EFFECT OF ANTI-ETHYLENE COMPOUNDS ON SELECTED FLOWERS

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VITA

Roger Don Anderson, son of Melvin and Donna (Blanchard) Anderson, was born February 19, 1947, in Delta, Utah. He worked on his father's farm in Oak City, attended Millard County Public Schools, and graduated from Delta High School in 1965. He attended one year at Snow College, Ephraim, Utah, served two years as a missionary for the Church of Jesus Christ of Latter-Day Saints in Canada, served active duty in the United States Army in Viet-Nam, and received the degree of Bachelor of Science (Horticulture) from Brigham Young University, Provo, Utah, in May, 1973. After several years in horticultural teaching and business he entered graduate school at Auburn University in September, 1979. He married Marzilla, daughter of Seth and Effie Ester (McCollough) Wright in March, 1973. They have three children: two daughters, Marleah and Ester, and one son, Hyrum Seth.

THESIS ABSTRACT

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Browning of camellia flowers due to vacuum infiltration or treatment with a ketone containing floral spray was reduced by the use of 1.0 mM aminoethoxyvinylglycine, AVG. Premature petal drop of individual geranium florets treated with 0.02 ml of 2000 pm 2-chloroethylphosphonic acid (ethephon) spray was reduced by 0.5-2.0 mM AVG, 0.1-1.0 mM aminooxyacetic acid, AOA, and 0.05 - full strength CT 2000, a silver thiosulfate product. Floret drop of snapdragons treated with 150 ppm ethephon in holding solution was prevented and vase life extended by a 12 h pulse of full strength CT 2000 prior to treatment.

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I. INTRODUCTION

The postharvest physiology of flowers has been under intensive investigation in order to lengthen the time consumers may enjoy floricultural crops. Rogers (35) in 1973 and Mayak and Halevy (18, 30) in 1980 reviewed the literature concerning factors which reduce the keeping quality of cut flowers. These factors included both physiological and microbial stem plugging, excess dessication, low respirable substrate, poor color stability, lack of control of flower opening and development, and ethylene injury.

Staby, et al. (42) estimated that 30 per cent of all floriculture crops die prematurely due to ethylene-induced disorders. Many flowers are adversely affected by endogenous as well as exogenous ethylene. Under some conditions plants may produce sufficient quantities of ethylene to induce senescence. As early as 1908, it was understood that the petal in-rolling, "sleepiness" in carnations, Dianthus caryophyllus L., was due to ethylene (11). Poinsettias, Euphorbia pulcherrima Wild, develop stress induced epinasty (3, 38, 39). Orchid, Cattleya sp. Lindl., sepals can fade, wilt, and develop 'dry sepal' (2, 12). Snapdragons, Anthirrhinum majus L., calceolarias, Calceolaria crenatiflora Cav. (16), delphiniums, Delphinium sp. L., and sweet peas, Lathyrus odoratus L. (40), abscise florets. Hybrid geraniums, Pelargonium x hortorum Bailey, drop petals (5, 27, 28) when exposed to ethylene. Mechanically damaged tissue biosynthesize more ethylene than

healthy tissue (23, 48). Chrysanthemum, Chrysanthemum x morifolium Ramat., flowers when infected with ray blight fungi produced more ethylene than noninfected flowers (15, 48). Some sources of exogenous ethylene commonly found in proximity to floriculture crops are: ripening fruit, natural gas leaks, improperly vented greenhouse heaters, exhaust fumes from internal combustion engines, and by-products of polyethylene manufacturing (35).

Several chemicals have been reported to reduce ethylene induced disorders: benzylisothiocyanate or BITC (32), hydroxyquinoline citrate or 8HQC (33), two analogs of rhizobitoxine (47), aminoethoxyvinylglycine or AVG (3, 38, 39), aminooxyacetic acid or AOA (4, 8, 19, 43, 46), silver nitrate or $AgNO_3$ (7, 10, 17), silver thiosulfate or STS, a complex of $AgNO_3$ and $Na_2S_2O_3$ (7, 13, 25, 34, 37, 41, 45), thiabendazole (6), ethanol (20), and cobalt nitrate (26).

