alter Brix, titratable acidity or their ratio. Wax treatments did not alter Brix, but had a small effect on titratable acidity (*P=0.0452*). Titratable acidity rapidly declined in waxed fruit, which resulted in a faster increase in the Brix/titratable acidity ratio (Figure 6). In both years, titratable acidity of waxed fruit decreased more rapidly than unwaxed fruit. This could be related to poor air exchange of the waxed fruit.

Shelf life

Holding fruit at room temperature and under ambient conditions for seven days stimulated additional degreening, but the effect was marginal. In 2003 hue angle of untreated fruit declined from 67.5 at harvest to 64.7 degrees seven days later, and in 2004 hue angle declined from 67.0 degrees to 62.3 degrees seven days later. This was not enough of a color change to change the marketability of the fruit. Ethylene had an early effect on promoting degreening early in storage, but the effect diminished during storage. In 2003, there was a significant ethylene * week interaction (*P=0.0308*), with ethylene treated fruit having slightly lower hue angle than untreated fruit one week after harvest, but the additional benefit of ethylene treatment was not apparent by the end of the experiment (Figure 7). In 2004, there was a significant ethylene * wax * week interaction (*P=0.0088*). Ethylene decreased the hue angle one week after harvest, but the effect diminished by the end of the experiment. Wax delayed degreening of untreated fruit.

As with the stored fruit, waxed fruit retained fruit turgidity better than unwaxed fruit maintained under simulated retail conditions for seven days. There was a significant week * wax interaction in 2003 (*P=0.0207*) and in 2004 (*P=0.0713*). Waxed fruit maintained fruit turgidity when held on the shelf, although the results were inconsistent with respect to time in storage and between years (Figure 8). As with stored fruit, fruit length declined (*P=0.0120*) rather than fruit width (*P=0.1566*) in 2004. The lack of a difference on length or width decrease in 2003 indicates that the fruit lost size uniformly.

There was a significant week * ethylene * fungicide interaction on fruit weight in 2004 (*P=0.0290*). Most treatments responded similarly with exception of the non-ethylene treated fruit the received TBZ fungicide treatments, which lost weight more rapidly than other treatments (Figure 9).

In 2003, there were significant week * ethylene * wax interactions for Brix (*P=0.0338*), titratable acidity (*P=0.0016*) and the Brix/titratable acidity ratio (*P=0.0072*). Ethylene-treated fruit enhanced Brix more rapidly than untreated fruit with wax treatment allowing faster increases in Brix (Figure 10). Non-ethylene treated fruit with wax had the fastest decrease in titratable acidity. Ethylene treated fruit without wax had lower initial titratable acidity, which largely remained unchanged during storage. The net effects of ethylene and wax treatments on the Brix/titratable acidity ratio resulted in the fastest increase for the non-ethylene treated waxed fruit; although the ethylene treated non-waxed fruit had the highest initial Brix/titratable acidity ratio.

In 2004, there was a significant week * ethylene * wax interaction on Brix (P=0.0225) (Figure 11), but titratable acidity and the Brix to titratable acidity ratio were unaffected. Brix for the waxed fruit increased at a faster rate than the Brix for the non-waxed fruit.

Discussion

Ethylene treatments of 5ppm failed to advance degreening to a level that would justify use at this rate. Treatment at 5ppm did not degreen the fruit adequately to improve marketability. The minor effect of ethylene seen on color at this rate was diminished in both the storage and shelf-life studies since the fruit continued degreening naturally, even in cold storage. Consumers prefer uniform yellow-orange or orange color fruit with no appearance of green in the peel (Campbell et al., 2004). Postharvest treatments of ethylene at higher concentrations may be necessary for Satsuma mandarins in order to obtain full color development. A study from Japan, reported a significant positive coloration of Satsuma mandarin exposed to 120ppm for 12 hours at 20°C (Yamauchi et al., 1997).

In addition, the duration that the fruit are exposed to ethylene may also need to be increased and postharvest temperatures during degreening may also need to be increased. According to a study by Beever (1990), lower temperatures (<5°C) tend to delay the effects of degreening of Satsuma mandarins. In the current experiment, fruit were placed directly into cold storage after exposure which likely diminished the effects of ethylene in reference to color development. Ethylene did not influence color, effect size, or disease development; however, it did affect internal quality. Ethylene has been shown to affect internal quality on 'Shamouti' oranges. In this study, fruit were exposed to 10ppm of ethylene for 60 hours. There were no significant effects on Brix, TA, or their ratio; however, ethylene did affect the accumulation of off-flavors (Porat et al., 1999).

Waxing treatment had the greatest effect on preserving fruit quality initially, especially weight and size. This was particularly true for the shelf-life treatments as time progressed. The longer the fruit were placed in cold storage the more magnified the effect of wax treatment became when the fruit were placed on the shelf. Surface coatings have been shown to reduce weight loss by as much as 20% during storage (Lawes and Prasad, 1999).

Fungicide had a minor effect on preserving fruit quality, in part because disease incidence was low. D'Aquino et al. also found no fungicidal effect on fruit quality in tangelos using Imazalil and other fungicides (1998.)

According to subjective measurements fruit lost marketability around week 8 in both years for the storage and the shelf-life studies. During this time period fruit began to become unappealing in appearance and taste. Unwaxed fruit failed to maintain fruit turgidity and usual appearance, while waxed fruit still looked fresh.

Waxed fruit began to develop off-flavors beginning around the week 8 or 9 of storage. Unwaxed fruit also developed off flavors at this time, but to a lesser extent. Waxed fruit development of off-flavors was most likely due to fermentation within the fruit caused by poor air exchange. Permeability is one of the most important factors in reducing off-flavors in the fruit (D'Aquino et al., 1998). During periods of poor air exchange anaerobic respiration increases ethanol, acetaldehyde and internal carbon dioxide concentrations within the fruit (Hagenmaier, 2002). In a study using different coatings on Minneola tangelos, D'Aquino et al. found an increase in fermentation products in fruit held at 20°C after only two weeks (1998). The poor air exchange in this experiment may be due to the thickness of the wax layer. A thinner wax application may be necessary in the future to prevent anaerobic respiration (alcoholic fermentation).

The ethylene concentration used in this study did not degreen to an acceptable level for market use. Ethylene, however, did affect internal quality of the fruit and should be monitored in future studies. Waxed fruit held their appearance for longer than the unwaxed fruit. Thickness of the wax was detrimental to fruit market life because of the off-flavors induced. Future studies need to evaluate the role wax plays on storage life and how it relates to the development of off-flavors. In this study, disease incidence was low for both years. Because of this fact there was no proper evaluation of fungicide effectiveness. Futures studies need involve fruit that are intentionally inoculated with pathogens to determine the effectiveness of fungicides and how they relate to fruit quality.

III. INTERACTION OF WAX, FUNGICIDE, AND ETHYLENE TREATMENTS ON STORAGE AND SHELF-LIFE OF SATSUMA MANDARINS: EFFECT ON VITAMIN C CONTENT

Introduction

Globally, especially the United States, there is a growing trend by consumers to purchase healthier foods that offer essential vitamins and nutrients. Despite the growing health consciousness of many consumers, fruit and vegetable consumption remains below the recommended average in most countries (Ragaert, 2004). Citrus fruit are a prime source of Vitamin C, antioxidants, phenolics, carotenoids and fiber. Vitamin C is one of the most important essential vitamins. Vitamin C is known to reduce the risk of cancers (Van Duyn and Pivonka, 2000; Chen et al., 2002; Jacobs et al., 2002), prevent scurvy, help with the maintenance of organs, and sustain many biological functions (Lee and Kader, 2000). High amounts of Viatmin C have also been linked to the prevention and relief of common cold symptoms (Hemila, 1996).

The cold and flu season usually coincides with the Satsuma mandarin harvest season along the Gulf Coast. Since Vitamin C's important role in nutrition is widely known by consumers, Gulf Coast Satsuma growers need to better understand how Vitamin C responds to postharvest handling techniques so they can provide the most nutritious fruit to their customers. Growers will then be able to use Vitamin C content as a marketing tool. Vitamin C content and health statements can be placed on boxes to enhance consumer knowledge. The objective of this study was to determine the efficacy of ethylene exposure, fungicide application, and wax application effect Vitamin C content during storage and on the shelf.

Materials and Methods

Fruit utilized within this study were from mature Satsuma mandarin trees (*Citrus unshiu*, Marc. ‘Owari’) budded onto *Poncirus trifoliata* (L.) Raf. ‘Rubidoux’ rootstock. Satsumas from the Gulf Coast Research and Extension Center in Fairhope, AL were monitored weekly, starting in October, in order to determine optimum harvest date. Satsumas were harvested after the fruit reached a 10:1 soluble solids to titratable acidity ratio. The optimum (10:1) ratio was reached in mid-November and harvest followed, which is the typical harvest period for this cultivar in this region (Ebel et al. 2004). During harvest, care was taken to prevent puncturing or causing damage to the fruit. After harvest, the fruit were transported to a grading house where they were washed and graded for size. Fruit with large blemishes and deformities were culled. Fruit from the various trees were randomly mixed in order to minimize tree effect among treatments. Fruit were boxed and transported to Auburn University where they were placed in cold storage (4^{o}C) overnight.

Approximately 800 fruit were placed in 9 kg corrugated ventilated boxes and placed in the center of an atmospheric controlled chamber where 5 ppm of ethylene gas was supplied by a compressed gas cylinder (Airgas Gas; Radnor, PA) at a rate of one room volume exchange each hour for 24 hours at room temperature. Ethylene application varied between years. In 2003, ethylene was applied directly into the headspace at one end of the chamber and exited an 8cm port at the other end of the lid. Air was circulated in the chamber and around the boxes of fruit by a fan (Model:

HANF75, the Holmes Group, Inc., Millford, MA). In 2004, ethylene was applied through the bottom of the chamber via a manifold of 2 cm PVC pipe with exit holes drilled 10 cm apart to release the ethylene gas uniformly throughout the chamber. A fan was used to circulate air and a ventilation hole cut in the top of the chamber. The concentration of ethylene in the headspace was measured every 6-10 hours to ensure it was maintained near 5ppm (Table 1). A 1cm^3 gas volume was withdrawn for determination of ethylene concentration by gas chromatography (Perkins Elmer Sigma 3B Model Gas Chromatograph) fitted with a flame ionization detector. Ethylene was separated on a Porepak-Q column (76cm x 1.6 cm) at 70ºC with helium as the carrier gas. Fruit not treated with ethylene remained in cold storage.

After ethylene treatment, ethylene and non-ethylene treated fruit were treated with wax (Sta-Fresh 4201, FMC Technologies; Riverside, California) and fungicides (Table 2). Imazalil (Freshgard 700, FMC Technologies; Riverside, California) and 2-(4-thiazolyl) benzimidazole (TBZ) (Freshgard 598, FMC Technologies; Riverside, California), the fungicides used in this experiment, were applied at the labeled rates for citrus. For the non-waxed treated fruit, fungicides were applied at a rate of 1000ppm a.i. in water. Groups requiring wax were treated with a 1:3 dilution wax/water solution. For treatments requiring a wax and fungicide combination, the fungicides were mixed directly in the wax/water solution. Imazalil was mixed with wax at a rate of 2000ppm a.i., while TBZ was mixed at a rate of 1000ppm a.i. The treatments were applied to the peel of the fruit using a rough cloth to simulate a brush application typical of commercial grading machines.

