DEVELOPMENT AND EVALUATION OF AN ANTISENSE ACC-OXIDASE (CMACO-1) 'GALIA' F1 HYBRID MUSKMELON (*Cucumis melo* L. var. *reticulatus* Ser.)

By

JEANMARIE MINK HARTY

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DEVELOPMENT AND EVALUATION OF AN ANTISENSE ACC-OXIDASE (CMACO-1) 'GALIA' F1 HYBRID MUSKMELON (*Cucumis melo* L. var. *reticulatus* Ser.)

By

Jeanmarie Mink Harty

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Recognized for its flavor, the Galia muskmelon (*Cucumis melo* L. var. *reticulatus* Ser.) is also known to have a short shelf-life. To address this concern, previous work transformed the 'Galia' male parental line with an antisense ACC oxidase (CMACO-1) gene. The gene inhibited the last step in ethylene biosynthesis, resulting in transgenic male parental lines that produced less ethylene and were firmer, subsequently possessing a longer shelf-life than the non-transgenic fruit. These lines were used to create antisense 'Galia' (ASG) hybrids (ASxWT, ASxAS, WTxAS).

During fall 2006, preliminary ASG lines were evaluated with the original 'Galia' muskmelon. Fruits were harvested at four stages: stage 1.) zero-slip, green (ZG); 2.) zero-slip, yellow-green (ZYG); 3.) half-slip (HS); and 4.) full-slip (FS). Data were recorded for days to harvest, fruit size, quality, ethylene evolution and respiration. At stage ZG, all ASG muskmelons were similar in size, quality, ethylene and respiration to 'Galia'. At stage ZYG, ASxAS and ASxWT muskmelons were significantly firmer than 'Galia' and produced less ethylene yet were similar in soluble solids content (SSC). On average,

ASG melons remained on the vine six days longer than ‘Galia’ and were similar in quality to ‘Galia’ at stages HS and FS.

Aroma volatiles were identified in order to determine what sets ‘Galia’ flavor apart from look-a-like cultivars. The compounds considered important in ‘Galia’ muskmelons were benzyl acetate, ethyl-2-methyl butyrate, methyl 2-methyl butyrate, ethyl isobutyrate, 2-methylbutyl acetate, hexyl acetate, ethyl butyrate, ethyl caproate, cis-3-hexenyl acetate, and isovaleronitrile.

ASG and ‘Galia’ muskmelon aroma was evaluated in 2006 and 2007. The greatest differences in aroma among ASG and ‘Galia’ were at stage ZYG, where volatiles were greatest in ‘Galia’. After five-days storage at 20 C in fall 2007, line ASxAS fruit remained firmer, when harvested at stage ZYG. At stages ZG, HS and FS, aroma and quality differences were few. On average, ASG fruit remained on the vine four days longer than ‘Galia’, suggesting a wider harvest window. Even though there were some differences in volatiles at stage ZYG, in order to enhance shipping quality, it is recommended that line ASxAS be harvested at stage ZYG, where SSC was acceptable and fruit firmness was greatest at harvest and after storage.

CHAPTER 1
INTRODUCTION

The Galia (*Cucumis melo* L. var. *reticulatus* Ser.) muskmelon was developed in Israel by breeder Zvi Karchi and released in 1973. As a F_1 hybrid, 'Galia' has specific female and male parental lines. The female parental line of 'Galia' is a Ha' Ogen type melon cultivar called 'Noy Yizre'el' (Karchi, personal comm., 2004), which is green-fleshed with a smooth, sutured skin (Karchi, 2000). The male parental line of 'Galia' was originally from the Peninsula of Crimea, in the Ukraine, and is a cultivar called 'Krymka', in which fruits were round with a golden, netted skin and light green, firm flesh (Karchi, personal comm., 2004). The resulting hybrid cross—the 'Galia' muskmelon, has round fruits with an orange-netted skin and a green, soft textured flesh with a unique, musky aroma, high soluble solids content (13 to 15° Brix), and is a high yielding cultivar (Karchi, 2000). As a result of marketing campaigns with Agrexco and sales at the British food chain, Marks and Spencer, 'Galia' muskmelons became popular all over Western Europe, except France (Karchi, 2000).

Although it is a high quality fruit, 'Galia' muskmelon has some limitations. Besides being highly susceptible to powdery mildew (*Podosphaera xanthii* (formerly *Sphaerotheca fuliginea* Schlech ex Fr. Poll.)) (Mitchell et al. 2006 and 2007a), another main disadvantage is its short shelf-life (Mitchell et al. 2007a and 2007c; Nuñez-Palenius et al., 2005). 'Galia' muskmelons may last two to three weeks if harvested at a pre-slip stage and stored at low temperatures (Aharoni et al., 1993). Exporters have increased shelf-life by harvesting fruit at an immature (pre-ripe/slip) stage; however this leads to lower quality fruit (Fallik et al., 2001; Canliffe and Shaw, 2002; Pratt. 1971). In order to achieve the best flavor, 'Galia' must be harvested at full-slip. To address 'Galia's

limitations, breeders have created a whole new market class called ‘Galia’-type (GT) muskmelons (Karchi, 2000). These GT cultivars have improved disease resistance/tolerance as well as an increased shelf-life (Karchi, 2000; Mitchell et al, 2006 and 2007a). Unfortunately, although these GT cultivars are firm, many often lack the flavor, aroma, and high sugar content of the original ‘Galia’ hybrid (Mitchell et al. 2006 and 2007a).

In order to attain an increased shelf-life and maintain the high quality and flavor of the original ‘Galia’ muskmelon, researchers have transformed the ‘Galia’ male parental line (cv. ‘Krymka’) with an antisense ACC-oxidase gene (CMACO-1) (Nuñez -Palenius et al., 2006a). ACC oxidase is the catalyst in the last step in the biosynthetic pathway of ethylene (Yang and Hoffman, 1984), a hormone that has a major role in fruit ripening and senescence (Abeles et al., 1992). This hormone initiates fruit softening, changes in carbohydrate metabolism, aroma volatile production, and abscission, but does not regulate fruit size and sugar content (Pech et al., 1999; reviewed by Nuñez-Palenius et al., 2008).

The work from Nuñez -Palenius et al. (2006a) produced two independent antisense ‘Krymka’ lines named TGM-AS-1 and TGM-AS-2. Fruits from TGM-AS-2 line produced less ethylene and were firmer than untransformed fruits at half and full-slip stages (Nuñez -Palenius et al., 2006b). TGM-AS-1 fruits also exhibited lower ethylene production, but only during the half-slip stage (Nuñez -Palenius et al., 2006b). Conversely, the female parental line of ‘Galia’ muskmelon, cultivar ‘Noy Yizre’el’ was unable to be transformed with an antisense ACC-oxidase (CMACO-1) gene (Nuñez -Palenius et al., 2005).

Nonetheless, an additional result of Nuñez -Palenius's work was the development of antisense 'Galia' muskmelon hybrids (T_0GMH-AS-1 and T_0GMH-AS-2) produced from the T_0 transgenic male parental lines (TGM-AS-1 and TGM-AS-2) (Mitchell et al., 2007c). During fall 2004, T_0 TGMH-AS-1 and T_0 TGMH-AS-2 lines remained on the vine longer than 'Galia' (Mitchell et al., 2007c). However, in a previous crop, a severe powdery mildew (*P. xanthii*) epidemic led to no differences in days to harvest (DTH) between AS lines and 'Galia' (Mitchell et al., 2007b). Due to these challenges, it was desirable to obtain AS 'Galia' hybrids where both the male and female line incorporated the antisense ACC-oxidase (CMACO-1) gene. With both parents positive for the transgene, it was hypothesized that the F_1 hybrid progeny fruit would have reduced ethylene production and therefore a longer shelf-life.

The objectives of this research were three-fold. The first research objective was aimed at producing an elite stock of the antisense male TGM-AS-1 and TGM-AS-2 lines provided by Nuñez-Palenius, and use these lines to produce an antisense ACC-oxidase (CMACO-1) female parental line through backcrossing. A second objective was to produce antisense 'Galia' (ASG) hybrid muskmelons through traditional breeding methods where both the female and male parental lines possess the antisense ACC-oxidase (CMACO-1) gene. The third and final objective was to evaluate the ASG muskmelons by collecting fruit quality data (fruit size, firmness, soluble solids content (SSC), titratable acidity, pH, and aroma) and ethylene and respiration rates. These data were to be collected at different stages of growth to establish fruit quality characteristics of ASG muskmelons and to develop optimal harvest time guidelines. It is also important to note that, because the fruit quality factor, aroma, had not previously been evaluated on

muskmelon at the University of Florida, Horticultural Sciences Department, aroma volatile collection and analysis procedures were determined. Aroma is an important fruit quality factor that is involved in fruit flavor and may be negatively affected by low ethylene production since increased aroma coincides with fruit ripening.

In order to accomplish these objectives, research was conducted from 2004 through 2007 and is summarized in the following chapters. Chapter 2, Literature Review, summarizes the past and current literature that surrounds this research. Chapter 3, Production, Evaluation and Selection of Antisense ACC-oxidase (CMACO-1) Galia Muskmelon (*Cucumis melo* L. var. *reticulatus* Ser.), describes the development of elite parental lines, bearing an antisense ACC-oxidase gene. The elite parental lines were crossed in multiple combinations (AS♀ x WT ♂, AS ♀ x AS ♂, WT ♀ x AS ♂), and antisense 'Galia' (ASG) hybrid seed was produced. ASG hybrids then were evaluated and selections were made for additional studies. Chapter 4, Galia Muskmelon Fruit Quality and Flavor (*Cucumis melo* L. var. *reticulatus* Ser.), describes fruit quality factors and characterizes 'Galia' and GT muskmelon aroma volatiles and flavor. Chapter 5, Aroma Volatile and Fruit Quality Evaluation of Antisense ACC-Oxidase (CMACO-1) Galia Muskmelons (*Cucumis melo* L. var. *reticulatus* Ser.), compares the ASG and 'Galia' muskmelons over three seasons, evaluating fruit quality, including aroma, both at harvest and after storage. And lastly, Chapter 6, Conclusions, summarizes the results and discusses the significant outcome of this work.

CHAPTER 2
LITERATURE REVIEW

Introduction

Classification and Origin

Cucumis melo L., commonly called melon, belongs to the *Cucurbitaceae* or gourd family, a large family that consists of about 120 genera and over 800 species, including pumpkin and squash (*Cucurbita spp*.), cucumber (*Cucumis sativa*) and watermelon (*Citrullus lanatus*) (Whitaker and Davis, 1962; Jeffrey, 1990; Seymour and McGlasson, 1993; Nayar and More, 1998; Robinson and Decker-Walters, 1999). Within the *Cucumis melo* species there are numerous genetically diverse, polymorphic, interfertile subspecies or groups (Whitaker and Davis, 1962; Seymour and McGlasson, 1993; Nayar and More, 1998). These subspecies or groups have been analyzed and categorized by several scientists, including Naudin,1859; Whitaker and Davis, 1962; Smith and Welch, 1964; Munger and Robinson, 1991; Kirkbride, 1993; Robinson and Decker-Walters, 1999; Guis et al., 1998; and Pitrat et al., 2000.

In 1859, Naudin divided *Cucumis melo* into ten groups. Eventually, they were regrouped into seven (Whitaker and Davis, 1962; Smith and Welch, 1964; Guis et al., 1998). These seven groups include variations of *cantalupensis, reticulatus, inodorus, saccharinus, chito, dudaim, conomon and flexuosus.*

The *cantalupensis* group, which is believed to have originated from Cantaluppe, Italy (Robinson and Decker-Walters, 1997) are rarely grown in the U.S. and are true cantaloupes (Whitaker and Davis, 1962). They are usually non-netted, have a rough or warty skin with prominent sutures (Whitaker and Davis, 1962), and flesh is orange or

green. However, the name (cantaloupe) is incorrectly attributed to netted melons in the U.S. (Bailey and Bailey, 1976).

Reticulatus or muskmelon and also called rockmelons in Australia (Aubert and Bourger, 2004) have netted skin, though some may have shallow sutures; flesh color ranges from green to deep orange (Whitaker and Davis, 1962). These melons are sweet with a musky aroma (Whitaker and Davis, 1962).

Inodorus or winter melon is a smooth-skinned fruit with minimal aroma, but has sweet white or green flesh and have a long shelf-life (Whitaker and Davis, 1962; Seymour and McGlasson, 1993). These include Canary, Casaba, Crenshaw and Honeydew (Seymour and McGlasson, 1993).

Saccharinus melons are very sweet with smooth skin that has green spots and some grey (Guis et al., 1998).

Chito, or 'Mango melon' or 'Garden Lemon' are small, smooth and mottled fruits with an acidic flavor that are used as ornamentals or for pickling (Whitaker and Davis, 1962).

Dudaim or Pomegranate melons are also sometimes grouped with *chito* (Naudin, 1859). These are small fruits with a yellow rind, white to pink flesh and have a musky odor (Whitaker and Davis, 1962; Guis et al., 1998). The most common type of *dudaim* is Queen Anne's Pocket Melon, which has become localized in Louisiana and Texas (Whitaker and Davis, 1962).

Conomon, also called Chinese cucumber or Oriental Pickling Melon, are small, oblong fruits with white, crisp flesh, a smooth exterior and have minimal aroma (Naudin, 1859; Guis et al., 1998; Whitaker and Davis, 1962).

And *Flexuosus* (syn. *Utilissimus)* is a long, serpent-like melon used in salads in the Indo-Gangetic plains (Nayar and More, 1998). It is also called Snake Melon or Serpent Melon (Whitaker and Davis, 1962).

The *cantalupensis*, *reticulatus* and *saccharinus* groups have been suggested to be grouped together (Munger and Robinson, 1991). *Dudaim* and *chito* have also been grouped as one under the *chito* name (Naudin, 1859; Munger and Robinson, 1991; Guis et al., 1998). However in 2000, Pitrat and his colleagues suggested only two groups: sweet melons, which included *cantalupensis*, *reticulatus*, *inodorous* and *makuawa* groups; and non-sweet, which included *chate* (*chito*), *flexuosus* and *conomon* groups (Burgur et al., 2003). Nonetheless, it remains debatable if these groups serve any useful purpose except for horticultural characteristics (Whitaker and Davis, 1962).

All groups of *Cucumis melo* easily hybridize together (Whitaker and Davis, 1968); and *Cucumis melo* is a cytologically stable diploid crop with 12 chromosomes (2n = 24) (Nayar and More, 1998; Doijode, 2001).

Most muskmelons are herbaceous annuals, few are perennials, and all are frost sensitive (Nayar and More, 1998; Bailey and Bailey, 1976). They are predominantly warm-season crops and are popular summer fruits (Nayar and More, 1998) and desserts (Bailey and Bailey, 1976). Origins stem from tropical and temperate subtropical areas of Africa, Asia and India, many of which thrive in hot and humid or desert conditions (Whitaker and Davis, 1962; Robinson and Decker-Walters, 1999; Munshi and Alvarez, 2005). Muskmelons can be traced as far back as 3000 B.C.E. and 2400 B.C.E. when the Ancient Egyptians cultivated and illustrated this fruit in paintings (Robinson and Decker-Walters, 1999; reviewed by Walters, 1989; Mills, 2000). They are found in the wild

throughout Africa and Asia, although truly wild forms and the largest concentration of non-cultivated species are found in Africa (Whitaker and Davis, 1962; Karchi, 2000). It is believed melons were introduced into Asia around 2000-1500 B.C.E. by way of the Silk Road (Karchi, 2000; reviewed by Walters, 1989), but culture of melons is thought to have started independently in both Africa and Asia, thus a center of origin is difficult to identify (Kerje and Grum, 2000).

As melon was domesticated in Africa and Asia, it is thought to have then spread into Europe and throughout much of the world (Kerje and Grum, 2000). Reportedly, Columbus brought muskmelon seed to the New World on his second voyage, and it was grown on Isabella Island, of the Galápagos Islands (Robinson and Decker-Walters, 1999; Boswell, 2000). The Spaniards introduced it to California in 1683 (Robinson and Decker-Walters, 1999). There are records of production dated back to 1609 in Bermuda and 1650 in Brazil (Boswell, 2000). In 1796, the first cantaloupe seeds from Tripoli arrived in Philadelphia via Bernard McMahon of Ireland (McDonald and Copeland, 1997). In 1881, a green-fleshed, netted (*reticulatus*) cultivar, 'Netted Gem', was introduced by W. Atlee Burpee Co. (Mills, 2000; Blinn, 1906). It is believed that many modern muskmelons are derived from Burpee's introduction (Mills, 2000). One such derivative, 'Rocky Ford' was originally from Rockyford, Co and was important in the commercial cantaloupe industry in the 1800s (Robinson and Decker-Walters, 1999; Blinn, 1906). 'Burpee Hybrid' was the first melon F_1 hybrid introduced in 1955 (Robinson and Decker-Walters, 1999). Today, the U.S. classifies melons by area-Western and Eastern shipper-types, and specialty, which include 'Galia' and other high quality melons suitable for niche markets (Shellie and Lester, 2004). The Western shipper

types are grown in Arizona, California, and Texas for both domestic and export markets (Shellie and Lester, 2004). However, Eastern shipper types, which are grown throughout the Eastern U.S. are more perishable and are primarily used loacally (Shellie and Lester, 2004). Both the Western and Eastern types are of the *reticulatus* group. Specialty type melons consist of many diverse groups such as *reticulatus* (Galia, Persian, and Ananas types) and *cantalupensis* (Charentais types) (Shellie and Lester, 2004). Another popular melon in the U.S. is the Honeydew (*inodorus),* which has a longer storage life than *reticulatus* and *cantalupensis* (Mills, 2000).

Consumption and Production

The U.S. ranks 4th in production of melons with 4.2% of the world market, behind China (>50%), Turkey (6.1%) and Iran (4.4%) (Borriss et al., 2006). From 1990 to 2002, the United States saw a 27 % increase in melon consumption due to healthier eating, year-round availability, economic growth, and improved cultivars (ERS/USDA, 2003). Since 2002, melon consumption still remains high (Lucier and Dettman, 2008). However, melon production in the U.S. decreased 17% from 1992 to 2004 (Borriss et al., 2006). In 2004, total U.S. cantaloupe production was valued at $300.6 million and honeydew melon at $89.7 million (Borriss et al., 2006). The U.S. is the largest importer of melons worldwide (Borriss et al., 2006). In 2004, the U.S. imported $117.3 million of melons from Mexico and Central America from December to April and exported melons valued at $91 million to Canada and Japan (Boriss et al., 2006). U.S. domestic melon production is from April to December in most states, with California, Texas and Georgia the top producers (Borriss et al., 2006; ERS/USDA, 2003). Florida is not among the top producers in melons, but it is the leading producer of watermelons in the U.S (Boriss et al., 2006).

Characteristics

Cucumis melo L. is an indeterminate vine crop that has tendrils and a prostate or climbing nature that produces soft, herbaceous stems and branches (Munshi and Alvarez, 2005). It is an andromonoecious species, first producing male (staminate) flowers on the main stem and later producing perfect (hermaphroditic) flowers on the lateral vines (Munshi and Alvarez, 2004; Whitaker and Davis, 1962). Although predominantly andromonoecious, there are some melon cultivars that are monoecious or gynomonoecious (Peterson et al., 1983; More et al., 1980; Munshi and Alvarez, 2005; Whitaker and Davis, 1962).

Fruits are produced through cross-pollination by bees. One flower requires 10 to 15 bee visits for adequate pollination to occur (Mills, 2000). Anthesis occurs in the morning between 5:30 and 6:30 a.m. at temperatures between 22-29 ℃ (Munshi and Alvarez, 2005; Nanpuri and Brar, 1966). Flowers are open for one day and pollen viability decreases as the day progresses (More and Seshadri, 1998). After pollination, the ovary wall expands and develops into pericarp (fruit wall) with an exocarp (skin), mesocarp (flesh) and endocarp (Munshi and Alvarez, 2005). Melon fruits are classified as an indehiscent pepo with three locule sections or ovaries (Robinson and Decker-Walters, 1999).

Muskmelon fruit growth and development follows a sigmoidal growth curve (reviewed by Pratt, 1971). Fruit set of melon is cyclic; one to four fruit are set in each cycle (Mills, 2000). After initial fruit set, flowers abort for five to eight nodes and then set fruit again. Fruit are ready to harvest about 80 to 120 days after planting seeds. Seeds are borne internally in the locular cavity mucilage along the receptacle tissue (Mills, 2000). Seed are viable at full maturity (Whitaker and Davis, 1962; Robinson and

Decker-Walters, 1997). Tens to hundreds or more of seeds are produced per fruit and will remain viable for many years if stored in dry and cool conditions (Robinson and Decker-Walters, 1997; Mills, 2000; Doijode, 2001).

Climacteric and Non-Climacteric Fruit

Fruits had been classified either 'climacteric' or non-climacteric based on their pattern of respiration levels during fruit ripening (Kidd and West, 1925). After the introduction of gas chromatography, the plant hormone ethylene could be detected and was found to have a large increase during the climacteric in fruits (Burg and Thimann, 1959, 1960). Fruit ripening patterns that are climacteric exhibit a rise in respiration concurrent with an autocatalytic production of ethylene (Abeles et al., 1992) known as the climacteric peak (Seymour and McGlasson, 1993). Examples of climacteric fruits are apple (*Malus domestica*), avocado (*Persea americana*), banana (*Musa*), and tomato (*Solanum lycopersicum*) (Kader, 2002a). Non-climacteric fruits do not have a rise in respiration or ethylene during fruit ripening. Such non-climacteric fruits include citrus (*Citrus*), cucumber (*Cucumis sativus*) and strawberry (*Fragaria x anannasa*) (Kader, 2002a).

The *cantalupensis* and *reticulatus* melon groups are climacteric (Lyons et al., 1962; Seymour and McGlasson, 1993; Flores et al., 2002); and have a moderate respiration rate (10 to 20 mg CO_2 kg^{-1} hr^{-1}) and high ethylene production rate (10.0 to 100.0 µl C_2H_4/ kg^{-1} hr^{-1}) (Kader, 2002a). Muskmelons and cantaloupes were reported to have a rapid climacteric at or near full maturity and abscission, with an interval from the pre-climacteric to the climacteric peak being 24 to 48 hours (Lyons et al., 1962). However, melons of the *inodorus* group are non-climacteric (Pratt et al., 1977) and have a low respiration rate (5 to 10 mg CO_2 kg^{-1} hr^{-1}) and produce moderate amounts of

ethylene (1.0 to 10.0 µl C_2H_4 kg^{-1} hr^{-1}) (Kader, 2002a). Kendall and Ng (1988) reported high amounts of ethylene in netted melons (*reticulatus*) at or near harvest and while non-netted melons (*inodorous*) did not produce ethylene until about 20 days postharvest.

The rise in ethylene production during ripening is thought to control changes in color, aroma, texture and flavor (Lelièvre et al., 1997; Hadfield et al., 1995; Alexander and Grierson, 2002; reviewed by Nuñez-Palenius et al., 2008). These changes have promoted extensive research on ethylene and fruit ripening (reviewed by Theologis, 1992; Shellie and Saltveit, 1993; Yamamoto et al., 1995; Lelièvre et al., 1997; Hadfield et al., 1995; Alexander and Grierson, 2002; reviewed by Nuñez-Palenius et al., 2008) and fruit quality (reviewed by Saltveit, 1999; reviewed by Nuñez-Palenius et al., 2008).

There are both ethylene dependent and independent events associated with ethylene production (Pech et al., 1999; reviewed by Nuñez-Palenius et al., 2008). In Charentais cantaloupes (*cantalupensis*), ethylene production during melon fruit ripening were reported to stimulate carbohydrate metabolism, yellowing of the rind, fruit softening, respiration, aroma volatile production and abscission (Pech et al., 1999; reviewed by Nuñez-Palenius et al., 2008). Ethylene independent activities include flesh color development, sugar and organic acid accumulation, loss of acidity and accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC) (Pech et al., 1999). In 'Krymka' muskmelons (*reticulatus*), which are also the male parental line of 'Galia' muskmelon, ethylene during ripening is associated with yellowing of the rind and fruit firmness loss while ethylene independent events are fruit weight and size, titratable acidity, seed number, mesocarp size,+ and total soluble solids (reviewed by Nuñez-Palenius et al., 2008). Ethylene production has also been associated with postharvest

decay (Li et al., 2006) and chilling injury (Pech et al., 1999), thus reducing ethylene biosynthesis could be a method of reducing postharvest losses and extending shelf-life in fruits (reviewed by Nuñez-Palenius et al., 2008).

Ethylene biosynthesis follows the pathway from methionine via *S*-adenosylmethionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC). The enzyme responsible for catalyzing the conversion of SAM to ACC, is ACC synthase (ACS) and the enzyme catalyzing ACC to ethylene is ACC oxidase (ACO) (Yang and Hoffman, 1984).

There are five ACS genes (*CMe-ACS1* to *CMe-ACS5*) that have been isolated from melon (Yamamoto et al., 1995; Ishiki et al., 2000; reviewed by Li et al., 2006; reviewed by Nuñez-Palenius et al., 2008; reviewed by Ezura et al., 2008). *CMe-ACS1* is expressed in the mesocarp tissue of ripening fruit and is a wound responsive gene (Yamamoto et al., 1995). *CMe-ACS2* is expressed in the fruit at the early stages of ripening (pre-climacteric) and in seedlings (Yamamoto et al., 1995). *CMe-ACS3* is also expressed in the mesocarp at the pre-climacteric stage, but at lower levels than *CMe-ACS2* (Ishiki et al., 2000). The role of *CMe-ACS4* is not clear, but *CMe-ACS5* is expressed in ripened fruit and is ethylene independent (reviewed by Li et al; reviewed by Nuñez-Palenius et al., 2008; reviewed by Ezura et al., 2008).

There are three ACO genes in melon (reviewed by Li et al, 2006; reviewed by Nuñez-Palenius et al., 2008; reviewed by Ezura et al., 2008). *CM-ACO1* is expressed in etiolated hypocotyls, roots, leaves flowers and fruit; and it has a role in fruit ripening and senescence as well as in response to wounding (Lasserre et al., 1996; Guis et al., 1997; reviewed by Li et al., 2006; reviewed by Nuñez-Palenius et al., 2008; reviewed by Ezura

et al., 2008). *CM-ACO2* is expressed in etiolated hypocotyls and *CM-ACO3* is expressed in many tissues, but not in fruit (Lasserre et al., 1996; Guis et al., 1997; reviewed by Li et al; reviewed by Nuñez-Palenius et al., 2008; reviewed by Ezura et al., 2008). The suppression of ethylene via blocking ACS and/or ACO in fruits has resulted in inhibition of the ripening process (reviewed by Pech et al., 2008).

In higher plants, there are two systems of ethylene production. System I occurs in all vegetative tissues and in the fruit before it ripens; and produces basal ethylene levels (reviewed by Jakubowicz, 2002; Alexander and Grierson, 2002). System II occurs once the fruit reaches its climacteric phase, when ethylene production is autocatalytic and during petal senescence (Alexander and Grierson, 2002; reviewed by Jakubowicz, 2002).

Not only is ethylene considered the "ripening hormone", but it is also responsible for many other physiological and developmental processes such as adventitious root formation, flower opening, leaf/flower senescence/abscission, seed germination and responses to both biotic and abiotic stresses (Yang and Hoffman, 1984; Abeles et al., 1992; Yang and Oetiker, 1998; Saltveit, 1998; reviewed by Ezura et al., 2008).

Essentially, the effect of ethylene on the fruit ripening process prepares the fruit for seed dispersal, by facilitating the visual and olfactory responses that make the fruit attractive to animals that will assist in releasing the seed (reviewed by Nath et al., 2006; Adams-Philips et al., 2004; reviewed by Ezura et al., 2008).

Maturity and Ripening

Fruit quality and shelf-life is dependent on maturity at harvest. Fruits that are harvested either immature or overripe have poor quality and subject to damage (Kader 2002b). The maturation of melons depends on the fruit type (Seymour and McGlasson, 1993). As muskmelons mature and ripen they develop a full net, the background rind

color will change and an abscission zone will develop (Shellie and Lester, 1996). The fruit will begin to separate from the stem at the abscission zone as it becomes fully ripe. Fruits are fully ripe when the stem separates completely from the fruit, also called 'full-slip' stage (Sykes, 1990; George, 1999). However, most muskmelons are commonly harvested when the stem separates half way, or 'half-slip' stage (Kasmire et al., 1970). Muskmelons harvested at peak of ripening will have high soluble solids and produce aroma, while those picked too early (prior to abscission zone development) will have reduced soluble solids, aroma and flavor, and those harvested too late (overmature) will have reduced quality (Seymour and McGlasson, 1993; reviewed by Pratt, 1971; Rosa, 1928; Lloyd, 1928). Generally, muskmelons are ready to harvest at about 42 days after anthesis (Agblor and Waterer, 2001).

Inodorus types, such as honeydew, do not 'slip' from the vine until they are overripe and instead, are harvested by cutting the melon from the vine (Pratt et al., 1977; Seymour and McGlasson, 1993). There are many types of melons with characteristics between that of muskmelon and honeydew, often making it difficult to determine the optimum harvest time (Seymour and McGlasson, 1993).

Fruit Quality

Since melons are often consumed as a dessert, optimum fruit quality is essential (Lloyd, 1928). Quality can be divided into both internal (aroma, flavor, texture) and external (color, firmness, size) factors, which changes depending on one's perspective (Shewfelt, 1999; Cause et al., 2003; Wills et al., 2007). In general, optimum fruit quality depends on fruit flavor which comprises the organoleptic attributes of taste (including fruit sweetness and acidity), aroma, texture, and color (Yamaguchi et al., 1977; Li et al., 2006; Goff and Klee, 2006; Causse et al., 2002). Flavor is perceived predominantly by

aroma receptors in the nose and taste receptors in the mouth (Fisher and Scott, 1997). Dependent on many variables, flavor is therefore a complex trait determined by many factors including culture, environment, genetics, production, and postharvest handling (Baldwin, 2002).

Sweetness

Fruit sweetness has been determined to be the most important fruit quality component (Yamaguchi et al., 1977). In the U.S., melons with a soluble solids content (SSC) of 9 °Brix are considered grade 'No. 1' and those with a SSC of 11 °Brix or higher are grade 'Fancy' (Lester and Shellie, 2004). Sucrose is the dominant sugar in ripe fruits, while glucose and fructose are the dominant sugars in fruits during the first 24 days after anthesis (McCollum et al., 1988). Since muskmelons do not have a starch reserve and therefore, no carbohydrates to convert to sugar, they will not increase in soluble sugars after harvest; thus, it is essential to harvest muskmelons at their optimum maturity (reviewed by Pratt, 1971; Burger et al., 2006). The longer the fruit remains on the plant accumulating sugars, the higher the SSC (Bianco and Pratt, 1977). Sucrose accumulation in melons is regulated by the inverse relationship of the enzymes, sucrose phosphate synthase (SPS), which accumulates during melon development and acid invertase (AI), which decreases (Burger and Schaffer, 2007; Lester et al., 2001; Hubbard et al., 1991; Hubbard et al., 1990; Hubbard et al., 1989; McCollum et al., 1988; Schaffer et al., 1987; reviewed by Seymour and MacGlasson, 1993; reviewed by Nuñez-Palenius et al., 2008). In a study that evaluated both sweet and non-sweet melon cultivars, the enzymes, SPS, sucrose synthase and neutral invertase were also positively correlated with sucrose accumulation in all melon types, though there was low activity of these enzymes in the non-sweet melons types (Burger and Schaffer, 2007). Although sucrose accumulation is

associated with the biochemical processes involved in ripening, it is also related to cultural factors (reviewed by Pratt, 1971). Fruits can obtain a high SSC with low night temperatures, a longer maturation period, large leaf area, and harvesting fully ripe fruits (Welles and Buitelaar, 1988). Conversely, low SSC can be attributed to harvesting fruit too early, such as prior to development of the abscission layer (Seymour and Mcglasson, 1993).

Acids

Acids, though of high importance in many other fruits such as tomato (*Solanum lycopersicum*) (Burger et al., 2003; Cause et al., 2003), have minimal effect on melon fruit quality (Yamaguchi et al., 1977). Sweet melons (*reticulatus*, *cantalupensis*, *inodorus* and *makuwa* groups) have a low organic acid content, with citric acid being the major organic acid (reviewed by Burger et al., 2006; Burger et al., 2003; Leach et al., 1989). Malic acid is also present in the mesocarp, but at much lower levels than citric acid (reviewed by Burger et al., 2006). Titrable acidity (% citric acid) in various melon types can range from 0.054% in 'Galia' muskmelons to 0.138% in 'Tendril' winter melons, with no differences in pH (range: 5.74 to 5.65) (Artes et al., 1993).

Texture

Melon firmness or texture is a critical characteristic since it directly relates to the postharvest shelf-life, transportability and pathogen susceptibility of the fruit (reviewed by Li et al., 2006). Texture preferences vary among consumers, as muskmelons that received poor ratings in sensory panels have been described as being too soft as well as too hard (Yamaguchi et al., 1977; Pardo et al., 2000). Nonetheless, it is considered to be the third most important attribute (fruit sweetness is first, followed by aroma and/or flesh color) that affects eating quality in muskmelons (Yamaguchi et al., 1977). Pectins are the

primary component in the cell wall contributing to fruit texture (reviewed by Prasanna et al., 2007). Muskmelon fruit softening is not clearly defined as it could be the result of ethylene production during ripening (Lester and Dunlap, 1985; Pratt et al., 1977; Bianco and Pratt, 1977) and/or the result of cell wall degradation due to enzymes (Rose et al., 1998; Seymour and McGlasson, 1993; reviewed by Nuñez-Palenius et al., 2008). Water loss after harvest is another factor that may contribute to fruit softening (Lester and Bruton, 1986), as well as the loss of mesocarp membrane integrity as determined by high electrolyte leakage (Lester, 1988). Melon softening during fruit ripening has also been related to changes in pectic and hemicellulosic polysaccharides as well as a net-loss of non-cellulosic neutral sugars (McCollum et al., 1989). In 'Charentais' cantaloupe fruits, there are also modifications in pectic and hemicellulosic polymers during ripening; and changes in the hemicelluose, xyloglucan were closely associated with cellulose microfibrils that may have an affect in early softening (Rose et al., 1998). The enzyme polygalacturonase (PG) is also attributed to pectin disassembly in melon (Hadfield et al., 1998), though the role and presence of PG in melon has also been disputed (McCollum et al., 1988, 1989; Lester and Dunlap, 1985; reviewed by Pratt, 1971).

Color

Flesh color in melon varies among types, contributing to their uniqueness and importance in fruit quality (reviewed by Pratt, 1971; Yamaguchi et al., 1977). Flesh pigments in melon can be many colors and vary from orange, light orange, pink, green, and white (reviewed by Nuñez-Palenius et al., 2008) or magenta/red-fleshed (Mitchell-Harty et al., 2009a). In orange fleshed melons, the predominant pigment is beta-carotene, though other pigments found include alpha-carotene, delta-carotene, lutein, phytofluene, phytoene, violaxantin, xanthophylls and traces of other carotenoids (reviewed by Pratt,

1971; Watanabe et al., 1991). Flesh coloring begins in the center of the fruit and progresses outward until color is uniform at maturity (Reid et al., 1970). Carotenoids have been detected in muskmelons as young as 10 days, but visual detection did not occur until 20 days post-anthesis (Lester and Dunlap, 1985). At 30 days post-anthesis, a two to three-fold increase in beta-carotene levels was also observed (Lester and Dunlap, 1985). And as carotenoids accumulate during melon development, chlorophyll content decreases (Reid et al., 1970; reviewed by Pratt, 1971). In a study of both light orange and orange flesh melons, the light orange flesh melons had about 50% less beta-carotene than the orange flesh types, while green and white flesh melons had the least amount of carotenoids (Watanabi et al., 1991).

Aroma

The characteristic flavor that is associated with melons is also dependent on its aroma. Aroma volatiles are released as the fruit ripens and their presence, absence and quantity characterize each melon type (Pratt, 1971; Teranishi, 1971; reviewed by Engel et al.,1990). According to Pratt (1971) research on muskmelon aroma began in the 1930's when Rakitan (1935, 1945) identified and noted increases during ripening of the compounds acetaldehyde and ethanol in muskmelons. In 1957, the compounds acetoin and 2, 3-butylene glycol were measured in muskmelon and revealed different volatile patterns. Acetoin was detected in fruits when they were over-ripe, while 2, 3-butylene glycol was found at harvest and increased until ideal eating stage, but disappeared when they were over-ripe (Serini, 1957 reviewed by Pratt, 1971).

Today, over 240 aroma compounds have been identified in muskmelon and the predominant aromatic compounds are esters, alcohols and aldehydes (Obando-Ulloa et al., 2008; Beaulieu, 2006; Lamikanra, 2002; Beaulieu and Grimm, 2001 and 2002;

Nijssen and Visscher, 1996; Njissen et al., 1996). Esters have a major role in fruit flavors, and are responsible for most of the flavor in melons and many other fruits (Fisher and Scott, 1997). Alcohols are less important to flavor, as they have a higher odor threshold value (Fisher and Scott, 1997), but play an important role in melon aroma (i.e. cis-6-nonen-1-ol, a green, melon aroma compound (Kemp et al., 1972, 1973, 1974). Aldehydes are also responsible for fruity aromas and may provide a characteristic flavor (Fisher and Scott, 1997). The aroma volatiles that are considered to be the most important compounds are determined by their odor value (OV) (also called log odor units), which is the ratio of the concentration of the compound to it's known odor threshold value (OTV) (Bauchot et al., 1998; Berger, 1995; Buttery 1993; Teranishi et al., 1991). When the OV of a compound is greater than 1.0, that compound is considered to be a 'significant contributor' to the overall aroma (Bauchot et al., 1998; Berger, 1995; Buttery 1993; Teranishi et al., 1991). Odor threshold values (OTVs) are usually determined in air, water, or mineral oil by several investigators with different test methods; thus OTVs can vary by as much as 1000 (Fischetti, 1994).

Aroma volatiles are believed to be under genetic control as there are noticeable differences between melon cultivars (Yahyaoui et al., 2002; Wyllie and Leach, 1992; Wyllie et al., 1996a). Seeds can also have an influence on aroma as pollinated fruits have a favorable aroma over parthenocarpic fruits, probably due to higher carbohydrate levels in pollinated fruits (Li et al., 2002). And higher amounts of aroma volatile compounds have been reported in mature cantaloupes compared to fruits at immature stages (Beaulieu, 2006; Senesi et al., 2005; Beaulieu and Grimm, 2001; Wang et al., 1996; Horvat and Senter, 1987; Yabumoto et al., 1977).

In other early studies of muskmelon aroma, over 50 volatile compounds were identified in muskmelons (Kemp et al., 1971, 1972a 1973; Kemp et al., 1972b). The compounds, cis-6-nonen-1-ol and cis-6 nonenal were associated with the muskmelon-like, musky or green melon aroma (Kemp et al., 1972, 1973, 1974). However, Yabumoto et al. (1977) suggested that dimethyl disulfide and other sulfur compounds may also be important components in muskmelon fruit flavor. This was also confirmed by Wyllie and Leach (1992) and Wyllie et al. (1993) who evaluated additional sulfur compounds. Sulfur compounds have a high impact on flavor since they bind to olfactory receptors (Fisher and Scott, 1997).

Yabumoto et al., (1978) reported that large quantities of volatile esters were found to be critical for the characteristic fruity aroma in muskmelon and that there were at least three different volatile groups (acetaldehyde and ethanol; ethyl esters; and acetate esters), that could be determined based on their specific patterns; and that acetate esters increased rapidly and plateaued while the other two groups exhibited a continuous accelerating rate of production. Differences in aroma were also observed in two cantaloupe cultivars, where 'PMR-45' was more aromatic than 'Top Mark' (Yabumoto et al., 1978). Yabumoto et al. (1978) concluded that the more aromatic 'PMR-45' was attributed to its higher ethylene production than 'Top Mark'.

An additional eight compounds were identified by Horvat and Senter (1987) from cantaloupes at different maturity stages. Schieberle et al. (1990) concluded that the major aromatic compounds of muskmelon were methyl 2-methylbutanoate, (*Z*)-3-hexenal, (*E*)-2-hexenal and ethyl 2-methylpropanoate. Wang et al. (1996) reported that ethanol was the only volatile in immature muskmelons whereas other volatiles such as ethyl acetate

and 2-methylbutyl acetate developed after 32 days post-pollination. Esters are considered to be 'positive' aroma compounds and alcohols 'negative' due to their fermented note (LoScalzo et al., 2001). Senesi et al. (2002) also concluded that ethyl esters such as ethyl acetate were highly correlated with high total aroma and consumer acceptance; and therefore, could be a marker of an optimum-quality melon.