In these studies the effect of selected anti-ethylene compounds (AVG, AOA, and STS) were investigated on senescence of selected flowers: Camellia japonica L. cvs. White Empress and Pink Perfection, Pelargonium x hortorum Bailey cv. Jackpot, and Antirrhinium majus L. cvs. Houston and Virginia.

Geraniums and snapdragons were chosen because of their particular responses to ethylene, snapdragons also because of inconsistant results obtained with current commercial preservatives, and camellias because of the lack of information on the cause of their premature petal browning.

II. REVIEW OF LITERATURE

Since the reviews of Rogers (35) and Mayak and Halevy (18, 30), the direction or research on postharvest physiology of flowers has been toward the inhibition of ethylene induced disorders. Senescence in carnations is preceded by a constant low level production of ethylene referred to as stage 1. At the onset of senescence a rapid increase in ethylene production takes place (stage 2). This is followed by a decrease in petal cytokinin activity and dry weight, and conversely, an increase in gynecia cytokinin activity and dry weight (13, 44). In stage 3 the cell walls begin to breakdown, the ethylene production decreases but the ethylene precursor remains high (9). Petals begin in-rolling, and the gynecia increase in size and begin to green (21). In roses, Rosa sp. L., senescence is marked by an increase in ethylene followed by a rapid increase in abcissic acid (ABA). Such a rise in ABA can be obtained by exogenous ethylene, but a rise in ethylene production cannot be obtained with ABA application (29).

The pathway of ethylene synthesis was described by Adams and Yang (1) in 1979 as follows: methionine→S-adenosylmethionine (SAM)→1-amino-cyclopropane-1-carboxylic acid (ACC)→ethylene. Inhibition by AOA has been shown to take place at the 1-amino-cyclopropane-1-carboxylic acid forming enzyme (1). Konze and Kwiatkowski (24) found manganese to be the co-factor for ACC synthesis.

Amrhein and Wenker (4) reported AVG, AOA, and similar aminooxy-compounds to inhibit ethylene synthesis. Wang and Baker (47) reported analogs of rhizobitoxine to appreciably lengthen vase life of carnations and snapdragons. The analogs are aminooxy-compounds similar to AOA and AVG (4). Miranda (28) recently reported AVG to be effective in reducing petal drop in hybrid geraniums. Shanks (39) found AVG effective in inhibition of stress induced epinasty in poinsettia. The action of AVG and AOA is theorized to be the blockage of the ACC producing enzyme which not only reduces ACC production but reduces the basic level of ethylene in the tissue. A difference in the pathways of the aminooxy-compound and silver ion action has been noted. AOA inhibition of ethylene is reduced with time, and AOA must be provided on a constant basis to insure continued inhibition of ethylene synthesis (8). Such a response indicates metabolism of the chemicals to an inactive state in the plant. Such an inactivation does not take place when silver compounds are used.

In 1977 Halevy and Kofranek (17, 19) used silver nitrate to reduce the effect of ethylene on carnations. Similar usage of $AgNO_3$ has been reported effective on roses (10), and geraniums (28). However, silver nitrate is not freely absorbed into plant tissue through the vascular system, and causes unsightly spotting when applied as a spray. In 1978 Veen and Van de Geijn (45) published results showing that an anionic silver complex, STS, could be freely absorbed through cut ends of carnation stems. Le Masson and Norwak (25) found the complex $AgNO_3$ + ethylene-diamine-tetra-acetic acid, di-sodium salt (EDTA) also reduced ethylene production.

In the presence of STS, silver accumulated in gynecia, ethylene synthesis decreased, and action of exogenous ethylene was inhibited (9). Beyer (7) proposed that the mode of action of Ag ions as that of inhibiting ethylene action rather than that of production because the low concentration of ethylene during stage 1 is not altered by STS treatment. STS was also found to inhibit ethylene action in carnations (8, 9, 26, 45), hybrid geraniums (28), and snapdragons (31).

III. MATERIALS AND METHODS

Experiment 1. Chemical reduction of Clear Life induced browning of 'Pink Perfection' camellia.

