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EFFECT OF ETHYLENE INHIBITORS ON LONGEVITY OF CUT CARNATIONS (*Dianthus caryophyllus* L.) AND ETHYLENE PRODUCTION BY FLOWERS

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A B S T R A C T

The effect of 24-h pulse treatment with STS, AOA, AIB, and ATA on the longevity of cut carnations (*Dianthus caryophyllus* L.) cultivars 'Dolce Vita', 'Impala', 'Domingo', 'Tanga', and 'Charlotte' was investigated. Among all the inhibitors tested, STS was the most effective in prolonging the vase life of carnations. A marked increase in flower longevity of all tested cultivars was also found when AOA or AIB were applied. ATA had a positive effect on vase life of four cultivars, but did not extend the longevity of 'Domingo' flowers. STS and AOA were most active in inhibiting ethylene production in 'Dolce Vita' carnations. Application of AIB or ATA delayed by 2 days the onset of climacteric ethylene production in flowers and decreased its level.

Key words: carnations, cut flowers, ethylene inhibitors, ethylene production, vase life

INTRODUCTION

The gas ethylene, a naturally occurring plant hormone, enhances senescence and shortens the vase life of many flowers (Reid and Wu, 1992). Carnations are very sensitive to ethylene, which in a concentration of 1 μl dm^{-3} for 24 hours causes irreversible wilting of open flowers (Camprubi and Nichols, 1978). Ethylene is responsible for inducing many of the biochemical processes leading to programmed cell death, including an activation of the senescence-related gene transcription (Woodson and Lawton, 1988; Lawton et al., 1990).

During the course of senescence, carnations exhibit a climacteric increase in ethylene evolution. In flower buds and young flowers its production is very low. During flower senescence

a sharp increase in generation of C_2H_4 is observed. Afterwards, ethylene production decreases and again remains stable at a low level (Borochov and Woodson, 1989). It is autocatalytically produced during carnation petal senescence. It means that the exposure to ethylene stimulates its biosynthesis (Woodson and Lawton, 1988; Savin et al., 1995). Autocatalytic ethylene production develops gradually with an advancement in the senescence of tissues. During the climacteric, there is a coordinate increase in the activities of ACC synthase and ACC oxidase (Woodson et al., 1992; Ten Have and Woltering, 1997), which convert S-adenosylmethionine (SAM) to 1-aminocyclopropanecarboxylic acid (ACC) and ACC to ethylene, respectively (Yang and Hoffman, 1984). Expression of the ACC synthase and ACC oxidase genes in carnation petals depends on the presence of ethylene (Savin et al., 1995).

The effects of ethylene can be reduced by pretreating flowers with inhibitors of ethylene biosynthesis or action. It has been found that pulse treatment with silver thiosulphate (STS) prolongs vase life of cut carnation flowers (Reid et al., 1980; Altman and Solomos, 1995). Silver ions in the form of silver thiosulphate delay carnation flower senescence, and also appear to block the ethylene receptor site and inhibit autocatalytic ethylene production (Veen, 1979). Although STS has become an essential tool for the delay of senescence of climacteric flowers, it contains a heavy metal, which is a potent environmental pollutant. Thus, other substances with effects similar to STS may be desirable. There are several inhibitors of ethylene biosynthesis, including aminooxyacetic acid (AOA) (Fujino et al., 1980), α-aminoisobutyric acid (AIB) (Satoh and Esashi, 1980), 3-amino-1,2,4-triazole (ATA) (Altman and Solomos, 1993).

The purpose of the present work was to examine the effect of different ethylene inhibitors such as STS, AOA, AIB, and ATA, used as a pulse treatment, on the longevity of 5 carnation cultivars. In 'Dolce Vita' treated with those compounds ethylene production during flower vase life was also determined.

MATERIAL AND METHODS

Experiments were conducted with carnation flowers (*Dianthus caryophyllus* L.) cultivars 'Dolce Vita', 'Impala', 'Domingo', 'Tanga' and 'Charlotte'. They were obtained from a local grower in the autumn and winter (from September to March). Flowers were harvested at the paint brush stage and transported dry to the laboratory where the stems were cut at 40 cm length and subjected to pulse treatment for 24 hours.

Treatments of cut flowers

Ends of freshly cut stems were held in glass vases containing the test solutions: silver thiosulphate (STS) at a concentration of 0.2 mM, aminooxyacetic acid (AOA) at 2 and 4 mM, α-aminoisobutyric acid (AIB) – 2 and 4 mM, 3-amino-1,2,4-triazole

(ATA) – 50 and 100 mM. Distilled water was used for the controls and to prepare the test solutions. After 24 hours of treatment the stems were recut and flowers were transferred to distilled water. During treatments and vase-life observations the carnations were kept in a climate-controlled room at 20°C, 60% relative humidity with a 16 hours photon flux density of 10 μmol $m^{-2}s^{-1}$ provided by fluorescent lamps.

