

STUDIES ON FRAGRANCE, VASE LIFE AND ETHYLENE REGULATION OF VOLATILE  
PRODUCTION IN ROSE FLOWERS

By

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This work is dedicated to my husband Ernesto and my son Nicolás

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This research determined the amounts of the main volatile compounds produced by fragrant and non-fragrant cut rose cultivars during vase life together with flower ethylene production, sensitivity respiration rate, weight loss and opening. No relationship was observed between the amount of volatile compounds produced and the longevity of the flower. Short vase life of fragrant rose cultivars was directly related with endogenous ethylene production, external ethylene sensitivity and quick fresh weight loss during vase life. Alternatively, fragrant roses with ethylene production below detection levels, insensitivity to external ethylene and slow fresh weight loss during vase life had acceptable vase lives. Respiration rate was not directly related with vase life of the flowers. Ethylene did not play a role on the regulation of volatile emission of the main biosynthetic pathways in rose flowers. Endogenous ethylene was very low in most of the cultivars and it was not autocatalytic. Ethylene had differential effect within flower petals since it regulated petal abscission and had no effect on volatile emission of fragrant rose flowers. Specific volatile compounds were produced in different amounts by each cut rose cultivar resulting in variable types of fragrance intensities on each flower. This research studied production of volatiles in relation to human detection and rose fragrance preferences. Human

panelists detected different rose fragrance intensities that corresponded to the total amount of volatiles produced by each cultivar. Panelists gave the highest rank to a rose fragrance constituted mainly by 2-phenylethanol, 3,5 dimethoxytoluene, (-) transcaryophyllene, geranyl acetate and neryl acetate. The ability of human subjects to detect different fragrance intensities between flowers from the same cultivars at different stages of development depended on the type of volatile, its amount and the composition given by the specific volatile compounds emitted. The results of the volatile compounds emitted by rose flowers together with the ethylene production and sensitivity that lead to short vase life and the human ability to detect fragrance intensities and preferences can be used by rose breeders and flower producers to select long lasting fragrant roses.

## CHAPTER 1 LITERATURE REVIEW

### **Introduction**

Fragrance is one of the most desirable traits of roses and fragrant roses are considered a high quality consumer luxury product that is in increasing demand. However, fragrance has not been a trait that predominates on the currently available cut rose cultivars. The reason for the loss of fragrance is still unknown (Schulz, 2003; Verhoeven et al., 2003). Most of the cut rose cultivars come from *Hybrid Tea* roses which have inadvertently lost their fragrance during the breeding process since characters such as vase life and flower form have been more intensively selected (Korban, 2007). Rose breeders have tried to explain the loss of fragrance during the breeding process. They agreed that fragrance is a characteristic difficult to predict. Two scented parents can produce an unscented or unpleasantly scented offspring (Cherri-Martin et al., 2007). For example, studies on the volatile composition of an *Hybrid Tea* progeny obtained from *Hybrid Tea* parents (Anna and Bo) that emitted European and Chinese scents respectively, demonstrated that even though each of the parents had a pleasant fragrance, a large proportion of the progeny lacked the volatile compounds that produced the pleasant fragrance of the parents. Moreover, it has been hard for rose breeders to obtain the combination of fragrance and good vase life in rose flowers using conventional breeding techniques (Bent, 2007). For years rose fragrance has been negatively correlated with vase life (Barletta, 1995). Studies performed in rose fragrance have described the volatile compounds emitted by rose flowers, some of their biochemical pathways and genes however these studies have not related the amount of volatiles produced by cut rose cultivars with flower vase life (Flament et al., 1993; Oka et al., 1999; Kim et al., 2000; Lavid et al., 2002; Scalliet et al., 2002; Joichi et al., 2005). The purpose of this research was to study the vase life of a variety of commercially available fragrant and non-fragrant cut rose cultivars in

relation with the longevity of the flower. The amount of volatile compounds produced by the flower was study in relation with factors that affect vase life such as ethylene production, sensitivity and respiration rate. This research was important as it described volatile emission and associated this process with flower longevity.

Then the research focused on studying the possible ethylene effects on volatile emission on cut rose cultivars. Understanding ethylene effects allows generating alternatives to control it. Volatile emission and ethylene effects were studied on commercial cut roses describing the processes as they occur at the consumer level. Volatile emission and ethylene effects were also studied on locally grown roses determining volatile emission during all stages of rose flower development without possible shipping and handling effects. This work is important because it provides the physiological guidelines to select cut rose cultivars with long vase life.

The volatile emission from rose flowers was related with the capacity of human subjects to detect differences in fragrant intensity from commercially available cut rose cultivars and from the same cultivar with different stages of development. The preference for rose fragrance from these rose samples was determined by scent panel tests. The panel tests were important because they characterized the differences in rose fragrance intensities that human subjects were able to detect and the fragrance type that they liked most. It is possible to modify the fragrant note of a flower by introducing the genes that encode the enzymes responsible for producing the specific substrates and also by optimizing the expression of the *novo* biosynthetic genes. Studies performed in flowers species such as petunia (Lucker et al., 2001) and carnation (Lavy et al., 2002) have demonstrated that the fragrance biosynthetic genes can be introduced into a transgenic plant leading to the modification of the floral scent. However, the work of making a flower fragrant is not complete until the resulting fragrance is detected and liked by human

subjects. In some cases, even if the fragrant gene is present in the new species it does not generate the desired fragrance because the volatile production in the transgenic plant cannot be detected by the human nose. This result was observed when carnation flowers were transformed with the *Clarkia breweri* linalool synthase gene (Lavy et al., 2002). The information of this research is important because it describes the volatile compounds and its amounts emitted by flowers and relates it with the ability to detect them by human subjects. This information is critical for breeders interested on selecting roses with fragrances that are detected and liked by humans.

### **History of Cultivation of Roses**

Roses (*Rosa*) have a long history of cultivation since they have been cultivated as ornamentals for more than 2,000 years. Roses have been cultivated since the times of Chinese dynasties (141-87 BC) where wild roses were common in the gardens of the imperial palace. Moreover, the importance of roses since ancient times is proven by the existence of rose diagrams in the ancient Chinese paintings, pottery and books. Chinese roses were introduced to Europe in 1792 and its importance in Europe can be traced back to the Renaissance by its presence in European paintings (Guoliang, 2003).

After the 1800's, the cultivation of roses increased dramatically due to intense breeding programs. Rose breeding focused on 5 major industries: garden, cut flower, house plant (pot rose), oil production and medicinal use. The cultivation of cut roses evolved rapidly since the first time of cultivation. The first cut roses started to be cultivated outdoors in several European countries such as Germany, France and Italy where the climate was favorable for their production. Thereafter, the modern cut flower industry started in 1896 in Holland when the first greenhouse was constructed. The cultivars cultivated at that time were short lived and the production was concentrated in local markets. The cut flower industry rapidly grew, expanding

in Europe and the USA. The quality of the roses rapidly increased due to extensive testing of new cultivars. Later, vase life and productivity started to increase due to improved breeding techniques that were being used intensively in the 1950's (Marriott, 2003).

Currently, breeding for cut rose cultivars continues to be intensive. During all these years of breeding selections have focused on characteristics such as long vase life, high productivity and novel colors. Fragrance has not been in the list of top desirable characters and consequently only a few cultivars are highly fragrant (Marriott, 2003).

### **Breeding Rose Cultivars**

Roses belong to the family Rosaceae and are classified in the genus *Rosa* which includes from 150 to 300 species and thousands of cultivars. Most of the modern roses are complex hybrids derived from only 10-20 genotypes of the former species. It is thought that the selection of this 10-20 species was based on easy availability, attractive characters or favorable seed set (Korban, 2007). Therefore, only a restricted number of progenitors are available to obtain desirable seedlings with specific characteristics for cut roses leading to a reduction in genetic variation. Wild species are often diploids and almost all cultivated roses are tetraploids (Korban, 2007).

Cultivars with new characteristics such as flower color and flowers shape were created by spontaneous mutations (de Vries et al., 1996). Characteristics such as plant morphology, recurrent flowering and scent have been introduced to new cultivars by rose genetics and molecular biology techniques. Currently, breeding greenhouse roses is more prevalent, due to the fact that the royalties for greenhouse cultivars are 3 to 5 times that for garden roses. Greenhouse roses can be classified as small-flower, medium-large flower and large flower cultivars. Cultivars in general show more uniformity in bud shape, flower shape, flower size, number of petals and stem length and thorniness within each flower color (de Vries et al., 1996).



Currently, breeding efforts are concentrated towards cultivars that allow a reduction in fungicide and pesticide applications and water optimization parameters that are critical for the environment (de Vries et al., 1996). However, other characters such as fragrance that have a high economic value are also being studied to be introduced in the cultivars (Pichersky et al., 2007). Probably the inclusion of fragrance in new cut rose cultivars in the future will be performed by introduction of fragrant genes using biotechnological tools. The aim will be to introduce fragrance without compromising other important characters such as long vase life.

Recent studies have shown that roses are an important source of novel genes involved in the biosynthesis of important volatile compounds (Guterman et al., 2002). There is a rose database with 877 genes from which some of them accumulate only in petals and stamens and are involved in the biosynthesis of volatile compounds characteristic of the rose floral scent (Channeliere et al., 2002). As an example, two novel fragrance related genes (OOMT1 and OOMT2) were described by (Guterman et al., 2002). These genes are involved in the production of 3,5 dimethoxytoluene a volatile compound that is a major contributor to the scent of *Rosa hybrida* cultivars. These genes are localized in the petals and are more abundant in the adaxial epidermal cells. Its regulation is critical in the evolution of scent production (Scalliet et al., 2006). Another gene identified as RhAAT1 is expressed in developing petals and its expression coincides with peak scent emission in floral petal tissue (Shalit et al., 2003). The discovery of these rose fragrance genes has provided the bases for the possibility of using biotechnology to create very fragrant cut rose cultivars. However, physiological studies are needed to introduce these genes without compromising other important characteristics such as vase life, productivity and color.

Even though rose fragrance contributes a significant commercial value to the cut rose crop, sufficient studies have not yet been performed to understand the physiological and biochemical

relationships between fragrance and postharvest performance. Thus, there is no solid genetic basis established for breeders to knowingly incorporate fragrance into commercial cultivars without affecting postharvest performance. In order to describe any possible relationship between flower fragrance and vase life, this study will evaluate the fragrance by determining the volatile compounds emitted from several commercial cultivars and will relate this production with the longevity of the flower under conventional postharvest conditions.

### **Biosynthesis of Fragrant Compounds**

Fragrance of flowers results from the biosynthesis and emission of low molecular weight compounds. These compounds have a high vapor pressure sufficient to be released and dispersed into the air under room temperature conditions. Most of the volatile compounds are products of three main biosynthetic pathways: terpenoids, fatty acid derivatives and phenylpropanoids (Knudsen et al., 1993). This study will focus on the volatile compounds from these main biosynthetic pathways on different rose cultivars.

#### **Terpenes**

Terpenes are produced by the terpenoid pathway which utilizes Acetyl CoA to produce isopentenyl diphosphate (IPP) one of the precursors of all terpenes. IPP is synthesized in the cytosol by the mevalonic acid (MVA) pathway and in the plastid is derived from pyruvate and glyceraldehyde-3-phosphate via the methyl-erythritol-phosphate (MEP) pathway. The other main precursor for terpenes is dimethylallyl diphosphate (DMAPP) which is synthesized from the MAP pathway in the plastids (Lichtenthaler et al., 1997). Terpenes are the major class of secondary metabolites present in plants. These compounds are generally insoluble in water and can be classified according to the number of carbons present in its structure as monoterpenes (10 carbons), sesquiterpenes (15 carbons) and diterpenes (20 carbons) (Taiz et al., 2002; Dudareva et al., 2006b). Some of the enzymes involved in the further biosynthesis of specific terpenes have

been characterized and these include prenyltransferases, farnesyl pyrophosphate synthase (FPPS), geranyl pyrophosphate synthase (GPPS) and terpene synthases (TPS)(Dudareva et al., 2006a). These enzymes produced a wide range of monoterpenes and sesquiterpenes. Terpene synthases have the ability to produce multiple products from one substrate (Taiz et al., 2002). Apart from the regular terpenoids, plants can produce irregular acyclic terpenoids such as homoterpenes and terpenoids with carbon skeletons ranging from C<sub>8</sub> to C<sub>18</sub> derived from carotenoids (C<sub>40</sub>). Some common volatile compounds derived from carotenoids include β – ionone and β – damascenone, compounds that are present in the fragrant profiles of *Rosa hybrida* (Dudareva et al., 2006b).

### **Fatty Acid Derivates**

Volatiles derived from fatty acids constitute the second largest group of floral volatiles. The main precursors of these volatiles are membrane lipids such as linoleic acid or linolenic acid. The synthesis of fatty acid derivate volatiles occurs by the lipogenase pathway (Feussner et al., 2002). Lipogenases (LOX) are the main enzymes involve in the synthesis of these compounds. Examples of volatiles synthesized via this pathway include *trans*-2-hexenal, *cis* -3-hexenol and methyl jasmonate (Dudareva et al., 2006b).

### **Phenylpropanioids**

The volatiles produced in this pathway are volatile compounds mainly involved in plant reproduction and defense. The precursor for phenylpropanoids is phenylalanine (Phe) and the main enzyme that catalyzes the biosynthetic reaction is l-phenylalanine ammonia-lyase (PAL). A side branch of the general phenylpropanoid pathway produces other important volatile compounds known as benzenoids which originate from *trans*-cinnamic acid (Boatright et al., 2004). Some of the compounds originated from this pathway include eugenol, phenyl ethanol and methyl benzoate. Besides phenylalanine other amino acids such as alanine, valine, leucine,

isoleucine and methionine are precursors of more volatile compounds including aldehydes, alcohols and esters (Dudareva et al., 2006b).

Even though three main biosynthetic pathways exist, the diversity of volatile compounds emitted from flowers is very large. For example, more than 400 floral volatile compounds have been identified for roses (Flament et al., 1993). The enormous diversity of volatile compounds comes from the enzymatic modifications such as hydroxylations, acetylations and methylations that occur in conjunction with the main biosynthetic pathways.

### **Fragrance Biosynthesis in Roses**

Rose fragrance is a trait that is specific for each rose species. Humans associate rose fragrance with the typical fragrance of “old” European garden roses. But there is more than just the fragrance of a garden rose. The fragrance of roses can be classified in five major fragrance groups: fruity, myrrh, old rose, musk and tea (Bent, 2007). Each group is characterized by the composition of different volatile compounds produced in different amounts. The fragrant note of a rose comes from the emission of a wide range of volatile compounds including alcohols, aldehydes, alkenes, monoterpenes, sesquiterpenes, ester, ethers and ketones (Flament et al., 1993; Kim et al., 2000). Some of these volatile compounds are produced in certain rose species while some are not present thus making each rose species very specific in its fragrance note. What makes a volatile to be emitted in a rose species and not in another are the expression of specific genes that code for enzymes and the presence of the required substrates. The enzyme that catalyzes the conversion of one compound may be present in rose species and this species emits the compound but when the enzyme is absent the rose species does not emit the specific compound. All the enzymes that play a role in the biosynthesis of rose volatiles are present in the petals that are the main site of emission of these compounds (Guterman et al., 2002; Lavid et al., 2002).

The biosynthetic pathways from some of the major components of rose fragrance have been studied in detail expanding the knowledge of the main genes and enzymes that contribute to the scent of this special flower (Lavid et al., 2002; Scalliet et al., 2002; Watanabe et al., 2002; Wu et al., 2004; Scalliet et al., 2006). For example, one of the major volatiles of European rose scent such as *Rosa damascene* is 2-phenylethanol. Two hypothetical biochemical pathways have been suggested for the production of 2-phenylethanol. One is the production of this compound from L-phenylalanine mainly by its conversion into phenylpyruvic acid which later transforms into either phenylacetaldehyde or phenyllactic acid which then converge into 2-phenylethanol (Watanabe et al., 2002). A more recent study suggested the conversion of 2-phenylethanol from shikimic acid via chorismic acid, L-phenylalanine and phenylacetaldehyde (Yang et al., 2009). Another important component of rose scent is 3,5 dimethoxytoluene (orcinol dimethyl ether) which is present in almost all the modern Hybrid Tea roses and gives the characteristic tea note fragrance for this group (Scalliet et al., 2008). The biosynthesis of this compound occurs by the action of O-methyltransferases (OMT) which act on non methylated precursors such as orcinol, orcinol monomethyl ether guaiacil and eugenol (Lavid et al., 2002; Scalliet et al., 2002; Wu et al., 2003). Volatile acetate esters such as geranyl acetate are other major components of the fragrant note of *Rosa hybrida*. These compounds are produced from the monoterpene geraniol by the action of RhAAT1 enzyme (Shalit et al., 2003). The volatile 1,3,5-trimethoxybenzene a major compound of *Rosa Chinensis* scent is synthesized in three methylation steps from phloroglucinol. The first step is catalyzed by (POMT) phloroglucinol o-methyltransferase enzyme and the second and third are catalyzed by OMT (Wu et al., 2004). Besides the biochemical and genetic approaches, volatile compounds have been studied at the level of regulation of their production and emission.

## Regulation of Volatile Biosynthesis and Emission

Most volatile compounds are synthesized *de novo* in the tissues from which they are emitted (Dudareva et al., 2000). The emission of volatiles generally occurs at very specific tissues, at certain stages of development and at specific times of the day. The mechanisms that regulate its emission are very complex, and involve the expression of certain genes, the activity of specific enzymes and the availability of substrates.

In general, volatile production and emission are regulated spatially and temporarily. The spatial regulation is performed by the presence of specific structures involved in the production and emission of certain volatile compounds. Volatiles are produced exclusively in the cells of the epidermal layer of the parts of the plant so they can easily be released into the atmosphere. All flower parts including petals, sepals, anthers and gynoecia are sources of volatiles (Dobson et al., 1990). Spatial regulation occurs as different volatiles are produced from different parts of the flower. For example, studies performed in *Rosa rugosa* flower parts (petals, sepals, androecium and gynoecium) showed that different volatiles were emitted in each one of the flower parts (Dobson et al., 1990). For example, terpenoids and benzenoid alcohols were the main volatiles present in the petals, sepals, and less present in androecium and gynoecium. Benzenoids were highly present in the anthers and fatty acid derivatives were present most specifically in the pollen. Interestingly, in rose flowers sesquiterpenes were mainly present in sepals and the gynoecium (Dobson et al., 1990). However petals were the flower part where the majority of the volatiles were biosynthesized and emitted (Bergougnoux et al., 2007). The petals have most of the enzymes present predominantly in the epidermal cells making them the principal site of biosynthesis and emission of the volatile compounds (Scalliet et al., 2006). In roses, both epidermal layers of the petals are capable of producing and emitting scent volatiles

(Bergougnoux et al., 2007). To avoid spatial variability, this research will describe the volatile compounds emitted only from the petals and flower buds from different rose cultivars.

In plants, the structures involved in production and emission of the volatile compounds include both external and internal secretory structures. External secretory structures include glands which are highly differentiated structures formed by many cells, or glandular hairs and glandular epidermis which are simpler structures. Most terpenes, specifically monoterpenes, are secreted by special external structures called glandular trichomes. Internal secretory structures include idioblasts, secretory cells, cavities and ducts (Evert, 2006). To further understand any differences in volatile emission among cultivars, this study will study the morphology of the petals using electron microscopy. Any morphological differences such as presence of secretory structures and differences in cell shape will be correlated with the different type and amounts of volatile compounds emitted by each cultivar.

### **Temporal Regulation**

Volatile emission is regulated in time as the amount of volatiles generally changes with the development of the flower. Generally volatiles increase as the flower opens, its emission peaks when the flower is ready to be pollinated and decreases as the flower dies (Dudareva et al., 2006a). The major source of regulation during development is the availability of substrates and activity of enzymes at specific stages of development. This pattern of developmental regulation was studied in gardenia and jasmine flowers (Watanabe et al., 1993). During development of these flowers the volatile precursors were present in the buds and as the flower opened they were transformed by specific enzymes into volatile compounds. Also the enzymes were newly induced and expressed or were in inactive forms and then activated as flower opened. Moreover, the production of substrates for volatile compounds increased during flower opening (Watanabe et al., 1993). The production of fragrance compounds has been studied in cut flower species such

as carnations. In carnations, thirteen major volatiles related to fragrance have been described (Schade et al., 2001). Synthesis of these volatiles appears to be developmentally regulated, with maximum synthesis corresponding to developmental stages just after flower bud opening (Schade et al., 2001). Volatile regulation during development was also studied in *Rosa damascena* flowers. In this study, different volatile compounds were emitted at different stages of development (citronellol emission peaked at stage 4 while 2-phenylethanol peaked at stage 6) (Oka et al., 1999). Volatile emission reached the highest level at late opening stages (5 and 6) and then decreased during the latest stages of development as a result of an increase of enzymatic hydrolysis (Oka et al., 1999). Moreover, studies have shown how specific volatiles can have different emission peaks at different stages of development. For example in *Rosa hybrida* cv 'Honesty', 2-phenylethanol had maximum emission times about 6 hours in the light period in early developmental stages but it changed to be maximum at night in later stages of development (Helsper et al., 1998). The regulation during development was also observed in the emission of acetate volatile such as 2-phenyl ethylacetate and cis-3-hexenyl acetate from *Rosa hybrida*. The production of these volatiles was very low at stages 1 and 2 (immature bud), became apparent at stage 3 (flower starting to open) and then rapidly increases and peaks at stage 5 of development (fully open flower). In *Rosa hybrida* 'Fragrant Cloud' the maximum volatile emission at stage 5 corresponded with maximum enzymatic activity and the main reason for the lack of emission of the volatiles at early stages was the limited availability of the corresponding substrates (Shalit et al., 2003). To expand the knowledge on the developmental regulation of volatile emission as occurs on commercial cultivars, this research will describe volatile emission on flowers that will receive conventional commercial harvest, shipping and handling practices, and will develop and open under postharvest room conditions simulating consumer's vase conditions.



## **Rhythmic Emission of Volatiles**

As described above, volatiles are emitted in different amounts at different stages of development. However, there is another source of variability in volatile emission. This source is the different emission patterns observed during the day. Volatiles are produced rhythmically and in different amounts in a 24 hr photoperiod. In roses, the rhythmic emission of volatiles can be controlled by a circadian clock or by light as demonstrated by several studies performed with different rose species. The emission of volatiles from *Rosa hybrida* cv 'Honesty' were studied under a constant 12 hour photoperiod and under conditions of constant light and constant dark. The results showed that individual volatiles differed considerable in emission patterns but in general all were controlled by a circadian rhythm (Helsper et al., 1998). Under a 12 hour photoperiod the maximum emission was observed during the light period for most of the compounds analyzed. When flowers were exposed for a 12 hour photoperiod followed by constant light or dark the rhythmicity of the emission continued however with a tendency to decline with time. On the other hand no rhythmicity was observed on the volatile emission when rose flowers were exposed to continuous dark conditions under early stages of development (Helsper et al., 1998). Interestingly, when the 12 hour photoperiod was restored some volatiles recover the rhythmicity after 2 days, other volatiles didn't recover the rhythmicity and others recovered it in an abnormal pattern (Helsper et al., 1998). Moreover, the circadian rhythm emission was also observed in *Rosa damascene semperflorens* cv 'Quatre Saisons' flowers which showed a rhythmic emission of volatiles with a maximum emission observed after 8-10 hours into a 12 hour photoperiod. The rhythmicity was maintained when flowers were kept under constant dark or light conditions implying a circadian regulation (Picone et al., 2004). Generally, as occurs with the developmental regulation, the rhythmic emission of volatiles coincided with a rhythmic expression of the genes that encode the enzymes or precursors for specific compounds

(Hendel-Rahmanim et al., 2007). For example, geranyl acetate emission in *Rosa hybrida* cv 'Fragrant Cloud' has a rhythmic pattern as observed in other rose species and its rhythmicity coincided with the expression of its biosynthetic gene, alcohol acetyl transferase. Both the emission of the compound and the expression of the gene were maintained even under constant dark or light conditions (Hendel-Rahmanim et al., 2007). The emission of the volatiles also depended on the availability on the substrate. When the rose flowers were stored under constant light conditions the concentration of the substrate declined as was the emission of the compound. However, the expression of the biosynthetic gene germacrene D synthase was constant during the day even though the compound germacrene D emission had a peak during the light period (Hendel-Rahmanim et al., 2007). These examples illustrated the complexity of regulation of scent emission on flowers. In order to minimize temporal variation in this study, volatile production from different cultivars will be conducted at the same time of the day on all experiments.

### **Conditions that Affect the Cultivation and Selection of Fragrant Cultivars**

Fragrant roses are considered luxury products since they are distributed at very high prices and at very low volume (Mouchette, 2001; Bent, 2007). Even though the fragrance is a characteristic that is very desirable by consumers, the majority of cut rose cultivars lack this important trait. From the 3,900 rose cultivars described back in 1956 only 20 % of them were described as strongly fragrant (Bent, 2007). Flower fragrance is a complex trait. Factors such as the capability of fragrance detection by humans, the developmental stage of the flower, the physiology of volatile emission, the postharvest and transport conditions and the physiology of roses during senescence are some of the reasons that may explain why fragrance is not as predominant as it should be in cut rose flowers.

## **Human Perception**

It's important to note that fragrance is a subjective trait. Not all human individuals are capable of detecting the same concentration and volatiles emitted from flowers. A rose can be classified as a nice fragrant flower by one individual while another may not even consider the same flower as fragrant. Many factors influence the response of humans to fragrances including the mood, the innate physiological sensitivity for the specific compound being exposed and the past history and familiarity with similar fragrance stimulus (Lawless et al., 1998). The physiology of human smell does not contribute to the optimum detection of flower volatiles. The olfactory receptors in humans are located very high in the nasal cavity. This remote location may influence the proper detection of the volatiles at the time of exposure to them since only a small percentage of the volatiles flowing through the nose actually reaches the sensory organs (Lawless et al., 1998). Some organic molecules stimulate the olfactory sense of humans more than others some being recognized at part per billion concentrations while others detected at part per thousand. Rose volatiles differ in their perception; some small quantities are needed for its detection while others need large quantities to be detected. The threshold may also vary between different individuals. Threshold levels may increase following periods of constant stimulation and rating therefore the perceived intensity may decrease over time (Lawless et al., 1998). Moreover, one person can be sensitive to a particular scent and be completely insensitive to another. Therefore, at the time of asking human individuals to classify a rose as fragrant or not will depend on many factors including the ability to sense the major components of the floral trait and to the person's fragrance educational experience. In order to determine the ability of human subjects to detect fragrances from commercially available cultivars, scent panel studies will be conducted. The preference for different rose fragrance types will be also described by scent panel studies.

## Flower Development

Fragrant production is influenced by the flower developmental stage. If we consider that fragrance has evolved in nature to attract pollinators, it is important to understand that it is emitted at the time of flower development that maximizes this event. Maximum emission of volatile compounds is an event that happens once during development and at a very specific time. In the case of roses, it generally occurs during the day at maximum flower opening stage when the stamens are visible, however this event may vary among cultivars. Studies have confirmed that different times of maximum volatile emission during flower opening varies on different rose individuals (Watanabe et al., 1993; Oka et al., 1999). Studies performed on diverse rose species have shown differences in maximum emission during the day and in the time of emission of specific volatiles. For example, studies of volatile emission from *Rosa damascene* showed that all volatiles were emitted rhythmically with maximum peaks at 8-10 hours into a 12 hour photoperiod (Picone et al., 2004) while studies of volatiles from *Rosa hybrida* showed that they have a peak early in the light period (Helsper et al., 1998; Hendel-Rahmanim et al., 2007). Therefore, the fragrant perception from a rose flower may depend on the developmental stage at which it is smelled. In the flower industry, the developmental stage at harvest is critical to ensure further flower opening and development. If a rose is harvested too tight it will never open and if it is harvested too open it will not last long enough to cover the transport and consumer time. Most cut rose cultivars are harvested at a stage that is close enough to stand the long handling and shipping times but mature enough to open during the consumer stage. The problem is that the commercial harvest stage may not be the optimum stage for volatile emission to occur. Therefore, this research will correlate the volatile emission from commercially handled rose flowers at different stages of flower opening during vase life with human subject's detection of these volatile compounds and their preferences.

## **Conditions During Handling and Distribution**

Roses are currently ranked among the top three cut flowers in the world. Together with other cut flower products roses worth around US\$ 1 billion in export to North America (Asocolflores, 2007). The industry trend is to produce the cultivars in regions where the production costs are low. Then flowers are transported to key markets around the world. Therefore, roses are grown in many countries of the world with a diversity of climates and environmental conditions. This has created pressure to develop new cultivars that adapt to the variable and demanding growing and shipping conditions.

The distribution of cut roses include packaging the flowers in boxes and keeping the flowers in cold rooms at temperatures generally below 5 ° C in the dark. These conditions are definitively not favorable for volatile production and emission. A rose is going to emit less volatiles, therefore be less fragrant, on a cloudy, rainy, windy day while conditions of few air currents, high air humidity and warm temperatures will favor the emission of fragrance, therefore increasing the fragrance trait of a specific rose. These conditions may affect the fragrance note since they may influence the inherent rhythmic volatile biosynthesis and emission from the flower. Therefore when a consumer receives the flower the fragrant note may be altered from its original rhythm affecting the volatile emission. Then, the strong fragrant rose cultivars are generally produced and offered in local markets avoiding the long shipping and storage conditions. However, formal studies on fragrance produced under consumer conditions have not been conducted. This research will study the fragrance emission from cultivars treated with the conventional harvest, packaging and shipping procedures to determine the fragrance emission as it occurs at the consumer level.

Studies performed on fragrant cultivars have focus on elucidating the physiology of fragrance emission during development (Shalit et al., 2004), biosynthesis of the main volatile

compounds present in fragrant roses (Lavid et al., 2002; Scalliet et al., 2002; Watanabe et al., 2002; Shalit et al., 2003; Wu et al., 2003; Wu et al., 2004; Scalliet et al., 2006) characterization of the main volatile compounds emitted by rose species (Flament et al., 1993; Kim et al., 2000; Joichi et al., 2005) and regulation of the emission of these volatiles (Helsper et al., 1998; Picone et al., 2004; Bergougnoux et al., 2007; Hendel-Rahmanim et al., 2007). Few physiological studies have been conducted on commercially available cut rose fragrant cultivars, thus little is understood on the many processes involved and the reasons for the lack of fragrance in most rose cultivars. Therefore the studies conducted in research will focused on studying the physiology including ethylene production and sensitivity, respiration, volatile emission and vase life of fragrant and non –fragrant cut rose cultivars to provide additional knowledge about cut fragrant cultivars. The results of this work will provide the physiological parameters to assist rose producers to select cut fragrant roses with long vase life. This work will provide additional knowledge to the scientific community to understand the physiology of a cut fragrant rose and will assist cut rose breeders in selecting long lasting, fragrant cut rose cultivars.

### **Ethylene**

Ethylene is a gaseous plant hormone that can act at very low concentration and plays a central role in the regulation of physiological and developmental processes including seed germination, fruit ripening, leaf abscission and flower senescence (Abeles et al., 1992).

Ethylene is formed from methionine via S-adenosyl-L-methionine (AdoMet) and the cyclic non-protein aminoacid 1-aminocyclopropane 1-carboxylic acid (ACC). The enzymes that catalize the conversion of AdoMet to ACC and ACC to ethylene are ACC synthase and ACC oxidase respectively. The genes encoding these enzymes belong to a multigene family (Yang et al., 1984).

Ethylene biosynthesis is regulated both positively and negatively. The positive regulation occurs as exogenous ethylene stimulates the tissue's ability to convert ACC to ethylene by stimulating the development of ACC oxidase activity leading to an autocatalytic production of ethylene. This condition is known as system II of ethylene production. This system generally occurs in mature fruits and in mature / open floral structures (Kende, 1993). The negative regulation occurs as exogenous ethylene inhibits the ethylene biosynthesis from the tissue by negatively regulating the ACC synthase genes limiting the availability of ACC synthase. This condition is known as System I of ethylene production and occurs in immature fruits and early stages of flower development. Therefore, fruits and flowers generally pass from producing very low ethylene levels (System I) in the early stages of development to a rise in ethylene production (System II) as development progresses. The shift to autocatalytic ethylene production requires the induction of genes that encode the ethylene biosynthetic enzymes couple with increase ethylene sensitivity in the tissues (Yang et al., 1984). Both the regulation of ethylene biosynthesis and sensitivity are key process to maintain product quality. This research will study ethylene production and external ethylene sensitivity to determine ethylene's role on the quality and vase life of fragrant cut rose cultivars.

Ethylene biosynthesis can be reduced by chemicals such as aminoethoxyacetic acid (AOA) and aminoethoxyvinylglycine (AVG) which inhibit the action of ACC synthase. ACC synthase is the rate limiting enzyme that converts S-adenosyl-methionine (SAM) into the ethylene precursor ACC (Yang et al., 1984). However, flowers treated with AOA and AVG can be sensitive to external ethylene sources (Serek et al., 1993; Staby et al., 1993). Therefore, the tendency in the industry has been to use chemicals that inhibit ethylene action instead of ethylene biosynthesis. Ethylene action can be prevented by the effect of chemicals such as silver thiosulphate (STS) and

1-methylcyclopropane (1-MCP). These compounds bind to ethylene receptors in plants avoiding ethylene responses to occur thus extending the vase life of flowers (Sisler et al., 2003). STS is applied as hydration solution taken up through the stem. Therefore, STS is a compound that stays in the tissue and is able to bind to new receptors as they are produced. STS has been used with success in a wide number of ethylene sensitive cut flowers such as carnations (Veen, 1979), alstromeria (Chanasut et al., 2003) and roses (Mor et al., 1989; Lukaszewska et al., 1990). However the use of silver in several countries is restricted due to environmental regulations. The compound 1-MCP is applied as a gas and does not have the capability of binding to the new receptors as they are produced as the tissue grows, therefore its effect is transitory. 1-MCP has become an alternative nontoxic inhibitor of ethylene action because it is not toxic to living organisms or the environment (U.S. Environmental Protection Agency, 2007).

In order to understand the mechanism of action of inhibitors of ethylene action it is important to understand the ethylene signaling pathway. The signaling pathway starts as ethylene binds to the receptors (ETR) which are proteins embedded in the membrane. The ethylene receptors belong to the histidine kinase receptor family and are negative regulators of ethylene response. As ethylene binds to the receptor the signal is repressed. When ethylene is not present in the environment the receptor is active and activates the CTR (constitutive triple response) protein. This protein is also negatively regulated by ethylene. When ethylene is not present, CTR is active and represses further signaling. When ethylene is present CTR is inactive, therefore the signal transduction is no longer repressed. This leads to the activation of transcription factors such as EIN and genes that are responsible for the ethylene effects reviewed by (Kieber, 1997; Stepanova et al., 2005). Therefore when STS or 1-MCP molecules bind to the receptor instead of ethylene, CTR is active and the signaling ethylene cascade is repress.



