

Short communication

The role of ethanol or acetaldehyde in the biosynthesis of ethylene in carnation (*Dianthus caryophyllus* L.) cv. Yellow Candy

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Abstract

Carnation (*Dianthus caryophyllus* L.) cv. Yellow Candy flowers were treated with ethanol solution (4%) in the absence or presence of 1 or 10 mM L-methionine, or ethanol or acetaldehyde solution (0.05%), in the absence or presence of 1 mM aminocyclopropane carboxylic acid (ACC). Spermidine in petals was measured over time in flowers treated with 4% ethanol or distilled water. Ethanol treatment significantly increased vase life by 10 days; methionine had no significant effect. The vase life of ACC-treated flowers was reduced by 4 days, the ethylene climacteric peak advanced by 5 days and ethylene production was increased in comparison with flowers not treated with ACC. In the absence of ACC, ethanol solution significantly increased vase life of carnation cv. Yellow Candy by 5 days and inhibited ethylene production, whereas in the presence of ACC, ethanol neither increased vase life nor inhibited ethylene production. Acetaldehyde also failed to increase vase life of carnation cv. Yellow Candy either in the absence or presence of ACC. There was no difference between spermidine content of ethanol- or distilled water-treated flowers. It was concluded that 4% ethanol failed to inhibit conversion of methionine to S-adenosyl methionine (SAM) because ethanol failed to inhibit production of spermidine. Furthermore, ethanol or acetaldehyde also failed to inhibit conversion of ACC to ethylene. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Ethanol inhibits ethylene biosynthesis and sensitivity to ethylene action in carnation (*Dianthus caryophyllus* L.) cvs. Yellow Candy, Sandrosa and

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White Candy flowers (Pun et al., 1999). However, acetaldehyde, a metabolite of ethanol, has also been reported to increase flower longevity and has been described as the causal agent for retardation of carnation cv. White Sim flower senescence (Podd and Van Staden, 1999a) by inhibiting ethylene production (Podd and Van Staden, 1999b). This conflicts with earlier research results where ethanol was described as the main agent responsible for decreasing ethylene production or sensitivity to ethylene (Heins and Blakely, 1980; Wu et al., 1992). A similar controversy has been reported in research on tomatoes. Ripening of tomato cv. Romanesco fruit could be prevented by inhibiting ethylene production with ethanol vapour in the presence of alcohol dehydrogenase inhibitor (Massantini et al., 1995). However, in a similar experiment, Beaulieu et al. (1997) suggested that it was acetaldehyde and not ethanol which was effective in inhibiting ripening in tomato cv. Castelmart fruit. The difference between these two experiments could be due to differences in age of the fruit used (pink in the former and mature green stage in the latter) and the cultivar. The difference in results from research on carnations cannot be explained so easily.

Current information from research on carnation supports the idea that ethanol inhibits production of aminooxycyclopropane carboxylic acid (ACC) (Wu et al., 1992). However, there are no data to suggest how or where this inhibition is occurring. From the ethylene biosynthesis pathway, ethylene production could be inhibited either at the methionine to *S*-adenosyl methionine (SAM) step or the SAM to ACC step or both. There is also some suggestion (Heins and Blakely, 1980; Podd and Van Staden, 1999a) that ethanol inhibits the conversion of ACC to ethylene. Ethanol at 2% with an unknown cultivar (Heins and Blakely, 1980) or 3% with 'White Sim' (Podd and Van Staden, 1999a) inhibited conversion of ACC to ethylene. However, measurement of ethylene from carnation flowers was taken for only 27 h after ACC treatment in the earlier experiment (Heins and Blakely, 1980) and not over the entire vase life of the flowers. In the experiment with 'White Sim' (Podd and Van Staden, 1999a), no

data were presented. The role of acetaldehyde in the inhibition of conversion of exogenously applied ACC in carnation flowers has not yet been tested.

SAM is a precursor of ACC, as well as of the polyamine spermidine (Serrano et al., 1999). Ethylene and spermidine have opposite effects on fruit ripening and senescence (Kakkar and Rai, 1993). Ethylene is the hormone of senescence, whereas spermidine is considered to be related to young or actively growing tissues (Smith, 1985; Kakkar and Rai, 1993). Low but stable concentrations of spermidine during vase life have been found in the long lasting carnation cv. Killer; low, stable concentrations of spermidine have been suggested to be connected with flower longevity (Serrano et al., 1991).

This research on cv. Yellow Candy was established to elucidate the role of ethanol and acetaldehyde in the biosynthesis of ethylene (methionine to SAM or ACC to ethylene). Firstly the effect of methionine on the vase life of flowers treated with or without ethanol solution using exogenously applied L-methionine was determined. Secondly, inhibition of the conversion of ACC to ethylene with ethanol or acetaldehyde was tested. Thirdly, inhibition of the conversion of methionine to SAM with ethanol was examined by measuring the concentration of spermidine.