C. japonica cv. Pink Perfection flowers were harvested from field grown plants at Auburn University, February 20, 1981. Single flowers, selected for uniformity and lack of blemishes, were placed in 5 ml plastic vials containing one of the following treatments: 1) deionized water check, 2) chlormequat (2-chloroethylmethylammonium chloride) 0.01%, 3) STS 5 mM, 8 mM silver nitrate and 32 mM sodium thiosulfate dissolved separately, then combined and diluted, and 4) AVG 1 mM. Each solution was prepared using deionized water. Twenty-four flowers were used in each treatment. One half of the flowers in each treatment were treated with Clear Life, a commercial floral spray manufactured by Colorado Dye and Chemical, Inc., Boulder, CO, for 3 seconds with the spray can held approximately 4.6 cm from the flower surface. This spray induced browning of camellia flowers similar to that caused by mechanical injury. Clear Life contains: a wax, ketones as a solvent, and a carrier. Treatments were randomly placed in a growth chamber at a constant temperature of 22 $C^{o} \pm 1^{o}$ and an irradiance of 9.15 klx. Injury data was recorded after 8 h in the chamber. Flowers were recorded as either brown or non-browned, a flower being considered browned if any visible discoloration was present.

Experiment 2. Reduction of browning of 'White Empress' camellia by vacuum infiltration.

C. japonica L. cv. White Empress flowers were harvested and selected as in Experiment 1. A solution of 5 per cent Rogard (a trademarked commercial floral preservative manufactured by Gard Products, Arlington Heights, IL) was used as the control and the same solution plus 1 mM AVG was used as the treatment. Solutions were placed in 6.5 cm x 17 cm x 3.8 cm deep plastic boxes fitted with a lid. Flowers (one flower per box) were submerged upside down in a solution and then placed in a vacuum chamber using 1 control and 1 AVG treated flower per run. Flowers were exposed to 381 mm Hg vacuum for 15 sec. After infiltration flowers were removed from the solution and placed in dry containers to evaporate off excess solution. Flowers were evaluated one hour after infiltration as in Experiment 1.

Experiment 3. Chemical reduction of premature petal abscission in 'Jackpot' geraniums.

Seedling P. x hortorum Bailey cv. Jackpot flowers were harvested from outdoor grown plants at Auburn University on July 14, 1981. Individual, newly opened, florets were selected for uniformity and placed in 2 ml vials filled with one of the following treatments: AVG at 0.5 mM, 1.0 mM, or 2.0 mM; AOA at 0.1 mM, 0.5 mM, 1.0 mM or 1.5 mM; and CT 2000 (a commercial STS compound sold as a pretreatment for carnations, manufactured by Smithers Oasis Co., Kent, OH) at full strength, .05%, 0.1%, or 0.5% solutions. Deionized water was used in

each treatment formulation and also served as the control. Florets were kept in these treatment solutions in a room maintained at 24° C ± 1° for 12 h under 0.43 to 1.25 klx irradiance. Then, using a 3.65 to 4.8 liter orifice mist blower, 5 ml of 2000 ppm 2-chloroethylphosphonic acid (ethephon) (14, 49) was misted over the top of all florets at a rate of 5 ml per 1.5 m^2. The number of florets abscised was determined before and after ethephon application. A floret was considered abscised when one or more petals had fallen.

Experiment 4. Extension of vase life and reduction of floret abscission of 'Virginia' snapdragons.

A. majus L. cv. Virginia flowers grown in the university greenhouse were harvested May 8, 1981 and graded Society of American Florist (S.A.F.) fancy or better (36). Stems were cut to uniform 60 cm length, placed in 250 ml Erlenmyer flasks (3 stems per experimental unit) containing 200 ml of one of the following treatments: STS at 0.5 mM, 1.0 mM, or 2.0 mM; AVG at 0.5 mM, 1.0 mM, or 2.0 mM; and deionized water control. Flowers were pulsed in treatment solution for 12 h in a room at 23° C ± 1° and 12 h of 0.43 to 1.25 klx irradiance. Treatment solutions were replaced with deionized water which was changed daily for the remainder of the experiment. Evaluation was made every day and flowers were considered unacceptable when foliage wilted, or the first florets browned, abscised or wilted.

Experiment 5. Extension of vase life and reduction of floret abscission of 'Houston' snapdragon.

