

Effects of 2, 5-Norbornadiene and Ethylene on the Induction of Activity of 1-Aminocyclopropane-1-carboxylate (ACC) Synthase, and on Increases in the ACC Content and the Rate of Ethylene Production in Petals of Cut Carnation Flowers during Senescence

Hiroshi HYODO, Yoshinori TERADA and Sanae NODA

Department of Horticulture, Faculty of Agriculture, Shizuoka University, Ohya, Shizuoka 422

Summary

A marked increase in ethylene production occurred in cut carnation flowers (*Dianthus caryophyllus* L. cv. Izu-Pink) during senescence. The application of ethylene hastened senescence by promoting ethylene production, while the applied 2,5-norbornadiene (NBD) retarded the progress of senescence of flowers by reducing the rate of ethylene production, such that no initiation of wilting of flowers was observed 8 d after harvest when untreated flowers had completely wilted. Most of the ethylene produced by senescing carnation flowers was derived from petals.

The activity of 1-aminocyclopropane-1-carboxylate (ACC) synthase was markedly induced in petals of carnation flowers during senescence, and was accompanied by increases in the level of ACC and the rate of ethylene production. This induction was promoted by ethylene and suppressed by NBD. It is considered that ethylene accelerates ethylene biosynthesis by promoting the induction of ACC synthase, while NBD reduces ethylene biosynthesis by suppressing the induction of ACC synthase, which may result from inhibition of ethylene action by NBD.

Introduction

The plant hormone ethylene is closely associated with senescence of flowers (7). In cut carnation flowers ethylene production occurs remarkably during senescence (5, 9), and the evolved ethylene plays an essential role in petal wilting (6).

In higher plants ethylene is synthesized from methionine, and 1-aminocyclopropane-1-carboxylic acid (ACC) serves as an intermediate of ethylene biosynthesis (17). This methionine and ACC pathway was shown to operate in carnation flowers during senescence (1). In this pathway where the formation of ACC is a rate-limiting step, the induction and level of ACC synthase which catalyzes the conversion of S-adenosylmethionine (SAM) to ACC may play an important role in inducing and regulating the rate of ethylene production (17). Peiser demonstrated that ACC synthase activity that increased in petals during senescence correlat-

ed with ethylene production by flowers (10).

It has been shown that ethylene biosynthesis is regulated by ethylene (17). In the autocatalytic ethylene production the rate of ethylene synthesis is promoted by either endogenous or exogenous ethylene. In this mechanism ethylene may enhance the induction of activity of ACC synthase and/or ethylene-forming enzyme (EFE). In the study on the mode of action of silver thiosulfate (STS), Veen stated that STS reduced the rate of ethylene production by preventing ethylene from binding to the receptor site, thus inhibiting autocatalytic ethylene action (13, 14).

It has been reported that the application of 2,5-norbornadiene (NBD), a competitive inhibitor of ethylene action (12), reduced the rate of ethylene production, resulting in a large increase in the vase life of cut carnation flowers (11, 16), and that in the tissue treated with NBD, the activities of ACC synthase and EFE were greatly reduced (16).

The present study deals with the induction of the activity of ACC synthase and increases in the

ACC content and the rate of ethylene production in petals of cut carnation flowers during senescence. Also, the effects of NBD and ethylene on the development of ACC synthase activity, ACC level and ethylene production were studied in senescing petals.

Materials and Methods

Carnation flowers (*Dianthus caryophyllus* L. cv. Izu-Pink) were obtained from a commercial grower in Shizuoka one or two d before full opening. Flower stems were trimmed to 8 cm prior to treatment. Flowers were placed individually in 63 ml Erlenmeyer flasks containing 50 ml deionized water and held at 20°C. Flower senescence was scored with a rating scale of 1 to 6: score 1, before full opening; 2, full opening (petals fully expanded); 3, beginning of wilting (initiation of petal inrolling); 4, moderate wilting; 5, complete wilting; 6, complete senescence (browning of the petal edges).

The flowers in flasks were sealed daily for 1 to 3 h in 1.22 l glass jars and 1 ml of head space gas was withdrawn by syringe for ethylene analysis. The sample gas was assayed for ethylene in a Hitachi 163 gas chromatograph equipped with a flame ionization detector and a 1 m activated alumina column at 70°C.

After determination of the rate of ethylene production of whole flowers, the flowers were separated into petals and pistils, which were subjected to determination of ethylene production, ACC content and ACC synthase activity. Seven petals isolated from both outer and inner whorls of flowers were used as the petal materials. Those were enclosed in a 140 ml Erlenmeyer flask for 1 h and ethylene concentration in the head space was measured by gas chromatography.

