Progress Report

Understanding the relationship between floral fragrance, ethylene production and vase life of cut rose flowers

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Summary

The three main objectives of this research project are:

I. Determine the main volatile organic compounds that characterize the fragrance profile of fragrant and non fragrant cut rose cultivars.
II. Describe the fragrance profile of cut roses during vase life and the effect of external and internal ethylene on the fragrance profile and vase life of cut roses.
III. Study the effects of anti-ethylene treatments on the fragrance profile and vase life of fragrant and non-fragrant cut rose flowers.

During the period from January through July, 2008 the effects of external ethylene and anti-ethylene treatments were evaluated on ('Allure', 'Lovely Dream', 'Erin', 'Osiana' and 'Red Sensation'). The ethylene synthesis inhibitor (AVG) and the ethylene sensitivity inhibitor (STS) were evaluated to determine whether ethylene synthesis or sensitivity exerts the most control over vase life of cut roses. During the course of these experiments, we evaluated the effects of external ethylene and anti-ethylene treatments on the fragrance volatile profile of cut roses.

MATERIALS AND METHODS

Flowers were harvested between 8 -10 am at the same day at a commercial stage from farms located in Ecuador. Flowers were taken into the postharvest room (14-18 ^{o}C) within 30 minutes from harvest, where they were placed into a fresh hydration solution (water, 60 ppm chlorine and citric acid to adjust pH to 4.5) for 2 hours. After hydration flowers were processed and placed into the cooler at 3^{o}C in the same solution for 12 hours. The next day, flowers were packaged into 2 commercial half boxes. In order to protect the flowers from an eventual ethylene exposure during shipment, 1-MCP sachets (EthylBloc ^{TM}, Floralife® , Inc. , Walterboro, SC) were included inside one shipping box. Two 1-MCP sachets were dipped in water prior placement inside one box. The box was closed immediately after the 1-MCP sachet placement. The other box contained no 1-MCP sachets. Each box contained 7 bunches each with 25 stems each (50 cm in length). A temperature recorder was placed inside one box. The boxes were send to the carrier agency and then via FedEx to Gainesville, Fl within 3-4 days.

Boxes generally arrived to Gainesville, Fl between 10am to 1 pm. The boxes were opened and flowers were processed immediately after arrival. Flowers were re-cut to 45 cm in length and placed into different hydration solutions (Table 1). Flowers that received the 1-MCP treatment during shipment were placed in a bucket with a solution of 0.2 mM Silver thiosulphate (STS). The STS solution was prepared following the protocol from Esmaeli *et al* 2005. Flowers with no 1-MCP were hydrated either in di water or 100 ppm aminoethoxyvinylglycine (AVG) (Valent U.S.A Corporation, Walnut Creek, CA). The total volume of hydration solution was 5 liters and the hydration was performed at 21 ^{o}C for 3 hours. After 3 hours, the flowers that were in STS solution were transferred to a solution of di water. Then flowers from all treatments were placed at 3 ^{o}C overnight.

Table 1. Hydration solutions and ethylene treatments performed.

Anti-ethylene treatment during shipment	**Hydration after shipment**	**Hydration during external ethylene exposure**	**External ethylene application**	**Hydration during vase life, ethylene, CO_2 and volatile determination**
Plus 1-MCP	0.2mM STS	Di water	1ppm	Di water
			air	
No 1-MCP	Di water	Di water	1ppm	Di water
			air	
	100 ppm AVG	100 ppm AVG	1ppm	100 ppm AVG
			air	

The next day, flowers were placed into two different vase solutions: di water (for di and sts pre-treated flowers) or 100 ppm AVG (for AVG pre-treated flowers) (Table 1). The solutions were kept the same throughout the vase life, ethylene, CO_2 and volatile collection evaluations. The vases were placed into sealed aquarium glass tanks and exposed to either 1ppm ethylene or air for 24 hours at 21^{o}C and 12 hour photoperiod. After the ethylene/air exposure flowers were taken out of the tanks and randomly distributed for vase life, ethylene, CO_2 and volatile production determination.