A. majus L. cv. Houston flowers were harvested July 23, 1981, graded, S.A.F. fancy or better (36) and placed in tap water by the commercial grower for 1 h before being transported and placed in treatment solutions. Stems were cut to uniform 60 cm length, placed in 250 ml Erlenmyer flasks (4 stems per experimental unit) containing 200 ml of one of the following treatments; AOA at 0.1 mM, 1.0 mM, or 2.0 mM; CT 2000 full strength; Silflor 50 2% (a commercial STS formulation used as a pretreatment for carnations, manufactured by Florlife Inc., Chicago, IL), and deionized water control. Flowers were pulsed in the treatment solutions for 12 h. The pulse solution was replaced with senescence induction solution consisting of 150 ppm ethephon, for 24 h. This solution was then replaced with deionized water, which was changed daily for the remainder of the experiment. Evaluation was made every day, and flowers were considered unacceptable for vase life when foliage wilted, or the first florets browned, abscised, or wilted. Per cent flowers non-abscised was recorded when less than 5 florets on a stem remained turgid.

Count data were analyzed by contingency table analysis. Percentage data were transformed (arcsin of square root) and analyzed by analysis of variance and Duncan's multiple range tests.

IV. RESULTS AND DISCUSSION

Experiment 1. Chemical reduction of Clear Life induced browning of 'Pink Perfection' camellia.

Browning of 'Pink Perfection' camellia was induced by application of Clear Life spray (Table 1). The proportion of flowers browned by Clear Life was reduced by application of 0.1 mM AVG (Table 2). No difference was observed between 0.5 mM STS, 0.01% chlormequat, or check treatments. The response of camellia flowers to the Clear Life spray could be caused by the ketone content of the spray damaging cell membranes. If the damage to the tissue causes a rise in ACC and in turn ethylene then both STS and AVG should give similar results as both inhibit the "autocatalytic" rise in ethylene (8). The lack of effect by STS could indicate other systems are involved in the AVG inhibition of browning. Aminooxy-compounds such as AOA and AVG have been reported to inhibit other enzyme systems such as phenylalanine ammonia-lyase (PAL) and phenylproponoid synthesis (19). AOA inhibits pyridoxal-phosphate-containing enzymes such as the transaminases (22).

Table 1.

Effects of Clear Life on 'Pink Perfection' Camellia.
Data Recorded 8 h after Treatment.
Experiment 1.

Treatment[z]	Number of flowers	
	Brown	Non-brown
Clear Life	38[y]	10
No Clear Life	14	34

[z] Clear Life, a commercial floral spray (manufactured by Colorado Dye and Chemical, Inc., Boulder, CO) containing a wax, ketone solvent and carrier.

[y] Chi-square 11.60 significant at 0.1%.

Table 2.

Chemical Reduction of Clear Life Induced Browning
on 'Pink Perfection' Camellia.
Data recorded after 8 h.
Experiment 1.

Treatment[z]	Number of flowers	
	Brown	Non-brown
Check	12[y]	0
Chlormequat 0.01%	12	0
STS 0.5 mM	9	3
AVG 0.1 mM	5	7

[z] Chlormequat = 2-chloroethylmethylammonium chloride; STS = Silver thiosulfate; AVG = Aminoethoxyvinylglycine.

[y] Chi-square 16.67 significant at 0.5%. AVG 1.0 mM responsible for most of the deviation.

Experiment 2. Reduction of petal browning of 'White Empress' camellia by vacuum infiltration of AVG.

All of the nonAVG-treated flowers exhibited browning within 15 min of removal from the vacuum chamber (Figure 1). None of the AVG treated flowers exhibited any browning during the duration of the 8 h testing period. All flowers exhibited the same translucent appearance after infiltration, and the infiltration solution surrounding all flowers became discolored indicating the removal of some tissue fluids. The extraction of tissue fluids and rapid entrance of solution into the tissue could cause mechanical damage and thus induce ethylene production; however, the rapid browning of nonAVG-treated flowers would more likely indicate polyphenyol oxidase (PPO) activity. In tomatoes, the time required to show rapid rise in ACC production after mechanical injury was 90-120 min (22). Further investigation of AVG and AOA on PPO should be undertaken to determine their effect on camellia browning and the enzyme systems responsible for browning.