The day after treatments were applied (Day 0) five fruit from each treatment group were carefully hand peeled, sectioned, and placed in freezer bags. Fruit were then stored at -80°C until chemical analysis were preformed.

Chemicals and reagents.

All solvents used were of analytical or HPLC grade and purchased from Fisher Scientific (Fisher Scientific, Raleigh, NC) and all chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ultrapure bidistilled deionized water from a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout the study.

Vitamin C extraction and analysis.

Reduced ascorbate (AA), dehydroascorbate (DHA) and total ascorbate (AA + DHA) were determined spectrophotometrically by a modified method from Gossett et al. (1994). All extraction and quantification procedures were performed under amber lighting conditions, to avoid photo-oxidation of antioxidants (GE Gold F40/GO, 40W), and under chilled conditions (4°C). Approximately 5g of frozen juice sacs were homogenized using a Virtis Shear homogenizer Model 225318 (The Virtis Co., Inc., Gardiner, N.Y.) in a pre-chilled Virtis glass macro flask 50 - 400ml, cat # 171439 containing 30 ml of pre-chilled 4°C 5% (v/v) *m*-phosphoric acid for approximately 1 minute at a setting of 70. The homogenate was centrifuged at 22,000g for 20 minutes in a Beckman Model J2-21 refrigerated high speed centrifuge using a JA 20 rotor (Beckman Instruments, Inc. Palo Alto, CA) at 4°C. The clarified supernatant was filtered through Miracloth (Calbiochem, La Jolla, CA, USA) and subsequently processed utilizing solid-phase extraction (SPE) to elute undesired components such as sugars, acids, and other water-soluble compounds. Approximately 5.0 ml aliquot of clarified juice filtrate was loaded onto a preconditioned Sep-Pack C_{18} cartridge (Waters Technologies Corporation, Milford, MA) and activated as outlined by (Albrecht et al., 1990). Samples were used immediately or stored at -80^{o}C until further analysis.

Total ascorbate (AA + DHA) was determined in a reaction mixture consisting of 200µl of SPE eluant, 500µl of 150 mM KH_2PO_4 buffer (pH 7.4) containing 5 mM EDTA and 100µl of 10 mM dithiothreitol (DTT) to reduce DHA to AA. After 10 minutes at room temperature, 100µl of 0.5% (w/v) N-ethylmaleimide (NEM) was added to remove excess DTT.

AA was assayed in a similar manner except that 200µl of bidistilled deionized water was substituted for DTT and N-ethylmaleimide. Color was developed in both series of reaction mixtures with the addition of 400µl of 10% (w/v) trichloroacetic acid (TCA), 400µl of 44% (v/v) o-phosphoric acid, 400µl of α,ά-dipyridyl in 70% (v/v) ethanol and 200µl of 30 g l^{-1} $FeCl_3$. The reaction mixtures were incubated at 40^{o}C in an Isotemp water bath Model 228 (Fisher Scientific, Suwanee, GA) for 1h and quantified spectrophotometrically using a Synergy HT ELX 808 IU Ultra Microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT) at 525nm. A standard curve was generated based on pure L-ascorbate standards between 1 and 50µmol in 5% (v/v) *m*-phosphoric acid. For each sample, DHA was estimated from the difference of total ascorbate and AA. Vitamin C content was expressed as mg / 100gfw of juice.

Storage and Shelf-life Treatments

For this study, storage was defined as the amount of time a fruit was held in cold storage at 4^{o}C, which is the industry standard for storing citrus fruit (Plaza et al., 2003).

Fruit were sampled once every two weeks. Ten fruit from each treatment were randomly selected from cold storage and processed in the same manner as the Day 0 fruit.

Shelf-life was defined as fruit held in ambient condition for one week in addition to the storage time held at 4°C. Fruit from each treatment were randomly selected from cold storage during the same week as the storage fruit and held at room temperature and ambient relative humidity for one additional week in order to simulate the effects of being placed on a grocery store shelf.

Statistical Analysis

Storage and shelf life data were evaluated separately. Data were analyzed as a 2 (ethylene treatment) by 2 (wax treatment) by 3 (fungicide treatment) factorial, split-plot (over time) using the general linear model (GLM) procedure of the Statistical Analysis System (SAS Institute, 1985). Due to the difference in ethylene application procedure, data were analyzed separately for each year. The analyses were conducted on treatment means for AA, DHA, and TAA. Only potential interactions with ethylene were tested. Means for AA, DHA, and TAA were determined using the SAS means procedure before applying the GLM procedure. Where all interactions were not significant, means were separated using Duncan's multiple range test ($P \leq 0.05$).

Results

Storage life

Fungicides did not have an effect on Vitamin C content in either year for the storage or the shelf-life studies. There was a significant week * ethylene * wax interaction for AA in 2003 ($P=0.0261$) (Table 5). Fruit treated with ethylene and wax declined the most in AA while fruit with the non-ethylene, unwaxed treatment increased the most over time (Figure 12). This effect is most likely due to poor gas exchange within the fruit caused by excessive concentrations of wax and the added stress the fruit endured due to the ethylene exposure.

In 2004, a significant week * ethylene * wax interaction for DHA occurred (*P=0.0324*). DHA declined for all treatments (Figure 13). However the ethylene wax treatment declined at the fastest rate.

Shelf-life

There was a significant week * ethylene interaction for DHA in 2003 (*P=0.0406*) and 2004 (*P=0.0018*) (Table 6). In 2004, there was also a significant interaction between week and ethylene on TAA (*0.0261*).

<u>Discussion</u>

Cultivar, environmental conditions, and cultural practices are all important factors which influence Vitamin C content in a fruit. According to Lee and Kader (2000) light intensity during the growing season is one of the most important factors affecting Vitamin C content. In general fruit that are exposed to higher light intensities have increased amounts of Vitamin C. Another important factor is temperature before harvest during the maturation period. Satsumas grown under cool weather conditions (20-22°C day, 11-13°C night) had a higher Vitamin C content than Satsuma fruit grown under warmer conditions (30-35°C day, 20-25°C night) (Lee and Kader, 2000).

The loss of Vitamin C can also be minimized by careful postharvest handling. This includes not damaging the fruit physically during postharvest treatments and also proper temperature storage. Improper postharvest techniques can contribute greatly to the degradation of Vitamin C during storage. Physical injury to the fruit, either during or after harvest, causes Vitamin C to metabolize at a faster rate (Lee and Kader, 2000). By limiting physical injury, such as puncturing the peel and bruising, excessive Vitamin C loss from this type of injury can be avoided. In general, Vitamin C decreases over time in storage (Adisa, 1998). High temperatures can reduce the retention of Vitamin C especially if the fruit are going to be stored for any length of time. In a study by Shaw et al. (1993), Vitamin C decreased by 22% for fruit stored at 50°C after the third week and by the 12th week Vitamin C retention was less than 32%.

The USDA's recommended daily allowance of Vitamin C is 85-90 mg per day for an adult (Anonymous, 2001). Other publications have reported mandarins contain approximately 24-38mg/100g FW AA (Lee & Kader, 2000; Piga et al., 2002; Ladaniya et al., 2003). In this study the average AA at harvest for untreated fruit was 36mg/100g FW at harvest. Duration of storage only affected Vitamin C concentration in 2004 for the storage experiment. Fruit Vitamin C content was reduced 8% by the end of the experiment.

Information on how Vitamin C changes over the postharvest life of the fruit can be an important marketing tool that growers need to utilize. Consumers are becoming increasingly aware of what is in their food. Vitamin C is an important antioxidant that consumers desire because the human body does not produce it (Antonelli et al., 2002). Table 7 lists some commonly consumed fruits and their comparative Vitamin C values. Satsuma mandarins are a good source of Vitamin C. The information obtained from this study regarding Vitamin C content will be useful for labeling and promoting Satsuma mandarins.

IV. EVALUATION OF SATSUMA MANDARINS IN A COMMERCIAL DEGREENING ROOM

Introduction

There is a growing interest in promoting the redevelopment of the Satsuma mandarin industry along the Northern Gulf Coast, in part because the climate in this region promotes excellent flavor and appearance (Ebel et al., 2004). The Satsuma harvest season usually occurs around late October through early December. Exact timing of the harvest is largely dependent on two factors: market conditions and weather preceding harvest.

In order to remain profitable, Satsuma growers must offer a consumer acceptable product. Most consumers desire a ripe fruit that has a uniform orange peel color (Campbell et al., 2004). However, peel color does not necessarily correlate with internal quality (Pool and Gray, 2002). As shown by Ebel et al. (2004), the minimum internal fruit quality is reached before complete coloration of the fruit peel. Therefore, fruit harvest is dependant on internal quality as well as exterior peel color, unless the latter can be artificially modified.

The industry standard for measuring internal quality is the Brix (soluble solids) to titratable acidity ratio (Spiegel-Roy and Goldschmidt, 1996). Certain states, such as Louisiana, have instituted a minimum ratio of 10:1 in order to eliminate lower quality fruit from entering the market. In Alabama, ‘Owari’ is the most commonly grown variety and usually reaches maturity in early November (Ebel et al., 2004). In most years, the fruit is harvested before complete degreening occurs. In years where temperatures are constantly high, carotenoid accumulation in the peel of Satsumas may be significantly delayed (Tuset et al., 1988). In order to ensure Satsumas meet color requirements established by the market, degreening is often required. In a previous study 5ppm of ethylene gas for 24 hours at room temperature was used to degreen fruit. However, the results from this study showed that this concentration and duration was not effective. Peel color did not develop adequate color to increase marketability of the fruit, therefore alternative procedures for degreening Satsumas need to be developed.

The objective of this pilot study was to evaluate the effects of longer exposure of ethylene on Satsuma mandarins in an existing degreening room currently used for banana degreening at a commercial farmer's market. Ethylene is known to aid in the process of peel coloration in citrus fruit causing chlorophyll in the peel to breakdown, which in turn, causes the naturally occurring peel pigments to become visible (Porat et al., 1999). Fruit degreened after harvest tends to have less orange color than fruit allowed to develop color on the tree (Plaza et al., 2004). The secondary objective of this study was to determine the best means of short-term storage after removing fruit from the ethylene chamber.

Materials and Methods

Fruit from mature Satsuma mandarin trees (*Citrus unshiu*, Marc. 'Owari') on *Poncirus trifoliate* (L.) Raf. 'Rubidoux' rootstock were used in the study. Fruit were harvested from the Gulf Coast Research and Extension Center in Fairhope, AL on November 15, 2004. On the same day as harvest, the fruit were transported to a degreening room at the Alabama State Farmers Market in Montgomery, Alabama. At the time of the study, the room was empty with the exception of the fruit utilized for this study. The temperature of the room was maintained at 17.8°C.

Ethylene Fluid (Precision Generators, Inc; Virginia Beach, VA) was placed in the degreening room to provide ethylene gas. Three-nine kilogram boxes of Satsumas were placed in the degreening room. The Satsumas were exposed to ethylene for: 24, 48 and 72 hours. Ethylene concentration in the room was measured daily for three days (Table 7). The concentrations ranged from approximately 270ppm to 20ppm over the three day period. A control sample was also taken in an empty room. Ethylene concentration in the control room ranged from 4 to 10ppm.