Vase life

Flowers selected for vase life evaluation were placed into vases with 1 l of either di or AVG solution (Table 1). Flowers were maintained at 21 ^{o}C, 50-60 % RH and 10 μmol m^{-2} s^{-1} of light (12 hrs/day). Flower vase life was determined as the time from placement in the vase (day0) to the appearance of visual senescence symptoms (i.e. petal wilting, abscission, bluing and disease). Flower opening was rated at day 1, 2 and then every two days until day 8 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^{o} to stem, 4 = outer petals reflexed at approximately 115 ^{o} to stem, 5 outer petals reflexed at 90 ^{o} to stem (modified from (Kuiper et al., 1996)).

Ethylene production and Respiration rate

Flowers selected for ethylene and CO_2 production were cut to 6 cm in length and placed into 22 ml glass vials with di water (sts and controls) or AVG (Table 1). For ethylene production determination flowers were placed into 900ml glass mason jars with 8ml of 1 M KOH to maintain CO_2 concentration below 0.1 %. Jars were closed for 21 hours at 21 ^{o}C. Then 1 ml gas sample was taken from the jar using a 1 ml syringe and analyzed with a FID gas chromatograph (Hewlett-Packard 5890 Series II). Injector, detector and oven temperatures were 110, 150 and 130 ^{o}C respectively. Different flowers were used to determine CO_2 production. In this case the jars were closed for 2 hours at 21 ^{o}C. Then, 1mL of gas was taken out of the jar and analyzed on a Gow-Mac gas chromatograph (Series 580, Bridge water, NJ) equipped with a thermal conductivity detector (TCD). The carrier gas (helium) flow rate was 0.5mLs−1. The oven was set at 40 ◦C and the detector and injector were operated at ambient conditions. Jars were then opened and identical measurements were taken the following day. Once the jars were opened the fresh

weight of each flower and the weigh of the vial were determined. Ethylene and CO_2 was measured during 7 days.

Volatile collection

Volatiles were collected after 0, 2, 4 and 6 days of the ethylene/air treatment. Flowers were re-cut to 6 cm in length and placed into 22 ml glass vials with di water (di and STS treatment) or AVG (Table 1). Then flowers were placed into 900 ml glass jars that were connected with a continuous air flow system. Carbon filtered air was pumped/pulled through the jars for one hour and trapped on a sorbent Super Q column to concentrate the volatiles. 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 21 ^{o}C between 3:30 and 4pm at each sampling date. At each sampling time, 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^{o} to stem, 4 = outer petals reflexed at approximately 115 ^{o} to stem, 5 outer petals reflexed at 90 ^{o} to stem (modified from (Kuiper et al., 1996)). The same flowers from each treatment were used during the 6 day collection.

Statistical analysis

Three replicate vases containing eight stems each were used per treatment for vase life evaluation. Flowers were arranged in vases for evaluation in a completely randomized block design. Data were analyzed by ANOVA using SAS® Version 8 (SAS Institute Inc., Cary, NC, USA). The significance of differences among treatment data means were determined using LDS multiple comparison test at p=0.05. Four stems were used per treatment for ethylene detection and three stems per treatment were used for respiration determination. Two flowers were used per treatment for volatile extractions.

RESULTS

Vase life

External ethylene (1ppm for 24 hours at 21 ^{o}C) had a negative effect on the vase life of 'Allure', 'Lovely dream', 'Erin', 'Osiana' and 'Red sensation' varieties compared to air exposure. 'Osiana' flowers hydrated in di water and treated with ethylene had the highest external ethylene sensitivity in terms of reduction in vase life. Vase life of 'Osiana' was reduced in 10 days with external ethylene application compared to air exposure. 'Allure' had a reduction of 3 days, 'Red Sensation' and 'Erin' 2 days and 'Lovely Dream' 1 day.
The negative effects of external ethylene on vase life were prevented when flowers were treated with STS. The inhibition of ethylene perception improved vase life on 'Allure', 'Lovely Dream', 'Erin', 'Osiana' and 'Red sensation' flowers compared to controls. However, these effects were not prevented by the inhibition of ethylene synthesis. AVG did not produce any affect on the vase life of 'Allure', 'Osiana' and 'Red Sensation' compare to controls. However, it improved vase life on 'Lovely Dream' and 'Erin' flowers compared to controls (Figure 1).