Experiment 3. Chemical reduction of premature petal abscission of 'Jackpot' geranium.

Geraniums differ greatly in response to ethylene with 'Jackpot' being moderately susceptible to petal abscission (5). Flowers treated with STS, AOA, and AVG abscised less than the check flowers after ethephon treatment (Table 3). Differences between treatment concentrations of AOA and AVG were not significant in this experiment. The STS product CT 2000 at the 0.1% level gave good response, and increasing

Table 3.

Effect of Anti-Ethylene Compounds on Petal Abscission on 'Jackpot' Geranium with Ethephon Spray Treatment Experiment 3.

Treatment[z]		Flowers per treatment: Number abscised	Number non-abscised	Test x^2
Deionized water		16	0	
AVG		25	23	
AOA		30	33	
CT 2000		31	32	15.29*
CT 2000	0.05%	12	4	
	0.1%	4	12	
	0.5%	6	9	
	Full strength	9	7	8.84*
AVG	0.5 mM	10	6	
	1.0 mM	7	9	
	2.0 mM	8	8	1.17 N.S.
AOA	0.1 mM	11	5	
	0.5 mM	7	8	
	1.0 mM	6	10	
	1.5 mM	6	10	4.18 N.S.

[z] CT 2000 = a silver thiosulfate compound manufactured by Smithers Oasis, Kent, OH; AVG = aminoethoxyvinylglycine; AOA = aminooxyacetic acid.

* Significant at 5% level.

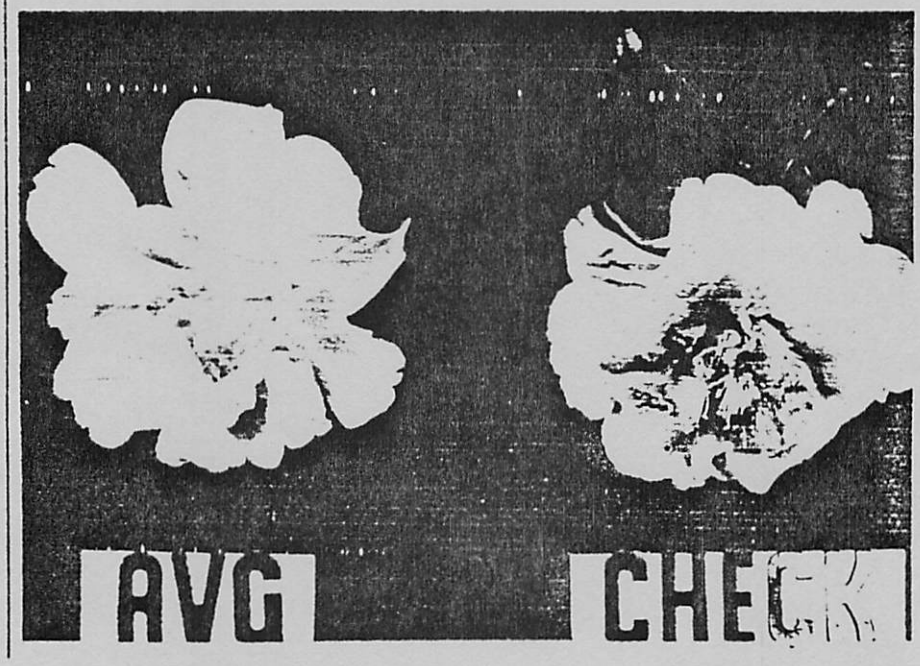

Figure 1. 'White Empress' camellias vacuum infiltrated with 0.5% Rogard floral preservative (check), plus 1.0 mM aminoethoxyvinylglycine (AVG).

the concentration did not improve response. Staby and Reid (41) reported that an effective range exists below which STS activity increases with concentration and above which life decreases with concentration due to phytotoxicity. Results of the present research agree with recent work by Miranda and Carlson (23) which reported STS, and AVG as effective chemicals in reducing abscission in geraniums. The current research also indicates AOA to be effective in reducing petal abscission.

Experiment 4. Extension of vase life and reduction of floret abscission of 'Virginia' snapdragons.

No differences were noted between treatments. All flowers dried on the stem indicating a lack of ethylene induced senescence (Appendix Table 5).