Also, the method that volatiles are measured is another factor to consider when determining aromatic profiles (Jordan et al., 2001). Volatile collection from muskmelon essence and fruit puree demonstrated differences in compound in detection (Jordan et al., 2001).

Aroma volatiles vary not only among different melon cultivars (Senesi et al., 2002), but also among different melon types such as honeydew melons (*Cucumis melo* L. var. *inodorous*), which have at least three unique compounds, (*Z*)-6-nonenyl acetate, (*Z*)-3-nonenyl acetate and (*Z, Z*)-3, 6-nonadienyl acetate compared to other melons (Buttery et al., 1982). Aroma volatiles from cultivar 'Golden Crispy' melons, which have a smooth yellow skin and white flesh, revealed the presence of thioether esters and dioldiesters, not previously reported in melons (Wyllie and Leach, 1990). 'Queen Anne's Pocket' melons (*Cucumis melo* L. var. *dudaim*) are noted for their aroma, not taste, which corresponded to higher levels of volatiles found in the skin rather than the pulp (Aubert and Pitrat, 2006).

True cantaloupes (*Cucumis melo* L. var. *cantalupensis*) are another melon type where over 100 volatile compounds, which include sulfur compounds such as 2-(methylthio)ethanol have been reported (Homatidou et al., 1992). Another type of cantaloupe, Charentais (*Cucumis melo* L. var. *cantalupensis*), are prized and for their

aroma and sweet flavor (Aubert and Bourger, 2004; Goldman, 2002). More than 80 compounds have been identified in Charentais melons, with the esters, ethyl acetate, 2-methylpropyl acetate and 2-methylbutylacetate comprising 60% of the total identified volatiles (Bauchot et al., 1998). However, aroma development in Charentais cantaloupes that have been transformed with an antisense ACC-oxidase gene had a significant decrease in volatiles, having only 20-30% of the total quantity of acetates as compared with nontransformed fruit (Bauchot et al., 1998 and 1999). Ethylene, therefore, is also believed to have an effect on aroma, as the ethylene inhibited melons demonstrated reduced aroma (reviewed by Zhu et al., 2005; Bauchot et al, 1998 and 1999). This reduction in aroma volatiles could be due to the reduction in esters, which are catalyzed by an alcohol acetyltransferase (AAT) enzyme, which is regulated by ethylene (Bauchot et al., 1998). Analysis of different Charentais cantaloupe cultivars with wild, mid, and long storage shelf-life concluded that long shelf-life (LSL) types were the least aromatic, probably due to their lower ethylene production (Aubert and Bourger, 2004). Saftner et al. (2006) also reported higher volatiles in cantaloupe (climacteric) versus honeydews (non-climacteric).

Aroma volatiles have also been studied on various ‘Galia’-type cultivars (Leach et al., 1989; Wyllie and Leach, 1992; Fallik, et al., 2001 and 2005; Hoberg et al., 2003; Obando-Ulloa et al., 2008; Shalit et al., 2001; Kourkoutas et al., 2006). Wyllie and Leach (1992) concluded that sulfur-containing compounds in the aroma volatiles of muskmelon were important and found that the ‘Galia’ muskmelon used in their study had relatively intense amounts of 2-(methylthio)ethyl acetate. Research on ‘Galia’-type muskmelon aroma found that in the GT cultivars, ‘C8’ and ‘5080’, butyl acetate, 2-

methylbutyl acetate and hexyl acetate were the most abundant compounds (Fallik et al., 2001). Fallik et al. (2001) also reported how higher aroma does not necessarily imply higher consumer preference. Cultivar 'C8' had stronger aroma than '5080', but contained less sugar and was not preferred as often as '5080' by a taste panel. Shalit et al. (2001) reported that volatile acetates were higher in ripe 'Arava' fruits than in unripe ones; and that volatile acetates were correlated with total soluble solids. Volatile esters are formed via the enzymes, alcohol acetyltransferasaes (AAT) (EC 2.3.1.84), which catalyze the reaction between acyl CoA and alcohol during ripening (El-Sharkawy et al., 2005). Khanom and Ueda (2008) reported that the esters isobutyl acetate and benzyl acetate were produced the most in melons (cv. 'Earl's faveorite and cv. 'Prince'). Shalit et al. (2001) found that AATs were present in 'Arava' fruits and increased as they ripened. This was in contrast to a non-climacteric, casaba-type melon, which was a volatile-acetate-lacking nonaromatic melon with negligible AAT activity. Hoberg et al. (2003) did not measure actual quantities of aroma, but their studies with sensory panels reported that fruits with negative characters such as 'greeny', 'solvent' and 'unpleasant' odors were not preferred by consumers and could be differentiated from pleasant or 'fruity' odors on the basis of retro-nasal odor. Hoberg et al. (2003) concluded that cultivars 'C8' and 'Ideal' had more negative characters, especially after 16 days in storage; however, they are no longer grown in Israel as a result of their findings. Obando-Ulloa et al. (2008) reported that cultivar 'Fado' was highest in propyl acetate, methyl 2-methylbutanoate and hexyl acetate. Kourkoutas et al. (2006) found 'Galia' contained higher levels of the acetate esters isobutyl, butyl, 2-methylbutyl and hexyl acetate, than cantaloupe and honeydew.

Although there has been research on 'Galia'-type muskmelon aroma, there are no known reports on the aroma of the original 'Galia' muskmelon. Although work by Leach et al, 1989 and Wyllie and Leach (1992) may have been with the original 'Galia' cultivar, however they did not specify so, and the work by Kourkoutas et al. (2006) reported results on 'Galia' muskmelons with no report of the actual cultivar name.

Taste and Sensory Analysis

Organoleptic quality involves not only color, texture and aroma, but also taste (Causse et al., 2002). There are five major taste sensations: saltiness, sweetness, sourness, bitterness and umami (savory) (Fisher and Scott, 1997). Taste sensations occur upon contact with food in the mouth (Fisher and Scott, 1997). Therefore, sensory measurements of quality attributes can also provide an approximation of consumer acceptability (Abbott, 1999). Since muskmelons tend to be quite variable in quality, sensory analyses are another method used to determine muskmelon fruit quality (Aulenbach and Worthington, 1974; Yamaguchi et al., 1977). Sensory panels can be performed with both trained and untrained panelists (Fisher and Scott, 1997). Trained sensory panels can be expensive and slow (Studman, 2001), but information on both consumer satisfaction as well as identification of organoleptic qualities of the product can be obtained (Guérineau et al., 2000). There are different types of sensory panels that include discrimination tests- where a triangle test, paired comparison test, duo-trio test, and ranking test may be used; or affective tests (hedonic tests), which assess consumer preference and/or acceptance and rate samples according to a hedonic category scale (Fisher and Scott, 1997). Panelists can be asked to rate and comment on a variety of quality attributes including: firmness/texture, flavor, sweetness, appearance, acceptability, juiciness and eating quality (Senesi et al, 2002; Saftner et al., 2006).

‘Galia’ Muskmelon

‘Galia’ and ‘Galia’-Type Specialty Cultivars

Developed in Israel in 1973 by plant breeder Zvi Karchi, the ‘Galia’ muskmelon (*Cucumis melo* L. var. *Reticulatus* Ser.) was the first F_1 hybrid melon developed in Israel. It was the product of ‘Noy Yizre’el’ and ‘Krymka’ cultivars. ‘Noy Yizre’el’ was a Ha’Ogen type, which was a common Hungarian open-pollinated cultivar grown throughout Israel and valued for its high yield and quality. ‘Krymka’ was an early producing Ukrainian cultivar, which was selfed for ten generations and selected for uniformity (Karchi, 2000; Karchi, personal communication, 2004). The result of this cross, the ‘Galia’ muskmelon, was a superior quality fruit with sweet, green flesh, a yellow netted exterior and a fragrant, musky aroma (Karchi, 2000).

After its introduction, ‘Galia’ muskmelon quickly became a popular new market name throughout Europe and the Mediterranean by way of an intense marketing campaign with Agrexco and sales at the popular British food chain, Marks and Spencer (Karchi, 2000). By the 1980s, ‘Galia’ muskmelons were sold all over Western Europe, except France (Karchi, 2000). Its popularity is attributed to its intense flavor, aroma and sweetness. In effect, ‘Galia’ F_1 hybrid melon production was one of the factors that helped revive Israel’s agricultural production, breeding and research as well as increase it’s competive advantage in world markets (Karchi, 2000).

Although it is a high quality melon, ‘Galia’ muskmelon has some limitations. Besides being highly susceptible to powdery mildew (*Podosphaera xanthii*) (Mitchell et al. 2006 and 2007), another main disadvantage is its short shelf-life (Mitchell et al. 2007a and 2007b; Nuñez-Palenius et al., 2005). ‘Galia’ muskmelons may last two to three weeks if harvested at a pre-slip stage and stored at low temperatures (Aharoni et al.,

1993). In order to achieve the best flavor however, 'Galia' must be harvested at full-slip. Exporters have increased shelf-life, but decreased quality by harvesting fruit at an immature stage; however this leads to lower quality fruit (Fallik et al., 2001; Cantliffe and Shaw, 2002; Pratt. 1971). As a result of 'Galia's limitations and its popularity, breeders have worked to improve disease resistance/tolerance as well as improve shelf-life (Mitchell at al., 2007). Today 'Galia' is a trade name for other look-a-like melon cultivars, commonly called 'Galia'-type melons. Unfortunately, although these 'Galia'-type cultivars are firm, they often lack the flavor, aroma, and high sugar content of the original 'Galia' hybrid (Mitchell et al., 2006, 2007a and 2007b). According to a survey of seed companies throughout the world, today there are over 75 'Galia'-type muskmelons. At least 60 'Galia'-types have been released as cultivars (Table 1). Eight seed companies currently sell the original 'Galia' F_1 hybrid (Bakker Brothers, Genesis, Golden Valley Seed, Hazera, Seeds of Change, Thompson and Morgan and Zeraim Gedera). Of these eight companies, it is known that Hazera, Genesis and Zeraim Gedera actually produce the 'Galia' F_1 hybrid. At least two of the companies, Golden Valley Seed and Thompson and Morgan purchase the seed from an outside source and re-sell it (J. Harty, survey, 2008).

'Galia' Muskmelon Production

'Galia' muskmelon is grown primarily in Israel, Morocco, Turkey, and Spain, being exported principally to the U.K. and Europe where it is in high demand (Rodriguez et al., 2002). 'Galia' muskmelons grown in the Mediterranean for export to Europe usually weigh 0.9 to 1.5 kg and can have a SSC up to 14 °Brix (Rodriguez, 2003). 'Galia' muskmelons are also produced in Central America (Guatemala/Honduras) and some parts of U.S. (J. Ortiz, personal comm., 2008). The 'Galia' muskmelon can be found in U.S.

markets, but quality is low due to production and harvesting practices (Rodriguez et al., 2002). Generally, 'Galia' muskmelons are field-grown and imported from countries where it is picked immature, at a zero-slip stage, to ensure a longer shelf-life (Cantliffe and Shaw, 2002).

The 'Galia' cultivar is especially adapted to intensive irrigation and fertilization where yields of up to 50 Mt/ha of high quality fruits (13 to 15 °Brix) have been recorded under arid environments (Karchi, 2000). During the 1970's, 'Galia' muskmelon was produced in northern Israel under dry land farming conditions where nitrogen (N) was only applied prior to rainfall during the winter (Rodriguez et al, 2005). Production in southern Israel used different types of irrigation, which included rain-fed cultivation, complimentary and complete irrigation; and also required N applications throughout the season, depending on the crop growth stage (Hecht, 1998). Producers in the north of Israel grew under open field conditions while producers in the south grew under protected tunnels (D. J. Cantliffe, personal comm., 2008).

Although 'Galia' muskmelon is adapted for intensive field cultivation, production in the Arava desert in Israel under protected structures, such as tunnels, is also used to protect crops from wind, rain and low temperatures (Rodriguez, 2003). 'Galia' muskmelon production under protected structures is also employed in the U.S. with the use of passively-ventilated tunnels and greenhouses (Jett, 2004; Shaw et al., 2001; Rodriguez et al., 2002; Rodriguez, 2003; Waldo et al., 1997 and 1998). 'Galia' muskmelons are sensitive to rainfall during flowering and fruit set, making them difficult to produce in the field in places such as Florida (Rodriguez et al, 2002). Rainfall is also a problem at harvest time, as it has been found that SSC can decrease in some muskmelons

after rain (Wells and Nugent, 1980; Bouwkamp et al., 1978). Therefore, the protected structures are a method to ensure highest quality 'Galia' muskmelons (Rodriguez et al, 2002).

Production of 'Galia' muskmelons in passively-ventilated greenhouses is accomplished by trellising plants in an upright vertical fashion and careful pruning of lateral shoots (Shaw et al., 2001; Rodriguez, 2003). Plants are grown in pots or bags with a soilless media and, twine, strung down from a steel cable, is clipped under the cotyledons and the plant is twisted up and around the twine as the plant grows (Rodriguez, 2003). The soilless media used can be a variety of types, including composted pine bark and perlite (Rodriguez et al., 2006). During the first three to four weeks after planting, or the vegetative stage, all lateral branches are pruned up to the eighth node to allow for optimum plant growth that will improve fruit load support (Shaw et al., 2001; Rodriguez, 2003). After this stage, female flowers develop on the lateral branches and are pollinated via bumble bees (*bombus impatiens*) (Shaw et al., 2001; Rodriguez, 2003). Pruning off lateral branches that do not set fruit as well as trimming lateral branches that have developing fruits (leaving only one or two leaves to serve as a source of assimilates) continues throughout the season (Rodriguez, 2003).

Plant spacing in protected culture is important as growers make efficient use of production space in order to help off-set high greenhouse costs (Rodriguez et al., 2006). Studies of 'Galia'-type muskmelons planted at densities of 1.7, 2.5, 3.3 and 4.1 plants m^{-2} demonstrated that yield increased linearly with increasing plant density, without negatively affecting fruit quality (Rodriguez et al., 2006). Therefore, if market prices for 'Galia' muskmelons are $1.44 per kg, plants grown at a density of 3.3 plants m^{-2} will

have almost double the returns than those at a density of 1.7 plants m^{-2} (Rodriguez et al., 2006).

Irrigation and fertilization of soilless grown muskmelons is accomplished through drip irrigation (Shaw et al., 2001). Just as 'Galia' muskmelon production in southern Israeli soils required applications N throughout the season, it is also important in soilless production (Rodriguez et al., 2005). Adjusting N concentrations in the fertilizer solution according to different growth stages (flowering, flowering to fruit set, fruit development, and fruit ripening) is recommended to reduce over-fertilization (Rodriguez et al., 2005). Excess N causes plants to become more vegetative and reduces the potential to maximize fruit set (D. J. Cantliffe, personal comm., 2008).

'Galia' Muskmelon Postharvest Practices

In order to achieve peak flavor, 'Galia' must be picked at a fully ripe or 'full-slip' stage (Karchi, 1979; Mitchell et al. 2007a; Nuñez-Palenius et al., 2005), which reduces storage life. Exporters have increased shelf-life, but decreased fruit quality by harvesting at an immature stage (Fallik et al., 2001; Cantliffe and Shaw, 2002; Pratt. 1971). Although there are 'Galia'-type (GT) cultivars available that have an extended shelf-life, they often lack the flavor, aroma and high soluble solids content of the original 'Galia' hybrid (Mitchell et al., 2007a).

In addition to production of GT cultivars, several methods have been used to extend the postharvest shelf-life of 'Galia' and GT muskmelons. They can be harvested early, at a green, pre-slip or half-slip stage when fruits are firmer, but this often results in reduced sweetness and flavor (Fallik et al., 2001; Cantliffe and Shaw, 2002; Pratt. 1971). Muskmelons can also be stored at low temperatures (2.5 to 5 ºC) to maintain firmness (Asghary et al., 2005) or rinsed with hot water to reduce both fruit softening and decay

development (Fallik et al., 2000; Lalaguna, 1998; Teitel et al, 1989). 'Galia' muskmelons subjected to < 1.0 kGy of irradiation combined with a hot-water dip protected fruit from decay and did not affect quality (Lalaguna, 1998). Waxes have also been used to maintain internal and external melon fruit quality (Fallik et al., 2005; Aharoni et al., 1992). Sodium bicarbonate has been reported to reduce decay as well as maintain firmness (Aharoni et al., 1997). Moreover, a combination of hot water and a wax treatment with sodium bicarbonate may also be used to reduce decay and increase fruit quality (Illić and Fallik, 2007). Other postharvest treatments of GT muskmelons have included applications of hydrogen peroxide or treatments of hinokitiol (β-thujaplicin, a chelating agent that inhibits microbial enzymes) (Aharoni et al., 1994; Aharoni et al., 1993). Storage of 'Galia' muskmelons in a controlled atmosphere of 10% CO_2 and 10% O_2 both with and without an ethylene absorbent decreased fruit softening and decay (Aharoni et al., 1993). The use of 1-methylcyclopropene (1-MCP), an ethylene action inhibitor, suppressed softening of 'Galia' muskmelons at both green and yellow maturity stages (Ergun et al., 2006).

Although these methods can be effective in extending the postharvest shelf-life of melon, many result in an extra step that producers and distributors would most likely choose to avoid. Therefore, instead of further complicating the postharvest handling process, another means could be to modify the innate quality of the fruit itself. To accomplish this, it is necessary to understand key features of the fruit.

The 'Galia' muskmelon is a climacteric fruit. It has a burst of respiration concurrent with an autocatalytic production of ethylene (Abeles et al., 1992; Seymour and McGlasson, 1993). This is a defining feature of ripening in fruits such as melons

(Bower et al., 2002) and causes the fruit to ripen, abscise, and soften very quickly (Abeles et al.,1992). This is why 'Galia' has a short shelf-life. 'Galia' are best when harvested fully ripe, when the climacteric peak and abscission occur (Cantliffe and Shaw, 2002; Mitchell et al., 2007c). Therefore, knowledge about the ethylene biosynthetic pathway is important since it is essential to the fruit ripening process. Furthermore, the last step in the ethylene biosynthetic pathway can be inhibited using antisense technology, which blocks ethylene production in the fruit, therefore, making the fruit firmer (reviewed by Nuñez-Palenius et al., 2008). This has been accomplished in tomato (*Lycopersicon esculentum*) (Hamilton et al., 1990), 'Charentais' and 'Vedrantais' cantaloupes (Ayub et al., 1996; Guis et al, 1997; Guis et al, 2000; Silva et al., 2004) and plums (*Prunus domestica* L.) (Callahan and Scorza, 2007). In addition, the most recent work using antisense technology, by Nuñez-Palenius et al. (2006a), was able to transform the male parental line of 'Galia' muskmelon (cv. 'Krymka') with an antisense ACC-oxidase gene (CMACO-1). Antisense 'Krymka' fruits produced less ethylene and were firmer than wild-type (WT) fruits, yet soluble solids content (SSC) was similar to WT fruits (Nuñez-Palenius et al., 2006b). The work from Nuñez-Palenius et al. (2006a and 2006b) provided an essential step towards the goal of improving the shelf-life, while maintaining the high quality and flavor of the original 'Galia' muskmelon.

Table 2-1. List of 'Galia'-type muskmelon (*Cucumis melo* L. var. *reticulatus* Ser.) cultivars.

Seed Company	Cultivar Name
BakkerBrothers	Antalya, Bardot, Bastogne
D. Palmer Seed	Gala, Girlie, GM-04-22, Sigal
De Ruiter	Abellan, Ajax, Cyro, Riscal, Supra, Zondra
Emerald Seeds	Melon - EM 740 F-1, Melon - EM 782 F-1
Enza Zaden	Sembol, Sereen
Golden Valley Seed	Alia, Amur, GVS 125, GVS 205, GVS 206
Hazera Genetics	Elario, Galante, Galapago, Jalisco, Lavi gal, Veronica, Vitorio, Nestor, Gal-52, Gal 152
Hygrotech	Omega
Namdhari Seed	NS 923, NS 929, NS 931
Nirit Seed	Nirit, Gilat
Nunhems	Solarnet, Solarking, Esmeralda, Estoril, Malika, Solarprince
Rogers/Syngenta	Vicar, Galileo
Seigers Seed (Seminis)	Gallicum
United Genetics	Early Gal, Galistar, Green-Go, Green Star, R's Flavorite
Technisem	Caline
Vigour Seeds	NiZ 52-07 F1
Zeraim Gedera/Syngenta	Arava, Don Juan, Inbar, Royal, Campeon, Jazmo, Pharis, Royal, Boa Vista,

CHAPTER 3
PRODUCTION, EVALUATION, AND SELECTION OF ANTISENSE ACC-OXIDASE (CMACO-1) GALIA F1 HYBRID MUSKMELON (*Cucumis melo* L. var. *reticulatus* Ser.)

Introduction

The Galia (*Cucumis melo* L. var. *reticulatus* Ser.) muskmelon was developed in Israel by breeder Zvi Karchi and released in 1973. It was bred for production in the warm, dry desert conditions in Israel (Karchi, 2000). The female parental line of 'Galia' is a Ha' Ogen type melon cultivar called 'Noy Yizre'el' (Karchi, personal comm., 2004). 'Noy Yizre'el' fruit are green-fleshed with a smooth, sutured skin (Figure 3-1) (Karchi, 2000). Ha' Ogen melons were introduced to Israel from Hungary and were a popular cultivar adapted to the intensive agricultural practices, which included the use of plasticulture with irrigation and fertilization (Karchi, 2000). The smooth skin of Ha' Ogen melon made it susceptible to damage and therefore, it was difficult to ship. Additionally, it was open-pollinated, which made it easy for others to grow and save seeds of these melons (Karchi, 2000).

The male parental line of 'Galia' was originally from the Peninsula of Crimea, in the Ukraine, and is a cultivar called 'Krymka', in which fruits were round with a golden, netted skin and light green, firm flesh (Karchi, personal comm., 2004). 'Krymka' was selfed over 10 generations and selected for uniformity (Karchi, personal comm., 2004).

The resulting hybrid cross—the 'Galia' muskmelon (Figure 3-2) had round fruits with an attractive orange-netted skin and a green, mellow textured flesh with a unique aroma and high soluble solids content (13 to 15 °Brix) and was a high yielding cultivar (Karchi, 2000). In addition, 'Galia' muskmelon was considered to have a longer shelf-life compared with the Ha' Ogen types and therefore was able to compete in the European

markets (Karchi, 2000). Furthermore, 'Galia' muskmelon was an F_1 hybrid, an exclusive, Israeli cultivar (Karchi, 2000). As a result of marketing campaigns with Agrexco and sales at the British food chain, Marks and Spencer, 'Galia' muskmelons became popular all over Western Europe, except France (Karchi, 2000).

The popularity of this melon made it into a new market class of melons as breeders later released 'Galia'-type (GT) cultivars with improved shelf-life and disease resistance (Karchi, 2000). At present, there are over 75 GT cultivars available from numerous seed companies (Harty, 2009).

Today 'Galia' muskmelons have a reduced shelf-life as compared with newer GT cultivars, which often lack the flavor and high SSC of the original 'Galia' (Mitchell et al., 2007). In order to maintain the high quality and flavor of the original 'Galia' muskmelon, the 'Galia' male parental line (cv. 'Krymka') was transformed with an antisense ACC-oxidase gene (CMACO-1) (Nuñez -Palenius et al., 2006a). ACC oxidase is the catalyst in the last step of the ethylene biosynthetic pathway (Yang and Hoffman, 1984). Ethylene is a hormone that has a major role in fruit ripening and senescence (Abeles et al., 1992), initiates fruit softening, changes in carbohydrate metabolism, aroma volatile production and abscission. Fruit size and sugar content are not regulated by ethylene (Pech et al., 1999; reviewed by Nuñez-Palenius et al., 2008).

The work of Nuñez -Palenius et al. (2006a) produced two independent antisense 'Krymka' lines named TGM-AS-1 and TGM-AS-2. Fruits from TGM-AS-2 line produced less ethylene and were firmer than untransformed fruits at half and full-slip stages (Nuñez -Palenius et al., 2006b). TGM-AS-1 fruits also exhibited lower ethylene production, but only during the half-slip stage (Nuñez -Palenius et al., 2006b).

Conversely, the female parental line of 'Galia' muskmelon, cultivar 'Noy Yizre'el' was unable to be transformed with an antisense ACC-oxidase (CMACO-1) gene (Nuñez - Palenius et al., 2005). Nuñez-Palenius developed antisense 'Galia' muskmelon hybrids (T_0GMH-AS-1 and T_0GMH-AS-2) produced from the T_0 transgenic male parental lines (TGM-AS-1 and TGM-AS-2) (Mitchell et al., 2007b). During fall 2004, T_0 TGMH-AS-1 and T_0 TGMH-AS-2 lines remained on the vine five days longer than 'Galia' (Mitchell et al., 2007b). However, in a previous crop, a severe powdery mildew (*Podosphaera xanthii*) epidemic led to no differences in days to harvest (DTH) between antisense lines and 'Galia' (Mitchell et al., 2007b). Due to these challenges, it was desirable to obtain AS 'Galia' hybrids where both the male and female line incorporated the antisense ACC-oxidase (CMACO-1) gene. With both parents positive for the transgene, it was hypothesized that there would be an increased chance of the F_1 hybrid progeny fruit to have reduced ethylene production and therefore a longer shelf-life.

As an alternative to plant transformation, since that previously did not work in the female line (Nuñez-Palenius et al., 2005), the backcross method was used to insert the antisense ACC oxidase (CMACO-1) gene into the female line. Pollen from transgenic F_1 'Galia' (TGH1 and TGH2) was used to pollinate the wild-type female 'Galia' parental line. Female transgenic backcross 1 (BC_1) seeds were produced during the summer of 2004. Repeated backcrossing continued until the genetic background of the female was 97% (BC_4). Backcrossing is a type of recurrent hybridization used to add a desirable allele to an already adapted and productive cultivar that is lacking in the desired allele (Poehlman and Sleper, 1995). Therefore, the objectives of this research were to produce an elite stock of the TGM-AS-1 and TGM-AS-2 lines, use these lines to produce an

antisense ACC-oxidase (CMACO-1) female parental line through backcrossing, produce an antisense 'Galia' (ASG) hybrid muskmelon with both female and male transgenic parental lines through traditional breeding methods, and collect fruit quality data (fruit size, firmness, soluble solids content (SSC), ethylene and respiration) at different stages of growth on preliminary lines for selection of ASG hybrids to be used for further research. Future research would include evaluation of fruits at different harvest stages for fruit quality, including size, SSC, firmness, and aroma volatiles. Ethylene and respiration rates will be observed in order to verify reduced ethylene in transgenic lines as well as track the climacteric. These results will help establish fruit quality characteristics of ASG muskmelons and develop guidelines of when to harvest the ASG muskmelons in order to benefit from the transgenic modification.

Materials and Methods

Antisense ACC-oxidase 'Galia' F_1 Hybrid Development

Development of elite parental lines, bearing an antisense ACC-oxidase gene was done throughout 2004 and 2005. TGM-AS-1 and TGM-AS-2 (T_0 generation) were selfed and selected for the delayed ripening phenotype until a homozygous T_4 generation was produced. At the same time, the T_0 hybrid lines, TGH-AS-1 and TGH-AS-2 lines were crossed to the female parental line and an antisense (AS) backcross 1 (AS BC_1 generation) was also produced. The AS BC_1 was used to continue to backcross the transgene into the female line until a backcross 4 (AS BC_4) female population was produced. Parental line production was done in an evaporative-cooled fan and pad glasshouse according to the methods of Nuñez -Palenius et al. (2006a). Plants were grown using commercial production, pruning and nutrient requirements according to the methods of Shaw et al. (2001).

Selfing and backcrossing was completed each morning, during flowering, between 7:30 a.m. and 11:00 a.m. To self, three male flowers were removed from the plant and all three flowers were used to pollinate one female flower on the same plant. To backcross, three male flowers were picked from a designated backcross plant. These three flowers were then used to pollinate one female flower on a wild-type female plant. Prior to the backcross-pollination, the female flower was emasculated with sterile tweezers. All pollinated flowers were tagged with the date.

Two crops of selfed males and backcrossed females were able to be produced each year, one from January to May and again from August to October. Production continued until AS male T_4 and AS female BC_4 populations were produced in fall 2005. Data were recorded during each season for days to harvest (DTH) for the male transgenic lines. Every fruit from a plant was given a reference number. The fruits with the longest DTH were selected for the next generation. Also, during fall 2005, T_3 TGM-AS-1 and TGM-AS-2 male lines and a wild-type (WT) male were screened for ethylene and respiration data. This was done to observe both the amount of ethylene evolved from the new lines and track the climacteric peaks in these lines, which would provide a new method of when to harvest the antisense hybrids. Fruits were harvested at different days after anthesis. Ethylene and respiration were collected according to the procedures as outlined for the hybrid evaluation.

During spring 2006, the female AS BC_4 and AS T_4 male were crossed and AS 'Galia' (ASG) hybrid seeds were produced. The ASG hybrids were made in different combinations (AS♀ x WT ♂, AS ♀ x AS ♂, WT ♀ x AS ♂). Since there were initially

two independent transgenic male lines from Nuñez-Palenius et al. (2006a), these two lines resulted in two main ASG hybrid family lines, ASG-1 and ASG-2.

Transgene Detection for Antisense Male, Female and Hybrid Lines

All seedlings were produced at the University of Florida, Gainesville, FL campus in a Conviron plant growth chamber (Controlled Env. Ltd.,Winnipeg, Manitoba, Canada) according to Mitchell-Harty et al. (2009a). DNA extraction was done when seedlings had one true leaf, according to Mitchell-Harty et al. (2009b). A polymerase chain reaction (PCR) analysis was used to identify the seedlings with the transgene. The PCR reaction was conducted in a DNA Thermal Cycler 480 (Applied Biosystems, Foster City, CA, U.S.A.) according to the parameters of Nuñez -Palenius et al. (2006a). Following amplification, the PCR products were viewed on a 1% agarose gel by ultraviolet (UV) light according to Nuñez -Palenius et al. (2006a). PCR analysis was completed on every putative transgenic seedling prior to planting.

ASG Muskmelon Production

Two ASG muskmelon trials were conducted, in fall 2006 and spring 2007. The first trial, completed in fall 2006, involved the screening of multiple lines of the ASG material. Seeds were sown on 7 July 2006. The commercial 'Galia' and ASG lines used were 'Galia'muskmelon (Hazera Genetics, Israel), and three lines each of ASG-1 hybrid combinations of ASxWT (ASxWT(ASG-1a), ASxWT(ASG-1b) and ASxWT(ASG-1c)), WTxAS (WTxAS(ASG-1d), WTxAS (ASG-1e) and WTxAS (ASG-1f)) and ASxAS (ASxAS(ASG-1g), ASxAS(ASG-1h) and ASxAS(ASG-1i)), and one ASxAS line of ASG-2, labeled ASxAS2 was also sown. After the fall 2006 trial, one ASG line from each cross (ASxWT, WTxAS and ASxAS) were selected and evaluated with the original

'Galia' hybrid (Hazera Genetics, Israel) in spring 2007. In spring 2007, seeds were sown on 19 Jan. 2007.

Once transgenic seedlings were identified through PCR analysis and all seedlings had three true leaves, they were transplanted on the 3 Aug. 2006 and 27 Feb. 2007. The plants were grown according to the production and nutrient requirements of Shaw et al. (2001) in a saw-tooth style, passively-ventilated greenhouse (TOP greenhouses, Ltd., Barkan, Israel), located at the University of Florida, Plant Science Research Education Unit located in Citra, FL. ASG hybrids were not self-pollinated. Bumble bees from Class A research hives (*Bombus impatiens*, Natupol, Koppert Biological Systems, Inc., Romulus, MI) were used for cross-pollination. All flowers were tagged with the date of anthesis to track the days to harvest (DTH).

An integrated pest management (IPM) approach, which used scouting, biological control and sprays, was used for management of arthropod pests according to Mitchell-Harty et al. (2009a).

Fruit Harvest Procedure

Fruits were harvested from 29 Sept. to 30 Oct. 2006 and 10 May to 18 June 2007 according to the methods of Mitchell-Harty et al. (2009a) at four stages of ripening. Stage: 1.) zero-slip green (ZG): external skin still green in color with no abscission layer development; 2.) zero-slip, yellow-green (ZYG): external skin green and yellow, with no abscission layer development; 3.) half-slip (HS): fruit abscising half-way; and 4.) full-slip (FS): fruit separates easily from the stem. During each harvest period, fruits were picked each afternoon, transported to the Gainesville campus and stored at 20 ºC for 12 hours. All postharvest variables were measured the following morning, 12 hours after harvest.

Ethylene, Respiration and Fruit Quality Measurements

Ethylene and respiration measurements were measured on whole fruits consistent with methods and equipment as described by Mitchell-Harty et al. (2009a). After the fruits had their 12-hour storage interval (following harvest), ethylene and respiration rates were measured from all fruits.

Following ethylene and respiration measurements, fruit quality variables, which included flesh thickness, firmness, and SSC were measured from a 2.5-cm slice of fresh pulp from the equatorial region of the fruit according to the methods of Mitchell et al. (2007a). Pulp firmness was determined at two equidistant points on the equatorial region of each fruit slice using the Instron Universal Testing Instrument (Model 4411-C8009, Canton, MA), which was fitted with a 50-kg load cell and an 11-mm convex probe with a crosshead speed of 50 mm min^{-1}. SSC (°Brix) was measured with a temperature-compensating, handheld refractometer (Model 10430, Reichert Scientific Instrument, Buffalo, NY) from fresh juice expressed from two pulp samples; and flesh thickness was measured with a caliper (Digimatic Mycal, Mitutoyo, Japan) from peel to cavity.

Statistical Analysis

The ASG hybrid trials were conducted in a randomized complete block design (RCBD) with four replications. Number of fruits per treatment (n) ranged from one to 10 fruits. However, in fall 2006, some of the ASG lines did not produce sufficient fruits to be harvested at stage ZG. Therefore, fall 2006 ZG results are presented as grouped data (ASxWT (ASG-1a,b,c), WTxAS (ASG-1d,e,f) and ASxAS (ASG-1g,h,i)). For stages ZYG, HS and FS, results are presented by the individual lines. After promising individual ASG lines were selected from the fall 2006 results, the selections were evaluated again during spring 2007. Fall and spring results from the selections were then

analyzed together at each stage. All data were subject to analysis of variance (ANOVA) using the GLM procedure (SAS Institute, Version 9, Cary, NC, U.S.A.). Significant means were separated with Fisher's Least Significant Difference ($P<0.05$). Standard error (SE) values were calculated for each ethylene and respiration data point.

Results and Discussion

Parental Line Production

Selfed AS male line TGM-AS-1 over the four generations, averaged an extra 22 days on the vine as compared with the wild-type male (Table 3-1 and Figure 3-3). DTH for T_2 to T_4 generations of TGM-AS-2 decreased over the four generations, which indicated that the delayed-ripening phenotype was not expressed. The progressed failure of line TGM-AS-2 is interesting, since this line had previously demonstrated the greatest inhibition of ethylene production in the original T_0 work by Nuñez-Palenius et al., (2006b). Review of the southern blot analysis by Nuñez -Palenius et al. (2006a) illustrated a single copy of the transgene for both TGM-AS-1 and TGM-AS-2 lines. However, the photograph (Nuñez -Palenius et al., 2006a) portrays line TGM-AS-2 with a higher expression of the transgene as compared with line TGM-AS-1. Possibly, multiple insertions of the transgene occurred, which could lead to gene silencing (Vaucheret and Fagard, 2001). This could be a possible explanation for the failure of this line to express the desired reduced ethylene delayed ripening characteristic.

Backcross 4 (BC_4) female lines with the antisense ACC-oxidase (CMACO-1) were produced from both TGM-AS-1 and TGM-AS-2 male parental lines. AS BC female lines were selected based on transgene detection only. Comparison of the antisense (AS) BC female lines to a wild-type female was not easily measured by DTH. Wild-type fruit from female line plants averaged 47 to 57 DTH, as did DTH for fruits

from AS BC female lines (Table 3-2). Production of female backcross lines was also difficult as compared with selfed male production, as many female line backcrossed flowers did not set. This may have been due to damage to the ovary while being emasculated.

Ethylene and respiration rates were to be analyzed from T_3 TGM-AS-1, TGM-AS-2 and wild-type males at different days after anthesis. However, since these days varied for each fruit, as did the number of fruit collected for each day, results were presented according to different stages of ripening—stage ZG: zero-slip with green skin ; stage ZYG: zero-slip with yellow/green skin; stage HS: half-slip; stage FS: full-slip; and stage PS: 1 day after full-slip, or post-slip. Ethylene and respiration from T_3 and wild-type (WT) fruit from male line plants generally increased during stage ZYG (Figs. 3-4 to 3-6). TGM-AS-1 fruits had low ethylene production at stages ZG, ZYG and HS, which was followed by increases at FS and PS (Fig. 3-4). Compared to fruit from WT male lines, TGM-AS-1 had lower ethylene production at all stages except stage PS. TGM-AS-2 male fruits had diverse amounts of ethylene production (Fig. 3-5), which were similar to 'Galia' at stages ZYG and FS. Due to the continued decline in delayed-ripening for TGM-AS-2 fruits and the varied ethylene production at all stages, only an ASxAS hybrid combination of line TGM-AS-2 was considered useful for subsequent screening. The instability of the T_3 TGM-AS-2 line is interesting, considering that the T_0 TGM-AS-2 line from Nuñez-Palenius et al. (2006b) produced the least amount of ethylene.

Hybrid Muskmelon Results and Selection, Fall 2006

Although there was some variation among the lines in DTH during every stage, there was a general pattern in the ASG-1 lines in which they had with the greatest DTH, while line ASG-2 and 'Galia' had the lowest DTH. At stage ZG (Table 3-3), no

significant differences in DTH occurred among the lines. Fruit quality at stage ZG illustrated a difference in only SSC among the lines (Table 3-3). The ASG-1 lines, ASxWT and ASxAS had the lowest SSC. Stage ZG muskmelons from all lines weighed greater than 1 kg and were firm with low ethylene and respiration rates (Table 3-3).

At stage ZYG (Table 3-4), DTH was greatest for ASG-1 lines, ASxWT (c), and ASxAS (h,i) while 'Galia' had the least DTH. Line ASxAS2 (ASG-2) was similar in DTH to 'Galia'. Stage ZYG fruit exhibited the most differences among lines in fruit quality (Table 3-4). Mean fruit weight for all lines was again greater than 1 kg. All lines, except ASxWT (ASG-1d) and ASxAS (ASG-1i), had SSC above 9 °Brix, deeming them acceptable to meet the standards for U.S. Grade No.1 fruit (Lester and Shellie, 2004). Fruits were generally still firm at this stage; however the ASG-1 lines, ASxAS (g,h,i) and ASxWT (b) were firmer than 'Galia'. Lines WTxAS (ASG-1d) and ASxAS2 (ASG-2) were similar in firmness to 'Galia'. Ethylene production rates were lowest for the ASG-1 lines, ASxWT (b,c) and ASxAS (h,i) and highest for 'Galia' and lines WTxAS (ASG-1d) and ASxAS2 (ASG-2). The similarities in quality and ethylene production between 'Galia' and line ASxAS (ASG-2) continued to verify that the transgene in line ASG-2 has probably been silenced.

At stage HS, DTH was greatest for the ASG-1 lines, ASxAS (i) and WTxAS (g) (Table 3-5). Fruit weight and size varied among the lines, however, all lines weighed greater than 1.2 kg (Table 3-5). Fruit SSC was at or above 9 °Brix for all lines. Ethylene and respiration rates increased for all lines from stage ZYG (Table 3-5).

For fruits harvested at stage FS, all ASxAS (ASG-1g,h,i) lines remained on the vine longer than 'Galia' (Table 3-6). Overall, the ASG-1 lines, ASxAS (g,h,i) remained

on the vine an average of eight days longer than 'Galia'. The maximum DTH for lines of ASxAS (ASG-1) ranged from 57 to 60 DTH—10 to 13 days longer than the max DTH for 'Galia'. DTH for line ASxAS2 (ASG-2) was similar to 'Galia', which continued to confirm gene silencing in this line.