After 24 hours of exposure, one box of fruit was removed and transported to the Department of Horticulture at Auburn University, Auburn, Alabama to determine peel color. Forty fruit from the 24-hour exposure treatment were randomly divided into two groups of twenty fruit each. Forty fruit from the control treatment were divided in the same manner. Peel color readings were taken for all fruit using an electronic colorimeter (Model CM-2002, Minolta Camera Co., Japan). Color readings consisted of an average of two opposing sides of each fruit. Readings included hue angle and chroma. Hue angle is the measure of color and decreases as the peel turns from green to yellow to orange. Green is in the range of 160°, yellow is 90° and orange is 45°. Chroma is a measurement of color intensity from near white to pure color (McGuire, 1992; Voss, 1992).

After color measurements were taken, one group of twenty fruit from both the control and the 24-hour exposure time was placed on a lab bench top at room temperature, while the other group was placed in a cold storage unit at 4°C. After 48 hours, the second box of fruit was removed from the degreening room. Forty fruit were removed from the 48-hour exposure treatment and divided in the same manner as the 24-hour treatment. Color readings were taken on the cold storage and the bench storage groups for the control, 24-hour, and 48-hour exposure treatments. After 72 hours, the process was repeated with measurements taken for both storage and bench stored fruit of all treatments. On the fourth and fifth days, all treatments and their corresponding storage groups were again measured for color.

Results

Hue angle showed a dramatic difference between the storage conditions (Figure 14). The hue angle of the fruit stored on the bench top decreased at a much faster rate for all treatments compared to the fruit stored in the cold storage unit. Indicating the fruit stored on the bench top a higher accumulation of carotenoids resulting in a more orange color by the end of the experiment. Chroma readings increased over time for each ethylene exposure and storage condition (Figure 15). The 48 and 72 hour fruit stored on the bench top degreened to a rate suitable for a commercial retail store.

When consumers evaluate the external appearance of a fruit in the grocery store, they use visual observations to determine whether or not to purchase. Visual examination of the different ethylene exposure treatments indicated there was a color difference with regards to both the duration of ethylene exposure and the storage conditions. Fruit placed in the cold storage unit immediately after ethylene exposure developed less uniform orange color than fruit left at room temperature. Fruit exposed to ethylene for 72-hours produced a visually less green fruit compared to the 0, 24, and 48-hour exposure levels. Fruit in this treatment group was degreened to a level that would increase the marketability of the fruit.

Discussion

Temperatures lower than 28 to 29°C have been shown to slow the degreening process (Ritenour et al., 2003). Fruit maintained at room temperature developed better coloration indicating that higher temperatures maintained in the degreening rooms are necessary for optimal effects of ethylene exposure on peel color.

Color readings obtained from the Minolta colorimeter verifed visual evaluations. Exposure to ethylene for two days (48 hours) and then two additional days at room temperature was the best treatment for degreening Satsuma mandarins.

An unexpected result of this experiment was that there was no physical apparent damage observed on the fruit even though the fruit were exposed to extremely high concentrations of ethylene (Table 8). Degreening with ethylene has been known to increase the rate of decay and cause cold sensitivity to fruit (Palou et al., 2003, Porat et al. 1999 and Cohen, 1977). It has also been reported that increased concentrations (greater than 5ppm) can cause damage on fruit and that low temperatures retard color development (Ritenour et al., 2003), while other studies have determined the effects of ethylene at temperatures as high as 40°C and reported positive fruit quality results (Plaza et al., 2004). Positive color development has been reported for ethylene exposure duration as high as six days (Tuset et al., 1988). This is in contrast to recommendations from Florida, which warns that exposure longer than 36 hours on mandarin will result in dramatically increased decay (Ritenour et al., 2003). From the data collected in this experiment, two days of ethylene exposure and two additional days at room temperature provided the best results. Before any firm conclusions are made or any recommendations given to growers it should be noted that the fruit were only observed for five days and no internal quality attributes were measured. In the future a study needs to be done on a larger scale and more quality aspects of the fruit need to be measured.

V. APPLICATIONS AND SUGGESTIONS FOR FUTURE RESEARCH

Research implications

In order to maintain a viable citrus industry, Satsuma growers must sell enough fruit to make it economically feasible to continue producing Satsumas. Most of the growers in Alabama sell their fruit on a weight basis regardless of distribution method, whether it is sold on-farm, at a roadside stand, through a governmental program or in a retail environment. On-farm and roadside stand distributors are not as concerned with fruit weight loss since their fruit is harvested in smaller increments and sold quickly after harvest. Growers opting to sell their fruit in this manner are also not as concerned with fruit appearance or postharvest fungicide applications due to the short amount of time it takes to get the fruit from producer to consumer.

Growers selling their fruit in either a government program or in a retail environment must be concerned about fruit weight loss and appearance since fruit must be harvested in large quantities and delivered farther distances to their final destination, all the while maintaining a consumer acceptable product. Therefore, these growers need to better understand which postharvest handling practices allow fruit to maintain weight, minimize disease and keep fruit consumer friendly.

Results of this project will allow growers choosing to market through retail market channels to better understand the most effective means to maintain the fruit quality desired by consumers: large, orange, non-blemished fruit (Campbell et al., 2004).

In order to achieve uniform peel coloration, degreening with ethylene must be used for Alabama fruit since the internal quality is acceptable before the peel completely is completely orange. Ethylene provides a more efficient means of coloring than natural coloring on the tree. Since retail stores demand orange citrus, growers are advised to utilize ethylene to advance color development in a timely and efficient manner. Taking the fruit off the tree as early as possible also aids in hardening off the tree to prevent freeze damage in the winter.

Fruit exposed to 5ppm of ethylene for 24 hours showed a lower hue angle (more orange color) in both the storage and shelf-life experiments compared to fruit not exposed to ethylene and at 5ppm, there was no observable damage to the fruit associated with the ethylene treatment. The effects were not large enough to increase marketability of the fruit, therefore, the color was not enhanced enough to warrant use at this level.

Damaged fruit is another problem for Satsuma growers. Damage can be caused by numerous factors, including insects, disease, and physical damage during harvest. Most of the insect damage occurs before harvest. Physical damage and disease, however, occurs during and after harvest. Better handling of the fruit during harvest grading and packing can prevent physical damage and lessen the chance of problems during storage from water loss and disease. The use of preventative fungicides can also be used to prevent fungal diseases.

A key element of the storage/shelf-life project was to examine the effect of wax on fruit. Growers must be conscious of both weight loss and external appearance, since a decrease in weight may result in a reduction in price from the retailer and a hard, shriveled looking fruit available to the consumer. Implications regarding waxed versus non-waxed fruit indicate that waxed fruit performed better in both storage and shelf-life experiments for 2003. With respect to shelf-life, non-waxed fruit lost weight at a significantly faster rate than fruit treated with wax. However, in 2004 there was no difference. Fruit treated with wax maintained turgidity and a glossy peel color when compared to the non-waxed fruit in both years. Non-waxed fruit shriveled and were unacceptable for consumer purchase. This would be especially true if fruit were stored for several days or weeks prior to being sold or consumed. Waxed fruit developed off-flavors and released their peel from the flesh of the fruit. This could have been due to the wax layer being applied to thick and promoting poor gas exchange within the fruit. Unwaxed fruit declined in marketability about the same time as the waxed fruit, week 8-9 in both years, due to loss of turgidity in the peel. Based on the conservation of weight and overall retention of quality, growers are recommended to implement fruit waxing in order to extend both storage and shelf-life.

As well as looking at the physical appearance of the food, consumers are becoming increasingly concerned with labels on the products they buy. The knowledge gained by the Vitamin C study can be used to give consumers a Satsuma that has the optimal health benefits. Fruit packaging can also have a more effective label that details Vitamin C content. Better labeling can then be used as a marketing tool in order to increase product awareness, garner first time users, and thereby, increase Satsuma sales.

In summary, utilizing ethylene, wax, and fungicides, will allow growers to market a product that is able to compete with similar mandarins in both appearance and quality. By giving the consumer what they want, growers can increase demand for their product.

Future Research

The results obtained from this research are part of the puzzle, but more research is needed to allow growers to provide a high quality product that can be marketed over the longest amount of time. Future research is needed to take the postharvest handling to the next step with regards to degreening, fungicide application, and internal antioxidant content.

Although these projects indicate ethylene can be used to degreen the fruit, more work should be conducted in several areas. First, future research should examine varying ethylene concentrations in order to understand which concentration degreens most efficiently and poses an insignificant risk for fruit damage. Other avenues of research should concentrate on the optimal duration of ethylene exposure and storage temperatures after exposure. Along with external quality measures, fruit should also be monitored for internal quality both during and after the study to determine if increased duration results in any detrimental effect to the fruit.

Research should also focus on fungicide usage. During the two years this study was conducted fungal disease pressures were low. Future fungicide research should be conducted during years of high disease incidence. By working with plant pathologists, years lending themselves to high disease concentrations can be identified. Other research can identify the proper application method for applying fungicides to Satsumas. Examples of application methods to be looked at include: dipping, application as part of a wax solution, and spraying a solution onto the fruit.

Waxed fruit developed off-flavors and showed signs of alcoholic fermentation late in the storage/shelf-life study. One possible cause of this could be poor gas exchange within the fruit. The wax application causes a modified atmosphere within the fruit. Thinner applications of the wax may relieve the problem promoting an even longer shelf/storage life for the fruit.

Future research should also continue to evaluate the health benefits associated with Satsumas. Phenolics, nitrogen compounds, carotenoids and some vitamins are some of the other compounds found in citrus (Piga et al., 2002). These compounds should be measured throughout storage. There are also a number of value-added opportunities that growers should also consider. The peel contains a high nutrient value as well as the flesh; although, the peel of the fruit is discarded while flesh or juice is used.

By obtaining a better understanding of internal content, growers can more effectively promote the health benefits of Satsumas. This information can be used in both package labeling and informational literature.

In summary, the storage and shelf-life of fruit in this experiment was between 8 and 9 weeks for both years. However, future research may be able to extend the life of the fruit even longer. Conducting future research with ethylene, wax, and fungicides will allow growers to more effectively color and protect their valuable crop. Research on the internal components of a Satsuma will allow growers to better promote their product. Increased research will allow growers to offer a product that is more competitive and profitable.

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VII. APPENDIX A: TABLES

Time after start of ethylene treatment (hours)	Ethylene concentration (ppm)
2003	
0	4.40
1	4.68
5	4.81
10	4.44
19	5.00
24	5.16
2004	
0	5.43
4	5.27
14	4.89
20	3.69

Table 1. Ethylene concentration during 24-hour exposure treatment for the storage and shelf-life experiments in 2003 and 2004.

Treatment number	Ethylene Treatment	Wax Treatment	Fungicide Treatment
1	Ethylene	Wax	Imazalil
2	Ethylene	Wax	TBZ
3	Ethylene	Wax	No Fungicide
4	Ethylene	No Wax	Imazalil
5	Ethylene	No Wax	TBZ
6	Ethylene	No Wax	No Fungicide
7	No Ethylene	Wax	Imazalil
8	No Ethylene	Wax	TBZ
9	No Ethylene	Wax	No Fungicide
10	No Ethylene	No Wax	Imazalil
11	No Ethylene	No Wax	TBZ
12	No Ethylene	No Wax	No Fungicide

Table 2. Treatments applied for the storage and shelf-life studies.