The flower longevity was recorded daily and the average vase life was calculated. Flower vase life was terminated when petal inrolling or wilting occurred. Three replicates with at least five flowers were used for each treatment. The results were tested by an analysis of variance and the Duncan's t-test at P=0.05.

Determination of ethylene production

The experiment was carried out with 'Dolce Vita' carnations. After a chemical treatment, the stems were recut to a length of 5 cm. Individually weighed flowers were placed in vials containing distilled water and were enclosed in 1 l glass jars for 2 h at 20°C. After this time, 1 ml gas samples were withdrawn from the head space for ethylene determination by gas chromatography (Hewlett Packard 5890 equipped with a flame ionization detector). After each determination, the jars were aerated. Results are expressed as nanoliters of ethylene produced per 1 gram of fresh weight per hour (nl $g^{-1}h^{-1}$) and are the mean ±SD of six flowers.

RESULTS AND DISCUSSION

Immediately after harvest, cut 'Dolce Vita' carnations produced just small amounts of ethylene. In untreated flowers placed in distilled water, the peak of ethylene generation was observed a few days after harvest, just before petals began to wilt (Fig. 1). This autocatalytic production is the result of a feedback mechanism in which the produced ethylene, through the interaction with a binding site, stimulates the activity of the enzymes involved in its biosynthesis. The produced ethylene initiates the biochemical changes leading to petal senescence (reviewed by Van Altvorst and Bovy, 1995). Silver ions block the ethylene receptor site and inhibit autocatalytic ethylene production in flowers (Veen, 1979). Silver chelated to an anionic silver thiosulphate complex, is very mobile in plant tissues and moves very quickly (about 2 m h^{-1}) to the floral parts (Veen and Van de Geijn, 1978). Thus it is beneficial for the ethylene sensitive flowers such as carnations. It has been shown by several authors (Veen, 1979; Reid et al., 1980; Altman and Solomos, 1995) that a short time treatment of cut carnations with STS improved flower longevity. It was also effective in our experiment. Pulsing with STS at a concentration of 0.2 mM markedly (from 73 to 135% over the control) prolonged the vase life of all tested cultivars (Tab. 1). The best positive effect on improving flower longevity, 135% over the control, was obtained

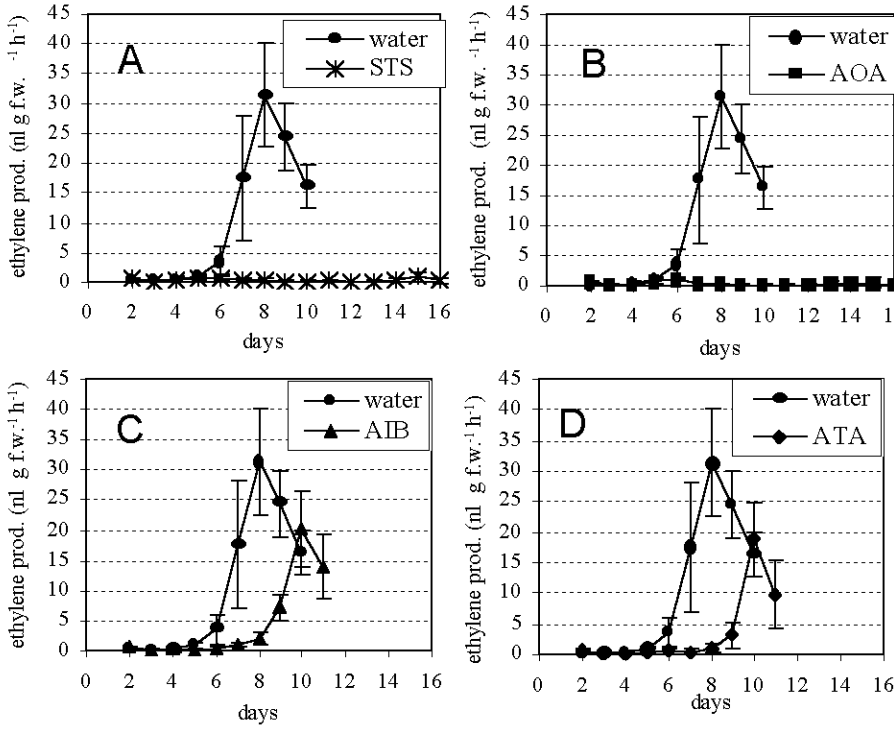

Figure 1. Effect of pulse treatments with 0.2 mM STS (A), 4 mM AOA (B), 4 mM AIB (C), and 50 mM ATA (D) on ethylene production in cut 'Dolce Vita' flowers