Stage FS fruit exhibited few differences in fruit quality among all the ASG lines and 'Galia' (Table 3-6). Fruit SSC was again above 9 °Brix for all lines, though many were at or above 11 °Brix. Muskmelon SSC of 11 °Brix or higher meet the criteria of U.S. Grade 'Fancy' (Lester and Shellie, 2004). Fruit firmness and ethylene production rates varied among lines. 'Galia' was among the firmest fruits, though it also produced among the greatest amount of ethylene evolution. This was most likely due to the fruits being at or near their climacteric peak (Lyons et al., 1962). Line ASxAS (ASG-1i) was among the least firm fruit, and also had the lowest ethylene production. This was probably due to the longer time on the vine and failure of the ASxAS (ASG-1i) fruit to slip early during its peak climacteric phase. Delay in abscission zone development was also observed in T_0 TGM-AS-2 muskmelons (Nuñez-Palenius et al. (2006b) as well as in antisense ACO Charentais cantaloupes (Flores et al., 2001). Respiration rates were similar among all lines (Table 3-6).

Since the ASG-1 lines, particularly those in lines ASxAS and ASxWT, had differences in DTH, fruit firmness and ethylene production at stage ZYG, this stage appeared to be the ideal time to harvest ASG-1 fruit as to obtain optimum quality and perhaps a longer shelf-life. At stage ZYG, all ASG-1 muskmelons had acceptable fruit weight and size as well as acceptable SSC (9 °Brix). Fruit firmness for ASG-1 lines, ASxWT and ASxAS was also greatest and ethylene production was low. If ASG-1 fruits

are not harvested at stage ZYG, then by stages HS and FS, they are equal in fruit quality (especially firmness) to 'Galia' and present no added postharvest benefits. Therefore, the antisense 'Galia' hybrid fruits could be harvested early (prior to full-slip), shipped, and then consumed at the high fruit quality standards of wild-type 'Galia'.

Within line ASxAS (ASG-1 g,h,i) each of the three individual lines tested exhibited the qualities desired for a longer shelf-life 'Galia', especially if harvested at stage ZYG (Table 3-4). One of these lines (ASG-1h) was selected for further research due to its high SSC (12 °Brix) and low ethylene production (0.69 ng kg^{-1} s^{-1}) at stage ZYG as well as averaging 46 DTH by stage FS. Within the ASxWT (ASG-1 b,c) lines, at stage ZYG, two of the lines tested exhibited lower ethylene production than 'Galia' and also had a high SSC, but only line, ASG-1b, was significantly firmer than 'Galia' (Table 4). Fruits from line WTxAS (ASG-1 d,e) did not demonstrate many significant differences from 'Galia' in terms of firmness and ethylene production. The WTxAS line, perhaps behaved similar to the first T_0 antisense hybrids grown in 2004 (Mitchell et al., 2007b), where only the male parental line incorporated the transgene. However, in order to confirm that line WTxAS (ASG-1 d,e) is not expressing the desired characteristics, a selection will be taken from this line and it will be included in future studies for additional analysis.

The ASG-2 line, ASxAS2 displayed characteristics of 'Galia' as it was similar in firmness and in ethylene production to 'Galia' at most stages. Since this line stopped expressing the desired characteristics, was not used in subsequent ASG research. Only ASG-1 lines will be included in future research.

The ASG-1 lines selected and produced in spring 2007 were ASxAS (ASG-1h), ASxWT (ASG-1b) and WTxAS (ASG-1d). These selections were also used to compare fall 2006 and spring 2007 results.

Fall 2006 and Spring 2007 ASG-1 Results

Stage ZG

At stage ZG, only flesh thickness and SSC differed among lines, however there were seasonal differences for most variables. Spring 2007 fruits were generally smaller and lower in SSC, averaging 8 °Brix (Table 3-7). There were no line x season interactions for any stage ZG variables over fall 2006 and spring 2007. This was probably due to the few differences observed among the lines, as all fruits were immature at this stage.

Stage ZYG

At stage ZYG, DTH differences occurred among the lines, where all ASG-1 lines were harvested later than 'Galia'. Also, the ASG-1 lines were overall 50% firmer than 'Galia'. Seasonal differences were also observed for every variable. Spring 2007 fruits were smaller and 60% less firm than fall 2006 fruits (Table 3-8). There were also line x season interactions for SSC, ethylene and respiration (Table 3-9). Fruit SSC for line WTxAS was not affected by season, whereas all other lines were sweetest in the fall. Fall melon crops have the potential of having higher brix values because of lower night temperatures than spring crops (Lester et al., 2007; Lester et al., 2006; Beaulieu et al., 2003). Both ethylene and respiration rates were increased from stage ZG fruit. Ethylene rates also varied between fall and spring, while respiration rates remained consistent, both among lines and seasons. The variation in ethylene rates might be due to an atypical series of wildfires that occurred during spring 2007 in north Florida. The occurrence of

smoke, which consists of ethylene (Rodriguez, 1932), is known to induce ripening and reduce chlorophyll in fruits and leaves (Kader, 2002). During the week of the first harvest, smoke from wildfires was in close proximity to the production site (Fig. 3-7), this could have led to some of the ASG-1 fruits ripening more rapidly, and also to some plant damage that was later observed on the muskmelon leaves. In spring 2007, ASG-1 and 'Galia' fruits had similar ethylene rates at stage ZYG, though in fall 2006, lines ASxWT and ASxAS produced less ethylene than 'Galia'. Respiration rates were generally similar among all lines in both seasons.

Stage HS

At stage HS, all ASG-1 lines were harvested later than 'Galia'. ASG-1 lines, ASxWT and ASxAS had the firmest fruits. While the ASG-1lines, ASxWT and ASxAS were firmer than 'Galia', the TGM-AS-1 half-slip fruits in the work reported by Nuñez-Palenius et al. (2006b) were not firmer than the wild-type. Thus, perhaps the addition of the antisense female parental line in the ASG-1 hybrids has aided in the improvement of the antisense hybrid. The fall 2006 fruits were again, larger and firmer compared to spring 2007 (Table 3-10), which again alluded to the poor environmental conditions that occurred in spring 2007. Line x season interactions for length, SSC and ethylene occurred (Table 3-11). Ethylene rates were either increased or remained at levels similar to stage ZYG. Fall 2006 ethylene rates at stage HS were higher than spring 2007 rates for all lines except 'Galia'. There were no differences in ethylene among all lines.

Stage FS

'Galia' and ASG-1 fruits at stage FS were similar in many quality variables, expect in size, where 'Galia' muskmelons were larger and weighed more (Table 3-12). Stage FS fruit firmness decreased for all lines from stage HS. Ethylene and respiration

rates were increased from stage HS, yet there were no differences among the lines. Fruit quality in fall 2006 was generally better as compared with spring 2007. Fruits were larger in fall 2006 and SSC and firmness were also higher as compared with spring 2007 (Table 3-12). The only line x season interaction was for DTH, where during spring 2007, 'Galia' averaged the same DTH as all ASG-1 lines. However, in fall 2006, all ASG-1 lines had longer DTH than 'Galia', ranging from three to seven more days on the vine (Table 3-13). Antisense ACO Charentais cantaloupes also displayed a delay in slipping from the vine (Ayub et al., 1996; Flores et al., 2001). The spring 2007 result of 'Galia's similarity in DTH among the ASG-1 lines could be due to the effects of the wildfire/smoke event. The damaged plants may have caused 'Galia' to ripen slower in this case, as ethylene and respiration rates of 'Galia' and ASG-1 lines were also similar. Ethylene rates were higher in fall 2006 at stage FS than spring 2007. Again, the fact that fruits remained on the plant longer in the spring as compared with fall may have attributed to the lower ethylene rates at stage FS. By the time fruits reached stage FS in spring 2007, they were most likely post-climacteric and produced less ethylene as compared to fall 2006 fruits, which ripened without any plant stress.

ASG-1 muskmelons harvested at stages ZG, HS and FS exhibited many similarities to the original 'Galia' muskmelon, especially in firmness and ethylene production. Only stage ZYG ASG-1 muskmelons from lines ASxAS and ASxWT demonstrated increased firmness and lower ethylene, but only when grown in optimal conditions, such as good temperatures (Min: 18 °C to Max: 35 °C), minimal disease/insect pressure, and no wildfires or hurricanes. There was also no difference in respiration among the ASG-1 lines and 'Galia' at stage ZYG, or at any other stage. Other

reports of antisense ACO Charentais cantaloupes report a lack of a climacteric rise in the antisense fruit compared with wild-type fruit (Bower et al., 2002). Perhaps both reduced ethylene and respiration may be observed in the ASG-1 muskmelons during a storage treatment where the gas emissions can be tracked over time.

The ASG-1 lines of ASxAS and ASxWT demonstrated the most potential for a longer shelf-life 'Galia' muskmelon if harvested at stage ZYG and produced in an optimal environment (such as acceptable temperatures and low insect/disease pressures). These ASG-1 lines exhibited similar fruit size (greater than 1 kg), similar SSC (ranged from 10.5 °Brix to over 12 °Brix) to original 'Galia', yet were firmer than 'Galia' at stage ZYG. The ASG-1 lines, ASxAS and ASxWT also produced less ethylene than 'Galia' during fall 2006. Even though reduced ethylene was not observed again in spring 2007, most likely due to the environmental problems associated with that season, the ASG-1 lines ASxWT and ASxAS were again firmer than 'Galia' in spring 2007 and had a later DTH than 'Galia'. These results are similar to what Nuñez-Palenius et al. (2006a and 2006b), Ayub et al. (1996) and Guis et al. (1997) reported in other antisense acc-oxidase melons in terms of firmer fruit, low ethylene and similar fruit quality (fruit size and SSC).

Summary

Renowned for their flavor and sweetness, Galia (*Cucumis melo* L. var. *reticulatus* Ser.) melons have a reduced shelf-life as compared with standard melons. The objective of this research was to develop a true 'Galia' F_1 hybrid with a longer shelf-life while maintaining sweetness. The 'Galia' male parental line was previously transformed with an antisense ACC-oxidase gene (CMACO-1),which produced two independent T_0, TGM-AS-1 and TGM-AS-2, transgenic lines (Nuñez-Palenius et al., 2006a). These lines were used in a traditional plant breeding program to produce antisense (AS) 'Galia' F_1 hybrids

(AS ♀ x WT ♂, AS ♀ x AS ♂, WT ♀ x AS ♂). During fall 2006, several lines of the AS 'Galia' F_1 (ASG) muskmelon crosses were grown and evaluated with the original 'Galia' cultivar. After the fall 2006 trial, one ASG line from each cross (ASxWT, WTxAS and ASxAS) was selected and evaluated again with 'Galia' in spring 2007. Fruits were harvested at four stages of growth: stage 1.) zero-slip, green (ZG); 2.) zero-slip, yellow-green (ZYG); 3.) half-slip (HS); and 4.) full-slip (FS). Data were recorded for days to harvest, fruit weight, size, flesh thickness, soluble solids content (SSC), firmness, ethylene and respiration (CO_2). At stage ZG, all antisense (AS) muskmelons were similar in size, quality, ethylene and CO_2 production to 'Galia.' At stage ZYG, ASxAS and ASxWT melons were significantly firmer than 'Galia' in fall and spring, yet only produced less ethylene than 'Galia' in fall 2006. Also at stage ZYG, there were no differences in SSC among all lines during each season. ASxAS, WTxAS, ASxWT melons remained on the vine an average of three to five days longer than 'Galia' and were similar in quality, ethylene, and CO_2 to 'Galia' at the HS and FS stages. The differences that occurred in lines ASxAS and ASxWT at stage ZYG indicated a potential that a longer shelf-life 'Galia' muskmelon were achieved when harvested at stage ZYG.

Table 3-1. Days to harvest (DTH) results of the transformed (T) antisense male (TGM-AS-1 and TGM-AS-2) lines that were selfed from spring 2004 through fall 2005.

	Days to harvest (DTH)			
Line	T_1	T_2	T_3	T_4
TGM-AS-1	51	52	59	59
TGM-AS-2	46	42	37	36
Wild-type	30	38	30	35
LSD (0.05)[z]	8.4	10.9	2.9	4.6

[z], Mean separation by Fisher's least significant difference test (P=0.05).

Table 3-2. Days to harvest (DTH) results of backcrossed (BC) antisense female (TGF-AS-1 and TGF-AS-2) lines from spring 2004 through fall 2005.

	Days to harvest (DTH)			
Line	BC_1	BC_2	BC_3	BC_4
TGF-AS-1	51	50	51	48
TGF-AS-2	53	54	49	44
Wild-type	57	51	51	47
LSD (0.05)[z]	3.2	1.9		

[z], Mean separation by Fisher's least significant difference test (P=0.05).

Table 3-3. Stage ZG means of days to harvest, fruit quality, ethylene production and respiration rates of 'Galia' and grouped Antisense 'Galia' (ASG -1 and 2) muskmelons, fall 2006.

Line	Days to harvest (DTH)	Weight (kg)	Length (mm)	Width (mm)	Flesh thickness (mm)	Soluble solids content (ºBrix)	Firmness (N)	Ethylene ($ng\ kg^{-1}\ s^{-1}$)	Respiration ($\mu g\ CO_2\ kg^{-1}\ s^{-1}$)
Galia	38	1.53	155	143	35.9	10.5	39.8	0.21	9.2
ASxWT (ASG-1)	40	1.09	138	129	28.5	9.9	43.9	0.09	8.2
WTxAS (ASG-1)	39	1.40	153	141	30.7	6.3	37.3	0.22	8.0
ASxAS (ASG-1)	43	1.12	137	129	30.9	7.6	43.7	0.10	6.9
ASxAS (ASG-2)	40	1.18	138	129	31.1	10.1	37.7	1.40	10.0
LSD (0.05)[z]						2.0			

[z], Mean separation by Fisher's least significant difference test (P≤0.05).

Table 3-4. Stage ZYG means of days to harvest, fruit quality, ethylene production and respiration rates of 'Galia', and individual lines of Antisense 'Galia' (ASG -1 and 2) muskmelons, fall 2006.

Line	Days to harvest	Weight (kg)	Length (mm)	Width (mm)	Flesh thickness (mm)	Soluble solids content (°Brix)	Firmness (N)	Ethylene (ng kg^{-1} s^{-1})	Respiration (µg CO_2 kg^{-1} s^{-1})
Galia	38	1.50	151	143	30.5	11.8	24.9	2.86	10.9
ASxWT (ASG-1b)	41	1.30	145	136	31.2	12.2	38.1	1.41	11.7
ASxWT (ASG-1c)	43	1.67	142	138	30.3	11.2	28.0	1.40	13.4
WTxAS (ASG-1d)	42	1.42	149	140	30.5	9.5	28.0	2.52	16.0
ASxAS (ASG-1g)	41	1.45	149	145	30.4	8.5	32.8	1.97	15.8
ASxAS (ASG-1h)	43	1.30	144	134	30.5	11.6	33.4	0.69	10.4
ASxAS (ASG-1i)	45	1.31	156	137	32.9	6.75	31.3	0.69	8.8
ASxAS2 (ASG2)	40	1.60	157	144	35.8	12.0	21.7	1.95	13.4
LSD (0.05)[z]	3.5					1.8	6.4	1.2	

[z], Mean separation by Fisher's least significant difference test (P=0.05).

Table 3-5. Stage HS means of days to harvest, fruit quality, ethylene production and respiration rates of 'Galia', 'Galia'-type (MG10183) and Antisense 'Galia' (ASG -1 and 2) muskmelons, fall 2006.

Line	Days to harvest	Weight (kg)	Length (mm)	Width (mm)	Flesh thickness (mm)	Soluble solids content (ºBrix)	Firmness (N)	Ethylene (ng kg^{-1} s^{-1})	Respiration (µg CO_2 kg^{-1} s^{-1})
Galia	40	1.43	143	141	34.2	12.3	21.4	2.84	12.1
ASxWT (ASG-1b)	43	1.80	162	148	36.4	12.3	24.3	3.53	12.7
ASxWT (ASG-1c)	42	1.29	143	137	26.5	10.0	9.1	4.21	14.9
WTxAS (ASG-1d)	42	1.75	162	149	31.9	10.0	15.3	5.66	12.7
WTxAS (ASG-1e)	45	1.49	155	142	31.6	10.4	20.3	2.99	24.7
ASxAS (ASG-1g)	41	1.48	157	140	33.8	9.6	17.7	3.93	10.7
ASxAS (ASG-1h)	43	1.45	147	140	32.8	11.8	25.6	2.37	12.3
ASxAS (ASG-1i)	51	2.05	166	157	36.8	9.0	11.2	1.79	14.9
ASxAS2 (ASG2)	37	1.72	163	146	33.8	11.0	18.0	6.93	12.7
LSD (0.05)[z]	4.6	0.4	12.9			1.9	7.8	2.3	

[z], Mean separation by Fisher's least significant difference test (P≤0.05).

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Table 3-6. Stage FS means of days to harvest, fruit quality, ethylene production and respiration rates of 'Galia' and Antisense 'Galia' (ASG -1 and 2) muskmelons, fall 2006.

Line	Days to harvest	DTH Min	DTH Max	Weight (kg)	Length (mm)	Width (mm)	Flesh thickness (mm)	Soluble solids content (ºBrix)	Firmness (N)	Ethylene (ng kg^{-1} s^{-1})	Respiration (µg CO_2 kg^{-1} s^{-1})
Galia	39	32	46	1.68	157	147	33.2	11.3	18.1	4.97	14.0
ASxWT (ASG-1b)	43	34	53	1.36	145	136	33.2	12.1	18.9	5.07	13.7
ASxWT (ASG-1c)	42	37	47	1.12	141	131	29.2	9.9	12.3	3.08	13.2
WTxAS (ASG-1d)	43	38	49	1.31	145	133	29.4	10.3	13.1	5.83	15.0
WTxAS (ASG-1e)	41	37	47	1.41	155	140	30.4	11.1	16.4	4.34	14.6
ASxAS (ASG-1g)	47	37	58	1.86	165	154	37.8	9.2	14.2	3.36	11.8
ASxAS (ASG-1h)	46	40	57	1.45	150	137	34.2	11.1	16.2	3.03	11.8
ASxAS (ASG-1i)	47	43	60	1.54	157	142	37.3	9.5	9.7	1.13	10.7
ASxAS2 (ASG2)	40	34	47	1.60	157	144	36.3	12.1	13.0	5.55	12.0
LSD (0.05)[z]	5.3							1.8	4.7	1.8	

[z], Mean separation by Fisher's least significant difference test (P≤0.05).

Table 3-7. Stage ZG means of days to harvest, fruit quality, ethylene production and respiration rates of 'Galia' and Antisense 'Galia' (ASG -1) muskmelons, fall 2006 and spring 2007.

Line	Days to Harvest	Weight (kg)	Length (mm)	Width (mm)	Flesh thickness (mm)	Soluble solids content (°Brix)	Firmness (N)	Ethylene (ng kg^{-1} s^{-1})	Respiration (µg CO_2 kg^{-1} s^{-1})
Galia	47	1.34	148	134	32.7	9.6	36.5	0.13	6.91
ASWT	46	1.03	135	123	26.3	8.8	41.8	0.07	6.26
WTAS	49	1.27	146	132	29.5	7.1	37.6	0.11	5.50
ASAS	51	1.26	139	130	28.8	9.0	41.6	0.04	5.50
LSD (0.05) [z]					3.8	1.6			
Fall 2006	40	1.34	145	136	30.9	9.4	40.8	0.15	7.89
Spring 2007	57	1.11	139	124	27.8	7.8	37.9	0.02	4.19
Significance[y]	**	**	**	**	**	**	NS	**	*

[z], Mean separation by Fisher's least significant difference test (P≤0.05).
[y], NS, *, ** Non-significant (NS) or significant F-test at P≤0.05 and P≤0.01, respectively.

Table 3-8. Stage ZYG means of days to harvest, fruit quality, ethylene production and respiration rates of 'Galia' and Antisense 'Galia' (ASG -1) muskmelons, fall 2006 and spring 2007.

Line	Days to Harvest	Weight (kg)	Length (mm)	Width (mm)	Flesh thickness (mm)	Firmness (N)
Galia	41	1.24	142	132	29.0	17.4
ASWT	45	1.13	137	126	28.7	32.5
WTAS	46	1.08	133	127	29.2	21.2
ASAS	45	1.08	135	125	27.7	28.8
LSD (0.05) [z]	2.3					3.3
Fall 2006	41	1.38	147	139	30.8	30.7
Spring 2007	47	0.89	127	117	26.5	19.2
Significance[y]	**	**	**	**	*	**

[z], Mean separation by Fisher's least significant difference test (P≤0.05).
[y], *, **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table 3-9. Stage ZYG line x season (L x S) interaction means of soluble solids content, ethylene production and respiration rates.

	Soluble solids content (°Brix)		Ethylene ($ng\ kg^{-1}\ s^{-1}$)		Respiration ($\mu g\ CO_2\ kg^{-1}\ s^{-1}$)	
Line	Fa06	Sp07	Fa06	Sp07	Fa06	Sp07
Galia	11.8	9.8	2.86	1.07	10.89	9.64
ASWT	12.2	8.9	1.41	1.38	11.71	10.94
WTAS	9.2	9.4	2.75	1.89	16.99	8.55
ASAS	11.6	8.7	0.68	1.18	10.34	10.29
LxS LSD (0.05)[z]	0.95		0.74		2.9	

[z], Mean separation for line x season interaction by Fisher's least significant difference test (P≤0.05).

Table 3-10. Stage HS means of days to harvest, fruit quality, ethylene production and respiration rates of 'Galia' and Antisense 'Galia' (ASG -1) muskmelons, fall 2006 and spring 2007.

Line	Days to Harvest	Weight (kg)	Width (mm)	Flesh thickness (mm)	Firmness (N)	Respiration ($\mu g\ CO_2\ kg^{-1}\ s^{-1}$)
Galia	42	1.21	130	31.9	16.2	12.20
ASWT	46	1.39	133	31.4	20.7	11.43
WTAS	49	1.28	132	30.2	14.6	13.67
ASAS	45	1.14	127	28.7	20.9	11.81
LSD (0.05) [z]	2.1				4.0	
Fall 2006	42	1.56	142	33.8	22.1	13.50
Spring 2007	47	0.94	118	27.2	14.1	11.05
Significance[y]	**	**	**	**	**	*

[z], Mean separation by Fisher's least significant difference test (P≤0.05).
[y], *, **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table 3-11. Stage HS line x season (L x S) interaction means of fruit length, soluble solids content and ethylene.

	Length (mm)		Soluble solids content (°Brix)		Ethylene ($ng\ kg^{-1}\ s^{-1}$)	
Line	Fa06	Sp07	Fa06	Sp07	Fa06	Sp07
Galia	143	134	12.3	9.8	2.84	2.48
ASWT	162	131	12.3	8.7	3.54	1.8
WTAS	157	133	9.3	8.9	3.89	1.65
ASAS	147	122	11.8	9.0	2.37	1.9
LxS LSD (0.05)[z]	7		1.1		0.7	

[z], Mean separation for line x season interaction by Fisher's least significant difference test (P≤0.05).

Table 3-12. Stage FS means of days to harvest, fruit quality, ethylene production and respiration rates of 'Galia' and Antisense 'Galia' (ASG -1) muskmelons, fall 2006 and spring 2007.

Line	Weight (kg)	Length (mm)	Width (mm)	Flesh thickness (mm)	Soluble solids content (°Brix)	Firmness (N)	Ethylene ($ng\ kg^{-1}\ s^{-1}$)	Respiration ($\mu g\ CO_2\ kg^{-1}\ s^{-1}$)
Galia	1.39	148	135	32.5	10.9	14.6	3.89	13.4
ASWT	1.12	136	126	30.5	10.9	16.6	4.03	13.6
WTAS	1.08	136	125	29.4	9.19	12.3	3.78	13.3
ASAS	1.15	137	126	30.1	10.1	13.6	3.01	12.0
LSD (0.05) [z]	0.2	6.9	6.4		0.9	2.0		
Fall 2006	1.46	151	139	33.1	11.2	17.0	4.38	13.6
Spring 2007	0.91	128	117	28.1	9.32	11.5	2.98	12.6
Significance[y]	**	**	**	**	**	*	**	*

[z], Mean separation by Fisher's least significant difference test ($P \leq 0.05$).

[y], NS, *, ** Non-significant (NS) or significant F-test at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 3-13. Stage FS means of significant line*season interaction means of days to harvest.

	Days to harvest	
Line	Fa06	Sp07
Galia	39	46
ASWT	43	47
WTAS	42	47
ASAS	46	49
LxS LSD (0.05)[z]	1.5	

[z], Mean separation for line x season interaction by Fisher's least significant difference test (P≤0.05).

Figure 3-1. 'Galia' muskmelon parental lines. The female parental line, 'Noy Yizre'el' (Left) and the male parental line, 'Krymka' (right).

Figure 3-2. The 'Galia' F_1 hybrid muskmelon.

Figure 3-3. TGM-AS-1 fruit from a T_4 generation still green and on the vine after pollination on 3-23-06; and a wild-type male fruit ready to be harvested at full-slip that was pollinated on 3-25-06.

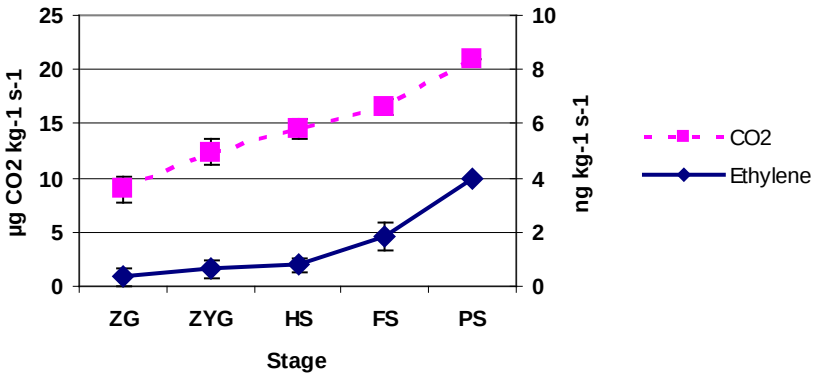

Figure 3-4. Ethylene evolution and respiration (CO_2) rates of T_3 TGM-AS-1 muskmelons harvested at different stages (ZG= zero-slip, green; ZYG= zero-slip, yellow/green; HS= half-slip; FS= full-slip; PS= post-slip) of ripening, fall 2005.

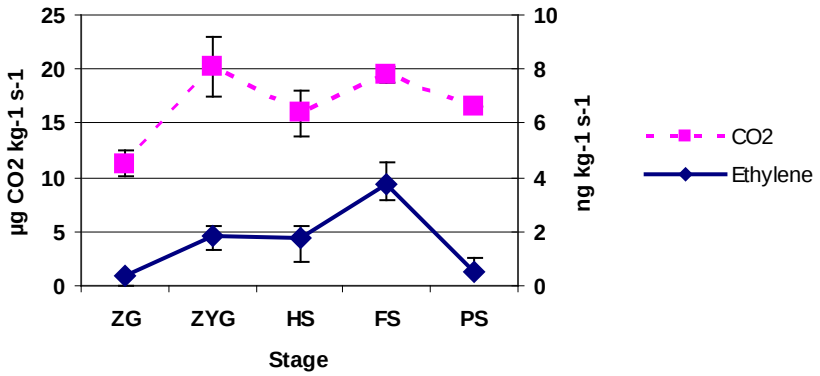

Figure 3-5. Ethylene evolution and (CO_2) respiration rates of T_3 TGM-AS-2 muskmelons harvested at different stages (ZG= zero-slip, green; ZYG= zero-slip, yellow/green; HS= half-slip; FS= full-slip; PS= post-slip) of ripening, fall 2005.

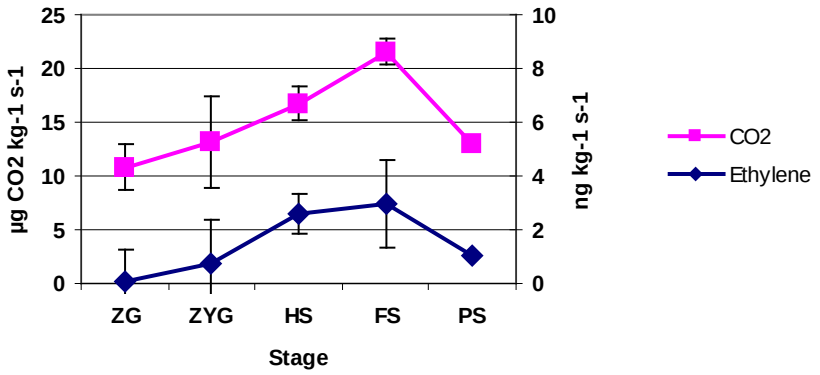

Figure 3-6. Ethylene evolution and respiration (CO_2) rates of wild-type male ('Krymka') muskmelons harvested at different stages (ZG= zero-slip, green; ZYG= zero-slip, yellow/green; HS= half-slip; FS= full-slip; PS= post-slip) of ripening, fall 2005.

Figure 3-7. The Protected Agriculture Greenhouse site enveloped in smoke from nearby wildfires during the week of May 8, 2007.

CHAPTER 4
GALIA MUSKMELON FRUIT QUALITY AND FLAVOR (*Cucumis melo* L. var. *reticulatus* Ser.)

Introduction

The 'Galia' muskmelon features a golden-netted exterior, sweet, green flesh and a musky fragrance (Karchi, 2000). It is an F_1 hybrid that was developed by Israeli breeder, Zvi Karchi, who named it after his daughter; it was released in 1973 (Karchi, 2000). After its introduction, 'Galia' muskmelon quickly became a popular market name throughout Europe and the Mediterranean by way of an intense marketing campaign with Agrexco- an Israeli agricultural exporter, and sales at the popular British food chain, Marks and Spencer (Karchi, 2000). By the 1980s, 'Galia' muskmelons were distributed all over Western Europe, except France (Karchi, 2000). Its popularity was attributed to its intense flavor, aroma, and sweetness. In effect, 'Galia' F_1 hybrid melon production was one of the factors that helped revive Israel's agricultural production, breeding and research as well as increase it's competive advantage in world markets (Karchi, 2000).

Although 'Galia' muskmelon is an exceptional fruit, it has some limitations. Besides being highly susceptible to powdery mildew (*Podosphaera xanthii*) (Mitchell et al. 2006, 2007a, and 2007b), another main disadvantage is its short shelf-life (Mitchell et al. 2007b; Nuñez-Palenius et al., 2006a and 2006b; Aharoni et al., 1993). In order to achieve peak flavor, 'Galia' must be picked at a fully ripe or 'full-slip' stage (Mitchell et al. 2007b; Nuñez-Palenius et al., 2005), which reduces storage life. Exporters have increased shelf-life, but decreased quality by harvesting fruit at an immature stage; however this leads to lower quality fruit (Fallik et al., 2001; Cantliffe and Shaw, 2002; Pratt. 1971).

As a result of ‘Galia’s limitations and its popularity, breeders have worked to improve disease resistance/tolerance as well as improve shelf-life (Mitchell et al., 2007a), resulting in a “new generation” of improved ‘Galia’ muskmelons (MOAG, 2006). Today ‘Galia’ is a trade name for other “look-a-like” melon cultivars, commonly called ‘Galia’-type melons. There are over 75 ‘Galia’-type cultivars available in the market (Harty, 2008). Unfortunately, although these ‘Galia’-type cultivars are firm, they often lack the flavor, aroma, and high soluble solids content of the original ‘Galia’ hybrid (Mitchell et al., 2007a and 2007b). This is also true for other crops, such as tomato (*Solanum lycopersicum*), where breeders have improved traits such as yield and disease resistance, but have fallen short on improving fruit quality (Causse et al., 2002).

Although flavor comprises taste, texture, and aroma (Goff and Klee, 2006), it is a complex trait also determined by many factors including genetics, environment, culture, production and postharvest handling (Baldwin, 2002). In order to improve taste, breeders must consider all this information to gain the desired quality (Causse et al., 2003). This includes consideration of the developmental and biochemical changes in fruit color, texture, flavor, and aroma (Li et al., 2006).

Sweetness, however, is often considered to be one of the most important fruit quality components in muskmelon (Yamaguchi et al., 1977). Nevertheless, since muskmelons tend to be quite variable in quality, aroma and sensory analyses are also used to determine muskmelon fruit quality (Beaulieu and Lea, 2003; Aulenbach and Worthington, 1974; Yamaguchi et al., 1977). Sensory measurements of quality attributes can provide an approximation of consumer acceptability (Abbott, 1999) as taste is important aspect of organoleptic quality (Causse et al., 2002).

The characteristic flavor that is associated with melons is dependent on its aroma. Aroma volatiles are released as the fruit ripens and their presence, absence, and quantity characterize each melon type (Pratt, 1971; Teranishi, 1971). According to Pratt (1971) research on muskmelon aroma began in the 1930's when Rakitan (1935, 1945) identified and noted increases during ripening of the compounds acetaldehyde and ethanol in muskmelons. Today, over 240 aroma compounds have been identified in muskmelon and the predominant aromatic compounds are esters, alcohols, and aldehydes (Obando-Ulloa et al., 2008; Lamikanra, 2002; Beaulieu, 2006; Beaulieu and Grimm, 2001, 2003; Njissen et al., 1996). Aroma volatiles are believed to be under genetic control as there are marked differences between melon cultivars (Yahyaoui et al., 2002; Wyllie and Leach, 1992). Higher concentrations of aroma volatile compounds have been reported in mature cantaloupes (muskmelons) as compared with fruits at immature stages (Beaulieu, 2006; Senesi et al., 2005; Beaulieu and Grimm, 2001; Horvat and Senter, 1987).

Aroma volatiles have been studied on various 'Galia'-type (GT) cultivars such as 'Arava', 'Fado', 'C8', '5080', '7302' (Leach et al., 1989; Wyllie and Leach, 1992; Fallik, et al., 2001 and 2005; Hoberg et al., 2003; Obando-Ulloa et al., 2008; Shalit et al., 2001; Kourkoutas et al., 2006).

To date, there are no known reports on the aroma volatiles of the original 'Galia' muskmelon. The volatile work by Leach et al., 1989 and Wyllie and Leach (1992) may have been with the original 'Galia' cultivar, but it was not stated; and the work by Kourkoutas et al. (2006) reported results on 'Galia' muskmelons with no report of whether it was 'Galia' or a GT.

As it is already known and confirmed that 'Galia' is a high-quality fruit with excellent flavor (Karchi, 2000; Shaw et al., 2001; Fallik et al., 2001; Aharoni et al., 1993), it is not known what distinguishes the original 'Galia' muskmelon apart from some GT cultivars. To determine what may set 'Galia' flavor apart from GTs, the present study focused on aroma, and sought to identify volatiles of the true 'Galia' F_1 hybrid.

Materials and Methods

Three experiments were conducted during fall 2006, spring 2007 and fall 2007. Seeds of 'Galia' (Hazera Genetics, Israel) and 'MG10183' (Zeraim Gedera/Syngenta, Israel) were planted on 7 July 2006, 19 Jan. 2007 and 31 July 2007. An additional 'Galia'-type (GT), 'Elario' (Hazera Genetics, Israel) was only grown in fall 2007. 'Elario' was added to the research experiment since it was identified as being one of the standard fall cultivars commonly grown in Israel (A. Gadiel, ARAVA, personal communication, 2007), whereas 'MG10183' has not yet been released (M. Peretz, Zeraim Gedera, personal communication, 2008). 'MG10183' was selected due to previous studies where it was found to be a higher quality (good SSC and firmness) GT muskmelon (Mitchell et al., 2007a; Mitchell et al., 2006)

Seedlings were produced at the University of Florida, Gainesville, FL campus in Model 128A polystyrene plug trays (Speedling, Bushnell, FL) with a commercial fine-grade plug growing medium (Premier ProMix 'PGX,' Quakertown, PA). Seedlings were grown in a Conviron plant growth chamber (Controlled Env. Ltd.,Winnipeg, Manitoba, Canada) at temperatures of 28 $^{\circ}$C (day) and 22 $^{\circ}$C (night) with 16 hour daily artificial lighting**.** Seedlings were fertilized after the first true leaf expanded, and fertilization

continued once per week with Peters Professional All Purpose Plant Food (Spectrum Group, St. Louis, MO) at the rate according to Mitchell et al. (2007).

Once seedlings had three true leaves, they were transplanted on the 3 Aug. 2006, 27 Feb. and 15 Aug. 2007. Plants were grown in a saw-tooth style, passively-ventilated greenhouse (TOP greenhouses, Ltd., Barkan, Israel), located at the University of Florida, Plant Science Research Education Unit located in Citra, FL. The plants were grown using commercial greenhouse muskmelon production techniques and nutrient requirements according to the recommendations of Shaw et al. (2001). Plant spacing was 48 cm between plants and 90 cm between rows. Plant density was 2.5 plants $\cdot$ m^{-2}. Pollination was achieved via bumble bees from Class A research hives (*Bombus impatiens*, Natupol, Koppert Biological Systems, Inc., Romulus, MI). In fall 2006 one hive was released on 23 Sept. 2006. In the spring there were three hives released on 29 March, 6 April and 26 April 2007; and in fall 2007 two hives were released on 29 Aug. and 20 Sept. 2007. All flowers were tagged with the date of anthesis.

Temperature and photosynthetic photon flux (PPF) at the canopy level were recorded daily at 30-min. intervals by HOBO data loggers (Onset Comp. Corp., Bourne, MA). Within canopy temperatures were also recorded at 15 minute intervals by WatchDog data loggers (Spectrum Tech., Plainfield, IL). The monthly temperature averages were taken as an average of the 'within' and 'at' canopy readings.

Insect pests were monitored weekly by scouting one plant from each plot per block. Biological control was used for management of arthropod pests in all three seasons. During all three seasons, whiteflies (*Bemisia tabaci* biotype B), flower thrips (*Frankliniella tritici* (Fitch)), two-spotted spider mites (*tetranychus urticae*) and red

mites (O*ligonychus ilicis*) were observed in the crop. As a result, *Erotmocerus eremicus* and *Encarsia formosa* (ENERMIX, Koppert Biological Systems, Inc., Romulus, MI) and *Erotmocerus mundus* (BEMIPAR, Koppert Biological Systems, Inc., Romulus, MI) were released to control whitefly at an average release rate of 7.4 wasps · m^{-2}; *Orius insidiosus* (THRIPOR-I, Koppert Biological Systems, Inc., Romulus, MI), a predatory bug for control of flower thrips was released at an average rate of 4.5 bugs · m^{-2}; *Amblyseius swirskii* (SWIRSKI-MITE PLUS, Koppert Biological Systems, Inc., Romulus, MI), predatory mites of thrips and whitefly, were released at an average rate of 97 mites · m^{-2}; *Neoseiulus californicus* (Biotactics Inc., Perris, CA) predatory mites, used to control two-spotted spider mites were released at an average rate of 28 mites · m^{-2} , and *Neoseiulus fallacis* (Biotactics Inc., Perris, CA) predatory mites, used to control red mites, were released at an average rate of 40 mites · m^{-2}. Also released was a parasitic wasp, *Aphidius colemani* (APHIPAR, Koppert Biological Systems, Inc., Romulus, MI) of the green peach aphid (*Myzus persicae* [Sulzer]), as a preventative measure at an average rate of 3.3 wasps · m^{-2}.

Due to a spider mite infestation in certain areas of the crop during all three seasons, Abamectin miticide (Agri-Mek, Syngenta Crop Prot., Inc., Greensboro, NC) was sprayed (rate: 30 oz. ha^{-1}) on 26 Sept., 2006, 3 Oct., 2006 and 17 Sept. 2007. In spring 2007, an insecticidal soap, Mpede (Mycogen Corp., San Diego, CA) was sprayed to control spider mites (rate: 2% v/v solution). No preventative fungicides were sprayed for powdery mildew (*Podosphaera xanthii*). Once powdery mildew was evident in the crop, plants were sprayed weekly with potassium bicarbonate (Milstop, BioWorks Inc., Fairport, NY; rate: 2.8 kg ha^{-1}), a foliar fungicide that suppresses powdery mildew and assists in

keeping the plants productive. Milstop applications began on 3 Oct. 2006, 14 May 2007 and 8 Oct. 2007.