	Peel color		Fruit size				Fruit flavor		
	Hue angle	Chroma	Weight	Length	Width	Height to Diameter ratio	Brix	Titratable acidity	Brix/ Titratable acidity ratio
					2003 harvest				
Ethylene (E)	0.1860	0.1750	0.6979	0.4271	0.6495	0.2363	0.3712	0.4553	0.1569
Wax (W)	0.0337	0.6363	0.5453	0.1909	0.2421	0.0289	0.9693	0.1334	0.0568
Fungicide (F)	0.0304	0.0628	0.4147	0.7387	0.6979	0.5316	0.1892	0.3624	0.3771
Week	<0.0001	0.3414	<0.0001	<0.0001	<0.0001	0.0006	<0.0001	0.0439	<0.0001
Week * E	0.8544	0.8887	0.8248	0.6364	0.7366	0.4704	0.6222	0.3554	0.3323
Week * W	0.2741	0.9917	0.0692	0.0174	0.0875	0.1244	0.6785	0.1754	0.1242
Week * F	0.1182	0.1016	0.2587	0.4377	0.1746	0.8017	0.3963	0.5538	0.8277
Week * E * W	0.3588	0.9052	0.2993	0.8965	0.0824	0.2939	0.2338	0.0001	<0.0001
Week * E * F	0.0826	0.1299	0.3949	0.5034	0.1622	0.0986	0.1642	0.0042	0.0012
					2004 harvest				
Ethylene (E)	<0.0001	0.5766	0.2082	0.2430	0.6033	0.3283	0.0318	0.0137	0.2019
Wax (W)	0.6323	0.4223	0.0351	0.3683	0.0011	0.0909	0.4964	0.0343	0.0230
Fungicide (F)	0.4618	0.8207	0.1741	0.7336	0.3905	0.6889	0.7267	0.8161	0.8287
Week	<0.0001	0.0250	<0.0001	0.6124	0.0285	0.2241	0.0001	0.0669	0.0005
Week * E	0.0445	0.2578	0.4844	0.5950	0.7257	0.7291	0.0085	0.0080	0.1949
Week * W	0.7242	0.7919	0.4322	0.7885	0.0901	0.0917	0.8332	0.0669	0.0452
Week * F	0.7277	0.9371	0.4083	0.8096	0.7486	0.9890	0.9436	0.8092	0.7818
Week * E * W	0.1007	0.7790	0.5976	0.7936	0.3466	0.6593	0.7128	0.7132	0.8013
Week * E * F	0.9483	0.3268	0.3480	0.8596	0.0429	0.0416	0.1520	0.6796	0.8865

Table 3. P values for an analysis of variance for selected interactions and main effects to determine effect of ethylene, wax and fungicide treatments on fruit quality during storage at (4 ^{o}C) for two years.

	Peel color		Fruit size				Fruit flavor		
	Hue angle	Chroma	Weight	Length	Width	Height to Diameter ratio	Brix	Titratable acidity	Brix/ Titratable acidity ratio
				2003 harvest					
Ethylene (E)	0.0064	0.0987	0.7916	0.7617	0.2101	0.2516	0.5420	0.7434	0.4231
Wax (W)	0.4350	0.0025	0.6726	0.7398	0.3934	0.1828	0.4802	0.0211	0.0865
Fungicide (F)	0.8372	0.7533	0.1328	0.3897	0.3405	0.2185	0.3483	0.7787	0.9614
Week	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.3566	0.0194	0.0001	<0.0001
Week * E	0.0308	0.7055	0.7848	0.9808	0.2042	0.1182	0.5806	0.7093	0.7414
Week * W	0.7586	0.0001	0.0207	0.0120	0.1566	0.2111	0.4028	0.0641	0.2334
Week * F	0.7089	0.5840	0.7031	0.9470	0.3237	0.1359	0.2982	0.5743	0.7341
Week * E * W	0.9730	0.6303	0.9121	0.3990	0.2379	0.5950	0.0338	0.0016	0.0072
Week * E * F	0.0963	0.3347	0.8221	0.8253	0.7909	0.3516	0.6091	0.1979	0.2495
				2004 harvest					
Ethylene (E)	0.0011	0.0616	0.1865	0.3037	0.4381	0.5801	0.4134	0.3941	0.3054
Wax (W)	0.4059	0.0011	0.1961	0.6478	0.8888	0.3624	0.1602	0.5726	0.2239
Fungicide (F)	0.5484	0.6653	0.4862	0.9700	0.6938	0.6606	0.4832	0.6294	0.9232
Week	<0.0001	0.7245	<0.0001	0.0029	0.3766	0.0005	0.0037	0.0015	<0.0001
Week * E	0.0545	0.0617	0.4672	0.3510	0.7114	0.3277	0.8062	0.1163	0.1232
Week * W	0.0294	0.0126	0.0713	0.7142	0.2635	0.3216	0.0614	0.3932	0.0887
Week * F	0.9416	0.7245	0.9447	0.9525	0.9684	0.9640	0.8626	0.8774	0.8993
Week * E * W	0.0088	0.5241	0.4708	0.9838	0.9266	0.9271	0.0225	0.2751	0.8700
Week * E * F	0.4288	0.0181	0.0290	0.3538	0.3315	0.0008	0.1979	0.1807	0.4683

Table 4. P values for an analysis of variance for selected interactions and main effects to determine effect of ethylene, wax and fungicide treatments on fruit quality during storage then one week on the shelf.

	AA	DHA	TAA
		2003 harvest	
Ethylene (E)	0.0065	0.7113	0.3852
Wax (W)	0.1470	0.9641	0.5649
Fungicide (F)	0.4263	0.9807	0.8652
Week	0.2932	0.8402	0.5212
Week * E	0.0531	0.5957	0.7492
Week * W	0.1992	0.9011	0.6712
Week * F	0.5333	0.9803	0.9381
Week * E * W	0.0261	0.9361	0.3690
Week * E * F	0.6331	0.9983	0.9259
		2004 harvest	
Ethylene (E)	0.8765	0.8869	0.8605
Wax (W)	0.3462	0.6062	0.7564
Fungicide (F)	0.6415	0.8714	0.6832
Week	0.0170	0.0138	0.0053
Week * E	0.5311	0.6715	0.8733
Week * W	0.1214	0.4725	0.5519
Week * F	0.5449	0.9305	0.6812
Week * E * W	0.9437	0.0324	0.2388
Week * E * F	0.6975	0.6738	0.5968

Table 5. P values for an analysis of variance for selected interactions and main effects to determine effect of ethylene, wax and fungicide treatments on Vitamin C content during storage at (4 ^{o}C) for two years.

	AA	DHA	TAA
		2003 harvest	
Ethylene (E)	0.5900	0.0597	0.4144
Wax (W)	0.1250	0.5180	0.5104
Fungicide (F)	0.2348	0.3477	0.7916
Week	0.0207	<0.0001	0.0908
Week * E	0.6756	0.0406	0.3141
Week * W	0.1891	0.2573	0.8359
Week * F	0.4920	0.3359	0.9038
Week * E * W	0.3037	0.6628	0.3678
Week * E * F	0.8960	0.7995	0.9813
		2004 harvest	
Ethylene (E)	0.4652	0.0078	0.0502
Wax (W)	0.3097	0.1550	0.9744
Fungicide (F)	0.7592	0.1459	0.7719
Week	0.4575	0.7278	0.6980
Week * E	0.5206	0.0018	0.0261
Week * W	0.1951	0.2661	0.6617
Week * F	0.7172	0.1700	0.7634
Week * E * W	0.4108	0.1098	0.8178
Week * E * F	0.5180	0.2432	0.9863

Table 6. P values for an analysis of variance for selected interactions and main effects to determine effect of ethylene, wax and fungicide treatments on Vitamin C content during storage then one week on the shelf.

Fruit	*Approximate Vitamin C content (mg/100 g FW)*
Apple (Fuji), with skin	3 *
Apple (Red Delicious), with skin	3 *
Grapefruit (ruby red)	48 *
Grapefruit (white)	29 *
Orange (Navel)	59 *
Pomelo	32 *
Satsuma Mandarin	36 **
Strawberry	80 *
Tangerine	22 *

Table 7: Approximate Vitamin C content in common fruit crops.
* Franke et al., 2004
**An average of the 2003 and 2004 control group fruit

	Concentration after exposure (ppm)		
	24-hours	48-hours	72-hours
	130.11	267.48	17.42
	123.69	259.85	16.86
	119.55	266.21	25.58
	131.05	267.26	
	128.06	279.88	
Average concentration	*126.49*	*268.14*	*19.95*

Table 8. Concentration of ethylene in a commercial degreening room during an ethylene degreening experiment.

VIII. APPENDIX B: FIGURES

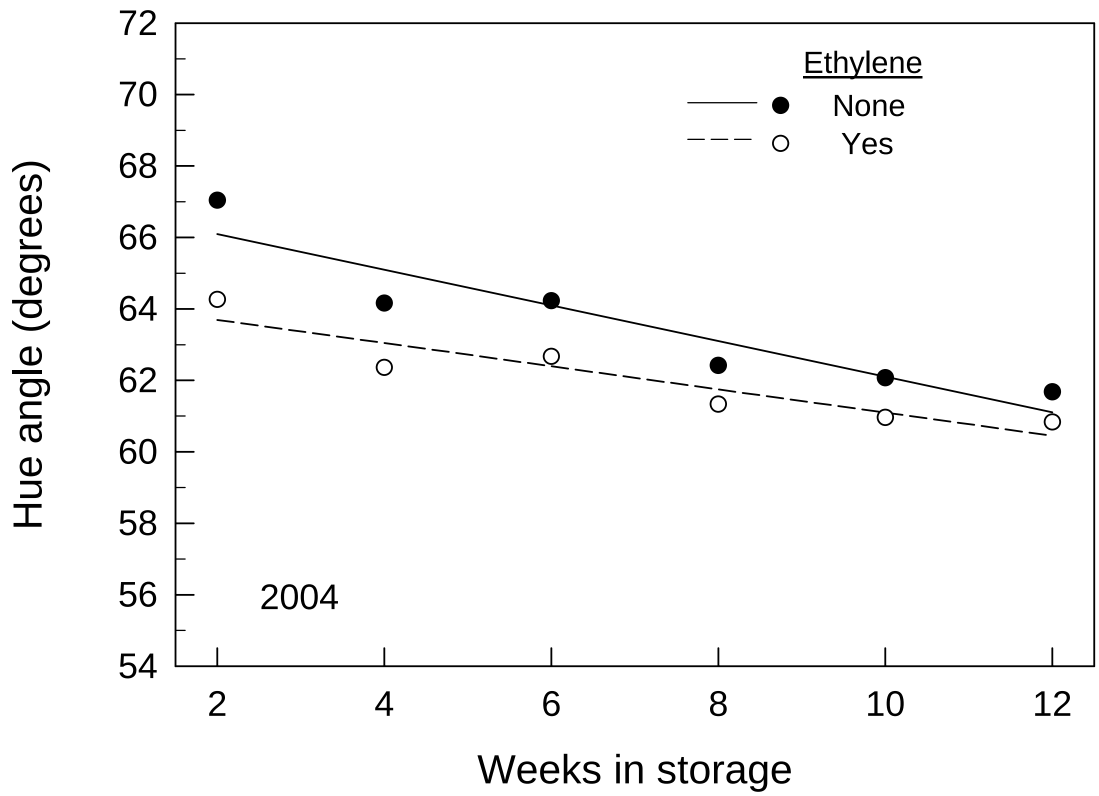

Figure 1. The effect of ethylene treatments on hue angle during storage at (4^{o}C) in 2004.