Ethylene production

Ethylene production depended on the variety and on the inhibition of ethylene perception. The highest ethylene production was observed in 'Lovely Dream' with a peak observed after 2 days. The production then declined to low levels towards the 7 day. 'Allure', 'Erin', Osiana' and 'Red Sensation' produced significantly lower amounts of ethylene compared to 'Lovely Dream' during the 7 day period. No ethylene production peaks were observed on these varieties. Ethylene production was lower than controls on 'Lovely Dream' flowers with no ethylene perception. On the other hand, ethylene production was higher than controls on flowers of 'Allure', 'Erin' and 'Osiana' with no ethylene perception. Ethylene production was very low in 'Red sensation' and it did not varied with inhibition of ethylene perception (Figure 2).

Respiration rate

Respiration rate depended on the variety. The inhibition of ethylene perception produced low respiration rates on some varieties. The inhibition of ethylene synthesis produce higher respiration rate in one cultivar but it did not had any effect on the other 4. The highest respiration rate was observed on 'Allure' followed by 'Lovely dream', 'Osiana'. 'Erin' and 'Red Sensation' flowers hydrated in di water and treated with air (Figure 3).
Inhibition of ethylene perception decreased the respiration rate in 'Lovely Dream', 'Erin' and 'Red Sensation' after external ethylene exposure. The same trend was observed in 'Red Sensation' after 4 and 6 days of ethylene exposure. Contrary, the inhibition of ethylene synthesis increased the respiration rate in 'Lovely Dream'. 'Lovely Dream' treated with AVG transpired higher than control after 5 days of external ethylene exposure. Additionally, the inhibition of ethylene synthesis had no effects on the respiration rate of 'Allure', 'Erin', 'Osiana' and 'Red Sensation'(Figure 4).

Volatile collection

Volatile production changed over time on all varieties. After external ethylene exposure, total volatile production decreased over time in ‘Allure’, ‘Osiana’ and ‘Red Sensation’ flowers hydrated in STS, AVG and di water. Contrary, volatile production increased over time on ‘Lovely Dream’ and ‘Erin’ on flowers treated with STS, AVG and di water (Figure 5).

Inhibition of ethylene synthesis and production slightly changed the volatile production of rose flowers over time. After external ethylene exposure, AVG treated flowers of ‘Allure’, ‘Lovely Dream’ and ‘Red Sensation’ produced less fragrance than control immediately after external ethylene exposure. This tend continued over time in ‘Lovely Dream’ and ‘Red Sensation’. ‘Erin’ flowers treated with AVG produced higher amount of total volatiles than the control immediately after ethylene exposure. Then at day 2 the volatile production was lower than the control and continued low over time. ‘Osiana’ volatile production was not different from control on AVG treated flowers.
After external ethylene exposure, the total volatile production on ‘Allure’ ‘Osiana’ and ‘Red Sensation’ flowers treated with STS was not different from the control over the six days of measurements. However, ‘Lovely Dream’ and ‘Erin’ flowers treated with STS and ethylene produced less fragrance than the control at day 0, then the volatile production increased and was higher than controls on days 2 and 4. Finally volatile production was again lower than the control at day 6 for ‘Lovely Dream’ and not different from control on ‘Erin’ (Figure 6).

Figure 1. Vase life of fragrant and non fragrant cut rose cultivars after ethylene exposure (1pmm) for 24 hours at 21°C. Prior to external ethylene exposure flowers were hydrated in di water, 2.0mM STS or 100 pmm AVG. Means and standard error of 24 flowers. Different letters = significant difference between means $\alpha = 0.05$.