Fruit Selection and Postharvest Treatments

Fruits were harvested at full maturity, but at four different stages of ripening. Stage: 1.) zero-slip green (ZG): external skin still green in color with no abscission layer development; 2.) zero-slip, yellow-green (ZYG): external skin green and yellow, with no abscission layer development; 3.) half-slip (HS): fruit abscising half-way; and 4.) full-slip (FS): fruit separates easily from the stem. Fruits were harvested from 29 Sept. to 30 Oct. 2006; 10 May to 18 June 2007 and 2 Oct. to 26 Oct. 2007. During each harvest period, fruits were harvested daily, in the afternoon and separated into two groups. In the first group, the harvest treatment, all postharvest variables were measured 12 hours after harvest. In the second group, the storage treatment, fruits were stored at 20 °C and 85% relative humidity (RH). Storage days varied for the different stage fruits. This was done to be able to track the climacteric phase (ethylene and respiration was measured daily from each fruit while in storage) and fruit quality data was measured at an appropriate edible time (not over-ripe). Stages ZG and ZYG were stored for five days, stage HS was stored for three days, and stage FS fruit was stored for two days. Only fruits harvested during spring and fall 2007 were subjected to a storage treatment. The fall 2006 trial was used to determine proper aroma volatile collection methods at harvest only, a storage treatment was not a part of that research. Immediately after harvest, fruit weight and size were recorded. Fruits were then transported to campus and put in 20 °C storage. The next morning, 12 hours after harvest, ethylene, and respiration rates were measured from all fruits.

Ethylene and Respiration Measurements

To determine ethylene evolution and respiration rates, each melon was sealed in an airtight 3 L Tupperware container for one hour at 20 ºC allowing ethylene and carbon dioxide to accumulate. Two samples were taken from the headspace using a hypodermic syringe through a rubber septum. A 1.0 ml sample for ethylene was injected into a Tracor 540 Gas Chromatograph (Tremetrics Analytical Division, Austin, TX) equipped with a photoionization detector (PID) and a stainless steel alumina F1 column (Supelco, Sigma-Aldrich, Bellefonte, PA), with a mesh size of 80/100 and was 914 x 3.18 mm (length x diam.). The detector and injector operated at 100 °C and the oven was 50 °C. The carrier gas was helium with a flow rate of 40 ml min^{-1}. A 0.5 ml sample for carbon dioxide was injected into in a Gow-Mac, Series 580 gas chromatograph (GC) (Gow-Mac Instruments, Bridgewater, NJ, U.S.A.). The Gow-Mac was equipped with a thermal conductivity detector (TCD) and a 80/100 mesh Porapak Q column (Agilent Tech., Inc., Santa Clara, CA) that was 1219 x 3.18 mm (length x diam.). The carrier gas was helium at a flow rate of 30 ml min^{-1}. The detector and injector operated under ambient conditions (26 to 27 ºC) and the oven was at 40 ºC.

After ethylene and respiration measurements, fruits from the harvest treatment were removed from storage. Fruits in the storage treatment remained at 20 °C.

Fruit Quality Measurements

Directly following storage for all treatments, fruit quality variables, which included flesh thickness, firmness, and SSC were measured on fresh fruit according to Mitchell et al. (2007). A 2.5 cm slice was taken from the equatorial region of each fruit and flesh thickness, firmness, and SSC were measured. A caliper (Digimatic Mycal, Mitutoyo, Japan) was used to measure flesh thickness from peel to cavity. Pulp firmness

was determined at two equidistant points on the equatorial region of each fruit slice using an Instron Universal Testing Instrument (Model 4411-C8009, Canton, MA). The Instron was fitted with a 50 kg load cell and an 11 mm convex probe with a crosshead speed of 50 mm min^{-1}. Firmness data were expressed as the maximum force (bioyield point, in Newton) attained during deformation. Soluble solids content (SSC) (°Brix) was measured with a temperature-compensating, handheld refractometer (Model 10430, Reichert Scientific Instrument, Buffalo, NY) from fresh juice expressed from two pulp samples taken from the equatorial slice.

Remaining pulp was used for aroma volatile collection and a quantity was frozen for Total Titratable Acidity (TTA) and pH measurements. Titratable Acidity (TTA) and pH were measured with a 719 S Titrino (Metrohm Ltd., Hersisau, Switz.) from mesocarp flesh that was macerated, centrifuged (Beckman, Model J2-21) for 15 min. at 15, 000 x *g* and the supernatant was filtered through cheese clothe. TTA was determined by pH titration to 8.1 with 0.1 N NaOH and calculated as percent malic acid equivalents.

Aroma Volatile Collection and Analysis

Aroma volatiles were collected from 100 g of fresh, chopped mesocarp flesh (2-cm (L) x 1-cm (w)) from each fruit. Volatile collection was done according to Schmelze et al. (2003) with nonyl acetate as an internal standard. Fruit was inserted into Simex glass tubes (28 x 1.5 x 610 mm; Pegasus Glass). With the aid of a vacuum pump, air, filtered through a hydrocarbon trap (Agilent Technologies, Palo Alto, CA), flowed through the tubes for 1-hour at 618 ml min^{-1}. Volatiles were collected on a Super Q column (30 mg Altech® resin) and eluted with methylene chloride. Volatiles were separated on an Agilent Technologies DB-5 column (length x diam.: 30 x 0.25 mm) and

analyzed on an Agilent Technologies 6890N (7683 Series) Gas Chromatograph (GC) (Agilent Tech., Inc., Santa Clara, CA). The GC was equipped with a flame ionization detector (FID) with a detection temperature of 280 °C. The GC/FID had an inlet temperature of 220 °C and an oven ramp from 40 to 250 °C at 5° per minute; the carrier gas was helium. Retention times were compared with known standards and quantified with Agilent ChemStation software. Volatile peak identities were confirmed by an Agilent Technologies 5975 Gas Chromatograph/Mass Spectrometer (GC/MS).

Thirty-eight volatile compounds were identified. Of the 38 compounds, 11 to 19 compounds, depending on cultivar and harvest stage, were considered to be 'significant contributors' (SCs) to the overall aroma of 'Galia' and both GTs. Total SCs for 'Galia' by stage FS were 18 while 'MG10183' and 'Elario' had 17 and 19 SCs, respectively (Table 4-1). Significant contributors to aroma were identified as a result of dividing the concentration of the compound (determined with GC/FID) by its known odor threshold value (OTV), resulting in the odor value (OV) of the compound (Bauchot et al., 1998; Teranishi et al., 1991). Compounds with OVs greater than one were considered significant contributors to the aroma. Odor threshold values (OTVs) were obtained in the literature. The SC compounds for all cultivars consisted of over 90% of the total identified volatiles (TIV) in every season at harvest and after storage, with the exception of fall 2006 harvest volatiles, where the SC compounds consisted of only 78% of the TIV.

Sensory Evaluation

A sensory analysis was done in spring 2008 with cultivars 'Galia', 'MG10183', 'Elario' and an additional cultivar, 'Red Moon' was included as a control. The same production practices were used in spring 2008. The 'Red Moon' cultivar is a type of

melon called European netted cantaloupe. These are characterized by their deep, orange/magenta pigmented flesh and a sutured/netted exterior. The 'Red Moon' is currently trademarked the 'Perfect Melon™', boasting a guaranteed high SSC of up to 14 °Brix (Mitchell-Harty et al., 2008).

For the sensory evaluation, all fruits were harvested at their recommended harvest stage. Cultivars 'Galia', 'MG10183', and 'Elario' were harvested at stage FS. 'Red Moon' was harvested at its recommended stage- just when a crack at the abscission layer (or ¼ slip) begins to develop (Mitchell-Harty et al., 2008). SSC and firmness values were also obtained. The evaluation was performed at the University of Florida Food Science and Human Nutrition taste panel laboratory. Panelists consisted of random students, employees and visitors to the UF campus.

The day before the taste panel, fruits were harvested at stage FS for cultivars 'Galia', 'MG10183' and 'Elario'. 'Red Moon' was harvested at the onset of abscission layer development at the stem (the recommended harvest stage for 'Red Moon' according to Mitchell-Harty et al., 2008). Fruits were stored at 20 °C over night and transported to the Food Science laboratory the next morning. For the evaluation, two fruits of each type were selected and sliced into 15 g pieces (on average). Fruits were kept on ice until served to the panelist. In order to continuously serve fresh fruit throughout the day, two new fruits of each cultivar were sliced and served every 1.5 hours.

Fruit from the four cultivars (samples) were each given a unique code and panelists were asked to taste each sample according to the code. Panelists rated the samples on a 9-point hedonic scale (1= dislike extremely, 2= dislike very much, 3= dislike moderately, 4= dislike slightly, 5 = neither like or dislike, 6= like slightly, 7= like

moderately, 8= like very much, 9 = like extremely) for appearance, overall acceptance, sweetness, flavor and firmness. Panelists were also asked to comment on each sample. Water and unsalted crackers were provided to cleanse the palate between each sample. A total of 74 panelists, consiting of random visitors, staff and students, participated in the sensory evaluation.

After the sensory panel was closed, aroma volatiles and SSC were collected on the remaining fruit that was used in the sensory evaluation, after the evaluation was completed. Fruit firmness was measured on remaining fruits.

Statistical Analysis

A randomized complete block experimental design (RCBD) with four replications were used in fall 2006 and spring 2007, and three replications were used in fall 2007. Number of fruits per plot (n) ranged from three to 12 fruits. Data were analyzed using the GLM procedure (SAS Institute, Version 9, Cary, NC, U.S.A.). All data presented were subjected to analysis of variance (ANOVA) and significant treatment means separated by Fisher's least significant difference ($P \leq 0.05$). Data was analyzed within season and harvest stage. Standard error (SE) values were calculated for each ethylene and respiration data point.

Analysis for the melon sensory evaluation was done in a randomized complete block design (RCBD) with 74 panelists as the 'blocks'. Means were separated using Tukey's HSD ($P<0.05$).

Results

Days to Harvest (DTH)

Days to harvest (DTH) for 'Galia' was generally later than the GT cultivars (Tables 4-2, 4-4, 4-6, 4-8). This indicated the speed in growth and development of the GT

cultivars could be a beneficial trait for producers who require a fast-growing or short season crop. DTH in fall 2006 and fall 2007 were 5 to 14 days less as compared to spring 2007 possibly due to greater temperatures compared to spring 2007 (Table 4-10). Also, the shortest DTH occurred during fall 2007, where the greatest average, maximum and minimum temperatures as well as the greatest monthly solar radiation or photosynthetic photon flux (PPF) was recorded of all three seasons (Table 4-10). The increased temperature and light were due to the replacement of the greenhouse roof plastic, which was completed during summer 2007.

Fruit Quality and Aroma Volatiles

Stage ZG

Stage ZG fruit at harvest indicated no differences in fruit quality for fall 2006 fruit (Table 4-2). Spring and fall 2007 fruit had some variation in weight and size, but most quality variables were similar among the cultivars (Table 4-2). All cultivars were generally firm at stage ZG, and in fall 2007, 'Elario' also had the highest ethylene production (Table 4-2).

Fruits harvested at stage ZG exhibited low volatile emissions at harvest for all cultivars, though increases in appeared after the five day storage period (at 20 °C) in spring and fall 2007 (Table 4-3). Of the 19 significant contributor (SC) compounds, there were only 11 to 15 found at harvest and 15 to 16 found after storage at stage ZG. The lowest number of SC compounds, with only Nos. 1-10, 15 (Table 4-1) was observed in fall 2006 at harvest. In spring 2007, there were 14 SC compounds (Nos. 1-4 and 6-15, Table 4-1) while fall 2007 had 15 SC compounds (Nos. 1-12, 16-18; Table 4-1), although compound Nos. 16 and 17 were only present in significant amounts in 'Elario'. Few

differences were seen in the 38 identified volatiles among the cultivars at harvest *(data shown in Appendix B-1, Tables 1 to 3, not needed for manuscript).*

After storage for five days in spring and fall 2007, TIV for stage ZG fruits increased over 200% for 'Galia' and 'MG10183', while 'Elario' had no significant increase in TIV (Table 4-3). TIV for 'Galia' at stage ZG were similar to harvest levels (Table 4-3). 'MG10183' was greatest in TIV after storage in spring 2007, but not in fall 2007, where 'Elario' was greatest in TIV (Table 4-3). The increases in TIV after storage led to differences in 11 of the 38 identified volatile compounds between 'Galia' and 'MG10183' in spring 2007, and differences in four compounds in fall 2007 *(data shown in Appendix B-1, Tables 4 and 5, not needed for manuscript)*. Fruit firmness decreased from harvest in spring and fall 2007, to an average of 19 N and 13 N, respectively.

Throughout the five days storage in spring and fall 2007 for stage ZG fruits, ethylene and respiration were generally low for all cultivars. In spring 2007, ethylene and respiration rates for 'Galia' averaged 0.2 ng kg^{-1} s^{-1} and 7.1 µg CO_2 kg^{-1} s^{-1}, respectively throughout storage. Also in spring 2007, ethylene and respiration rates for 'MG10183' averaged 0.3 ng kg^{-1} s^{-1} and 10.6 µg CO_2 kg^{-1} s^{-1}, respectively, but also included a 69% increase in CO_2 from day one to day two, followed by a decline. In fall 2007, ethylene and respiration rates were higher for both GT cultivars as compared with 'Galia' (Fig. 4-1A). 'Elario' had an increase in ethylene and CO_2 on day two and then declined. 'Galia' and 'MG10183' had a general increases in ethylene and CO_2 throughout storage. The increases in both ethylene and respiration rates for both 'MG10183' and 'Elario' at stage ZG, could represent the climacteric.

Stage ZYG

Fruit quality and aroma at stage ZYG was generally improved as compared with stage ZG as the fruits were in the process of ripening at this stage. Fruit quality factors, such as SSC increased while firmness decreased compared to stage ZG. Ethylene and respiration rates also increased from stage ZG as did aroma volatile emissions and additional differences were seen in the 38 volatiles among the cultivars. The total number of SC compounds at harvest ranged from 14 to 17.

Fruit quality differences at stage ZYG within each season were minimal, variations occurred during fall 2006 and spring 2007 (Table 4-4). 'MG10183' was consistently firmer than 'Galia' in fall 2006 and spring 2007. Differences in TIV were only observed in fall 2007, where 'Elario' was greatest (Table 4-5). 'Elario' was also over 500x greater in the compound methyl 2-methyl butyrate, as compared to the other cultivars.

SC compounds were lowest during fall 2006, with only 14, while spring 2007 and fall 2007 had 15 and 17, respectively. The SC compounds consistent in all cultivars were compound nos. 1-11, 13-14 (Table 4-1), while No. 12 (ethyl isobutyrate) was not an SC in fall 2006 and No. 15 (cis-3-hexenyl acetate) was not an SC in fall 2007. Fall 2007 also had compound No. 17, which was only significant in 'Galia' and Elario' and Nos. 18 and 19 were only significant in 'Elario'.

Among the 38 volatile compounds at harvest, there were only three differences among them in fall 2006 results. In spring and fall 2007, differences among the 38 volatile compounds increased to 16 and 17 among the 38 compounds *(data shown in Appendix B-2, Tables 1 to 3, not needed for manuscript).*

Following the five day storage period in spring and fall 2007, there were no differences in TIV among the cultivars (Table 4-5). There were 15 SC compounds (No's

1-15, Table 4-1) present in all cultivars after storage in both seasons, however in fall 2007 ethyl-3-(methylthio)propionate (No. 17, Table 4-1) was also an SC. During spring 2007, after storage TIV increased for 'MG10183' while 'Galia' was similar to its harvest TIV. Differences among the 38 compounds decreased to six between 'Galia' and 'MG10183' *(data shown in Appendix B-2, Tables 4 to 5, not needed for manuscript).* Fruit quality after storage in spring 2007 was similar to harvest results, except in firmness, which decreased and 'MG10183' remained a firmer fruit (20 N) after the five day storage period compared to 'Galia' (4 N).

After storage five days in fall 2007, TIV increased for all cultivars after storage as compared to harvest TIV. The compound methyl 2-methyl butyrate increased and was similar among all cultivars. Differences among the 38 compounds remained similar to harvest levels with 15 compounds exhibiting variation *(data shown in Appendix B-2, Tables 4 to 5, not needed for manuscript)*. The only difference in fruit quality was again with firmness, as 'Elario' (18 N) was firmer than 'MG10183' (10 N) and 'Galia' (4 N).

During the five day storage period in spring and fall 2007, ethylene and respiration rates and patterns varied. In spring 2007, ethylene rates were averaged 0.6 and 0.7 ng kg^{-1} s^{-1} for 'Galia' and 'MG10183', respectively throughout storage. Whereas during fall 2007, ethylene rates were over 200% greater and averaged 2 ng kg^{-1} s^{-1} for all cultivars throughout storage (Fig. 4-1B). Respiration rates were similar in both seasons, averaging 10 to 12 µg CO_2 kg^{-1} s^{-1} for all cultivars during storage. As discussed earlier, the climacteric for the GT cultivars most likely began during stage ZG. As ethylene and respiration rates were increased at stage ZYG, the climacteric, most likely continued. The greatest ethylene and respiration rates were observed on either day one or two for all

cultivars in both seasons, which was then followed by a continual decline through storage.

Stages HS

When results of stage HS fruit were compared to stage ZYG, it was found that most fruit quality factors and TIV remained the same except for firmness, which continued to decrease and also for TIV during fall 2006, when 'MG10183' TIV increased from stage ZYG at harvest. The total number of SC compounds ranged from 14 to 18 at harvest and were similar to stage ZYG during all seasons, with the exception of fall 2007, where cis-3-hexenyl acetate was also an SC. Differences among the 38 compounds were less compared to stage ZYG, as there were differences in only five of the 38 compounds among the cultivars in fall 2006 and spring 2007, and only four in fall 2007 *(data shown in Appendix B-3, Tables 1 to 3, not needed for manuscript)*. Ethylene rates at stage HS increased from stage ZYG in both spring and fall 2007 whereas respiration rates were similar to stage ZYG.

Fruit quality at stage HS resulted in remarkable differences in SSC and firmness. 'MG1018' was consistently sweeter than 'Galia', while 'Elario' had the lowest SSC in fall 2007. The GT cultivars were also again firmer than 'Galia'. TIV at stage HS were similar among all cultivars except for 'Elario', which was again greatest in TIV at harvest in fall 2007 (Table 4-7). 'Elario' was also greatest again in methyl 2-methyl butyrate at harvest.

After three days storage in spring and fall 2007, TIV was similar among cultivars (Table 4-7). The SC compounds after storage in spring 2007 (stage HS) were similar to those at harvest. SC compounds in fall 2007 after storage were also similar to harvest SC compounds, except for compound No. 18 (isobutyl propionate), which was not a SC in

'Elario' after storage. In spring 2007 there were no differences in the 38 volatile compounds between cultivars, however, in fall 2007 there were differences in 10 compounds after storage *(data shown in Appendix B-3, Tables 4 to 5, not needed for manuscript*). Compared to harvest, TIV remained at similar levels for spring 2007 fruits, while in fall 2007, 'Galia' and 'MG10183' increased over 100% in TIV after storage (Table 4-7). After three days storage for stage HS fruits in spring and fall 2007 the average SSC of all cultivars was 10.1 °Brix and 9.3 °Brix, respectively. Fruits firmness decreased from harvest for all cultivars and the GT cultivars grown in both seasons were firmer than 'Galia'.

Throughout the three day storage period during both seasons, ethylene rates were greatest on day one for 'Galia' and 'MG10183' then declined during storage. In fall 2007, cultivar 'Elario' was greatest in ethylene on day two (Figure 4-1). Respiration rates over the three day storage period were similar in both spring and fall 2007, data from fall 2007 is presented (Figure 4-1C). The general respiration pattern during both seasons illustrated a decrease in CO_2 by the end of the storage period.

Stage FS

By stage FS, fruit quality factors were similar to stage HS, and TIV was greatest at stage FS for only fall 2006 and 2007 'MG10183' fruits. Stage FS TIV was similar to stage HS for all other cultivars. Fruit quality patterns within stage FS were also similar to stage HS as 'MG10183' was sweeter and firmer than 'Galia' (Table 4-8). The number of SC compounds varied among seasons with 15 SC compounds in fall 2006 and spring 2007 and 16 in fall 2007. Differences in the 38 compounds were increased from stage HS and varied from 8, 6 and 10 through fall 2006, spring and fall 2007, respectively *(data shown in Appendix B-4, Tables 1 to 3, not needed for manuscript).* Interestingly, of

the 11 SC compounds in fall 2007, two compounds with a 'green' aromatic scent (cis-6-nonen-1-ol and cis-3-hexenyl acetate) were greatest in both GT cultivars.

TIV at harvest were similar among the cultivars, although 'Elario' still had the greatest amount of methyl 2-methyl butyrate. After the two day storage period, 'Elario' had the greatest TIV in fall 2007 and again, the greatest amount of methyl 2-methyl butyrate (Table 4-9). SC compounds after storage were similar to harvest in both seasons, except for 'Elario', which increased 42% in TIV as compared to harvest TIV. There were differences in only two of the 38 compounds after storage in spring 2007 *(data shown in Appendix B-4, Tables 4 to 5, not needed for manuscript)*. Each fruit quality variable, ethylene and respiration rates prior to or after the two day storage treatment were similar among cultivars in spring 2007.

After storage two days in fall 2007, there were differences in 11 compounds among the cultivars *(data shown in Appendix B-4, Tables 4 to 5, not needed for manuscript)*, and cultivar 'Elario' had the highest values for most of the compounds. Also during fall 2007, differences after the two day storage period were observed in fruit quality where 'MG10183' (11.3 °Brix) was greater in SSC than 'Elario' (8.7 °Brix), but similar to 'Galia' (9.6 °Brix). Also, both 'Elario' (15 N) and 'MG120183' (17 N) were firmer than 'Galia' (9 N). There were no differences in ethylene or respiration rates on day one or two of storage. All cultivars decreased in both ethylene and CO_2 rates from day one (Figure 4-1D). The decreases observed in ethylene and respiration could be indicative of these fruits in their post-climacteric stage.

Sensory analysis

A sensory evaluation was conducted on the 'Galia' and GT muskmelons to further evaluate fruit quality and taste, and compare the panelist results to the fruit quality data. The sensory evaluation, which included 'Galia', both GT cultivars and 'Red Moon' (Figure 4-2) melon as a control, indicated that taste panelists preferred the appearance of 'Red Moon' melon the most (Figure 4-3). Comments by panelists stated 'Red Moon' flesh had "great orange color". Overall acceptability (a general category of how much panelists liked the sample overall) was greatest for 'MG10183'. Both sweetness and flavor were greatest for 'MG10183' and 'Galia', which also were similar in SSC, which was above 11 °Brix (Table 4-11). Firmness, though greatest in Newtons for 'Red Moon' (Table 4-11), was less liked by panelists who rated 'MG10183' greatest followed by 'Red Moon'. 'Galia' was the least firm fruit and was also rated low, probably because of this.

Although SSC was greatest for 'Galia', 'MG10183' and 'Red Moon' (Table 4-11), panelists preferred 'MG10183' the most, followed by 'Galia'. Comments by panelists indicated that 'Galia's reduced firmness contributed to its low overall acceptance. However, the increased firmness of 'Red Moon' also may have contributed to its low acceptance. Comments stated that 'Red Moon' was "too firm". Also, TIV was lowest for 'Red Moon' (Table 4-11) and panelists commented that it had "little flavor". This could indicate that the low TIV of 'Red Moon' was not adequate enough to sustain a great acceptance, even though it had a high SSC. However, a high TIV, as seen in 'Elario', which produced the greatest concentration of volatiles at every stage, except stage FS, did not result in good flavor as observed in the sensory panel. 'Elario' received the lowest score by panelists for flavor and sweetness, and correspondingly, also had the

lowest SSC. Fallik et al. (2001) also reported that higher aroma does not necessarily mean higher consumer preference. In their study, the GT cultivar 'C8' had stronger aroma than cultivar '5080', but contained less sugar and was not preferred as much as '5080' by a taste panel. Therefore, the results from the spring 2008 sensory panel proved that high sugar, such as with 'Red Moon', does not necessarily equate to high consumer acceptance and that both increased aroma as observed in 'Elario' and decreased aroma as observed in 'Red Moon' also does not indicate high consumer acceptance. Firmness is another important issue as illustrated between 'Galia' and 'MG10183' where both cultivars were sweet, but 'MG10183' was accepted over 'Galia' due to 'Galia's texture, which was considered "too soft."

Although no storage test was conducted with 'Red Moon', 'Galia' and the GT cultivars in spring 2008, previous research demonstrated that 'Red Moon' TIV decrease after storage (Mitchell-Harty et al., 2008) while 'Galia' and the GTs, as discussed in spring and fall 2007, generally increased in TIV following storage. This could also be an advantage to 'Galia's consumer acceptance over 'Red Moon' as the aroma of a food has an important influence in people choices (Lewinsohn et al., 2001).

Differences occurred in 23 of the 38 identified compounds among the cultivars in the sensory analysis (Table 4-11). There were 13 SC compounds in all cultivars (Table 4-11). The compounds, isobutyl acetate, propyl acetate and ethyl-3-(methylthio)propionate were not SC compounds in spring 2008 in any cultivar. Also, isobutyl propionate was not an SC in 'Elario' as in other seasons. The lower number of SC compounds during spring 2008 may be due to a slight variation in aroma volatile collection, due to the timing of the sensory analysis. In spring 2008, volatiles were collected on fresh, stage FS

fruit that was used in the sensory evaluation, after the evaluation was completed. This resulted in aroma volatiles being collected later in the day, after the fruit was chopped and remained out while the sensory evaluation was in session. Whereas in the previous seasons, aroma volatile collection was done immediately the day after harvest, with no extensive time elapsing once fruit was chopped. Therefore, these results stressed the importance of timely volatile data collection. Due to the volatile nature of these compounds, variation is often common in modifications to aroma volatile collection (Reguso and Pellmyr, 1998). However, measuring aroma volatiles on fresh fruit gives the closest representation as a consumer would find of what compounds are emitted. Of the SC compounds in spring 2008, benzaldehyde (almond scent), was greatest in 'Red Moon' compared to the other cultivars. 'Red Moon' also emitted high levels of, cis-6-nonen-1-ol (green, melon-pumpkin scent) as did 'Elario'. Isovaleronitrile (oniony, solvent scent) was once again a unique SC to 'Galia'.

Throughout the stages and in the sensory panel, the aroma volatile analysis of the original 'Galia' muskmelon and GT cultivars in this research revealed few distinctions among these cultivars, perhaps since they are derived from a limited germplasm base. TIV was mostly similar among cultivars 'Galia' and 'MG10183' while 'Elario' had greater in TIV at stages ZYG and HS at harvest, and stage FS after storage. The pattern of aroma development generally increased as fruits ripened for all cultivars and continued to increase after storage (Fig. 4-3).

Stage ZG had 12 SC compounds whereas this increased at stages ZYG, HS and FS, where all had at least 14 SC compounds among all cultivars. The compound, isovaleronitrile was a unique SC to 'Galia'. Isovaleronitrile is a nitrile with an oniony or

solvent aroma (Khiari et al., 2003) that is thought to be derived from the amino acids leucine or isoleucine (Tieman et al., 2006). The compound isobutyl propionate was a unique SC to 'Elario'. Isobutyl propionate is a methylpropanoate ester with a fruity or rum-like odor (Smiley and Jackson, 2002). Furthermore, a SC unique to both GT cultivars was benzaldehyde, a compound with an almond aroma (Fischetti, 2000). Benzaldehyde, is also a SC compound found in red-fleshed melons such as 'Red Moon' (Mitchell-Harty et al., 2008).

Of the 19 SC compounds found in the 'Galia' and GT cultivars used in this study, all except isovaleronitrile of these have been reported in muskmelon. The SC compounds, isobutyl acetate, butyl acetate, 2-methylbutyl acetate and hexyl acetate have been previously reported as the most abundant volatile compounds in other GT cultivars (Fallik et al., 2001). Additionally, Obando-Ulloa et al. (2008) reported that the GT cultivar 'Fado' was greatest in propyl acetate, methyl 2-methylbutanoate as well as hexyl acetate, which is similar to what was measured in this study. Kourkoutas et al. (2006) found 'Galia' contained greater levels of the acetate esters isobutyl, butyl, 2-methylbutyl and hexyl acetate, than cantaloupe and honeydew. These compounds were also present in high concentrations in this study. Ethyl-3-(methylthio)propionate, a SC compound found in 'Galia' and 'Elario' only, is a sulfur compound with a fresh, melon-like aroma (Jordán et al., 2001), which may be important to the musky odor of muskmelon. Wyllie and Leach (1992) concluded that sulfur-containing compounds in the aroma volatiles of muskmelon were important and found that the 'Galia' muskmelon used in their study had relatively intense amounts of another sulfur compound, 2-(methylthio)ethyl acetate. Ethyl-3-(methylthio)propionate, though present in this study, may not have been

contributing much to the aroma. The SC compounds with high OVs, were benzyl acetate, ethyl-2-methyl butyrate, ethyl isobutyrate, methyl 2-methyl butyrate, ethyl butyrate, hexyl acetate, 2-methylbutyl acetate and ethyl caproate (Table 4-11).

The outcome of few differences in TIV and individual volatile compounds, especially between 'Galia' and 'MG10183' may be attributed to the minimal difference between these cultivars or also, perhaps the sampling method may have to be altered. More samples as well as pooled samples may account for reduced variation and more significance, revealing a better picture of the true differences. However, volatile collection using fresh samples, as in this study, has probably resulted in a higher degree of true aromatic compounds identified.

Not only are the amounts of individual volatile compounds important, but also the unique combinations of these volatiles that determine the aromatic properties (Thomson, 1987; Lewinsohn, et al., 2001) and add to the overall flavor. Perhaps the few differences observed in this research, such as isovaleronitrile as a SC compound in 'Galia' only and the absence of ethyl-3-(methylthio)propionate as a SC compound in 'MG10183' may be sufficient to account for a subtle difference in flavor and therefore, consumer preference.

Based on this research, the compounds considered to be the most important to the high-quality original 'Galia' muskmelon were benzyl acetate, ethyl-2-methyl butyrate, methyl 2-methyl butyrate, ethyl isobutyrate, 2-methylbutyl acetate, hexyl acetate, ethyl butyrate, ethyl caproate and cis-3-hexenyl acetate due to their high OVs over a three-season average. Additionally, isovaleronitrile and ethyl-3-(methylthio)propionate may also be noteworthy; as isovaleronitrile was only a SC in 'Galia' and ethyl-3-(methylthio)propionate was only a SC in 'Galia' and 'Elario'.

As research continues on aroma, collection methods are improved and more threshold levels are determined, other compounds will be revealed to play a greater role in the aromatic profile of 'Galia' and other muskmelons, which will be useful in attaining an exceptionally high-quality melon. 'Galia' and GT muskmelons improved in overall fruit quality as the fruits ripened. Harvesting fruits prior to the climacteric phase, such as in stage ZG, resulted in increased firmness, but lower aroma. Harvesting at stage ZYG or later resulted in softer flesh, but there was increased aroma as fruits were at their climacteric peak.

As illustrated in the sensory panel, sweetness, texture and aroma were key factors to the overall acceptance. Acceptable muskmelons must be sweet, but they must also be firm- but not too firm (as with 'Red Moon'). And good flavored melons must have sweetness, but also acceptable aroma, which must be at an acceptable level as was observed with fruits where aroma totals that were both high ('Elario') and low (Red Moon') were not favored.

Summary

Galia muskmelon (*Cucumis melo* L. var. *reticulatus* Ser.) is a world renowned cultivar, which has been popular for over 35 years and has generated its own market class of specialty melons called 'Galia'-type (GT) cultivars. The GT muskmelons are firmer that the original 'Galia', but flavor has been compromised in breeding efforts to increase firmness. Since flavor is important to the quality of muskmelons, and includes the organoleptic traits of taste, aroma and texture, it is important to determine factors that characterize a muskmelon with excellent quality, especially 'Galia', which is prized for its sweet, green, aromatic flesh. The high-quality 'Galia' muskmelon and its GT relatives are therefore excellent candidates to study fruit flavor. To determine what has set 'Galia'

flavor apart from GT cultivars, this research focused on aroma, and sought to identify volatiles of the true 'Galia' F_1 hybrid. To evaluate aroma development, fruits were harvested at four stages: 1.) zero-slip, green (ZG); 2.) zero-slip, yellow-green (ZYG); 3.) half-slip (HS); and 4.) full-slip (FS). At each stage, quality factors (size, soluble solids content (SSC), firmness, pH, titratable acidity, and aroma), ethylene and respiration rates were measured. 'Galia' muskmelon results were compared to the GT cultivar, 'MG10183' in fall 2006, spring and fall 2007; and to another GT, 'Elario' in fall 2007. GC/MS and GC/FID verified 38 aroma compounds. Of these, 11 to 19 compounds significantly contributed to the aromatic profile, depending on stage and cultivar. Increases in aroma volatiles were observed as fruits ripened and after storage at 20°C. Total identified volatiles (TIV) were lowest at stage ZG, where ethylene and respiration rates were also lowest. Stage ZG TIV at harvest was similar for all cultivars during every season and fruit quality was also similar. As ethylene and respiration rates increased in stages ZGY, HS and FS, TIV also increased. The most differences in individual volatiles and TIV were seen during stage ZYG. Fruit quality differences were observed in firmness, where GTs were firmer than 'Galia'. In spring 2008, A sensory evaluation was conducted on stage FS fruit at harvest and an additional cantaloupe cultivar, 'Red Moon' which was marketed as the 'Perfect Melon', was included as a control and compared with 'Galia', 'MG10183' and 'Elario'. Although 'Red Moon' had the greatest firmness and had high SSC, taste panel preference was highest for 'MG10183', followed by 'Galia', then 'Red Moon'. The least favorite cultivar was 'Elario'. Based on this research, the compounds considered to be the most important to high-quality 'Galia' muskmelons were benzyl acetate, ethyl-2-methyl butyrate, methyl 2-methyl butyrate, ethyl isobutyrate, 2-

methylbutyl acetate, hexyl acetate, ethyl butyrate, ethyl caproate, cis-3-hexenyl acetate, isovaleronitrile, and ethyl-3-(methylthio)propionate.

Table 4-1. Volatile compounds considered to be significant contributors to the aroma of 'Galia' and GT cultivars, 'MG10183' and 'Elario', fall 2006, spring 2007 and fall 2007.

Ref No.	Volatile compound	Scent	Odor threshold value (OTV) (ppb)	Air/ water[z]	OTV Ref.[y]
1	cis-6-nonen-1-ol	waxy, melon, green, pumpkin	1	water	2, 6
2	ethyl caproate	powerful fruity, pineapple, banana	1	water	6
3	benzyl acetate	sweet, jasmine, apple, pear	0.04; 2-270	air; ?	1,2
4	ethyl propionate	sweet, fruity, ethereal	10	water	6
5	isobutyl acetate	fruity	66	water	6
6	hexyl acetate	fruity, green, pear (apple-like)	2	water	6
7	ethyl butyrate	fruity, pineapple, cognac	1	water	6
8	ethyl-2-methyl butyrate	sharp, sweet, green, apple, fruity	0.1 – 0.3	water	6
9	2-methylbutyl acetate	fruity	5	water	6
10	methyl 2-methyl butyrate	sweet, fruity	0.25	water	6
11	amyl acetate	bananas	7.5; 0.095	?; air	5
12	ethyl isobutyrate	sweet, rubber	0.1	water	6
13	propyl acetate	nail-polish remover	40-700	water	3
14	butyl acetate	fruity	66	water	6
15	cis-3-hexenyl acetate	powerful green, fruity, floral, banana, melon	1.2; 7.8	water	10, 11
16	isovaleronitrile[x]	oniony, solvent, fruity	3.2; 1000	water	8, 9
17	ethyl-3-(methylthio)propionate[w]	fruity, pineapple, tropical	7	water	6
18	benzaldehyde[v]	bitter almond, almond	4.25; 350-3000	water	4, 9
19	isobutyl propionate[u]	rum-like, fruity	20	air	7

[z], OTV as determined in air or water; ? = unknown.[y], 1.) Waldhoff and Spilker. 2005; 2.) Burdock, 2005; 3.) SIS, 2007; 4.) Fischetti, 1994.; 5.) Ladd. Res. 2006; 6.) Leffingwell; 7.) Nagata, 1990 8.) Khiari et al., 2002, 9.) Buttery et al., 1991.[x], Significant contributor in 'Galia' only.[w], Significant contributor in 'Galia' and 'Elario' only. [v], Significant contributor in 'Elario' and 'MG10183' only. [u], Significant contributor in 'Elario' only.

Table 4-2. Stage ZG days to harvest and fruit quality means at harvest of 'Galia' and 'Galia'-type muskmelons.

Cultivar	Days to Harvest			Weight (kg)			Length (mm)			Width (mm)			Flesh thickness (mm)		
	Fa06[z]	Sp07[z]	Fa07[y]	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07
Galia	38	56	28	1.53	1.15	0.91	154	142	128	143	126	118	35.9	29.4	22.9
MG10183	35	39	27	1.31	0.68	0.95	140	109	123	141	110	124	32.5	25.8	25.1
Elario	n/a	n/a	30	n/a	n/a	1.29	n/a	n/a	135	n/a	n/a	141	n/a	n/a	29.5
Significance[z]	*	**		NS	*	^{LSD}0.27	NS	*	^{LSD}10.8	NS	*	^{LSD}14	NS	NS	^{LSD}3.4

Cultivar	Soluble solids content (°Brix)			pH			Total titratable acidity (TTA)			Firmness (N)			Ethylene (ng kg^{-1} s^{-1})			Respiration (µg CO2 kg^{-1} s^{-1})		
	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07
Galia	10.5	9.5	6.3	7.17	6.55	6.15	0.11	0.14	0.14	39.8	46.2	42.0	0.21	0.05	0.2	9.18	4.63	11.7
MG10183	9.5	8.7	8.8	7.12	6.4	6.44	0.10	0.13	0.14	36.2	33.3	40.6	0.58	0.17	0.5	11.5	8.17	8.1
Elario	n/a	n/a	8.0	n/a	n/a	6.21	n/a	n/a	0.13	n/a	n/a	51.0	n/a	n/a	1.2	n/a	n/a	10.7
Significance[z]	NS	NS		NS	NS		NS	NS		NS	*		NS	NS	^{LSD}0.5	NS	NS	

[z], Non-significant (NS) or significant F-test at P≤0.05 (*) and P≤0.01 (**), respectively in fall 2006 and spring 2007; n/a= not applicable, cultivar not grown. [y], Mean separation by Fisher's least significant difference test (P≤0.05) in fall 2007.

Table 4-3. Stage ZG means of total identified volatiles (TIV), measured in ng gFW^{-1} h^{-1}, from 'Galia' and 'Galia'-type muskmelons.

	TIV at Harvest (ng gFW^{-1} h^{-1})			TIV after Storage (ng gFW^{-1} h^{-1})	
Cultivar	Fa06[z]	Sp07[z]	Fa07[y]	Sp07	Fa07
Galia	336	453	145	627	1410
MG10183	553	450	352	2271	1187
Elario	n/a	n/a	903	n/a	1679
Significance[z]	NS	NS		**	^{LSD}267

[z], Non-significant (NS) or significant F-test at P≤0.01 (**), respectively in fall 2006 and spring 2007; n/a= not applicable, cultivar not grown. [y], Mean separation by Fisher's least significant difference test (P≤0.05) in fall 2007.

Table 4-4. Stage ZYG fruit quality means of 'Galia' and 'Galia'-type muskmelons.

Cultivar	Days to Harvest			Weight (kg)			Length (mm)			Width (mm)			Flesh thickness (mm)		
	Fa06[z]	Sp07[z]	Fa07[y]	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07
Galia	38	44	32	1.49	1.00	1.02	151	133	128	143	122	124	31.9	28	23.7
MG10183	35	38	29	1.24	0.65	1.20	135	111	135	136	107	132	30.5	25	28.5
Elario	n/a	n/a	29	n/a	n/a	1.61	n/a	n/a	150	n/a	n/a	148	n/a	n/a	34.8
Significance[z]	*	*	2.6	NS	*		NS	*		NS	*		NS	NS	

Cultivar	Soluble solids content (°Brix)			pH			Total titratable acidity (TTA)			Firmness (N)			Ethylene (ng kg^{-1} s^{-1})			Respiration (µg CO2 kg^{-1} s^{-1})		
	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07
Galia	11.8	9.8	8.7	7.19	6.53	6.45	0.09	0.14	0.15	24.9	9.9	20.8	2.86	1.07	3.40	10.89	9.62	12.9
MG10183	12.5	10.6	8.9	7.35	6.77	6.35	0.10	0.12	0.14	32.1	36.1	35.1	5.31	1.87	3.80	16.61	13.3	11.9
Elario	n/a	n/a	6.8	n/a	n/a	6.44	n/a	n/a	0.12	n/a	n/a	34.9	n/a	n/a	5.70	n/a	n/a	14.1
Significance[z]	NS	NS		NS	*		NS	NS	^{LSD}0.02	**	**		*	NS		NS	**	

[z], Non-significant (NS) or significant F-test at P≤0.05 (*) and P≤0.01 (**), respectively in fall 2006 and spring 2007; n/a= not applicable, cultivar not grown. [y], Mean separation by Fisher's least significant difference test (P≤0.05) in fall 2007.