Inhibition of ethylene responses also occurs when the receptor is defective and does not bind to ethylene as in the case of mutants *etr-1*. This mutant was first described in *Arabidopsis* seedlings which lack the denominated triple hook response when grown in the dark (Chang et al., 1993). This response consists of a seedling that grows with a pronounced curvature and shorter length of the hypocotyl under an ethylene rich environment. Seedlings with the defective receptor *etr-1* do not present any curvature in an ethylene rich environment. It is possible to breed plants that express a mutant receptor *etr-1* that does not bind to ethylene repressing all ethylene responses. Plants such as petunia and carnation have been successfully transformed with the *etr-1* gene resulting in transgenic plants with delayed senescence. The transformed petunia plants presented a delayed natural and pollinator-induced senescence compared to wild type plants (Wilkinson et al., 1997). The same results were observed on the transformed carnations which showed a delayed senescence by at least 6 days compared to control flowers (Bovy et al., 1999). Additionally transgenic carnations did not showed the typical ethylene symptom of petal inrolling. The petals stayed firm and finally decolorized. Furthermore, the transgenic carnation flowers presented longer vase life than carnations treated with either inhibitors of ethylene biosynthesis (aminoacetic acid) or inhibitor of ethylene perception (silver thiosulphate) (Bovy et al., 1999). In order to confirm the role of ethylene of flower quality, vase life and flower opening of fragrant roses will be determine after treatment with inhibitors of ethylene production such as AVG and inhibitors of ethylene perception such as STS.

### **Roses and Ethylene**

Ethylene has a negative effect on postharvest quality of roses and can be either present in the environment or produced internally by the flower. External ethylene decreases vase life of miniature and cut rose cultivars (Mor et al., 1989; Serek et al., 1996; Muller et al., 1998; Muller et al., 2000; Muller et al., 2001; Chamani et al., 2005). Studies have shown that nearly 80% of

fresh cut rose cultivars are ethylene sensitive, as measured by reduced vase life or reduced flower opening after treatment with exogenous ethylene (Nell et al., 2009). The differences in ethylene sensitivity and vase life between rose cultivars can be explained by differences in the receptor levels (Muller et al., 2002). Ethylene sensitive cultivars have an increase expression of the ethylene receptor (RhETR3) as they senesce and display a short vase life. While ethylene insensitive cultivars present low levels of expression of (RhETR3) throughout development and displayed a long vase life (Muller et al., 2002), external ethylene can also alter rose bud opening (Reid et al., 1989; Chamani et al., 2005). Ethylene can regulate flower opening in roses by either enhancing the process or inhibiting it according to the variety. *Rosa hybrida* cv 'Samantha' presented accelerated flower opening after exposure to ethylene while the cultivar 'Kardinal' showed reduced flower opening. The different response of flower opening to ethylene exposure between the cultivars is explained by difference in the expression of ethylene receptor gene RhETR1 and RhETR-3 (Ma et al., 2006) and the ethylene biosynthetic gene RhACS3 (Ma et al., 2005).

In cut rose cultivars, external ethylene has several effects including increased petal abscission, premature wilting, fresh weight loss during vase life and alteration in bud opening and increase of autocatalytic ethylene production (Reid et al., 1989; Serek et al., 1995; Muller et al., 1998; Muller et al., 2001; Chamani et al., 2005; Tan et al., 2006; Xue et al., 2008). Endogenous ethylene can also be produced by rose flowers as part of their senescence process (Mor et al., 1989; Muller et al., 2001; Chamani et al., 2005). For example, some miniature rose cultivars such as 'Bronze' produce a clear rise in ethylene production observed as the flower senesced even in the absence of exogenous ethylene (Muller et al., 2001). This rise in ethylene production results from increasing ACC synthase activity that leads to high ACC content in the

flowers (Muller et al., 2001). Internal ethylene can also be produced as result of stress conditions like cold storage (Mor et al., 1989; Muller et al., 2000). Roses exposed to stress conditions such as storage at 1 °C can produce two to four times the maximum level of ethylene produced by fresh non-stored flowers when transferred to ambient conditions. This increase of autocatalytic ethylene production results from an increase in ACC content in the petals (Faragher et al., 1987b). Internal ethylene production can also occurred after external ethylene exposure as observed in the cut rose variety 'First Red' (Chamani et al., 2005).

The rise in ethylene production at the onset of senescence or under external ethylene exposure can be accompanied by a rise in respiration rate, a process defined as a climacteric response (Kays et al., 2004) . Short vase life of some miniature rose cultivars has been associated with high respiration rates that occur postharvest (Monteiro et al., 2001). Respiration is a process that occurs during flower development and its rate after harvest is influenced by temperature. The rate of flower development and aging as well as vase life are parameters closely related to respiration rate (Reid, 2003). This study will measure ethylene production and respiration rate from cultivars to further understand the ethylene physiology on these cut flowers and the factors that may relate with the short vase life of some fragrant cultivars.

### **Ethylene and Fragrance Biosynthesis**

The production of floral volatile organic compounds is tightly associated with developmental processes such as flower opening and events related to pollinator syndromes. Ethylene is a hormone that regulates senescence and development in flowers and plays a role in the emission of volatiles in certain flower species such as petunia and sweet pea flowers (Negre et al., 2003; Sexton et al., 2005; Underwood et al., 2005). Studies performed in petunia, carnation and sweet pea flowers have elucidated some ideas of how ethylene plays a role in volatile emission in some flower species. Petunias are flowers that produce maximum levels of

volatiles at night when the flowers are open. Petunias are pollinated by moths which are nocturnal insects. When pollination occurs there is an increase ethylene production and sensitivity in the flower that leads to senescence (O'Neill, 1997). The increase in ethylene production is related with a decrease in volatile synthesis and emission from the flower (Underwood et al., 2005). Additionally, cut sweet pea flowers also produce increasing amounts of ethylene during development and exogenous ethylene accelerated wilting and petal abscission (Sexton et al., 2005). Volatile production of cut pea flowers declined exponentially as the flower senesce. When flowers were treated with external ethylene the decline in volatile production was accelerated compared to controls. The role of ethylene in the decline of fragrance of cut pea flowers was evident as cut pea flowers treated with 1-MCP and STS had a delayed reduction in volatile emission compared with controls (Sexton et al., 2005). On the other hand, carnations which are flowers that exhibit a climacteric-like peak in ethylene production just before the onset of petal senescence and exhibit changes in volatile composition as the flower senesces did not showed a direct role of ethylene with volatile emission (Schade et al., 2001). In carnations, individual volatiles changed dramatically and independently during flower development. However the volatile composition was not directly regulated by ethylene since a flower that had senesce naturally produced a very different fragrant note compared to a young ethylene treated flower (Schade et al., 2001). In roses, volatile composition declines during development (Oka et al., 1999; Lavid et al., 2002; Shalit et al., 2003). However, these studies have not determined the role of ethylene on the fragrant emission of cut roses. Therefore, this work will concentrate on describing the composition of rose fragrance during development and the ethylene effect on the fragrance composition of several rose cultivars.

## CHAPTER 2 DETERMINATION OF VOLATILE COMPOUNDS AMOUNTS AND VASE LIFE OF CUT ROSES

### **Introduction**

Exciting new cut flower cultivars drive retail flower sales, and are released every year to satisfy the demands of the world-wide market. Cut rose breeding focuses on increasing qualitative and quantitative traits such as stem length, flower bud size, productivity, disease and pest resistance, and vase life. Over the last 30 – 40 years, development of these traits has resulted in a reduction in flower fragrance, and the belief that fragrance is negatively correlated with vase life. However, the specific reasons explaining the loss of typical fragrance in cut rose flowers and its relation with vase life have not yet been determined (Schulz, 2003). Even though rose fragrance contributes significant commercial value to cut roses, sufficient studies have not been performed to understand the physiological and biochemical relationships between fragrance and postharvest performance. Thus, there is no solid genetic basis for breeders to knowingly incorporate fragrance into commercial cultivars without affecting postharvest performance.

More than 400 floral volatile compounds have been identified for roses (Schulz, 2003). Most of the volatiles are products of three main biosynthetic pathways: terpenoids, phenylpropanoids and fatty acid derivatives (Knudsen et al., 1993). Floral fragrances differ between rose species (Kim et al., 2000). Specifically, the fragrance profile of *Rosa hybrida* L. is characterized by 41 compounds including alcohols, aldehydes, alkanes, monoterpenes, sesquiterpene, esters, ethers and ketones (Kim et al., 2000). The main volatile esters emitted by fragrant garden roses are 2-phenylethyl acetate, *cis*-3-hexenyl acetate, geranyl acetate and citronellyl acetate (Shalit et al., 2003). Main fragrance rose components are produced primarily during the day. Volatile regulation is linked to a natural circadian rhythm with maximum

emission during the light period (Helsper et al., 1998). Maximum emission and rhythm differs between compounds (Hendel-Rahmanim et al., 2007).

Vase life of cut rose flowers is affected by both growing conditions (light, temperature, relative humidity, fertilization) and postharvest procedures (shipping and storage temperature, sanitation and hydration solutions). Biotic and abiotic conditions during shipping and handling such as water deficit, darkness, high and low temperatures are detrimental to vase life and flower quality (Muller et al., 2000). These factors induce ethylene biosynthesis which promotes leaf and petal abscission, accelerated or delayed opening and accelerated wilting, conditions that affect the vase life of the flower (Lukaszewska et al., 1990; Liao et al., 2000; Chamani et al., 2005). Longevity of commercially important cut rose cultivars can be negatively affected by external ethylene (Chamani et al., 2005). Symptoms of ethylene sensitivity include accelerated petal wilting, petal abscission and acceleration or retardation in flower opening. Some cut rose cultivars show internal ethylene production peaks characteristic of climateric commodities such as carnations (Vanaltvorst et al., 1995; Chamani et al., 2005). The relationship between ethylene sensitivity, production and fragrance has not been studied for cut roses. The objective of this work is to describe the main volatile compounds, patterns of ethylene production and sensitivity and vase life of several commercial fragrant and non-fragrant cut rose cultivars.

### **Materials and Methods**

A selection of fragrant and non-fragrant cut rose cultivars *Rosa hybrida* L. ('Allure', 'Avan Garde', 'Coolwater', 'Ectasi', 'Erin', 'Freedom', 'Lovely Dream', 'Osiana', 'Peckoubo', 'Plaza Roja', 'Red Sensation', 'Savoy', 'Yabadabadoo') were harvested early in the morning at commercial mature stage in Ecuador and Colombia. Flowers stayed in the field for a maximum time of 30 minutes before being transferred to the postharvest room and graded according to flower color, bud size and stem length. Then, they were grouped into 25 stem bunches, re-cut

and hydrated for 18 hours in a commercial hydration solution. Flowers were shipped to the University of Florida, Gainesville by airplane and refrigerated truck within 4 days. At arrival, 32 flowers were randomly selected from bunches and placed into vases containing a commercial vase solution (Chrysal Clear Professional 3, Pokon Chrysal). Flower vase life was determined as the time from placement in the vase to the appearance of visual senescence symptoms (i.e. petal wilting, abscission, bluing and disease). Flowers were held at 21 °C, 40-50 % RH and under 10  $\mu\text{mol s}^{-1}\text{m}^{-2}$  of light (12 hrs/day) for evaluation. One group of flowers was randomly selected for vase life and others were designated for destructive test for volatile collection, ethylene and respiration.

### **Volatile Collection**

Volatiles were collected from flowers at day 0, 2, 4 and 6 of vase life. At each sampling time, petals were removed from the flower head and weighed. Petals were then placed into glass tubes connected to a continuous air flow system. Carbon filtered air was pumped/pulled through the tubes for one hour and trapped on a sorbent Super Q. Volatiles collected on the sorbent were eluted with methylene chloride (Fisher Scientific). Nonyl acetate was added as a standard after collection. Samples were analyzed by gas chromatography and mass spectrometry as described by Underwood et al., (2005). Volatiles were collected at 4pm at each sampling date. At each time volatiles were measured, flower opening was rated using the following scores: 1 = outer petals tightly wrapped around bud, 2 = outer petals starting to reflex from bud, 3 = outer petals reflexed approximately 135° to stem, 4 = outer petals reflexed at approximately 115° to stem, 5 outer petals reflexed at 90° to stem.

## **Ethylene and Carbon Dioxide Measurements**

Ethylene and CO<sub>2</sub> production was measured for 7 days by placing individual flowers in 0.48 l glass mason jars. Each flower was placed inside the jar with a plastic vial containing 8 ml deionized water. Jars were closed for 4 hours, then one ml gas sample was taken from the jars and analyzed with a gas chromatograph (Hewlett-Packard 5890 Series II). Jars were then opened and identical measurements were taken the following day.

## **Statistical Analysis**

Four replicate vases containing 8 stems were used for vase life evaluation. Five stems per cultivar were used for ethylene detection and respiration. Three flower buds were used for volatile extractions at each sampling time. Flowers were arranged in vases for evaluation in a completely randomized block design. Data were analyzed by ANOVA using SAS<sup>®</sup> Version 8 (SAS Institute Inc., Cary, NC, USA). The significance of differences among treatment data means were determined using a Tukey multiple comparison test at  $p=0.05$ .

## **Results**

### **Volatile Collected on Fragrant and Non-Fragrant Cut Rose Cultivars**

Highly fragrant cultivars (based on smell and amount of volatiles produced during vase life) ‘Allure’, ‘Ectasi’ and ‘Lovely Dream’ produced 3,5 dimethoxytoluene in combination with cis-3 hexenyl acetate, 2-phenylethyl acetate and phenylethyl alcohol. Moderately fragrant cultivars produced many of the same volatile compounds as highly fragrant cultivars with the addition of benzoic acid, eicosene, and caryophyllene. Cultivars with little or no fragrance produced 3,5 dimethoxytoluene or eicosene only (as in ‘Red Sensation’, ‘Peckoubo’ and ‘Yabadabadoo’) or in combination with other volatiles (cis-3 hexenyl acetate, eicosene) in ‘Coolwater’, (benzoic acid methyl ester) ‘Freedom’ and ‘Plaza Roja’ (cis-3 hexenyl acetate, caryophyllene) (Table 2-1).



The volatile 3,5 dimethoxytoluene was produced by all cultivars except 'Red Sensation'. Phenylethyl alcohol was produced by fragrant cultivars 'Erin', 'Lovely Dream' and 'Avan Garde' while cis – 3 hexenyl acetate was produced by the fragrant cultivars 'Allure', 'Erin', 'Lovely Dream', and non-fragrant cultivars 'Plaza Roja' and 'Coolwater'. 2-phenylethyl acetate was produced by two fragrant cultivars 'Erin' and 'Lovley Dream'. 3-exen-1-ol acetate was produced by fragrant 'Savoy' and 'Avan Garde'.

The maximum production of each volatile compound depended on the developmental stage of the flower. For example the maximum production of the two major volatiles (2-phenylethyl alcohol and 3,5 dimethoxytoluene) in the fragrant cultivar 'Lovely Dream' varied according to the developmental stage of the flower. The maximum production of phenylethyl alcohol was observed at day 4 in 'Lovely Dream' (Figure 2-1). Day 4 was also the time of maximum flower opening registered for this cultivar (Figure 2-1 and Figure 2-2). Contrary, maximum production of 3,5 dimethoxytoluene was observed at day 0 that corresponded to the lowest opening stage in 'Lovely Dream' (Figure 2-1).

### **Vase Life**

Fragrant and non-fragrant cut rose cultivars presented all ranges of vase life. Long vase life was observed on fragrant cultivars 'Avan Garde', 'Erin', 'Osiana' and 'Savoy' and non-fragrant cultivars 'Freedom', 'Red Sensation' and 'Yabadabadoo'. Short vase life was observed on fragrant cultivars 'Allure' and 'Ectasi' and the non fragrant cultivar 'Coolwater' (Table 2-2).

### **Opening**

The opening stage for the non fragrant cultivars 'Plaza Roja', 'Coolwater' and 'Yabadabadoo' was less over time than the opening stage observed for fragrant cultivars 'Allure', 'Lovely Dream' and 'Erin'. A low opening stage observed in non fragrant cultivars 'Plaza Roja', 'Coolwater' and 'Yabadabadoo' was related with long vase life (Figure 2-2 and

Table 2-2). Additionally, rose cultivars that reached maximum opening stages early during vase life presented short vase life. Contrary, cultivars that reached maximum opening stages later during vase life presented long vase life. For example, fragrant cultivar ‘Allure’ presented a maximum opening of 4 at day 2 while fragrant cultivars ‘Lovely Dream’ and ‘Erin’ presented a maximum opening stage of 5 at day 4. Also, ‘Allure’ and ‘Lovely Dream’ presented short vase life while ‘Erin’ presented a long vase life (Table 2-2).

### **Internal Ethylene Production and Respiration**

The most ethylene was produced by the fragrant cultivar ‘Lovely Dream’. Moderate levels of ethylene production were observed on fragrant cultivars ‘Allure’ and ‘Ectasi’ and the non-fragrant cultivar ‘Coolwater’. Low level ethylene production was observed on fragrant cultivars ‘Avan Garde’, ‘Erin’, ‘Osiana’ and ‘Savoy’ and non-fragrant cultivars ‘Freedom’, ‘Red Sensation’, and ‘Yabadabadoo’. Cultivars with low ethylene production had long vase life except ‘Savoy’ which had long vase life with a moderate ethylene production rate (Table 2-2).

High respiration rates were observed on fragrant cultivars ‘Allure’, ‘Avan Garde’ and ‘Lovely Dream’ and ‘Savoy’. Low respiration rates were observed on the fragrant cultivar ‘Erin’, and non-fragrant cultivars ‘Freedom’, ‘Red Sensation’ and ‘Yabadabadoo’. Low respiration was associated with long vase life (Table 2-2). Cultivars with short vase life such as ‘Allure’ had higher respiration rates than cultivars with long vase life such as ‘Red Sensation’ over 7 days.

### **Discussion and Conclusions**

In this study, vase life of cut rose flowers was short, moderate or long, and was independent of how much fragrance a flower emitted. Fragrant and non-fragrant cultivars had short vase life (e.g. ‘Allure’, ‘Coolwater’) and long vase life ( e.g. ‘Erin’, ‘Freedom’). Vase life was short for those cultivars that produced high levels of ethylene (e.g. ‘Lovely Dream’) and had high or moderate respiration rates (e.g. ‘Allure’). Vase life was long for cultivars with low

ethylene production and low to moderate respiration rates (eg. ‘Erin’, ‘Red Sensation’). Thus, we observed no direct relationship between vase life and fragrance for the cut rose cultivars evaluated in this study. Vase life was more tightly associated with ethylene production and respiration.

Ethylene production rate was high in the fragrant cultivar ‘Lovely Dream’ and low in fragrant cultivar ‘Erin’ and non-fragrant cultivars ‘Freedom’, ‘Plaza Roja’, ‘Red Sensation’ and ‘Yabadabadoo’ (Table 2-3). Ethylene production has been reported in other rose cultivars (Liao et al., 2000) but ethylene production in cut rose has not been related with fragrance. Studies have reported a relationship between maximum volatile emission and maximum ethylene sensitivity in petunia flowers (Underwood et al., 2005). In our study we detected high ethylene and fragrance production in the fragrant cultivar ‘Lovely Dream’ at day 4 (Figure 2-1). High fragrance and medium ethylene production was observed on ‘Allure’ and ‘Ectasi’. The cultivars ‘Avan Garde’ and ‘Erin’; ‘Coolwater’, ‘Peckoubo’ and ‘Plaza Roja’; ‘Red Sensation’ and ‘Yabadabadoo’ had medium low and non-fragrance production respectively with low ethylene production (Table 2-2). Further studies were performed to describe and understand the relation between maximum volatile production and ethylene production and sensitivity in the cultivars included in this study (Chapter 3).

Respiration is a mechanism by which the energy stored in the form of carbon compounds is released by plants. As energy reserves are released the tissue can experience depletion on carbon compounds and accelerated senescence. This process is critical in structures such as flowers which are organs where carbon is not stored (Kays et al., 2004). Under the same temperature conditions, respiration rate was high in ‘Allure’, ‘Avan Garde’ ‘Lovely Dream’ and ‘Savoy’ and low in ‘Erin’, ‘Freedom’, ‘Red Sensation’ and ‘Yabadabadoo’. High respiration

rates were associated with a short vase life in 'Allure' and 'Lovely Dream' and with a long vase life in 'Avan Garde' and 'Savoy'. Low respiration was associated with long vase life in 'Erin', 'Freedom', 'Red Sensation' and 'Yabadabadoo' (Table 2-2). Therefore the short vase life observed in cultivars with high respiration rates may be explained by an accelerated senescence that occurs due to a quicker depletion of energy reserves.

Phenylethyl alcohol is a compound characterized by a mild rose odor and together with other components such as geraniol, citronellol and nerol is the major component of rose fragrance (Bauer, 1990). In our study this compound was present in all fragrant cultivars and absent in non-fragrant ones (Table 2-2). Particularly high amounts of phenylethyl alcohol were detected in the most fragrant cultivar (based on smell) 'Lovely Dream'. Maximum emission of phenylethyl alcohol in 'Lovely Dream' at day 4 coincided with maximum flower opening (Figure 2-1). Maximum production of phenylethyl alcohol also coincided with maximum production of total volatiles reported at maximum stage of development in other fragrant *Rose hybrida* cultivars such as 'Fragrant Cloud' (Shalit et al., 2004). The timing between maximum volatile production rate with maximum flower opening is likely explained by a pollinator biology relationship. Generally, volatile compounds are produced in flowers as a means to attract pollinators. Phenylethyl alcohol was produced at flower opening stage 4-5, a time at which stamens were visible and available to possible pollinators.

Volatile compounds such as 3,5 dimethoxytoluene, were present in both fragrant and non-fragrant cultivars in high amounts. The presence of this compound in high amounts was expected because 3,5 dimethoxytoluene is reported as a major scent compound of many modern rose cultivars and its fragrance provides the classic 'tea scent' that characterizes the Tea and Hybrid tea roses (Scalliet et al., 2008). Moreover, studies have reported that this compound is almost

imperceptible to the human nose but is perceived by honey bees (Shalit et al., 2004). Therefore it is not appropriate to categorize a fragrant cultivar based on simple human perception. Much work needs to be conducted to determine which fragrances are most desirable to humans. The human preferences for the rose fragrances from commercially available cultivars will be discussed in chapter 5.

In general, vase life of cut rose cultivars is affected by numerous physiological factors. In our study we addressed the relationship between ethylene, respiration and vase life with volatile composition. We concluded that ethylene and respiration had more clear association with vase life than volatile composition. Further studies will help to better understand the effects of these parameters on cut rose flower vase life.

Table 2-1. Main volatiles produced by cut rose cultivars during vase life.

Cultivar	Main volatile compounds (In order by concentration high to low)
'Allure'	3,5 dimethoxytoluene; cis-3hexenyl acetate,
'Avan Garde'	3,5dimethoxytoluene; benzoic acid, methyl ester; phenylethyl alcohol; 1-nonadecene; 3-hexen-1-ol,acetate;
'Coolwater'	3,5 dimethoxytoluene, cis 3 hexenyl acetate, Eicosene
'Ectasi'	nerol(cis-gernaiol);geranil acetate; 1-nonadecane; 3,5 dimethoxytoluene; acetic acid hexyl ester
'Erin'	Eicosene; 3,5 dimethoxytoluene; 2-phenylethyl acetate;cis-3hexenyl acetate; phenylethyl alcohol
'Freedom'	3,5 dimethoxytoluene; benzoic acid,methyl ester;caryophyllene
'Lovely Dream'	3,5 dimethoxytoluene; Phenylethyl alcohol; 2-phenylethyl acetate; cis3-hexenyl acetate
'Osiana'	3,5 dimethoxytoluene; benzoic acid, methyl ester; caryophyllene; 3-Hexen-1-ol,acetate
'Peckoubo'	3,5 dimethoxytoluene
'Plaza Roja'	cis-3hexenylacetate;caryophyllene; 3,5 dimethoxytoluene,
'Red Sensation'	Eicosene
'Savoy'	3,5 dimethoxytoluene; benzioc acid; 1-nonene; benzene, 1-ethenyl-4-methoxy beta-Ionone ; 3-hexen-1-ol,acetate
'Yabadabado'	3,5 dimethoxytoluene

Table 2-2. Vase life of cut rose cultivars associated with fragrance, ethylene production and respiration.

Cultivar	Color	Fragrance *	Ethylene *	Respiration *	Vase life *
'Allure'	lavender	+++	++	+++	+
'Avan Garde'	lavender	++	+	+++	+++
'Coolwater'	lavender	+	+	-	+
'Ectasi'	dark pink	+++	++	++	+
'Erin'	yellow	++	+	+	+++
'Freedom'	red	-	+	+	+++
'Lovely Dream'	pink	+++	+++	+++	++
'Osiana'	peach	++	+	++	+++
'Peckoubo'	pink	+	++	++	++
'Plaza Roja'	red	+	+	-	++
'Red Sensation'	red	-	+	+	+++
'Savoy'	pink	++	++	+++	+++
'Yabadabadoo'	orange	-	+	+	+++

		Fragrance perception by human smell	Ethylene (ulC2H4/Kg-hr)	Respiration (mlCo2/Kg-hr)	Vase life (average days)
+	low	Hardly perceived	< 2	< 50	< 6
++	medium	Slightly perceived	3-4	60	7
+++	high	Strongly perceived	> 5	> 70	> 8
-	no production				

\* Rating conventions for ethylene, respiration and vase life.

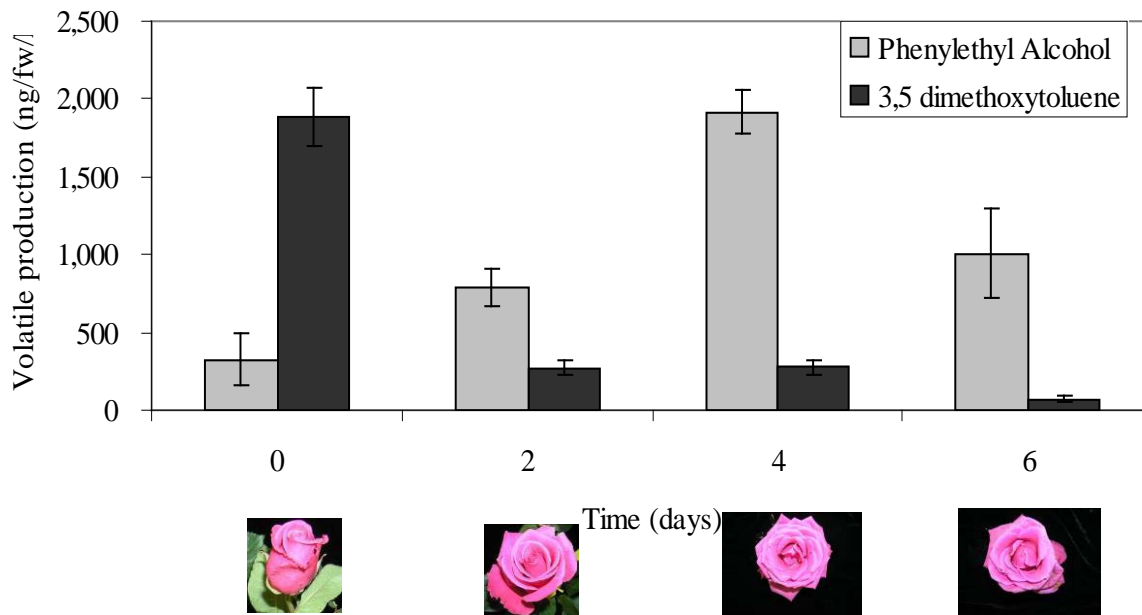


Figure 2-1. Production of 2-phenylethanol and 3,5 dimethoxytoluene in 'Lovely Dream' over time. Pictures represent opening stage at the time of volatile collection. Means and standard error n=5.

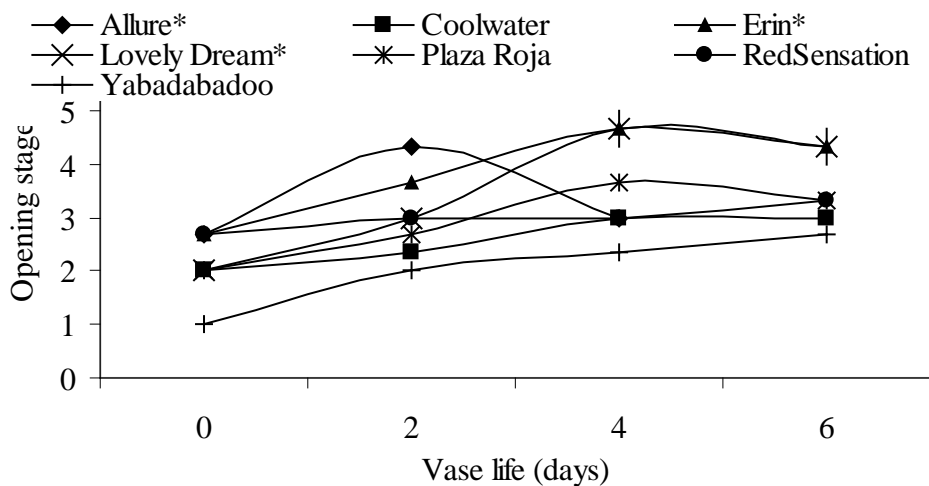


Figure 2-2. Opening stage of cut rose cultivars at the time of volatile collection. Flower opening was registered for each flower at each time volatiles were measured following rating score: 1 = outer petals tightly wrapped around bud, 2 = outer petals starting to reflex from bud, 3 = outer petals reflexed approximately 135° to stem, 4 = outer petals reflexed at approximately 115° to stem, 5 outer petals reflexed at 90° to stem. Means and standard error, n= 3.



CHAPTER 3  
RELATIONSHIP BETWEEN FRAGRANCE, VASE LIFE AND THE ROLE OF ETHYLENE  
IN VOLATILE AMOUNTS ON CUT ROSE CULTIVARS

**Introduction**

Vase life is a trait that influences cut flower quality. Vase life of cut roses has been negatively correlated with fragrance (Barletta, 1995). Most modern cut rose cultivars come from *Hybrid Tea* roses which have inadvertently lost their fragrance during the breeding process since characters such as longevity, flower form, flower shape, flower size, number of petals and productivity have been more intensively selected (Korban, 2007). Vase life is affected by several pre-harvest and postharvest factors, and by the genetic constitution of the flower (Halevy et al., 1979). Studies have been conducted to determine the production factors affecting rose vase life such as light intensity, temperature, relative humidity and carbohydrate concentration (Mortensen et al., 2000; Marissen, 2001; Mortensen et al., 2007; Pettersen et al., 2007). Short vase life of roses has been associated with factors such as excessive water loss that occurs after harvest due to the inability of stomata to function proper after harvest (Mortensen et al., 2000; Mortensen et al., 2007; Pettersen et al., 2007) and with high respiration rates that occur during postharvest (Monteiro et al., 2001). Respiration rates are important in cut flowers because they provide an indication of the vase life of the product: the higher the respiration rate the shorter the vase life; the lower the rate, the longer the vase life (Reid, 2003). Postharvest factors such as temperature during shipping, storing time, constitution and quality of the hydration solutions during the distribution chain and at the consumer level have also been studied in relation to vase life (Halevy et al., 1979; Leonard et al., 2001; Marissen, 2001; Garcia et al., 2003; Nell et al., 2005). However, none of the above studies have reported specific physiological conditions that explain the presumably short vase life of fragrant cut roses.

Ethylene has a negative effect on postharvest quality of roses. External ethylene decreases vase life of miniature and cut rose cultivars (Mor et al., 1989; Serek et al., 1996; Muller et al., 1998; Muller et al., 2000; Muller et al., 2001). Studies have shown that nearly 80% of fresh cut rose cultivars are ethylene sensitive, as measured by reduced vase life or reduced flower opening after treatment with exogenous ethylene (Nell et al., 2009). In cut rose cultivars, external ethylene has several effects including increased petal abscission, premature wilting, fresh weight loss during vase life and alteration in bud opening and increase of autocatalytic ethylene production (Reid et al., 1989; Serek et al., 1995; Muller et al., 1998; Muller et al., 2001; Chamani et al., 2005; Tan et al., 2006; Xue et al., 2008). Endogenous ethylene can also be produced by rose flowers as part of the senescence process (Mor et al., 1989; Muller et al., 2001; Chamani et al., 2005) or as result of stress conditions like cold storage (Mor et al., 1989). The increase of internal ethylene production in rose flowers occurs as a result of the up-regulation of the enzymes involve in ethylene biosynthesis 1-Aminocyclopropane 1-carboxylic acid (ACC) oxidase and ACC synthase (Muller et al., 2001). The rise in ethylene production at the onset of senescence or under external ethylene exposure can be accompanied by a rise in respiration rate, a process defined as a climacteric response (Kays et al., 2004).

Ethylene effects can be controlled by altering either ethylene biosynthetic or signaling pathways. Ethylene biosynthesis can be reduced by chemicals such as aminoethoxyvinylglycine (AVG), which inhibits the action of 1-Aminocyclopropane1-carboxylic acid (ACC) synthase, the rate limiting enzyme in ethylene synthesis (Abeles et al., 1992). Flowers treated with AVG can be sensitive to external ethylene (Serek et al., 1993; Staby et al., 1993). Ethylene action can be prevented by the effect of chemicals such as silver thiosulphate (STS) and 1-methylcyclopropene (1-MCP). These compounds bind to ethylene receptors in plants preventing ethylene responses to

occur and extending the vase life of cut flowers (Sisler et al., 2003). STS and 1-MCP have been useful to increase vase life in a wide variety of ethylene sensitive cut flowers such as carnations (Veen, 1979), alstroemeria (Chanasut et al., 2003) and roses (Mor et al., 1989; Lukaszewska et al., 1990; Serek et al., 1995).