Petals were homogenized with 80 % ethanol (10 ml/g tissue) for extraction of ACC in a mortar and pestle. After centrifugation at $8,000 \times g$ for 15 min, the supernatant was concentrated to dryness in vacuo at 45°C in a rotary evaporator. The residue was taken up in 3 ml of water, and an aliquot of 0.5 ml was assayed for ACC by the method described by Lizada and Yang (4).

ACC synthase was prepared and assayed by the following procedures. Seven petals were homogenized at 2°C in 5 ml of 100 mM EPPS (N-(2-hydroxyethyl) piperazine-N'-3-propanesulfonic acid) buffer, pH 8.5, containing 10 mM

2-mercaptoethanol and 5 μ M pyridoxal phosphate by mortar and pestle. The homogenate was centrifuged at $16,000 \times g$ for 25 min at 2°C. The supernatant was dialyzed for 1 d at 2°C against three changes of dialyzing buffer consisting of 5 mM EPPS buffer, pH 8.5, 5 mM 2-mercaptoethanol and 5 μ M pyridoxal phosphate. The dialyzed solution was used as an enzyme preparation for ACC synthase. ACC synthase activity was assayed with a reaction mixture consisting of 50 mM EPPS buffer, pH 8.5, 50 μ M SAM, 4 μ M pyridoxal phosphate and enzyme preparation in a total volume of 1 ml. The reaction mixture was incubated at 30°C for 30 min, and the reaction was stopped by addition of 0.1 ml 20 mM HgCl₂. ACC formed from SAM by ACC synthase was assayed by the method of Lizada and Yang (4).

Treatment of flowers with ethylene was as follows. Flowers in Erlenmeyer flasks were placed in a 12 to 13 l desiccator into which ethylene was injected through a syringe to the desired concentration (0.5 μ l/l), which was monitored by gas chromatography. For control flowers, a beaker containing a 0.25 M solution of mercuric perchlorate was placed in the desiccator to absorb ethylene.

For treatment of flowers with NBD, liquid NBD was applied with a micropipette onto a sheet of filter paper in a small petri dish in the desiccator, which was immediately sealed with a cover. Liquid NBD readily volatilized at 20°C. The amount of liquid applied was calculated to give the desired gaseous concentration (1,000 μ l/l) in the desiccator. Flowers were treated with ethylene for 2 d at the beginning and with NBD for a whole period of experiments. Everyday desiccators were opened and ventilated with fresh air, and then ethylene or NBD was reintroduced. In all desiccators a beaker containing 5 N KOH was placed to absorb CO₂ evolved from flowers.

EPPS, pyridoxal phosphate, SAM and ACC were purchased from Sigma Chemical Co. NBD and 2-mercaptoethanol were purchased from Wako Pure Chemical Industries, Ltd. Ethylene was obtained from Iwatani & Co. Ltd..

Results

A marked increase in ethylene production occurred during senescence of carnation flowers 'Izu-

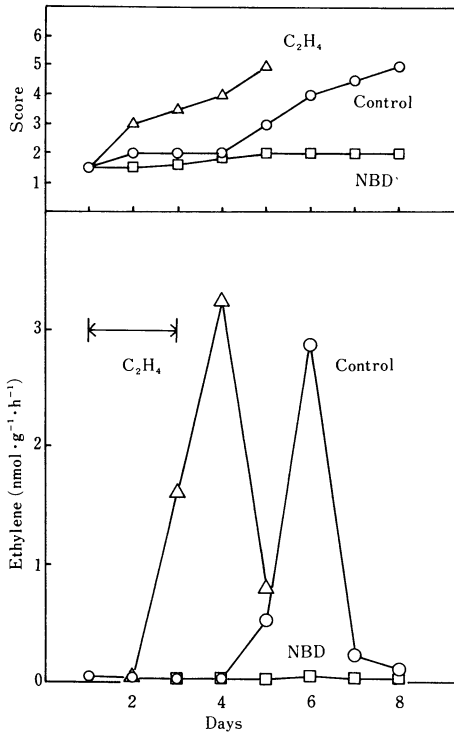


Fig. 1. Effects of NBD and ethylene on rate of ethylene production and petal wilting of cut carnation flowers during senescence at 20°C. (○), control flowers; (Δ), flowers treated with ethylene from day 1 to 3; (□), flowers treated with NBD.

Pink'. The rate of ethylene production reached a peak 6 d after harvest at 20°C, followed by a sharp decline to a low level on the following day when petals almost wilted (Fig. 1). The surge of ethylene production was accompanied by a climacteric rise in respiration and a decrease in fresh weight of flowers (data not shown). The initiation of wilting (petal inrolling) coincided with the steep rise in ethylene production and the wilting advanced as petals continued to produce a large amount of ethylene.