Table 4-5. Stage ZYG means of total identified volatiles (TIV), measured in ng gFW^{-1} h^{-1}, from 'Galia' and 'Galia'-type muskmelons.

	TIV at Harvest (ng gFW^{-1} h^{-1})			TIV after Storage (ng gFW^{-1} h^{-1})	
Cultivar	Fa06[z]	Sp07[z]	Fa07[y]	Sp07	Fa07
Galia	1646	2616	1387	2103	4877
MG10183	1322	1418	715	2246	4452
Elario	n/a	n/a	4790	n/a	4384
Significance[z]	NS	NS	^{LSD}1870	NS	

[z], Non-significant (NS) F-test at P≤0.05 in fall 2006 and spring 2007; n/a= not applicable, cultivar not grown. [y], Mean separation by Fisher's least significant difference test (P≤0.05) in fall 2007.

Table 4-6. Stage HS fruit quality means of 'Galia' and 'Galia'-type muskmelons.

Cultivar	Days to Harvest			Weight (kg)			Length (mm)			Width (mm)			Flesh thickness (mm)		
	Fa06[z]	Sp07[z]	Fa07[y]	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07
Galia	40	45	33	1.42	1.00	1.15	143	134	136	141	120	125	34.2	30	27.0
MG10183	34	40	31	1.62	0.70	1.38	150	114	143	151	108	139	36.4	25	29.6
Elario	n/a	n/a	31	n/a	n/a	1.86	n/a	n/a	154	n/a	n/a	154	n/a	n/a	35.3
Significance[z]	**	**	^{LSD}1.2	NS	NS	^{LSD}0.6	NS	*		*	*	^{LSD}21.3	NS	NS	

Cultivar	Soluble solids content (°Brix)			pH			Total titratable acidity (TTA)			Firmness (N)			Ethylene (ng kg^{-1} s^{-1})			Respiration (µg CO2 kg^{-1} s^{-1})		
	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07
Galia	12.3	9.8	9.4	7.2	6.73	6.46	0.10	0.13	0.12	21.5	10.9	11.7	2.84	2.48	3.6	12.2	12.3	10.5
MG10183	12.9	11.2	10.8	7.28	6.71	6.63	0.11	0.12	0.13	27.6	27.5	22.8	6.17	3.84	4.4	13.4	13.3	13.0
Elario	n/a	n/a	8.6	n/a	n/a	6.67	n/a	n/a	0.11	n/a	n/a	23.8	n/a	n/a	4.4	n/a	n/a	13.4
Significance[z]	NS	**	^{LSD}1.1	NS	NS		NS	NS	^{LSD}0.02	NS	**	^{LSD}6.5	*	NS		NS	NS	^{LSD}1.5

[z], Non-significant (NS) or significant F-test at P≤0.05 (*) and P≤0.01 (**), respectively in fall 2006 and spring 2007; n/a= not applicable, cultivar not grown. [y], Mean separation by Fisher's least significant difference test (P≤0.05) in fall 2007.

Table 4-7. Stage HS means, measured in ng gFW^{-1} h^{-1}, of total identified volatiles (TIV), from 'Galia' and 'Galia'-type muskmelons.

	TIV at Harvest (ng gFW^{-1} h^{-1})			TIV after Storage (ng gFW^{-1} h^{-1})	
Cultivar	Fa06[z]	Sp07[z]	Fa07[y]	Sp07	Fa07
Galia	1624	1914	1545	2294	3756
MG10183	1656	2384	1007	2395	3208
Elario	n/a	n/a	5702	n/a	4439
Significance[z]	NS	NS	^{LSD}3297	NS	

[z], Non-significant (NS) F-test at P≤0.05 in fall 2006 and spring 2007; n/a= not applicable, cultivar not grown. [y], Mean separation by Fisher's least significant difference test (P≤0.05) in fall 2007.

Table 4-8. Stage FS fruit quality means of 'Galia' and 'Galia'-type muskmelons.

Cultivar	Days to Harvest			Weight (kg)			Length (mm)			Width (mm)			Flesh thickness (mm)		
	Fa06[z]	Sp07[z]	Fa07[y]	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07
Galia	39	46	34	1.68	1.10	1.22	157	138	141	146	123	126	33.2	32	29.9
MG10183	38	43	32	1.44	0.80	1.10	147	119	131	145	112	129	32.9	28	27.9
Elario	n/a	n/a	32	n/a	n/a	1.50	n/a	n/a	144	n/a	n/a	144	n/a	n/a	32.9
Significance[z]	NS	**	[LSD]0.1	NS	**	[LSD]0.2	NS	**	[LSD]4.8	NS	**	[LSD]14.2	NS	NS	

Cultivar	Soluble solids content (°Brix)			pH			Total titratable acidity (TTA)			Firmness (N)			Ethylene (ng kg^{-1} s^{-1})			Respiration (µg CO2 kg^{-1} s^{-1})		
	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07
Galia	11.3	10.4	10	7.23	6.76	6.57	0.1	0.13	0.14	18.1	11.1	11.5	4.97	2.83	3.70	14.1	12.8	12.1
MG10183	13.1	12	11.2	7.26	6.76	6.61	0.09	0.11	0.13	24.1	22.7	20.4	5.76	3.34	5.10	13.6	12.7	10.8
Elario	n/a	n/a	9	n/a	n/a	6.83	n/a	n/a	0.09	n/a	n/a	19	n/a	n/a	3.90	n/a	n/a	11.8
Significance[z]	*	*	[LSD]1.3	NS	NS		NS	NS	[LSD]0.03	NS	**	[LSD]6.8	NS	NS		NS	NS	

[z], Non-significant (NS) or significant F-test at P≤0.05 (*) and P≤0.01 (**), respectively in fall 2006 and spring 2007; n/a= not applicable, cultivar not grown. [y], Mean separation by Fisher's least significant difference test (P≤0.05) in fall 2007.

Table 4-9. Stage FS means of total identified volatiles (TIV), measured in ng gFW^{-1} h^{-1}, from 'Galia' and 'Galia'-type muskmelons.

	TIV at Harvest (ng gFW^{-1} h^{-1})			TIV after Storage (ng gFW^{-1} h^{-1})	
Cultivar	Fa06[z]	Sp07[z]	Fa07[y]	Sp07	Fa07
Galia	2190	1034	1616	2330	2645
MG10183	2446	1464	1710	2828	2981
Elario	n/a	n/a	2700	n/a	3822
Significance[z]	NS	NS		NS	[LSD]866

[z], Non-significant (NS) F-test at P≤0.05 in fall 2006 and spring 2007; n/a= not applicable, cultivar not grown. [y], Mean separation by Fisher's least significant difference test (P≤0.05) in fall 2007.

Table 4-10. Temperatures and photosynthetic photon flux (*PPF*) during fall 2006, spring and fall 2007 of 'Galia' and 'Galia'-type muskmelons grown in a passively-ventilated greenhouse.

Production month	Average Temp. (°C)	Max. Temp. (°C)	Min. Temp. (°C)	Avg. Daily *PPF* (µmol m^{-2} s^{-1})	Max. Daily *PPF* (µmol m^{-2} s^{-1})
Aug. 2006	29.6	45.3	23.2	25.2	99.5
Sept. 2006	30.9	49.6	15.9	26.9	90.0
Oct. 2006	24.6	48.2	9.3	25.5	85.2
Feb/Mar. 2007	25.1	32.4	9.1	40.3	86.8
Apr. 2007	24.0	46.0	10.4	42.7	152
May. 2007	26.2	44.2	12.0	37.9	104
Jun. 2007	29.4	50.7	17.9	28.7	89.9
Aug. 2007	34.3	52.0	22.0	42.3	1937
Sept. 2007	29.6	54.0	20.4	308	1937
Oct. 2007	28.0	44.8	12.9	127	867

Table 4-11. Stage FS means of soluble solids content (SSC, °Brix), firmness (N) and aroma (ng gFW^{-1} h^{-1}) of 'Galia', 'Galia'-type and 'Red Moon' melons, spring 2008.

Fruit quality	Galia	MG10183	Elario	Red Moon	LSD(0.05)[Z]
Soluble solids content (°Brix)	11.3	11.5	8.5	11.7	0.91
Firmness (N)	10.0	24.6	17.9	41.1	4.16
Aroma Compound	Galia	MG10183	Elario	Red Moon	LSD(0.05)[Z]
propyl butyrate	2.04	2.42	2.74	0.63	
tiglic aldehyde	0.31	1.51	2.14	0.58	0.67
4-methyl-1-cyclohexene	0.07	0.07	0.02	0.04	
cis-3-hexen-1-ol	0.85	1.90	2.01	0.50	0.80
trans-cyclodecene	0.08	0.16	0.50	0.50	0.10
cinnamyl acetate	0.13	0.34	0.42	0.24	0.19
2-methyl-1-butanol	14.7	3.72	3.98	1.01	2.82
furfuryl acetate	0.49	0.50	0.53	0.23	
methyl isobutyrate	3.60	2.91	3.15	8.89	4.26
allyl methyl sulfide	0.67	0.08	0.47	0.39	
butyl propionate	2.38	1.08	1.21	0.38	1.10
cyclooctene	0.70	0.68	1.07	1.13	
isobutyl butyrate	3.32	2.49	2.14	0.81	0.94
Benzaldehyde* [GTs and R]	1.90	4.46	4.56	7.15	2.41
3-phenylpropylacetate	2.30	9.71	5.40	1.60	4.27
methyl caprylate	11.5	4.07	6.59	6.35	
methyl caproate	5.26	6.90	7.83	9.44	
isobutyl propionate	8.09	5.08	7.89	4.89	2.42
ethyl-3-(methylthio)propionate	1.76	2.29	2.84	0.19	0.74
heptyl acetate	8.80	27.6	25.8	1.62	7.23
phenethyl acetate	3.36	6.02	6.68	18.6	2.56
amyl acetate*[G and GTs]	19.6	36.6	32.7	6.3.0	13.6
methyl butyrate	20.4	9.90	15.6	13.0	
cis-6-nonen-1-ol*	24.4	98.5	117.0	172.5	57.6
Isovaleronitrile*[G]	14.1	0.07	0.08	0.08	5.27
ethyl caproate*	13.8	44.2	78.5	14.1	20.6
cis-3-hexenyl acetate	32.9	15.5	74.1	22.0	38.7
benzyl acetate*	0.70	1.2	32.4	59.6	
ethyl propionate*	21.4	66.9	77.6	30.6	
ethyl isobutyrate*	0.26	78.7	65.0	0.36	21.4
isobutyl acetate	32.4	10.8	21.9	9.33	9.66
propyl acetate	6.16	2.84	6.50	2.62	
hexyl acetate*	24.2	18.0	27.0	7.76	
ethyl butyrate*	39.1	8.00	12.4	28.7	17.3
butyl acetate*[R]	47.9	39.2	35.3	78.5	39.2
ethyl-2-methyl butyrate*	12.9	55.7	67.1	15.6	
2-methylbutyl acetate*	138.1	64.0	96.9	33.2	
methyl 2-methyl butyrate*	1636	1242	1581	11.7	407
Total Volatiles	1884	1877	2450	571	482
*Signif. Contributors	1726	1735	2224	472	489

[z], Mean separation by Fisher's least significant difference test ($P=0.05$) . Units= ng gFW^{-1} h^{-1}.*, denotes a significant contributor to the aroma. [G], significant contributor to 'Galia' only. [GT], significant contributor to 'Galia'-types only. [R], significant contributor to 'Red Moon' only.

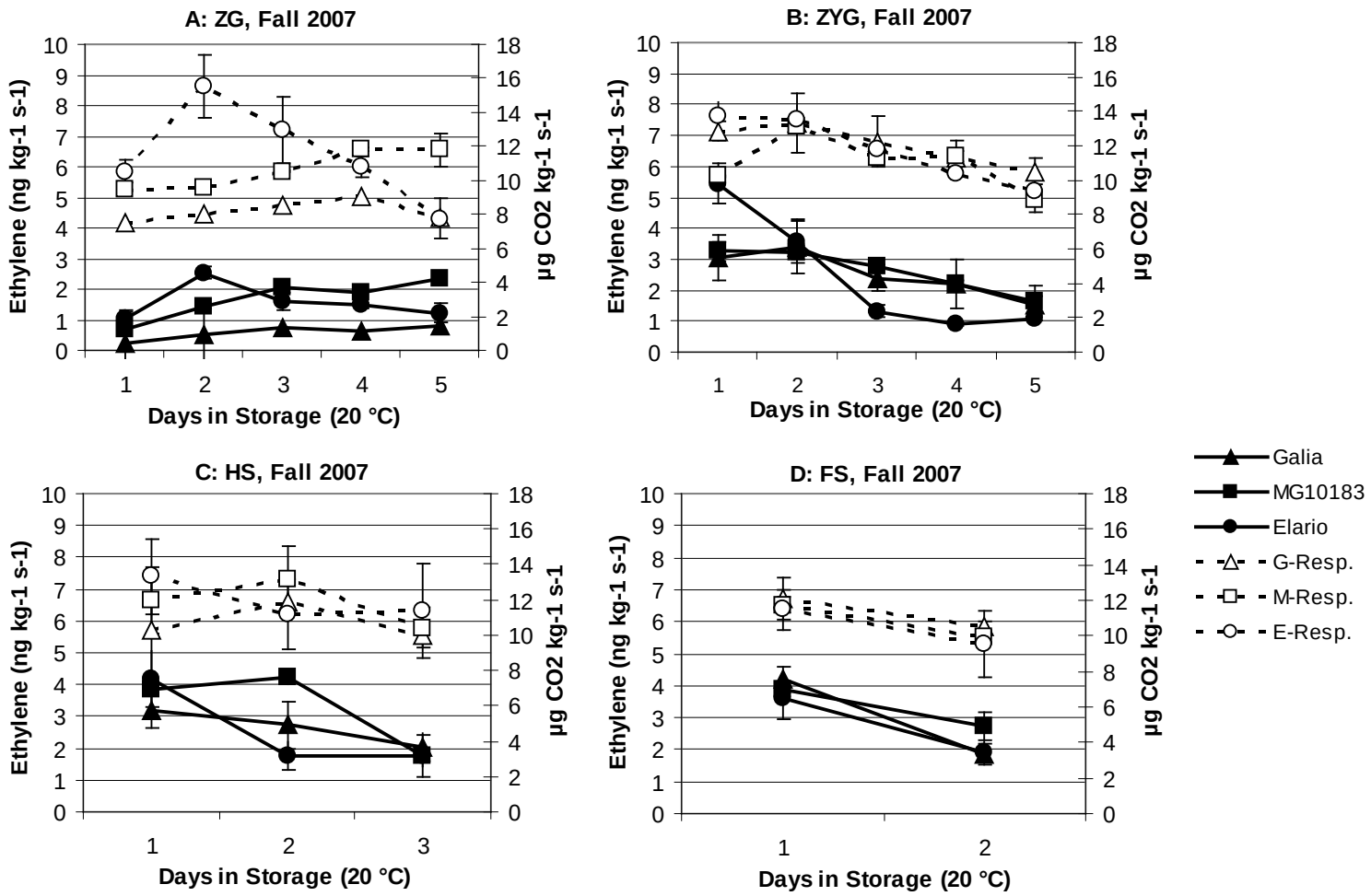

Figure 4-1. Ethylene and respiration rates during storage at 20 °C , harvested at stages ZG, ZYG, HS and FS for 'Galia' and GT muskmelons, fall 2007.

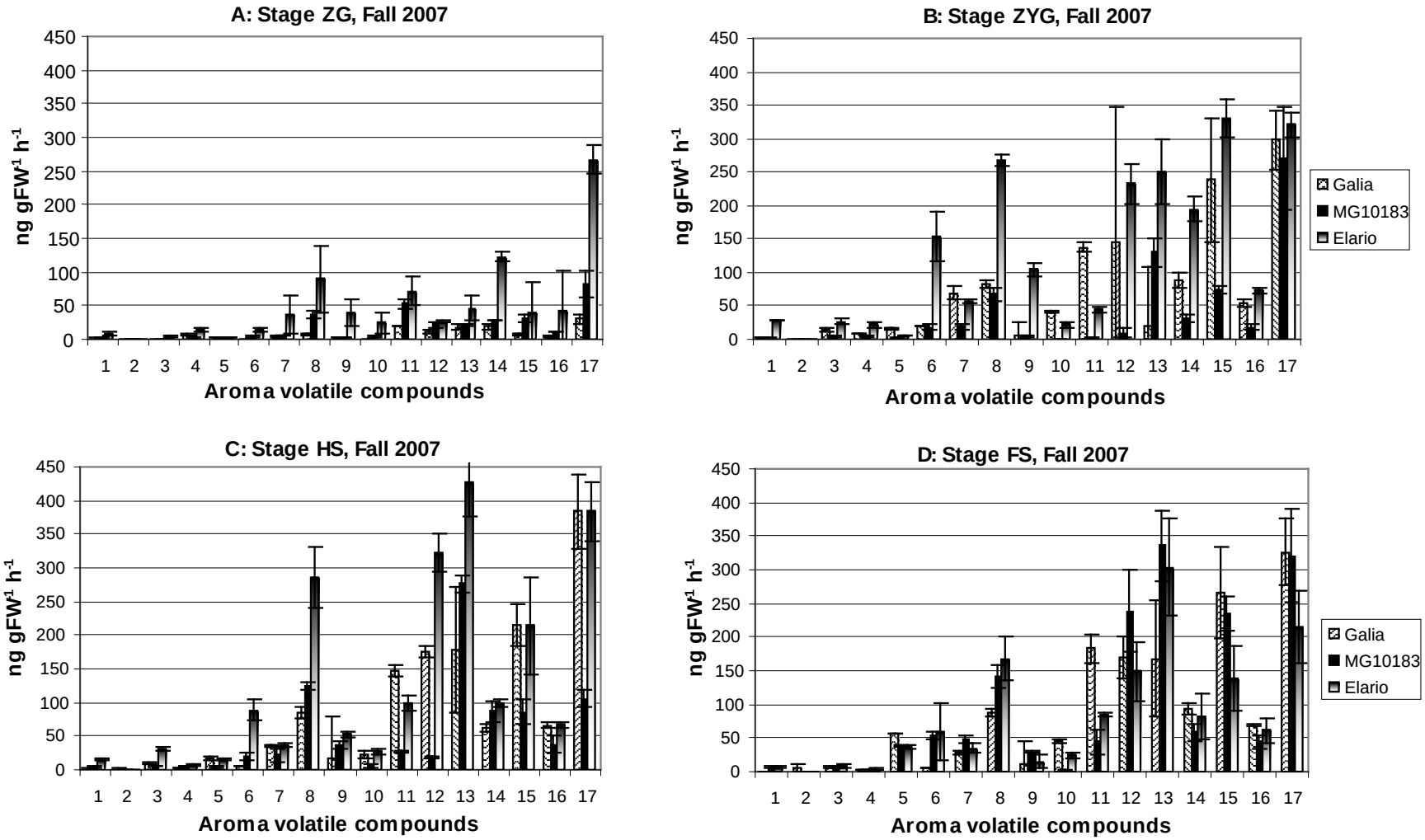

Figure 4-2. Aroma volatile emissions of 17 SC compounds found in 'Galia', 'MG10183' and 'Elario', fall 2007, (Number (n) of fruits ranged from 1 to 12). 1= benzaldehyde, 2= isovaleronitrile, 3= isobutyl propionate, 4= ethyl-3-(methylthio)propionate, 5= amyl acetate, 6= cis-6-nonen-1-ol, 7= ethyl caproate, 8= benzyl acetate, 9= ethyl propionate, 10= ethyl isobutyrate, 11= isobutyl acetate, 12 = propyl acetate, 13= hexyl acetate, 14= ethyl butyrate, 15= butyl acetate, 16= ethyl-2-methyl butyrate, 17= 2-methylbutyl acetate.

Figure 4-3. 'Galia', 'MG10183', 'Elario' and 'Red Moon' melons (*Cucumis melo* L.)used in the sensory panel, spring 2008.

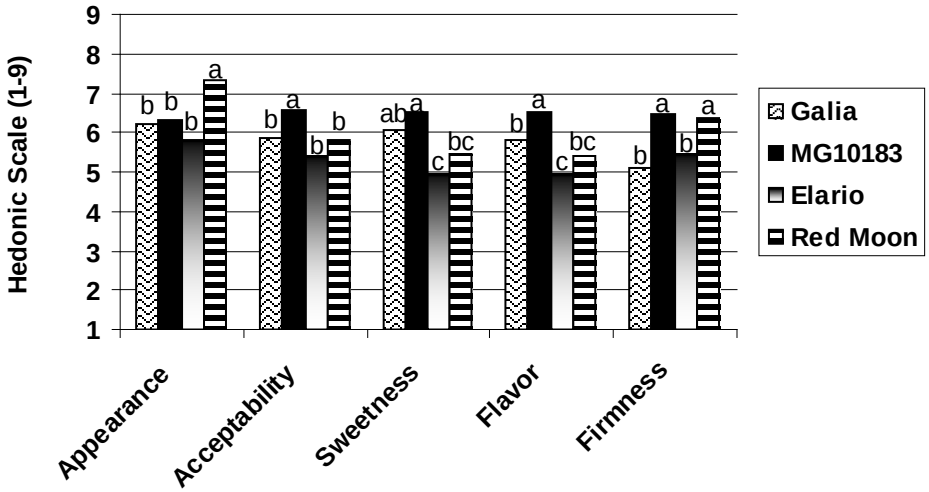

Figure 4-4. Sensory evaluation results from fruit harvested at the recommended stage, FS for 'Galia', 'MG10183' and 'Elario'; and the recommended harvest stage for 'Red Moon', which is when a crack begins at the abscission layer, spring 2008.

CHAPTER 5
AROMA VOLATILE AND FRUIT QUALITY EVALUATION OF ANTISENSE ACC-OXIDASE (CMACO-1) GALIA F_1 HYBRID MUSKMELONS (*Cucumis melo* L. var. *reticulatus* Ser.)

'Galia' muskmelon, a high quality specialty melon that features a characteristic golden netted exterior and a sweet, green flesh, is renowned for its flavor (Aharoni et al., 1992; Karchi, 2000). In order to achieve the distinct flavor of 'Galia', it is best eaten when harvested at the fully ripe or full-slip stage (Karchi, 1979), thus its shelf-life is limited. Due to this limitation, 'Galia'- type (GT) cultivars have been bred, which are firmer than the original 'Galia' muskmelon (Mitchell et al., 2007a). Although GT cultivars may have a longer shelf-life than true 'Galia', many lack the high soluble solids content (SSC) and unique aroma of the original hybrid (Mitchell et al., 2007a; Cantliffe et al., 2001).

Additionally, several methods have been used to extend the postharvest shelf-life of 'Galia' and GT muskmelons. They can be harvested early, at a green, pre-slip or half-slip stage when fruits are firmer, but this often results in poor sweetness and flavor (Fallik et al., 2001; Cantliffe and Shaw, 2002; Pratt. 1971). Muskmelons can also be stored at low temperatures (2.5 to 5 ºC) to maintain firmness (Asghary et al., 2005) or rinsed with hot water to reduce both fruit softening and decay development (Fallik et al., 2000; Lalaguna, 1998; Teitel et al, 1989). 'Galia' muskmelons subjected to < 1.0 kGy of irradiation combined with a hot-water dip protected against decay and did not affect fruit quality (Lalaguna, 1998). Waxes have also been used to maintain internal and external melon fruit quality (Fallik et al., 2005; Aharoni et al., 1992). Sodium bicarbonate has been reported to reduce decay as well as maintain firmness (Aharoni et al., 1997). Moreover, a combination of hot water and a wax treatment with sodium bicarbonate may also be used to reduce decay and increase fruit quality (Illić and Fallik, 2007). Other postharvest treatments of GT muskmelons have included applications of hydrogen peroxide or

hinokitiol hinokitiol (β-thujaplicin, a chelating agent that inhibits microbial enzymes) treatments (Aharoni et al., 1994; Aharoni et al., 1993). Storage of 'Galia' muskmelons in a controlled atmosphere of 10% CO_2 and 10% O_2 both with and without an ethylene absorbent decreased fruit softening and decay (Aharoni et al., 1993). The use of 1-methylcyclopropene (1-MCP), an ethylene action inhibitor, suppressed softening of 'Galia' muskmelons at both green and yellow maturity stages (Ergun et al., 2006).

Although these methods can be effective, many result in an extra step that producers and distributors would most likely choose to avoid. Therefore, instead of further complicating the postharvest handling process, another means could be to modify the ripening pattern of the fruit itself. To do this, it is necessary to understand key features of the fruit such as the ethylene biosynthesis and its effect on fruit quality.

The 'Galia' muskmelon is a climacteric fruit and has a burst of respiration concurrent with an autocatalytic production of ethylene (Abeles et al., 1992; Seymour and McGlasson, 1993). This is a defining feature of ripening in fruits such as melons (Bower et al., 2002) and causes the fruit to ripen, abscise and soften very rapidly in most cases (Abeles et al., 1992). This has attributed to the short shelf-life of 'Galia' muskmelon. 'Galia' are best when harvested fully ripe, when the climacteric peak and abscission occur (Cantliffe and Shaw, 2002; Mitchell et al., 2007c). Therefore, knowledge about the ethylene biosynthetic pathway is important since it is essential to the fruit ripening process (Seymour and McGlasson, 1993).

Ethylene biosynthesis follows the pathway from methionine via *S*-adenosylmethionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC). The enzyme responsible for catalyzing the conversion of SAM to ACC, is ACC synthase (ACS) and the enzyme catalyzing ACC to ethylene is ACC oxidase (ACO) (Yang and Hoffman, 1984). However, the last step in

the ethylene biosynthetic pathway can be inhibited using antisense technology and has been accomplished in tomato (*Lycopersicon esculentum*) (Hamilton et al., 1990), 'Charentais' and 'Vedrantais' cantaloupes (Ayub et al., 1996; Guis et al, 1997 and 2000; Silva et al., 2004) and plums (*Prunus domestica* L.) (Callahan and Scorza, 2007). Furthermore, work by Nuñez-Palenius et al. (2006a) transformed the male parental line of 'Galia' muskmelon (cv. 'Krymka') with an antisense ACC-oxidase gene (CMACO-1). Antisense 'Krymka' fruits produced less ethylene and were firmer than wild-type (WT) fruits, yet soluble solids content (SSC) was similar to WT fruits (Nuñez-Palenius et al., 2006b).

Nuñez-Palenius et al. (2006a and 2006b) research provided an essential step towards the goal of improving the shelf-life, while maintaining the high quality and flavor of the original 'Galia' muskmelon. Nuñez-Palenius (2006a and 2006b) determined that fruit quality factors such as SSC, TTA and pH were unaffected by the transgene and the ethylene inhibition by the antisense (CMACO-1) gene resulted in a firmer muskmelon. From that work, antisense ACC-oxidase (CMACO-1) 'Galia' (ASG) hybrid muskmelons were developed (Mitchell et al., 2007b). Although ASG muskmelons are firmer than 'Galia' and have similar SSC, aroma volatile production associated with the ASG muskmelons has not yet been studied. This is important as changes in aroma also occur during ripening and can affect fruit quality (Wang et al., 1996).

The objective of this research was to determine whether or not adding an antisense ACC-oxidase (CMACO-1) gene to the parental lines of 'Galia' muskmelon give use to a longer shelf-life 'Galia' that retained all of the outstanding fruit quality characteristics.

Materials and Methods

Three experiments were conducted during fall 2006, spring and fall 2007. Seeds of the original 'Galia' (Hazera Genetics, Israel) and antisense 'Galia' (ASG) lines (ASxAS, ASxWT and WTxAS) were planted on 7 July 2006, 19 Jan. 2007 and 31 July 2007. ASG hybrid lines

were produced from the 'Galia' male parental line (cv. 'Krymka') that was previously transformed with an antisense ACC-oxidase gene (CMACO-1) by Nuñez-Palenius et al. (2006a). Lines of antisense (AS) male parents were selfed and selected for the delayed ripening phenotype and a backcross 4 (AS BC4) female population was also produced. The AS T_4 male and AS BC_4 lines were crossed (ASxWT, WTxAS, ASxAS) and AS 'Galia' F_1 hybrid seed were produced in spring 2006 (Mitchell et al., 2007b).

Seedlings were produced at the University of Florida, Gainesville, FL campus according to Mitchell-Harty et al. (2008). Seedlings were grown in a Conviron plant growth chamber (Controlled Env. Ltd.,Winnipeg, Manitoba, Canada) and fertilized once per week (after expansion of first true leaf) with Peters Professional All Purpose Plant Food (Spectrum Group, St. Louis, MO) at the rate according to Mitchell et al. (2007a). When seedlings had one true leaf, a polymerase chain reaction (PCR) analysis was used to identify the antisense seedlings with the transgene. DNA was extracted from a 1.5 cm sample that was sliced from the youngest leaf tissue of each seedling. The DNA extraction method used was the 'Shorty' buffer procedure for DNA isolation, from the University of Florida, Hansen Lab, Nucleic Acid Isolation website (http://www.hos.ufl.edu/meteng/HansonWebpagecontents/NucleicAcidIsolation.html). The PCR reaction was conducted in a DNA Thermal Cycler 480 (Applied Biosystems, Foster City, CA, U.S.A.) according to the parameters of Nuñez -Palenius et al. (2006a). Also according to Nuñez -Palenius et al. (2006a), electrophoresis of the amplified PCR products was done on a 1% agarose gel and viewed by ultraviolet (UV) light. PCR analysis was completed on every putative transgenic 'Galia' seedling.

After transgenic seedlings were identified and all seedlings had three true leaves, they were transplanted on the 3 Aug. 2006, 27 Feb. and 15 Aug. 2007. All three trials were

conducted in a saw-tooth style, passively-ventilated greenhouse (TOP greenhouses, Ltd., Barkan, Israel), located at the University of Florida, Plant Science Research Education Unit located in Citra, FL. The plants were grown using commercial greenhouse muskmelon production techniques and nutrient requirements according to the recommendations of Shaw et al. (2001). Pollination was achieved via bumble bees from Class A research hives (*Bombus impatiens*, Natupol, Koppert Biological Systems, Inc., Romulus, MI). All flowers were tagged with the date of anthesis. Temperature and photosynthetic photon flux (PPF) at the canopy level were recorded daily at 30-min. intervals by HOBO data loggers (Onset Comp. Corp., Bourne, MA). Within canopy temperatures were also recorded at 15 minute intervals by WatchDog data loggers (Spectrum Tech., Plainfield, IL). The monthly temperature averages were taken as an average of the 'within' and 'at' canopy readings.

Insect pests and diseases were monitored weekly by scouting one plant from each plot per block. An integrated pest management (IPM) approach, which included the use of biological control and sprays, was used for management of arthropod pests in all three seasons and released at the rates according to Mitchell-Harty et al. (2008).

Fruit Selection and Postharvest Treatments

Fruits were harvested consistent with the methods Mitchell-Harty et al. (2008) from 29 Sept. to 30 Oct. 2006; 10 May to 18 June 2007 and 2 Oct. to 26 Oct. 2007 at four different stages of ripening. Stage: 1.) zero-slip green (ZG): external skin still green in color with no abscission layer development; 2.) zero-slip, yellow-green (ZYG): external skin green and yellow, with no abscission layer development; 3.) half-slip (HS): fruit abscising half-way; and 4.) full-slip (FS): fruit separates easily from the stem. During each harvest period, fruits were harvested each day, in the afternoon, and fruit weight and size (length and width) were recorded immediately.

The fruit was transported to campus and separated into two groups- harvest and storage. In the harvest group, all postharvest variables were measured 12 hours after harvest. In the storage group, fruits were stored at 20 °C and 85% relative humidity (RH). Storage days varied for the different stage fruits. This was done to be able to track the climacteric phase (ethylene and respiration rates were measured daily from each fruit while in storage) and collect fruit quality data at an appropriate edible time. Stages ZG and ZYG were stored for five days, stage HS was stored for three days, and stage FS fruit was stored for two days. Only fruits harvested during spring and fall 2007 were subjected to a storage treatment. However, due to limited fruit number, line WTxAS was not included in the storage treatment.

Ethylene and Respiration Measurements

Ethylene and respiration measurements were measured on whole fruits from both H and S treatments consistent with methods and equipment as described by Mitchell-Harty et al. (2008). After fruit were harvested, they were transported to Gainesville and stored at 20 °C and 85% RH. The next morning, 12 hours after harvest, ethylene and respiration rates were measured from all fruits.

Fruit Quality Measurements

Fruit quality variables, which included firmness, SSC and flesh thickness were measured on fresh fruit according to Mitchell et al. (2007a) directly following storage for all treatments. Pulp firmness, SSC and flesh thickness data were recorded from a 2.5 cm slice, taken from the equatorial region of each fruit. These measurements were done using an Instron, refractometer and caliper. Pulp firmness was determined at two equidistant points on the equatorial region of each fruit slice using the Instron Universal Testing Instrument (Model 4411-C8009, Canton, MA), which was fitted with a 50 kg load cell and an 11 mm convex probe with a crosshead speed of 50 mm min^{-1}. SSC (°Brix) was measured with a temperature-compensating, handheld

refractometer (Model 10430, Reichert Scientific Instrument, Buffalo, NY) from fresh juice expressed from two pulp samples and a caliper (Digimatic Mycal, Mitutoyo, Japan) was used to measure flesh thickness from peel to cavity.

The remaining pulp from the 2.5 cm slice was used for aroma volatile collection and a quantity was frozen for Total Titratable Acidity (TTA) and pH measurements. Aroma volatile collection, TTA and pH measurements were all performed in accordance with methods and equipment as stated by Mitchell-Harty et al. (2008). In brief, methods for aroma volatile collections were performed according to Schmelze et al. (2003) and Tieman et al. (2006) with nonyl acetate as an internal standard. Aroma volatiles were collected from 100 g of fresh, chopped mesocarp pulp from each fruit that was inserted into Simex glass tubes (28 x 1.5 x 610 mm; Pegasus Glass). Air, via a vacuum pump, filtered through a hydrocarbon trap (Agilent Technologies, Palo Alto, CA), flowed through the tubes for one hour at 618 ml min^{-1}. Volatiles were collected on a Super Q column (30 mg Altech® resin) and eluted with methylene chloride. Volatiles were separated on an Agilent Technologies DB-5 column (length x diam.: 30 x 0.25 mm) and analyzed on an Agilent Technologies 6890N (7683 Series) Gas Chromatograph (GC) (Agilent Tech., Inc., Santa Clara, CA). The GC was equipped with a flame ionization detector (FID). Retention times were compared with known standards and quantified with Agilent ChemStation software. Volatile peak identities were confirmed by an Agilent Technologies 5975 Gas Chromatograph/Mass Spectrometer (GC/MS).

As a result of GC/MS, 38 volatile compounds were identified and standards were procured. Of the 38 compounds, 10 to 17 were considered to be significant contributors to the aroma, depending on stage and season (Table 5-1). Significant contributors to aroma were identified as a result of dividing the concentration of the compound (determined with GC/FID)

by its known odor threshold value (OTV), resulting in the odor value (OV) of the compound (Bauchot et al., 1998; Berger, 1995; Buttery 1993; Teranishi et al., 1991). Compounds with OVs greater than one were considered to be SCs to the aroma (Bauchot et al., 1998). Odor threshold values (OTVs) were obtained through a literature search (Table 5-1).

Statistical Analysis

All trials were conducted in a randomized complete block design (RCBD) with three replications. Number of fruits per treatment (n) ranged from three to 12 fruits. Data were analyzed using the GLM procedure (SAS Institute, Version 9, Cary, NC, U.S.A.). All data presented were subject to Fisher's least significant difference ($P \leq 0.05$). Data were separated into the two groups, harvest and storage, and analyzed by stage. A split-block experiment design with season as the main block and line as the split block were used for the combined analysis. An analysis of variance (ANOVA) was conducted using SAS (SAS Institute, Version 9, Cary, NC, U.S.A.).

Results and Discussion

Days to Harvest

At stage ZG, only line ASxAS was harvested later than 'Galia', all other lines were harvested at similar times. By stages ZYG and HS, all ASG lines were harvested later than 'Galia' (Tables 5-2, 5-3, 5-5). For stage FS fruit, there was a significant line x season interaction (Table 5-8). Although stage FS DTH varied among the seasons, generally, ASG lines remained on the vine longer than 'Galia', which is a significant feature of the delayed ripening characteristic. The delayed ripening and development of the abscission zone of the ASG fruit was also reported in antisense (AS) 'Krymka' muskmelons (Nuñez-Palenius et al., 2006b). The AS 'Krymka' from Nuñez-Palenius's research were used to develop these ASG lines (Harty et al., 2009). Throughout the three seasons, ASG lines remained on the vine an average of four days

longer than ‘Galia’. However, this also depended on season and/or environmental conditions. Environmental influences commonly affect melon production and days to harvest (Bower et al., 2002). Generally, muskmelon growth was at an increased rate in the fall seasons, most likely due to higher temperatures at the onset of fruit set and development.

In fall 2007, fruits matured much earlier due to higher temperatures and solar radiation as compared with fall 2006 and spring 2007 (Table 5-8; Fig. 5-1). The increased temperature and light were due to the replacement of the greenhouse roof plastic, which was completed during summer 2007. Conversely, the growth rate of the spring 2007 crop was decreased due to cooler temperatures at the onset of production and also to extended cloud cover during May due to wild fire smoke. During the spring, vast brush fires spread through Florida resulting in dramatic smoke levels near the greenhouse.

Fruit Quality and Aroma

Stage ZG

Within the harvest treatment, there was no line x season interaction for any fruit quality factors or TIV at stage ZG over the three seasons (Tables 5-2 and 5-9). Fruit quality at stage ZG indicated few differences among lines, although ‘Galia’ was a larger fruit. SSC was frequently lower for line WTxAS and fruit size also varied, but this could also be due to sampling variation. Fruits were firmest and SSC was lowest at stage ZG, which was analogous to fruit quality of other GT cultivars harvested at a green stage (Fallik et al., 2001). Ethylene and respiration rates were also lowest at this stage, demonstrating that stage ZG fruits were pre-climacteric as no significant increases in either gas occurred throughout the storage treatments.

All lines had identical significant contributor (SC) compounds, which generally consisted of at least 90% of the TIV, both at harvest and after storage at each stage. Aroma volatile production was lowest at stage ZG as compared with the other stages and the three season

average resulted in only 11 SC compounds at harvest, stage ZG. The compounds that were not contributing significantly (amyl acetate, ethyl isobutyrate, propyl acetate, butyl acetate, ethyl-3-(methylthio)propionate and isovaleronitrile) were mostly esters with a fruity aroma. Other research has also reported lower amounts of aroma volatile compounds in immature cantaloupes as compared with mature fruits (Beaulieu, 2006; Beaulieu and Grimm, 2001; Wyllie et al., 1996b). There were only two compounds with differences among the 38 volatiles from all lines: methyl isobutyrate was greatest in 'Galia' and cis-3-hexenyl acetate was greatest in line WTxAS.

For stage ZG fruits that were stored for five days in spring and fall 2007 there were no differences in any postharvest variables among the lines (data not shown). There was a seasonal difference for SSC only, where spring 2007 fruits were sweeter (8 °Brix) than fall 2007 (6.2 °Brix) fruits. There was a line x season interaction for firmness and ethylene production. For 'Galia' and line ASxWT, fruits were firmer overall (15 N in spring 2007 and 8 N in fall 2007) and produced less ethylene (0.48 ng kg^{-1} s^{-1}, spring 2007 and 1.75 ng kg^{-1} s^{-1}, fall 2007 throughout storage) in spring 2007. Whereas line ASxAS fruits had similar firmness (9 N) and ethylene (0.5 ng kg^{-1} s^{-1}) production in both seasons. Fruit firmness decreased after storage as compared with harvest levels in both seasons. The average respiration rate was 6.5 µg CO_2 kg^{-1} s^{-1} throughout storage for all lines, and no significant increases in ethylene or respiration occurred throughout the storage treatments (data not shown).

There was also a line x season interaction for TIV after storage (Table 5-10). After the five day storage treatment in fall 2007, 'Galia' and line ASxWT had the greatest TIV and were significantly greater than their TIV at harvest (Table 5-10). Fallik et al. (2001) also observed an increase in aroma after storage of GT fruits harvested at a green stage. This did not happen for line ASxAS after storage, where TIV for line ASxAS was similar to its TIV at harvest. The total

number of SC compounds increased as compared with those at harvest. There were 16 SC compounds for all lines in spring 2007, which included all SC compounds except isovaleronitrile and these consisted of over 90% of the TIV after storage at stage ZG. In fall 2007, all lines had 15 SCs. Isovaleronitrile and cis-3-hexenyl acetate were not considered SCs compound at this time.