Ethylene regulates the volatile emission in petunia flowers by down-regulating the genes involved in volatile biosynthesis, by decreasing the activity of the biosynthetic enzymes, processes that lead to decrease synthesis of substrates (Schuurink et al., 2006). In petunias, ethylene is produced after pollination events leading to decreased emission of volatiles as the flower senesces (Negre et al., 2003). The emission of volatiles from flowers generally occurs within very specific tissues, at certain stages of development and at specific times of the day. Most volatile compounds are synthesized *de novo* in the tissues from which they are emitted (Dudareva et al., 2000). Volatiles are produced exclusively in the cells of the epidermal layer of the plant so they can be easily released into the atmosphere. All flower parts including petals, sepals, anthers and gynoecia can be sources of volatiles (Dobson et al., 1990). However, the majority of the volatiles in flowers are biosynthesized and emitted in the petals (Bergougnoux et al., 2007) and more specific in the petal limb as observed in petunias (Schuurink et al., 2006). The petals have most of the enzymes present predominantly in the epidermal cells making them the principal site of biosynthesis and emission of the volatile compounds (Scalliet et al., 2006). In roses, both epidermal layers of the petals are capable of producing and emitting scent volatiles (Bergougnoux et al., 2007). Several volatile biosynthetic genes have been characterized in rose flowers (Guterman et al., 2002) and some common biochemical modifications such as hydroxylation, acetylation and methylation have been described for volatile production (Dudareva et al., 2006a). Volatile regulation in roses has been described at the developmental

level (Helsper et al., 1998; Oka et al., 1999; Shalit et al., 2003). For example, different volatile compounds (citronellol and 2-phenylethanol) were emitted at different stages of development (citronellol emission peaked at stage 4 while 2-phenylethanol peaked at stage 6) in *Rosa damascena* Mill (Oka et al., 1999). In *Rosa hybrida* cv ‘Honesty’, 2-phenylethanol was emitted at maximum during the day at early developmental stages but it changed to be maximum at night during later stages of development (Helsper et al., 1998). Volatile regulation in roses has also been described at the spatial level. Petals are the major sites of volatile production within the flower (Dobson et al., 1990), and the adaxial and abaxial epidermal tissues are sites of volatile production within the petals (Bergougnoux et al., 2007). Rhythmic emission of rose volatiles are reported to occur as a result of diurnal (Picone et al., 2004) and circadian regulation (Helsper et al., 1998). However, the role of ethylene on volatile regulation on rose flowers was not studied in these experiments.

This research investigated the relationship between ethylene production, ethylene sensitivity, respiration rate and fresh weight loss of flowers with the vase life and volatile emission of fragrant and non-fragrant cultivars. The primary focus was to determine which factors affect the vase life of fragrant rose flowers most significantly.

## **Materials and Methods**

### **Cultivar Screening Experiment**

In order to determine the relationship between vase life and fragrance, a selection of fragrant and non-fragrant cut rose cultivars *Rosa hybrida* L. ‘Allure’, ‘Avan Garde’, ‘Coolwater’, ‘Ectasi’, ‘Erin’, ‘Freedom’, ‘Lovely Dream’, ‘Osiana’, ‘Peckoubo’, ‘Plaza Roja’, ‘Red Sensation’, ‘Savoy’ and ‘Yabadabadoo’ were shipped to the University of Florida from commercial farms located in Colombia and Ecuador. Flowers from all cultivars were harvested between 8 -10 a.m. at normal commercial stages of development. Flowers were taken to a

postharvest room (14-18 °C) within 30 minutes of harvest, where they were cut to 50 cm and placed into fresh hydration solution (water, 60  $\mu\text{L L}^{-1}$  chlorine and 0.9g/l citric acid to adjust pH to 4.5) for 2 hours. After hydration, flowers were grouped per cultivar and placed at 3°C in the same solution for 12 hours. The next day, flowers were packaged into commercial corrugated boxes (104.1 cm long x 24.1 cm tall x 22.9 cm wide). Each box contained 8 bunches (25 stems each). The boxes were sent via FedEx to Gainesville, FL and arrived within 4 days of harvest. A temperature recorder (Hobo<sup>®</sup> Temp, Onset Computer Corporation, Bourne, MA, USA) was placed inside the boxes in order to register the shipping temperatures. The range of temperatures registered was from 3 °C to 30 °C during shipping and average and average 8 °C. Upon arrival, the boxes were opened and flowers were cut to 45 cm in length and placed for 3 hours at 21 °C into 5 liters of deionized (DI) water. All flowers were held at 3 °C overnight (17 hours) to follow retail handling procedures. The next day different flowers were selected for vase life/opening studies ( $n = 24$ ), ethylene production ( $n = 5$ ), respiration rate ( $n = 5$ ), and volatile emission ( $n = 5$ ) per cultivar.

### **Vase life**

Flowers were placed into vases containing one liter of DI water and maintained in a postharvest evaluation room at 21 °C and 40-60% relative humidity with a 12 hour photoperiod under 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of light provided by overhead cool white fluorescent bulbs (Sylvania, Danvers, MA). Vase life was determined from the time flowers were placed into the postharvest evaluation room until the first sign of visual flower senescence symptoms occurred (i.e. petal wilting, abscission, bluing and disease). Flower opening of each flower was determined in order to determine the difference in developmental stages between cultivars over time. Flower opening was rated immediately after placing flowers into the postharvest room (day 0) and every two

days using the following system: 1 = outer petals tightly wrapped around bud, 2 = outer petals starting to reflex from bud, 3 = outer petals reflexed approximately 135° to stem, 4 = outer petals reflexed at approximately 115° to stem, 5 outer petals reflexed at 90° to stem. A rating of 3 or above was considered acceptable opening for consumer satisfaction.

A total of two replications (studies) per cultivar, each with 24 stems (3 vases with 8 stems per vase), were used using a randomized complete block design. Vase life differences between cultivars were analyzed using SAS GLM procedure (SAS® Version 9 Institute Inc., Cary, NC, USA). When significant cultivar effects ( $p \leq 0.005$ ) were determined by GLM, data means were separated using Tukey's multiple comparison test at  $p = 0.05$ . Data are presented as the mean  $\pm$  standard error of the mean of one typical study.

### **Ethylene production and respiration rate**

Flowers were cut to 6 cm in length and each flower was placed into 22 ml glass vials containing DI water. Each flower maintained in the vial was then placed into 900 ml glass mason jars containing 8 ml of 1 M potassium hydroxide (KOH) to maintain carbon dioxide (CO<sub>2</sub>) concentration below 0.1 %. All jars were closed for 21 hours and maintained at postharvest condition described above for the vase life evaluations. A 1 ml gas sample was obtained from each jar and analyzed by gas chromatography (Hewlett-Packard 5890 Series II, Avondale, PA) fitted with a flame ionization detector (FID) and a 80/100 Alumina F-1, 106.7 cm long x 0.3 cm diameter column (Supelco, Bellefonte, PA). Injector, detector and oven temperatures were 110, 130 and 150 °C, respectively. Ethylene production was measured daily between 9 a.m. and 12 a.m. immediately after the gas aliquot was obtained, the fresh weight of each flower was determined and the jars were left opened for 3 hours to allow for a complete air exchange and then resealed, so daily ethylene measurements could be obtained. Ethylene production from each

flower was measured for 7 days or until flowers showed senescence symptoms. Ethylene rate was expressed as  $\mu\text{lC}_2\text{H}_4/\text{kg}\cdot\text{hr}$  and calculated from  $\mu\text{lC}_2\text{H}_4/\text{kg}\cdot\text{hr} = \text{ppm C}_2\text{H}_4 \times [(\text{void volume ml}/\text{min} \times 60 \text{ min}/\text{hr})/(\text{kg} \times 1000)]$ .

Respiration rate was measured by  $\text{CO}_2$  produced from the flower. Flowers were placed into sealed glass mason jars as described above for the ethylene analysis with the difference that KOH was not added to the jars. All jars were closed for 2 hours and maintained under postharvest conditions described above for vase life evaluations. A 1 mL gas sample was obtained from each jar and analyzed on a Gow-Mac gas chromatograph (Series 580, Bridge water, NJ) equipped with a thermal conductivity detector (TCD). Jars were then opened until the following day.  $\text{CO}_2$  was measured daily between 9 a.m. and 12 a.m. for up to 7 days or until flowers showed senescence symptoms. The fresh weight of each flower was determined daily immediately after the  $\text{CO}_2$  sample was obtained. The fresh weight was used to calculate the respiration rate. Respiration rate was expressed as  $\text{mlCO}_2/\text{kg}\cdot\text{hr}$  and calculated from  $\text{mlCO}_2/\text{kg}\cdot\text{hr} = \% \text{ Co}_2 \times [(\text{void volume ml}/\text{min} \times 60\text{min}/\text{hr})/(\text{kg} \times 1000)]$ .

A total of two replications (studies) per cultivar, each with 5 stems for ethylene and 5 stems for respiration rate determination were used using a randomized complete block design. Data was analyzed using SAS GLM procedure (SAS<sup>®</sup> Version 9 Institute Inc., Cary, NC, USA). When significant cultivar effects ( $p \leq 0.005$ ) were determined by GLM, data means were separated using Tukey's multiple comparison test at  $p = 0.05$ . Data are presented as the mean  $\pm$  standard error of the mean of one typical study.

### **Volatile collection**

Volatile compounds were collected from each cultivar immediately after placement into vases (day 0) and 2, 4 and 6 days later. Petals were removed from each flower, weighed and

placed into glass tubes (17 mm x 61 cm, 127 mL volume) that were connected to an air pump that provided a continuous air flow system. Filtered air passed through the tubes for one hour and volatiles were trapped on a sorbent Super Q column (Alltech, Nicholasville, KY) and eluted with 150  $\mu$ l of methylene chloride (Fisher Scientific, Pittsburgh, PA). Five  $\mu$ l of nonyl acetate (Aldrich Chemical Company, Inc. Milwaukee, WI) was added to the eluted volatile mix of each column after collection as an elution control and to standardize the quantity of the compounds from each sample. Samples were analyzed by gas chromatography and mass spectrometry as described by Underwood et al., (2005). In order to avoid variation in volatile emission due to temperature and diurnal regulation, volatiles were collected at 21 °C between 3:30 and 4 p.m. at each sampling date. In order to determine the developmental stage of the flower at the time of volatile collection, the opening stage of the flower was documented using opening scores described previously at each time volatiles were measured.

A total of two replications (studies) per cultivar were used for volatile collection, each with 20 stems per cultivar. Five different stems per cultivar were used on each day. Data were analyzed using SAS GLM procedure (SAS<sup>®</sup> Version 9 Institute Inc., Cary, NC, USA). When significant cultivar effects ( $p \leq 0.005$ ) were determined by GLM, data means were separated using Tukey's multiple comparison test at  $p = 0.05$ . Data are presented as the mean  $\pm$  standard error of the mean of one typical study.

### **Ethylene Experiments**

Fragrant 'Allure', 'Erin', 'Lovely Dream' and 'Osiana' and the non-fragrant 'Red Sensation' were selected from the screening experiment to further study the factors that lead to the differences in vase life observed among fragrant and non-fragrant cultivars. 'Allure' was selected as it was a very fragrant cultivar with a short vase life, low ethylene production and low



respiration rate. ‘Erin’ was selected as it was a fragrant cultivar with long vase life, low ethylene production and low respiration rate. ‘Lovely Dream’ was selected as it was a very fragrant cultivar with a short vase life and produced the highest amounts of ethylene during vase life. ‘Osiana’ was selected as a fragrant cultivar with long vase life, low ethylene and respiration rate and because in preliminary studies, it was extremely sensitive to exogenous ethylene which induced petal drop within 24 hours after being exposed to  $1 \mu\text{L L}^{-1}$  ethylene. ‘Red Sensation’ was selected from the non-fragrant cultivars as it had long vase life, the lowest ethylene production and lowest respiration rate (Table 3-1). Ethylene sensitivity, ethylene production, respiration rate and weight loss during vase life were determined for these five cultivars. To gain a better understanding of the relationship between ethylene, rose floral fragrance and vase life, the effects of ethylene on volatile production during flower development were determined on these cultivars.

In order to establish if ethylene played a role in flower fragrance and vase life, it was necessary to stop the perception and production of ethylene by treating flowers with anti-ethylene chemicals. Flowers from each of the five cultivars listed above were harvested, processed at the farm, packed and shipped as described above in the screening experiment. Two boxes per cultivar were shipped for this study. In order to protect the flowers from potentially being damaged by ethylene exposure during shipping, two 1-MCP sachets (EthylBloc<sup>TM</sup> Sachets, Floralife<sup>®</sup>, Inc. , Walterboro, SC) dipped in water were included inside one shipping box (104.1 cm long x 24.1 cm tall x 22.9 cm wide) and closed immediately. The other box contained no 1-MCP sachets and was used as a control. The boxes were sent via FedEx to Gainesville, FL within 4 days of harvest. A temperature recorder ((Hobo<sup>®</sup> Temp, Onset Computer Corporation, Bourne, MA, USA) was placed inside the boxes in order to register the shipping temperatures.

The range of temperatures registered was the similar to as the temperatures registered for the screening experiments.

Upon arrival, the flowers pre-treated with 1-MCP sachets were cut to 45 cm in length and placed for 3 hours at 21 °C into 5 liters of 0.2 mM (silver thiosulphate (STS)), a compound that prevented the flower to perceive ethylene during vase life. Flowers placed into the STS solution were transferred to a solution of DI water after 3 hours to avoid potential phytotoxicity. Flowers shipped with no 1-MCP sachets were cut to 45 cm and placed for 3 hours at 21 °C into 5 liters of deionized (DI) which was used as the control or in 0.7 mM (aminoethoxyvinylglycine (AVG)) (Valent U.S.A Corporation, Walnut Creek, CA), a solution that prevented the flower from producing internal ethylene during vase life. Flowers in DI water and AVG remained in these solutions. All flowers held at 3 °C overnight (17 hours) to follow retail handling procedures. The next day, flowers were placed into two different vase solutions: DI water (for DI and 1-MCP + STS pre-treated flowers) or 0.7 mM AVG (for AVG pre-treated flowers). Flowers were exposed to 1  $\mu\text{L L}^{-1}$  ethylene or air for 24 hours under postharvest conditions described above. Ethylene and air were applied by placing the vases into 99 L volume sealed glass chambers with a continuous airflow (0.6  $\text{L min}^{-1}$ ). The ethylene concentrations inside the chambers were monitored before placing stems in the chambers and after 4, 18 and 24 hours in the chambers using a gas chromatograph (Hewlett-Packard 5890 Series II) equipped with a flame ionization detector (FID) and a 80/100 Alumina F-1, 106.7 cm long x 0.3 cm diameter column (Supelco, Bellefonte, PA). Flowers were in the same hydration solution used during air/ethylene exposure for further evaluation. After hydrating with STS, AVG and DI water and the ethylene and air treatments, different flowers were selected to measure vase life and opening ( $n = 24$ ), ethylene production ( $n = 4$ ), respiration rate ( $n = 3$ ), and volatile emission ( $n = 2$ ) per treatment.

### **Vase life evaluations**

Flower vase life was determined using the same postharvest room conditions as described above in the vase life screening experiment. Flowers from each cultivar hydrated in either AVG, DI, STS and treated with ethylene or air were arranged in a 3 (hydration) x 2 (ethylene) factorial design. A total of two replications (studies) per cultivar were used, each with 24 stems (3 vases with 8 stems per vase) per treatment using a randomized complete block design. Data were pooled together from each study. The pooled data were analyzed using SAS GLM procedure (SAS<sup>®</sup> Version 9 Institute Inc., Cary, NC, USA). When significant treatment effects ( $p \leq 0.005$ ) were determined by GLM, data means were separated using Tukey's multiple comparison test at  $p = 0.05$ . Data are presented as the mean  $\pm$  standard error of the mean.

### **Ethylene effects on ethylene production and respiration rate**

Ethylene production and respiration rate were determined as described above in the screening experiment. Fresh weight of each flower was determined daily when jars were opened and used to calculate the ethylene and respiration rate as described in the screening experiment. Fresh weight data was also used to determine any ethylene effects on weight gain/loss of flowers and to register any differences in weight gain/loss during vase life that may explain the vase life differences among rose cultivars. Treatments were arranged in a 3 (hydration) x 2 (ethylene) factorial design. A total of two replications (studies) per cultivar were used, each with 4 stems per treatment for ethylene production determination and 3 stems per treatment for respiration rate determination using a randomized complete block design. Data were pooled together from each study. The pooled data was analyzed using SAS GLM procedure (SAS<sup>®</sup> Version 9 Institute Inc., Cary, NC, USA). When significant treatment effects ( $p \leq 0.005$ ) were determined by GLM, data

means were separated using Tukey's multiple comparison test at  $p = 0.05$ . Data are presented as the mean  $\pm$  standard error of the mean.

### **Ethylene effects on volatile production**

Volatile compounds were collected from flowers in all treatments for each cultivar immediately after the ethylene/air treatment (day 0) and 2, 4 and 6 days later of vase life. To measure the volatile emission from each flower throughout vase life, volatiles were collected as described above in the screening experiments with the difference that petals were not removed from the flower for volatile analysis. Therefore the same flower from each treatment was used during the entire 6 day collection. Flower stems were re-cut to 6 cm in length and placed individually into 22 ml glass vials with the same solution used during the ethylene/air treatment. Each flower was placed into 900 ml glass jars that were connected to the same air flow system described above in the screening experiment. Jars were opened and maintained at the postharvest conditions mention above. Treatments were arranged in a 3 (hydration) x 2 (ethylene) factorial design. A total of two replications (studies) were used, each with 2 stems per treatment per cultivar. Data were pooled together from each study. The pooled data was analyzed using SAS GLM procedure (SAS<sup>®</sup> Version 9 Institute Inc., Cary, NC, USA). When significant treatment effects ( $p \leq 0.005$ ) were determined by GLM, data means were separated using Tukey's multiple comparison test at  $p = 0.05$ .

### **Scanning Electron Microscopy Studies of the Rose Petal**

In order to determine if the differences in volatiles emitted from rose cultivars were due to differences in petal morphology, rose petal epidermal cells from all culitvars were analyzed by scanning electron microscopy. Two rose petals discs (2mm) from each variety were fixed in 4% glutaraldehyde in phosphate buffer saline (PBS). The following steps were with the aid of a Pelco Biowave Laboratory Microwave (Ted Pella, Redding, CA). The samples were washed in 1

X PBS and post fixed with 1% buffered osmium tetroxide. The fixed samples were washed in de-ionized water and dehydrated in a graded ethanol series (25%, 50%, 75%, 95%, 100%). The samples were then dried with a critical point dryer (Leica CPD 030, Bannokburn, IL) with bone dry carbon dioxide, mounted with carbon double sticky tabs (Ted Pella, Redding, CA) sputter coated with gold palladium (Denton Vacuum Desk II, Moorestown, NJ) and viewed on a S-4000 field emission SEM ( Hitachi High Technologies American, Inc. Schaumburg NJ). The scanning electron microscopy was performed by staff at the ICBR (Interdisciplinary Center for Biotechnology Research) Electron Microscopy & BioImaging Lab, University of Florida, Gainesville, FL

## **Results and Discussion**

### **Relationship Between Fragrance and Vase Life**

A rose must last at least seven days after handling and shipping as these number of days are the minimum time that a consumer expects a cut flower to last in a vase (Barletta, 1995). Vase life of fragrant ‘Allure’, ‘Ectasi’, ‘Lovely Dream’ and non-fragrant ‘Coolwater’ was less than 7 days. Vase life exceeded 7 days on fragrant ‘Avan Garde’, ‘Erin’, ‘Osiana’, ‘Savoy’, and non-fragrant ‘Freedom’, ‘Peckoubo’, ‘Plaza Roja’, ‘Red Sensation’ and ‘Yabadabadoo’. Cultivars with seven or more days of vase life were considered to have long vase life, while cultivars with less than seven days of vase life were considered to have a short vase life. Volatile amounts were high ( $> 4.4 \mu\text{g/gfw/h}$ ) and low ( $< 0.8 \mu\text{g/gfw/h}$ ) depending on the cultivar. High levels of volatile compounds were emitted from ‘Osiana’ and ‘Lovely Dream’, fragrant cultivars with long and short vase life, respectively. Low amount of volatiles were emitted from ‘Peckoubo’ and ‘Coolwater’, non-fragrant cultivars with long and short vase life, respectively (Table 3-1).

Significant ethylene was produced by fragrant ‘Lovely Dream’, a fragrant cultivar with short vase life. Less ethylene was produced by ‘Erin’, ‘Osiana’, ‘Allure’ and ‘Ectasi’ fragrant cultivars with long and short vase life and by non-fragrant ‘Freedom’, ‘Peckoubo’ and ‘Red Sensation’ cultivars with long vase life (Table 3-1). Increased ethylene production during development can occur in harvested commodities together with an increase in respiration rate at the end of development, condition defined as a respiratory climacteric response (Kays et al., 2004). Respiration is important for cut flower quality because it is highly correlated with vase life (Reid, 2003). Studies have demonstrated that high respiration rates are correlated with short vase life of cut roses during storage. High respiration rates were observed on ‘Peckoubo’ and ‘Ectasi’, non-fragrant and fragrant cultivars with long and short vase life, respectively. Low respiration was observed on ‘Erin’ and ‘Lovely Dream’, fragrant cultivars with long and short vase life respectively and on ‘Red Sensation’ non-fragrant cultivar with long vase life (Table 3-1). The vase life, volatile production, ethylene production and respiration rate results observed on these cultivars demonstrated that vase life of fragrant and non-fragrant cut rose cultivars was a complex parameter and was not directly related with the amount of volatile compounds, internal ethylene production or respiration rate (Figure 3-1). Further studies were conducted with selected cultivars to get a better understanding of the interaction between vase life, fragrance and ethylene sensitivity on cut rose flowers.

Based on the volatile emission, ethylene production, respiration rate and vase life of these cultivars, three cultivars with acceptable vase life, ‘Erin’, ‘Osiana’ and ‘Red Sensation’, and two cultivars with the shortest vase life, ‘Allure’ and ‘Lovely Dream’, were selected to further study factors that may affect vase life of fragrant cultivars, such as ethylene sensitivity, ethylene biosynthesis, respiration and fresh weight changes during flower development. These cultivars

were also used to study the effects of ethylene on volatile emission of rose flowers, because they provided fragrant ( ‘Allure’, ‘Lovely Dream’, ‘Erin’, ‘Osiana’) and non-fragrant cultivars ( ‘Red Sensation’) and a range of ethylene production and respiration rates (Table 3-1).

### **Volatile Compounds Emitted by Rose Flowers During Vase Life**

Volatile compounds observed in rose flowers consisted of compounds derived from three main biosynthetic pathways: phenylpropanoids, lipid derivatives and terpenoids (Knudsen et al., 1993). Fragrant ‘Allure’, ‘Erin’, ‘Lovely Dream’, ‘Osiana’ and non-fragrant ‘Red Sensation’ produced mostly phenylpropanoids (Figure 3-2) and lower levels of lipid derivatives (Figure 3-3) and terpenoids (Figure 3-4). Considering the range of vase life of these cultivars (Table 3-1), the fragrance of rose cultivars was not related to the individual levels of phenylpropanoids, lipid derivatives or terpenoid compounds. For example, ‘Allure’ and ‘Lovely Dream’, highly fragrant cultivars with short vase life and ‘Erin’ and ‘Osiana’, less fragrant cultivars with long vase life, produced mainly phenylpropanoids followed by terpenoids and lipid derivatives (Table 3-2). Non-fragrant ‘Red Sensation’, cultivar with long vase life produced mainly phenylpropanoids followed by lipid derivatives and terpenoids (Table 3-2). Studies have reported high levels of phenylpropanoids in ‘Sandra’ and ‘Silva’ rose cultivars and high levels of terpenoids in ‘Cardinal’ flowers, however the volatile levels were not related to vase life in the study (Kim et al., 2000).

Results from the current research are the first to demonstrate that the amount of phenylpropanoid, lipid derivative and terpenoid volatile compounds did not relate with vase life. The production of volatiles from each biosynthetic pathway was regulated differentially in all cultivars through development independently of their vase life. For example, phenylpropanoids and lipid derivatives increased during development as observed in the compounds 2-phenylethanol in ‘Erin’ and ‘Lovely Dream’ cultivars with long and short vase life, respectively

(Figure 3-2) and 3-cis-hexen-ol in 'Osiana' (long vase life) and 'Allure' and 'Lovely Dream' (short vase life) (Figure 3-3). Amounts of phenylpropanoids, lipid derivatives and terpenoids decreased during development as observed in the compounds methylbenzoate in 'Allure' (short vase life) and 3,5 dimethoxytoluene in 'Erin', 'Osiana' (long vase life) and 'Lovely Dream' (short vase life) (Figure 3-2), eicosane in 'Red Sensation' (long vase life) (Figure 3-3) and transcaryophyllene in 'Osiana' (long vase life) and 'Lovely Dream' (short vase life) (Figure 3-4). Amounts of phenylpropanoids, lipid derivatives and terpenoids remained constant during development as occurred on the compounds phenethyl benzoate in 'Erin' and 'Red Sensation', cultivars with long vase life (Figure 3-2), eicosane and 3-cis-hexen-ol in 'Erin' (Figure 3-3), (1R)(+)  $\alpha$ -pinene in 'Erin', 'Red Sensation' (long vase life) and 'Lovely Dream' (short vase life) and terpeniol in 'Red Sensation' cultivar with long vase life (Figure 3-4). Other volatiles such as the terpenoids s(-)-citronellol and linalyl acetate in 'Allure' (short vase life) (Figure 3-4) were produced in low amounts at early stages of development then increased at day 2 and decreased at later stages of development. Therefore, phenylpropanoids, lipid derivatives and terpenoids were produced in different amounts during development and the production pattern was variable in cultivars with both short and long vase life.

The differential production of diverse amounts and types of volatile compounds during development gave a characteristic fragrance that changed over time for each rose cultivar. For example, the fragrance of 'Allure' was fruity given by the production of methyl benzoate during the first day of vase life (Figure 3-2) and then changed to floral, fruity, sweet and citrus from (s)-(-)-B citronellol and linalyl acetate (Tandon et al., 2000; Jirovetz et al., 2002), compounds produced at the end of vase life (Figure 3-4). 'Erin' and 'Osiana' had a characteristic tea scent from 3,5 dimethoxytoluene (Figure 3-2), mixed with spicy note of trans caryophyllene (Figure 3-



4) (Shalit et al., 2004) which were produced at high amounts during early stages of vase life. At later stages of development the fragrance of ‘Erin’ changed to floral from the production of 2-phenylethanol, phenethyl benzoate (Figure 3-2) combined with green and grassy fragrance given from eicosane (Figure 3-3), compounds produced in higher amounts than other volatiles towards the end of vase life. The fragrance of ‘Osiana’ changed to a green, grassy fragrance given from increased production of cis-3-hexen-ol (Tandon et al., 2000; Jirovetz et al., 2002) at late stages of vase life (Figure 3-3). ‘Lovely Dream’ had the typical floral rose note during vase life from 2-phenylethanol (Figure 3-2) and alpha –pinene (Figure 3-4) (Watanabe et al., 2002) compounds produced constantly as the flower opened in the vase. At later stages, the floral fragrance of ‘Lovely Dream’ was combined with a green, grassy fragrant note given by the main lipid derivative cis-3-hexen-ol (Tandon et al., 2000; Jirovetz et al., 2002) produced in high amounts at this point (Figure 3-4).

In order to determine if the differences in volatile emission among rose cultivars were due to morphological differences between the petal cells, scanning electron microscopy was conducted on the petals of all cultivars (Figure 3-5). The adaxial petal surface had conical cells while the abaxial petal surface had flat cells on all cultivars. The surface of the cells on both sides was striated on all cultivars. No differences were observed on petal cell number or size among fragrant and non-fragrant cut rose cultivars. A slight difference in the adaxial cell shape was observed among cultivars. The cell shape was pentagonal on the fragrant ‘Allure’, ‘Erin’ and ‘Osiana’, and non-fragrant ‘Red Sensation’ flowers and dome and conical on the fragrant ‘Lovely Dream’ (Figure 3-5). The results of this study confirmed that the differences observed in the amount of volatiles emitted between cultivars were not due to external differences in petal morphology and suggest that they occurred as a result of internal biochemical differences.

Different volatile amounts were collected from petals of ‘Allure’, ‘Erin’, ‘Lovely Dream’, ‘Osiana’ and ‘Red Sensation’ all cultivars with petals with pentagonal and dome adaxial and flat abaxial cells.

The results of this study described the emission of volatile compounds as occurred in flowers that have passed through conventional handling and transportation processes. The volatile amounts through flower development reported in this study are important because they are more closely related to the amounts that rose consumers are being exposed to. The developmental regulation of phenylpropanoid, lipid derivatives and terpenoid volatiles as reported in this study agreed with other studies that have reported developmental regulation of volatiles in rose flowers (Helsper et al., 1998; Oka et al., 1999; Shalit et al., 2003). Furthermore, the results of volatile emission from rose flowers in this study together with the results of other flower species such as carnations, petunias and snapdragons (Schade et al., 2001; Negre et al., 2003) confirm that emission of volatile compounds from a flower is a complex process influenced by several factors (development, substrate availability, enzymatic activity, diurnal and circadian regulation between others). These factors are known to be affected by the senescence process of the flower and are regulated differentially in each flower species. Understanding senescence effects on rose volatile emission provides alternatives to delay the effects and allows maximizing floral fragrance emission at the consumer level.

### **Effects of Senescence on the Volatile Composition on Rose Flowers**

Studies have reported differences in gene expression, enzymatic activity and availability of substrates as causes for the different amount of volatiles emitted from different rose cultivars (Lavid et al., 2002; Scalliet et al., 2002; Watanabe et al., 2002; Wu et al., 2004; Scalliet et al., 2006). The current results demonstrate that fragrant and non-fragrant cultivars are producing volatiles at different levels as the flower senesces. Events such as membrane instability, changes

in activity of biosynthetic enzymes, reduced substrate availability and decreased gene expression may occur during flower senescence thus affecting volatile production. For example, results of this study showed that the lipid derivative cis-3-hexen-ol was produced at advanced stages of development in 'Allure', 'Lovely Dream' and 'Osiana' (Figure 3-3) when physiological processes related to senescence occurred. In these cultivars the changes in lipid volatile emission toward the end of development corresponded with visually senescing changes such as petal wilting as observed in 'Allure' and 'Lovely Dream' and petal drop as observed in 'Osiana'. Lipid derivatives are formed from linoleic and linoleic acids by the action of lipogenases and hydroperoxyliases enzymes that are thought to be associated with plastid envelope membranes (Blee et al., 1996). The lipid volatiles originate from membranes of petal tissue and are released from the membrane into the cytosol by hydrophobic lipid-protein particles (Hudak et al., 1997). One of the most important changes that occur during flower senescence is the change in the status of membrane lipids. As a flower senesces, the membrane lipids appeared in a disordered liquid-crystalline phase measured by x-ray diffraction analysis (Faragher et al., 1987a). During senescence, the membranes also lose their capacity to act as hydrophobic barrier (Faragher et al., 1987b). These changes of the physical state of membrane lipids are responsible in part for the increased membrane permeability which occurs prior to flower death (Faragher et al., 1987b). Therefore, these processes help supply the substrates for lipid derivative volatiles resulting in an increase production of these type of volatiles at later stages of development.

The decreased amounts of phenylpropanoids (Figure 3-2) and terpenoids (Figure 3-4) observed in 'Allure', 'Erin' 'Lovely Dream' and 'Osiana' through development was an event expected to occur because of physiological changes that take place during senescence directly affected the biosynthetic pathways in the flower. In rose flowers, changes of the enzymatic

activity and precursor availability occur with changes in the physiology of the flower as a result of the senescence process (Guterman et al., 2002; Dafny-Yelin et al., 2005). Studies of the volatile emission during flower opening suggest that the decline in emission of volatiles at late stages of development is a result of partial enzymatic hydrolysis of enzymes involved in the volatile biosynthetic pathways. Activity of the biosynthetic enzymes and availability of the precursors have been reported as factors regulating the emission of fragrant volatile compounds (Negre et al., 2003; Schuurink et al., 2006; van Schie et al., 2006). Analysis of the rose petal proteome during development have shown low activities of scent biosynthetic enzymes towards the late stages of development (Dafny-Yelin et al., 2005).

Further studies are needed to determine which specific senescence factors (i.e membrane degradation, availability of specific substrates, and changes of enzymatic activity) are affecting the volatile emission on rose flowers during development. For example, increased emission of cis-3-hexen-ol in senescing 'Allure', 'Lovely Dream' and 'Osiana' flowers may be related with availability of substrates such as linoleic and linolenic acids and action of biosynthetic enzymes such as lipogenases (Blee et al., 1996). Decrease of phenylpropanoid compounds such as 3,5-dimethoxytoluene, methyl benzoate and terpenoid (-) trans caryophyllene toward the advance stages of development in 'Allure', 'Erin', 'Lovely Dream' and 'Osiana' may be explained by the availability of substrates for each compound such as orcinol, benzoic acid and isopentenyl diphosphate (IPP) respectively, and to the activity of the respective enzymes such as O-methyltransferases, S-adenosyl-L-methionine:benzoic acid carboxyl methyltransferase (BAMT) and B-caryophyllene synthase CAS as described previously (Scalliet et al., 2002; Boatright et al., 2004; Dudareva et al., 2006a). These results will give a better understanding of the specific

biochemical changes that affect volatile emission in rose flowers that occur as a result of senescence.

### **Ethylene Regulation of Volatile Emission During Development**

To further understand the differences in volatile emission and regulation in roses, volatile emission was examined during flower opening and was related with endogenous ethylene production. Ethylene production during flower development was very low in ‘Allure’, ‘Erin’, ‘Osiana’ and ‘Red Sensation’, cultivars with short and long vase life. Ethylene production was high in ‘Lovely Dream’, a cultivar with short vase life (Figure 3-6). ‘Allure’ and ‘Erin’ were the only cultivars to have increased ethylene production at the end of vase life (Figure 3-6). Endogenous ethylene had no effect on the production of phenylpropanoids (Figure 3-7) lipid derivatives (Figure 3-8) or terpenoids (Figure 3-9) for any of the cultivars over time. Ethylene production was not autocatalytic as ethylene exposure did not increase internal ethylene production in all cultivars as flowers aged (Figure 3-6). The absence of autocatalytic ethylene production in these cut rose cultivars agree with the results observed in ‘Vanilla’ miniature flowers as these flowers did not exhibit an increase in ethylene production after ethylene exposure (Muller et al., 2001). The results of this study are different from the results reported on ‘Bronze’ miniature rose flowers which had an increase ethylene production after external ethylene exposure (Muller et al., 2001). Therefore these studies report the great variability that exists between rose cultivars in terms of autocatalytic ethylene production.