By treatment with 0.5 μ l/l ethylene for 2 d from day 1 to 3, the increase in ethylene production by flowers started two d earlier than in the control flowers (Fig. 1). The wilting of petals started shortly after the treatment with ethylene. In contrast to control flowers and those treated with ethylene, ethylene production was totally arrested by treatment with 1,000 μ l/l NBD. No progress in petal senescence was observed over the entire

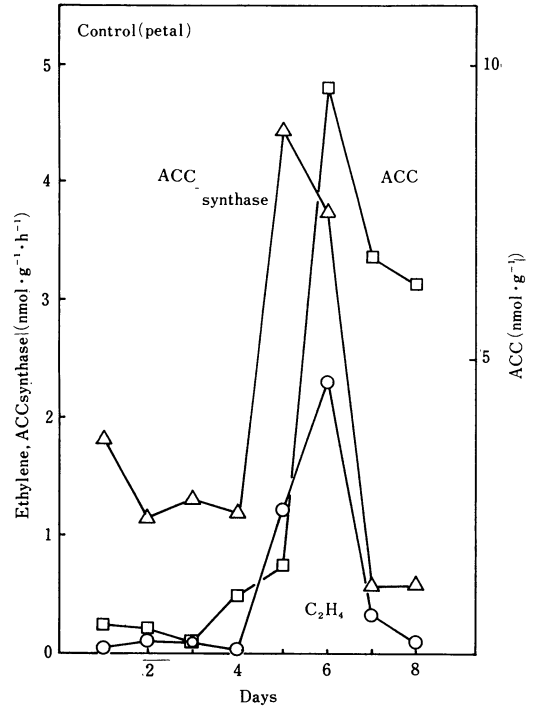


Fig. 2. Changes in ACC synthase activity, ACC content and rate of ethylene production in petals of cut carnation flowers during senescence at 20°C. (Δ), ACC synthase activity; (□), ACC content; (○), rate of ethylene production.

period of experiment (Fig. 1).

In the advanced stage of senescence most of the ethylene produced by carnation flowers was derived from petals, therefore petals were analyzed for ACC synthase activity, ACC content and ethylene production. The activity of ACC synthase rapidly increased in petals during senescence, followed by increases in the level of ACC and the rate of ethylene production (Fig. 2). After reaching a maximum, the activity of ACC synthase declined sharply. The application of exogenous ethylene to flowers induced the rapid increase in ACC synthase activity in petals, accompanied by increases in the ACC content and the rate of ethylene production (Fig. 3). By the application of exogenous ethylene, ACC synthase activity started to increase two d earlier than in the control. Moreover, the activity reached a level much higher than that of the control. It is evident that there is a close relationship between the increase in activity

of ACC synthase and the level of ACC and the rate of ethylene production in the petal tissue.

Exposure of flowers to 1,000 $\mu\text{l/l}$ NBD strongly suppressed the induction of the activity of ACC synthase and totally reduced the formation of ACC and ethylene in petals (Fig. 4). Although there appeared a somewhat gradual increase in ACC synthase activity, followed by a decline, neither ACC nor ethylene formation was significantly affected.

Discussion

During senescence of cut carnation flowers the activity of ACC synthase increased in petals as has been shown by Peiser (10). As a result the rate of ACC formation was enhanced, EFE activity also increased in petals during senescence (8). These led to the increased rate of ethylene production, which was responsible for petal wilting. The amount of conjugate-ACC (N-malonyl-ACC) also increased almost parallel with ACC (data not shown).

In the course of senescence most of ethylene produced was derived from petals, while there was an increase in ethylene production by the pistil in the initial stage of senescence. ACC synthase activity in the pistil also increased and reached a peak earlier than in the petal (data not shown).

The exogenous ethylene induced petal tissue to increase the activity of ACC synthase, followed by an increase in ACC and ethylene production. That ethylene promoted the development of ACC synthase activity was reported by Bufler (2). This may be one of the key steps in ethylene biosynthesis enhanced by ethylene (18).