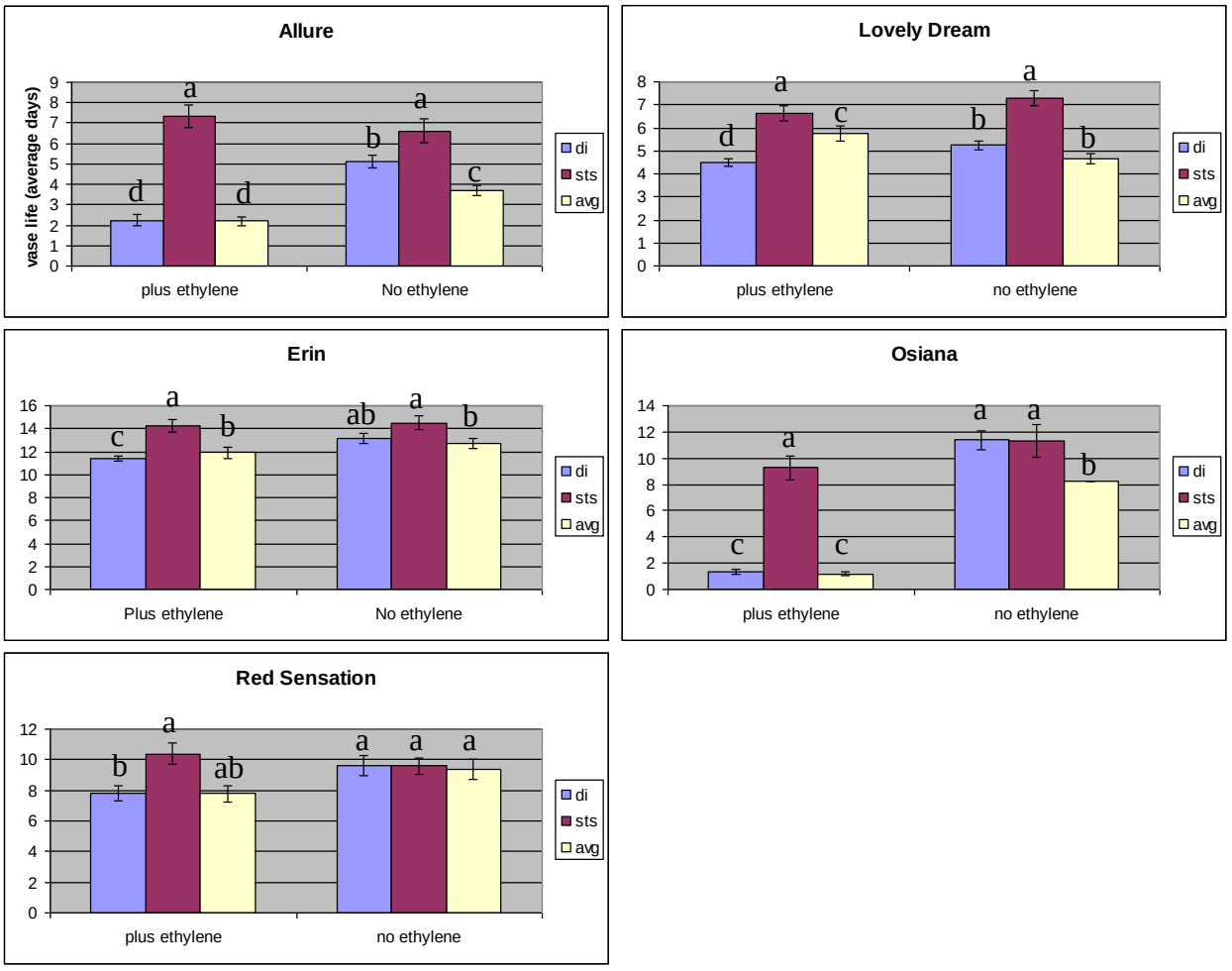


Figure 2. Ethylene production of cut rose cultivars. Flowers were hydrated in di water, 2.0mM STS or 100 pmm AVG. Means and standard error of 4 flowers.