Volatile emission was also studied after exogenous ethylene exposure. Exogenous ethylene had no effect on the production of phenylpropanoids (Figure 3-7) and lipid derivatives production (Figure 3-8) for any of the cultivars. Exogenous ethylene had an effect on the production of terpenoids in ‘Allure’ (Figure 3-9). The amount of terpenoid volatile compounds was not affected by ethylene treatment in ‘Lovely Dream’, ‘Erin’, ‘Osiana’ and ‘Red Sensation’

(Figure 3-9). The amount of terpenoid on 'Allure' flowers treated with the ethylene perception inhibitor (STS) was five times higher the amount produced by flowers that perceived ethylene (DI treated) after air exposure. After ethylene exposure, terpenoid production on STS treated 'Allure' flowers was ten times higher the amount produced by DI treated flowers (Figure 3-9). Reduction of volatile emission after ethylene application is likely due to a direct ethylene effect on the biosynthetic pathway for these volatiles as it has been reported in other flowers such as petunia. In petunia volatile production after ethylene exposure was reduced due to a decreased expression of the genes involved in the biosynthetic phenylpropanoid pathway such as (S-adenosyl-L-Met:benzoic/salicylic acid carboxyl methyltransferase) (BSMT), benzoyl-CoA:benzyl alcohol/phenylethanol benzoyltransferase (BPBT) and phenylalanine ammonia lyase PAL (Negre et al., 2003; Underwood et al., 2005). Ethylene regulation of volatile compounds in rose flowers, as reported in this study was different from petunia.

The physiological results reported in this research provide a better understanding of the ethylene effect on volatile compounds on rose flowers. These results provide the guidelines to use biochemical and biotechnological approaches to further study ethylene effect on volatile emission in rose flowers. Ethylene effect can be studied on the activity of enzymes such as orcinol O-methyltransferase (OMT) and the expression of genes such as germacrene D synthase reported to be involved in the phenylpropanoid/terpenoid pathway in rose flowers (Guterman et al., 2002; Lavid et al., 2002). This will expand the knowledge on the specific biochemical factors that are affected by ethylene and cause the ethylene effect of terpenoid volatile emission.

### **Ethylene Effects on the Physiology of Rose Flowers**

External ethylene decreased vase life of all flowers but the degree of ethylene sensitivity differed among cultivars (Figure 3-10). 'Osiana' vase life was most affected (10 day reduction in vase life) after ethylene exposure, followed by 'Allure', 'Red Sensation', 'Erin' and 'Lovely

Dream' which had reductions of three, two, two and one days of vase life, respectively. Vase life was significantly increased in flowers of all cultivars with inhibited ethylene perception (STS treated) after ethylene treatment (Figure 3-10). Inhibition of ethylene perception had no effect on weight loss on 'Allure', 'Osiana' and 'Red Sensation'. Long vase life of flowers with inhibited ethylene perception led to longer maintenance of fresh weight and decreased respiration rates during vase life in some cultivars. For example, inhibition of ethylene perception (STS treatment) allowed 'Erin' and 'Lovely Dream' to maintain fresh weight for 7 days and 6 days, respectively compared to 'Erin' and 'Lovely Dream' flowers hydrated in DI which started to lose weight 2 days after ethylene treatment (Figure 3-11). The delayed time of fresh weight loss observed in 'Erin' and 'Lovely Dream' flowers with inhibited ethylene perception, corresponded with a long vase life observed in these cultivars (Figure 3-10).

In 'Lovely Dream', inhibition of ethylene perception (STS treated) decreased respiration from 14 mlCO<sub>2</sub>/kg/h to 10 mlCO<sub>2</sub>/kg/h compared to 'Lovely Dream' flowers that perceived and synthesized ethylene (DI treated) in an air and ethylene environment (Figure 3-12). The decreased respiration rate observed in flowers with inhibited ethylene perception corresponded to an increase in vase life in 'Lovely Dream' (Figure 3-10). Inhibition of ethylene perception had no effect on the respiration rates on 'Allure', 'Erin', 'Osiana' and 'Red Sensation'.

Vase life was not increased by inhibiting ethylene biosynthesis (AVG treated) after ethylene or air treatment (Figure 3-10) in all cultivars. AVG inhibits ACC synthase which is the key regulator of ethylene biosynthesis (Abeles et al., 1992). Therefore AVG treated flowers were still sensitive to external ethylene (Figure 3-10). Furthermore, inhibition of ethylene biosynthesis (AVG treatment) had no effect on the time 'Lovely Dream' and 'Erin' flowers started to lose weight after ethylene exposure but delayed the fresh weight loss after air treatment (Figure 3-11).

AVG had no effect on weight loss on 'Allure', 'Osiana' and 'Red Sensation'. However, inhibition of ethylene biosynthesis (AVG treated) decreased respiration from 14 mlCO<sub>2</sub>/kg/h to 10 mlCO<sub>2</sub>/kg/h compared to flowers that synthesized ethylene (DI treated) in an air and ethylene environment in 'Lovely Dream' and had no effect on 'Allure', 'Erin', 'Osiana' and 'Red Sensation' (Figure 3-12). These results suggest that internal ethylene production did not play a role on controlling the fresh weight loss for these cut rose cultivars while internal ethylene reduced vase life of some cut rose cultivars by increasing respiration rates during vase life. These results are important because they illustrate the variable effects of ethylene on physiological parameters such as weight loss and respiration rate that affect vase life of cut rose flowers.

### **Factors Related with Vase Life of Roses**

Short vase life on fragrant flowers was often associated with an increase in ethylene production during vase life as observed in fragrant 'Lovely Dream' (Figure 3-6). Endogenous ethylene production in 'Lovely Dream' was low at early development then increased after 3 days of vase life with subsequent low production towards the end of development (Figure 3-6). 'Erin', 'Osiana' and 'Red Sensation' produced negligible amounts of ethylene during development (Figure 3-6) and had a long vase life (Figure 3-10). 'Allure' produced low amounts of ethylene during development and increased prior showing the first senescence symptoms (Figure 3-6) and had a short vase life (Figure 3-10). Short vase life was observed after ethylene exposure in highly ethylene sensitive cultivars such as 'Osiana' (Figure 3-10). Short vase life was also related to rapid fresh weight loss during vase life. After 4 days of vase life fragrant 'Allure' lost 60 % of its initial fresh weight while fragrant 'Erin', 'Lovely Dream', 'Osiana', and non-fragrant 'Red Sensation' lost 20, 10, 20 and 10 % of fresh weight respectively at the same time (Figure 3-11). 'Allure' also opened faster than 'Erin', 'Lovely Dream', 'Osiana', and 'Red Sensation' cultivars. 'Allure' opened to a developmental stage greater than 4 while 'Erin', 'Lovely Dream', 'Osiana',



'Red Sensation' did not open more than a developmental stage of 4 after 1 day of vase life. The faster opening rate of 'Allure' likely led to more exposed petal surfaced area that resulted in greater amount of water loss from this cultivar. Factors that increased water loss in cut flowers include continuous stomata opening, increase cuticular transpiration, reduced water uptake caused by vascular occlusion originated from bacteria or deposition of lignins, suberin and tannin (Van Doorn, 1997a). Stomata were not observed on the surface of the petals of any of the cultivars and no differences in epidermal morphology were observed by SEM studies (Figure 3-5). Therefore other causes different from stomata opening may contribute to the differential water loss observed in these cultivars. Further studies will be necessary to determine the water relation factors that are causing the increased water loss in this cultivar.

High respiration rates during development were not directly related with vase life in rose flowers. Short vase life occurred on fragrant 'Allure' and 'Lovely Dream', cultivars with high flower respiration rates. Long vase life was observed in 'Erin' and 'Osiana', flowers with high respiration rates during development (Figure 3-13). Fragrant 'Allure', 'Erin', 'Lovely Dream' and 'Osiana' respired more than non-fragrant 'Red Sensation' (Figure 3-13). Vase life of 'Red Sensation' was double the vase life of 'Allure', lower than 'Erin' and the same as 'Osiana' (Figure 3-10). High respiration rate was related with rapid flower development. Fragrant 'Allure', 'Erin', 'Lovely Dream' and 'Osiana' flowers had high respiration rates ( $> 12$  ml CO<sub>2</sub>/kg/h) and opened to stages greater than 3.5 while non-fragrant 'Red Sensation' had a low respiration rates (6 ml CO<sub>2</sub>/kg/h) and did not opened greater than 2.5 during development (Figure 3-13). This study demonstrated that if a rose flower develops quickly and has a high respiration rate, it will not necessarily senesce faster than a flower that develops slowly and has a low respiration rate. These results did not agree with the importance of respiration rate on vase

life that has been demonstrated with studies performed in other rose cultivars such as ‘Raphaella’ roses. A significant correlation between vase life and respiration rate during storage periods was reported for this cultivar, the highest the respiration rate the shortest its vase life (Reid, 2003). High respiration rates indicate high metabolic activity of the tissues that also includes loss of substrate, synthesis of new compounds and release of heat energy (Kays et al., 2004). Since fragrance biosynthesis and emission is a process that requires significant metabolic energy, these results suggest that one factor that may enable a rose flower to be fragrant is being highly metabolically active, since all fragrant cultivars respired at higher rates than the non-fragrant cultivar during vase life (Figure 3-13).

### **Conclusion**

Vase life was not related with the amount of volatile compounds emitted from rose flowers. Short vase life was observed in highly sensitive fragrant cultivars (‘Osiana’) and on cultivars with an increased ethylene production during vase life (‘Lovely Dream’). Short vase life was also related with rapid development and quick fresh weight loss during vase life (‘Allure’). Long vase life was related with slow development and slow fresh weight loss during vase life as in non-fragrant (‘Red Sensation’) and with low ethylene production as in fragrant ‘Erin’ and ‘Osiana’. Volatile emission was not regulated by endogenous or exogenous ethylene except for terpenoid emission in ‘Allure’. These results demonstrate that the processes of volatile synthesis, ethylene synthesis, ethylene perception, fresh weight loss and respiration are not linked in rose flowers. Factors such as low ethylene sensitivity, low internal ethylene production, and maintenance of fresh weight for more than 6 days during vase life should be considered when selecting cultivars with long vase life. Finally, this study provided the basic physiological guidelines that combined with biochemical and biotechnological techniques are the tools to produce long lasting fragrant rose cultivars.

Table 3-1. Total volatile emission ( $n = 5$ ), ethylene production ( $n = 5$ ), respiration rate ( $n = 5$ ) and vase life ( $n = 24$ ) of fragrant \* and non-fragrant cut rose cultivars. Data represent means and standard error. Different letters within column = significant different  $\alpha=0.05$

Cultivar	Color	Fragrance ( $\mu\text{g/gfw/h}$ )	Ethylene production ( $\mu\text{l/kg/h}$ )	Respiration ( $\text{mlCO}_2/\text{kg/h}$ )	Vase life (average days)
'Allure' *	lavander	$2.91 \pm 1.30$ b	$0.6 \pm 0.06$ b	$17.6 \pm 1.1$ fg	$3.8 \pm 0.1$ c
'Avan Garde' *	lavander	$2.88 \pm 1.29$ b	$1.7 \pm 0.41$ ab	$68.1 \pm 2.5$ d	$10.1 \pm 0.5$ b
'Coolwater'	lavander	$0.76 \pm 0.34$ cd	$3.9 \pm 2.68$ ab	$36.5 \pm 3.7$ e	$5.5 \pm 0.7$ bc
'Ectasi' *	dark pink	$0.27 \pm 0.12$ cd	$0.8 \pm 0.33$ b	$199.0 \pm 7.3$ a	$3.5 \pm 0.2$ c
'Erin' *	yellow	$1.38 \pm 0.62$ c	$0.6 \pm 0.11$ b	$13.0 \pm 0.4$ g	$10.3 \pm 0.5$ ab
'Freedom'	red	$0.40 \pm 0.18$ cd	$0.5 \pm 0.13$ b	$56.5 \pm 2.8$ d	$12.3 \pm 0.3$ a
'Lovely Dream' *	pink	$5.18 \pm 2.32$ a	$4.7 \pm 1.61$ a	$12.9 \pm 0.2$ g	$6.6 \pm 0.6$ bc
'Osiana' *	peach	$1.06 \pm 0.47$ cd	$0.6 \pm 0.02$ b	$68.9 \pm 3.4$ d	$12.1 \pm 0.5$ a
'Peckoubo'	pink	$0.12 \pm 0.07$ d	$0.4 \pm 0.06$ b	$157.1 \pm 4.0$ b	$9.0 \pm 0.5$ b
'Plaza Roja'	red	$0.03 \pm 0.00$ d	$3.0 \pm 1.27$ ab	$33.0 \pm 0.3$ ef	$8.7 \pm 0.5$ b
'Red Sensation'	red	$0.37 \pm 0.17$ cd	$0.2 \pm 0.04$ b	$7.9 \pm 0.3$ g	$8.4 \pm 0.8$ b
'Savoy' *	pink	$4.43 \pm 1.98$ a	$1.3 \pm 0.16$ b	$89.2 \pm 3.5$ c	$9.5 \pm 0.5$ b
'Yabadabadoo'	orange	$0.20 \pm 0.09$ cd	$0.9 \pm 0.17$ ab	$57.2 \pm 0.9$ d	$9.5 \pm 0.5$ b

Table 3-2 Percentage of phenylpropanoid, lipid derivative and terpenoid volatile production from total amount of volatiles ( $\mu\text{g/gfw/h}$ ) produced per cultivar

Cultivar	Volatile	Total amount ( $\mu\text{g/gfw/h}$ )	% of total
'Allure'	Lipid derivatives	0.09	3.0
	Phenylpropanoids	2.84	96.1
	Terpenoids	0.03	0.9
	Total	3.00	
'Erin'	Lipid derivatives	0.00	1.4
	Phenylpropanoids	0.29	93.7
	Terpenoids	0.02	4.9
	Total	0.31	
'Lovely Dream'	Lipid derivatives	0.00	0.4
	Phenylpropanoids	1.09	97.3
	Terpenoids	0.03	2.3
	Total	1.12	
'Osiana'	Lipid derivatives	0.22	22.3
	Phenylpropanoids	0.70	71.0
	Terpenoids	0.07	6.7
	Total	0.98	
'Red Sensation'	Lipid derivatives	0.00	14.0
	Phenylpropanoids	0.02	76.3
	Terpenoids	0.00	9.7
	Total	0.02	

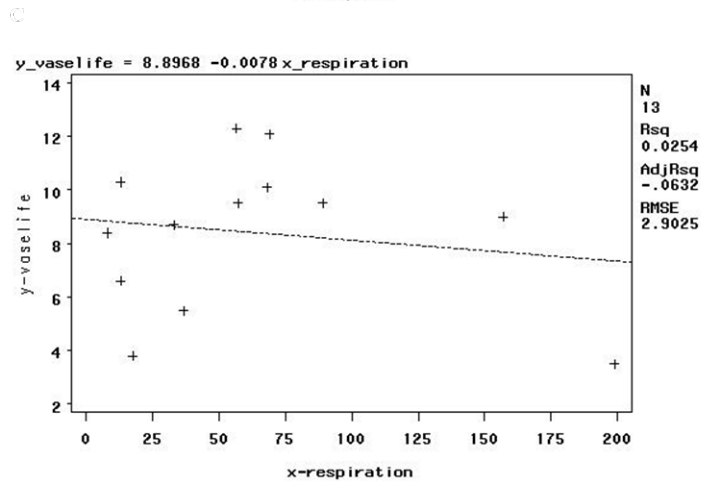
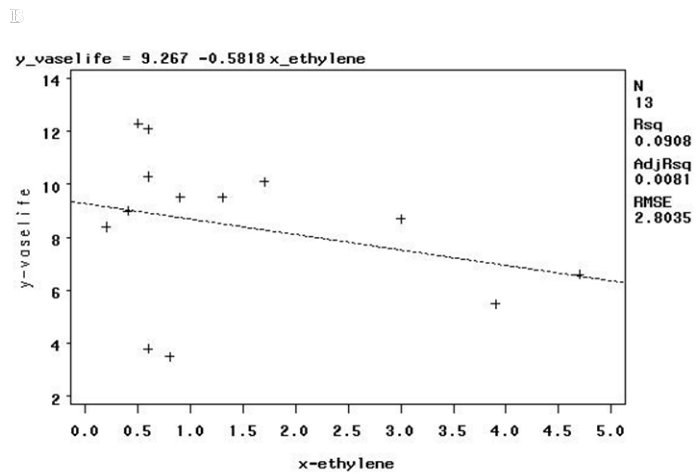
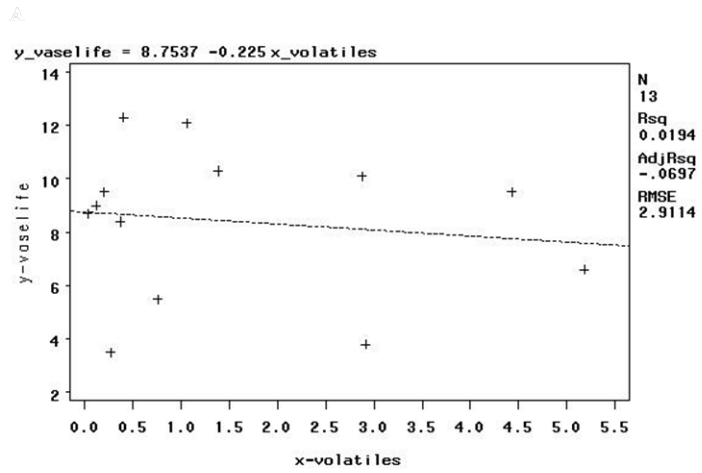


Figure 3-1. Regression analysis for vase life and A) volatile emission B) ethylene production C) respiration rate.

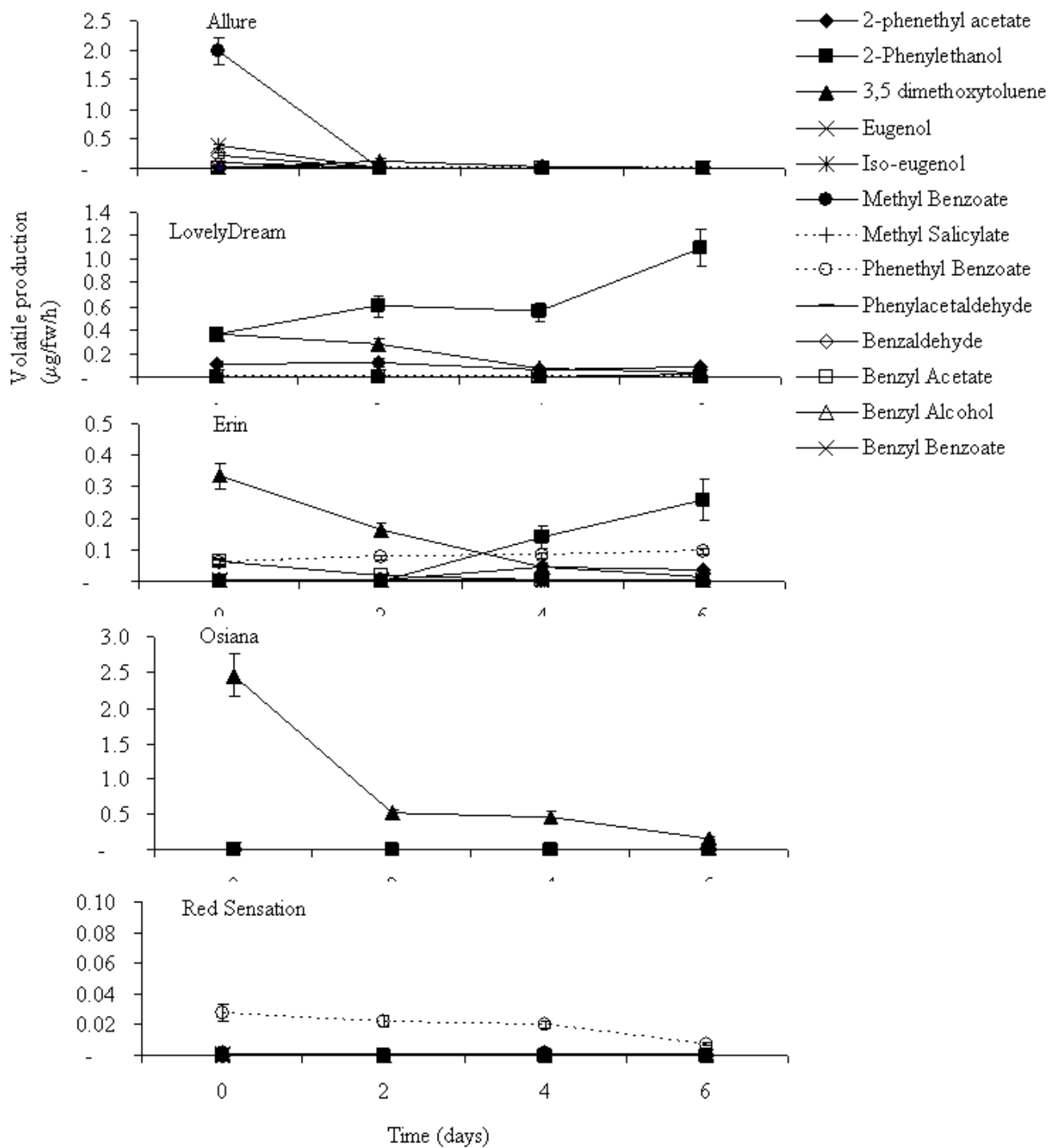


Figure 3-2 Phenylpropanoid production ( $\mu\text{g}/\text{fw}/\text{h}$ ) during development of *Rosa* cultivars. Data from each treatment were pooled to show total production of each compound during development. Means and standard error  $n = 12$ .

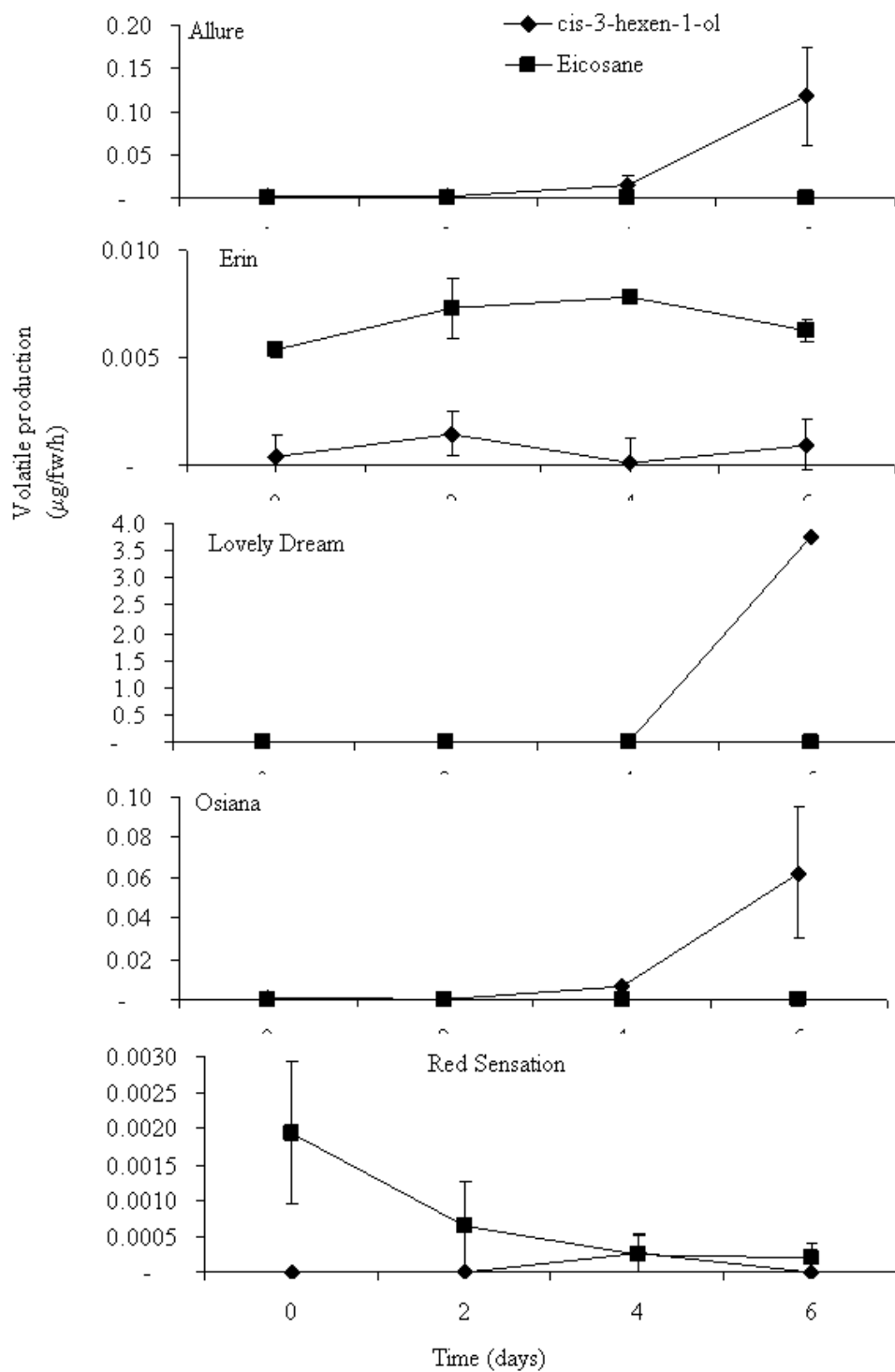


Figure 3-3 Lipid derivative production ( $\mu\text{g}/\text{fw}/\text{h}$ ) during development of *Rosa* cultivars. Data from each treatment were pooled to show total production of each compound during development. Means and standard error  $n = 12$ .

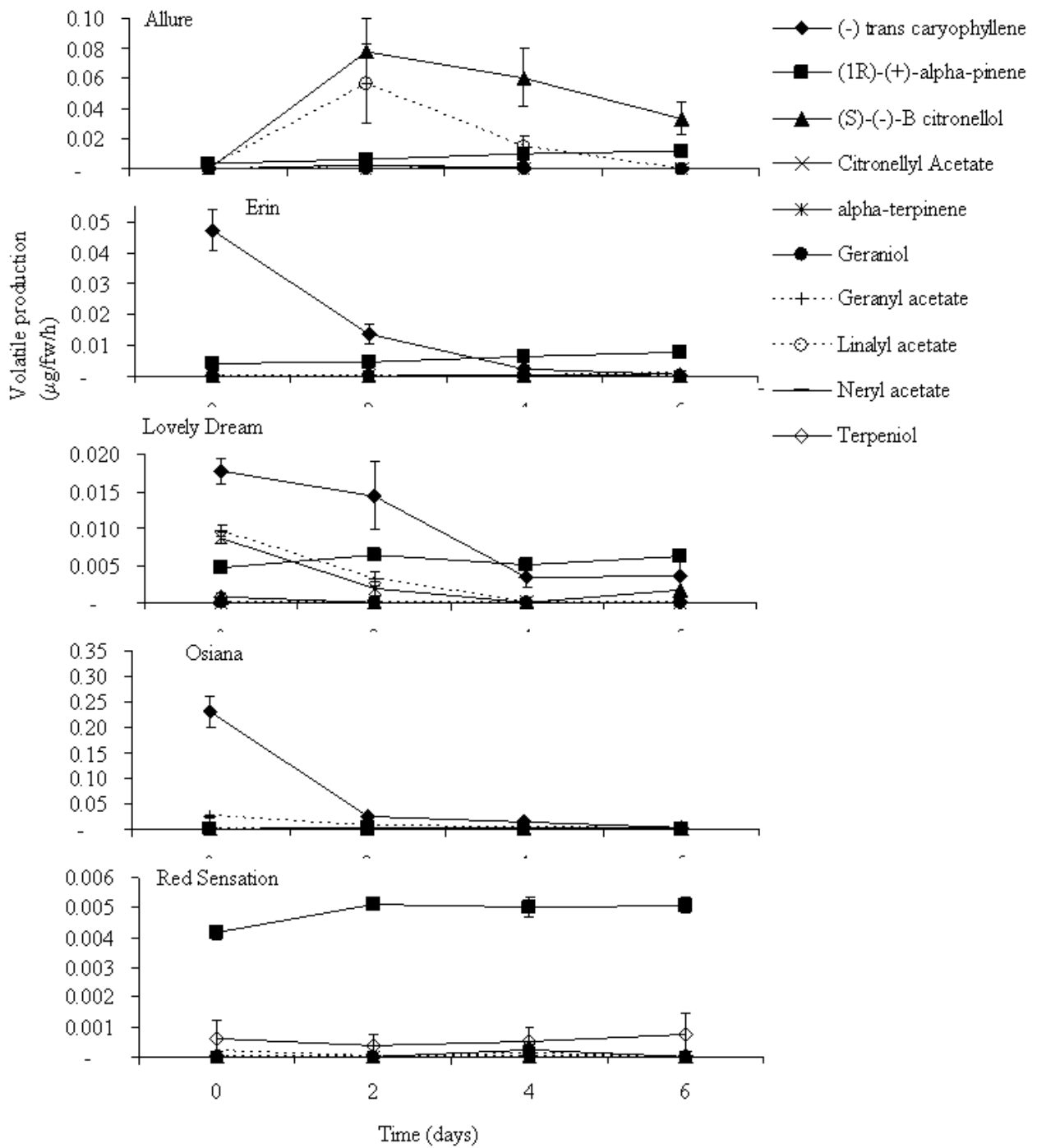


Figure 3-4 Terpenoid production ( $\mu\text{g}/\text{fw}/\text{h}$ ) during development of *Rosa* cultivars. Data from each treatment were pooled to show total production of each compound during development. Means and standard error  $n = 12$ .



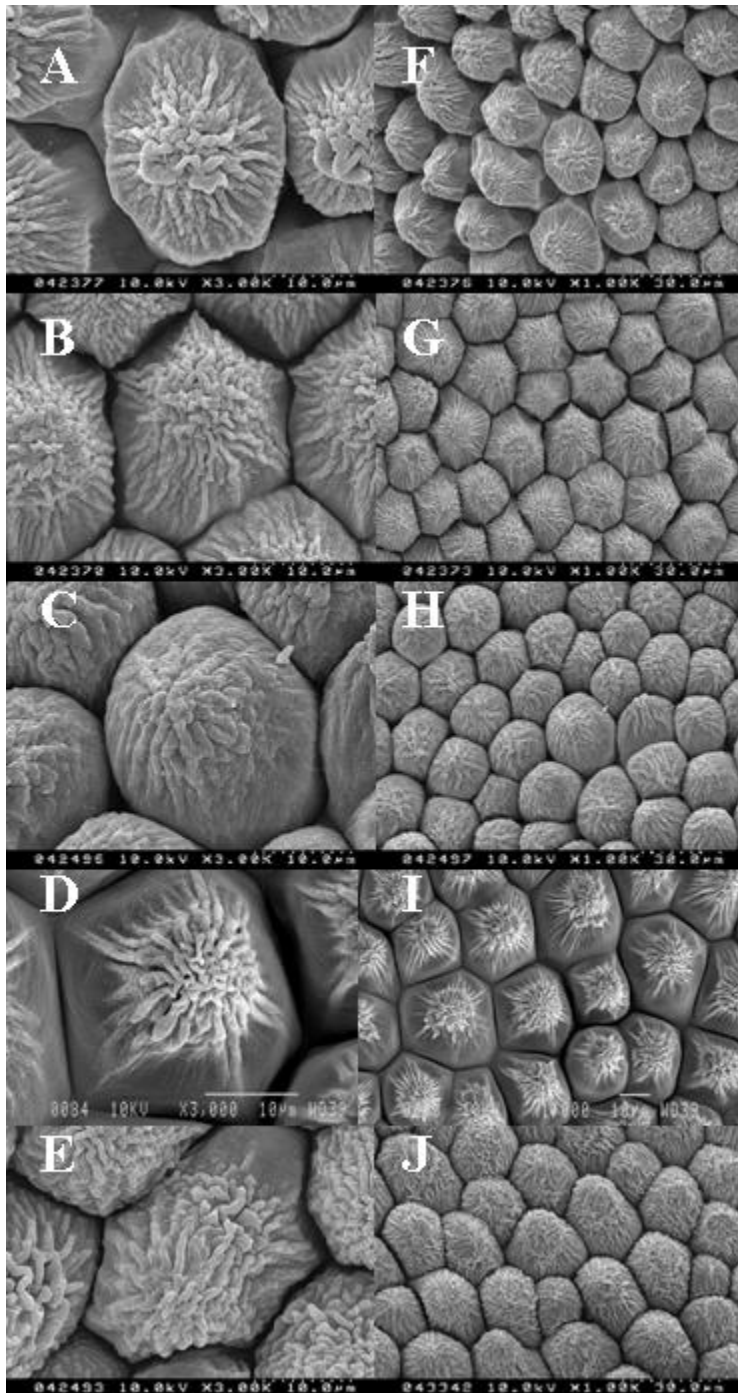


Figure 3-5. Petal cell shape of *Rosa* (A-E). 3000x, (F-J) 1000X 39mm working distance, 10keV, probe current of  $1 \times 10^{-10}$  amperes, aperture 3 (70 $\mu$ m), tilt of 0°. The specimen was prepared via glutaraldehyde fixation, ethanol dehydration, CPD, sticky tab mounting, and coated with 20nm of sputtered gold. The micrographs are centered on the conical

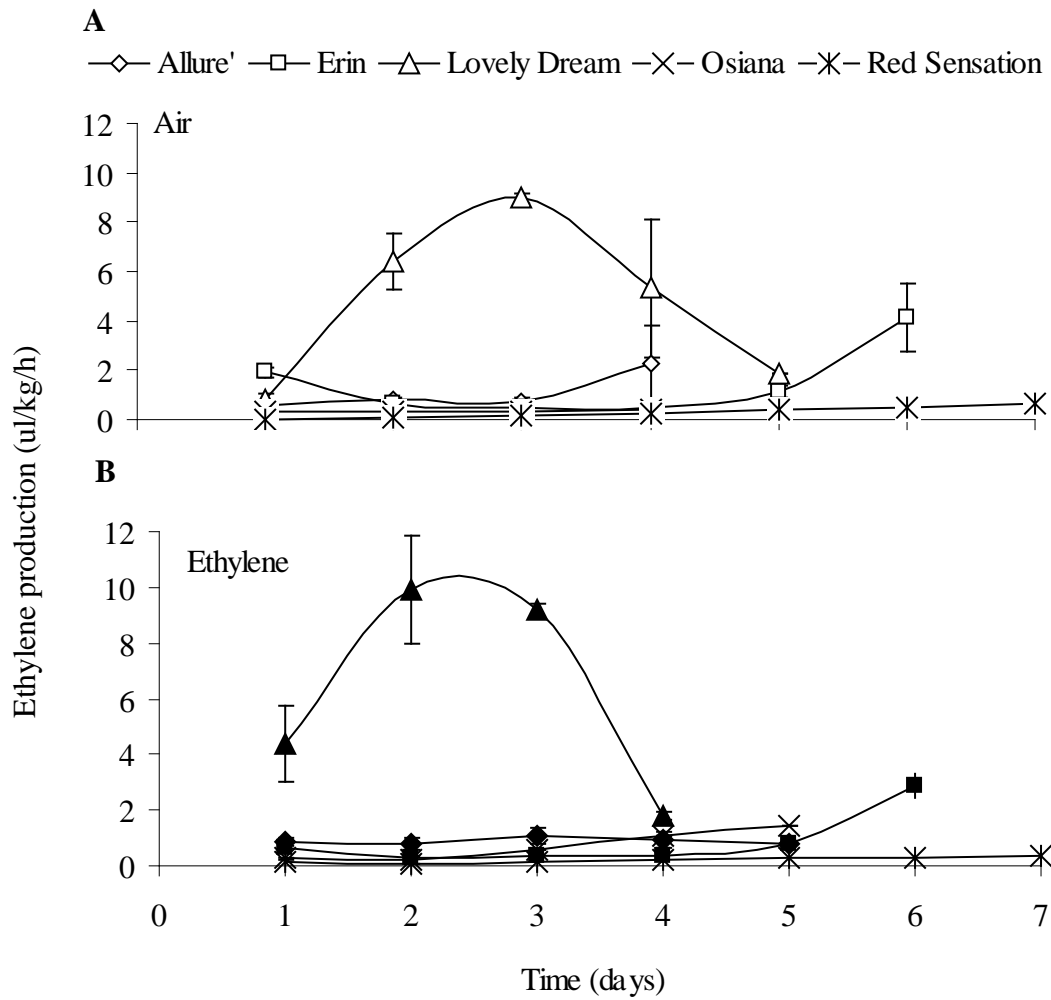


Figure 3-6. Endogenous ethylene production from rose cultivars during development (A) and after external ethylene exposure ( $1 \mu\text{LL}^{-1}$  for 24 hours) (B). Means and standard error  $n = 8$ .