NBD at a concentration of 1,000 $\mu\text{l/l}$ strongly inhibited ethylene production by petals. This may have resulted from the suppression of the induction of ACC synthase and the subsequent inhibition of ACC synthesis. Wang and Woodson reported that besides ACC synthase, NBD also strongly inhibited the development of EFE (16). Sisler and Yang

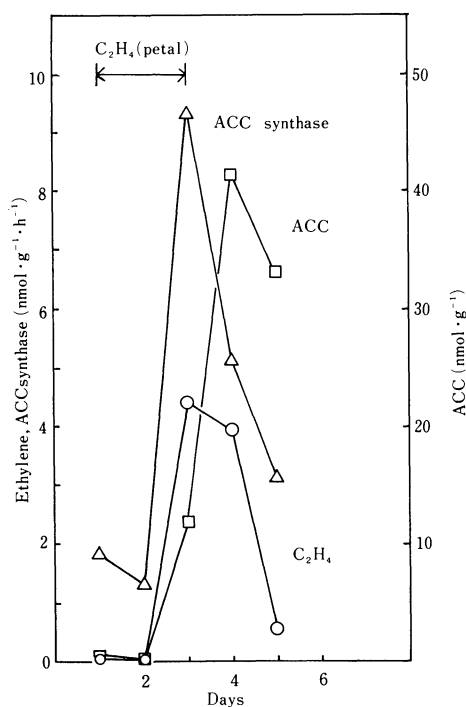


Fig. 3. Effects of ethylene on the induction of the activity of ACC synthase and on increases in the level of ACC and the rate of ethylene production in petals of carnation flowers during senescence. (Δ), ACC synthase activity; ($\square$), ACC content; ($\circ$), rate of ethylene production.

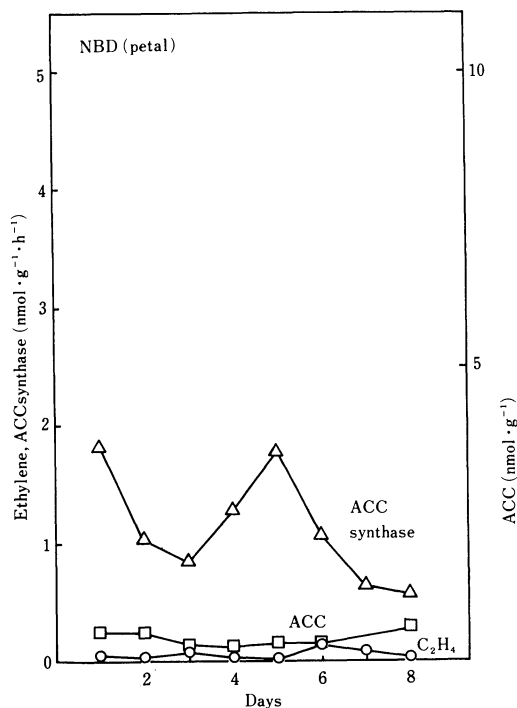


Fig. 4. Effects of NBD on the induction of the activity of ACC synthase and on increases in the level of ACC and the rate of ethylene production in petals of carnation flowers during senescence. (Δ), ACC synthase activity; ($\square$), ACC content; ($\circ$), rate of ethylene production.

showed that NBD competitively inhibited ethylene's action with a K_i value of $170 \mu\text{l/l}$ in pea epicotyl tissue (12). It is likely that endogenous ethylene is associated with the induction of ACC synthase in carnation petals during senescence as has been suggested with the climacteric fruits, and that by inhibiting the action of ethylene by NBD, the induction of ACC synthase is suppressed. We have recently demonstrated that the activity of ACC synthase induced in the wounded mesocarp tissue of *Cucurbita maxima* was enhanced by the applied NBD. In contrast to carnation petals the exogenous ethylene suppressed the induction of ACC synthase in the wounded mesocarp tissue, possibly due to the autoinhibitory action of ethylene. These results suggest that NBD may have promoted the induction of ACC synthase by releasing the suppression caused by endogenous ethylene (3).

It is evident that NBD can prolong the vase life of carnation flowers by reducing the rate of ethylene production. Sisler *et al.* reported that NBD at $2,000 \mu\text{l/l}$ doubled the vase life of the flowers (11). In the present experiments, too, no initiation of wilting was observed in the presence of $1,000 \mu\text{l/l}$ NBD on day 8 after harvest, when control flowers had completely wilted. However, NBD will not be as practical as STS in postharvest handling because of its gaseous nature and unpleasant smell (15).