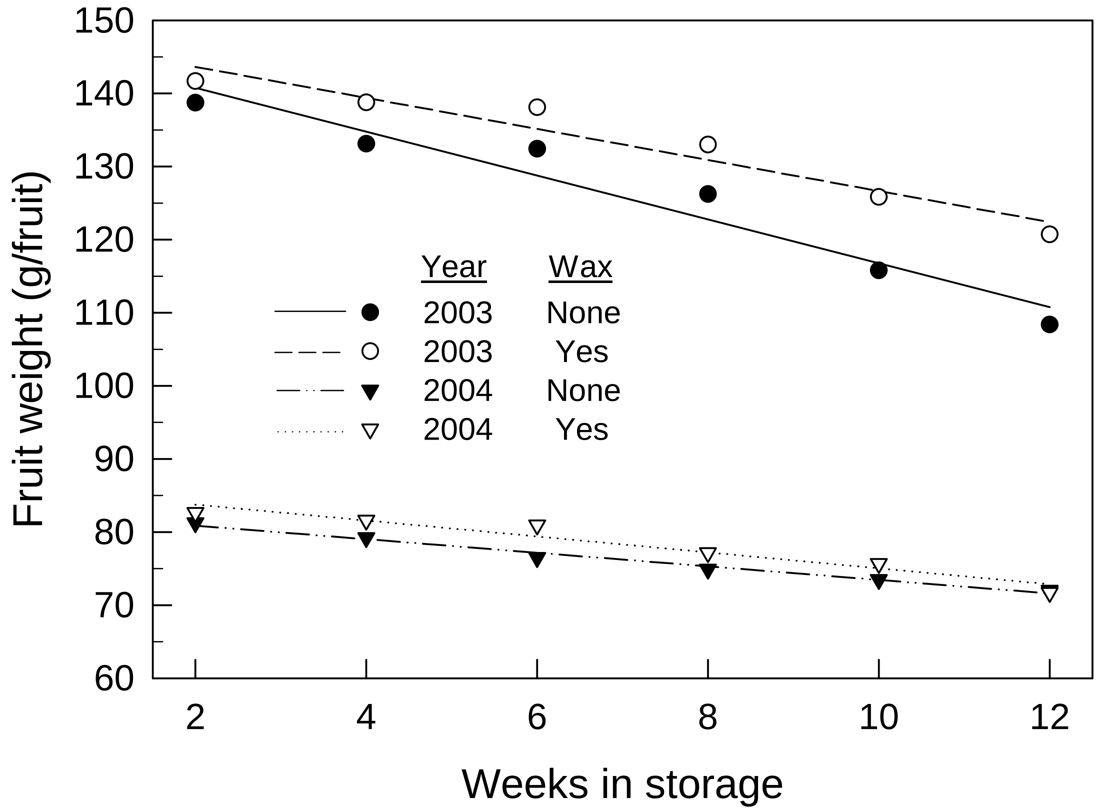

Figure 2. The effect of wax treatments on fruit weight during storage at (4^oC) in 2003 and 2004.

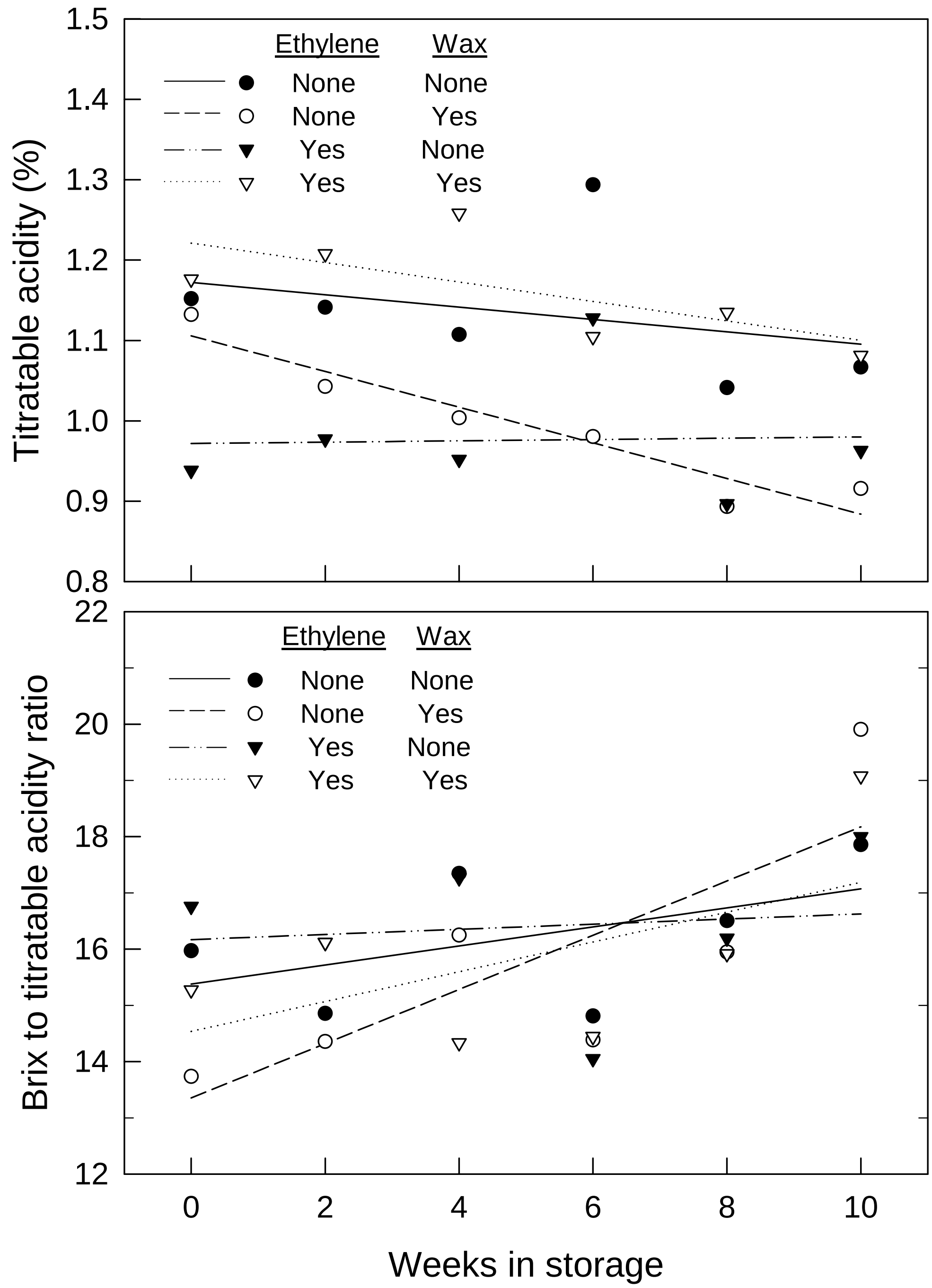

Figure 3. The effect of ethylene and wax treatments on titratable acidity and Brix during storage at (4^{o}C) in 2003.

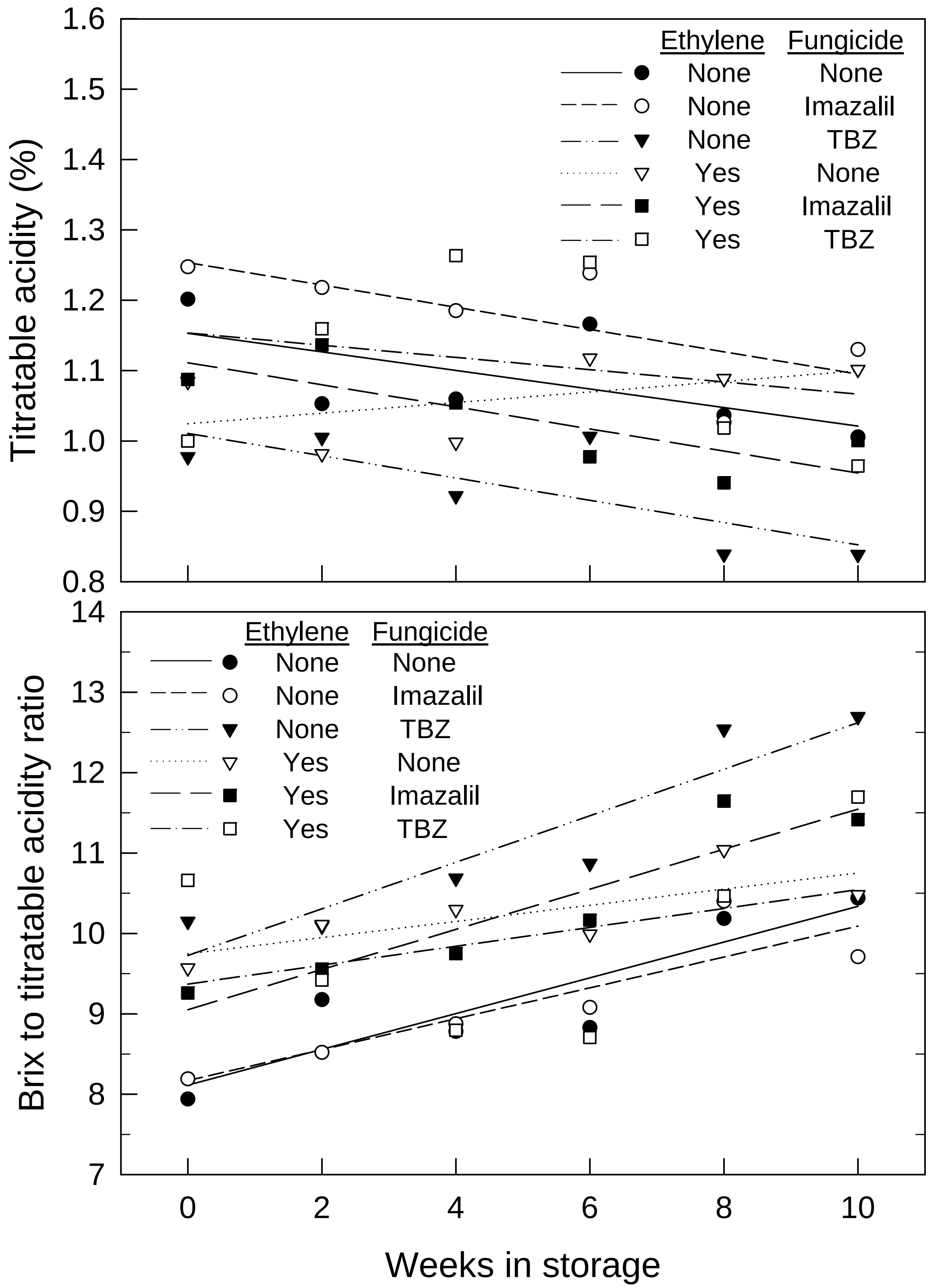

Figure 4. The effect of ethylene and fungicide treatments on titratable acidity and Brix during storage at (4^{o}C) in 2003.