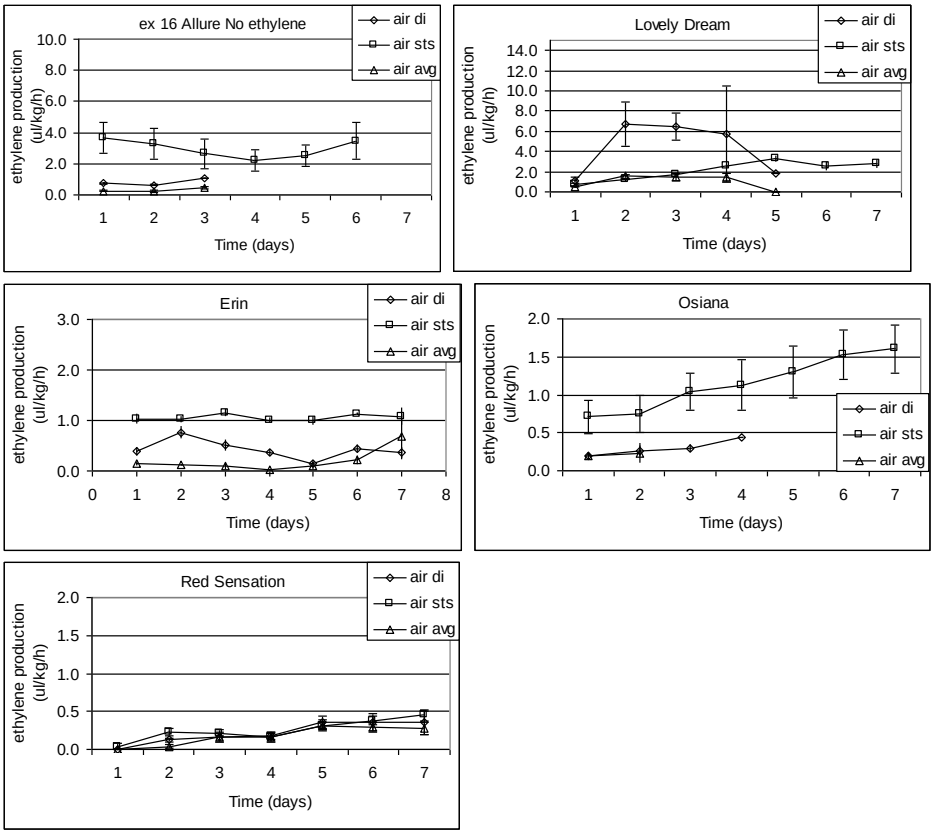


Figure 3. Respiration rate of cut rose cultivars over time. Flowers were hydrated in di water overnight, re-cut to 6 cm in length and placed inside glass mason jars in a vial with di water. Jars were closed for 2 hours at 21 ^{o}C.

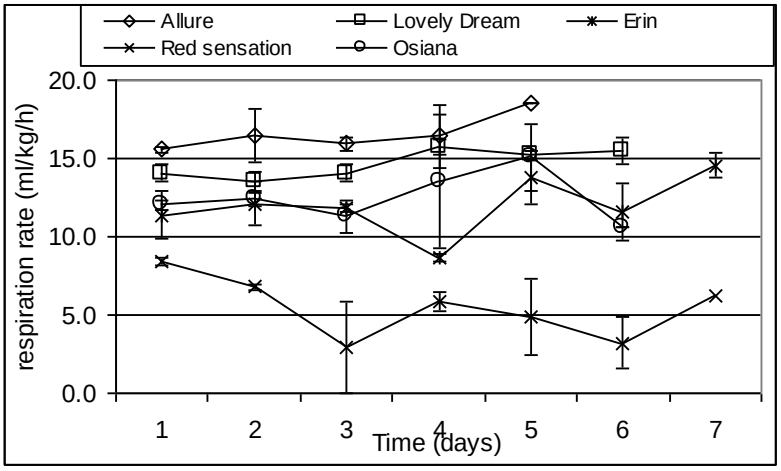


Figure 4 Respiration rate of cut rose cultivars after ethylene exposure (1pmm) for 24 hours at 21°C. Prior to external ethylene exposure flowers were hydrated in di water, 2.0mM STS or 100 pmm AVG. Means and standard error n=3.