Experiment 5. Extension of vase life and reduction of floret abscission of 'Houston' snapdragons.

The vase life of 'Houston' snapdragon was extended by the use of the STS product CT 2000 at full strength as a 12 h pretreatment (Table 4). Abscission of 'Houston' snapdragons was reduced by AOA concentrations above 1.0 mM and CT 2000. However, CT 2000 gave the greatest improvement in vase life with no floret abscission. Florets died and dried on the stem in the CT 2000 treatments. Furthermore, no stem browning took place in CT 2000 treated flowers as was present in all other treatments.

Wang, et al. (47) have documented floret abscission as being closely related to ethylene production, and found that abscission was inhibited by rhizobitoxine analogs.

Veen and Van de Geijn (45) reported STS as inhibiting ethylene induced senescence in carnations. Nowak (31) reported the same chemical as effective on senescence of snapdragons. The current research confirms the work of Nowak as to the effectiveness of STS inhibition of floret abscission and extension of vase life of snapdragons. However, in research conducted earlier (Appendix Table 5.) no floret abscission or vase life extension occurred without an ethylene source.

Differences in AOA results between the present research and those of Nowak (31) could be due to the differences in cultivar of snapdragon and concentration of ethylene. AVG and AOA reduce ACC production in the plant, thus reducing the climatric rise in ethylene. In contrast, STS blocks the action of ethylene (9). The present research results may be explained by Beyer's (7) conclusion that silver is similar to copper, a possible co-factor in ethylene incorporation. Butler (9) found no change occurred in the basal rate of ACC but the action of indogenous and exogenous ethylene was inhibited.

Table 4.

Effect of Anti-Ethylene Compounds on Vase Life and Floret Abscission of 'Houston' Snapdragons Treated with 150 ppm Ethephon. Experiment 5.

Treatment		Vase life (days)	Mean abscised (%)
Deionized water[z]		3.2 a[y]	48.4 a
AOA	0.1 mM	4.0 a	39.6 a
	1.0 mM	3.9 a	10.0 bc
	2.0 mM	4.0 a	8.2 c
CT 2000 full strength		7.1 b	0.0 c
Silflor 50. 2.0%		4.0 a	25.8 ab

[z] AOA = aminooxyacetic acid; CT 2000 = a silver thiosulfate compound, manufactured by Smithers Oasis, Kent, OH; Silflor 50 = a silver thiosulfate compound manufactured by Florlife Inc., Chicago, IL.

[y] Means in columns followed by different letters significant at 5% level, Duncan's new multiple range test using arcsin transforms.

V. CONCLUSION

Clear Life-and vacuum infiltration-induced browning of camellias was reduced by the use of 1 mM AVG. Such reduction of damage could be of value to the floral industry in reducing losses of camellia flowers due to brusing during handling and shipping. Geranium petal abscission was reduced by the use of known anti-ethylene chemicals: AVG, AOA, and STS. Such abscission reduction would permit long distance shipping of geraniums without loss of flower petals and prolong life in the marketplace and consumer environments. Snapdragon floret abscission was also reduced by the use of the above mentioned anti-ethylene compounds. CT 2000 at full strength was not only effective in reducing floret abscission, it was also effective in extending vase life of snapdragons exposed to an external source of ethylene in the form of a 150 ppm ethephon spray. The value of preventing damage due to external ethylene would facilitate storage with ethylene generating plant material, reduce ethylene injury in transportation due to internal combustion engines and other sources, and lastly, increase sales due to a longer lasting product.

This investigation shows effectiveness of 3 anti-ethylene chemicals, AVG, AOA, and STS, on flower life. However, further research is warranted on cultivars, concentrations, methods of application, mode of action, and environmental effects.

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Appendix Table 5.

Abscission of 'Virginia' Snapdragons Treated with Anti-Ethylene Compounds. Experiment 4.

Treatment[z]		Floret drop	Vase life (days)
Deionized water		0	8
STS	0.5 mM	0	8
	1.0 mM	0	8
	2.0 mM	0	8
AVG	0.5 mM	0	8
	1.0 mM	0	8
	2.0 mM	0	8

[z] STS = Silver thiosulfate; AVG = Aminoethoxyvinylglycine.