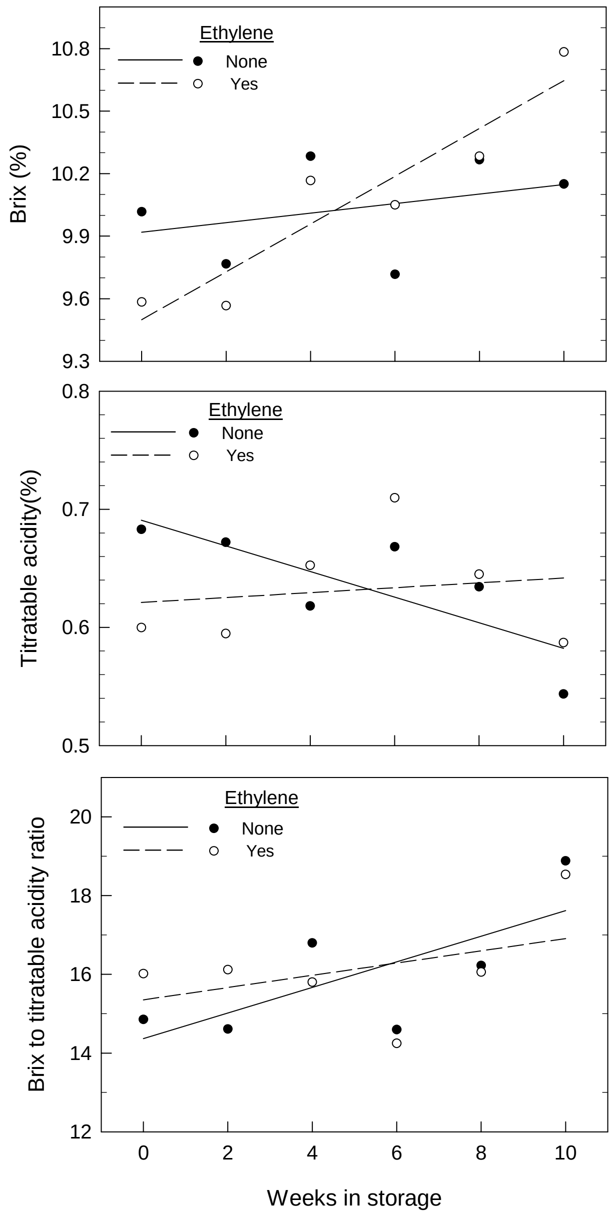

Figure 5. The effect of ethylene treatments on Brix, titratable acidity, and their ratio during storage at (4°C) in 2004.

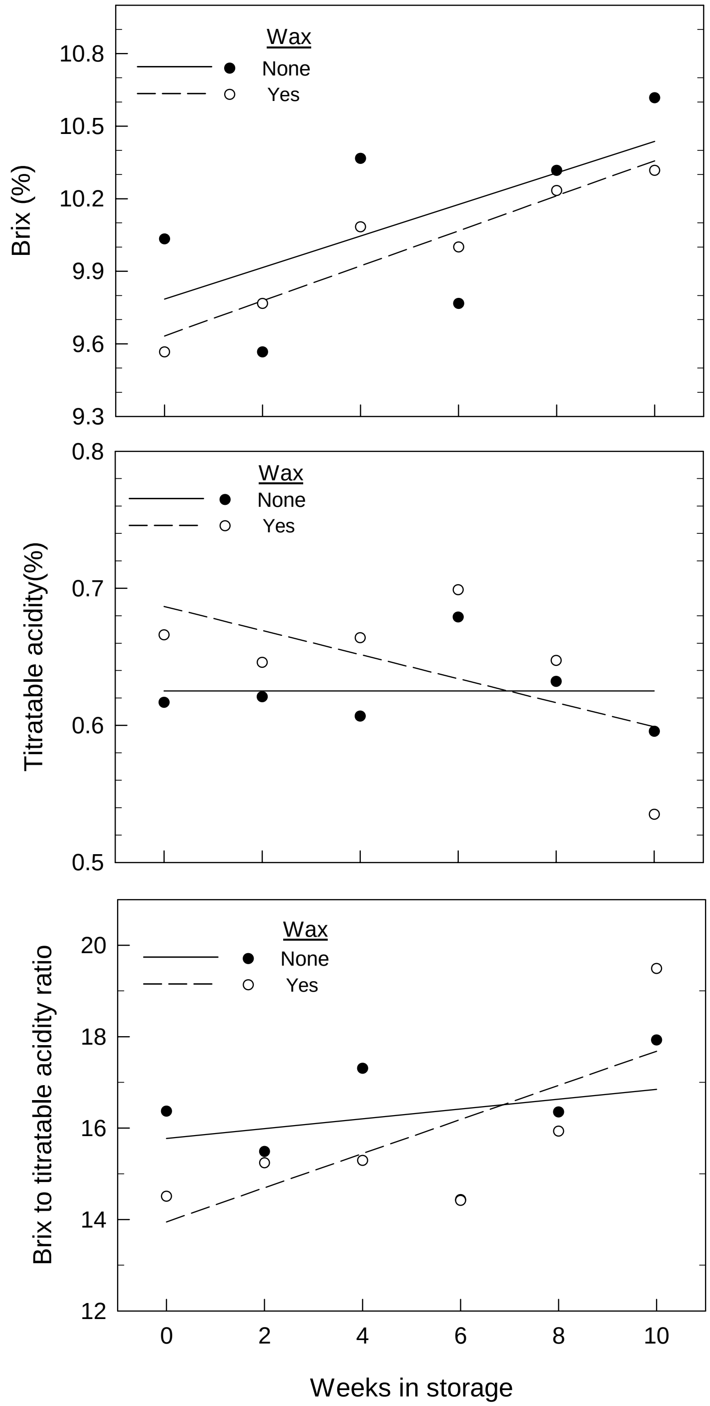

Figure 6. The effect of wax treatments on Brix, titratable acidity, and their ratio during storage at (4°C) in 2004.

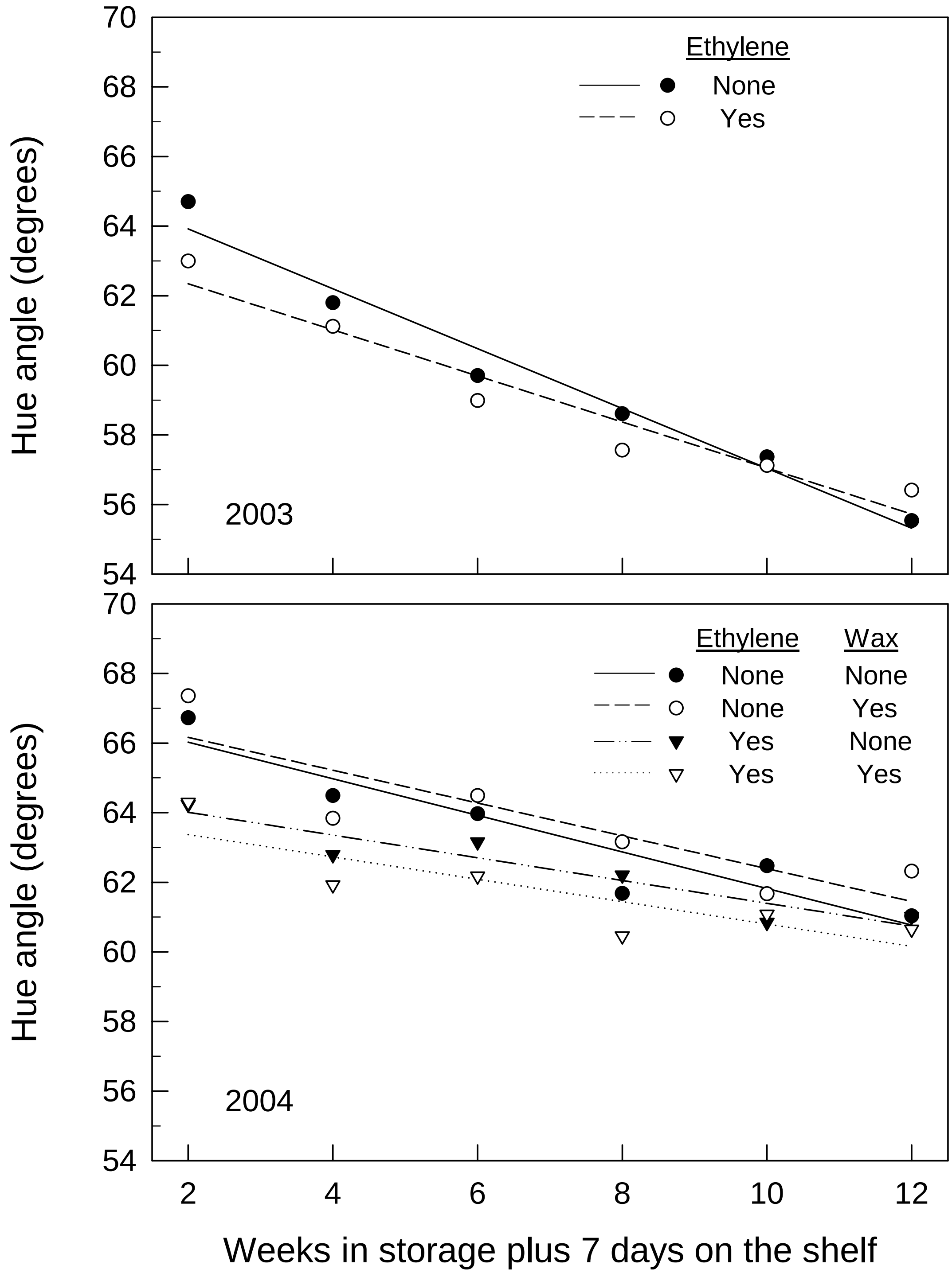

Figure 7. The effect of ethylene and ethylene by wax treatments on hue angle during the shelf-life experiment at ambient temperature in 2003 and 2004.