Table 1. Effect of pulse treatment with silver thiosulphate (STS) on longevity of cut carnations

Cultivar	Treatment	Vase life [days]	Vase life [%]*
'Dolce Vita'	water (control) STS 0.2 mM	7.0 a 14.9 b	100 213
'Impala'	water (control) STS 0.2 mM	5.5 a 9.5 b	100 173
'Domingo'	water (control) STS 0.2 mM	8.5 a 20.0 b	100 235
'Tanga'	water (control) STS 0.2 mM	7.7 a 13.9 b	100 180
'Charlotte'	water (control) STS 0.2 mM	11.4 a 20.3 b	100 178

*Values are percentages of the respective controls

when STS was used for 'Domingo' flowers. In 'Dolce Vita' carnations treated for 24 hours with STS solution vase life was increased by 113% over water control. During the senescence of STS-treated 'Dolce Vita' flowers, there was no ethylene climacteric and complete inhibition in ethylene evolution was observed (Fig. 1A).

Table 2. Effect of pulse treatment with aminooxyacetic acid (AOA) on longevity of cut carnations

Cultivar	Treatment	Vase life [days]	Vase life [%]*
'Dolce Vita'	water (control)	6.6 a	100
	AOA 2 mM	11.1 b	168
	AOA 4 mM	13.1 c	198
'Impala'	water (control)	5.5 a	100
	AOA 2 mM	7.5 b	136
	AOA 4 mM	9.3 c	169
'Domingo'	water (control)	11.1 a	100
	AOA 2 mM	17.7 b	159
	AOA 4 mM	17.6 b	158
'Tanga'	water (control)	7.1 a	100
	AOA 2 mM	9.7 b	137
	AOA 4 mM	10.7 b	151
'Charlotte'	water (control)	11.4 a	100
	AOA 2 mM	14.0 b	123
	AOA 4 mM	13.5 b	118

*Explanations see Table 1

AOA, an inhibitor of ACC synthase, has been shown to prolong the longevity of cut carnations by inhibiting ethylene synthesis. Yu et al. (1979) reported that AOA inhibits the activity of ACC synthase by complexing with the essential co-factor, pyridoxal phosphate. Fujino et al. (1980) found that when carnation flowers were continuously kept in a solution containing AOA, the production of ethylene was suppressed and wilting was delayed. The influence of pulse treatment with AOA at concentrations of 2 and 4 mM on the longevity of cut carnations is shown in Table 2. The application of AOA significantly prolonged the vase life of all tested cultivars when compared to the control. The concentration of 4 mM had a better effect on improving the vase life of 'Dolce Vita' and 'Impala' cultivars than pulsing with 2 mM solution. Similarly as in the experiment of Bichara and Van Staden (1993), our results showed that the pulse treatment with AOA increased flower longevity but not to the same extent as STS when 'Domingo', 'Tanga' and 'Charlotte' carnations were treated. However, in 'Dolce Vita' flowers pulsed with 4 mM AOA, ethylene production was completely inhibited as in carnations treated with STS (Fig. 1B). Also, Fujino et al. (1980) and Bartoli et al. (1996) found that AOA inhibited ethylene production in carnation flowers.

In our experiments, a structural analog of ACC, α-aminoisobutyric acid was also tested. Pulsing cut carnations with AIB prevented the senescence of flowers (Tab. 3). Flowers of all tested cultivars placed in 4 mM AIB for 24 hours showed a significant increase in their longevity in comparison to the control with distilled water, although the treatment

Table 3. Effect of pulse treatment with α-aminoisobutyric acid (AIB) on longevity of cut carnations

Cultivar	Treatment	Vase life [days]	Vase life [%]*
'Dolce Vita'	water (control)	6.6 a	100
	AIB 2 mM	8.5 b	129
	AIB 4 mM	9.1 b	138
'Impala'	water (control)	5.5 a	100
	AIB 2 mM	5.9 ab	107
	AIB 4 mM	6.7 b	122
'Domingo'	water (control)	11.1 a	100
	AIB 2 mM	11.1 a	100
	AIB 4 mM	13.1 b	118
'Tanga'	water (control)	7.6 a	100
	AIB 2 mM	9.3 b	122
	AIB 4 mM	9.9 b	130
'Charlotte'	water (control)	11.4 a	100
	AIB 2 mM	13.6 b	119
	AIB 4 mM	14.9 b	131

*Explanations see Table 1

with 2 mM AIB was without significant benefit in 'Impala' and 'Domingo' flowers. Pretreatment of cut 'Dolce Vita' carnations with 4 mM AIB delayed and reduced ethylene production as compared to those untreated (Fig. 1C). Improvement in carnation flower longevity 'Tanga' was also obtained in our previous experiment (Wawrzyńczak et al., 1996). Similarly, Yamamoto et al. (1995) and Onozaki et al. (1998) observed that the pulse or continuous application of AIB to cut carnations was effective in preserving the freshness of flowers, and Serrano et al. (1990) found that continuous AIB treatment delayed the peak of ethylene production. This is likely because AIB inhibits ethylene production by acting as a competitive inhibitor of ACC oxidase (Satoh and Esashi, 1983; Serrano et al., 1990).