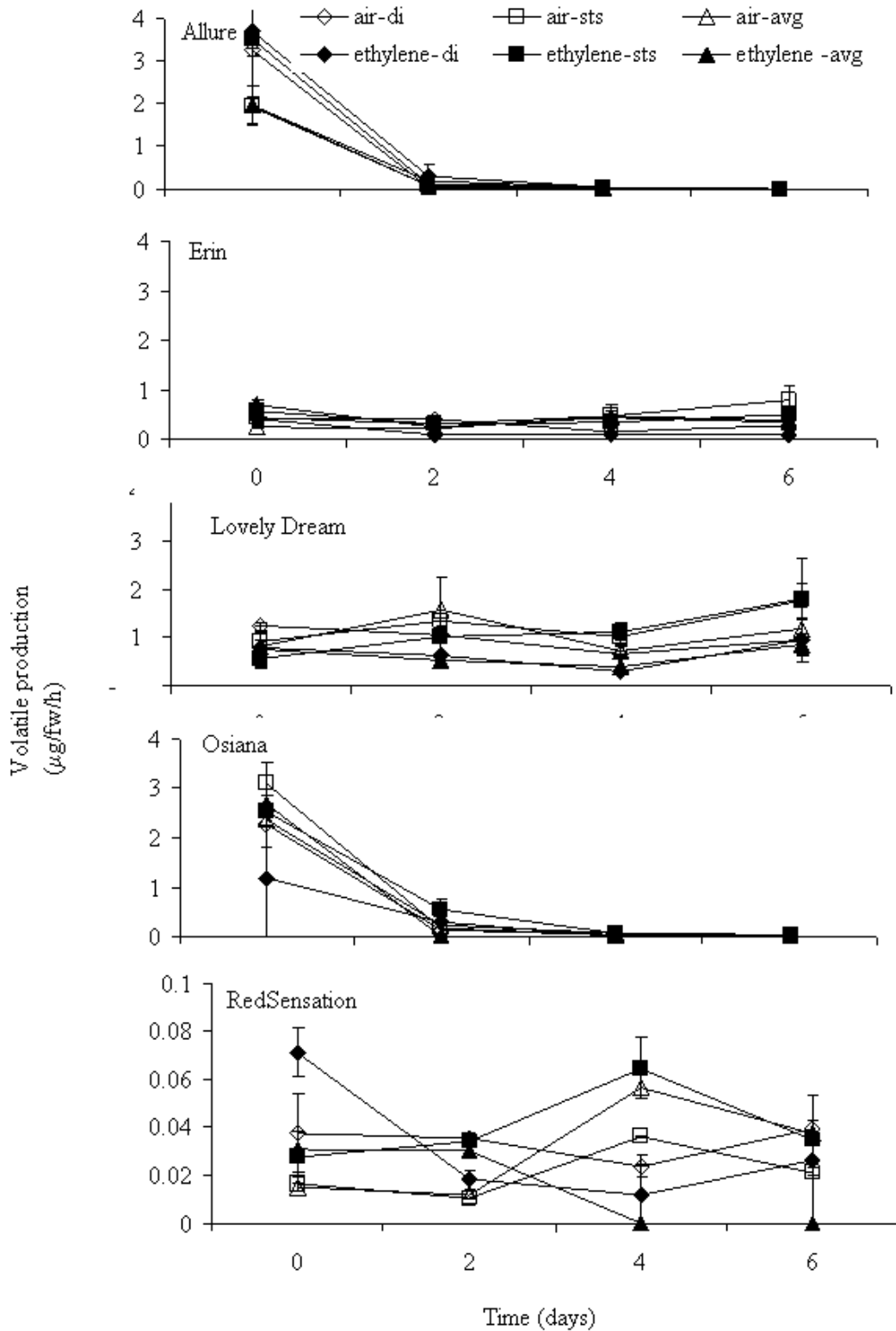


Figure 3-7. Phenylpropanoid production ( $\mu\text{g}/\text{fw}/\text{h}$ ) from *Rosa* cultivars. Flowers were treated with 0.7 mM AVG, 0.2 mM STS or DI water prior to exposure to ethylene or air. Means and standard error  $n = 4$ .

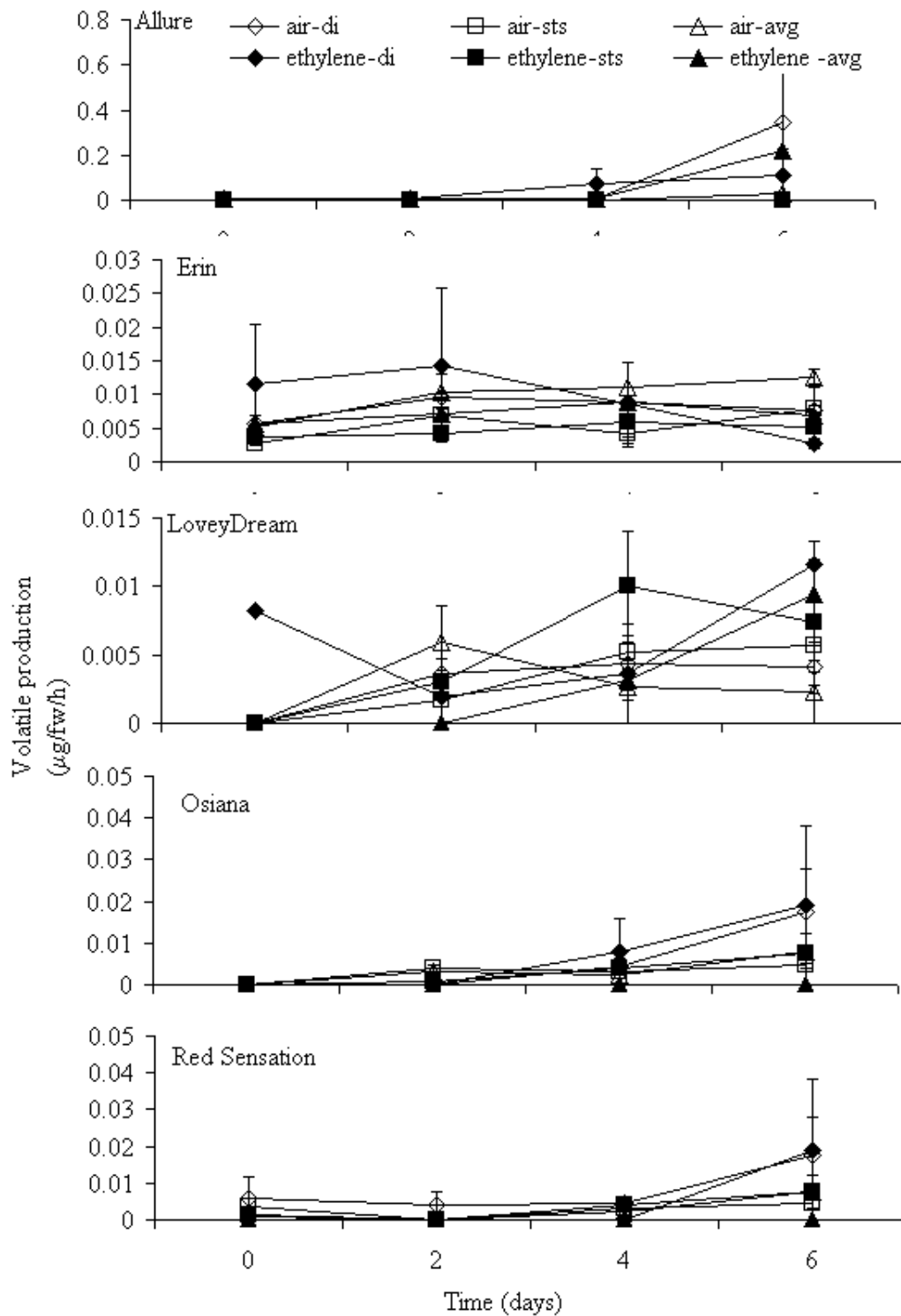


Figure 3-8. Lipid derivative production ( $\mu\text{g}/\text{fw}/\text{h}$ ) from *Rosa* cultivars. Flowers were treated with 0.7 mM AVG, 0.2 mM STS or DI water prior to exposure to ethylene or air. Means and standard error  $n = 4$ .

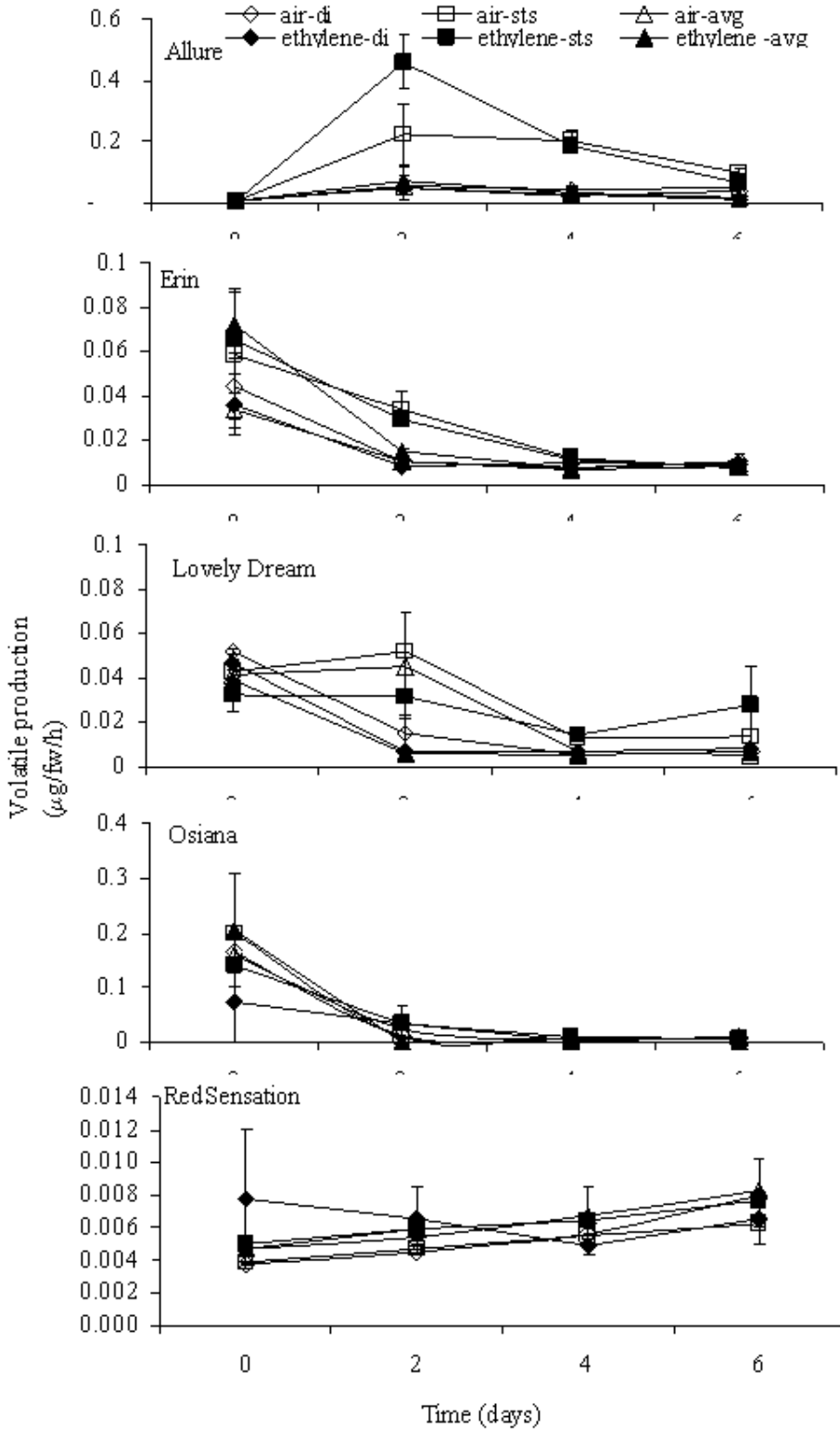


Figure 3-9. Terpenoid production ( $\mu\text{g}/\text{fw}/\text{h}$ ) from *Rosa* cultivars. Flowers were treated with 0.7 mM AVG, 0.2 mM STS or DI water prior to exposure to ethylene or air. Means and standard error  $n = 4$ .

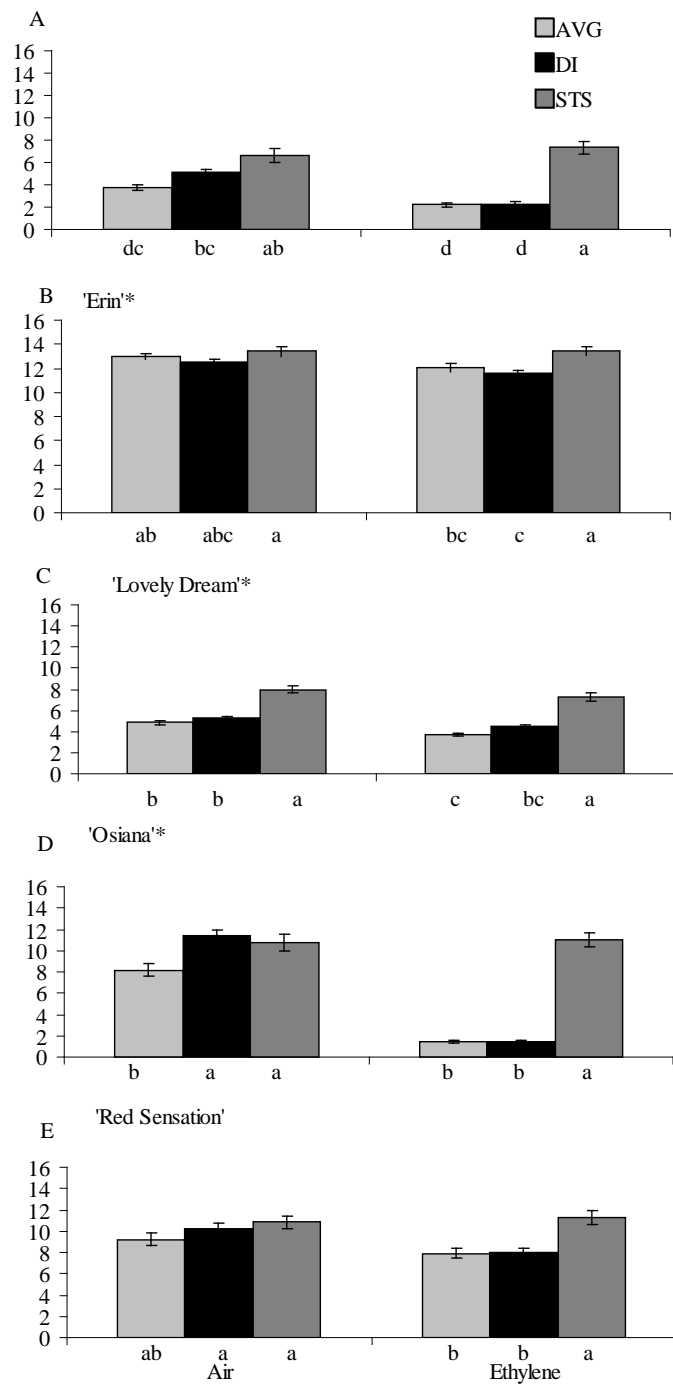


Figure 3-10. Ethylene effects on vase life of fragrant (\*) and non-fragrant flowers. Flowers were treated with 0.7 mM AVG, DI (control) or 0.2mM STS prior to exposure to either ethylene ( $1 \mu\text{LL}^{-1}$ ) or air for 24 hours. Means and standard error  $n = 48$ . Means within cultivar with the same letter are not significantly different  $\alpha = 0.05$ . A) 'Allure' \* B) 'Erin' \* C) 'Lovely Dream' \* D) 'Osiana' \* E) 'Red Sensation'

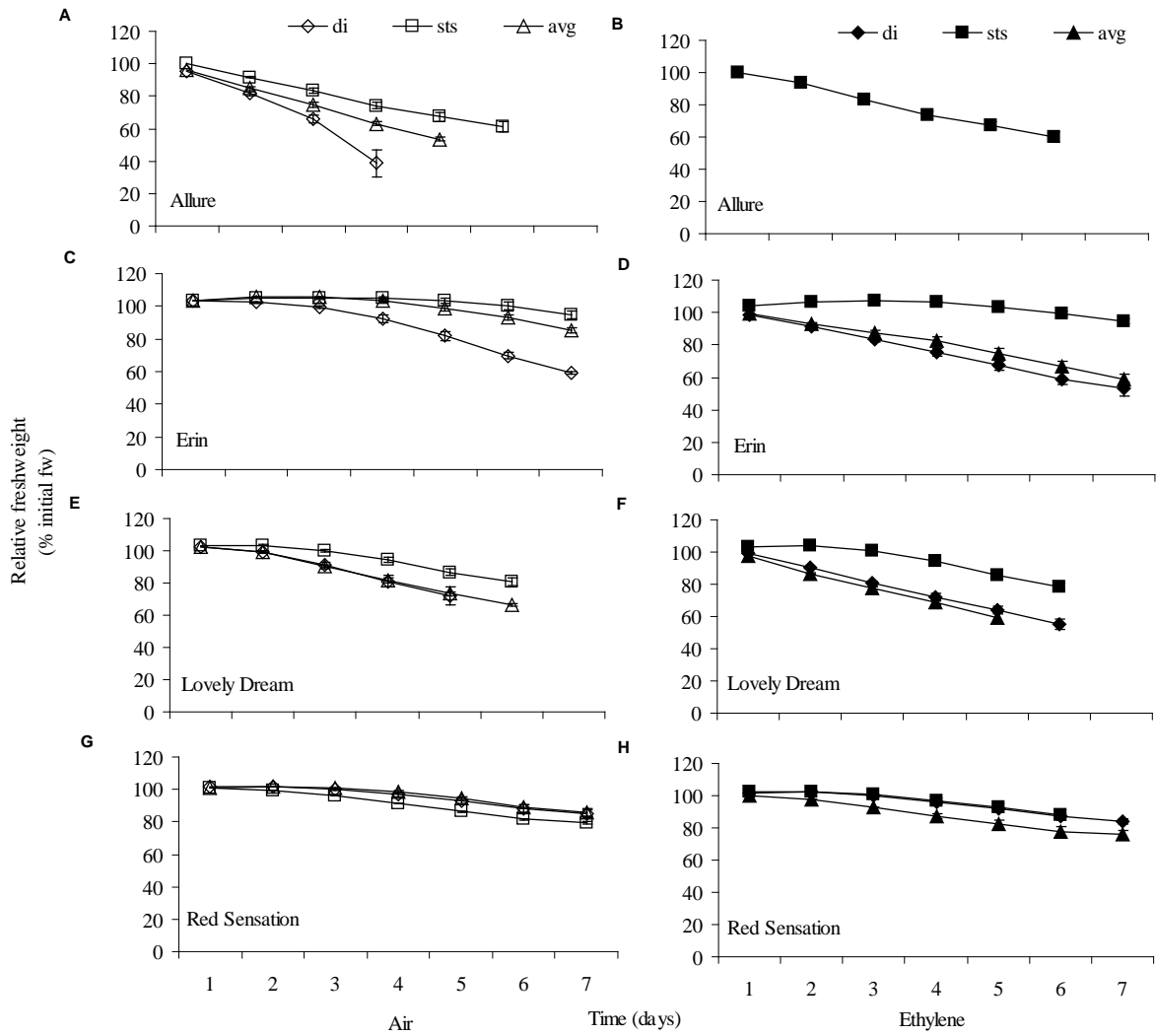


Figure 3-11. Effects of hydration treatment and ethylene on weight change during vase life of fragrant and non-fragrant flowers. Flowers were hydrated in 0.7 mM AVG, DI water or 0.2 mM STS prior to ethylene or air exposure. Means and standard error  $n = 6$ . 'Allure' hydrated with AVG and DI and treated with ethylene were all dead so no data was registered. A, B) 'Allure' C,D) 'Erin' E-F) 'Lovely Dream', G,H) 'Red Sensation'

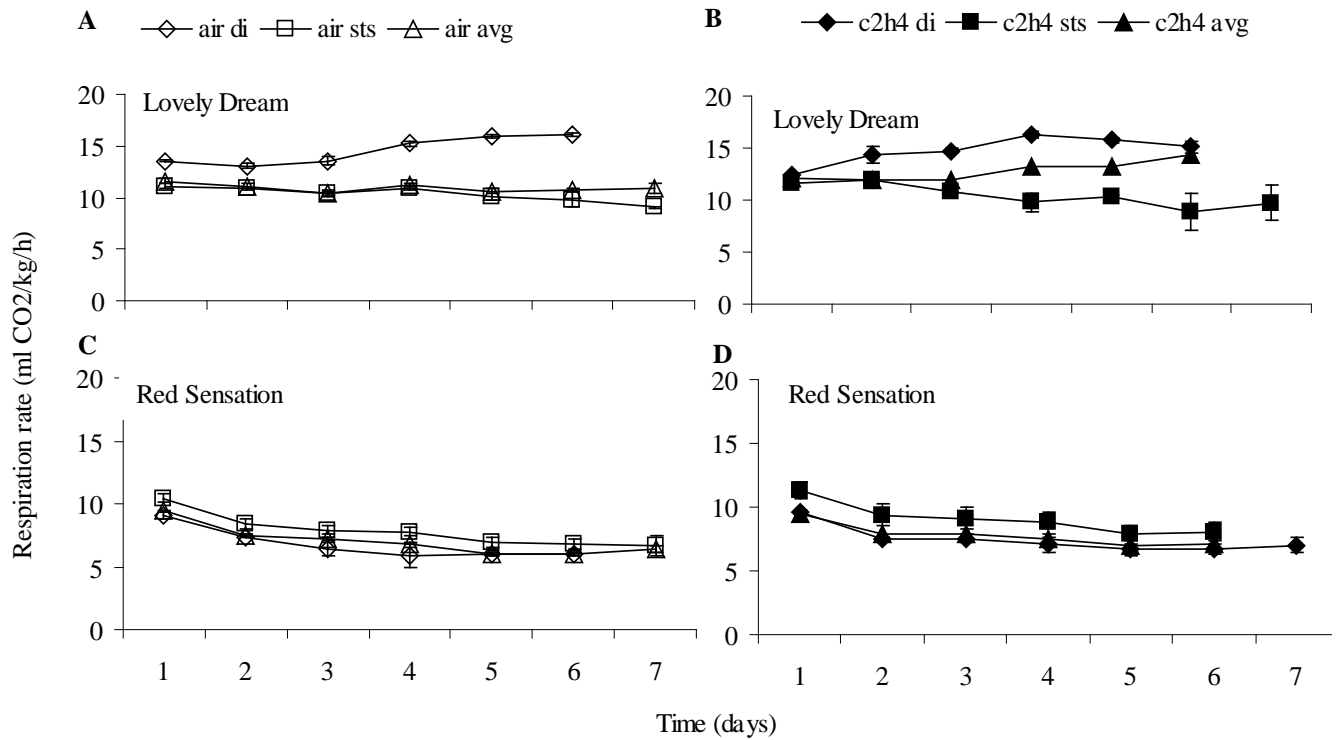


Figure 3-12 Effects of hydration treatment on the respiration rate of rose flowers. Flowers were hydrated in 0.7 mM AVG, DI water or 0.2 mM STS prior to ethylene or air exposure. Means and standard error  $n = 6$ . A, B) 'Lovely Dream' C,D) 'Red Sensation'



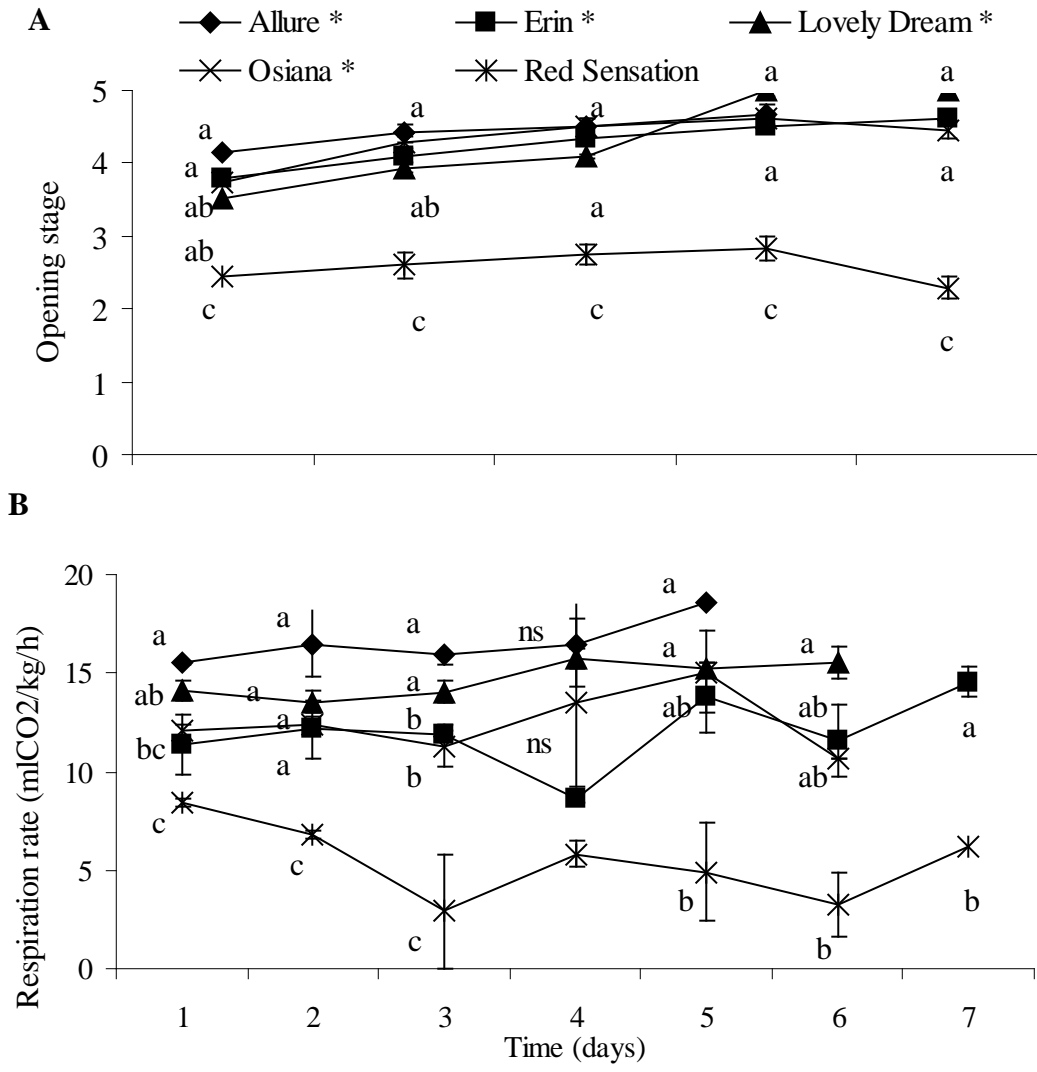


Figure 3-13. Opening ( $n = 48$ ) and respiration rate ( $n = 6$ ) of fragrant \* and non-fragrant cut rose cultivars during flower development. Means and standard error. Different letters within day represent significant differences between cultivars  $\alpha=0.05$

CHAPTER 4  
DEVELOPMENTAL AND ETHYLENE REGULATION OF VOLATILE PRODUCTION IN  
ROSE FLOWERS

**Introduction**

The mechanisms that regulate the emission of volatile compounds from flowers are very complex, and involve the expression of certain genes, the activity of specific enzymes and the availability of substrates in specific flower tissues, at certain stages of development and at specific times of the day. Most volatile compounds are synthesized *de novo* in the tissues from which they are emitted (Dudareva et al., 2000). The major sites of volatile emission in rose flowers are the petals (Dobson et al., 1990) and within the petals the adaxial and abaxial epidermis are tissues where volatiles are produced and emitted (Dobson et al., 1990; Bergougnoux et al., 2007). The enzymes and the substrates from the major biosynthetic pathways are present in these tissues and their activity and concentration are regulated through development (Watanabe et al., 1993; Schade et al., 2001).

As the flower develops the amount of volatile compounds produced by the flower usually increases, reaches its highest levels when the flower is receptive to pollination and decreases as the petals senesce (Dudareva et al., 2006a). However, this pattern of volatile emission during development is variable between flower species and varies with respect to specific volatile compounds emitted as observed in gardenia flowers (*Gardenia jasminoides*) (Watanabe et al., 1993), carnations (*Dianthus caryophyllus* L. cv White Sim) (Schade et al., 2001) and roses (Helsper et al., 1998; Oka et al., 1999). For example, in *Rosa damascena* Mill different compounds are emitted at different stages of development (citronellol emission peaked at stage 4 while 2-phenylethanol peaked at stage 6 ) (Oka et al., 1999). Moreover, the total volatile emission of this flower reaches the highest level at late opening stages (5 and 6) and then decreases during the last stages of development (Oka et al., 1999). In *Rosa hybrida* cv ‘Honesty’

2-phenylethanol was emitted at a maximum level during the day at early developmental stages but changed to emit maximum levels at night in later stages of development (Helsper et al., 1998). Developmental regulation was also observed in the emission of 2 phenylethylacetate and cis-3-hexenyl acetate from *Rosa hybrida* 'Fragrant Cloud' (Shalit et al., 2003). Production of these volatiles was very low at immature flower bud (stages 1 and 2), increased at stage 3 (mature closed flower) and then rapidly peaked at stage 5 of development (fully open flower). In *Rosa hybrida* 'Fragrant Cloud' the maximum volatile emission at stage 5 corresponded with maximum enzymatic activity and the main reason for the lack of emission of the volatiles at early stages was the limited availability of the respective substrates (Shalit et al., 2003). All of these studies report changes in enzymatic activities and availability of the substrates during flower development as the main explanation to the variability in volatile emission during development (Watanabe et al., 1993; Oka et al., 1999; Schade et al., 2001). However, substrate availability and enzymatic activity are not the only parameters that change during flower opening. Therefore, it is important to study other factors that occur and may affect volatile emission during flower development. As flowers develop changes in the activity and sensitivity to hormones such as ethylene are also observed (Borochoy et al., 1989). Therefore, this research focused on determining the ethylene effects on volatile emission on rose flowers.

Ethylene is a plant hormone that regulates several physiological processes like growth, seed germination, flower initiation, organ abscission, fruit ripening and stress responses (Abeles et al., 1992). Flowers usually become more sensitive to ethylene as they develop (Borochoy et al., 1989). Ethylene regulates the process of volatile emission in petunia (*Petunia X hybrida* cv Mitchell Diploid) flowers. Ethylene produced after pollination decreases the emission of volatile compounds, such as methylbenzoate by suppressing transcription of the gene benzoic acid

methyl transferase (BSMT) responsible for methylbenzoate synthesis. It also decreases the (BSMT) enzyme activity toward methyl benzoate substrate (benzoic acid) on wild type Mitchell diploid petunias. Ethylene produced after pollination had no effect of methyl benzoate emission, BSMT gene expression, BSMT enzymatic activity or amount of benzoic acid in ethylene insensitive transgenic *etr-1* petunias (Negre et al., 2003). In roses, ethylene effects have concentrated mainly on flower quality and have not included the role of ethylene on the volatile emission of fragrant roses (Mor et al., 1989; Reid et al., 1989; Serek et al., 2006). In roses, ethylene is produced as part of the senescence process and some rose cultivars are highly sensitive to external ethylene exposure (Serek et al., 1996). However, ethylene production and sensitivity are highly variable among rose cultivars (Muller et al., 1998; Muller et al., 2001). For example, ethylene was produced during senescence in rose cultivars such as ‘Bronze’ and ‘Vanilla’, while it was hardly detected in other cultivars such as ‘Charming’ and ‘Pink Marina’ (Muller et al., 1998). Ethylene symptoms in roses include yellowing and loss of buds, early senescence of the flower, senescence and abscission of petals and leaf discoloration (Serek et al., 1996). Biochemical, genetic and physiological studies have been conducted to find the methods to reduce ethylene effects at the level of ethylene biosynthesis and perception increasing flower quality (Faragher et al., 1987b; Reid et al., 1989; Lukaszewska et al., 1990; Ma et al., 2005). Commercially, chemical inhibitors of ethylene perception such as silver thiosulphate (STS) have been used successfully to help rose flowers avoid ethylene related symptoms (Lukaszewska et al., 1990).

This research studies the ethylene effect on volatile emission of locally grown rose flowers. The roses used in this research were an interest model since the flowers had a fast development, were very fragrant, sensitive to external ethylene and shatter all the petals at the end of

senescence. Thus, it was interesting to determine volatile emission from these rose flowers at all stages of development avoiding shipping and handling procedures observed in studies conducted with cut roses (Chapter 3) and to determine if ethylene played any role on volatile emission on rose flowers.

## **Materials and Methods**

### **Plant Material**

Rose (*Rosa sp* 'Louis Philippe') flowers were grown at the University of Florida greenhouses at 25°C day/ 21 °C night in 11.4 liter pots with Farfard 2B potting medium (Farfard, Apopka, FL). Plants were fertilized at every irrigation with Scott's 15-5-15 (Scotts, Marysville, OH). Flowers were collected for experiments when plants were seven months old.