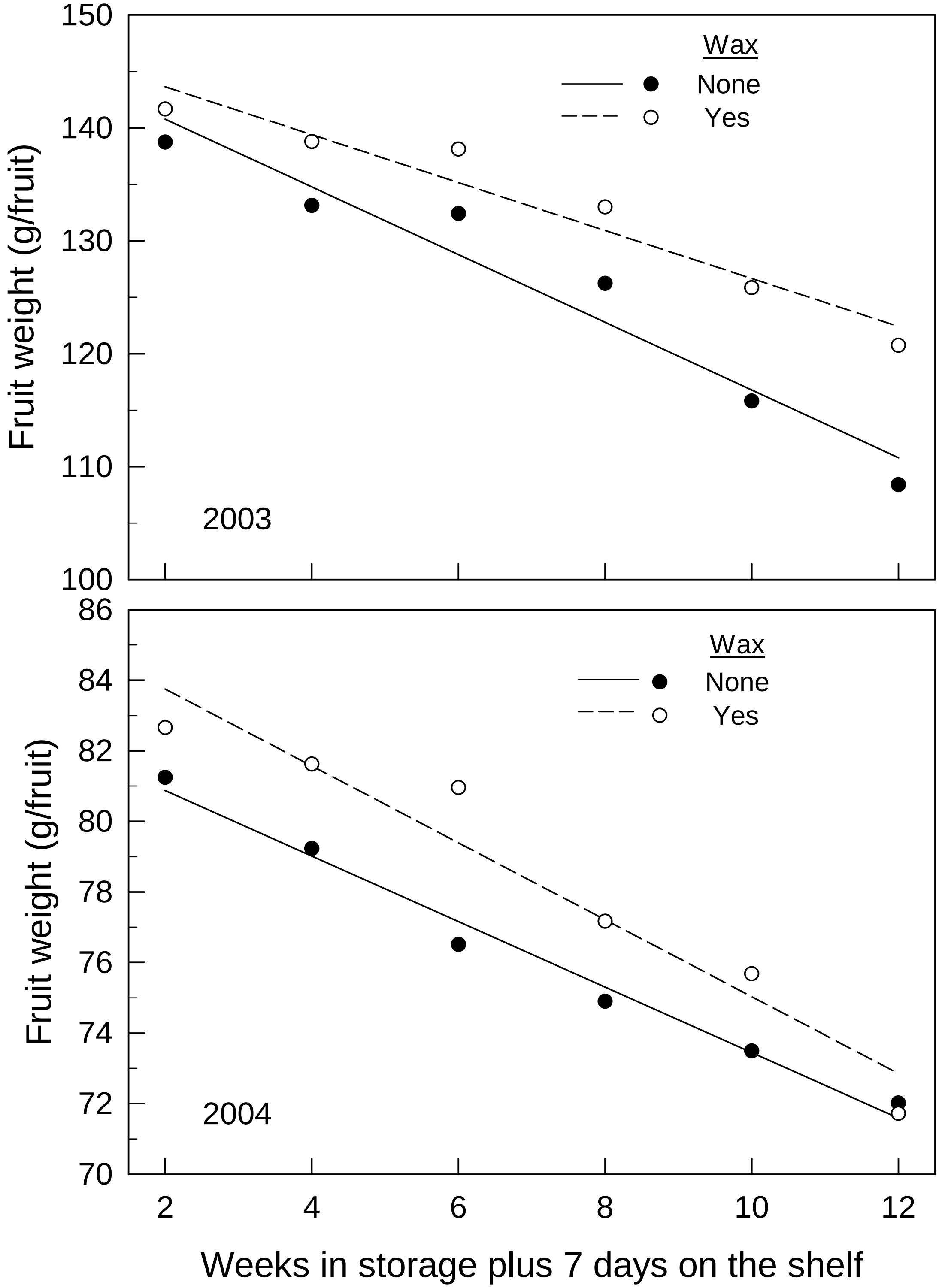

Figure 8. The effect of wax treatments on fruit weight during the shelf-life experiment at ambient temperature in 2003 and 2004.

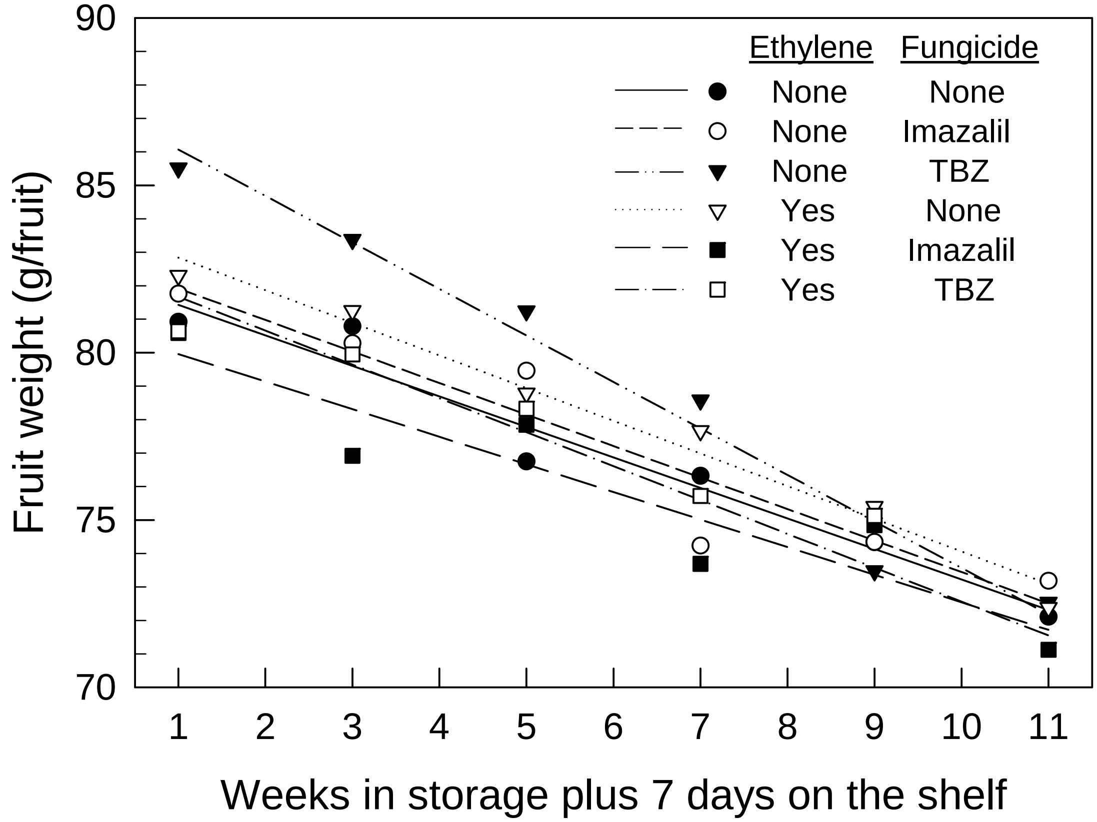

Figure 9. The effect of ethylene and fungicide treatments on fruit weight during the shelf-life experiment at ambient temperature in 2004.

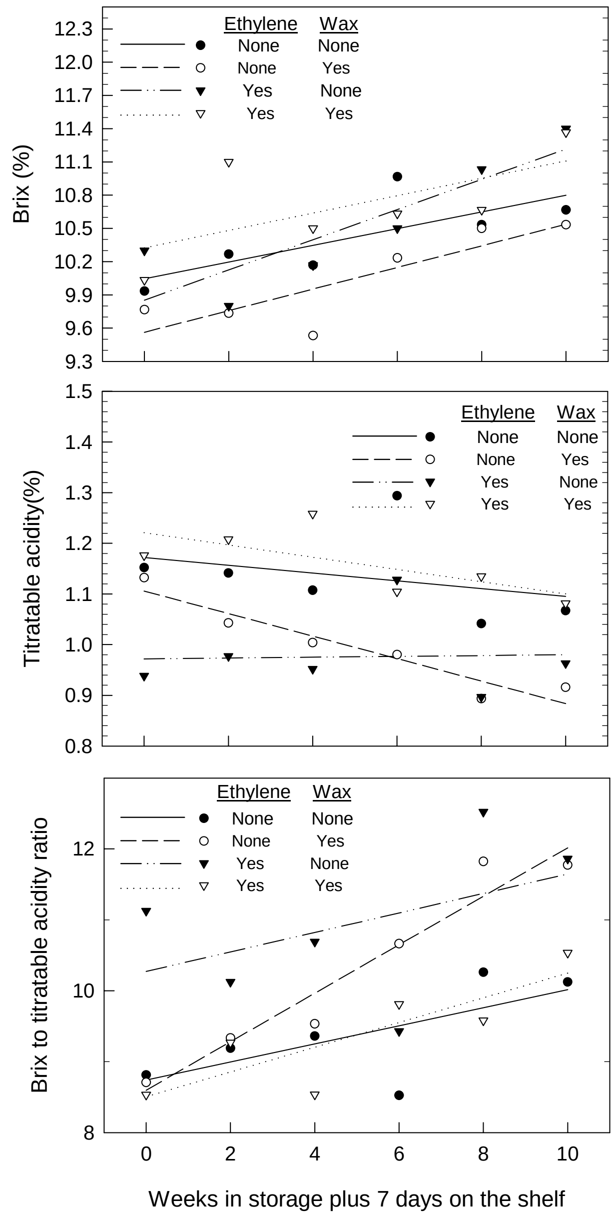

Figure 10. The effect of ethylene and wax treatments on Brix, titratable acidity, and their ratio during the shelf-life experiment at ambient temperature in 2003.

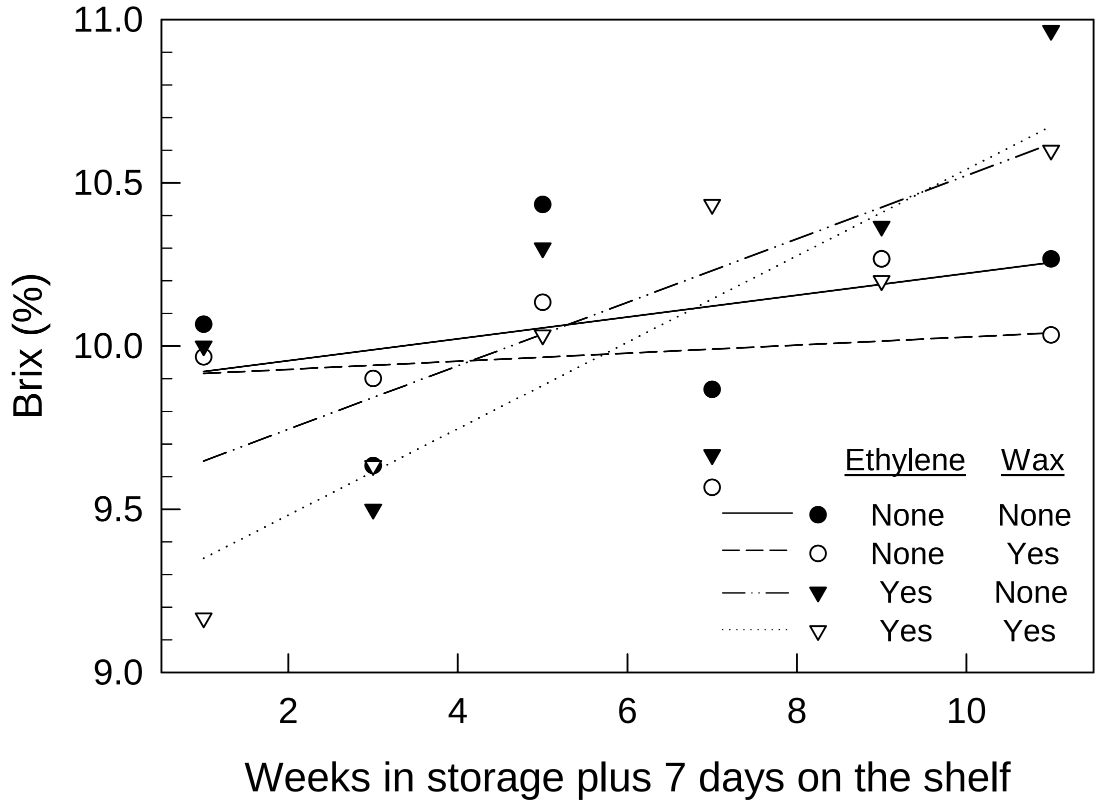

Figure 11. The effect of wax and ethylene treatments on Brix during the shelf-life experiment at ambient temperature in 2004.