2. Materials and methods

The cultivar 'Yellow Candy' was selected because of its positive response to ethanol and high sensitivity to ethylene (Pun et al., 1999). The flowers were grown commercially (Pun et al., 1999) and paint brush stage flowers (colour development of flower completed and partial unfurling of petals (Menguc and Usta, 1994)) were freshly harvested on the day the experiment started. The flowers were transported dry and non-refrigerated to the laboratory, cut in the air to 200 mm stem length and put in 100 ml distilled water or test solution.

Ethanol or acetaldehyde solutions were prepared using absolute ethanol or acetaldehyde. Prepared solutions of ethanol or acetaldehyde

were put into the refrigerator immediately and kept cool before use for faster absorption by the flowers when treated (Slootweg, 1995). Individual flowers were exposed to test solutions continuously by placing them into a conical flask containing 100 ml test solution. Parafilm (American National Cam™) was wrapped around the stem and over the top of the conical flask to restrict solution loss to the flower only. Vase life was evaluated under standard environmental conditions (temperature $20 \pm 1^\circ\text{C}$, day length 12 h (07:00–19:00), cool-white fluorescent light with photosynthetically active photon flux density (PPFD) of $15 \mu\text{mol m}^{-2} \text{s}^{-1}$, RH 60–70% (Reid and Kofranek, 1980)). Flower quality was assessed daily by visual scoring (using reference photographs) on a scale from 6 to 1, where 1 was fully senesced flowers. Flower vase life was considered terminated once the flower petals were noticeably wilted and/or showed necrotic marks (i.e. reached a score of 2 (Wu et al., 1989)).

The flowers were treated with or without 1 or 10 mM L-methionine (Heins and Blakely, 1980) in distilled water or ethanol (4%; v/v) (Pun et al., 1999). The flowers were first treated with the test solution for 48 h and, thereafter, were subjected to L-methionine treatment for 24 h. The cut end of the stem was recut before dipping into the L-methionine solution. After 24 h treatment with L-methionine solution, the end of the stem was recut again before replacing it in the test solution. Five individual flowers were used as replicates per treatment.

The flowers were treated with or without 1 mM ACC (Whitehead et al., 1984; Wu et al., 1989) in distilled water, 4% ethanol or 0.05% acetaldehyde (v/v) solution. The flowers were first treated with the test solution for 48 h as above and, thereafter, were subjected to ACC for 24 h. The cut end of the stem was recut before dipping into the ACC solution. After 24 h treatment in ACC, the end of the stem was recut again before replacing it in the test solution. Five individual flowers were used as replicates per treatment. Individual flowers were removed from the ACC solution and sealed for 30 min in a 500 ml plastic container fitted with a rubber sampling port. After 24 h and, thereafter, at 24 h intervals until the end of the flower vase

life, 1 ml of headspace gas was removed with a gas-tight syringe (Hamilton). Ethylene in the sample was then analysed by gas chromatography (Pun et al., 1999).

The flowers were treated with 4% ethanol or distilled water and placed in the controlled environment as in other experiments. Two flowers per treatment were sampled at intervals of 4 days to measure the concentration of spermidine during senescence. Spermidine was extracted with HClO_4 and analyzed by the benzoylation method (Serano et al., 1999). Benzoyl-polyamines were analyzed by HPLC using the Waters system. The elution consisted of acetonitrile:water (52:48, v/v, as solvent), run isocratically with a flow rate of 1 ml min^{-1} . The benzoyl-polyamines were eluted through a reverse-phase column (Spherisorb ODS 25 micron column with 250 mm length and 4.6 mm ID) and detected by absorbance at 254 nm. A relative calibration procedure was used to determine the amounts of polyamines in samples using standard curves of spermidine (Sigma).

2.1. Statistical analysis

ANOVA were performed using SYSTAT; means were separated using Fisher's LSD at 0.05%.

3. Results and discussion

Vase life of control flowers in water was 15 days. There was no reduction in vase life in the presence of methionine (data not presented). However, vase life of the flowers treated with ethanol, with or without methionine, was 25 days, which was significantly ($P < 0.05$) greater than the controls.

The vase life of the flowers treated with distilled water, ethanol or acetaldehyde was significantly reduced ($P < 0.001$) when ACC was applied exogenously; data are presented from a typical experiment (Fig. 1). Reduction in vase life of all ACC-treated flowers was associated with a significant ($P < 0.001$) increase in total ethylene production (μl per flower) (almost 2-fold in distilled water and 5-fold in treated flowers) and a reduc-

tion in time to the ethylene peak by 5 days in distilled water and 8 days in acetaldehyde-treated flowers (Fig. 2).