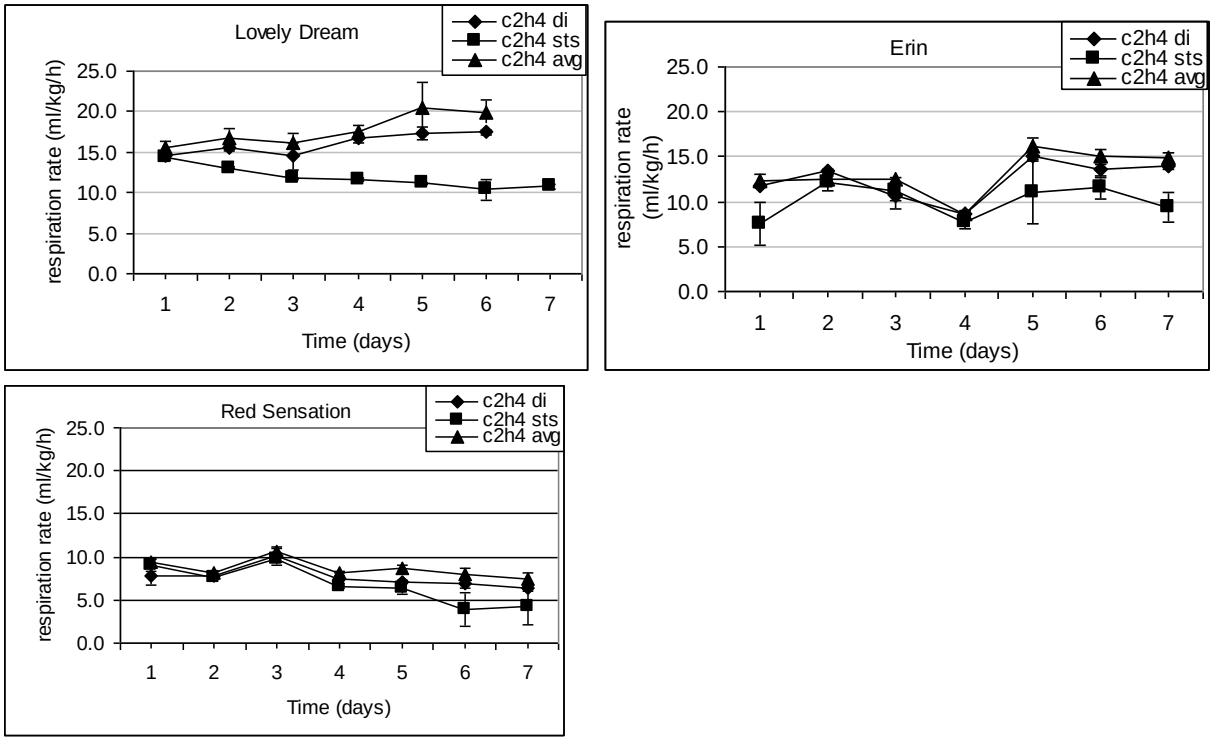
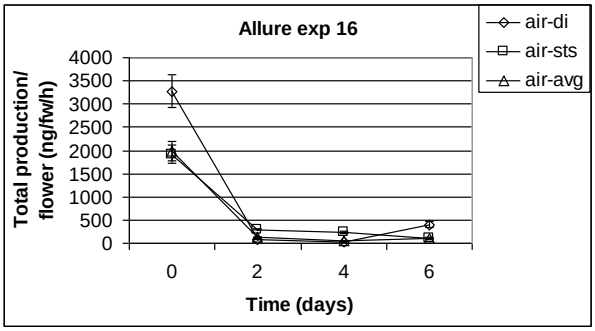
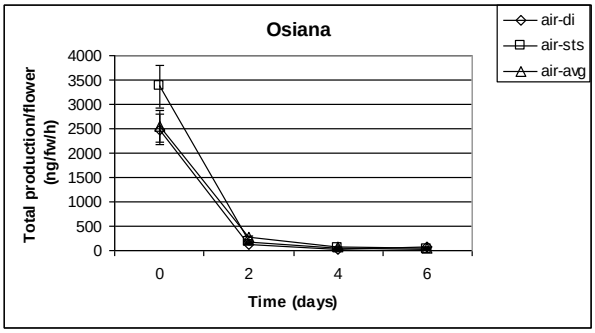
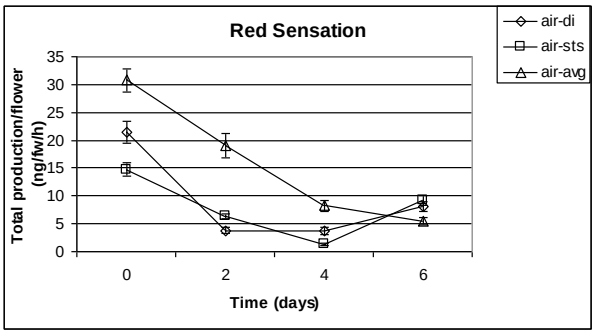


Figure 5. Total volatile production over time of cut rose cultivars over time. Flowers were placed in a solution of di water. Means and standard error of two flowers.