ATA is another compound that inhibits the climacteric peak of ethylene production and prolongs the vase life of carnation flowers (Altman and Solomos, 1993; 1994; Serrano et al., 1999). Altman and Solomos (1993) found that the continuous treatment of cut carnations with 50 or 100 mM aminotriazole extended their vase life to 18 days. The positive influence of ATA on flower longevity is likely associated with the inhibitory effect of ATA on ethylene biosynthesis and action (Altman and Solomos, 1994). Data presented in Table 4 show that the vase life was significantly improved in 'Dolce Vita', 'Impala', 'Tanga' and 'Charlotte' flowers pulsed with ATA at 50 and 100 mM concentrations but the vase life of 'Domingo' cultivar was not extended when compared to the control. Similarly, as in carnations treated with AIB, pulse treatment of 'Dolce Vita' flowers with 50 mM ATA reduced the level of ethylene production and delayed its peak by 2 days (Fig. 1D).

Table 4. Effect of pulse treatment with aminotriazole (ATA) on longevity of cut carnations

Cultivar	Treatment	Vase life [days]	Vase life [%]*
'Dolce Vita'	water (control)	6.6 a	100
	ATA 50 mM	8.2 b	124
	ATA 100 mM	7.9 b	120
'Impala'	water (control)	5.5 a	100
	ATA 50 mM	6.5 b	118
	ATA 100 mM	6.4 b	116
'Domingo'	water (control)	11.1 a	100
	ATA 50 mM	12.1 a	109
	ATA 100 mM	11.5 a	104
'Tanga'	water (control)	7.2 a	100
	ATA 50 mM	10.9 b	151
	ATA 100 mM	10.8 b	150
'Charlotte'	water (control)	10.8 a	100
	ATA 50 mM	19.5 b	180
	ATA 100 mM	20.1 b	186

*Explanations see Table 1

Based on the results obtained, we can conclude that the longevity of cut carnations can be improved by pre-treating flowers with inhibitors of ethylene biosynthesis or action. Generally, flowers pulsed with STS had the longer vase life (73-135% over the control) than any other tested. AOA and AIB also delayed flower senescence of all cultivars, but were less effective than STS solution. It is possible that the addition of cytokinins to AOA or AIB can increase flower vase life to the same extent as STS (Wawrzyńczak et al., 1996; Harkema et al., 1991). Moreover, Onozaki et al. (1998) reported that the addition of calcium nitrate to AIB solution prolonged the vase life of carnations as compared with a single application of AIB. ATA was effective in extending the longevity of four cultivars ('Dolce Vita', 'Impala', 'Tanga', 'Charlotte') and its effect on vase life of 'Charlotte' flowers was similar to that obtained with STS. The application of ethylene inhibitors such as STS, AOA, AIB, ATA to cut carnations slows or completely inhibits ethylene production in tissues. The-present study demonstrates that these compounds could be applied as a pulse treatment to extend the longevity of cut carnations.

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WPŁYW INHIBITORÓW ETYLENU NA TRWAŁOŚĆ CIĘTYCH GOŹDZIKÓW (*Dianthus caryophyllus* L.) I PRODUKCJĘ ETYLENU PRZEZ KWIATY

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S T R E S Z C Z E N I E

Badano wpływ 24-godzinnego traktowania STS, AOA, AIB i ATA na trwałość ciętych goździków (*Dianthus caryophyllus* L.) odmian 'Dolce Vita', 'Impala', 'Domingo', 'Tanga' i 'Charlotte'. Spośród wszystkich testowanych inhibitorów etylenu STS najefektywniej przedłużał trwałość goździków. Także zastosowanie AOA lub AIB wpływało na znaczne przedłużenie trwałości kwiatów wszystkich testowanych odmian. ATA wywierał pozytywny wpływ na trwałość kwiatów czterech odmian, ale nie przedłużał trwałości goździków 'Domingo'. STS i AOA najsilniej hamowały produkcję etylenu przez kwiaty goździków 'Dolce Vita'. Zastosowanie AIB lub ATA powodowało opóźnienie klimakterycznego wzrostu produkcji etylenu o 2 dni i zmniejszało poziom jego wydzielania przez kwiaty.

Słowa kluczowe: goździki, kwiaty cięte, inhibitory etylenu, produkcja etylenu, trwałość kwiatów