There was no significant effect of treatment or time on spermidine concentration in petals. The overall average spermidine content was 317 nmol gFW⁻¹ (range 175–490 nmol gFW⁻¹).

Ethanol solution at 4%, which has been reported to be the optimum ethanol concentration to increase vase life of several carnation cultivars (Pun et al., 1999), significantly increased vase life. The increase of 10 days was the same as that reported earlier (Pun et al., 1999). Exogenous methionine at 1 or 10 mM failed to change the vase life of carnation flowers treated with distilled water; similar results with 1 mM methionine have been reported for carnation cv. White Sim (Heins and Blakely 1980). Furthermore, spermidine concentration in petals was not affected by ethanol suggesting that ethanol is not involved in the inhibition of conversion of methionine to SAM. This could explain why there was no detrimental effect of exogenous methionine in the present experiment and that of Heins and Blakely (1980).

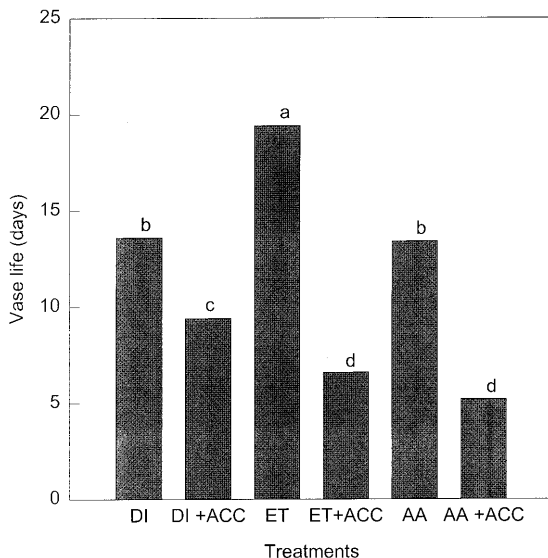


Fig. 1. Influence of 4% ethanol (ET) or 0.05% acetaldehyde (AA) on the vase life of carnation cv. Yellow Candy flowers in the absence or presence of exogenously applied ACC. DI, distilled water. Treatments which do not share the same letter are significantly different ($P < 0.05$).

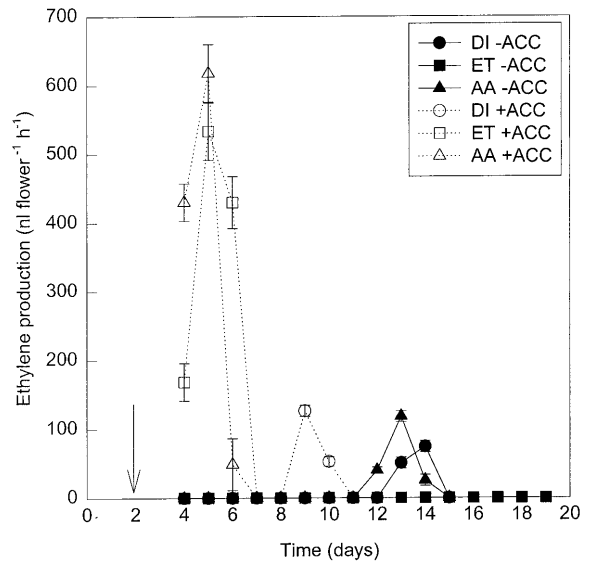


Fig. 2. Influence of 4% ethanol (ET) or 0.05% acetaldehyde (AA) on ethylene production of carnation cv. Yellow Candy flowers in the absence or presence of exogenously applied ACC. ↓ Indicates time of ACC application. Bars indicate S.E.M.

It could be possible that exogenously applied methionine is distributed to both ACC and spermidine, resulting in no net effect because of their opposite role in senescence (Kakkar and Rai, 1993). Although spermidine concentration between flowers was variable, the range of spermidine was similar to that of carnation cv. Master (Serrano et al., 1999) and cv. Arthur, but higher than in the non-climacteric cv. Killer (Serrano et al., 1991).

The vase life of carnation cv. Yellow Candy flowers was reduced when ACC was applied exogenously. The addition of exogenous ACC could have provided additional substrate for production of ethylene, thereby reducing vase life. Application of ACC exogenously in the presence of ethanol severely reduced the vase life of flowers in contrast to the vase life in distilled water with or without ACC. This reduction in vase life could be due to the failure of ethanol to inhibit ethylene production in the presence of ACC; this effect has been reported for mango (Burdon et al., 1996). In contrast, the flowers treated with ethanol in the absence of ACC showed total inhibition of ethyl-

ene production and the increased vase life of the flowers, supporting earlier reports (e.g. Heins and Blakely (1980), Pun et al. (1999)). The flowers in ACC with ethanol or acetaldehyde produced ethylene earlier than the flowers in distilled water and, therefore, showed no inhibition of conversion of ACC to ethylene in the 19 days of the experiment. This contrasts with results reported by Heins and Blakely (1980) where ethanol (2%) inhibited ethylene production until 27 h after application of ACC.