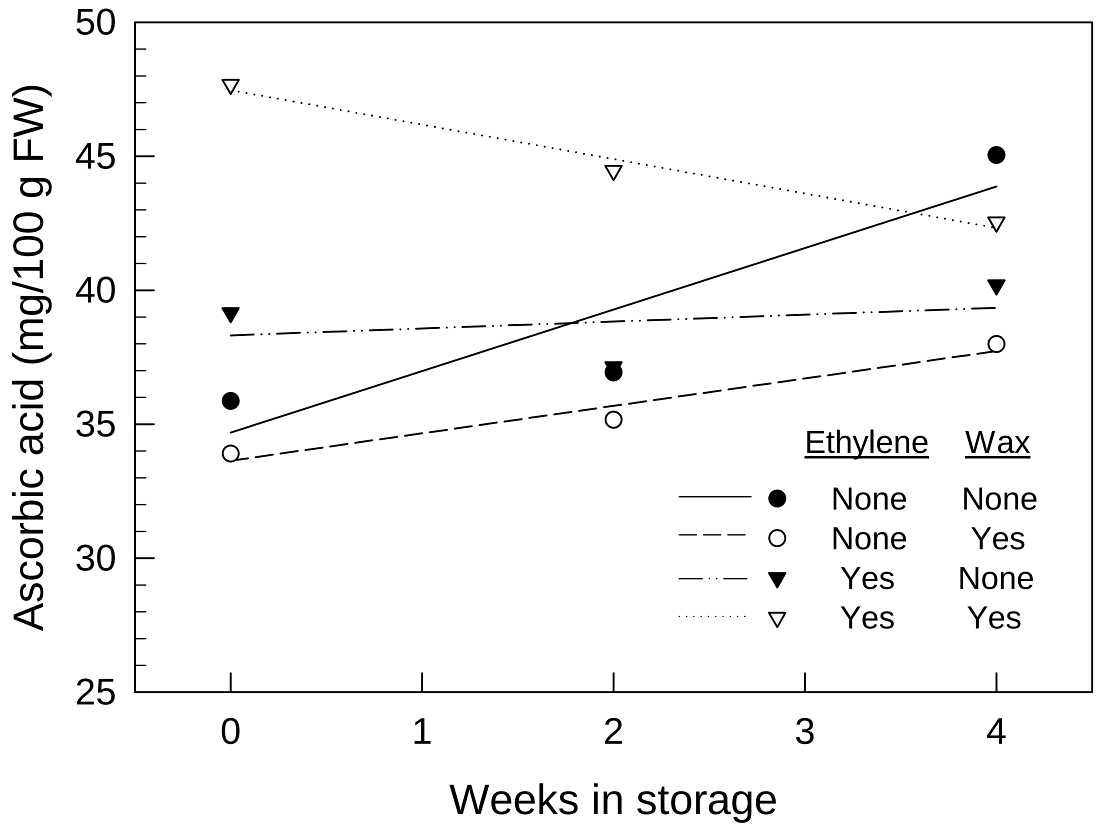

Figure 12. The effect of ethylene and wax treatments on ascorbic acid during storage at ($4^{o}C$) in 2003.

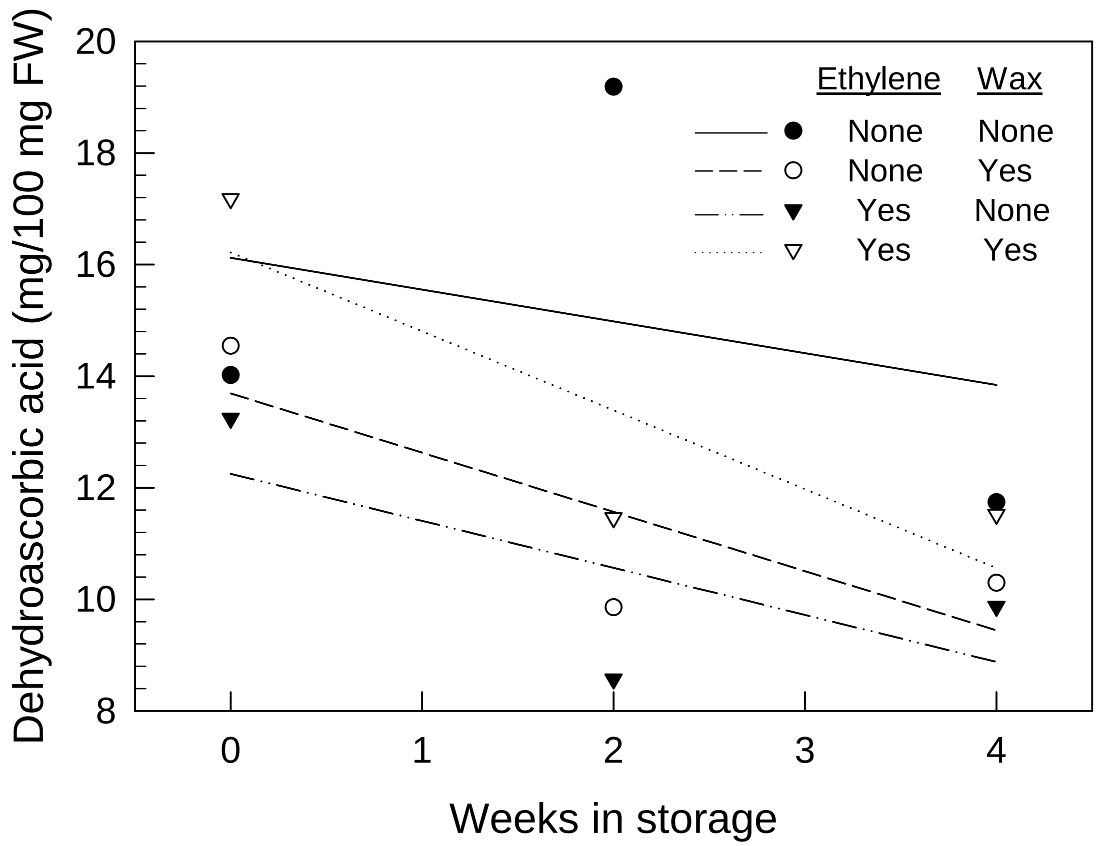

Figure 13. The effect of ethylene and wax treatments on dehydroascorbic acid during storage at (4°C) in 2004.

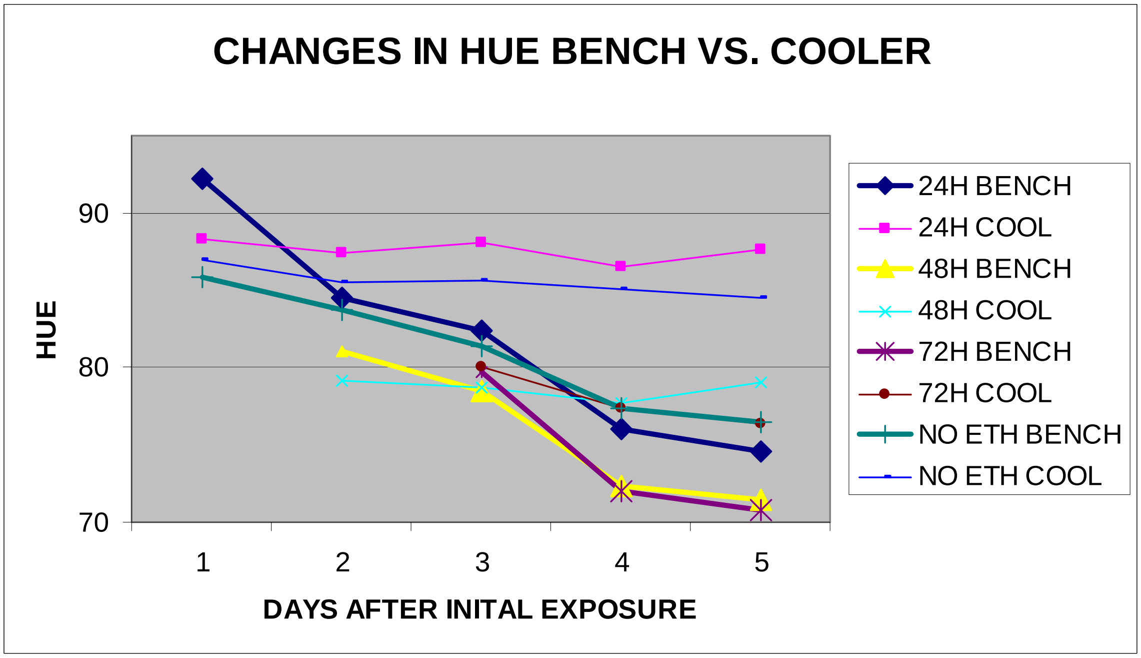

Figure 14. The effect of ethylene exposure duration and temperature treatments on hue angle.

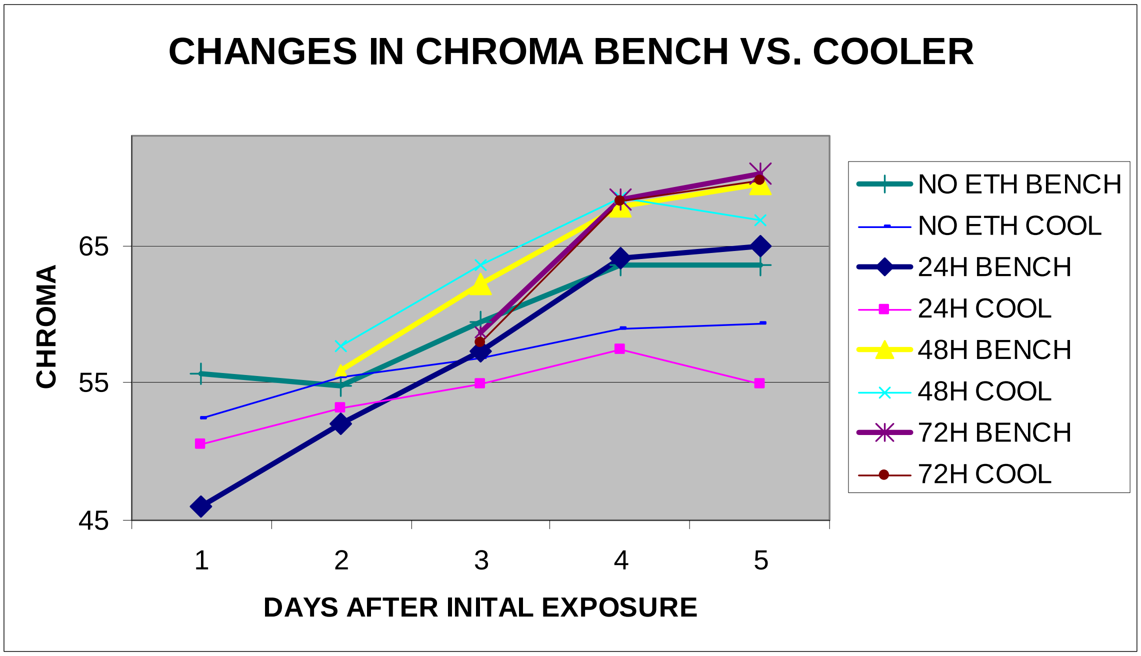

Figure 15. The effect of ethylene exposure duration and temperature treatments on chroma.