Stage ZYG

Within the harvest treatment, fruit weight, size and SSC were similar among all lines. All ASG lines were firmer than 'Galia' (Table 5-3). Compared to stage ZG, firmness decreased for all lines. The rate of fruit softening from stage ZG was greatest for 'Galia' with a 53% decrease in firmness; while all ASG lines averaged a 31% overall decrease in firmness. Seasonal differences occurred for every variable, though fall 2006, generally had the largest and sweetest fruits; while fruits were firmest in both fall seasons.

There was a line x season interaction for TTA, pH, ethylene and respiration rates as well as TIV at stage ZYG (Tables 5-3, 5-4 and 5-9). TTA and pH values were inconsistent throughout the seasons, but ASG fruits generally had a lower TTA and higher pH than 'Galia'. TIV increased for all lines from stage ZG, as did SSC (Tables 5-3, 5-4 and 5-9). Ethylene and respiration rates at stage ZYG, over the three seasons increased for all lines as compared with stage ZG (Table 5-4). However, ethylene rates for 'Galia' at stage ZYG increased over 1500% from stage ZG- double when compared to ASG lines, which had an average increase of 660%. The most differences in the volatile compounds among the lines were measured at stage ZYG, with differences in 20 of the 38 volatiles (Table 5-11). This may be attributed to the increases in ethylene and respiration rates as the fruits ripened. Other climacteric melons, such as the Charentais also exhibit an increase in aroma as they begin to ripen (Liu et al., 2004). However, as 'Galia' had greater amounts of the majority of the significant volatiles as compared with lines

ASxAS and ASxWT, it appeared to be more aromatic. Bauchot et al. (1998 and 1999) reported that antisense ACO Charentais ('Vedrantais') cantaloupes were less aromatic than the wild-type fruits with some esters reduced by 90% and total volatiles 60% to 85% lower. In this study, 'Galia' had greater amounts of many of the volatiles at stage ZYG (Table 5-11). Of the 17 SC compounds, there were 16 SCs in 'Galia' and 15 in the ASG lines. The SC compound, ethyl-3-(methylthio)propionate was not significant in 'Galia' nor in any ASG lines and isovaleronitrile was not a SC compound in any ASG lines at stage ZYG. Of the 16 SC compounds in the lines at stage ZYG, the five with the highest odor values (OVs), and therefore, contributing the most to the aroma profile, for 'Galia' were benzyl acetate, ethyl-2-methyl butyrate, methyl 2-methyl butyrate, ethyl isobutyrate, and, 2-methylbutyl acetate (Table 5-12). The top five SC compounds in the ASG lines were also similar, though lines ASxWT and WTxAS had ethyl butyrate with the fifth highest OV (Table 5-12). Of the five volatiles with the highest OVs, 'Galia' had 90% or more amounts of benzyl acetate, methyl 2-methyl butyrate, ethyl isobutyrate and both 'Galia' and line WTxAS were over 65% greater in 2-methylbutyl acetate as compared with the ASG lines (Table 5-12).

Due to the TIV line x season interaction, further analysis of each season revealed differences. In fall 2006, line WTxAS greatest; in spring 2007, 'Galia' was greatest and in fall 2007 'Galia' was only greater than line WTxAS (Table 5-9). Both lines ASxAS and ASxWT were only lower in TIV as compared with 'Galia' in spring 2007; line ASxAS also lower than 'Galia' in fall 2006. During fall 2006, at stage ZYG, there were differences in 26 of the 38 identified volatile compounds in fall 2006, stage ZYG fruits. Of the 26 volatiles with differences, line WTxAS produced the most in 19 volatiles; both line WTxAS and 'Galia' were greatest in four, all lines except ASxAS were greatest in three. There were 14 SC compounds in

all lines at stage ZYG, fall 2006 at harvest. The SC compounds, ethyl isobutyrate, ethyl-3-(methylthio)propionate and isovaleronitrile were not prominent at this stage.

In spring 2007, TIV was greatest for 'Galia' (Table 5-9). Of the 38 volatile compounds, differences occurred in 16 compounds among the lines, with 'Galia' greatest in the majority. However, there were 16 SC compounds found in all lines at stage ZYG. These were similar to stage ZYG, fall 2006 with the addition of ethyl isobutyrate and isovaleronitrile. Ethylene and respiration rates were similar among all lines during this season. No differences in ethylene among 'Galia' and the ASG lines in spring 2007 could be attributed to Florida wild fires, which spread through areas in close proximity to the greenhouse facilities. Smoke is well-known to have ethylene as a major component (Rodriguez, 1932). The ethylene produced from the wildfire smoke may have led to early ripening of all fruits and thus no differences overall in ethylene production. Furthermore, after the fire/smoke event subsided, the plants were yellowed and damaged as a result. This demonstrated the sensitivity of the ASG muskmelons to stress and emphasized that these ASG lines need to be produced under ideal conditions. Stressed plants can also produce more ethylene as a response (Srivastava, 2001; Morgan and Drew, 1997; McGlasson, 1970). This stress response was previously observed in studies on antisense ACC-oxidase (CMACO-1) hybrids (TGH-AS-1 and TGH-AS-2) developed from a first generation transgenic male parent, which performed similarly to original 'Galia' during an epidemic of severe powdery mildew (*Podosphaera xanthii* (formerly *Sphaerotheca fuliginea* Schlech ex Fr. Poll.)) (Mitchell et al., 2007c). The TGH-AS-1 and TGH-AS-2 muskmelons had similar DTH to 'Galia' in the diseased spring 2004 crop, while a fall 2004 crop was better managed for powdery mildew and differences were seen in DTH between 'Galia' and the transgenic fruits remained on the vine an average of five days longer than 'Galia' (Mitchell et al., 2007c).

In fall 2007, there were no differences in TIV among 'Galia' and lines ASxAS and ASxWT (Table 5-9). Of the 38 compounds, there were differences in 10, of which 'Galia' was greatest in most (data not shown). There were 16 SCs for all lines; isovaleronitrile was not significant in any line at this stage. All ASG lines produced less ethylene and CO_2 than 'Galia'. Ethylene rates at harvest increased from stage ZG for all lines except line ASxAS, which remained at levels similar to stage ZG and further demonstrated its low ethylene production even as the fruit began to ripen. Respiration rates at harvest for all lines were similar to stage ZG (harvest) rates (Table 5-4).

Stage ZYG fruits were stored for five days in spring and fall 2007. Differences were only seen in firmness among the lines where line ASxAS was firmer (6.3 N) after storage than lines ASxWT and 'Galia' (both averaged 4 N). Compared to stage ZYG at harvest, SSC remained at levels similar to harvest while fruit firmness decreased for all lines. Seasonal differences occurred in fruit weight, size and firmness as fall 2007 fruits were 30% larger and firmer than spring 2007 fruits. For all lines, the overall average SSC was 8 °Brix.

Also at stage ZYG, after storage, there was a line x season interaction for ethylene, respiration and TIV. For the ASG lines in fall 2007, ethylene and respiration rates were 70% and 25% lower, respectively, as compared with spring 2007 rates (Fig. 5-2). For 'Galia' however, ethylene and respiration rates were 48% and 14% higher, respectively in fall as compared with spring 2007 (Fig. 5-2). In spring 2007, average ethylene evolution rates throughout storage were low and similar for all lines (Fig. 5-2). Respiration rates were lower for 'Galia' and increases in CO_2 were observed on day two for all lines (Fig. 5-2). Whereas in fall 2007, ethylene and respiration rates were greatest for 'Galia', especially at days one through three (Fig. 5-2). Increases in ethylene were observed on day two for 'Galia' and ASxAS, while line ASxWT had

an increase on day four of storage. Increases in respiration (CO_2) were observed on day two for 'Galia' and line ASxWT; and on day four for line ASxAS. These increases in CO_2 could be representative of the climacteric occurring during stage ZYG. This is in contrast however, to antisense Charentais cantaloupes, which were reported to lack a climacteric rise in respiration (Bower et al., 2002). Although the ASG lines ASxWT and ASxAS in this study exhibited a slight rise in respiration during storage, respirations rates of these lines in fall 2007 were lower than 'Galia'. The lack of consistency among 'Galia' and the ASG lines in ethylene and respiration during both seasons after storage could be a result of the wildfire smoke during spring 2007.

TIV after storage in spring 2007 TIV was similar among all lines, though increased after storage for the ASG lines, while 'Galia' TIV remained at levels similar to harvest. Of the 38 volatiles, there were differences in only two among the lines- hexyl acetate was greatest in 'Galia', while 2-methyl-1-butanol was greatest in all ASG lines. All 17 SC compounds were present in all lines. In fall 2007, TIV was greatest for 'Galia' followed by line ASxAS. Line ASxWT was lowest in TIV (Table 5-10). Only the ASG lines increased in TIV compared to harvest TIV. There were differences in 15 of the 38 TIV compounds and 'Galia' was greatest in all except two, where line ASxAS was also greatest. There were 16 SC compounds for all lines, ethyl-3-(methylthio)propionate was not a SC at this time.

Stage HS

Analysis of the stage HS three season means at harvest indicated differences in firmness, where lines ASxWT and ASxAS had the firmest fruits. The stage HS firmness decreased from stage ZYG for all ASG lines, while 'Galia' remained the same. The continued firmness of these ASG lines at stage HS is an added highlight of these ASG hybrid muskmelons. Results from the antisense 'Krymka' muskmelons (the original T_0 antisense male parental line of the ASG lines)

did not have firmer fruits at stage HS as compared with wild-type fruits (Nuñez-Palenius et al., 2006b).

Seasonal differences occurred for all variables and there was a line x season interaction for TTA, pH and ethylene among the lines (Tables 5-5 and 5-6). TIV did not differ among the lines (Table 5-9), and there were differences in only six of the 38 volatile compounds (data not shown). Compared to stage ZYG, TIV increased for all lines except 'Galia'. Of the three season average, there were 16 SC compounds at stage HS in all lines. Ethyl-3-(methylthio)propionate was not a SC compound at stage HS.

Further analysis of the line x season interactions indicated that ethylene rates varied over the seasons, though during fall 2006 and spring 2007, all lines had similar ethylene production rates. In fall 2007, lines ASxAS and WTxAS produced less ethylene than 'Galia' while WTxAS produced the greatest amount of ethylene (Table 5-6). Compared with stage ZYG, stage HS ethylene production generally increased for all lines, except for 'Galia', in spring 2007, where there was a decrease in ethylene from stage ZYG. This decrease may also be related to no increase in TIV for 'Galia' from stage ZYG to stage HS. The aroma produced as a result of the ripening process is associated with ethylene evolution and respiration rates (Ayub et al., 2008).

Stage HS fruits were stored for three days in spring and fall 2007. Differences among the lines and seasons occurred for fruit weight and size, as line ASxWT had smaller fruits. There was a line x season interaction for firmness and TIV. In spring 2007, all lines had an average fruit firmness of 4 N, while in fall 2007, line ASxAS (10 N) had the firmest fruit as compared with 'Galia' (6 N) and ASxWT (7 N). The firm ASG muskmelons in fall 2007 as compared with spring 2007 fruits at stage HS after storage not only continued to validate the problems with the spring crop, but also indicated additional potential for the ASG muskmelons at stage HS.

As for TIV, in spring 2007, there were no differences in TIV among the lines (Table 5-10). There were differences in six of the 38 volatile compounds among the lines in spring 2007, stage HS fruits after harvest (data not shown). All 17 SC compounds were present at stage HS after storage in all lines. In fall 2007 after storage, TIV were greatest for 'Galia' and ASxWT (Table 5-10). There were differences in 11 compounds among the lines (data not shown). There were 15 SC compounds after storage, both isovaleronitrile and ethyl-3-(methylthio)propionate were not among SC compounds in any line. Average ethylene production and respiration rates for stage HS fruit in both seasons throughout storage were 1.13 ng kg^{-1} s^{-1} and 10.9 µg CO_2 kg^{-1} s^{-1}, respectively. Both ethylene and respiration rates generally decreased during storage (data not shown), indicating a decline in the climacteric during stage HS.

Stage FS

At harvest, there were differences in fruit size, SSC, TTA and firmness among the lines and seasonal differences in most variables as well. There was also a line x season interaction for weight and length (Tables 5-7 and 5-8). Firmness decreased from stage HS for all ASG lines and lines ASxAS and ASxWT were at a similar firmness to 'Galia'. Line WTxAS was lowest in firmness and SSC; while ethylene and respiration rates were lowest for line ASxAS (Table 5-7). The lack of differences among the ASG line and 'Galia' at stage FS is again in contrast to full-slip 'Krymka' fruits, which were significantly firmer than the wild-type (Nuñez-Palenius et al., 2006b). TIV at stages HS and FS were similar for all lines. There were no differences in TIV among lines (Table 5-9). There were differences in only five of the 38 compounds at stage FS and there were 16 SC compounds at stage FS overall seasons (data not shown). Ethyl-3-(methylthio)propionate was again, not a SC compound at stage FS. The SC compounds with the greatest OV were similar to those at stage ZYG for all lines (Table 5-12). Of these top five SC compounds, ethyl-2-methyl butyrate and 2-methylbutyl acetate were also considered important

to the aroma of GT cultivars ‘C8’ and ‘5080’ (Fallik et al., 2001). Additional volatiles reported in cultivars ‘C8’ and ‘5080’ (Fallik et al., 2001), which were also among the 17 SC compounds in this study, were isobutyl acetate, butyl acetate, ethyl butyrate, ethyl hexanoate and hexyl acetate.

Stage FS fruits were stored for two days in spring and fall 2007. There were no differences in fruit quality variable among the lines after storage and only a seasonal difference for fruit width, where fall 2007 fruits (129 mm) were wider as compared with spring 2007 (115 mm) fruits. Fruit SSC averaged 9 °Brix for all lines in both seasons after storage while firmness average 9N for all lines after storage in both seasons.

There was a line x season interaction for TIV after storage. Compared to harvest TIV, after storage stage FS TIV increased for only ‘Galia’ and line ASxWT. In spring 2007, TIV did not differ among lines; however fall 2007 TIV was greatest for line ASxWT (Table 5-10). In spring 2007 there were differences in seven of the 38 volatiles while in fall there were differences in four of the 38 compounds (data not shown). SC compounds totaled 17 after harvest for all spring 2007 fruit. There were only 15 SCs for ASG lines and 16 for ‘Galia’, fall 2007 fruit. Similar to fruit at harvest, ethyl-3-(methylthio)propionate was again not a SC compound in fall 2007 post-storage fruit, and isovaleronitrile was only an SC in fall 2007 ‘Galia’Both ethylene and respiration decreased in storage for all lines during both seasons (data not shown). The average ethylene production and respiration rates in both seasons were 2.57 ng kg^{-1} s^{-1} and 11.6 µg CO_2 kg^{-1} s^{-1}, respectively among all lines in storage. For stages HS and FS ethylene and respiration rates at harvest were elevated in comparison to stages ZG and ZYG, though both decreased in storage, suggesting the end of the climacteric.

Fruit harvest for all ASG lines was later than 'Galia' at stages ZYG, HS and FS. DTH for stage FS fruit over the three seasons provided the ASG lines a three to five day longer harvesting period. This indicated that the ASG lines ripened at a slower rate than 'Galia', which is a common characteristic for antisense melons (Nuñez-Palenius et al., 2006b; Ayub et al., 1996; Flores at al., 2001).

Over the three seasons at stages ZG, HS and FS, fruit quality variables, ethylene and respiration rates and TIV were mostly similar among all lines both at harvest and after storage. Though some differences did occur such as at stage HS in fall 2007, where line ASxAS was the firmest line after storage. Individual volatile and TIV differences at stages ZG, HS and FS were few, as all lines produced ethylene and respiration at similar rates. The fruit quality and TIV similarities among the ASG lines and 'Galia' at stages ZG, HS and FS suggest that at these stages, ASG lines are identical in quality to the original 'Galia'. The only difference was the length of time on the vine, which was longer for the ASG lines, particularly line ASxAS.

The SC compounds at all stages, as well as those with the greatest OVs (Table 5-12), were mostly similar among all lines and overall seasons, except for ethyl 3-(methylthio)propionate and isovaleronitrile. The reduced amount of these volatiles could be an environmental influence as they were mostly absent in fall 2006 and spring 2007 at harvest. However, both of these volatiles usually increased after storage, most likely due to the continued ripening in storage. Ethyl 3-(methylthio)propionate and isovaleronitrile are considered important to the aroma of true 'Galia' (Harty et al., 2009, unpublished). Also, TIV generally increased after storage at all stages, especially for 'Galia' and line ASxWT. Both at harvest and after storage TIV remained the same for line ASxAS at stages HS and FS. Wyllie et al. (1996b) also reported increases in total aroma volatiles after storage for 'Makdimon' muskmelons harvested before and at full-slip.

The majority of differences between the ASG and 'Galia' were observed during stage ZYG. At stage ZYG, fruit quality in terms of SSC, TTA and pH was generally similar among 'Galia' and lines ASxAS and ASxWT both at harvest and after storage. Firmness, however, was greatest for ASG lines at harvest and after storage. At harvest, ethylene rates were lowest for ASG lines at stage ZYG in fall 2006 and fall 2007, which could correspond to the firmer fruits. Low ethylene and firmer melon flesh was reported in antisense Charentais cantaloupes (Guis et al., 1997; Ayub et al., 1996) as well as antisense 'Kyrmka' muskmelons (Nuñez-Palenius et al., 2006b). As for aroma, TIV varied throughout the seasons at stage ZYG, though overall, 'Galia' was greatest in TIV at stage ZYG at harvest, and after storage 'Galia' had greater TIV in fall 2007. Low aroma volatiles have also been reported in antisense ACO 'Vedrantais' cantaloupes as compared with wild-type cantaloupes (Bauchot et al., 1998 and 1999).

While the reduction in aroma for the ASG lines at stage ZYG was not consistent in all seasons, this could be due not only to environmental effects, but also due to high variation of the 38 volatile compounds (data not shown). This variation also resulted in no correlations between the volatiles and other fruit quality factors. In the future, additional samples and/or pooled samples may be necessary to obtain a more complete analysis of the individual lines to reduce variation and gain more information.

Nonetheless, information obtained from these results suggested that fruits harvested at stage ZYG from line ASxAS exhibited the most potential for a longer shelf-life, high quality 'Galia' muskmelon. Line ASxAS was consistently firmer and produced less ethylene than 'Galia' at stage ZYG in fall 2006 and fall 2007 at harvest; and stage ZYG storage results demonstrated that line ASxAS remained a firmer fruit after five days at 20 °C. TIV for line ASxAS was reduced compared to 'Galia' at stage ZYG over all seasons, but this was not always

found in every season. Also, TIV for the ASG lines increased after storage indicating a more aromatic fruit. Although there was some reduction in aroma at stage ZYG for line ASxAS, overall, this line demonstrated positive results for an antisense ACC-oxidase 'Galia' hybrid muskmelon with good fruit quality, flavor and a longer storage life.

Summary

Galia muskmelon (*Cucumis melo* L. var. *reticulatus* Ser.) is recognized for its aroma, an important quality and flavor component. Although 'Galia'-type (GT) cultivars are available that are firmer than 'Galia,' they often have less flavor. In order to maintain the original 'Galia' flavor while increasing firmness, three antisense ACC-oxidase (CMACO-1) 'Galia' (ASG) hybrid lines were developed: ASxAS, ASxWT, WTxAS. The objective of this research was to evaluate fruit quality and aroma volatiles of 'Galia' and ASG lines at different stages of ripening and determine the effect of the genetic modification. During fall 2006, spring and fall 2007, fruits were harvested at four stages: stage 1.) zero-slip, green (ZG); 2.) zero-slip, yellow-green (ZYG); 3.) half-slip (HS); and 4.) full-slip (FS). Data were collected for fruit size, firmness, soluble solids content (SSC), pH and total titratable acidity (TTA). Aroma volatiles were collected from fresh pulp samples using the Super Q filter column method. GC/MS and GC/FID identified 38 aroma compounds, of which 18 were considered significant contributors to the aroma. Generally, aroma volatiles increased with maturity and after storage at 20°C. At harvest, fruit firmness was greatest at stage ZG while SSC, ethylene and respiration rates, and total identified volatiles (TIV) were low. All lines were similar in TIV at stage ZG. At stage ZYG, ethylene and respiration increased and SSC was a minimum of 9 °Brix for all lines while fruit firmness decreased. TIV was also lowest for lines ASxAS and ASxWT, while 'Galia' had the greatest TIV. The greatest differences were seen in the volatile compounds among the lines was at stage ZYG. At stages HS and FS, ethylene evolution and respiration rates continued to be

high, though individual volatile and TIV differences were few. After storage, there were no fruit quality differences at any stage among all lines in spring 2007. However, stage ZYG storage results from fall 2007 demonstrated that there is a potential for a longer shelf-life ASG muskmelon, as line ASxAS remained a firmer fruit after five days at 20 ℃. Fruit from the ASG lines remained on the vine an average of three to five days longer than 'Galia', suggesting a wider harvest window. Even though there were some differences in aroma volatiles at stage ZYG, it is recommended that line ASxAS be harvested at stage ZYG where SSC was acceptable and fruit firmness (for shipping) was greatest for fruits at harvest and after storage.

Table 5-1. Odor detection threshold levels (OTV) of 17 the significant contributor aroma compounds of 'Galia' and ASG muskmelons (adapted from Mitchell-Harty et al., 2008).

Volatile compound	Scent	Odor Threshold Value (OTV) (ppb)	Air/ water[z]	OTV Ref.[y]
cis-6-nonen-1-ol	waxy, melon	1	water	2, 5
ethyl caproate	powerful fruity, pineapple, banana	1	water	5
benzyl acetate	sweet, jasmine, apple, pear	0.04; 2-270	air; ?	1,2
ethyl propionate	sweet, fruity, ethereal	10	water	5
isobutyl acetate	fruity	66	water	5
hexyl acetate	fruity, green, pear (apple-like)	2	water	5
ethyl butyrate	fruity, pineapple, cognac	1	water	5
ethyl-2-methyl butyrate	sharp, sweet, green, apple, fruity	0.1 – 0.3	water	5
2-methylbutyl acetate	fruity	5	water	5
methyl 2-methyl butyrate	sweet, fruity	0.25	water	5
cis-3-hexenyl acetate	powerful green, fruity, floral, banana, melon	1.2; 7.8	water	8, 9
amyl acetate	bananas	7.5; 0.095	?; air	4
ethyl-3-(methylthio)propionate	fruity, tropical, grassy	7	water	5
ethyl isobutyrate	sweet, rubber	0.1	water	5
propyl acetate	nail-polish remover	40-700	water	3
butyl acetate	fruity	66	water	5
isovaleronitrile	oniony, solvent, fruity	3.2; 1000	water	6, 7

[z], OTV as determined in air or water; ? = unknown. [y], 1.) Waldhoff and Spilker, 2005; 2.) Burdock, 2005; 3.) SIS, 2007; 4.) Ladd. Res. 2006; 5.) Leffingwell; 6.) Khiari et al., 2002; 7.) Buttery et al., 1991; 8.) Khiari et al.,1999; 9.) Belitz et al., 2004.

Table 5-2. Stage ZG means of days to harvest and fruit quality variables for 'Galia' and ASG lines at harvest, fall 2006, spring and fall 2007.

Line	Days to harvest	Weight (kg)	Length (mm)	Width (mm)	Flesh thickness (mm)	Soluble solids content (°Brix)	TTA	pH	Firmness (N)	Ethylene (ng kg^{-1} s^{-1})	Respiration (µg CO_2 kg^{-1} s^{-1})
Galia	42	1.27	145	131	30.9	8.4	0.12	6.87	39.3	2.48	8.71
ASWT	40	0.93	130	118	24.8	7.8	0.11	6.85	41.0	0.16	7.35
WTAS	44	1.09	139	127	26.3	5.9	0.13	6.73	40.8	0.27	6.21
ASAS	45	1.23	135	126	27.8	8.0	0.11	7.01	41.9	0.17	6.04
LSD (0.05)[z]	2.9	0.24	9.7	7.4	2.7	1.1				0.18	3.5
Season											
Fall 2006	39	1.40	146	137	31.7	9.2	0.12	6.99	40.8	2.48	7.68
Spring 2007	57	1.13	139	125	28.0	7.8	0.12	6.70	39.0	0.13	4.40
Fall 2007	32	0.85	122	115	22.8	5.6	0.12	6.90	42.4	0.02	9.15
LSD (0.05)	6.0	0.25	6.3	6.5	2.7	1.5		0.3		0.4	4.2

[z], Mean separation by Fisher's least significant difference test ($P \leq 0.05$). [y], Line x season interaction not significant for all variables.

Table 5-3. Stage ZYG means of days to harvest and fruit quality variables for 'Galia' and ASG lines at harvest, fall 2006, spring and fall 2007.

Line	Days to harvest	Weight (kg)	Length (mm)	Width (mm)	Flesh thickness (mm)	Soluble solids content (°Brix)	Firmness (N)
Galia	38	1.16	138	129	27.5	10.1	17.9
ASWT	42	1.05	132	123	28.1	9.9	32.9
WTAS	42	1.18	136	129	30.4	9.2	22.2
ASAS	43	1.19	136	125	28.9	10.0	30.7
LSD (0.05)[z]	1.64						3.74
Season							
Fall 2006	41	1.37	147	137	30.8	11.4	30.9
Spring 2007	47	0.89	126	117	27.0	9.09	19.5
Fall 2007	38	1.17	132	128	28.2	8.91	27.6
LSD (0.05)	1.59	0.22	9.88	5.45	2.75	1.08	4.49

[z], Mean separation by Fisher's least significant difference test ($P \leq 0.05$).

Table 5-4. Stage ZYG means of significant line*season fruit quality parameters at harvest.

Line (L)	TTA			pH			Ethylene ($ng\ kg^{-1}\ s^{-1}$)			Respiration ($\mu g\ CO_2\ kg^{-1}\ s^{-1}$)		
	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07
Galia	0.09	0.14	0.15	7.19	6.53	6.45	1.38	3.59	2.89	10.9	10	12.8
ASWT	0.09	0.11	0.09	7.37	6.99	7.37	1.75	1.75	1.39	11.8	10.7	8.67
WTAS	0.11	0.12	0.1	6.8	6.91	7.06	1.16	1.33	2.97	18.2	9.42	7.19
ASAS	0.11	0.11	0.11	7.17	6.86	7.17	0.77	1.2	0.86	10.1	10.1	7.19
LxS LSD (0.05) [z]	0.01			0.1			0.88			3.1		

[z], Mean separation for line x season interaction by Fisher's least significant difference test ($P \leq 0.05$).

Table 5-5. Stage HS means of days to harvest and fruit quality variables for 'Galia' and ASG lines at harvest, fall 2006, spring and fall 2007.

Line	Days to Harvest	Weight (kg)	Length (mm)	Width (mm)	Flesh thickness (mm)	Soluble Solids Content (°Brix)	Firmness (N)	Respiration (µg CO_2 kg^{-1} s^{-1})
Galia	40	1.21	138	129	30.9	10.6	14.3	11.3
ASWT	42	1.30	142	130	29.5	9.9	21.8	11.3
WTAS	44	1.28	143	132	31.7	9.4	16.1	12.9
ASAS	43	1.27	140	131	30.0	10.2	18.6	10.9
LSD (0.05)[z]	1.9						3.0	
Season								
Fall 2006	42	1.61	154	144	34.7	11.3	21.8	13.6
Spring 2007	47	0.95	130	118	27.4	9.2	14.3	10.9
Fall 2007	37	1.23	139	129	29.5	9.5	17.0	10.2
LSD (0.05)	2.5	0.22	7.3	6.2	1.7	0.6	3.8	2.0

[z], Mean separation by Fisher's least significant difference test ($P \leq 0.05$).

Table 5-6. Stage HS means of significant line*season interaction of TA, pH and ethylene at harvest.

Line (L)	TTA			pH			Ethylene (ng kg^{-1} s^{-1})		
	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07
Galia	0.09	0.13	0.12	7.29	6.71	6.8	2.53	2.73	3.62
ASWT	0.1	0.12	0.11	7.17	6.72	7.2	3.67	1.81	4.56
WTAS	0.1	0.11	0.15	7.04	6.88	6.41	3.64	1.68	2.51
ASAS	0.11	0.11	0.11	7.19	6.96	7.18	2.47	1.9	1.33
LxS LSD (0.05)[z]	0.01			0.1			1.8		

[z], Mean separation for line x season interaction by Fisher's least significant difference test ($P \leq 0.05$).

Table 5-7. Stage FS means of fruit quality variables for 'Galia' and ASG lines at harvest, fall 2006, spring and fall 2007.

Line	Width (mm)	Flesh thickness (mm)	Soluble solids content (°Brix)	TTA	pH	Firmness (N)	Ethylene (ng kg^{-1} s^{-1})	Respiration (µg CO_2 kg^{-1} s^{-1})
Galia	133	31.9	10.6	0.13	6.91	14.0	3.78	13.1
ASWT	126	30.0	10.6	0.10	7.07	16.5	4.06	12.9
WTAS	125	27.3	8.2	0.11	7.10	11.0	4.06	13.2
ASAS	127	29.5	10.1	0.10	6.98	14.4	3.15	11.3
LSD (0.05)[z]	5.7	2.9	1.2	0.01		2.1		1.5
Season								
Fall 2006	139	33.0	11.5	0.10	7.16	17.0	4.51	13.8
Spring 2007	117	28.1	9.4	0.11	6.86	11.9	2.92	12.6
Fall 2007	128	27.9	8.7	0.11	7.04	13.1	3.89	11.4
LSD (0.05)	3.6	1.6	0.9		0.22	3.2	0.44	1.3

[z], Mean separation by Fisher's least significant difference test ($P \leq 0.05$).

Table 5-8. Stage FS means of significant line*season interaction for days to harvest, weight and length

Line (L)	Days to harvest			Weight (Kg)			Length (mm)		
	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07	Fa06	Sp07	Fa07
Galia	39	46	34	1.78	1.1	1.23	161	138	141
ASWT	44	47	37	1.29	0.83	1.3	142	126	143
WTAS	42	47	38	1.37	0.8	0.9	151	123	120
ASAS	46	49	38	1.35	0.87	1.23	148	126	138
LxS LSD (0.05)[z]		1.05			1.54			0.25	

[z], Mean separation for line x season interaction by Fisher's least significant difference test ($P \leq 0.05$).

Table 5-9. Means of total identified volatiles (TIV) (ng g FW^{-1} hr^{-1}) at harvest for 'Galia' and ASG muskmelons harvested at stages ZG, ZYG, HS and FS in fall 2006, spring and fall 2007.

Total Identified Volatiles (TIV) at Harvest (H)						
Line	ZG[z]	ZYG[y]			HS	FS
		Fa06	Sp07	Fa07		
Galia	287	1646	2616	1387	1589	1636
ASWT	333	1586	581	1087	1775	2089
WTAS	310	2491	1087	776	2397	2015
ASAS	273	1201	526	973	1427	1855
LSD (0.05)[x]		425	425	425		
Fall 2006	290				2214	2760
Spring 2007	240				1391	1500
Fall 2007	372				1786	1437
LSD (0.05)						891

[z] **,** ZG= stage zero-slip, green; ZYG= stage zero-slip, yellow/green; HS= stage half-slip; FS= stage full-slip. Stages ZG, HS and FS means are the three-season average.
[y] **,** There was a significant line x season interaction for stage ZYG only. LSD (0.05) for L x S interaction= 425 ng gFW^{-1} h^{-1}. [x] , Mean separation by Fisher's least significant difference test ($P \leq 0.05$). Units= ng gFW^{-1} h^{-1}.

Table 5-10. Means of total identified volatiles (TIV) (ng g FW^{-1} hr^{-1}) after storage at 20 °C for 'Galia' and ASG muskmelons harvested at stages ZG, ZYG, HS and FS in spring and fall 2007.

Total Identified Volatiles (TIV) after Storage (S)								
Line	ZG[z]		ZGY		HS		FS	
	Sp07	Fa07	Sp07	Fa07	Sp07	Fa07	Sp07	Fa07
Galia	627	1410	2103	4877	2294	3756	2330	2645
ASxAS	335	1722	1824	3034	1486	1389	2339	1742
ASxWT	467	680	2789	1436	1334	4324	1572	4240
WTxAS[y]	428	n/a	2249	n/a	2293	n/a	2086	n/a
LSD (0.05)[x]		610		1107		1106		962

[z] ZG= stage zero-slip, green; ZYG= stage zero-slip, yellow/green; HS= stage half-slip; FS= stage full-slip. [y] WTxAS was only stored in Spring 2007. [x] Means separated using Fisher's Least Significant Difference (LSD), $P<0.05$. Units= ng gFW^{-1} h^{-1}.

Table 5-11. Stage ZYG means, presented in ng gFW^{-1} h^{-1} of 'Galia' and Antisense 'Galia' (ASG) muskmelon aroma compounds measured at harvest, fall 2006, spring 2007 and fall 2007.

Aroma Compound	Galia	ASxAS	ASxWT	WTxAS	Line[z]	Season	L*S
propyl butyrate	0.98	0.75	0.54	1.39		*	
tiglic aldehyde	0.27	0.22	0.19	0.26		**	
4-methyl-1-cyclohexene	0.24	0.26	0.38	0.29		**	
cis-3-hexen-1-ol	1.12	0.55	0.37	0.41			
trans-cyclodecene	0.98	0.48	0.20	0.25			
cinnamyl acetate	0.32	0.33	0.29	0.15		*	
2-methyl-1-butanol	0.37	0.16	0.17	0.19			
furfuryl acetate	2.07	0.91	0.88	0.64	*	*	
methyl isobutyrate	2.32	2.22	1.92	2.14		**	
allyl methyl sulfide	1.51	0.39	0.35	0.72	*	**	**
butyl propionate	1.75	0.9	0.56	1.64	**	**	*
cyclooctene	0.94	1.42	0.63	1.45			
isobutyl butyrate	1.6	1.23	0.73	1.88		**	
benzaldehyde	1.04	1.15	0.93	0.67		**	
3-phenylpropylacetate	3.22	1.52	1.27	1.66	**	**	*
methyl caprylate	4.56	1.58	1.70	2.53	**	**	
methyl caproate	12.8	9.49	7.24	9.42	*	*	**
isobutyl propionate	7.23	7.39	3.71	6.92		**	
ethyl-3-(methylthio)propionate	4.33	2.46	2.44	3.48	*	**	**
heptyl acetate	6.69	2.8	2.58	3.22	**	*	**
phenethyl acetate	12.2	6.01	3.72	1.8	**	**	**
amyl acetate^	24.5	11.4	12.6	15.4	**	**	*
methyl butyrate	9.99	5.03	9.22	11.7	**	**	*
cis-6-nonen-1-ol^	26.8	15.8	13.1	9.41	*		**
Isovaleronitrile^	4.68	1.56	1.31	1.97		*	
ethyl caproate^	48.6	24.2	22.5	26.7	**	**	*
cis-3-hexenyl acetate^	19.3	6.14	10.1	16.1	**	**	**
benzyl acetate^	44.9	22.2	24.9	22.1	**	**	**
ethyl propionate^	40.9	33.1	42.7	28.7			
ethyl isobutyrate^	33.9	15.5	12.5	9.67	**	**	**
isobutyl acetate^	289	181	322	337		**	
propyl acetate^	127	67.5	120	86.1	**	**	*
hexyl acetate^	57	27.6	66.2	114.7	**		
ethyl butyrate^	56.6	44.6	42.4	42.8		**	**
butyl acetate^	157	85.7	103	132		*	
ethyl-2-methyl butyrate^	41.4	37.9	34.6	38.2			*
2-methylbutyl acetate^	328	167	243	357	**	**	**
methyl 2-methyl butyrate^	499	36.3	12.7	56.0	**	**	**
Total Identified Volatiles	1871	823	1123	1345	**	**	**
^Signif. Contributor (total)	1778	772	1074	1278	**	**	**

[z], Significance among lines, seasons and line x season interaction. Mean separation by Fisher's least significant difference test (*= P≤0.05, **=P≤0.01).^, Denotes a significant contributor (SC) compound.

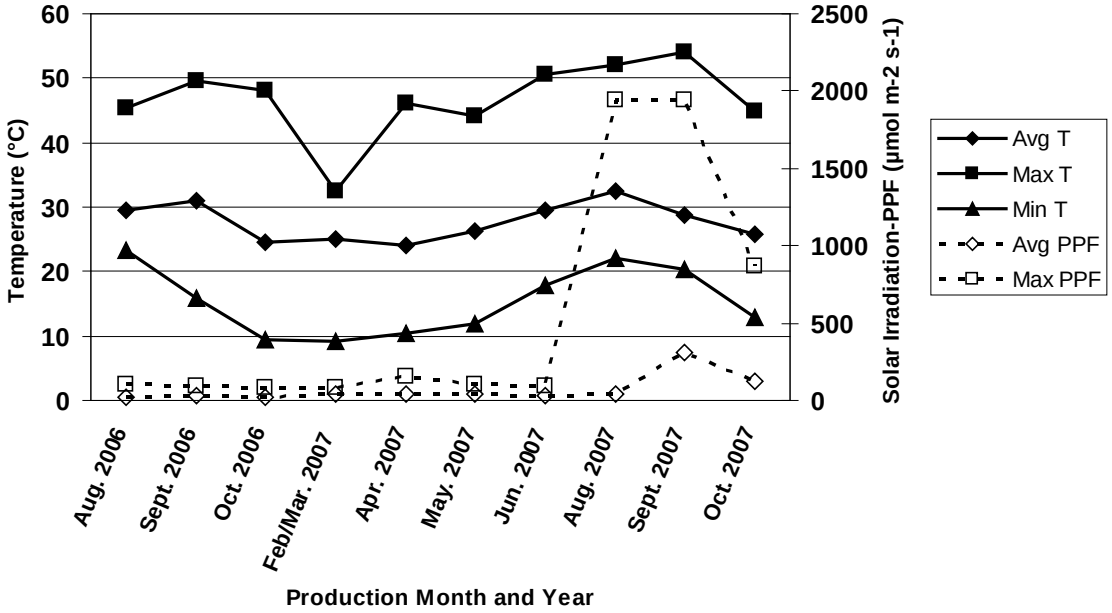

Figure 5-1. Average, maximum and minimum temperatures and solar radiation (Photsynthetic Photon Flux (PPF)) for 'Galia' and antisense 'Galia' (ASG) produced in a passively-ventilated greenhouse, fall 2006, spring and fall 2007.

CHAPTER 6
CONCLUSIONS

The original Galia muskmelon (*Cucumis melo* L. var. *reticulatus* Ser.) is recognized for its exceptional flavor and musky aroma. Newer 'Galia'-type (GT) muskmelons are firmer, but flavor has been compromised in breeding efforts to increase firmness. Therefore, a true 'Galia' muskmelon with a longer shelf-life is desirable to fully exploit flavor attributes while allowing longer distance shipping. The results of this research, which comprised the development, selection, and evaluation of an antisense ACC-oxidase (CMACO-1) 'Galia' F_1 hybrid muskmelon, from fall 2006 through spring and fall 2007 indicated that developing a longer shelf-life true 'Galia' muskmelon is possible.

Results from fall 2006 indicated that ASG-1 muskmelons harvested at stages ZG, HS and FS exhibited many similarities to the original 'Galia' muskmelon, especially in firmness and ethylene production. Only stage ZYG ASG-1 muskmelons from lines ASxAS and ASxWT demonstrated increased firmness and lower ethylene, however this only occurred when grown under optimal conditions (such as good temperatures (Min: 18 °C to Max: 35 °C), minimal disease/insect pressure, and no wildfires or hurricanes). There was also no difference in respiration among the ASG-1 lines and 'Galia' at stage ZYG, or at any other stage. Other reports of antisense ACO Charentais cantaloupes report a lack of a climacteric rise in the antisense fruit compared with wild-type fruit (Bower et al., 2002). The lack of reduced respiration in the fall 2006 study suggested that a storage treatment was necessary to track gas emissions over time to better evaluate the effect of the reduced ethylene on respiration rates in the ASG-1 muskmelons.