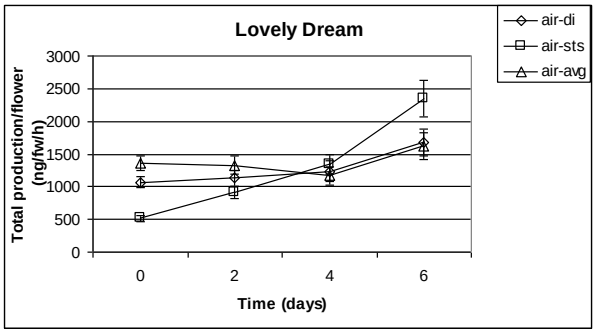
a. Decreased







b. Increased



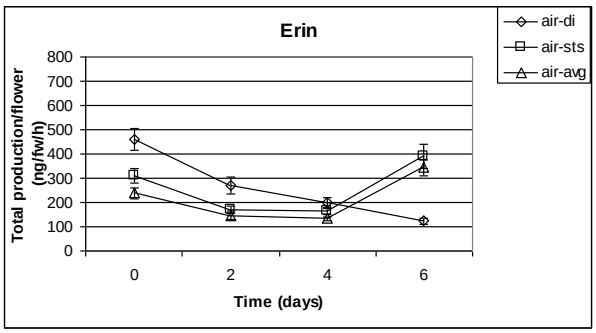
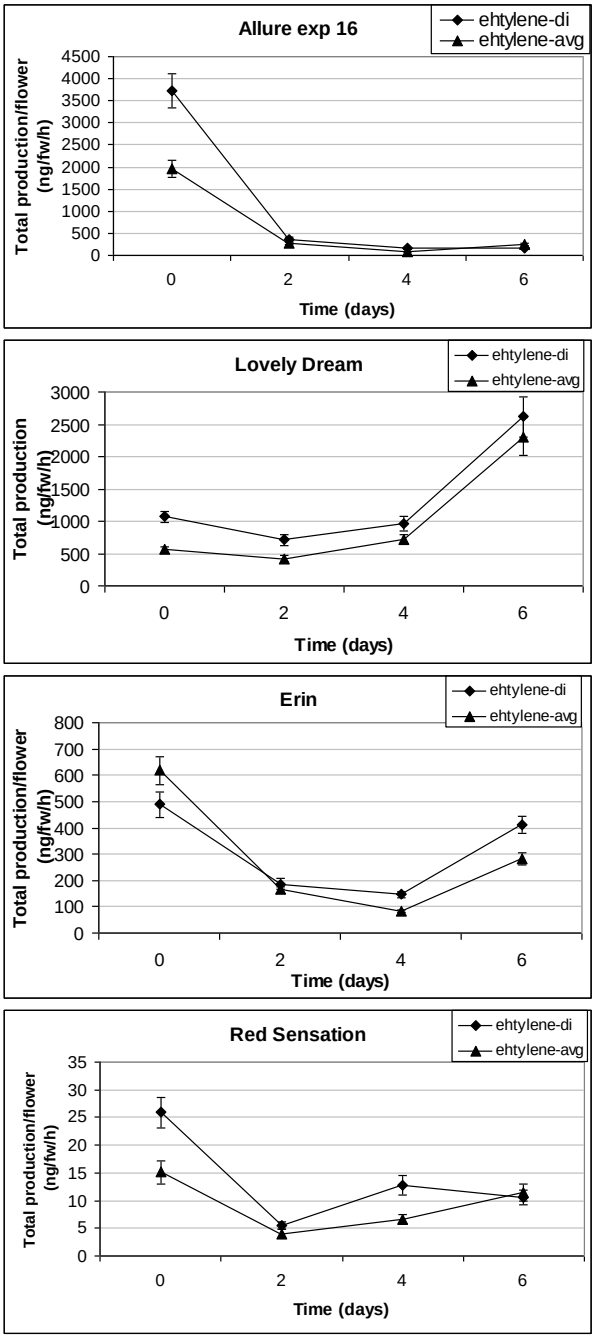


Figure 6. Effects of inhibitors of ethylene synthesis (AVG) and ethylene perception (STS) on total volatile production over time of cut rose cultivars after ethylene exposure (1pmm) for 24 hours. Prior to external ethylene exposure flowers were hydrated in di water, 2.0mM STS or 100 pmm AVG. Means and standard error n=2.

a. Effects of AVG on total volatile production



b. Effects of STS on total volatile production