### **Volatile Production During Flower Development**

Flower development stages were defined as: stage 1: immature bud (0.8mm diameter) no petals visible; stage 2: tight bud, sepals retracting petal whorl tightly closed; stage 3: sepals fully retracted outer petal whorl beginning to reflex from bud; stage 4: sepals fully retracted, outer petal whorl opened inner petal whorl closed; stage 5: inner and outer whorls opened, reproductive organs visible; stage 6: wilted or abscised petals (Figure 4-1). Flower development took 8 days from stage 1 to stage 6. Volatiles were collected from flower petals at each stage of development on the same day. Petals were weighed and placed into glass tubes (17 mm x 61 cm, 127 mL volume). Glass tubes were connected to an air pump and filtered air was passed through the tubes for one hour. Volatiles were trapped on a sorbent Super Q column (Alltech, Nicholasville, KY) and eluted with 150 µl of methylene chloride (Fisher Scientific, Pittsburgh, PA). Five µl of nonyl acetate (Aldrich Chemical Company, Inc. Milwaukee, WI) was added to the eluted volatile mix after collection as an elution control and to estimate the quantity of the compounds from each sample. Samples were analyzed by gas chromatography and mass

spectrometry as described by Underwood et al., (2005). In order to reduce environmental and temporal variability, volatiles were collected at 21 °C, at the same time of the day (between 3:30 and 4 p.m.) A total of two replications (studies), each replication with four flowers from each stage of development were used. Since no differences were observed between biological replicates, data were pooled from each study for analysis. Volatile emission data was analyzed using SAS GLM procedure (SAS® Version 9 Institute Inc., Cary, NC, USA). Data are presented as means ± standard error of the mean.

### **Effects of Ethylene on Volatile Emission and Petal Abscission**

Studies determined that ‘Louis Philippe’ were sensitive to ethylene at stage 4 of development (Table 4-1). Flowers were capable of producing volatiles at this and later stages of development (Figure 4-2). Therefore, this stage was selected to study ethylene effects on both volatile emission and petal abscission. Flowers were excised at stage 4 of development and taken immediately to the laboratory. The stem was re-cut to 4 cm and flowers were placed in either DI water or a solution of 0.2 mM Silver thiosulphate (STS), an inhibitor of ethylene perception (Chamani et al., 2005) and hydrated for three hours at room temperature (21 °C). After three hours, the flowers held in STS solution were transferred to a solution of DI water to avoid phytotoxicity. Next, flowers from both treatments were exposed to ethylene or air for 24 hours at 21 °C and 12 hr photoperiod under  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  of light provided by cool white fluorescent bulbs (Sylvania, Danvers, MA). Flowers from both treatments were placed into two glass aquarium tanks (37.85 L volume each). Tanks were sealed and potassium permanganate (ethylene scrubber) was included in the air treatment tank (control). The second tank (ethylene treatment) contained ethylene at a concentration of  $1 \mu\text{L L}^{-1}$ . Ethylene concentration was monitored in both tanks immediately after placing flowers in the tanks and after 4, 18 and 24

hours with gas a chromatograph (Hewlett-Packard 5890 Series II, Avondale, PA) equipped with a flame ionization detector (FID) and a 80/100 Alumina F-1, 106.7 cm long x 0.3 cm diameter column (Supelco, Bellefonte, PA). After 24 hours the tanks were opened, flowers were removed and the number of abscised petals was recorded for each treatment. The petals from flowers of each treatment were excised, weighed and placed into glass tubes (17 mm x 61 cm, 127 mL volume) for volatile analysis. Volatiles were collected as described previously. Treatments were arranged in a 2 (hydration) x 2 (ethylene) factorial design. A total of two biological replications (studies), each with 4 flowers per treatment were utilized in a randomized complete block design. Since no differences were observed between replicates, data from each study were pooled together. The pooled data was analyzed using SAS GLM procedure (SAS<sup>®</sup> Version 9 Institute Inc., Cary, NC, USA). When significant treatment effects ( $p \leq 0.005$ ) were determined by GLM, data means were separated using Tukey's multiple comparison test at  $p = 0.05$ . Data are presented as the mean  $\pm$  standard error of the mean.

### **Ethylene Production**

In order to determine the amount of ethylene produced and its effect on volatile emission, endogenous ethylene production was measured during flower development. Pilot studies determined that the earliest developmental stage that allowed the flower to open in the vase was stage 3 (data not shown). Flowers were harvested from the greenhouse at this stage of development and taken immediately to the laboratory. Stems were re-cut to 4 cm and placed into 22 ml glass vials with deionized (DI) water. Flowers were placed into 0.26 l glass mason jars with 8ml of 1 M potassium hydroxide (KOH) to maintain carbon dioxide (CO<sub>2</sub>) concentration below 0.1 %. Jars were closed for 21 hours and maintained under the postharvest conditions mentioned above. Next, a 1 ml gas sample was taken from each jar and analyzed with a gas

chromatograph (Hewlett-Packard 5890 Series II, Avondale, PA) equipped with a flame ionization detector (FID) and an 80/100 Alumina F-1, 106.7 cm long x 0.3 cm diameter column (Supelco, Bellefonte, PA). Injector, detector and oven temperatures were 110, 225 and 110 °C respectively. Immediately after the gas aliquot was obtained, the fresh weight and opening stage of each flower were recorded and the jars were left opened for 3 hours to allow for a complete air exchange and then resealed, so daily ethylene measurements could be obtained. Ethylene was measured daily between 9 a.m. and 12 a.m. until all petals abscised completely from the flower. A total of two replications (studies), each with 5 flowers were used with a randomized complete block design. Since no differences were observed between replicates, data were pooled together from each study. The pooled data was analyzed using SAS GLM procedure (SAS<sup>®</sup> Version 9 Institute Inc., Cary, NC, USA). Data are presented as the mean  $\pm$  standard error of the mean.

## **Results and Discussion**

### **Volatile Production During Flower Development**

This study expands the knowledge of the complex process of volatile emission in rose flowers ‘Louis Philippe’ by describing the production of lipid derivatives, terpenoids and phenylpropanoids volatile compounds during all stages of flower development. Fragrance was a result of the differential production of individual volatile compounds during development. Some volatile compounds were produced constantly during development as occurred with phenylpropanoids benzyl alcohol, benzyl benzoate, euglenol, isoeuglenol, phenethyl benzoate and phenylacetaldehyde and on the terpenoids (S)-(-)-B citronellol, geranyl acetate and neryl acetate. Other volatiles, such as the phenylpropanoid 2-phenylethanol and the terpenoid (-) trans caryophyllene, were produced at stage 2, increased at stage 3, then decreased at stages 4 and 5 and increased again at stage 6 when senescence symptoms, such as petal wilting and abscission occurred (Figure 4-2). Other volatile compounds such as the lipid derivatives cis-3-hexen-1-ol



and eicosane were produced at low levels at stage 2, gradually increased and peaked at stage 4, then gradually decreased toward later stages of development (Figure 4-2). The earliest developmental stage when volatile compounds from all types were produced was stage 2 (Figure 4-2). This stage corresponded with the earliest point at which petals were visible (Figure 4-1). Differential production of specific volatiles during development observed in rose flowers can be explained by differential gene expression and enzymatic activity during development (Guterman et al., 2002; Lavid et al., 2002; Shalit et al., 2003). Studies to uncover the identity of rose flower scent related genes have reported upregulation of 65 genes (18.5%), downregulation of 14 (4 %) genes and similar expression of approximately 277 (77%) genes during flower development (Guterman et al., 2002). Maximum scent emission at specific stages of development has been correlated with the increased expression of specific genes such as orcinol methyl transferases (OOMT) and alcohol acetyltransferase (RhAAT1) and maximum activity of the respective biosynthetic enzymes (Lavid et al., 2002; Shalit et al., 2003). Orcinol methyl transferases display activity towards substrates such as orcinol, precursor of 3,5 dimethoxytoluene, and euglenol, precursor for methyleuglenol. OMT activity peaks at stage 4 of flower development in 'Golden Gate' and 'Fragrant Cloud' cultivars (Lavid et al., 2002). Alcohol acetyltransferases activity (AAT) also peaked at stage 4 of development in 'Fragrant Cloud' utilizing the substrates geraniol and citronellol to form geranyl acetate and citronellyl acetate respectively (Shalit et al., 2003). The results of volatile emission from 'Louis Philippe' illustrate that the volatile production pattern as reported in 'Fragrant Cloud' and Golden Gate' does not always occur in other rose cultivars. For example, the levels of 3,5 dimethoxytoluene, citronellyl acetate and euglenol volatile compounds produced by 'Seven sisters' did not agree with the volatile production patterns of the respective compounds observed in 'Golden Gate' and 'Fragrant Cloud'. 'Louis

Philippe' did not produce 3,5 dimethoxytoluene and citronellyl acetate and produced constant low levels ( $< 0.1 \mu\text{g}/\text{fw}/\text{h}$ ) of eugenol during development (Figure 4-2). In contrast, the emission of geranyl acetate by 'Louis Philippe' did agree with the results reported in 'Fragrant Cloud'. Geranyl acetate was produced at maximum levels when the flower started to open (stage 4) (Figure 4-2). This pattern agreed with the increased enzymatic activity reported in 'Fragrant Cloud', where maximum levels of the enzyme AAT involved in production of geranyl acetate from geraniol occurred at stage 4 of development (Shalit et al., 2003). However, 'Louis Philippe' produced geraniol, a geranyl acetate precursor, at maximum levels when senescence symptoms occurred (stage 6) (Figure 4-2). The results reported in 'Louis Philippe' together with those reported in 'Fragrant Cloud' and 'Golden Gate' are important because they clearly demonstrate the complexity of developmental regulation in rose flowers and its variability between rose cultivars.

Differential production of specific volatiles during development observed in rose flowers can also be explained by changes in properties of membranes as the flower senesces (Faragher et al., 1987a; Fobel et al., 1987). Flower senescence involves changes, such as decreased in membrane lipid fluidity, increased on the activity of lipogenases, enzymes responsible of lipid degradation, and its respective substrates linoleic and linolenic acids substrates (Fobel et al., 1987). In this research, lipid derivative compounds such as cis3-hexen-ol and eicosane peaked at stage 4 of development and decreased when senescence symptoms (petal wilting/abscission) occurred (Figure 4-2). These results suggest a different regulation mechanism from senescence related changes for the lipid derivatives produced by this cultivar. Another important change that occurs as flower senescence is an increased endogenous ethylene production (Muller et al., 2001; Chamani et al., 2005) and ethylene sensitivity (Borochoy et al., 1989). Therefore, further studies

were conducted to determine if ethylene played a role in the regulation of volatile emission during rose flower development.

### **Ethylene Effects on Volatile Emission**

Amounts of lipid derivatived volatiles, phenylpropanoids and terpenoids did not change during development due to endogenous ethylene production from the flower (Figure 4-2). Internal ethylene was produced at negligible amounts at stages 3 and 4 of development then it gradually increased and was maximum when flowers dropped the petals (Figure 4-2). As described in the above section, volatile compounds increased, decreased or were constant over development. The ethylene production pattern confirms that the volatile compounds were produced independently of the endogenous ethylene produced by the flower (Figure 4-2).

Amounts of lipid derivatived volatiles after 24 hours of  $1 \mu\text{L L}^{-1}$  ethylene exposure, were 2 times less than the amounts produced after the same duration of air exposure (Figure 4-3). Inhibition of ethylene perception (STS treatment) did not increase the amounts of lipid derivatived volatiles, after ethylene treatment (Figure 4-3). Amounts of phenylpropanoids and terpenoids after ethylene exposure were no different from the amounts of the respective volatile groups after air exposure (Figure 4-3). These results suggest that the role of ethylene was variable among different biosynthetic groups. Ethylene did not play a role on the regulation of phenylpropanoids and terpenoids volatile emission while it had a negative effect on the emission of lipid derivatived volatiles in rose flowers.

Absence of an ethylene effect on volatile emission in rose flowers was different from the ethylene effect reported in other flowers species like petunia. In petunia, the amount of seven major volatiles was significantly reduced after internal ethylene production that occurred 36 hours after pollination (Underwood et al., 2005). External ethylene reduced all volatile compounds to negligible levels after 10 hours of ethylene treatment, but had no effect on volatile

emission from transgenic ethylene insensitive *etr-1* flowers (Underwood et al., 2005). The lack of ethylene regulation of volatile emission in rose flowers as reported in this study together with the ethylene effect on volatile emission reported in petunia, illustrate that volatile production is a process that is differentially regulated among flower species.

### **Ethylene Effects on Petal Abscission**

Rose flowers at stage 4 of development hydrated in DI water exposed to  $1 \mu\text{L L}^{-1}$  of ethylene shattered 100 % of their petals after 24 hours while flowers hydrated in DI water exposed to air for the same period of time shattered 0 % of their petals. Petal abscission was prevented when flowers were treated with an inhibitor of ethylene perception (STS). STS treated flowers at stage 4 abscised 0 % of their petals after 24 hours of ethylene and air treatment (Table 4-1). After ethylene treatment, flowers that abscised all petals (DI treated) produced the same amounts of volatile compounds as flowers that did not abscise petals (STS treated) (Figure 4-3). These results clearly show that ethylene regulates petal abscission but does not play a role in the regulation of volatile production in rose flowers.

Ethylene action may be restricted to specific biochemical pathways in petal tissues affecting petal abscission and volatile emission differentially. Petal abscission is regulated by changes that occur in the abscission zone at the base of the petal. Studies have reported that ethylene can increase the petal abscission process in *Pelargonium X hortorum* by affecting the structure of the mesophyll cells at the base of petals (Evensen et al., 1993). Volatile emission in roses occurs at the adaxial and abaxial epidermal tissues of the upper petal parts (Bergougnoux et al., 2007). Lack of ethylene effects on volatile emission in rose flowers can be a result of the lack of ethylene regulation on the volatile biosynthetic pathway at the level of gene expression and/or downstream levels in the epidermal cells. Genes involved in the biosynthesis of volatile compounds in rose flowers such as orcinol methyl transferases (OOMT) and alcohol

acetyltransferase (RhAAT1) and the respective biosynthetic enzymes have been characterized in roses, however the ethylene effect on these genes were not reported in these studies (Lavid et al., 2002; Shalit et al., 2003). Further studies are needed to determine the ethylene effect on the expression of genes involved in volatile emission in roses such as OOMT and RhAAT1 to further understand the reason for the lack of ethylene effect on volatile emission in rose petals.

In conclusion, the fragrance of the flower was a result of an increased, decreased or constant production of different volatile compounds from different biosynthetic groups during flower development. Volatile emission during development occurred independently of endogenous ethylene production. Rose flowers were extremely sensitive to ethylene in terms of flower appearance. All petals from stage 4 flowers abscised after ethylene exposure and at the same time were insensitive to ethylene in terms of volatile production. Volatile production was not different in flowers treated with ethylene or air. This study reported absence of ethylene regulation on volatile production in roses. The results show that volatile emission occurs independently of changes in petal appearance (wilting or abscission) resulting from the senescence/abscission process. These results provide a better understanding of the complex volatile emission in rose flowers.



Figure 4-1. Stages of development of fragrant rose *Rosa* ‘Louis Philippe’ 1: immature bud (0.8mm diameter) no petals visible; 2: close bud, sepals retracting petal whorl tightly closed; 3: sepals fully retracted outer petal whorl beginning to loosen; 4: sepals fully retracted, outer petal whorl opened inner petal whorl closed; 5: inner and outer whorls opened, reproductive organs visible; 6: wilted petals.

Table 4-1. Ethylene effects on petal abscission on ‘Louis Philippe’ at stage 4 of development. Flowers were treated with  $1 \mu\text{L L}^{-1}$  ethylene or air for 24 hours.

	Petal Abscission (%)	
	Air	Ethylene
DI	0	100
STS	0	0

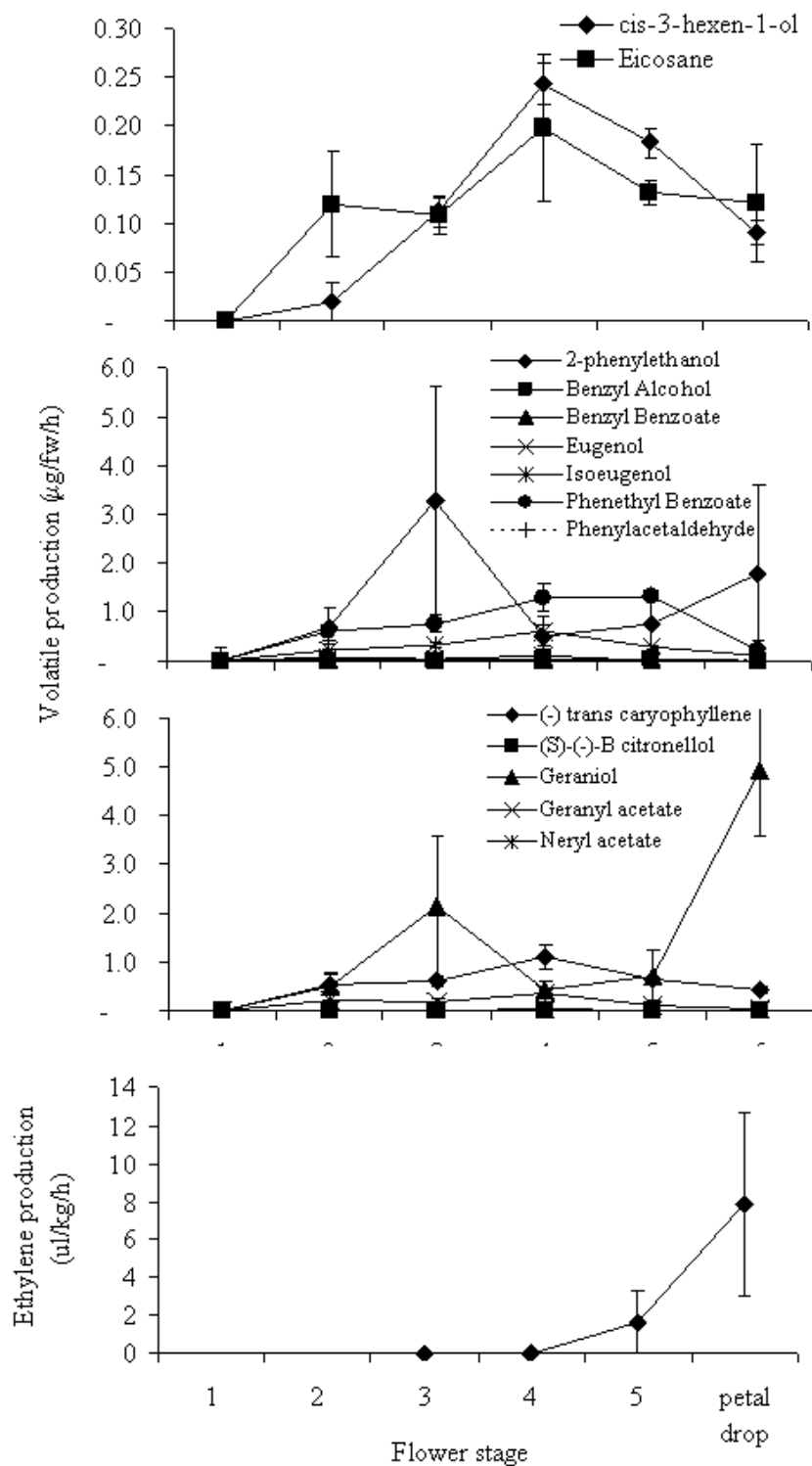


Figure 4-2. Changes in volatile amounts ( $\mu\text{g}/\text{fw}/\text{h}$ ) and ethylene production during rose flower development. Means and standard error  $n=8$ . A) Lipid derivatives B) Phenylpropanoids C) Terpenoids D) Ethylene production

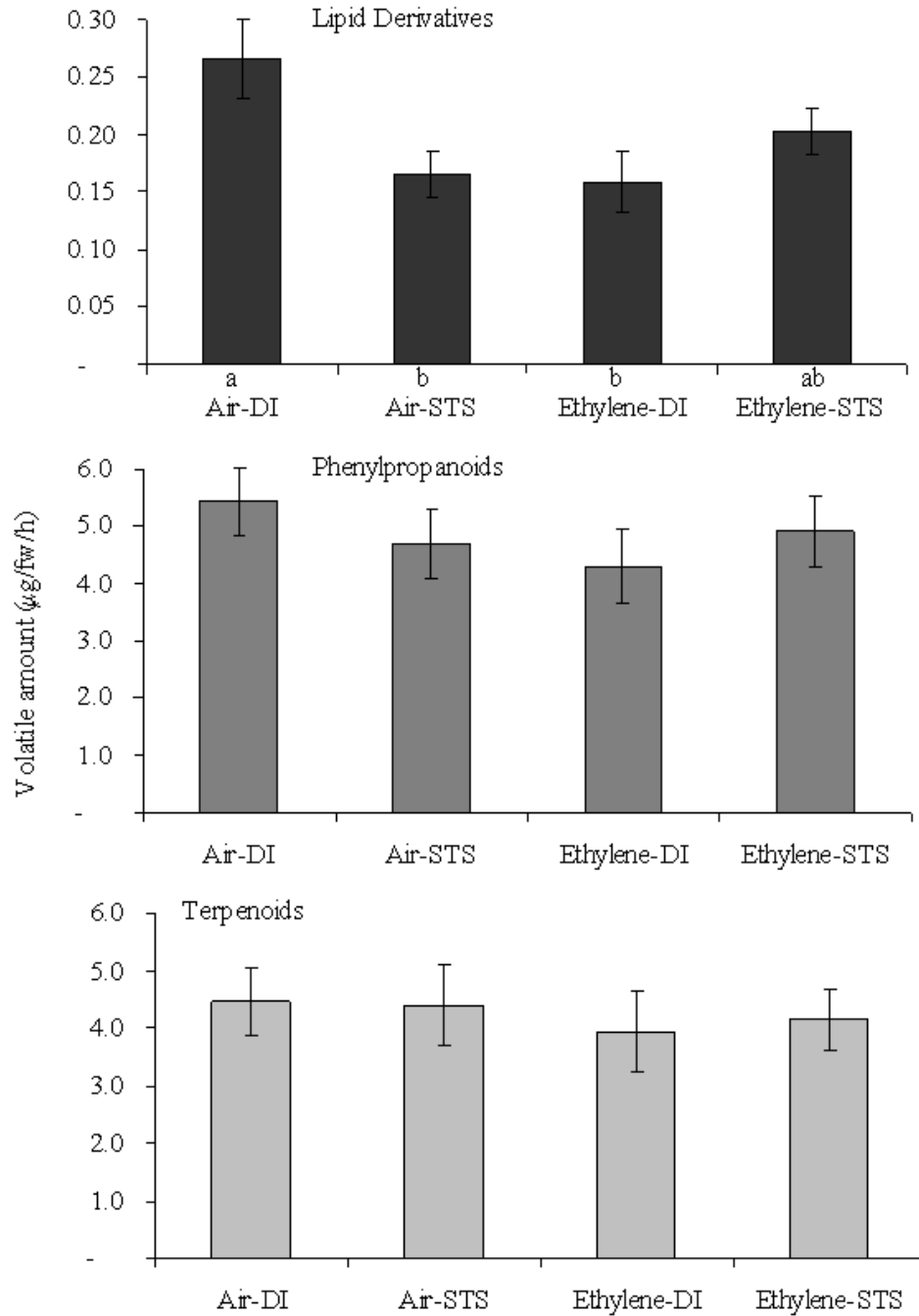


Figure 4-3. Ethylene effects on volatile emission on *Rosa* 'Louis Philippe' flowers at stage 4 of development. Means and standard error n=4. Different letters = significant difference between treatments  $\alpha = 0.05$ .



CHAPTER 5  
SENSORY EVALUATION OF ROSE FRAGRANCE INTENSITY REALTIVE TO  
VOLATILE EMISSION

**Introduction**

Fragrant roses are considered to be a luxury consumer product since they are sold at very high prices and at less volume than non-fragrant cut roses (Mouchette, 2001; Bent, 2007). The predominant traits breeders have selected for in modern cut rose cultivars are long vase life, big flower buds, high number of petals, long stems and resistance to pests and diseases (Korban, 2007). Since consumers buy flowers with these characteristics, these are the parameters that define cut rose quality. Even though fragrance is a very desirable feature by consumers, the majority of the modern cut rose cultivars lack this important trait. Of the 3,900 rose cultivars described back in 1956 only 20 % of them were described as strongly fragrant (Bent, 2007). Breeders have reported that most modern cut rose cultivars lost their fragrant quality as breeding efforts concentrated on flower color and shape (Zuker et al., 1998). Breeders and producers have tried to find an explanation for the loss of fragrance in modern cultivars. An explanation for the lack of fragrance is the rapid opening from the bud stage to a completely open flower that occurs in some fragrant roses (Mouchette, 2001). A rose flower that opens quickly does not perform well with the long shipping and handling schedule applied to modern cut roses since a couple of days up to several weeks can pass from harvest until the flower reaches the final consumer. This characteristic results in fragrant roses being distributed mainly in the local market (Mouchette, 2001). Therefore, modern cut roses with slow opening during the postharvest process does not favor volatile emission from the flower (Mouchette, 2001). Moreover, for years rose fragrance has been negatively associated with vase life (Barletta, 1995). One possible explanation for the short vase life of fragrant roses is the fact that fragrance tends to be linked to flowers with many thinner petals which are related with short vase life (Barletta, 1995; Bent, 2007). However,

studies have demonstrated that the thickness of the cuticle of petal epidermal cells is not different between fragrant and non-fragrant roses (Bergougnoux et al., 2007). Rose fragrance studies have concentrated on studying the main volatile compounds emitted from roses, their regulation and biosynthesis, however these studies have not related these conditions with flower vase life (Dobson et al., 1990; Flament et al., 1993; Oka et al., 1999; Kim et al., 2000; Guterman et al., 2002; Scalliet et al., 2002; Joichi et al., 2005; Cherri-Martin et al., 2007; Scalliet et al., 2008). Recent studies demonstrated that the reduced vase life observed in some fragrant cultivars resulted from an interaction between physiological parameters such as respiration, ethylene production and ethylene sensitivity and not from the ability of the flower to produce fragrance (Borda et al., 2007). All these studies demonstrated the importance of fragrance as a trait for cut rose cultivars and the interest of breeders on selecting fragrant roses. However, the physiological, biochemical and genetic studies should be complemented with studies on the ability of humans to detect volatile compounds emitted by rose flowers and their preferences because genes can be introduced and pathways can be activated resulting in rose flowers emitting certain volatile compounds, but if these are not detected or liked by humans the results obtained from these studies will be far from the goal of obtaining flowers with fragrances that suits consumer's preferences. Therefore this research studied the main volatile compounds emitted by commercial cut rose cultivars and related them with the ability of human subjects to detect differences in fragrance intensity and rate their preferences for different rose fragrance types and intensities.

Rose fragrance is a trait that is specific for each rose species. The fragrance of roses can be classified in five major fragrance groups: fruity, myrrh, old rose, musk and tea (Bent, 2007). Each fragrance group is characterized by the composition of different volatile compounds produced in different amounts. The fragrant note of a rose comes from the emission of alcohols,

aldehydes, alkenes, monoterpenes, sesquiterpenes, ester, ethers and ketones (Flament et al., 1993; Flament I. et al., 1993; Kim et al., 2000). Some of these volatile compounds are produced in certain rose species while they are not present in others, making the fragrant note of each cultivar very specific. For example, one of the major volatiles produced by *Rosa damascene* is 2-phenylethanol, a compound with a nice sweet floral note characteristic of European 'old rose' scent (Oka et al., 1999; Tandon et al., 2000). Another important component of rose scent is 3,5-dimethoxytoluene (orcinol dimethyl ether) which is present in almost all the modern *Hybrid Tea* roses and gives the characteristic 'tea note' fragrance for this group (Scalliet et al., 2008). Volatile acetate esters such as geranyl acetate are other major components of the fragrant note of *Rosa hybrida* 'Fragrant Cloud' (Shalit et al., 2003) and give a sweet fruity or citrus fragrance note to this cultivar (Fragrances, 2007). These studies illustrate how each fragrant cultivar has a specific fragrance depending on the type and amounts of volatile compounds produced. Therefore, this study determined the volatile compounds emitted by cut rose cultivars with different fragrance types and related them with human subject's preferences.

Flower opening is an important process to take into account when studying flower fragrance since flower fragrance changes as the flower develops. In most roses maximum volatile emission occurs during the day at the maximum flower opening stages when the stamens are visible for pollinators (Watanabe et al., 1993; Oka et al., 1999). Therefore, the perception of rose fragrance depends on the developmental stage at which it is detected. In the flower industry, the developmental stage at harvest is critical to ensure further flower opening and development. If a rose is harvested at the bud stage it will never open and if it is harvested when the flower is completely open it will not have the minimum vase life required to cover the transport and consumer time. Most cut rose flowers are harvested at a stage that is closed enough to withstand

the long handling and shipping times but mature enough to open during the consumer stage (Reid, 2004). Therefore this study evaluated fragrance emission and detection by human subjects at different stages of flower development after conventional harvest, shipping and handling procedures.

The fragrance of a flower perceived by a consumer is a subjective trait. A rose can be classified as a nice fragrant flower by one individual while other individual may not even consider the same flower as fragrant. Many factors influence the response of humans to fragrances including the disposition of the person at the time of detection, the innate physiological sensitivity for the specific compound being detected and the past history and familiarity with similar fragrance stimulus (Lawless et al., 1998). Moreover, the physiology of the sense of human smell does not contribute to the optimum detection of flower volatiles. The olfactory receptors in humans are located very high in the nasal cavity. This remote location may influence the proper detection of the volatiles at the time of exposure to them since only a small percentage of the volatiles flowing through the nose actually reaches the sensory organs (Lawless et al., 1998). Some organic molecules stimulate the olfactory sense of humans more than others, some being detected at part per billion concentrations while other detected at part per thousand. The perception of volatile compounds by humans is generally measured by odor units. An odor unit is a value assigned to a compound based on dividing the concentration of a compound (ppb) by the components detection threshold level (ppb), and indicates the contribution of certain compound to the fragrance of the flower (Leffingwell, 2001). For example, the odor unit reported from *Rosa damascene* for phenylpropanoids (i.e. phenethyl alcohol) is low ( $37 \times 10^{-3}$ ) compared to terpenoid (i.e. (-) citronellol) which is ( $9500 \times 10^{-3}$ )

(Leffingwell, 2001). Thus, rose volatiles differ in their perception; small quantities are needed for the detection of certain volatiles while large quantities are needed in others to be detected.

Some flower fragrances can have a positive effect on human emotions. Flower fragrance can increase interpersonal attraction and positive social perception (Baron, 1981; Haviland-Jones et al., 2005), and can affect a consumer's decision to buy a flower. For example, rose (*Rosa*) and clove (*Syringa*) fragrance added to carnation (*Dianthus*) compensated for color deficiency and significantly influenced purchase intention of consumers (Huang, 1997). Although fragrance is a very important trait for cut rose flowers few studies have described the ability of humans to detect differences in rose fragrance intensity from commercially available cut rose cultivars and the type of rose fragrances they prefer. Therefore this research studied the fragrance emission from rose flowers together with the ability of human subjects to detect different fragrance intensities and their preferences on commercially available cut rose cultivars.

### **Materials and Methods**

Previous studies reported that the amount and type of volatiles emitted from cut rose flowers varied as a result of differences among rose cultivars and opening stage of the flowers (Chapter 3). In order to determine if humans detected differences in volatiles emitted from different rose cultivars and at different developmental stages, two scent panel tests were performed. Subjects for the two tests were recruited on the University of Florida campus and consisted of males and females between the age of 18-24 years (test 1 n=99; test 2 n=89). For both panels, flowers were presented to panelists inside Styrofoam coffee cups covered with a plastic lid. Panelists were asked to open the lid, smell the sample, and close the lid before smelling the next sample. In order to minimize differences in appearance between samples, flowers were presented under red light conditions.

## Panel Test 1

Previous studies demonstrated that fragrant ‘Allure’, ‘Erin’, ‘Lovely Dream’ and non-fragrant ‘Red Sensation’ flowers produced lipid derivatives, phenylpropanoids and terpenoids in different amounts (Chapter 3). The first panel test measured the ability of people to detect differences in fragrance intensity and composition from these cultivars.

All cultivars were harvested between 8 -10 a.m. on the same day at a commercial stage from farms located in Ecuador. Flowers were taken into a postharvest room (14-18 °C) within 30 minutes of harvest, where they were placed into a fresh hydration solution (water, 60  $\mu\text{L L}^{-1}$  chlorine and 0.9g/l citric acid to adjust pH to 4.5) for 2 hours. After hydration flowers were grouped by cultivar and placed at 3°C in the same solution for 12 hours. The next day, flowers were packaged into commercial corrugated boxes (104.1 cm long x 24.1 cm tall x 22.9 cm wide). The boxes were sent via FedEx to Gainesville, FL within 4 days. Upon arrival, the boxes were opened and flowers were cut to 45 cm in length and placed by cultivar in vases containing 1 L of deionized water (DI) for 24 hours at 21 °C and 12 hr photoperiod under 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of light provided by overhead cool white fluorescent bulbs (Sylvania, Danvers, MA). After one day, flowers were offered to 99 people in one set of four samples (each sample corresponded to a rose cultivar). ‘Allure’, ‘Erin’, ‘Lovely Dream’ flowers were at developmental stage 4 (outer petals reflexed at approximately 115° to stem) and ‘Red Sensation’ was at developmental stage 3 (outer petals reflexed approximately 135° o to stem) when presented to panelists. Panelists were asked to smell each sample and rate the intensity of the fragrance using a low (0) - high (10) intensity linear scale. In order to detect which cultivar panelists preferred, they were asked to select how much they liked or disliked the fragrance of each flower using a 9-point balanced hedonic scale: 1:dislike extremely, 2:dislike very much, 3:dislike moderately, 4:dislike slightly, 5:neither like

nor dislike, 6:like slightly, 7:like moderately, 8:like very much, 9:like extremely for each sample. Panelists were also asked to rank each one of the samples from most preferred (1) to least preferred (4), to describe any differences between cultivars (if any), and to make additional comments about the fragrance of each sample. Likeability and preference results were analyzed by ANOVA and mean separation was determined using Tukey's HSD. Ranking was analyzed by Friedman analysis of Rank (Compusense).