Nevertheless, fall 2006 results of the ASG-1 lines of ASxAS and ASxWT demonstrated the greatest potential for a longer shelf-life 'Galia' muskmelon if harvested at stage ZYG and produced in an optimal environment. These ASG-1 lines exhibited similar fruit size (greater

than 1 kg), similar SSC (ranged from 10.5 °Brix to over 12 °Brix) to the original 'Galia', yet were firmer than 'Galia' at stage ZYG. The ASG-1 lines, ASxAS and ASxWT also produced less ethylene than 'Galia' during fall 2006. Even though reduced ethylene was not observed again in spring 2007, most likely due to the environmental problems of wildfire smoke and reduced light intensity associated with that season, the ASG-1 lines ASxWT and ASxAS were again firmer than 'Galia' in spring 2007 and had a later DTH than 'Galia'. These results are similar to what Nuñez-Palenius et al. (2006a and 2006b), Ayub et al. (1996) and Guis et al. (1997) reported in other antisense acc-oxidase melons in terms of firmer fruit, low ethylene and similar fruit quality (fruit size and SSC) to non-antisense fruit types. Selections from the ASG material were made and used to continue research.

Since flavor is important to the quality of muskmelons and includes the organoleptic traits of taste, aroma and texture (Goff and Klee, 2006), it was important to determine factors that characterize a muskmelon which might possess excellent quality or poor quality. The high-quality 'Galia' muskmelon and its GT relatives are therefore excellent candidates to study fruit flavor. To determine why 'Galia' flavor might be different from GT cultivars, this research also focused on aroma, in order to identify volatiles of the true 'Galia' F_1 hybrid. GC/MS and GC/FID verified 38 aroma compounds. Of these, 10 to 17 compounds significantly contributed to the aromatic profile, depending on stage and cultivar. Increases in aroma volatiles were observed as fruits ripened and after storage at 20°C. Based on this research, the compounds considered to be the most important to high-quality 'Galia' muskmelons were benzyl acetate, ethyl-2-methyl butyrate, methyl 2-methyl butyrate, ethyl isobutyrate, 2-methylbutyl acetate, hexyl acetate, ethyl butyrate, ethyl caproate and cis-3-hexenyl acetate due to their high OVs over a three-season average. OVs greater than one contribute the most to the aromatic profile and are

obtained as a result of dividing the concentration of the compound (determined with GC/FID) by its known odor threshold value (OTV (Bauchot et al., 1998; Teranishi et al., 1991). Additionally, isovaleronitrile and ethyl-3-(methylthio)propionate may also be noteworthy; as isovaleronitrile was only a SC in 'Galia' and ethyl-3-(methylthio)propionate is a sulfur compound known to be impart to the 'musky' aroma in muskmelons Wyllie and Leach (1992).

The final objective of this research was to evaluate fruit quality and aroma volatiles of 'Galia' and ASG lines at different stages of ripening and determine the effect of the genetic modification. During fall 2006, spring and fall 2007, fruits were harvested at four stages: stage 1.) zero-slip, green (ZG); 2.) zero-slip, yellow-green (ZYG); 3.) half-slip (HS); and 4.) full-slip (FS). Results demonstrated that fruit harvest for all ASG lines was later than 'Galia' at stages ZYG, HS and FS. Days to harvest (DTH) for stage FS fruit over the three seasons provided the ASG lines a three to five day longer harvesting period. This indicated that the ASG lines ripened at a slower rate than 'Galia', which is a common characteristic for antisense melons (Nuñez-Palenius et al., 2006b; Ayub et al., 1996; Flores at al., 2001).

Over the three seasons at stages ZG, HS and FS, fruit quality variables, ethylene and respiration rates and TIV were mostly similar among all lines both at harvest and after storage. Generally, aroma volatiles increased with maturity and after storage. Individual volatile and TIV differences at stages ZG, HS and FS were few, as all lines produced ethylene and respiration at similar rates. The fruit quality and TIV similarities among the ASG lines and 'Galia' at stages ZG, HS and FS suggest that at these stages, ASG lines are identical in quality to the original 'Galia'. The only difference was the length of time on the vine, which was longer for the ASG lines, particularly line ASxAS.

The greatest differences between the ASG and 'Galia' were observed during stage ZYG. At stage ZYG, fruit quality in terms of SSC, TTA and pH was generally similar among 'Galia' and lines ASxAS and ASxWT both at harvest and after storage. Firmness, however, was greatest for ASG lines at harvest and after storage. At harvest, ethylene rates were lowest for ASG lines at stage ZYG in fall 2006 and fall 2007, which could correspond to the firmer fruits. Low ethylene and firmer melon flesh was reported in antisense Charentais cantaloupes (Ayub et al., 1996) as well as antisense 'Kryrmka' muskmelons (Nuñez-Palenius et al., 2006b). As for aroma, TIV varied throughout the seasons at stage ZYG, though overall, 'Galia' was greatest in TIV at stage ZYG at harvest, and after storage 'Galia' had greater TIV in fall 2007. Low aroma volatiles have also been reported in antisense ACO 'Vedrantais' cantaloupes as compared with wild-type cantaloupes (Bauchot et al., 1998 and 1999).

After the five day storage period in spring and fall 2007 for stage ZYG fruits, differences were only seen in firmness among the lines where line ASxAS had the firmest fruits (6.3 N) after storage than lines ASxWT and 'Galia' (both averaged 4 N). TIV for line ASxAS was reduced compared to 'Galia' at stage ZYG over all seasons, but low TIV for line ASxAS as compared with 'Galia' was not consistent in every season. Also, TIV for line ASxAS muskmelons increased after storage, indicating a more aromatic fruit. Although there was some reduction in aroma at stage ZYG for line ASxAS, overall, this line demonstrated positive results for an antisense ACC-oxidase 'Galia' hybrid muskmelon with good fruit quality, flavor and a longer storage life.

This research presents the problem concerning the short-shelf life of the original 'Galia' muskmelon and proposes the antisense ACC-oxidase (CMACO-1) ASG muskmelon as an improved alternative. However, there are both advantages and disadvantages to this alternative.

The advantages are the delayed ripening characteristic, increased firmness at harvest and after storage as well as similar high quality to that of the original 'Galia' hybrid. The delay in ripening would give growers an extended harvest period and allow ASG fruits to be harvested at the most advantageous stage, stage ZYG. Harvesting at stage ZYG resulted in firmer fruits and therefore, a longer shelf life. Finally, the quality of the ASG muskmelons is similar to the original 'Galia', a fruit marketed in the United States, Europe and the Mediterranean (Shaw et al., 2001; Rodriguez et al., 2001; Karchi, 2000. Although there are these several advantages of the ASG muskmelons, the disadvantages cannot be ignored.

Disadvantages include the delayed ripening characteristic, environmental sensitivity, and the fact that this product is a genetically-modified organism (GMO). The delay in ripening, though also discussed to be advantageous, could also prove to be a burden on growers due to the longer production period. A longer growing season could result in additional expenses and time that growers may not have. The sensitivity of the ASG muskmelons, as observed in spring 2007 with the wildfire event and previously with pathogens such as powdery mildew, make production of ASG fruits a risky business. Lastly, GMO products are often not favorably viewed by consumers due to their unknown health and environmental effects (Whitman, 2000). This resistance could result in a limited or no market for the ASG muskmelon.

A non-GMO substitute for the ASG muskmelon could be newer 'Galia'-type (GT) cultivars such as 'MG10183' that was evaluated in this research. 'MG10183' demonstrated increased firmness as compared with the original 'Galia', yet was also preferred the most by consumers in a taste panel. In comparison with other GT cultivars, 'MG10183' was highly ranked in regard to fruit yield and quality (Mitchell et al., 2007a; Mitchell et al., 2006). Due to constant consumer complaints of fruits with little flavor, breeders today are beginning to focus

on quality in addition to yield for crops such as tomato (Causse et al., 2003). Melons, as well, are also being improved by breeders in terms of fruit quality as well as shelf-life and disease resistance (Hoberg et al., 2003; Cohen et al., 2000). As breeders employ traditional breeding strategies that integrate improved quality factors, the GM ASG muskmelon may merely serve as a scientific tool to investigate reduced ethylene in muskmelons.

APPENDIX A
CHAPTER 3 ANOVA TABLES

Table A3-1.

Source	DF	Mean square			
		T1	T2	T3	T4
Trt.	2	345**	164*	675**	537**
Rep	2	12.3	19.4	3.11	3.11
Error	4	13.8	23.1	1.61	4.11

* and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table A3-2.

Source	DF	Mean square			
		T1	T2	T3	T4
Trt.	2	25.4*	10.3*	3.44	14.8
Rep	2	5.78	4.33	10.1	20.1
Error	4	1.94	0.67	1.78	13.4

* and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table A3-3.

Source	DF	Mean square								
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	Firmness	Ethylene	Respiration
Trt.	4	15.8	136800	297	210	29.5	13.3**	39.3	10.4	18.7
Rep	3	15.1	34447	107	59.8	5.39	1.58	23.2	5.94	17.0
Error	12	4.93	81952	139	104	21.7	1.74	75.2	7.34	36.3

* and **, significant F-test at P≤0 .05 and P≤0.01, respectively.

Table A3-4.

Source	DF	Mean square								
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	Firmness	Ethylene	Respiration
Trt**	7	19.7**	81416	117.7	61.7	15.1	15.7**	108**	20.7**	89.1
Rep	3	3.6	111981	324.9	174	8.76	0.7	22.4	20.7	38.1
Error	21	5.64	72195	77.2	41.5	8.38	1.49	19.0	5.43	42.7

* and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table A3-5.

Source	DF	Mean square								
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	Firmness	Ethylene	Respiration
Trt	8	56.9**	225892	308	147	37	5.64*	124**	87.9*	231
Rep	3	14.7	134477	153	124	15.6	2.09	2.4	43.7	58.2
Error	24	9.8	89809	78.5	68.7	20	1.69	28.5	20.7	216

* and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table A3-6.

Source	DF	Mean square								
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	Firmness	Ethylene	Respiration
Trt	8	34.6*	191419	228	197	42.9	4.54*	35.2**	76.3	29.7
Rep	3	6.99	12873	4.48	34.2	4.01	2.13	27.4	11.5	3.37
Error	24	13.2	84512	140	86.4	20.6	1.46	10.2	12.5	19.7

* and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table A3-7.

Source	DF	Mean square								
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	Firmness	Ethylene	Respiration
Line	3	37.0	0.14	289.7	198	53.7*	9.17*	58.4	0.11	12.3
Season	1	2346**	0.4*	373*	1213**	80.0*	20.5*	68.2	1.13*	368**
L*S	3	11.7	0.06	96.2	68.6	10.2	2.38	22.3	0.09	22.4
Seas(Rep)	6	36.5	0.09	47.9	38.2	9.22	2.86	35.1	0.15	21.3
Error	18	16.1	0.08	140	82.9	13.1	2.27	57.1	0.16	30.9

* and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table A3-8.

Source	DF	Mean square								
		Days to harvest	Weight	Length	Width	Flesh	SSC	Firmness	Ethylene	Respiration
Line	3	30.6**	0.05	123.3	82.1	3.88	2.49*	380**	25.0*	35.8
Season	1	259**	1.92*	3188**	3804**	142**	32.4**	1066**	19.8	185*
L*S	3	1.78	0.03	72	41.7	3.72	4.84	11.8	16.6*	102**
Seas(Rep)	6	3.61	0.03	98	31.3	5.32	1.44	10.9	3.46	27.1
Error	18	4.7	0.04	100.9	41.5	7.99	0.82	10.1	4.08	25.8

*and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table A3-9.

Source	DF	Mean square								
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	Firmness	Ethylene	Respiration
Line	3	18.4*	0.09	247**	60.9	16.4	5.48*	80.4**	5.35	25.7
Season	1	205**	3.07**	4052**	4910**	345**	42.6**	516**	95**	163*
L*S	3	0.95	0.05	178*	40.7	18.5	3.45**	12.7	14.3*	55.1
Seas(Rep)	6	1.41	0.05	72.7	43.1	9.08	0.21	12.7	2.01	13.2
Error	18	4.13	0.04	44.2	38.1	16.9	1.04	14.2	3.44	29.1

* and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table A3-10.

Source	DF	Mean square								
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	Firmness	Ethylene	Respiration
Line	3	35.4**	0.15	251**	170*	13.9	5.25**	24.3**	14.1	13.4
Season	1	145**	2.47	4010**	3885**	195**	29.5**	241**	132**	27.7
L*S*	3	6.92	0.00	46.8	1.99	15.2	1.06	2.03	15.7*	14.9
Seas(Rep)	6	1.81	0.01	9.11	11.2	2.44	1.28	10.6	2.76	5.61
Error	18	2.03	0.03	42.8	36.9	8.7	0.77	3.77	7.87	6.31

* and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Additional Tables for Chapter 3

Table A1. Stage ZG external and internal color for 'Galia', 'Galia'-type (MG10183) and grouped ASG lines, fall 2006.

	External			Internal		
Line	Lightness[z]	Chroma[y]	Hue angle[x]	Lightness	Chroma	Hue angle
Galia	52.7	26.4	97.2	65.4	25.7	113.1
MG10183	55.5	36.3	102.1	76.4	24.9	112.2
ASWT (ASG-1)	47.4	22.4	93.1	74.6	23.5	111.7
WTAS (ASG-1)	50.6	38.3	107.0	71.8	25.8	112.2
ASAS (ASG-1)	55.1	23.5	102.3	73.2	25.2	111.3
ASAS2 (ASG-2)	46.8	16.9	101.9	71.3	28.9	112.5
LSD[w]	NS	NS	NS	NS	NS	NS

[z], Lightness expressed on a scale from 0=black to 100=white. [y], Chroma expressed on a scale from 0=gray to 60. [x], Hue angles: red, 0°; yellow, 90°; green, 180°; and blue, 270. [w], Means separated using Fisher's Least Significant Difference; * = significance at P<0.05; NS= not significant.

Table A2. Stage ZYG external and internal color for 'Galia', 'Galia'-type (MG10183) and grouped ASG lines, fall 2006.

	External			Internal		
Line	Lightness[z]	Chroma[y]	Hue angle[x]	Lightness	Chroma	Hue angle
Galia	52.6	32.18	97.8	73.2	25.1	110.6
MG10183	55.9	33.19	102.5	76.9	24.1	110.9
ASWT (ASG-1)	49.1	30.45	95.4	71.3	22.8	112.3
WTAS (ASG-1)	57.7	42.7	95.7	72.4	23.3	111.6
ASAS (ASG-1)	61.6	49.98	94.5	68.5	24.6	112.2
ASAS2 (ASG-2)	48.0	26.37	99.0	70.6	24.4	110.3
LSD[w]	NS	NS	NS	*2.95	NS	1.4

[z], Lightness expressed on a scale from 0=black to 100=white. [y], Chroma expressed on a scale from 0=gray to 60. [x], Hue angles: red, 0°; yellow, 90°; green, 180°; and blue, 270. [w], Means separated using Fisher's Least Significant Difference; * = significance at P<0.05; NS= not significant.

Table A3. Stage HS external and internal color for 'Galia', 'Galia'-type (MG10183) and grouped ASG lines, fall 2006.

	External			Internal		
Line	Lightness[z]	Chroma[y]	Hue angle[x]	Lightness	Chroma	Hue angle
Galia	59.8	41.6	93.6	71.9	25.4	111
MG10183	66.9	45.9	92.9	75.1	22.4	112
ASWT (ASG-1)	63.6	42.9	89.1	71.1	22.1	112
WTAS (ASG-1)	63.4	40.2	91.8	73.3	24.1	112
ASAS (ASG-1)	63.8	45.1	92.4	70.0	25.9	112
ASAS2 (ASG-2)	65.3	47.2	90.2	70.1	24.7	111
LSD[w]	*3.64	NS	NS	*2.72	NS	NS

[z], Lightness expressed on a scale from 0=black to 100=white. [y], Chroma expressed on a scale from 0=gray to 60. [x], Hue angles: red, 0°; yellow, 90°; green, 180°; and blue, 270. [w], Means separated using Fisher's Least Significant Difference; * = significance at P<0.05; NS= not significant.

Table A4. Stage FS external and internal color for 'Galia', 'Galia'-type (MG10183) and grouped ASG lines, fall 2006.

	External			Internal		
Line	Lightness[z]	Chroma[y]	Hue angle[x]	Lightness	Chroma	Hue angle
Galia	63.4	48.8	90.3	72.2	25.1	111
MG10183	56.9	37.9	94.7	73.4	23.4	112
ASWT (ASG-1)	62.2	41.6	91.7	68.6	23.0	112
WTAS (ASG-1)	60.1	45.4	85.9	70.2	25.8	111
ASAS (ASG-1)	60.7	38.3	96.1	68.8	24.5	112
ASAS2 (ASG-2)	65.4	48.9	90.5	71.1	23.3	111
LSD[w]	NS	NS	NS	NS	NS	NS

[z], Lightness expressed on a scale from 0=black to 100=white. [y], Chroma expressed on a scale from 0=gray to 60. [x], Hue angles: red, 0°; yellow, 90°; green, 180°; and blue, 270. [w], Means separated using Fisher's Least Significant Difference; * = significance at P<0.05; NS= not significant.

APPENDIX B
ADDITIONAL TABLES AND ANOVA TABLES FOR CHAPTER 4

Appendix B-1

Table B1-1: Stage ZG means of 'Galia' and 'Galia'-type melon aroma compounds, fall 2006.

Aroma Compound	Signif.[z]	'Galia'	'MG10183'
propyl butyrate	ns	0.88	0.61
tiglic aldehyde	ns	0.17	0.13
4-methyl-1-cyclohexene	ns	0.51	1.76
cis-3-hexen-1-ol	ns	0.02	0.04
trans-cyclodecene	ns	0.19	0.05
cinnamyl acetate	ns	0.06	0.03
2-methyl-1-butanol	ns	0.01	0.00
furfuryl acetate	ns	0.07	0.08
methyl isobutyrate	ns	2.92	2.39
allyl methyl sulfide	ns	0.04	0.34
butyl propionate	ns	0.03	0.06
cyclooctene	ns	0.10	0.30
isobutyl butyrate	ns	0.05	0.09
benzaldehyde	ns	0.31	0.53
3-phenylpropylacetate	ns	0.13	0.06
methyl caprylate	ns	0.79	0.91
methyl caproate	ns	5.32	4.31
isobutyl propionate	ns	0.21	0.41
ethyl-3-(methylthio)propionate	ns	0.66	1.38
heptyl acetate	ns	0.77	1.14
phenethyl acetate	ns	0.28	1.08
amyl acetate	ns	1.72	2.56
methyl butyrate	ns	7.52	11.1
cis-6-nonen-1-ol^	ns	6.81	11.9
isovaleronitrile	ns	0.10	0.04
ethyl caproate^	ns	10.2	7.27
cis-3-hexenyl acetate	ns	2.08	9.83
benzyl acetate^	ns	2.73	6.16
ethyl propionate^	ns	33.5	21.7
ethyl isobutyrate	ns	0.01	0.01
isobutyl acetate^	ns	103	162
propyl acetate	ns	38.8	59.2
hexyl acetate^	ns	10.5	24.2
ethyl butyrate^	ns	21.9	25.2
butyl acetate	ns	12.3	34.2
ethyl-2-methyl butyrate^	ns	21.9	12.8
2-methylbutyl acetate^	ns	48.0	115
methyl 2-methyl butyrate^	ns	25.0	24.6
Total Volatiles	ns	360	553
^Sig. Contributors	ns	283	420

[z], ns, non significant F-test at $P \leq 0.05$.

Table B1-2: Stage ZG means of 'Galia' and 'Galia'-type melon aroma compounds, spring 2007.

Aroma Compound	Signif.[z]	'Galia'	'MG10183'
propyl butyrate	ns	0.47	0.54
tiglic aldehyde	ns	0.01	0.00
4-methyl-1-cyclohexene	ns	0.12	0.03
cis-3-hexen-1-ol	ns	0.30	0.18
trans-cyclodecene	ns	0.16	0.09
cinnamyl acetate	ns	0.32	0.12
2-methyl-1-butanol	ns	0.75	0.03
furfuryl acetate	ns	0.51	0.47
methyl isobutyrate	ns	0.08	0.11
allyl methyl sulfide	ns	0.09	0.38
butyl propionate	ns	0.22	0.24
cyclooctene	ns	0.20	0.26
isobutyl butyrate	ns	0.23	0.25
benzaldehyde	ns	1.50	0.21
3-phenylpropylacetate	ns	0.75	0.42
methyl caprylate	ns	0.87	1.55
methyl caproate	ns	2.73	4.30
isobutyl propionate	ns	1.18	1.89
ethyl-3-(methylthio)propionate	ns	1.51	2.31
heptyl acetate	ns	1.63	2.05
phenethyl acetate	ns	0.10	0.09
amyl acetate^	ns	6.52	8.53
methyl butyrate	ns	8.18	0.19
cis-6-nonen-1-ol^	ns	1.82	2.23
isovaleronitrile	ns	0.03	0.01
ethyl caproate^	ns	15.6	8.01
cis-3-hexenyl acetate^	ns	16.4	19.4
benzyl acetate^	ns	19.6	20.3
ethyl propionate^	ns	36.4	40.4
ethyl isobutyrate^	ns	38.9	8.05
isobutyl acetate	ns	34.3	19.5
propyl acetate^	ns	63.1	74.8
hexyl acetate^	ns	31.8	44.5
ethyl butyrate^	ns	20.3	27.2
butyl acetate^	ns	27.1	91.6
ethyl-2-methyl butyrate^	ns	27.6	15.2
2-methylbutyl acetate^	ns	89.0	51.2
methyl 2-methyl butyrate^	ns	2.47	3.86
Total Volatiles	ns	453	450
^Sig. Contributors	ns	426	408

[z], ns, non significant F-test at $P \leq 0.05$.

Table B1-3: Stage ZG means of 'Galia' and 'Galia'-type melon aroma compounds, fall 2007.

Aroma Compound	LSD[z]	'Galia'	'MG10183'	'Elario'
propyl butyrate		0.36	0.16	0.89
tiglic aldehyde		0.27	0.04	7.79
4-methyl-1-cyclohexene		0.04	0.44	0.12
cis-3-hexen-1-ol		0.29	0.31	0.61
trans-cyclodecene		0.26	0.12	0.26
cinnamyl acetate		0.20	0.17	0.26
2-methyl-1-butanol		0.21	0.03	0.61
furfuryl acetate		0.17	0.18	0.36
methyl isobutyrate		0.39	0.07	0.07
allyl methyl sulfide		1.31	0.22	0.11
butyl propionate		0.33	0.17	0.44
cyclooctene		0.03	0.20	0.29
isobutyl butyrate		0.32	0.16	0.58
benzaldehyde		1.30	2.38	8.64
3-phenylpropylacetate		0.36	0.19	0.64
methyl caprylate		0.48	1.27	2.29
methyl caproate		1.57	3.07	9.06
isobutyl propionate		0.59	0.54	3.11
ethyl-3-(methylthio)propionate		5.97	1.83	13.9
heptyl acetate		0.78	0.88	1.75
phenethyl acetate		0.75	2.86	8.63
amyl acetate		3.09	3.10	3.26
methyl butyrate		0.11	0.34	19.4
cis-6-nonen-1-ol	5.30	0.39	4.06	14.8
isovaleronitrile		0.17	0.09	0.23
ethyl caproate		3.98	6.25	35.9
cis-3-hexenyl acetate		0.40	0.29	1.58
benzyl acetate		7.95	36.0	89.2
ethyl propionate		2.34	2.62	39.1
ethyl isobutyrate		0.24	3.35	24.2
isobutyl acetate		20.7	53.3	71.3
propyl acetate		11.0	22.0	26.4
hexyl acetate		17.2	21.1	45.6
ethyl butyrate		18.4	28.5	123
butyl acetate		7.11	30.3	39.8
ethyl-2-methyl butyrate		3.90	10.8	41.2
2-methylbutyl acetate		29.8	81.5	266
methyl 2-methyl butyrate		2.29	12.8	16.5
Total Volatiles		145	352	903
Signif. Volatiles		135	338	835

[z], Mean separation by Fisher's least significant difference test (P=0.05).

Table B1-4: Stage ZG means of 'Galia' and 'Galia'-type melon aroma compounds following storage for 5 days at 20°C, spring 2007.

Aroma Compound	Signif.[z]	'Galia'	'MG10183'
propyl butyrate	ns	1.24	1.35
tiglic aldehyde	ns	0.02	0.02
4-methyl-1-cyclohexene	ns	0.04	0.03
cis-3-hexen-1-ol	ns	0.26	0.75
trans-cyclodecene	ns	0.22	0.38
cinnamyl acetate	ns	0.31	0.32
2-methyl-1-butanol	ns	0.12	0.32
furfuryl acetate	*	0.79	1.58
methyl isobutyrate	ns	0.08	0.06
allyl methyl sulfide	ns	0.12	1.83
butyl propionate	ns	0.83	0.56
cyclooctene	ns	0.30	0.46
isobutyl butyrate	ns	0.62	0.54
benzaldehyde	ns	0.34	0.14
3-phenylpropylacetate	ns	0.93	1.43
methyl caprylate	ns	2.54	10.2
methyl caproate	ns	8.03	4.95
isobutyl propionate	*	0.92	2.57
ethyl-3-(methylthio)propionate	*	9.06	3.94
heptyl acetate	ns	1.78	3.55
phenethyl acetate	ns	0.30	0.58
amyl acetate*	*	6.0	13.4
methyl butyrate	ns	10.7	2.39
cis-6-nonen-1-ol	ns	2.11	3.75
isovaleronitrile	ns	0.04	0.05
ethyl caproate*	ns	21.3	5.15
cis-3-hexenyl acetate*	*	19.5	180
benzyl acetate*	*	12.7	77.1
ethyl propionate*	ns	49.7	68.4
ethyl isobutyrate*	ns	15.5	8.52
isobutyl acetate*	*	14.8	68.8
propyl acetate*	*	60.0	228
hexyl acetate*	ns	41.1	84.7
ethyl butyrate*	ns	89.4	70.4
butyl acetate*	ns	53.9	166
ethyl-2-methyl butyrate*	*	47.5	20.6
2-methylbutyl acetate*	ns	125	370
methyl 2-methyl butyrate**	*	22.3	855
Total Identified volatiles	*	627	2271
* Signif. contributors	*	568	2053

ns, * and **, not significant and significant F-test at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table B1-5: Stage ZG means of 'Galia' and 'Galia'-type melon aroma compounds measured after 5 days storage at 20°C and harvested at stage ZG, fall 2007.

Aroma Compound	LSD[z]	'Galia'	'MG10183'	'Elario'
propyl butyrate	2.31	5.92	2.63	4.74
tiglic aldehyde		0.01	0.02	0.03
4-methyl-1-cyclohexene		0.21	0.12	0.27
cis-3-hexen-1-ol		0.59	0.23	0.59
trans-cyclodecene		0.66	0.31	0.45
cinnamyl acetate		0.27	0.23	0.32
2-methyl-1-butanol		0.58	0.29	0.52
furfuryl acetate		1.61	1.64	2.30
methyl isobutyrate		0.01	0.02	0.09
allyl methyl sulfide		2.00	2.64	3.25
butyl propionate		3.22	1.55	2.20
cyclooctene		1.18	0.35	0.89
isobutyl butyrate		3.70	1.19	2.82
benzaldehyde	4.61	3.15	5.22	11.5
3-phenylpropylacetate		2.44	1.52	4.98
methyl caprylate		2.02	1.74	3.23
methyl caproate		5.01	2.67	5.69
isobutyl propionate		8.33	5.78	8.30
ethyl-3-(methylthio)propionate		7.91	5.78	6.67
heptyl acetate		2.81	1.83	3.71
phenethyl acetate		17.6	31.6	20.4
amyl acetate		7.41	8.06	10.8
methyl butyrate	3.18	10.7	0.71	0.59
pentyl acetate		21.3	14.3	22.9
cis-6-nonen-1-ol		5.81	4.84	50.5
isovaleronitrile		0.12	0.17	0.11
ethyl caproate		23.1	8.63	12.4
cis-3-hexenyl acetate		0.85	0.83	2.98
benzyl acetate	98.2	39.3	96.1	201
ethyl propionate		55.2	17.1	96.4
ethyl isobutyrate		16.2	0.45	23.8
isobutyl acetate		41.9	31.8	75.3
propyl acetate		209	231	161
hexyl acetate		170	100	246
ethyl butyrate		203	134	187
butyl acetate		179	228	107
ethyl-2-methyl butyrate		87.0	39.2	94.2
2-methylbutyl acetate		242	191	252
methyl 2-methyl butyrate		30.1	13.2	54.1
Total Volatiles	610	1410	1187	1679

[z], Mean separation by Fisher's least significant difference test ($P \leq 0.05$).

Appendix B-2

Table B2-1: Stage ZYG means of 'Galia' and 'Galia'-type melon aroma compounds, fall 2006.

Aroma Compound	Signif.[z]	'Galia'	'MG10183'
propyl butyrate	ns	0.27	0.24
tiglic aldehyde	ns	0.55	0.48
4-methyl-1-cyclohexene	*	0.51	1.75
cis-3-hexen-1-ol	ns	0.32	0.11
trans-cyclodecene	ns	0.14	0.15
cinnamyl acetate	ns	0.11	0.03
2-methyl-1-butanol	ns	0.00	0.00
furfuryl acetate	ns	0.34	0.20
methyl isobutyrate	ns	6.32	4.45
allyl methyl sulfide	*	0.18	2.26
butyl propionate	ns	0.31	0.14
cyclooctene	ns	0.60	0.50
isobutyl butyrate	ns	0.42	0.20
benzaldehyde	ns	0.39	0.93
3-phenylpropylacetate	ns	0.51	0.38
methyl caprylate	ns	1.62	1.64
methyl caproate	ns	10.6	7.33
isobutyl propionate	ns	1.34	0.91
ethyl-3-(methylthio)propionate	ns	1.81	2.33
heptyl acetate	ns	2.43	2.12
phenethyl acetate	ns	1.80	1.76
amyl acetate^	ns	8.19	5.08
methyl butyrate	ns	11.8	13.2
cis-6-nonen-1-ol^	ns	15.0	30.0
isovaleronitrile	*	1.08	0.05
ethyl caproate^	ns	26.7	13.2
cis-3-hexenyl acetate^	ns	18.8	11.8
benzyl acetate^	*	6.45	8.67
ethyl propionate^	ns	42.3	43.5
ethyl isobutyrate	ns	0.00	0.00
isobutyl acetate^	ns	690	538
propyl acetate^	ns	81.7	104
hexyl acetate^	ns	78.6	50.6
ethyl butyrate^	ns	36.8	32.7
butyl acetate^	ns	94.9	71.6
ethyl-2-methylbutyrate^	ns	49.27	25.9
2-methylbutyl acetate^	ns	408	307
methyl 2-methyl butyrate^	ns	46.9	39.1
Total identified volatiles	ns	1646	1322
^Sig. contributors	ns	1584	1269

ns and *, not significant and significant F-test at $P \leq 0.05$, respectively.

Table B2-2: Stage ZYG means of ‘Galia’ and ‘Galia’-type melon aroma compounds, spring 2007.

Aroma Compound	Signif.[z]	‘Galia’	‘MG10183’
propyl butyrate	ns	0.92	1.14
tiglic aldehyde	ns	0.03	0.01
4-methyl-1-cyclohexene	ns	0.07	0.07
cis-3-hexen-1-ol	ns	0.70	0.16
trans-cyclodecene	ns	0.30	0.32
cinnamyl acetate	*	0.08	0.20
2-methyl-1-butanol	*	0.35	0.07
furfuryl acetate	ns	2.23	0.84
methyl isobutyrate	ns	0.17	0.13
allyl methyl sulfide	*	0.12	2.78
butyl propionate	ns	0.97	0.50
cyclooctene	*	1.31	0.52
isobutyl butyrate	ns	0.97	0.78
benzaldehyde	*	0.12	0.03
3-phenylpropylacetate	*	4.43	1.39
methyl caprylate	*	5.37	14.2
methyl caproate	ns	12.9	14.8
isobutyl propionate	ns	5.75	3.96
ethyl-3-(methylthio)propionate	*	2.61	5.70
heptyl acetate	ns	7.80	4.93
phenethyl acetate	ns	0.74	0.25
amyl acetate^	*	21.6	15.4
methyl butyrate	*	6.08	2.38
cis-6-nonen-1-ol^	*	43.0	7.22
isovaleronitrile	ns	12.0	0.06
ethyl caproate^	ns	48.8	44.1
cis-3-hexenyl acetate^	ns	35.7	51.2
benzyl acetate^	*	44.3	84.1
ethyl propionate^	ns	72.6	63.6
ethyl isobutyrate^	*	60.0	0.70
isobutyl acetate^	*	42.1	124.3
propyl acetate^	*	156.5	189.9
hexyl acetate^	ns	76.1	172
ethyl butyrate^	ns	45.1	59.7
butyl acetate^	ns	143	188
ethyl-2-methyl butyrate^	ns	21.9	46.2
2-methylbutyl acetate^	ns	280	313
methyl 2-methyl butyrate^	*	1471	2.7b
Total Volatiles	ns	2616	1418
^Sig. Contributors	*	2565	1266

ns and *, not significant and significant F-test at $P \leq 0.05$, respectively.

Table B2-3: Stage ZYG means of 'Galia' and 'Galia'-type melon aroma compounds, fall 2007.

Aroma Compound	LSD[z]	'Galia'	'MG10183'	'Elario'
propyl butyrate		1.77	0.91	5.96
tiglic aldehyde		0.22	0.14	0.49
4-methyl-1-cyclohexene		0.15	0.07	0.27
cis-3-hexen-1-ol		2.32	0.12	2.42
trans-cyclodecene		2.49	0.28	0.30
cinnamyl acetate		0.78	0.33	0.32
2-methyl-1-butanol		0.78	0.02	7.88
furfuryl acetate		3.59	0.27	1.45
methyl isobutyrate		0.51	0.22	0.20
allyl methyl sulfide		4.24	3.36	4.74
butyl propionate	2.48	3.98	0.67	3.31
cyclooctene		0.91	0.48	2.71
isobutyl butyrate		3.42	0.92	9.75
benzaldehyde	22.6	2.61	1.00	28.4
3-phenylpropylacetate		4.65	0.49	6.02
methyl caprylate		6.75	7.49	7.77
methyl caproate		14.9	8.78	31.3
isobutyl propionate		14.7	3.59	26.5
ethyl-3-(methylthio)propionate	10.6	8.55	4.55	21.5
heptyl acetate	17.5	9.69	2.59	6.71
phenethyl acetate		33.9	21.6	60.5
amyl acetate		16.6	1.52	6.14
methyl butyrate	23.0	12.1	0.19	32.9
pentyl acetate		27.5	9.96	22.9
cis-6-nonen-1-ol	66.3	20.4	17.6	154
isovaleronitrile	0.24	0.53	0.10	0.18
ethyl caproate	35.3	69.6	19.5	58.3
cis-3-hexenyl acetate	2.50	2.99	0.74	5.13
benzyl acetate	48.9	83.8	67.3	267
ethyl propionate	71.5	6.60	4.49	105
ethyl isobutyrate	13.9	40.6	0.15	21.6
isobutyl acetate	27.6	137	1.84	44.9
propyl acetate	97.1	144	9.50	232
hexyl acetate	116	20.6	130	251
ethyl butyrate		87.9	31.7	195
butyl acetate		238	74.3	331
ethyl-2-methyl butyrate	29.6	53.7	17.4	73.4
2-methylbutyl acetate		298	271	320
methyl 2-methyl butyrate	987	6.26	0.7	2433
Total Volatiles	425	1387	715	4790

[z], Mean separation by Fisher's least significant difference test ($P \leq 0.05$).

Table B2-4: Stage ZYG means of 'Galia' and 'Galia'-type melon aroma compounds following storage for 5 days at 20°C, spring 2007.

Aroma Compound	Signif.[z]	'Galia'	'MG10183'
propyl butyrate	*	2.22	1.44
tiglic aldehyde	ns	0.03	0.01
4-methyl-1-cyclohexene	ns	0.08	0.03
cis-3-hexen-1-ol	ns	0.95	0.63
trans-cyclodecene	ns	0.19	0.32
cinnamyl acetate	ns	0.57	0.33
2-methyl-1-butanol	ns	19.2	0.51
furfuryl acetate	ns	1.60	1.71
methyl isobutyrate	ns	0.04	0.04
allyl methyl sulfide	*	0.62	1.49
butyl propionate	ns	1.74	0.68
cyclooctene	ns	0.33	0.69
isobutyl butyrate	*	1.50	0.69
benzaldehyde	ns	0.33	0.08
3-phenylpropylacetate	*	9.75	0.97
methyl caprylate	ns	2.65	5.89
methyl caproate	*	3.12	5.40
isobutyl propionate	ns	5.43	2.98
ethyl-3-(methylthio)propionate	ns	6.63	5.74
heptyl acetate	ns	7.02	2.68
phenethyl acetate	ns	0.13	0.86
amyl acetate^	ns	6.43	4.86
methyl butyrate	ns	14.1	7.53
cis-6-nonen-1-ol	ns	3.66	5.54
isovaleronitrile	ns	3.05	0.05
ethyl caproate^	ns	8.14	10.5
cis-3-hexenyl acetate^	ns	71.3	129
benzyl acetate^	ns	54.3	50.1
ethyl propionate^	ns	59.4	47.2
ethyl isobutyrate^	*	30.51	9.85
isobutyl acetate^	ns	71.3	62.7
propyl acetate^	ns	119	148
hexyl acetate^	ns	178	113
ethyl butyrate^	ns	87.2	69.4
butyl acetate^	ns	142	107
ethyl-2-methyl butyrate^	ns	65.9	33.5
2-methylbutyl acetate^	ns	289	274
methyl 2-methyl butyrate^	ns	803	1119
Total Identified volatiles	ns	2103	2246
^ Signif. contributors	ns	1954	2076

ns and *, not significant and significant F-test at $P \leq 0.05$, respectively.

Table B2-5: Stage ZYG means of 'Galia' and 'Galia'-type melon aroma compounds measured after 5 days storage at 20°C, fall 2007.

Aroma Compound	LSD[z]	'Galia'	'MG10183'	'Elario'
propyl butyrate		8.54	12.0	15.7
tiglic aldehyde		0.02	0.01	0.03
4-methyl-1-cyclohexene		0.18	0.17	0.26
cis-3-hexen-1-ol		3.52	2.06	4.17
trans-cyclodecene		0.44	0.71	0.35
cinnamyl acetate	1.42	3.04	0.25	0.45
2-methyl-1-butanol	17.5	60.3	1.92	1.88
furfuryl acetate		4.93	2.96	3.04
methyl isobutyrate		0.07	0.05	0.12
allyl methyl sulfide		3.47	7.03	4.44
butyl propionate		4.69	5.27	7.46
cyclooctene		0.52	0.67	1.20
isobutyl butyrate		7.00	3.85	7.25
benzaldehyde	4.22	7.60	9.35	23.7
3-phenylpropylacetate	11.9	28.2	3.91	41.3
methyl caprylate		3.53	4.25	3.86
methyl caproate		6.39	6.74	10.4
isobutyl propionate	4.90	11.8	7.67	15.7
ethyl-3-(methylthio)propionate	2.11	5.08	2.18	5.61
heptyl acetate	2.50	13.7	4.92	19.2
phenethyl acetate		23.0	19.7	21.3
amyl acetate		9.60	23.5	27.5
methyl butyrate	18.4	39.8	10.8	27.1
pentyl acetate		51.2	34.7	50.6
cis-6-nonen-1-ol	53.7	25.7	15.2	118.3
isovaleronitrile	3.73	12.6	0.14	0.09
ethyl caproate		22.6	18.3	19.7
cis-3-hexenyl acetate		4.26	2.25	2.39
benzyl acetate	126.3	105	92.8	259
ethyl propionate		28.7	31.5	20.3
ethyl isobutyrate		12.2	3.69	4.92
isobutyl acetate	55.3	120	49.0	33.1
propyl acetate		287	416	246
hexyl acetate	130	578	352	136
ethyl butyrate	44.9	226	294	79.5
butyl acetate		201	187	234
ethyl-2-methyl butyrate		62.4	68.4	64.2
2-methylbutyl acetate	152	551	390	308
methyl 2-methyl butyrate		2356	2368	2564
Total Volatiles		4877	4452	4384

[z], Mean separation by Fisher's least significant difference test ($P \leq 0.05$).

Appendix B-3

Table B3-1: Stage HS means of 'Galia' and 'Galia'-type melon aroma compounds, fall 2006.