The stimulation of ethylene production after ACC was added was largely responsible for the reduction of the flower vase life. The mechanism of stimulation of ethylene production with ethanol in the presence of exogenous ACC is unknown, but the site of activity is likely to be ACC oxidase because ethanol without ACC has been reported to result in total inhibition of ethylene production (Wu et al., 1992; Pun et al., 1999). This suggests that the inhibitory effect of ethanol on the biosynthesis of ethylene must occur before formation of ACC. This would explain the failure of ethanol to inhibit ethylene production in climacteric carnation flowers (Heins and Blakely, 1980); it is suggested that ACC accumulated in a climacteric flower and ethanol failed to inhibit the conversion of ACC to ethylene.

The results from the current research suggest that there are not different pathways of conversion of ACC to ethylene in non-climacteric and climacteric carnation petal tissue as proposed by Heins and Blakely (1980). However, the mechanism of stimulation of ethylene production with acetaldehyde in the presence of exogenous ACC, as yet, is unknown.

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References

- Beaulieu, J.C., Peiser, G., Saltveit, M.E., 1997. Acetaldehyde is causal agent responsible for ethanol-induced ripening inhibition in tomato fruit. *Plant Physiol.* 113, 431–439.
- Burdon, J.N., Dori, S., Marinansky, R., Pesis, E., 1996. Acetaldehyde inhibition of ethylene biosynthesis in mango fruit. *Postharvest Biol. Technol.* 8, 153–161.
- Heins, R.D., Blakely, N., 1980. Influence of ethanol on ethylene biosynthesis and flower senescence of cut carnation. *Sci. Hortic.* 13, 361–369.
- Kakkar, R.K., Rai, V.K., 1993. Plant polyamines in flowering and fruit ripening. *Phytochemistry* 33, 1281–1288.
- Massantini, M., Gobattoni, E., Botondi, R., Mencarelli, F., 1995. Role of alcohol dehydrogenase on ripening of ethanol-treated tomato and kiwifruit. *Acta Hortic.* 379, 297–304.
- Menguc, A., Usta, E., 1994. Research on the effects of silver thiosulphate plus sucrose pretreatment on the cold storage period and post storage vase life of cut flowers of carnation cv. Astor harvested at different maturities. *Acta Hortic.* 368, 802–807.
- Podd, L.A., Van Staden, J., 1999a. Is acetaldehyde the causal agent in the retardation of carnation flower senescence by ethanol? *J. Plant Physiol.* 154, 351–354.
- Podd, L.A., Van Staden, J., 1999b. The use of acetaldehyde to control carnation flower longevity. *Plant Growth Regul.* 28, 175–178.
- Pun, U.K., Rowe, R.N., Rowarth, J.S., Barnes, M.F., Dawson, C.O., Heyes, J.A., 1999. Influence of ethanol on climacteric senescence in five cultivars of carnation. *NZJ Crop Hortic. Sci.* 27, 69–77.
- Reid, M.S., Kofranek, A.M., 1980. Recommendations for standardised vase life evaluations. *Acta Hortic.* 113, 171–173.
- Serrano, M., Romojaro, F., Casas, J.L., Acosta, M., 1991. Ethylene and polyamine metabolism in climacteric and nonclimacteric carnation flowers. *HortScience* 26, 894–896.
- Serrano, M., Martinez-Madrid, M.A., Romojaro, F., 1999. Ethylene biosynthesis and polyamine and ABA levels in cut carnations treated with aminotriazole. *J. Am. Soc. Hortic. Sci.* 124, 81–85.
- Slootweg, G., 1995. Effect of water temperature on water uptake and vase life of different cut flowers. *Acta Hortic.* 405, 67–74.
- Smith, T.A., 1985. Polyamines. *Ann. Rev. Plant Physiol.* 36, 117–143.
- Whitehead, C.S., Halevy, A.H., Reid, M.S., 1984. Control of ethylene synthesis during development and senescence of carnation petals. *J. Am. Soc. Hortic. Sci.* 109, 473–475.
- Wu, M.J., van Doorn, W.G., Mayak, S., Reid, M.S., 1989. Senescence of Sandra carnation. *Acta Hortic.* 261, 221–225.
- Wu, M.J., Zacarias, L., Saltveit, M.E., Reid, M.S., 1992. Alcohols and carnation senescence. *HortScience* 27, 136–138.