Volatile compounds were collected from each cultivar on the same day of the panel evaluations in order to determine the volatile compounds and the amounts that the panelists were exposed on the panel test. For these measurements petals were excised from the flower, weighed and placed into glass tubes (17 mm x 61 cm, 127 mL volume). Tubes were connected to an air pump that provided a continuous air flow system. Filtered air was passed through the tubes for one hour and trapped on a sorbent Super Q column (Alltech) and eluted with 150 $\mu$ L methylene chloride (Fisher Scientific, Pittsburgh, PA). Five  $\mu$ L of nonyl acetate (Aldrich Chemical Company, Inc. Milwaukee, WI) was added to the eluted volatile mix after collection as an elution control and to estimate the quantity of the compounds from each sample. Samples were analyzed by gas chromatography and mass spectrometry as described by Underwood et al., (2005). A total of four flowers per cultivar were used for volatile analysis. Differences in volatiles emitted from flowers were analyzed by ANOVA using SAS<sup>®</sup> Version 9 (SAS Institute Inc., Cary, NC, USA). When significant treatment effects ( $p \leq 0.005$ ) were determined by ANOVA, data means were separated using Tukey's multiple comparison test at  $p = 0.05$ . Volatile amounts are presented as the mean  $\pm$  standard error of the mean for each cultivar. This experiment was conducted once.

## Panel Test 2

Previous results reported major differences in volatile compound's levels and composition between opened and closed 'Allure' and 'Lovely Dream' flowers (Chapter 3). Since these cultivars were very fragrant and presented major differences in volatile amounts and composition during development, they were selected to determine the ability of panelists to detect differences in fragrance intensity at different stages of development. 'Lovely Dream' and 'Allure' flowers were harvested, processed at the farm and shipped following the same procedures described previously. Upon arrival flowers were re-cut to 45 cm and hydrated in DI water overnight in the cooler (3.5 °C). The next day, half of the flowers were taken out of the cooler and placed into vases at room temperature (21°C) so flowers would develop and open while the other half were kept at 3.5 °C to prevent flower opening. Flowers were held in each of these conditions for four days and then presented to the panelists. A side by side comparison was performed with 82 panelists. The panelists received two sets of two flowers each. The first set consisted of one opened (stage 4.5) and one closed (stage 2.0) 'Lovely Dream' flower. The second set consisted of one opened (stage 4.5) and one closed (stage 2.5) 'Allure' flower. Stage 4.5 was defined as outer petals reflexed at approximately 115 ° to stem and stage 2.5 was defined as outer petals starting to reflex from bud in both cultivars. Panelists were asked to smell each sample and to rate the intensity of the fragrance using a low (0) – high (10) intensity linear scale. Panelists were asked to describe how much they liked or disliked the fragrance of each sample using the 9-point hedonic balanced scale described previously. Intensity and likeability results were analyzed by ANOVA and mean separation was determined using Tukey's HSD (Compusense). Volatile compounds were collected from opened and closed 'Allure' and 'Lovely Dream' flowers the same day of the panel as described above to determine the volatile compounds and the amounts that the panelists were exposed on the panel test. A total of four flowers from each cultivar at



each stage of development were used for volatile determination. Differences in the volatiles emitted from flowers between treatments were analyzed by cultivar with T-test  $\alpha = 0.05$  using SAS<sup>®</sup> Version 9 (SAS Institute Inc., Cary, NC, USA). This experiment was conducted once.

## **Results and Discussion**

### **Panel Test 1**

This study analyzed the amount and composition of volatile compounds emitted from fragrant and non-fragrant cut rose cultivars together with the ability of panelists to detect flower fragrance intensity from each flower. Fragrant cultivars ‘Allure’, ‘Lovely Dream’, and ‘Erin’ and the non-fragrant cultivar ‘Red Sensation’ produced different types and amounts of volatile compounds, resulting in different fragrance intensities that were detected by panelists (Table 5-1). The fragrance intensity of each flower corresponded to the total amount of volatiles produced. ‘Allure’ was the cultivar with the highest amount of total volatiles produced and the cultivar that received the highest intensity score (Table 5-1). After ‘Allure’, ‘Lovely Dream’ produced a lower amount of total volatiles and received the same intensity score as ‘Allure’. ‘Erin’ and ‘Red Sensation’ produced lower total volatile amounts and received lower intensity scores than ‘Allure’ and ‘Lovely Dream’ (Table 5-1). The highest intensity score did not correspond to the highest preference ranking score. The fragrance of ‘Lovely Dream’ received the highest likeability and preference ranking of all cultivars. ‘Lovely Dream’ produced 2-phenylethanol in higher amounts than the amounts produced by other cultivars (Table 5-1). The compound, 2-phenylethanol gives roses a floral ‘European old rose’ fragrance and is one of the major constituents of rose oil (Watanabe et al., 2002). This compound in combination with trans caryophyllene, 3,5 dimethoxytolunene, geranyl acetate and neryl acetate gave the fragrance note with the highest preference ranking (Table 5-1). Even though ‘Allure’ produced similar total amounts and received the same intensity score as ‘Lovely Dream’, it was less preferred than

'Lovely Dream' (Table 5-1). The fragrance of 'Allure' was described as intense and overwhelming by some panelists. 'Allure' produced isoeugenol, methyl benzoate, phenethyl benzoate, (S)-(-)- $\beta$ -citronellol and linalyl acetate which give a spicy, floral, fruity, floral and lavender fragrances, respectively (Jirovetz et al., 2002; National non-Food Crop, 2009; Sigma Aldrich, 2009) and were compounds not produced by 'Lovely Dream'. Methyl salicylate was also produced exclusively by 'Allure' and its levels were higher than levels from other compounds (Table 5-1). The compound methyl salicylate is a compound found in the oil of wintergreen which provides a sweet woody 'medicinal' smell (Sigma Aldrich, 2009). The medicinal fragrance of methyl salicylate in combination with the spicy, floral, fruity fragrance given by isoeugenol, methyl benzoate, phenethyl benzoate, (S)-(-)- $\beta$ -citronellol and linalyl acetate gave 'Allure' an unpleasant fragrance. These results clearly demonstrate that human subjects preferred rose fragrances given by few volatile compounds as in 'Lovely Dream' rather than fragrances that result from a combination of several volatile compounds as in 'Allure'.

'Erin' received a similar preference rank as 'Allure' (Table 5-1). Panelists described the fragrance of 'Erin' as a nice balance of flower and plant fragrance notes and a fresh natural light scent. 'Erin' produced benzyl acetate with a pleasant sweet aroma similar to jasmine (Schiestl et al., 2003), and this compound was not produced by any other cultivar (Table 5-1). 'Erin' produced nine and six times more isoeugenol, compound with a spicy clover like fragrance (Sigma Aldrich, 2009), than 'Allure' and 'Red Sensation', respectively (Table 5-1). The compound (-)-trans-caryophyllene, with a spicy woody fragrance (Jirovetz et al., 2002) was produced twice as much in 'Erin' compared the amount produced by 'Allure' and 'Lovely Dream' (Table 5-1). The combination of these compounds in 'Erin' gave a fragrance composition that was in the middle preference rank (Table 5-1). The fragrance of 'Red

Sensation' flowers received the lowest ranking. All volatile compounds produced by 'Red Sensation' were produced by other cultivars (Table 5-1). The amounts of all the compounds produced by 'Red Sensation' were less than 0.03  $\mu\text{g}/\text{fw}/\text{h}$  (Table 5-1). The panelist commented on 'Red Sensation' as a flower with almost no fragrance. The results observed in 'Red Sensation' suggest that volatile compounds produced by 'Red Sensation' in amounts less than 0.03  $\mu\text{g}/\text{fw}/\text{h}$  are out of the human's fragrance detectable range and will not contribute significantly to the fragrance note of a cut rose flower.

### **Panel Test 2**

The ability of panelists to detect different fragrance intensities between open and closed flowers varied according to the cultivar (Table 5-2). The different detection ability was a result of variable volatile amounts and composition between cultivars. Volatile compounds such as (-) trans caryophyllene, 3,5 dimethoxytoluene and geranyl acetate were produced in significant amounts by closed 'Allure' and 'Lovely Dream' flowers (Figure 5-1). The volatile compounds neryl acetate and phenethyl benzoate were produced in negligible amounts by both cultivars at both stages of development (Figure 5-1). Volatile compounds (S)-(-)-B citronellol and methyl salicylate were produced exclusively by closed 'Allure' flowers in considerable amounts. Volatile compounds 2-phenylethyl alcohol and geraniol were produced exclusively by opened 'Lovely Dream' in considerable amounts (Figure 5-1). Panelists did not detect differences in fragrance intensity from open versus closed 'Allure' flowers (Table 5-2). Open and closed 'Allure' produced mainly (S)-(-)-B citronellol, 3,5 dimethoxytoluene and methyl salicylate compounds with fruity, floral and medicinal fragrance respective (Figure 5-1). Volatile compounds such as methyl salicylate, which give an unpleasant fragrance, produced in high amounts at closed stages of development, possibly masked other volatiles such as (S)-(-)-B

citronellol which give a pleasant floral fragrance and were produced in less amounts. The combination of these volatiles and the amounts produced by 'Allure' were within human's detection levels in both open and closed flowers resulting in an inability to distinguish different fragrance intensities. The compound 3,5 dimethoxytoluene produced in highest amounts by 'Allure' has been described to be hardly detected by humans (Shalit et al., 2004). Panelists preferred the fragrance from open 'Allure' flowers over the fragrance of closed 'Allure' flowers (Table 5-2). Open 'Allure' produced lower amounts of compounds (S)-(-)-B citronellol, with pleasant floral, fruity fragrance and methyl salicylate compound with medicinal fragrance than amounts produced by closed flowers (Figure 5-1). The preference for open 'Allure' flowers with lower amounts of volatiles produced than closed flowers can also be explained by the fact that 'Allure's fragrance was described as overwhelming in the previous study. Panelists detected differences in fragrance intensity between open and closed 'Lovely Dream' (Table 5-2). Open 'Lovely Dream' flowers received a higher intensity score and produced higher amounts of 2-phenylethanol and geraniol than closed 'Lovely Dream' (Figure 5-1). The amounts of 2-phenylethanol and geraniol produced by open 'Lovely Dream' flowers may be in the human's detectable range while the amounts of the respective compounds in closed 'Lovely Dream' flowers may be outside the human detectable range allowing humans to detect the difference fragrance intensities. Panelist equally liked the fragrance from open and closed 'Lovely Dream' (Table 5-2). The compound 2-phenylethanol, which gives a characteristic rose-floral fragrance, was the most preferred by panelist in the previous study.

In conclusion, panelist's capacity to detect different fragrant intensities from rose flowers depended on the total amount of volatile compounds produced by each cultivar. High volatile amounts were related with high fragrance intensity as detected by human subjects. A high

fragrance intensity does not necessary correspond with a high preference. Preferences for rose fragrances by human subjects depend on the type of volatile compound, their amounts and interaction with other volatile compounds. A floral rose fragrance given by few volatile compounds such as 2-phenylethanol, 3,5 dimethoxytoluene, (-) transcaryophyllene, geranyl acetate and neryl acetate as occurred in 'Lovely Dream' is highly preferred by humans subjects. Fragrances given by a combination of several volatile compounds such as isoeugenol, methyl benzoate, methyl salicylate, phenethyl benzoate, (S)-(-)-B citronellol and linalyl acetate can be unpleasant to human subjects as in 'Allure'. The amounts and composition of eicosane, benzyl benzoate, isoeulgenol and phenethyl benzoate as occurred in 'Red Sensation' gave the fragrance least preferred by panelists. The levels of these compounds as produced in 'Red Sensation' were out of the range for fragrance detection by human subjects since 'Red Sensation' was described as a cultivar with a hardly detectable fragrance. The ability of panelists to detect different fragrance intensities from open versus closed flowers depended on type and amount of volatile and was variable between cultivars. Compounds such as (S)-(-)-B citronellol and methyl salicylate were in the consumer detectable levels in open and closed 'Allure' flowers making its intensity not detectable by panelists. Compounds such as 2-phenylethanol and geraniol were out of human detection levels in closed flowers and within detectable levels in open flowers allowing the consumer to get differences in fragrance intensities in open versus closed 'Lovely Dream'. These results illustrated how rose fragrance and the process of detection and liking by human subjects are events with complex interactions in cut rose flowers. In order to expand the knowledge about the rose fragrance preferences by human subjects, further studies should determine the specific odor units for 2-phenylethanol and other compounds found in 'Lovely Dream' since they were preferred by panelists. These results provided a guideline for breeders

willing to select specific fragrances for cut roses. Based on these results, breeders should work towards introducing compounds such as 2-phenylethanol that gives a typical rose fragrance in combination with few other floral type volatile compounds such as trans caryophyllene, geranyl acetate and neryl acetate. Breeders should not introduce several compounds in the same cultivar and should avoid compounds that produced medicinal fragrances such as methyl salicylate.

Table 5-1. Amount of volatile compounds ( $\mu\text{g}/\text{fw}/\text{h}$ ) produced from fragrant \* and non fragrant cut rose cultivars at developmental stage 4 ('Allure', 'Erin', 'Lovely Dream') and stage 3 ('Red Sensation'). Means  $\pm$  standard error n=4. Panelist intensity, likeability and ranking ratings. Means  $\pm$  standard error n= 99. Different letters = significant difference between means within parameter  $\alpha = 0.05$ .

Biochemical pathway	Compound	Cultivar			
		'Allure' *	'Erin' *	'Lovely Dream' *	'Red Sensation'
Lipid derivative	cis-3-hexen-1-ol	0.02 $\pm$ 0.00	0.00	0.00	0.00
	Eicosane	0.02 $\pm$ 0.00	0.11 $\pm$ 0.03	-	0.02 $\pm$ 0.00
Phenylpropanoid	2-phenylethanol	0.01 $\pm$ 0.00	-	0.06 $\pm$ 0.01	-
	3,5 dimethoxytoluene	2.67 $\pm$ 0.29	1.46 $\pm$ 0.22	2.65 $\pm$ 0.76	-
	Benzyl Acetate	-	0.15 $\pm$ 0.03	-	-
	Benzyl Benzoate	-	0.02 $\pm$ 0.01	-	0.02 $\pm$ 0.00
	Isoeugenol	0.02 $\pm$ 0.00	0.18 $\pm$ 0.05	-	0.03 $\pm$ 0.00
	Methyl Benzoate	0.01 $\pm$ 0.00	-	-	-
	Methyl Salicylate	0.29 $\pm$ 0.09	-	-	-
	Phenethyl Benzoate	0.02 $\pm$ 0.00	0.06 $\pm$ 0.01	-	0.02 $\pm$ 0.00
	Terpenoid	(-) trans caryophyllene	0.21 $\pm$ 0.02	0.49 $\pm$ 0.11	0.23 $\pm$ 0.06
(S)-(-)-B citronellol		0.16 $\pm$ 0.06	-	-	-
Geraniol		-	-	0.00	-
Geranyl acetate		0.01 $\pm$ 0.00	-	0.11 $\pm$ 0.03	-
Linalyl acetate		0.12 $\pm$ 0.03	-	-	-
Neryl acetate		0.02 $\pm$ 0.01	-	0.01 $\pm$ 0.00	-
Total amount		3.56	2.47	3.07	0.09
Panel parameter		Intensity	6.2 $\pm$ 0.2 a	4.1 $\pm$ 0.2 b	5.5 $\pm$ 0.2 a
	Likeability	6.5 $\pm$ 0.2 ab	6.1 $\pm$ 0.2 b	7.0 $\pm$ 0.2 a	4.81 $\pm$ 0.2 c
	Preference Rank(*)	230 b	238 b	181 c	341 a

(\*) Most preferred (1) – least preferred (4)

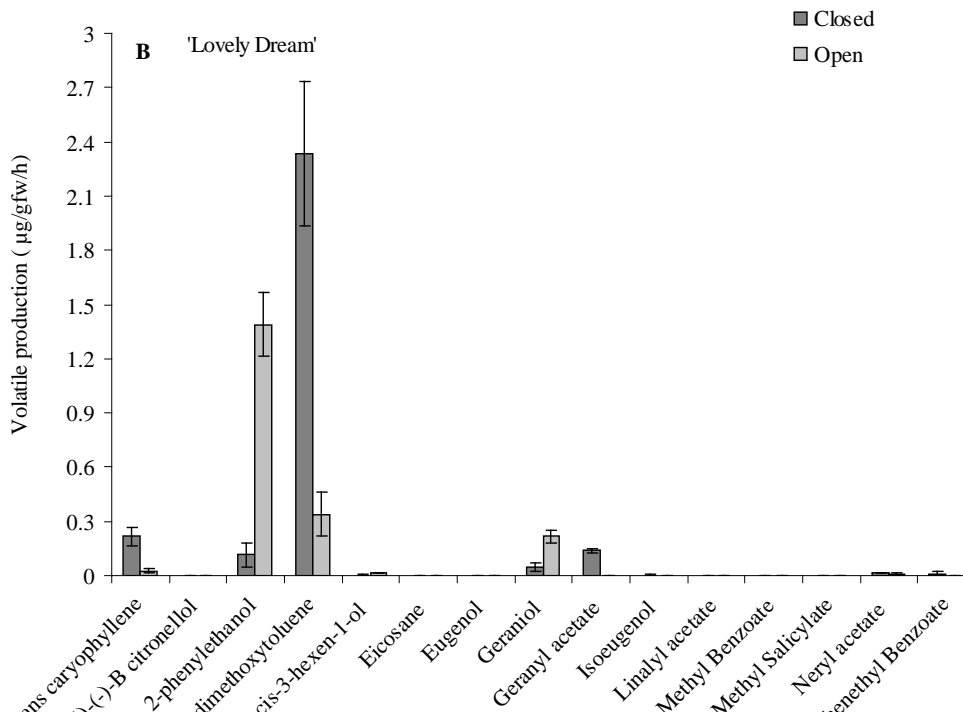
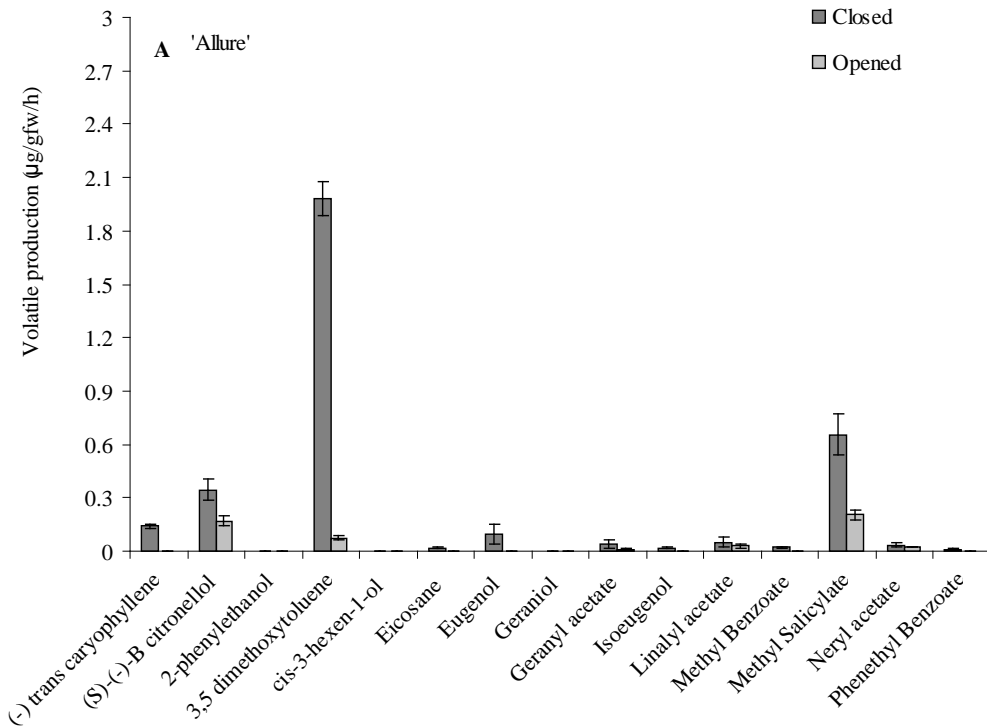


Figure 5-1. Differences in amount and composition of volatile compounds from fragrant cultivars at closed (stage 2) and opened (stage 4) developmental stage. Means  $\pm$  standard errors n=4. A) 'Allure' B) 'Lovely Dream'



Table 5-2 Intensity and likeability scores for open and closed ‘Allure’ and ‘Lovely Dream’ flowers. Means  $\pm$  standard error n=89.

	‘Allure’		‘Lovely Dream’	
	Open	Closed	Open	Closed
Intensity	4.9 $\pm$ 0.2 a	5.1 $\pm$ 0.2 a	3.2 $\pm$ 0.2 b	4.9 $\pm$ 0.2 a
Likeability	5.8 $\pm$ 0.2 b	6.4 $\pm$ 0.2 a	6.0 $\pm$ 0.2 a	6.5 $\pm$ 0.2 a

APPENDIX A  
DEHYDRATION LEVEL THAT AFFECTS VASE LIFE OF CUT ROSE (*ROSA HYBRIDA*  
L.) CULTIVARS AND ITS OCCURRENCE DURING SHIPPING AND STORAGE

**Introduction**

Few reports have described the effects of dehydration during shipping and storage on cut rose flower quality and vase life. In a screening study of 22 rose cultivars, Mokhtari and Reid (1995) reported that the ability of cut flowers to recover in terms of leaf water potential, water loss, water uptake and petal wilting from just 10 % weight loss varied greatly among cultivars.

Currently, flowers are produced long distances from market and transported at higher than recommended temperatures, conditions that likely promote flowers to water stress conditions not previously observed (Miller, 2001; Reid, 2001). Flowers are often stored before and/or after transport to regulate supply, particularly for major trading days like Valentine's Day and Mother's Day (Mastalerz, 1987). Roses are typically transported and stored dry inside fiberboard boxes often at temperatures between 5 to 10 °C (Reid, 2001). The resulting water stress reduces the ability of flowers to rehydrate completely and maintain optimal water relations during vase life (Faragher *et al.*, 1984; Mokharit *et al.*, 1995; van Doorn, 1997b).

Faragher *et al.* (1984) showed that dry storage of cut 'Mercedes' roses for 1.5 days at 22°C reduced flower weight and vase life by 19% and 33%, respectively, relative to control flowers kept in water. Similarly, Jin *et al.*, (2006) found that exposing cut 'Samantha' rose flowers for 1 day at 20°C induced a 29 % reduction in flower weight that resulted in water stress and a reduced vase life by 3 days compared to stems that were placed in water. Studies have demonstrated that storing roses for more than 3 days can decrease vase life up to 5 days (Leonard *et al.*, 2001). Long- term storage of roses has also been found to reduce vase life of 'Visa' roses (Serrano *et al.*, 1992) and 'Gabriella' roses (Mor *et al.*, 1989). The level of 1-aminocyclopropane-1-carboxylic acid (ACC) increased during the storage and flower senescence was found to be

higher in roses that were previously stored (Serrano et al., 1992). However, packaging type used during storage may alleviate negative effects of dry storage on rose flowers. For example, packaged 'Better Times' roses in closed cellophane bags for less than 5 days increased flower quality results (Hauge et al., 1947). These research attributed the increased in flower quality to increased carbon dioxide levels and maintenance of high relative humidity during storage.

Parameters such as weight loss and water potential have been used to study the water balance of cut flowers and have been found to reflect water deficit in plant tissues (Van Doorn, 1997a). These indicators of the water status of cut stems were not considered in early dry storage studies (Hauge et al., 1947; Serrano et al., 1992). A decline in water potential (increasing negative values) and fresh weight of rose petal tissue has been associated with wilting and was higher on cultivars with short vase lives (i.e. 'Golden Wave') compared to cultivars with long vase lives (i.e. 'Baccara' and 'Super Star') (Mayak et al., 1974).

Hydrating cut flower stems, a common practice used in the flower industry, is very effective in restoring the water status of stems that have been exposed to water stress during handling, shipping and storage (Halevy et al., 1981). Proper hydration practices include re-cutting the stems before placing them into solution to eliminate stem occlusion caused by microorganism and stem embolism and to maximize water uptake (Aarts, 1957; Burdett, 1970). Hydration capacity is variable among cultivars. For example, 'Cara Mia' roses hydrate easily in a solution with a wetting agent or citric acid, but are more difficult to hydrate than 'Sonia' (Mokharit et al., 1990). Hydration can be improved by using surfactants in the solutions (van Doorn et al., 1993). In contrast, hydrating 'Madame Delbard' rose for 1 hour after commercial transport had no effect on the daily water uptake, vase life or quality (Suzuki et al., 2001). This study did not evaluate the effect of different re-hydration times on vase life, water uptake and

quality, but found that solution uptake was 40 % greater when hydrated in solutions maintained at 34 °C compared to 2 °C during the first hour, but no differences were found in uptake after that time point nor in vase life or quality between the two temperatures.

This study was conducted to evaluate the impact of water status during shipping and/or storage on the subsequent vase life of cut rose flowers. Experiments were designed to define the maximum level of water loss tolerated by flowers without a loss of flower vase life. Effects of pre- and post-shipment hydration treatments or no hydration treatments on flower-recovery from shipment and storage-related weight loss are a prime focus of these studies.

## **Materials and Methods**

### **Plant Material**

Cut flowering stems of rose (*Rosa hybrida* L.) ‘Charlotte’, ‘Clear Ocean’, ‘Cristina’, and ‘Helio’ were obtained from a commercial farm near Bogotá, Colombia. These cultivars have red, cream white, pink, and yellow flowers, respectively. Flowers were harvested at commercial maturity from greenhouse-cultivated plants between 08:00 h and 11:00 h and transported dry to a postharvest room at each farm within 30 minutes of harvest. Flower heads were dipped for 10 sec in a fungicide solution (1 mL/L, Sportak<sup>®</sup>; Bayer CropScience AG, Monheim, Germany) and graded at ambient temperature (14 to 18°C) for uniformity of flower head development and stem length. Depending upon the cultivar and stem length at harvest, flower stems were cut to 50, 60 or 70 cm lengths, then combined into 25-stem bunches by cultivar within 1 to 2 h of harvest. Bunches were weighed and then flowering ends of each bunch were wrapped in a sheet of corrugated fiberboard and a polypropylene plastic sleeve for protection during transport.

### **Pre-Shipment Treatment**

Unless otherwise described, cut ends of flower bunches were placed into buckets containing a commercial hydration solution (2mL/L, Chrysal Clear Professional 1; Chrysal,

Naarden, The Netherlands) for 18 hours at 2°C. Immediately after hydration, bunches were weighed and wrapped in sleeves. Bunches were then packed into corrugated fiberboard boxes (dimensions: 104 cm-long, 25 cm-wide, 16 cm-high) for shipment. Each box was lined with a sheet of paper and contained eight bunches. A data logger (HOBO H08-001-02; Onset Computer Corporation, Bourne, MA, USA) was placed inside a randomly selected box to record air temperature during shipment. Boxes were commercially transported via airplane and truck from the farm to the University of Florida, in Gainesville, FL via Miami, FL within 6 days.

### **Post-Shipment Treatments**

#### **Experiment 1: Dehydration tolerance of cut rose flowers**

The objective of this experiment was to determine maximum weight loss and water potential tolerated by cut rose flowers before vase life was significantly reduced. All cultivars received the pre-shipment treatment described above. Upon arrival to the laboratory, bunches were removed from boxes and stems were randomly distributed into four dehydration treatments. Each stem was weighed, and then placed horizontally and separately on a wire mesh at 21°C, 50% relative humidity (RH) and under  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  of light (12 h/day). Stems remained on the mesh until they had lost 5, 10, 15 or 25 % of their harvest weight, which took approximately 1, 4, 8 and 24 hours, respectively. Control stems were rehydrated in a commercial hydration solution (Chrysal Clear Professional 1 solution 2mL/ L, Chrysal, Naarden, The Netherlands) mixed with deionized water when unpacked until they returned to harvest weight (0 % weight loss), then handled as outlined below. Leaf water potential was determined immediately after stems reached the designated de-hydration treatment (5, 10, 15 or 25%) as described in the section below.

Once stems loss the designated harvest weight (0, 5, 10, 15 and 25%), they were cut to 45 cm in length re-weighed and placed individually into 300ml volume glass vases containing

deionized water for vase life evaluation. Fresh weight was determined at harvest at the farm (day 0), after shipment (day 7), after de-hydration treatment (day 8), at the beginning of vase life (day 9) and then every two days during vase life until the flower showed the first signs of senescence.

A total of 10 stems per treatment per cultivar were used for fresh weight determination. The same 10 stems were used for vase life evaluation. A total of 10 different stems from those used for fresh weight and vase life were used for water potential determination. Vases were organized in a completely randomized design and data were analyzed by ANOVA using SAS<sup>®</sup> Version 8 (SAS Institute Inc., Cary, NC, USA). When significant ( $P \leq 0.05$ ) treatment effects were determined by ANOVA, differences among treatment data means were determined using Tukey's multiple comparison test at  $P \leq 0.05$ . Data are presented as means  $\pm$  standard error of the mean.

### **Experiment 2: Dehydration level after dry farm and shipment treatment**

The objective of this experiment was to determine if flower weight loss following hydration or no hydration at the farm prior to dry shipment affects vase life of cut roses. All cultivars were either hydrated or not using the conventional pre-shipment farm hydration solution as described above. Non-hydrated flowers were handled the same as hydrated flowers except the stems were placed upright into empty buckets for 18 hours at 2 °C. Four 25-stem bunches were assigned to each treatment. Bunches from both treatments were weighed before and after the farm hydration/no-hydration treatment. Flowers were sleeved, packaged and transported from Colombia to the University of Florida via Miami as described above.

Upon arrival to the laboratory, all bunches were weighed to determine weight loss from harvest during shipping. Stems were then randomly selected from the four bunches for each treatment. The basal 5 cm of each stem was removed with sharp, clean cutters. Freshly-cut ends

of stems of both treatments were placed into buckets containing a commercial hydration solution (2 mL/L, Chrysal Clear Professional 1; Chrysal, Naarden, The Netherlands) mixed with deionized water for 4 days at 1.5°C. These treatments simulated standard commercial rehydration protocols at the wholesale and retail level (Leonard *et al.*, 2001).

Stems were weighed after the 4 days of re-hydration treatments. Stems were then re-cut by removing an additional 5 cm and placed individually into 300 ml glass vases containing the commercial consumer preservative solution (15 mL/L, Chrysal Clear Professional 3; Chrysal, Naarden, The Netherlands) mixed with deionized for vase life evaluation. Fresh weight was determined at harvest (day 0), after farm hydration (day 1), after shipment (day 7), after re-hydration (day 11), at the beginning of vase life (day 12) and then every two days during vase life until the flower showed the first signs of senescence. Leaf water potential was determined after shipment (day 7), after re-hydration (day 11), at day 3 (day 15) and 5 (day 17) of vase life and at the end of vase life. A total of 10 stems per treatment per cultivar were used for fresh weight determination. The same 10 stems were used for vase life evaluation. A total of 10 different stems from those used for fresh weight and vase life were used for water potential determination. A completely randomized design was used for this experiment and data by ANOVA using SAS<sup>®</sup> Version 8 (SAS Institute Inc., Cary, NC, USA). When significant ( $P \leq 0.05$ ) treatment effects were determined by ANOVA, differences among treatment data means were determined using Tukey's multiple comparison test at  $P \leq 0.05$ . Data are presented as means  $\pm$  standard error of the mean.

### **Experiment 3: Effect of rehydration time**

The purpose of this experiment was to define the optimal rehydration treatment time that provided greatest recovery (weight and vase life) from shipment-related weight loss of flowers

receiving farm hydration followed by dry shipment as described on Experiment 1. Results from Experiment 1 showed that ‘Charlotte’ had the lowest water potential values indicative of low water status and a long vase life compared to the other cultivars. Therefore, this cultivar was selected to study the re-hydration effects on vase life.

Flowers received pre-shipment farm hydration as described above. Upon arrival to the laboratory, the basal 2 cm of stems was removed and placed into a commercial hydration solution (2 mL/L, Chrysal Clear Professional 1; Chrysal, Naarden, The Netherlands) for 0, 1, 24, 48 and 96 hours at 3.5°C and 94 % RH in the dark. Stems that received no rehydration treatment (0 hours) were re-cut and transferred directly to vases. In order to place stems from all treatments into vases at the same time, rehydration treatment of 96 hours were the first to be placed into hydration solution followed by 48, 24, 1 and 0 hours. Flowers were kept dry in the box maintained at 3.5 °C for 4, 2, 1 and 0 days while waiting to be re-hydrated for 0, 1, 24, 48 and 96 hours, respectively. After rehydration, stems were re-cut to 50 cm and placed individually into 300 ml glass vases containing the consumer solution (15 mL/L, Chrysal Clear Professional 3; Chrysal, Naarden, The Netherlands) mixed with deionized water for vase life evaluation.

A total of 7 stems per re-hydration time per cultivar were used for both fresh weight and vase life determination. A completely randomized design was used and data were analyzed by ANOVA using SAS<sup>®</sup> Version 8 (SAS Institute Inc., Cary, NC, USA). When significant ( $P \leq 0.05$ ) treatment effects were determined by ANOVA, differences among treatment data means were determined using Tukey’s multiple comparison test at  $P \leq 0.05$ . Data are presented as means  $\pm$  standard error of the mean.



#### **Experiment 4: Dehydration level during dry storage**

The objective of this experiment was to determine the fresh weight loss that occurs during dry storage and its effects on vase life of cut roses. ‘Charlotte’, ‘Cristina’ and ‘Helio’ were subjected to pre-shipment farm hydration treatment, handling and shipping conditions as described above. After shipment to Florida, stems from four 25-stem bunches were randomly divided into five dry storage treatments (0, 7, 14, 21 and 28 days). Stems were re-combined into 25-stem bunches and wrapped in polypropylene plastic sleeves. Bunches were then packed into corrugated fiberboard boxes and stored dry for 0, 7, 14, 21 and 28 days at 2.5°C and 96 % RH. At the end of each storage time, stems were re-cut to 45 cm-length by removing 5 cm of the base. Fresh weight was determined at the farm (day 0), after shipment (day 7), after each storage time and then every two days during vase life following the procedure described below. Stems were then placed into individual 300 ml glass vases containing deionized water for vase life evaluation.

A total of 9 stems per storage time per cultivar were used for fresh weight determination and 6 stems per cultivar were used for vase life evaluation. A completely randomized design was used and data were analyzed by ANOVA using SAS<sup>®</sup> Version 8 (SAS Institute Inc., Cary, NC, USA). When significant ( $P \leq 0.05$ ) treatment effects were determined by ANOVA, differences among treatment data means were determined using Tukey’s multiple comparison test at  $P \leq 0.05$ . Data are presented as means  $\pm$  standard error of the mean.