Aroma Compound	Signif.[z]	'Galia'	'MG10183'
propyl butyrate	ns	0.28	0.40
tiglic aldehyde	ns	0.97	0.80
4-methyl-1-cyclohexene	*	0.69	1.78
cis-3-hexen-1-ol	ns	0.27	0.09
trans-cyclodecene	ns	0.19	0.10
cinnamyl acetate	*	0.02	0.08
2-methyl-1-butanol	ns	0.01	0.01
furfuryl acetate	ns	0.43	0.39
methyl isobutyrate	ns	5.21	3.03
allyl methyl sulfide	*	0.12	3.57
butyl propionate	ns	0.19	0.21
cyclooctene	ns	0.69	0.49
isobutyl butyrate	ns	0.35	0.29
benzaldehyde	*	0.36	1.10
3-phenylpropylacetate	ns	0.75	0.63
methyl caprylate	ns	1.31	1.83
methyl caproate	ns	8.21	6.86
isobutyl propionate	ns	1.00	1.23
ethyl-3-(methylthio)propionate	ns	1.74	1.97
heptyl acetate	ns	2.64	2.53
phenethyl acetate	ns	3.48	5.45
amyl acetate^	ns	8.29	7.78
methyl butyrate	ns	11.7	10.3
cis-6-nonen-1-ol^	ns	19.9	41.8
isovaleronitrile	ns	3.05	0.02
ethyl caproate^	ns	17.0	12.7
cis-3-hexenyl acetate^	ns	13.6	31.0
benzyl acetate^	ns	4.43	8.97
ethyl propionate^	ns	33.16	25.5
ethyl isobutyrate	ns	0.01	0.01
isobutyl acetate^	ns	769	702
propyl acetate^	ns	91.3	101
hexyl acetate^	ns	73.9	86.1
ethyl butyrate^	ns	29.9	27.2
butyl acetate^	ns	95.1	118
ethyl-2-methylbutyrate^	*	29.9	17.1
2-methylbutyl acetate^	ns	361	405
methyl 2-methyl butyrate^	ns	37.2	29.7
Total identified volatiles	ns	1624	1656
^Sig. contributors	ns	1570	1582

ns and *, not significant and significant F-test at $P \leq 0.05$, respectively.

Table B3-2: Stage HS means of 'Galia' and 'Galia'-type melon aroma compounds, spring 2007.

Aroma Compound	Signif.[z]	'Galia'	'MG10183'
propyl butyrate	ns	0.70	1.16
tiglic aldehyde	ns	0.25	0.10
4-methyl-1-cyclohexene	ns	0.07	0.05
cis-3-hexen-1-ol	ns	0.35	0.34
trans-cyclodecene	ns	0.36	0.23
cinnamyl acetate	*	0.10	0.19
2-methyl-1-butanol	ns	0.24	0.23
furfuryl acetate	ns	1.14	1.51
methyl isobutyrate	ns	0.75	0.42
allyl methyl sulfide	ns	0.09	3.04
butyl propionate	*	0.96	0.35
cyclooctene	ns	0.88	0.53
isobutyl butyrate	ns	0.56	0.38
benzaldehyde	ns	0.36	0.48
3-phenylpropylacetate	ns	2.31	2.22
methyl caprylate	ns	2.85	3.11
methyl caproate	ns	6.61	6.98
isobutyl propionate	ns	4.32	2.21
ethyl-3-(methylthio)propionate	ns	3.82	3.93
heptyl acetate	ns	4.36	5.89
phenethyl acetate	ns	4.30	1.79
amyl acetate^	ns	13.7	26.0
methyl butyrate	ns	14.3	3.87
cis-6-nonen-1-ol^	ns	5.07	13.0
isovaleronitrile	*	13.5	0.08
ethyl caproate^	ns	23.9	23.7
cis-3-hexenyl acetate	*	20.6	142
benzyl acetate^	*	18.8	95.0
ethyl propionate^	ns	42.	75.4
ethyl isobutyrate^	ns	21.8	35.6
isobutyl acetate^	ns	26.7	53.0
propyl acetate^	ns	124	206
hexyl acetate^	ns	106	214
ethyl butyrate^	ns	46.8	39.3
butyl acetate^	ns	135	152
ethyl-2-methyl butyrate^	ns	33.3	102
2-methylbutyl acetate^	ns	173	234
methyl 2-methyl butyrate^	ns	1074	1184
Total Volatiles	ns	1914	2631
^Sig. Contributors	ns	1833	2384

ns and *, not significant and significant F-test at $P \leq 0.05$, respectively.

Table B3-3: Stage HS means of 'Galia' and 'Galia'-type melon aroma compounds, fall 2007.

Aroma Compound	LSD[z]	'Galia'	'MG10183'	'Elario'
propyl butyrate		2.18	1.84	6.08
tiglic aldehyde		0.01	0.06	0.09
4-methyl-1-cyclohexene		0.16	0.05	0.26
cis-3-hexen-1-ol		0.89	3.20	3.93
trans-cyclodecene		0.50	0.27	0.47
cinnamyl acetate		0.43	0.23	1.48
2-methyl-1-butanol		0.39	0.13	2.17
furfuryl acetate		1.79	1.37	2.69
methyl isobutyrate		0.10	0.17	0.24
allyl methyl sulfide		2.06	10.0	7.60
butyl propionate		2.49	3.44	6.70
cyclooctene		0.90	0.46	2.99
isobutyl butyrate		2.09	1.15	7.24
benzaldehyde		1.36	3.84	15.2
3-phenylpropylacetate		4.35	3.20	42.4
methyl caprylate		4.56	5.12	11.7
methyl caproate		13.8	9.33	19.5
isobutyl propionate		9.56	5.18	31.2
ethyl-3-(methylthio)propionate		3.54	5.29	7.02
heptyl acetate		10.1	6.88	31.7
phenethyl acetate		12.7	37.0	63.2
amyl acetate		56.9	28.4	69.3
methyl butyrate		15.1	12.5	20.
pentyl acetate		40.0	24.2	54.3
cis-6-nonen-1-ol	43.5	4.83	20.8	89.1
isovaleronitrile		1.23	0.04	0.18
ethyl caproate		35.5	21.2	36.1
cis-3-hexenyl acetate		2.18	2.24	9.08
benzyl acetate		85.4	125	286
ethyl propionate		17.9	37.6	53.2
ethyl isobutyrate		23.4	6.07	27.1
isobutyl acetate	49.1	147	27.1	99.7
propyl acetate		175	19.5	322
hexyl acetate		177	276	428
ethyl butyrate		62.1	86.5	99.5
butyl acetate		215	86.0	214
ethyl-2-methyl butyrate		65.8	37.1	67.2
2-methylbutyl acetate	205	384	106	384
methyl 2-methyl butyrate	1811	4.15	1.4	1854
Total Volatiles	3297	1545	1007	5702

[z], Mean separation by Fisher's least significant difference test ($P \leq 0.05$).

Table B3-4: Stage HS means of 'Galia' and 'Galia'-type melon aroma compounds following storage for 3 days at 20°C, spring 2007.

Aroma Compound	Signif.[z]	'Galia'	'MG10183'
propyl butyrate	ns	1.59	2.37
tiglic aldehyde	ns	0.06	0.02
4-methyl-1-cyclohexene	ns	0.05	0.10
cis-3-hexen-1-ol	ns	0.60	0.83
trans-cyclodecene	ns	0.16	0.28
cinnamyl acetate	ns	0.90	0.30
2-methyl-1-butanol	ns	3.96	1.11
furfuryl acetate	ns	0.88	1.48
methyl isobutyrate	ns	0.52	0.04
allyl methyl sulfide	ns	0.36	2.23
butyl propionate	ns	1.19	1.43
cyclooctene	ns	2.32	0.48
isobutyl butyrate	ns	1.37	1.21
benzaldehyde	ns	0.93	0.10
3-phenylpropylacetate	ns	4.72	3.73
methyl caprylate	ns	4.73	4.99
methyl caproate	ns	5.36	6.84
isobutyl propionate	ns	5.31	3.94
ethyl-3-(methylthio)propionate	ns	8.44	3.58
heptyl acetate	ns	5.43	9.45
phenethyl acetate	ns	1.48	0.19
amyl acetate^	ns	13.1	16.3
methyl butyrate	ns	13.4	8.98
cis-6-nonen-1-ol	ns	3.79	4.46
isovaleronitrile	ns	12.5	0.25
ethyl caproate^	ns	19.6	16.2
cis-3-hexenyl acetate^	ns	32.9	125
benzyl acetate^	ns	55.9	69.7
ethyl propionate^	ns	38.2	46.5
ethyl isobutyrate^	ns	16.2	19.8
isobutyl acetate^	ns	53.5	47.9
propyl acetate^	ns	135	121
hexyl acetate^	ns	159	166
ethyl butyrate^	ns	98.9	59.1
butyl acetate^	ns	120	126
ethyl-2-methyl butyrate^	ns	42.7	36.5
2-methylbutyl acetate^	ns	261	297
methyl 2-methyl butyrate^	ns	1167	1173
Total Identified volatiles	ns	2294	2395
^ Signif. contributors	ns	2198	2216

ns and *, not significant and significant F-test at $P \leq 0.05$, respectively.

Table B3-5: Stage HS means of 'Galia' and 'Galia'-type melon aroma compounds measured after 3 days storage at 20°C, fall 2007.

Aroma Compound	LSD[z]	'Galia'	'MG10183'	'Elario'
propyl butyrate		6.32	5.65	8.39
tiglic aldehyde		0.05	0.10	0.02
4-methyl-1-cyclohexene		0.32	0.17	0.11
cis-3-hexen-1-ol	0.67	1.68	1.00	2.37
trans-cyclodecene		0.50	0.45	0.23
cinnamyl acetate	0.41	1.06	0.14	0.74
2-methyl-1-butanol		2.72	1.15	6.37
furfuryl acetate		2.37	1.63	1.64
methyl isobutyrate		0.08	0.03	0.03
allyl methyl sulfide	2.52	2.59	8.51	5.65
butyl propionate		3.53	3.38	5.04
cyclooctene		0.76	0.42	1.30
isobutyl butyrate		4.18	2.16	6.24
benzaldehyde	7.35	3.78	6.39	17.1
3-phenylpropylacetate	6.98	9.70	5.00	34.9
methyl caprylate		3.97	3.78	10.1
methyl caproate		9.39	11.0	19.8
isobutyl propionate	3.20	8.96	7.62	13.7
ethyl-3-(methylthio)propionate		4.26	3.42	7.19
heptyl acetate	7.45	13.2	8.07	25.2
phenethyl acetate		17.3	37.2	39.4
amyl acetate		13.8	13.3	17.1
methyl butyrate		34.3	22.8	23.
pentyl acetate		39.4	35.9	45.2
cis-6-nonen-1-ol	20.5	15.7	15.0	69.3
isovaleronitrile		0.56	0.55	0.11
ethyl caproate		38.6	28.9	38.8
cis-3-hexenyl acetate		3.65	3.49	7.55
benzyl acetate	102	82.7	73.2	315
ethyl propionate	45.5	35.8	116	29.9
ethyl isobutyrate		9.35	16.68	16.55
isobutyl acetate		199	43.1	71.3
propyl acetate		222	218	239
hexyl acetate		501	426	463
ethyl butyrate		143	78.2	121
butyl acetate		426	90.3	173
ethyl-2-methyl butyrate		60.7	94.9	62.2
2-methylbutyl acetate		462	218	280
methyl 2-methyl butyrate		1374	1608	2262
Total Volatiles		3756	3208	4440

[z], Mean separation by Fisher's least significant difference test ($P \leq 0.05$).

Appendix B-4

Table B4-1: Stage FS means of wild-type (WT) 'Galia' and 'Galia'-type melon aroma compounds, fall 2006.

Aroma Compound	Signif.[z]	'Galia'	'MG10183'
propyl butyrate	ns	0.63	0.45
tiglic aldehyde	ns	1.80	1.11
4-methyl-1-cyclohexene	ns	0.80	1.33
cis-3-hexen-1-ol	*	0.50	0.09
trans-cyclodecene	*	0.16	0.11
cinnamyl acetate	ns	0.08	0.07
2-methyl-1-butanol	ns	0.007	0.01
furfuryl acetate	ns	0.65	0.66
methyl isobutyrate	*	5.39	3.35
allyl methyl sulfide	ns	1.32	4.23
butyl propionate	ns	0.63	0.26
cyclooctene	ns	0.94	0.75
isobutyl butyrate	ns	0.51	0.24
benzaldehyde	*	0.54	1.33
3-phenylpropylacetate	ns	1.46	0.97
methyl caprylate	ns	1.78	1.58
methyl caproate	ns	7.26	7.46
isobutyl propionate	ns	1.75	1.10
ethyl-3-(methylthio)propionate	ns	2.22	1.37
heptyl acetate	ns	4.29	3.67
phenethyl acetate	ns	3.79	9.20
amyl acetate^	ns	12.1	11.9
methyl butyrate	ns	14.4	13.4
cis-6-nonen-1-ol^	ns	31.9	64.1
isovaleronitrile	*	8.21	0.15
ethyl caproate^	ns	20.4	14.4
cis-3-hexenyl acetate^	ns	35.2	55.6
benzyl acetate^	*	5.95	11.0
ethyl propionate^	ns	33.9	30.6
ethyl isobutyrate	ns	0.03	0.01
isobutyl acetate^	ns	939	949
propyl acetate^	*	106	163
hexyl acetate^	ns	121	144
ethyl butyrate^	ns	39.5	33.4
butyl acetate^	ns	194	220
ethyl-2-methylbutyrate^	*	35.3	20.1
2-methylbutyl acetate^	ns	519	635
methyl 2-methyl butyrate^	ns	45.8	42.1
Total identified volatiles	ns	2190	2446
^Sig. contributors	ns	2104	2337

ns and *, not significant and significant F-test at $P \leq 0.05$, respectively.

Table B4-2: Stage FS means of 'Galia' and 'Galia'-type melon aroma compounds, Spring 2007.

Aroma Compound	Signif.[z]	'Galia'	'MG10183'
propyl butyrate	ns	0.75	0.68
tiglic aldehyde	ns	0.43	0.32
4-methyl-1-cyclohexene	ns	0.03	0.03
cis-3-hexen-1-ol	ns	0.36	0.19
trans-cyclodecene	ns	0.76	0.40
cinnamyl acetate	ns	0.16	0.13
2-methyl-1-butanol	ns	1.06	0.43
furfuryl acetate	*	1.27	1.60
methyl isobutyrate	ns	1.47	1.27
allyl methyl sulfide	ns	0.21	1.46
butyl propionate	*	0.98	0.34
cyclooctene	ns	2.61	1.50
isobutyl butyrate	ns	1.21	0.38
benzaldehyde	ns	1.36	1.14
3-phenylpropylacetate	ns	2.78	1.88
methyl caprylate	ns	2.42	3.54
methyl caproate	ns	8.40	6.44
isobutyl propionate	ns	3.33	2.07
ethyl-3-(methylthio)propionate	*	4.04	2.24
heptyl acetate	ns	7.17	5.30
phenethyl acetate	ns	4.47	4.66
amyl acetate^	ns	16.9	22.0
methyl butyrate	ns	15.2	8.43
cis-6-nonen-1-ol^	ns	7.57	6.72
Isovaleronitrile^	*	6.31	0.06
ethyl caproate^	ns	33.4	19.6
cis-3-hexenyl acetate^	*	20.1	84.4
benzyl acetate^	*	29.0	91.3
ethyl propionate^	ns	50.0	70.7
ethyl isobutyrate^	ns	51.7	33.2
isobutyl acetate^	ns	24.5	51.0
propyl acetate^	ns	49.9	129
hexyl acetate^	ns	84.8	104
ethyl butyrate^	ns	51.5	52.0
butyl acetate^	ns	84.1	122
ethyl-2-methyl butyrate^	ns	66.7	62.6
2-methylbutyl acetate^	ns	130	90.9
methyl 2-methyl butyrate^	ns	273	482
Total Volatiles	ns	1034	1464
^Sig. Contributors	ns	938	1306

ns and *, not significant and significant F-test at $P \leq 0.05$, respectively.

Table B4-3: Stage FS means of 'Galia' and 'Galia'-type muskmelon aroma compounds, fall 2007.

Aroma Compound	LSD[z]	'Galia'	'MG10183'	'Elario'
propyl butyrate	1.8	1.02	2.34	3.58
tiglic aldehyde		0.10	0.12	0.09
4-methyl-1-cyclohexene		0.18	0.29	0.07
cis-3-hexen-1-ol		0.58	0.56	0.81
trans-cyclodecene		0.21	0.51	0.23
cinnamyl acetate	0.39	0.26	0.41	1.72
2-methyl-1-butanol		0.39	0.29	1.09
furfuryl acetate		0.99	1.53	1.59
methyl isobutyrate		0.07	0.25	0.05
allyl methyl sulfide		1.54	7.01	4.25
butyl propionate		1.34	1.63	3.21
cyclooctene		0.54	0.41	0.79
isobutyl butyrate		1.20	1.39	2.33
benzaldehyde		1.16	6.45	6.82
3-phenylpropylacetate	17.4	2.84	4.09	25.1
methyl caprylate		2.09	4.96	4.58
methyl caproate		5.84	14.5	12.3
isobutyl propionate		7.02	6.43	7.65
ethyl-3-(methylthio)propionate		2.67	3.14	3.87
heptyl acetate	7.73	7.17	9.43	20.8
phenethyl acetate		14.4	40.4	23.6
amyl acetate		57.0	38.1	35.8
methyl butyrate		12.4	5.24	0.64
cis-6-nonen-1-ol	16.9	5.73	53.7	59.7
isovaleronitrile	1.83	4.44a	0.08b	0.11b
ethyl caproate		29.1	48.3	34.9
cis-3-hexenyl acetate	2.46	1.30	4.28	4.88
benzyl acetate		87.9	141	168
ethyl propionate		12.0	29.7	14.7
ethyl isobutyrate	22.8	46.5	1.98	24.2
isobutyl acetate		183	44.8	84.6
propyl acetate		170	239	150
hexyl acetate	114	168	336	304
ethyl butyrate		94.2	58.5	81.5
butyl acetate		266	235	138
ethyl-2-methyl butyrate		68.7	44.1	1.32
2-methylbutyl acetate		327	320	214.
methyl 2-methyl butyrate	270.7	34.5	3.68	1199
Total Volatiles		1616	1710	2700
Signif. Contributors	798.8	1542	1568	2559

[z], Mean separation by Fisher's least significant difference test (P=0.05).

Table B4-4: Stage FS means of 'Galia' and 'Galia'-type melon aroma compounds following storage for 2 days at 20°C, spring 2007.

Aroma Compound	Signif.[z]	'Galia'	'MG10183'
propyl butyrate	ns	2.40	1.55
tiglic aldehyde	ns	0.04	0.04
4-methyl-1-cyclohexene	ns	0.03	0.02
cis-3-hexen-1-ol	ns	0.72	0.23
trans-cyclodecene	ns	0.40	0.32
cinnamyl acetate	ns	0.32	0.27
2-methyl-1-butanol	ns	1.43	0.64
furfuryl acetate	ns	1.43	1.10
methyl isobutyrate	ns	0.37	1.23
allyl methyl sulfide	ns	0.17	3.00
butyl propionate	*	2.38	1.10
cyclooctene	ns	1.83	1.44
isobutyl butyrate	ns	1.61	0.83
benzaldehyde	ns	0.78	1.87
3-phenylpropylacetate	*	5.21	2.33
methyl caprylate	ns	2.57	2.84
methyl caproate	ns	7.55	8.00
isobutyl propionate	ns	6.35	4.11
ethyl-3-(methylthio)propionate	ns	6.17	3.07
heptyl acetate	ns	10.4	7.33
phenethyl acetate	ns	1.75	3.57
amyl acetate^	ns	20.0	15.8
methyl butyrate	ns	16.1	12.2
cis-6-nonen-1-ol	ns	5.14	4.90
Isovaleronitrile^	ns	24.3	0.72
ethyl caproate^	ns	22.8	22.2
cis-3-hexenyl acetate^	ns	77.2	176
benzyl acetate^	ns	46.2	66.3
ethyl propionate^	ns	39.7	37.1
ethyl isobutyrate^	ns	41.5	25.2
isobutyl acetate^	ns	36.5	52.7
propyl acetate^	ns	156	159
hexyl acetate^	ns	173	87.4
ethyl butyrate^	ns	103	53.0
butyl acetate^	ns	79.1	97.9
ethyl-2-methyl butyrate^	ns	56.4	50.6
2-methylbutyl acetate^	ns	196	206
methyl 2-methyl butyrate^	ns	1188	1701
Total Identified volatiles	ns	2330	2828
^Signif. contributors	ns	2183	2596

ns and *, not significant and significant F-test at $P \leq 0.05$, respectively.

Table B4-5: Stage FS means of 'Galia' and 'Galia'-type melon aroma compounds measured after 2 days storage at 20°C, fall 2007.

Aroma Compound	LSD[z]	'Galia'	'MG10183'	'Elario'
propyl butyrate		5.25	3.06	6.16
tiglic aldehyde		0.01	0.25	0.01
4-methyl-1-cyclohexene		0.18	0.13	0.27
cis-3-hexen-1-ol	1.27	1.02	0.82	2.85
trans-cyclodecene		0.35	0.55	0.44
cinnamyl acetate	0.99	0.98	0.20	2.17
2-methyl-1-butanol		0.30	0.19	0.44
furfuryl acetate		2.27	1.52	2.41
methyl isobutyrate	0.06	0.02	0.10	0.17
allyl methyl sulfide		2.81	8.66	3.41
butyl propionate		4.16	2.39	3.87
cyclooctene		0.81	0.72	0.30
isobutyl butyrate		3.46	1.46	3.16
benzaldehyde	4.33	2.9	4.99	9.99
3-phenylpropylacetate	6.76	7.94	5.90	33.5
methyl caprylate		4.31	7.24	4.35
methyl caproate		10.3	9.97	13.6
isobutyl propionate	4.22	7.38	6.03	13.3
ethyl-3-(methylthio)propionate	1.20	4.52	3.02b	4.72
heptyl acetate	12.9	20.2	13.6	33.1
phenethyl acetate		32.0	32.2	24.3
amyl acetate		22.8	9.97	8.53
methyl butyrate	26.3	28.4	12.8	0.20
pentyl acetate		27.8	30.8	31.9
cis-6-nonen-1-ol		7.09	42.4	36.9
isovaleronitrile		3.77	0.13	0.05
ethyl caproate		36.5	24.1	31.3
cis-3-hexenyl acetate		3.76	3.21	7.36
benzyl acetate		63.3	104	202
ethyl propionate		8.73	12.0	24.4
ethyl isobutyrate	1.51	10.4	2.44	0.94
isobutyl acetate		63.8	57.6	48.7
propyl acetate		234	159	215
hexyl acetate		486	470	168
ethyl butyrate		116	95.7	350
butyl acetate	ns	127	265	189
ethyl-2-methyl butyrate	ns	59.0	35.0	55.4
2-methylbutyl acetate	ns	392	245	222
methyl 2-methyl butyrate	913	848	1309	2067
Total Volatiles	866	2645	2981	3822

[z], Mean separation by Fisher's least significant difference test (P≤0.05).

Chapter 4 ANOVA Tables

Table 4-2, fall 2006.

Source	DF	Mean square										
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	pH	TTA	Firmness	Ethylene	Respiration
Trt	1	15.1*	0.1	360	5.61	23.1	2	0.0002	0.01	15.6	2.28	15.6
Rep	3	3.45	0.06	121	50.6	22.5	1.08	0.0001	0.00005	30.6	1.12	30.6
Error	3	1.45	0.17	420	232.2	18.3	0.75	0.0001	0.04	9.04	1.11	9.04

*, significant F-test at P≤0.05.

Table 4-2, spring, 2007.

Source	DF	Mean square										
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	pH	TTA	Firmness	Ethylene	Respiration
Trt	1	648**	0.45*	2145.1*	512*	26.6	1.28	0.04	0.0001	331.5*	0.21	83.9
Rep	3	16.5	0.03	56.1	207	8.01	2.3	0.02	0.0001	37.2	0.02	26.5
Error	3	13.3	0.03	172.8	41.7	20.2	0.56	0.01	0.0001	11.9	0.03	38.3

* and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table 4-2, fall, 2007.

Source	DF	Mean square										
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	pH	TTA	Firmness	Ethylene	Respiration
Trt	2	4.11	0.13*	117.5	413.8*	33.2	4.75	0.07	0.00001	94.7	5.78*	19
Rep	2	0.78	0.08	93.8	180.2	12.3	0.78	0.004	0.00001	32.2	0.04	82.1
Error	4	2.11	0.01	22.6	37.9	2.27	1.53	0.05	0.00001	25.5	0.33	87.7

*, significant F-test at P≤0.05.

Table 4-3. TIV, fall 2006.

Table 4-3. TIV, spring 2007.

At Harvest		
Source	DF	Mean square
Trt	3	74249
Rep	1	309861
Error	3	56086

At Harvest			After Storage	
Source	DF	Mean square	DF	Mean square
Trt	1	15.8	1	5408813
Rep	3	173582	3	138839
Error	3	63160	3	11056

Table 4-3. TIV, fall 2007.

	At Harvest		After Storage	
Source	DF	Mean square	DF	Mean square
Trt	2	460095	2*	182146
Rep	2	220817	2	15346
Error	4	148963	4	13855

Table 4-4, fall 2006.

Source	DF	Mean square										
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	pH	TTA	Firmness	Ethylene	Respiration
Trt	1	21.1*	0.13	544.5	98	4.06	0.91	0.00	0.05	103**	99.8*	220.6
Rep	3	1.46	0.03	58.8	44.3	28.2	0.68	0.0004	0.03	9.55	1.28	67
Error	3	1.46	0.08	86.8	100.3	23	1.71	0.00003	0.01	1.86	2.93	56.5

* and **, significant F-test at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 4-4., spring 2007.

Source	DF	Mean square										
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	pH	TTA	Firmness	Ethylene	Respiration
Trt	1	72*	0.25*	979*	420.5*	12.8	1.53	0.11*	0.001	1369**	10.6	91.1
Rep	3	3.5	0.04	111.2	100.5	32.5	0.61	0.01	0.0001	0.55	4.5	41.4
Error	3	4.33	0.01	59.9	25.8	4.66	0.53	0.01	0.0002	3.27	3.1	2.44

*and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table 4-4., fall 2007.

Source	DF	Mean square										
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	pH	TA	Firmness	Ethylene	Respiration
Trt	2	10.1*	0.31	353.2	438.4	91.8	3.92	0.01	0.0008**	251.8	5.78*	11.8
Rep	2	0.78	0.04	48.1	89.9	12.9	0.68	0.02	0.00001	83.1	0.04	14.6
Error	4	1.28	0.08	191.6	82.9	28.6	1.19	0.02	0.00004	38.4	0.33	35.8

*and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table 4-5. TIV, fall 2006.

At Harvest		
Source	DF	Mean square
Trt	3	209217
Rep	1	125334
Error	3	292381

Table 4-5. TIV, spring 2007.

At Harvest			After Storage	
Source	DF	Mean square	DF	Mean square
Trt	1	2871429	1	40792
Rep	3	170127	3	90822
Error	3	292802	3	177041

Table 4-5. TIV, fall 2007.

	At Harvest		After Storage	
Source	DF	Mean square	DF	Mean square
Trt**	2	14314161	2	2135686
Rep	2	445716	2	774529
Error	4	680519	4	65565

**, significant F-test at P≤0.01.

Table 4-6, fall 2006.

Source	DF	Mean square										
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	pH	TTA	Firmness	Ethylene	Respiration
Trt	1	66.1**	0.08	84.5	215.3*	10.6	0.78	0.0000	0.00001	76.6	184.4*	10.4
Rep	3	8.79	0.13	130.3	137.1	23.7	1.53	0.0002	0.01035	5.32	26.8	14.2
Error	3	1.13	0.04	64.6	13.6	42.5	1.78	0.00003	0.00301	12.4	16.3	5.04

*and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table 4-6, spring 2007.

Source	DF	Mean square										
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	pH	TTA	Firmness	Ethylene	Respiration
Trt	1	60.5**	0.18	780.1*	318.8*	49.0	3.78	0.002	0.0002	548.6**	30.4	7.8
Rep	3	5.0	0.003	24.5	13.3	1.54	0.6	0.008	0.0002	1.04	2.22	2.27
Error	3	0.17	0.02	52.5	24.1	13.6	0.08	0.003	0.00003	2.7	4.87	4.19

*and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table 4-6, fall 2007.

Source	DF	Mean square										
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	pH	TA	Firmness	Ethylene	Respiration
Trt	2	5.44*	0.39	258.5	615.1	54.1	3.48*	0.03	0.0004	134.8*	5.67	25.6*
Rep	2	0.11	0.01	3.94	17.2	8.34	0.11	0.05	0.0002	24.2	0.15	5.89
Error	4	0.28	0.07	161.1	88	13.4	0.21	0.15	0.0003	8.12	1.24	1.43

*, significant F-test at P≤0.05.

Table 4-7. TIV, fall 2006.

	At Harvest	
Source	DF	Mean square
Trt	3	2068
Rep	1	218821
Error	3	155854

Table 4-7. TIV, spring 2007.

	At Harvest		After Storage	
Source	DF	Mean square	DF	Mean square
Trt	1	1028317	1	20423
Rep	3	52498	3	278739
Error	3	521031	3	271895

Table 4-7. TIV, fall 2007.

	At Harvest		After Storage	
Source	DF	Mean square	DF	Mean square
Trt*	2	19804441	2	1141930
Rep	2	252598	2	197615
Error	4	2115017	4	510556

*, significant F-test at P≤0.05.

Table 4-8. TIV, fall 2006.

Source	DF	Mean square										
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	pH	TTA	Firmness	Ethylene	Respiration
Trt	1	1.13	0.13	214.2	2.65*	0.28	6.3	0.0002	0.002	68.4	10.1	1.2
Rep	3	0.46	0.03	72.1	32	3.58	0.64	0.00008	0.01	7.77	7.99	2.22
Error	3	0.13	0.09	59.3	69.2	11.9	0.37	0.001	0.01	22	10.9	9.87

*, significant F-test at P≤0.05.

Table 4-8. TIV, spring 2007.

Source	DF	Mean square										
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	pH	TTA	Firmness	Ethylene	Respiration
Trt	1	12.5**	0.18	743.1**	268*	34.9	4.81	0.00001	0.0006	267.4**	4.35	0.05
Rep	3	0.33	0.01	33.2	22.3	14	0.06	0.002	0.0001	3.89	1.07	3.86
Error	3	0.17	0.003	6.76	8.49	7.3	0.27	0.003	0.0001	3.3	0.68	1.19

*and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table 4-8. TIV, fall 2007.

Source	DF	Mean square										
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	pH	TA	Firmness	Ethylene	Respiration
Trt	2	4**	0.12**	141.3**	292.9*	18.8	3.63*	0.06	0.002**	68.4*	14.9	4.91
Rep	2	1	0.03	38.1	17.3	0.44	0.15	0.02	0.001	0.02	3.22	4.74
Error	4	0	0.01	4.51	39.5	6.04	0.33	0.02	0.0001	9.09	2.24	11.1

*and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table 4-9. TIV, fall 2006.

	At Harvest	
Source	DF	Mean square
Trt	3	130812
Rep	1	286230
Error	3	29992

Table 4-9. TIV, spring 2007.

	At Harvest		After Storage	
Source	DF	Mean square	DF	Mean square
Trt	1	370536	1	497379
Rep	3	285832	3	79320
Error	3	142306	3	1311701

Table 4-9. TIV, fall 2007.

	At Harvest		After Storage	
Source	DF	Mean square	DF	Mean square
Trt	2	1081594	2	1102426
Rep	2	213342	2	153950
Error	4	157529	4	155945

Table 4-11, spring 2008.

Source	DF	Mean square								
		SSC	Firmness	Methyl isobutyrate	Allyl methyl sulfide	ethyl propionate	Propyl acetate	Methyl butyrate	Isovaleronitrile	2-methyl 1-butanol
Trt	3	6.57**	524**	24.3*	0.18	2239	13.1	59	148**	110**
Rep	2	0.08	13.8	1.36	0.06	485	14.5	21.7	6.88	0.42
Error	6	0.21	4.34	4.54	0.33	534	6.35	13.7	6.96	1.99

*and **, significant F-test at P≤0.05 and P≤0.01, respectively.

Table 4-11, spring 2008.

Source	DF	Mean square								
		Tiglic aldehyde	4-methyl-1-cyclohexene	Ethyl isobutyrate	Isobutyl acetate	Methyl 2-methyl butyrate	Ethyl butyrate	Butyl acetate	Ethyl 2-methyl butyrate	Cis-3-hexen 1-ol
Trt	3	2.13**	0.002	5213**	347	1495818**	628	1148	2292	1.71**
Rep	2	0.14	0.002	223	89.4	9062	38.2	2.22	1651	0.17
Error	6	0.11	0.001	115	23.4	41438	75.1	386	786	0.16

*and **, significant F-test at P≤0.01.

Table 4-11, spring 2008.

Source	DF	Mean square								
		Isobutyl propionate	2-methyl butyl acetate	Propyl butyrate	Butyl propionate	Amyl acetate	Cyclooctene	Methyl caproate	Isobutyl butyrate	Benzaldehyde
Trt	3	9.08*	6078	2.59**	2.08*	569**	0.17	9.17	3.28**	13.8*
Rep	2	1.38	4388	0.16	0.05	51.6	0.15	5.7	0.38	0.16
Error	6	1.47	3662	0.17	0.31	46.5	0.11	2.68	0.22	1.45

*and **, significant F-test at P≤0.05 and P≤0.01, respecti vely.

Table 4-11, spring 2008.

Source	DF	Mean square								
		Ethyl caproate	Cis-3-hexenyl acetate	Hexyl acetate	Ethyl-3-(methylthio) propionate	Heptyl acetate	Methyl caprylate	Trans-cyclodecene	Cis-6-nonen-1-ol	3-phenyl propylacetate
Trt	3	2834**	2078*	218	3.93**	490**	29.6	0.15**	11220**	366**
Rep	2	123	213	108	0.17	22.1	7.83	0.001	732	7.16
Error	6	106	375	327	0.14	13.1	8.43	0.002	832	4.56

*and **, significant F-test at P≤0.05 and P≤0.01, respecti vely.

Table 4-11, spring 2008.

Source	DF	Mean square				
		Benzyl acetate	Phenethyl acetate	Cinnamyl acetate	Total volatiles	Signif. Contribs.
Trt	3	2402	139**	0.05*	1902374**	1681572
Rep	2	1356	1.37	0.01	7736	5165
Error	6	820	1.64	0.01	58115	59794

*and **, significant F-test at P≤0.05 and P≤0.01, respectively.

APPENDIX C
CHAPTER 5 ANOVA TABLES

Table C5-2.

Source	DF	Mean square										
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	TTA	pH	Firmness	Ethylene	Respiration
Line	3	40.3*	0.22*	416*	260*	60.8*	11.6*	0.01*	0.12	11.2	0.2	46.3
Seas	2	1919**	0.88**	1911**	1412**	240**	38.4**	0.001**	0.26**	34.0	4.57**	239**
L*S	6	126	0.03	55.1	23.1	18.8	3.07	0.001	0.08	18.0	0.44	25.4
Seas(rep)	6	219	0.06	39.2	42.3	7.2	2.27	0.001	0.1	26.8	0.13	60.5
Error	18	8.39	0.06	95.7	53.4	7.35	1.18	0.0002	0.06	55.2	0.25	42.7

*and **, significant F-test at P≤0.05 and P≤0.01, respecti vely.

Table C5-3.

Source	DF	Mean square										
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	TTA	pH	Firmness	Ethylene	Respiration
Line	3	38.4**	0.04	54.9	89.2	14.4	1.38**	0.001**	0.44**	449**	37.3**	36.9
Seas	2	352*	0.71**	1461**	1387**	46.6*	23.1	0.001*	0.29**	407**	7.7	154**
L*S	6	1.93	0.08	164.6	60	14.6	1.84	0.001**	0.16**	5.8	16.8*	88.7**
Seas(rep)	6	2.53	0.05	97.8	29.7	7.63	1.17	0.001	0.03	20.2	5.94	26.3
Error	18	2.75	0.04	76.4	41.8	10.1	1.06	0.0001	0.01	14.3	4.36	21.9

*and **, significant F-test at P≤0.05 and P≤0.01, respecti vely.

Table C5-5.

Source	DF	Mean square										
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	TTA	pH	Firmness	Ethylene	Respiration
Line	3	26.1**	0.01	43.2	10	8.18	2.32	0.0002	0.2**	93.6**	28.2**	22.8
Seas	2	342**	1.33**	1868**	2077**	170**	15.8**	0.001**	0.4**	174**	34.1**	132**
L*S	6	7.03	0.1	178	102	37.2	2.96	0.001**	0.14**	17.5	18.5**	46.6
Seas(rep)	6	6.14	0.05	53.9	38.7	2.83	0.41	0.0001	0.03	14.8	1.97	14.1
Error	18	3.47	0.05	79	48.5	18.9	1.15	0.0002	0.02	9.1	2.73	23.1

*and **, significant F-test at P≤0.05 and P≤0.01, respecti vely.

Table C5-7.

Source	DF	Mean square										
		Days to harvest	Weight	Length	Width	Flesh thickness	SSC	TTA	pH	Firmness	Ethylene	Respiration
Line	3	35**	0.19**	351**	111*	32.9*	11.9**	0.001	0.07	46.3**	14.6	25.5
Seas	2	335**	0.91**	1518**	1449**	97.3**	25.9**	0.0005**	0.28*	86.8*	64.9**	58.6**
L*S	6	4.64*	0.06*	149*	73.2	7.76	1.56	0.002	0.06	6.32	9.49	10.1
Seas(rep)	6	1.58	0.005	9.55	12.6	2.58	0.81	0.0003	0.05	10.3	1.62	5.58
Error	18	1.62	0.02	50.4	33.1	8.53	1.37	0.0002	0.05	4.68	7.25	7.71

*and **, significant F-test at P≤0.05 and P≤0.01, respecti vely.

Table C5-9.

Source	DF	Mean square			
		Stage ZG TIV at Harvest.	Stage ZYG TIV at Harvest	Stage HS TIV at Harvest.	Stage FS TIV at Harvest.
Line	3	6198	1761672**	1621190	363025
Seas	2	52671	1285478*	2033139	6685365*
L*S	6	50924	1092316**	1134116	325884
Seas(rep)	6	22444	162349	625156	796406
Error	18	31354	122644	615920	263086

*and **, significant F-test at P≤0.05 and P≤0.01, respecti vely.

Table C5-10.

Source	DF	Mean square			
		Stage ZG TIV after Storage, Fa07	Stage ZYG TIV after Storage, Fa07.	Stage HS TIV after Storage, Fa07.	Stage FS TIV after Storage, Fa07.
Rep	2	1104	81327	49234	55406
Line*	2	1715599*	8894705**	7273443**	4800206**
Error	4	72464	238363	238152	180146

*and **, significant F-test at P≤0.05 and P≤0.01, respecti vely.

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BIOGRAPHICAL SKETCH

Jeanmarie Mink Harty (née Mitchell) was born in 1975 to, Edward A. and Eileen J. Mitchell. Raised in Collegeville, PA, Jeanmarie was active throughout elementary and high school in her local 4-H Club, which sparked her interest in agriculture. She received her Bachelor of Science degree in turfgrass science from the Pennsylvania State University in 1997. Instead of becoming a golf course superintendent, she traveled throughout Europe in fall 1997 and joined the U.S. Peace Corps in May 1998. As a Schools' Self-Reliance Project Officer in the Kingdom of Lesotho, Southern Africa, Jeanmarie worked to ensure food-security at local primary schools. After successfully completing Peace Corps in June 2000, Jeanmarie's love for Lesotho led her to remain in the country for a third year and work as the Agriculture Director of a local NGO called GROW. While at GROW, Jeanmarie assisted and educated farmers in sustainable agricultural practices and worked to improve the Basotho's livelihoods. In 2001, Jeanmarie left Lesotho and moved to St. Croix, USVI where she was a Research Analyst II at the Agricultural Experiment Station of the University of the Virgin Islands (UVI). At UVI, Jeanmarie conducted research on sustainable agricultural practices. While on St. Croix, Jeanmarie met her husband, Cheyenne. In fall, 2003, Jeanmarie and Cheyenne moved to Gainesville, Florida where Jeanmarie was accepted to graduate school at the University of Florida, Department of Horticultural Sciences. Under the superior guidance of Daniel J. Cantliffe, Jeanmarie successfully learned how to grow melons, conduct research, and meet the requirements of a Ph.D. Upon graduation, it is the goal of Jeanmarie to continue to work in agriculture with a focus on improving production, quality, and postharvest practices for growers world-wide.