Literature Cited

1. BUFLER, G., Y. MOR, M. S. REID and S. F. YANG. 1980. Changes in 1-aminocyclopropane-1-carboxylic acid content of cut carnation flowers in relation to their senescence. *Planta* 150 : 439-442.
2. BUFLER, G. 1984. Ethylene-enhanced 1-aminocyclopropane-1-carboxylic acid synthase activity in ripening apples. *Plant Physiol.* 75 : 192-195.
3. HYODO, H. and H. FUJINAMI. 1989. The effects of 2, 5-norbornadiene on the induction of the activity of 1-aminocyclopropane-1-carboxylate synthase and of phenylalanine ammonia-lyase in wounded mesocarp tissue of *Cucurbita maxima*. *Plant Cell Physiol.* 30 : 857-860.
4. LIZADA, M. C. C. and S. F. YANG. 1979. A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. *Anal. Biochem.* 100 : 140-145.
5. MAYAK, S. and D. R. DILLEY. 1976. Regulation of senescence in carnation (*Dianthus caryophyllus*). Effect of abscisic acid and carbon dioxide on ethylene production. *Plant Physiol.* 58 : 663-665.
6. MAYAK, S., Y. VAADIA and D. R. DILLEY. 1977. Regulation of senescence in carnation (*Dianthus caryophyllus*) by ethylene. Mode of action. *Plant Physiol.* 59 : 591-593.
7. MAYAK, S. and A. H. HALEVY. 1980. Flower senescence. p. 131-156. In: K. V. Thimann (ed.) *Senescence in plants*. CRC Press, Boca Raton, Florida.
8. MAYAK, S., R. L. LEGGE and J. E. THOMPSON. 1981. Ethylene formation from 1-aminocyclopropane-1-carboxylic acid by microsomal membranes from senescing carnation flowers. *Planta* 153 : 49-55.
9. NICHOLS, R. 1966. Ethylene production during senescence of flowers. *J. Hort. Sci.* 41 : 279-290.
10. PEISER, G. 1986. Levels of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity, ACC and ACC-conjugate in cut carnation flowers during senescence. *Acta Hort.* 181 : 99-104.
11. SISLER, E. C., M. S. REID and D. W. FUJINO. 1983. Investigation of the mode of action of ethylene in carnation senescence. *Acta Hort.* 141 : 229-234.
12. SISLER, E. C. and S. F. YANG. 1984. Anti-ethylene effects of *cis*-2-butene and cyclic olefins. *Phytochemistry* 23 : 2765-2768.
13. VEEN, H. 1979. Effects of silver on ethylene synthesis and action in cut carnations. *Planta* 145 : 467-470.
14. VEEN, H. 1983. Silver thiosulphate: an experimental tool in plant science. *Scientia Hort.* 20 : 211-224.
15. VEEN, H. 1987. Use of inhibitors of ethylene action. *Acta Hort.* 201 : 213-222.
16. WANG, H. and W. R. WOODSON. 1989. Reversible inhibition of ethylene action and interruption of petal senescence in carnation flowers by norbornadiene. *Plant Physiol.* 89 : 434-438.
17. YANG, S. F. and N. E. HOFFMAN. 1984. Ethylene biosynthesis and its regulation in higher plants. *Ann. Rev. Plant Physiol.* 35 : 155-189.
18. YANG, S. F. 1987. The role of ethylene and ethylene synthesis in fruit ripening. p. 156-166. In: W. W. Thomson, E. A. Nothnagel and R. C. Huffaker (eds.) *Plant senescence : its biochemistry and physiology*.

カーネーションの切り花の老化の過程における花弁中の1-アミノシクロプロパン-
1-カルボン酸(ACC)合成酵素の活性の誘導, ACC 含量, エチレン生成量
の増大に及ぼす 2,5-ノルボルナジエンとエチレンの影響

兵藤 宏・寺田吉徳・野田早苗

静岡大学農学部 422 静岡市大谷 836

摘 要

カーネーション(*Dianthus caryophyllus* L. ‘伊豆ピンク’)の切り花は老化の過程でエチレン生成量が著しく増大し, そのエチレンにより花の萎凋がひき起こされた。花にエチレンを与えるとエチレン生成が促進され, 2,5-ノルボルナジエン(NBD)を与えるとエチレン生成が抑制され, 花の老化は著しく抑制された。

老化の進行中, 花の生成するエチレンは大部分花弁に由来した。1-アミノシクロプロパン-1-カルボン酸(ACC)合成酵素の活性は花弁において老化の過程で著しく増大し, それに伴い ACC の含量, エチレン生成量

も顕著に増加した。ACC 合成酵素の活性の誘導はエチレンにより促進され, 一方 NBD により抑制された。花弁においてエチレン(内生, 外生ともに)は ACC 合成酵素の誘導を促進することにより, エチレン生合成速度を高めると考えられる。一方エチレンの拮抗的阻害剤とみなされている NBD は, エチレンの作用を阻害することにより ACC 合成酵素の誘導を抑制し, その結果 ACC の生成を抑え, エチレン生成を低下させるものと考えられた。