#### **Vase Life Evaluation**

Flower vase life was measured as the time in days from placement of stems in vase solution to the appearance of visual senescence symptoms such as petal wilting, abscission, bluing, disease and bent neck. Flowers were held at 21 °C, 40-50 % RH and under 10  $\mu\text{mol m}^{-2}$

s<sup>-1</sup> of light (12 h/day) for evaluation. Opening of flowers was assessed every two days using the following rating score: 1 = outer petals tightly wrapped around bud, 2 = outer petals starting to reflex from bud, 3 = outer petals reflexed approximately 135° to stem, 4 = outer petals reflexed at approximately 115° to stem, 5 outer petals reflexed at 90° to stem.

### **Fresh Weight Measurements**

Cut flower stems were weighed in bunches immediately after harvest at the farm, after shipment and at arrival to the laboratory. Stems were individually weighed after de-hydration treatments in Experiment 1, after re-hydration solutions in Experiment 2, after re-hydration times in Experiment 3, and after storage times in Experiment 4 and during vase life on all experiments. Flower stem weight was expressed relative to initial harvest weight (%).

### **Water Potential Measurements**

Leaf water potential ( $\Psi_L$ ) was determined with a pressure chamber (Model 3000, Soil Moisture Equipment Corp., Santa Barbara, CA, USA) as described by (Scholander et al., 1965). In preliminary experiments, leaf water potential was found to be similar for each of the compound leaves on the same stem (data not shown), therefore a designated leaf was chosen to determine the water potential at each sampling time. At each sampling time, one leaflet from each stem was selected starting at the fifth compound leaf on the stem (going down from the bud). In order to equilibrate the leaf water potential with the stem water potential, leaflets were enclosed for 1 hour at 21°C in individual plastic stem water potential bags (17 cm deep x 9 cm wide) (PMS Instrument Company, Albany, OR, USA) with the open end of each bag closed tightly around the petiole (Fulton *et al.*, 2001). Balance pressure was determined using compressed nitrogen gas and increased at a rate of 25 kPa s<sup>-1</sup>. Cut flower stems used for  $\Psi_L$  measurements were considered destructive samples.

## Results and Discussion

### Experiment 1: Dehydration Tolerance of Cut Rose Flowers

Visible leaf and petal wilting became apparent when stems lost 10 % weight, in all cultivars and was severe when stems lost 15 % weight or more, in all cultivars (data not shown). However, loss of 5, 10 and 15 % from initial harvest weight did not reduce vase life for any of the cultivars tested in the present study (Table A-1). Dehydrating flower stems to 25 % of harvest weight reduced vase life by 5.4, 5.0 and 5.1 days for ‘Charlotte’ ‘Cristina’ and ‘Helio’ flowers respectively, compared to stems that were not dehydrated (0 % weight loss). ‘Clear Ocean’ was less sensitive to weight loss and tolerated losing 25 % without a significant reduction in vase life. Mokhtari and Reid (1995) reported that dehydrating cut ‘Cocktail’ and ‘Marlyse’ cultivars by 10% significantly reduced subsequent vase life. Cultivars such as ‘Royal Red’ and ‘Sonia’ were more tolerant to this dehydration treatment. Taken collectively, our results and those of Mokhtari and Reid (1995) indicate there is considerable variation in the dehydration tolerance among cut rose cultivars. Additionally, the results of this study show that visible wilt is not an indicator of loss of vase life. Differences in the dehydration tolerance or rehydration capacity of flowers may relate to morphological and physiological variation between cultivars such as the degree of lignification of the peduncle (Parups et al., 1976) , transpiration and water uptake rates (Zieslin *et al.*, 1978), xylem vessel diameter (van Meeteren et al., 2001; van Ieperen et al., 2002) and stomata structure and function (van Doorn, 1997b).

The amount of weight that was initially regained by stems on day 1 of vase life was proportional to the level of dehydration for all cultivars. For example, ‘Clear Ocean’ flowers that were dehydrated to 5, 10, 15 and 25% of harvest weight had an increase in weight of 6, 18, 26 and 35 % above harvest weight respectively, after one day in vase solution. A similar trend occurred for all cultivars (Figure A-1). The ability to recover fresh weight on the first day of vase

life was inversely related with vase life in ‘Charlotte’, ‘Cristina’ and ‘Helio’. At 25 % weight loss, these cultivars gained more weight (Figure A-1) and had a shorter vase life (Table A-1) than the weight recovered and the vase life of these cultivars at 0, 5 10 and 15 % weight loss. The ability to maintain stem weight above harvest weight during vase life was cultivar dependent and appeared to be related to vase life. The relatively long-lived cultivars ‘Charlotte’ and ‘Clear Ocean’ remained at or above harvest weight for 8 days while ‘Cristina’ and ‘Helio’ stems, which exhibited a shorter vase life, remained above harvest weight for only 4 to 5 days of vase life (Figure A-1).

Leaf water potential ( $\Psi_L$ ) significantly decreased as stems were progressively dehydrated (Figure A-2). The leaf water potential of stems subjected to a dehydration level of 5 % or more was significantly lower than stems hydrated to harvest weight (0% weight loss) for all cultivars. At maximum dehydration level (25 %), leaf water potential was lower by 1.5, 1.6, 1.8 and 2.2 MPa from stems that were not dehydrated (0 % weight loss) on ‘Charlotte’, ‘Clear Ocean’, ‘Cristina’, ‘Helio’, respectively. Immediately after reaching the maximum de-hydration level (25%) ‘Charlotte’, ‘Cristina’, and ‘Helio’, cultivars which vase life was reduced by 25 % weight loss, had  $\Psi_L$  of -1.8, -1.3 and -1.6 MPa, respectively (Figure A-2). These values are consistent with those reported by Thompson et al., (1983) who showed that dehydrating cut ‘Forever Yours’ rose stems to a level associated with reduced vase life had a  $\Psi_L$  of -1.73 MPa. In this study, leaf water potential of ‘Clear Ocean’ was -1.4 MPa at 25 % de-hydration treatment and vase life was not reduced, suggesting that water potential may not always be a valid indicator of the relationship between water stress of flowers and vase life.

## **Experiment 2: Dehydration Level after Dry Farm and Shipment Treatment**

After an 18-hour postharvest hydration treatment at the farm, fresh weight of all cultivars increased by 1.5 to 3.9%, while non-hydrated stems lost 0.3 to 0.7% of their harvest fresh weight

(Figure A-3). During the subsequent 6-day commercial shipment where air temperatures fluctuated from 3.4 to 21.8°C (average  $9.7 \pm 4.5$  °C), the hydrated flower stems lost 1.4 to 2.5% of their harvest weight, depending on the cultivar. In contrast, the non-hydrated stems lost an additional 0.9 to 1.3% of harvest weight during the 6-day shipment. The total weight loss from the farm to our laboratory following the 6 day shipment averaged 2.0 % for hydrated compared to 2.5% for flowers not hydrated at the farm. Therefore, neither hydrated or non-hydrated stems at the farm followed by the 6 day dry shipment did not reach a 25% loss in fresh weight which resulted in reduced vase life in Experiment 1.

Vase life of all cultivars was not affected by the hydration treatments following harvest. Presumably, vase life was not reduced because desiccation of farm hydrated and non-farm hydrated stems (0.9 to 2.5 % weight loss) was well below the critical 25% weight loss. Dry-handling stems at the farm and during shipment may have also contributed to maintaining subsequent vase life by reducing rates of flower tissue growth and development (Evans et al., 1986) or by preventing bacteria accumulation in xylem vessels at the cut stem base (van Doorn et al., 1991). Hydrating freshly-harvested rose stems at the farm was reported to enhance subsequent rates of opening of ‘Madame Delbard’, ‘Classy’, ‘First Red’ and ‘Leonidas’ rose flowers by 10-20% relative to stems not hydrated at the farm (Leonard *et al.*, 2001). However in this study, dry handling treatment did not significantly affect rates of ‘Charlotte’, ‘Clear Ocean’, ‘Cristina’ and ‘Helio’ flower opening during vase life compared to conventionally hydrated stems (data not shown).

‘Charlotte’, ‘Clear Ocean’, ‘Cristina’ and ‘Helio’ flower stems that received hydration treatment at the farm gained 15.8, 10.6, 17.3 and 9.2 % above harvest weight, respectively, after a 4-day post-shipment rehydration treatment. Non- hydrated ‘Clear Ocean’ and ‘Helio’ flower

stems gained significantly more weight (11.1 and 13.0% weight, respectively) during the 4-day post-shipment rehydration than stems that were hydrated at the farm (5.1 and 9.2% weight, respectively). Weight gain of 'Charlotte' and 'Cristina' stems was similar regardless of farm hydration treatment (Figure A-3). These results illustrate that a 4 day re-hydration treatment enabled the fresh weight of the stems to return to or increase from their initial harvest weights whether stems were hydrated or not at the farm. 'Charlotte', 'Clear Ocean', 'Cristina' and 'Helio' continued to increase in weight to a peak of 20.8, 5.9, 21.8 and 10.1 % respectively, above harvest weight by day 2 of vase life. Farm hydration treatment did not affect maximum weight gain during vase life on any cultivar. Maximum fresh weight gain, however, was different among the cultivars. Maximum weight gain from harvest during vase life was 12 % for 'Clear Ocean', 14 % for 'Helio', 22% for 'Charlotte' and 23 % for 'Cristina'.

The re-hydration recovery level obtained after 4 day re-hydration treatment was confirmed by high leaf water potential values on all cultivars. For example, upon arrival, the leaf water potential  $\Psi_L$  of 'Charlotte' with no farm hydration was lower ( -1.2 MPa ) compared to the water potential of stems hydrated at the farm (-1.0 MPa). After the 4 day re-hydration treatment (day 11 after harvest), leaf water potential values of farm hydrated and non-hydrated 'Charlotte' stems were significantly higher (-0.1 MPa) than those obtained upon arrival of farm hydrated and non-hydrated stems (Figure A-4). Leaf water potential values were similar to 'Charlotte' in hydrated and non-hydrated stems after shipment and re-hydration for the other cultivars. Leaf water potential gradually decreased during the vase life evaluation period and was not affected by the farm treatment during vase life on any cultivar. These results suggest that a re-hydration treatment is an effective practice to recover de-hydrated stems ( -1.24 MPa) to well hydrated

stems (-0.1 MPa) regardless of the farm treatment. Further research is needed to determine the minimum hydration time needed to optimize water status.

The lowest leaf water potential values registered were -1.20 MPa for ‘Charlotte’ and -1.24 MPa for ‘Clear Ocean’ on non-hydrated flowers. The lowest water potential values corresponded to the water potential values registered in Experiment 1 on stems exposed to 5 to 10 % de-hydration level. These values did not affect vase life of any cultivar. Vase life of all cultivars was not affected by the farm hydration treatment. Flower opening was not affected by farm hydration treatment on any cultivar (data not shown). Therefore, skipping hydration at the farm could be acceptable provided flowers are not subject to extend storage and are hydrated properly following transport. These results are likely due to the fact that rose cultivars of this study did not reach critical de-hydration levels measured by fresh weight and leaf water potential.

### **Experiment 3: Effects of Rehydration Time**

Rehydration period had no significant effect on vase life of ‘Charlotte’ flowers. Vase life of ‘Charlotte’ flowers that were rehydrated for 0, 1, 24, 48 or 96 hours varied from 6.9 to 8.3 days. The rehydration treatment time did not significantly affect rate of flower opening during vase life (data not shown). Fresh weight gained during vase life was affected by re-hydration treatment. Flower stems that received 0 and 1 hour rehydration treatment gained significantly more weight during vase life than stems that were re-hydrated for 24, 48 and 96 hours (Figure A-5 ). Flower stem fresh weight began to decrease after 2 days of vase life for stems rehydrated for 24, 48 and 96 hours, while the weight of stems rehydrated for 0 and 1 hours remained almost constant until the end of vase life (Figure A-5). The increased fresh weight gained during vase life on stems receiving 0 and 1 hour re-hydration was most likely due to lower water content in the stems resulting from the longer dry storage time treatment received by these stems. Stems with 0 and 1 hour re-hydration treatment were stored for 4 and 3 days, respectively before

receiving the re-hydration treatment. An increased fresh weight during vase life as a result of dry storage observed on stems hydrated for 0 and 1 hours (stored for 4 and 3 days) corresponded with an increased weight observed on stems de-hydrated to 15 % and 25 % of initial harvest weight in Experiment 1. Stems with 15 % and 25 % de-hydration levels gained significant more weight when placed into vase solution compared to stems that received de-hydration levels of 0 and 5 % (Figure A-1).

Fresh weight gained during vase life had no effect on the longevity of the flower. These results suggest that different re-hydration times are not critical for 'Charlotte' vase life. Re-hydrating 'Charlotte' flowers after shipping may be omitted only if flowers are stored at the correct temperature for no longer than 4 days and stems are placed immediately into appropriate vase hydration solution after being removed from these conditions.

#### **Experiment 4: Dehydration Level after Dry Storage**

'Charlotte' flowers could be stored dry at 2.5°C for 7 days without a significant reduction in vase life compared to non-stored flowers while 'Cristina' and 'Helio' could be stored for 14 and 21 days, respectively, before subsequent vase life was significantly reduced (Table A-2). Excessive storage significantly reduced vase life 2.6 to 4.0 days for 'Charlotte', 2.5 to 3 days for 'Cristina' and 5 days for 'Helio'. In earlier research, Faragher et al. (1984) showed that storing freshly-harvested 'Mercedes' rose flowers dry at 2°C for 10 days was sufficient to reduce subsequent vase life by 2 days and reduced maximum flower diameter. Leonard *et al.* (2001) showed that 'Classy', 'First Red' and 'Madame Delbard' rose flowers could be stored for only 0, 6, and 6 days, respectively, after a 4 to 6-day dry shipment without a significant loss in vase life. Storage of rose flowers at low temperature has become a common practice used by the floral industry to facilitate consistent supply to markets (Nell et al., 2005). Differences in the dehydration and dry storage tolerance observed among cut rose cultivars highlights the importance



of selecting and marketing cultivars suited to particular postharvest handling and storage practices.

'Charlotte', 'Cristina', and 'Helio' flowers lost 3.5, 5.1, and 3.1% of initial fresh weight at harvest, during the 6-day dry shipment where air temperatures fluctuated from 4.1 to 13.2°C (average  $8.8 \pm 2.8$  °C). When subjected to post-shipping storage, all cultivars continued to lose fresh weight which increased as storage time increased. For example, after 28 days of dry storage 'Charlotte', 'Cristina' and 'Helio' stems lost 18.5%, 14.9% and 18.6 % fresh weight, respectively, compared to 2.7%, 5.5%, and 2.3 % fresh weight, respectively, lost when not stored (Table A-3).

The amount of weight loss by each cultivar (18.5%, 14.9% and 18.6 %) at maximum storage time (28 days) was less than the weight loss that affected vase life (25 %) in Experiment 1. However, vase life of 'Charlotte', 'Cristina' and 'Helio' was reduced 3.3, 2.5 and 5 days respectively, after 28 days of storage compared to storage for 0 days. Upon placement into vase solutions, all flower stems increased in weight during the first day regardless of the preceding storage treatment time. Stem weight for all cultivars then gradually decreased until the end of vase life (data not shown). These results suggest that fresh weight loss during dry storage may not be the only factor that affects vase life. A rise in ethylene production may explain the reduction in vase life of cultivars after long periods of dry storage. Ethylene production and sensitivity can occur in some rose cultivars as a result of prolonged dry storage time, thus, negatively affecting vase life (Mor et al., 1989).

In conclusion, the present study established that extensive weight loss (25% from harvest weight) significantly reduced vase life of four rose cultivars. The de-hydration level that affected vase life (25 %) was not reached when stems were not hydrated at the farm, dry shipped or dry

storage up to 28 days. Dry farm treatment and dry shipping did not affected vase life of the cultivars of this study. Dry storage time affected vase life differentially among cultivars. Even though different re-hydration times had an effect on the amount of fresh weight gained by stems during vase life, they did not affect the longevity of the stems. Moreover, considerable variation in the dehydration and dry storage tolerance, rehydration capacity, water potential, and water balance during vase life existed among the studied cultivars.

Our findings confirm that de-hydration level in terms of fresh weight loss from harvest and leaf water potential of commercially handled stems that affects vase life is higher than values reported in early studies. This finding highlights the importance of selecting new rose cultivars with dehydration and dry storage tolerance characteristics suited to current commercial handling systems to obtain long vase life.

Table A-1. Vase life of four cut rose varieties after subjected to de-hydration levels of 0, 5, 10, 15 and 25 % weight loss relative to harvest weight. Means  $\pm$  standard error n= 10. Means with the same letter within variety are not significantly different  $\alpha= 0.05$ . (Experiment 1).

De-hydration treatment	Vase life (days)			
Weight loss from harvest weight (%)	'Charlotte'	'Clear Ocean'	'Cristina'	'Helio'
0	10.0 $\pm$ 1.2 a	9.9 $\pm$ 2.8 a	7.4 $\pm$ 2.5 a	7.4 $\pm$ 2.4 a
5	11.1 $\pm$ 1.5 a	9.9 $\pm$ 2.8 a	7.0 $\pm$ 2.9 a	8.4 $\pm$ 2.8 a
10	10.4 $\pm$ 1.2 a	8.2 $\pm$ 2.6 a	6.3 $\pm$ 3.0 a	8.3 $\pm$ 1.6 a
15	9.0 $\pm$ 1.4 a	9.4 $\pm$ 1.1 a	6.5 $\pm$ 1.9 a	7.5 $\pm$ 2.3 a
25	4.6 $\pm$ 4.1 b	7.8 $\pm$ 2.5 a	2.4 $\pm$ 2.2 b	2.3 $\pm$ 2.5 b

Table A-2. Vase life of cut ‘Charlotte’, ‘Cristina’ and ‘Helio’ rose flowers when dry stored for 0, 7, 14, 21 and 28 days in corrugated boxes at 2.5°C and 96% RH after a 6-day shipment from Colombia. Data are presented as means  $\pm$  standard errors (n=6). Data within cultivar followed by a different letter are significantly different at  $\alpha=0.05$ . (Experiment 4).

Storage time (days)	Vase life (days)		
	‘Charlotte’	‘Cristina’	‘Helio’
0	11.6 $\pm$ 0.4 a	7.0 $\pm$ 1.2 a	9.0 $\pm$ 1.0 a
7	10.5 $\pm$ 0.2 ab	6.1 $\pm$ 0.6 ab	6.3 $\pm$ 1.3 ab
14	9.0 $\pm$ 0.5 bc	5.5 $\pm$ 0.5 ab	6.5 $\pm$ 1.0 ab
21	7.6 $\pm$ 1.0 c	4.0 $\pm$ 0.0 b	5.0 $\pm$ 0.8 ab
28	8.3 $\pm$ 0.4 bc	4.5 $\pm$ 0.3 ab	4.0 $\pm$ 0.6 b

Table A-3. Effect of dry storage on percent fresh weight loss from harvest on ‘Charlotte’, ‘Cristina’ and ‘Helio’ rose flower stems when dry stored for 0, 7, 14, 21 and 28 days in corrugated boxes at 2.5°C and 96% RH after a 6-day shipment from Colombia. Data are presented as means  $\pm$  standard errors (n=6). Data within cultivar followed by a different letter are significantly different at  $\alpha=0.05$ . (Experiment 4).

Storage Treatment (days)	Fresh weight change (% of harvest weight)		
	‘Charlotte’	‘Cristina’	‘Helio’
0	-2.7 $\pm$ 0.1 a	-5.5 $\pm$ 2.1 a	-2.3 $\pm$ 0.7 a
7	-4.7 $\pm$ 0.6 a	-5.8 $\pm$ 0.6 a	-8.6 $\pm$ 1.0 b
14	-8.7 $\pm$ 0.4 b	-10.1 $\pm$ 1.1 ab	-8.8 $\pm$ 1.0 b
21	-16.0 $\pm$ 1.0 c	-13.3 $\pm$ 1.2 b	-15.0 $\pm$ 0.7 c
28	-18.5 $\pm$ 1.3 c	-14.9 $\pm$ 1.0 b	-18.6 $\pm$ 0.7 d

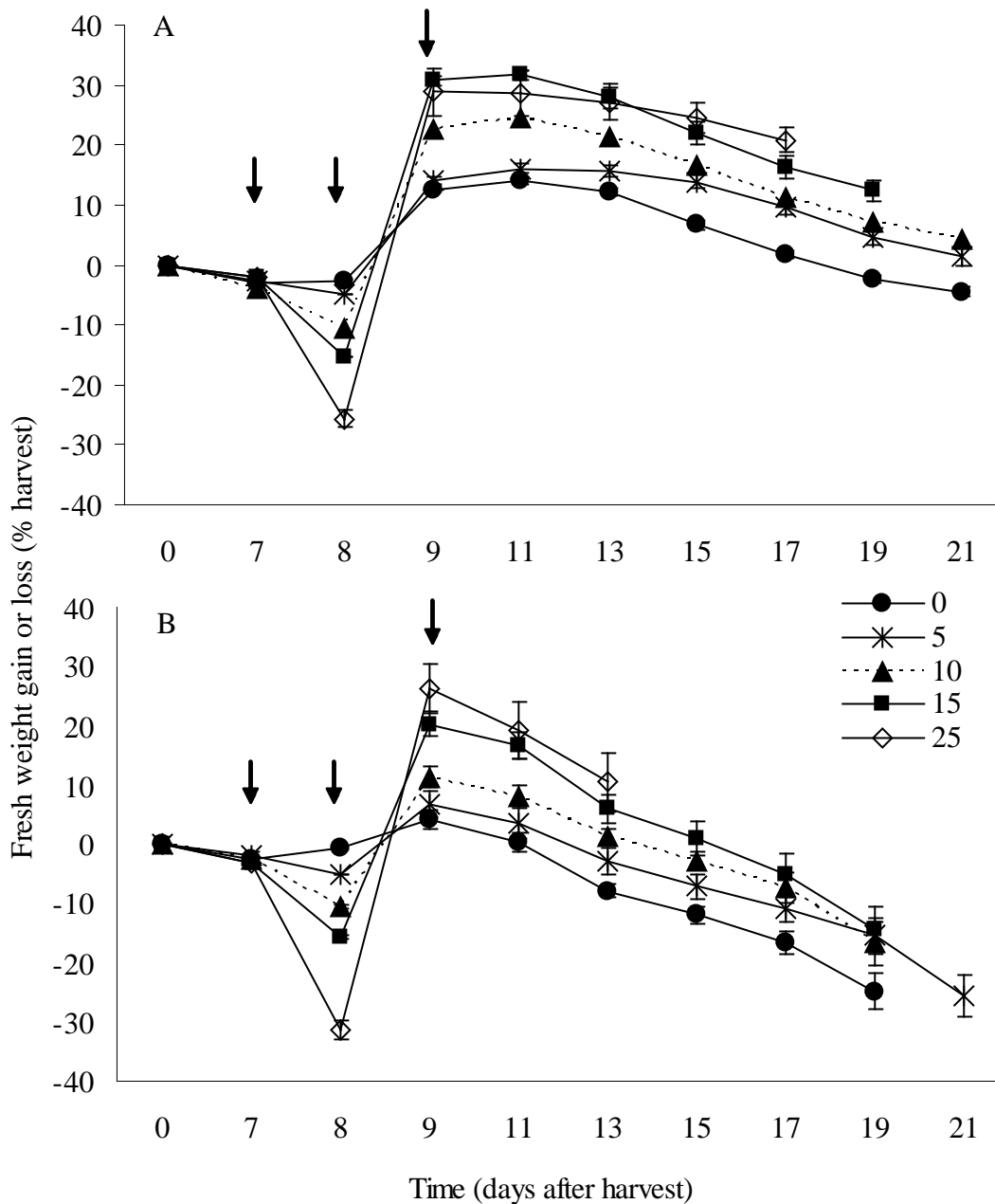


Figure A-1. Fresh weigh change from percent harvest weight of A) 'Charlotte' and B) 'Helio' during postharvest handling and vase life. Stems were de-hydrated to 0, 5, 10, 15 and 25 % of their harvest weight then placed into individual vases for vase life assessment. Means  $\pm$  standard error of the mean n= 10. Arrows represent from left to right fresh weight after shipment (day 7), de-hydration treatment (day 8), and day 0 of vase life (day 9 from harvest). (Experiment 1).

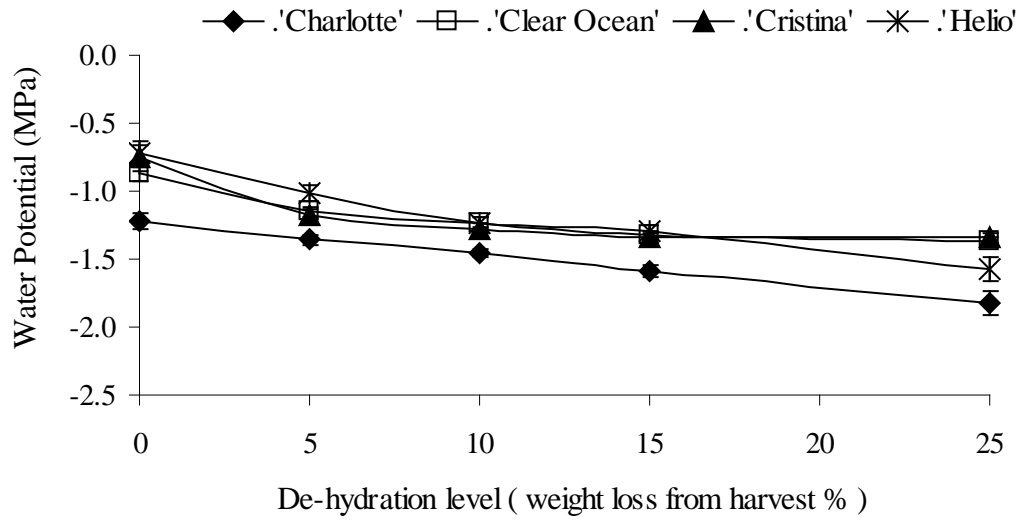


Figure A-2. Leaf water potential (MPa) of stems de-hydrated to 0, 5, 10, 15 and 25 % of their harvest weight measured immediately after stems reached each de-hydration treatment. Means  $\pm$  standard error of the mean n= 10. (Experiment 1).

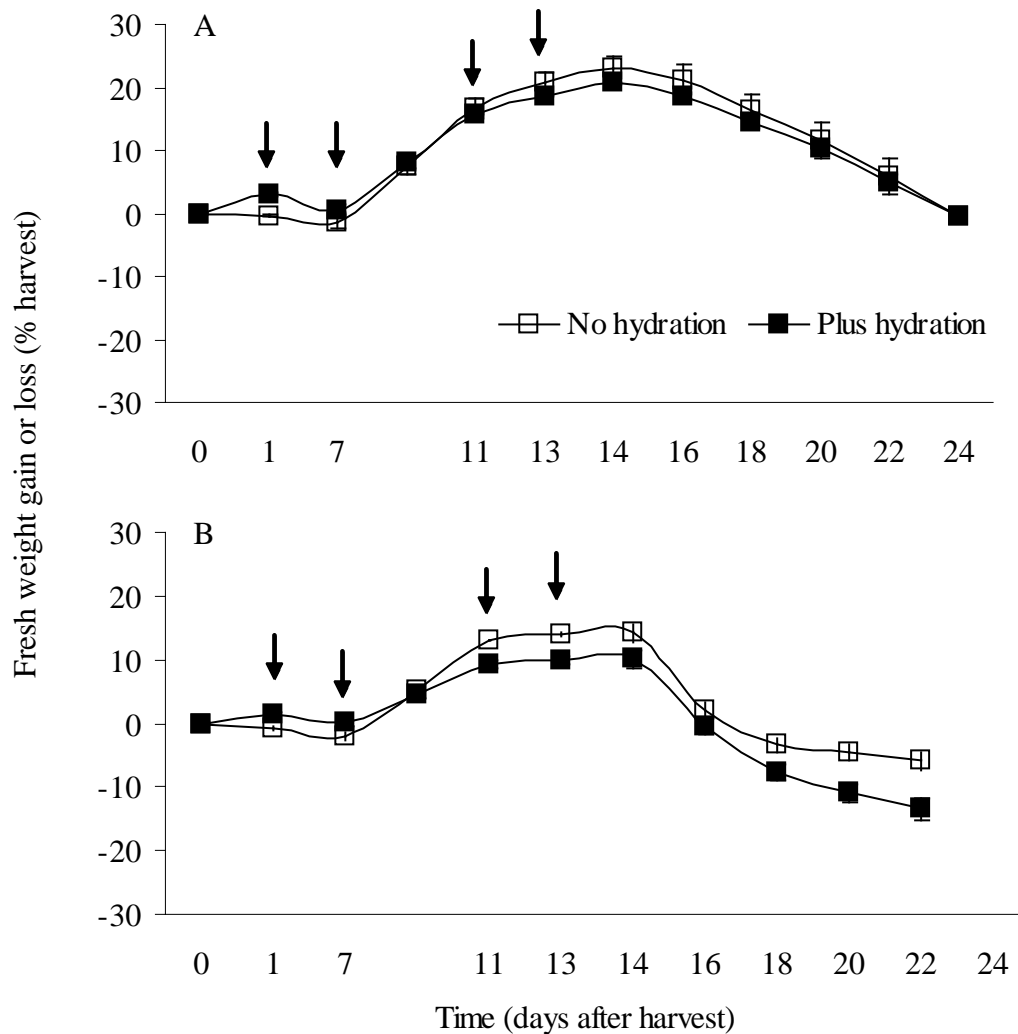


Figure A-3. Fresh weigh gained or loss (from % harvest weight) of A)'Charlotte' and B) 'Helio' during postharvest handling and vase life. Stems received two different hydration treatments (dry vs hydrated) at the farm for 18 hours at 2 °C. After farm treatment, stems were dry shipped within 7-days. After shipment, flowers were re-hydrated for 4 days, then placed into individual vases for vase life assessment. Means  $\pm$  standard error of the mean  $n= 10$ . Arrows represent from left to right fresh weight after farm treatment (18 hours), shipment (day 7), re-hydration (day 11) and day 1 of vase life (13 days after harvest). (Experiment 2).

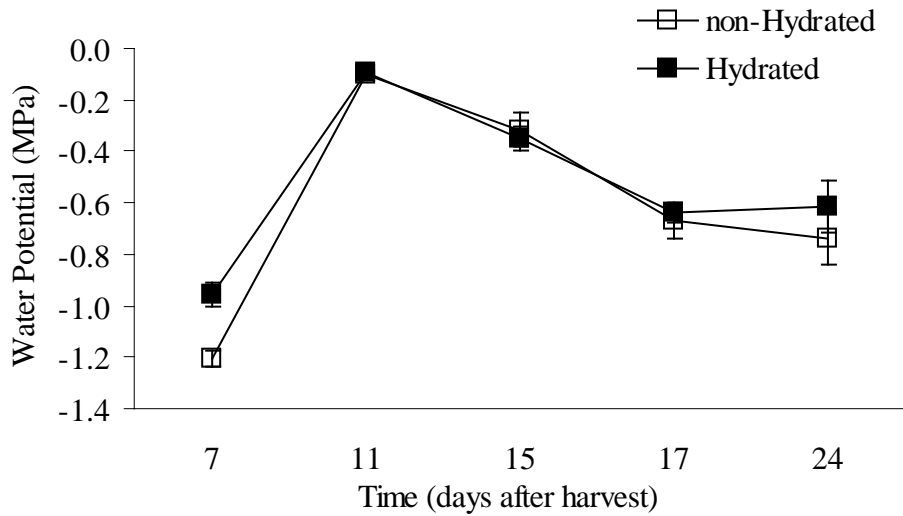


Figure A-4. Leaf water potential (MPa) of 'Charlotte' stems after dry shipment (day 7), re-hydration (day 11) at day 3 (day 15), day 5 (day 17) of vase life and at the end of vase life (day 24). Stems received either hydration or no hydration for 18 hours at 2 °C at the farm. Means  $\pm$  standard error of the mean of 10 stems. (Experiment 2).

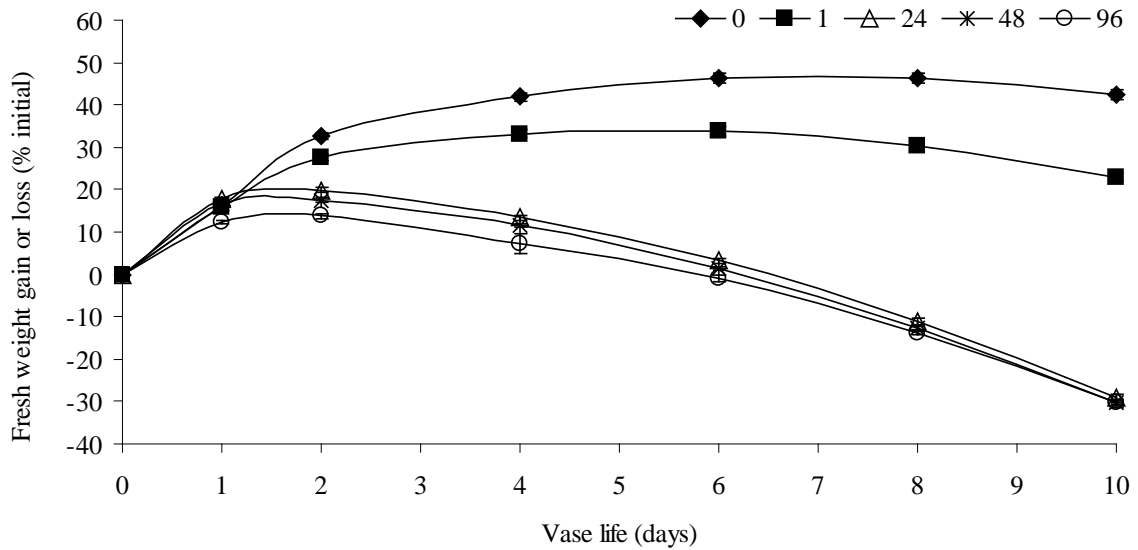


Figure A-5. Fresh weigh (% of initial weight) of 'Charlotte' roses during vase life. Stems were re-hydrated for 0, 1, 24, 48 and 96 hours after dry shipment and prior to vase life evaluation. Means  $\pm$  standard error of the mean of 7 stems. (Experiment 3).

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## BIOGRAPHICAL SKETCH

Ana Maria Borda was born in Bogotá, Colombia. She grew up in Colombia in her family's cut flower farm. She earned her Bachelor of Science from the University Los Andes, Bogotá, Colombia. She received her master's degree from The University of Queensland, Australia (2002). Upon graduating from her master's degree, she went back to Colombia and worked as a professor at Universidad Militar Nueva Granada. She joined the Environmental Horticulture Department on July 2005 at The University of Florida as a Doctor of Philosophy (PhD) student. Her specific field of research was studying the effect of ethylene biosynthesis and sensitivity on cut rose volatile compounds and its relation with vase life. She received her PhD from the University of Florida in the fall of 2009. Upon completion of her program she went back to Colombia to work in the cut flower industry.