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Changes in 1-Aminocyclopropane-1-carboxylic-acid Content of Cut Carnation Flowers in Relation to Their Senescence

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Abstract. The rise in ethylene production accompanying the respiration climacteric and senescence of cut carnation flowers (*Dianthus caryophyllus* L. cv. White Sim) was associated with a 30-fold increase in the concentration of 1-aminocyclopropane-1-carboxylic acid (ACC) in the petals (initial content 0.3 nmol/g fresh weight). Pretreatment of the flowers with silver thiosulfate (STS) retarded flower senescence and prevented the increase in ACC concentration in the petals. An increase in ACC in the remaining flower parts, which appeared to precede the increase in the petals, was only partially prevented by the STS pretreatment. Addition of aminoxyacetic acid (2 mM) to the solution in which the flowers were kept completely inhibited accumulation of ACC in all flower parts.

Key words: 1-Aminocyclopropane-1-carboxylic acid – *Dianthus*-Ethylene synthesis – Flower (senescence) – Senescence (flowers)

Introduction

The senescence of carnation flowers (*Dianthus caryophyllus* L.) is accompanied by a marked increase in the synthesis of ethylene, and a concomitant climacteric rise in respiration (Nichols 1966). Pretreatment of carnations with a silver thiosulfate complex (STS) prevents the climacteric rise in ethylene production (Veen 1979) and delays senescence of the flowers (Reid et al. 1980).

It has been shown that 1-aminocyclopropane-1-carboxylic acid (ACC) is an intermediate in the biosynthesis of ethylene (Adams and Yang 1979) and that the increase in ethylene synthesis during the ripening of various climacteric fruits is accompanied by increased levels of ACC in the tissue (Hoffman and Yang 1980). Aminoxyacetic acid (AOA), an inhibitor of ACC synthase (Yu et al. 1979) has been shown to increase the longevity of cut carnations by inhibiting their ethylene production (Fujino et al. 1980).

The purpose of the studies reported in this paper was to follow the changes in the level of ACC during senescence of carnation flowers, and to determine the effect of STS and AOA on these changes.

Material and Methods

1. Flowers. Carnation flowers (*Dianthus caryophyllus* L., cv. White Sim) were grown in a greenhouse, harvested at normal commercial maturity, and trimmed to 40 cm. The flowers were pretreated either with deionized water or with a 4 mM solution of silver-thiosulfate complex (STS) for 10 min and transferred to 200 μg/ml Physan (Consan Pacific, Whittier, Cal., USA) as a bacteriocide. The silver-thiosulfate complex (4 mM) was prepared by combining 8 mM $AgNO_3$ and 32 mM $Na_2S_2O_3$ with rapid stirring. In experiments examining the effects of AOA, flowers were kept continuously in solutions containing 200 μg/ml Physan and 2 mM AOA. The flowers were held under continuous light (1.5 W m^{-2} photosynthetically active radiation, measured with a Lambda, New York, N.Y., USA, photoradiometer) from fluorescent lamps (General Electric, Cleveland, O., USA; F96T12-CW-1500) at $25 \pm 2°$ C. At daily intervals, three flowers from each treatment were taken for measurement of ethylene and CO_2 production and estimation of ACC content.

2. Ethylene and CO_2 Measurement. Flowers were cut directly below the calyx and placed in closed jars (500 ml) for 30 min. The ethylene and CO_2 concentration in the jars were then measured by gas chromatography, using a Carle, Anaheim, Cal., USA analytical gas chromatograph equipped with a thermal conductivity detector for measurement of CO_2 and a photoionization detector (HNU Systems, Newton, Mass., USA) coupled to a high-sensitivity electrometer (Keithley, Cleveland, O., USA) for measurement of low concentrations of ethylene. These manipulations did not appear to cause wound-ethylene production, since the ethylene production

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Abbreviations: ACC = 1-aminocyclopropane-1-carboxylic acid; AOA = α-aminoxyacetic acid; STS = silver thiosulfate complex

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rate did not rise even 1 h after placing the flowers in the jars. The CO_2 concentration in the jars was never more than 0.5%.

3. ACC Extraction and Determination. Immediately after measurement of CO_2 and ethylene production, the flowers were separated into petals and the remaining parts (gynoecium, receptacle and sepals). After determination of the fresh weight of the organs, ACC was extracted from the tissue and determined by a modification of the method of Lizada and Yang (1979).

The flower tissue was ground in liquid nitrogen, then extracted with two to three times its weight of 80% ethanol for at least 16 h at 4° C with constant stirring. The resulting extract was centrifuged at 25,000·*g* for 15 min (Beckman, Fullerton, Cal., USA; model J-216). The pellet was resuspended in 80% ethanol and recentrifuged, and the combined supernatants were evaporated to drynesss in vacuo. The dry residue was dissolved in a known amount of water (half to one time the original fresh weight of the tissue, according to the anticipated concentration of ACC) and usually assayed for ACC without further purification. To 0.1 ml of the aqueous extract in a test tube was added 1 µmol of $HgCl_2$, and sufficient water to bring the volume to 0.8 ml. The test tubes were sealed with serum caps and 0.2 ml of an ice-cold mixture (2:1, v/v) of commercial bleach (5% NaOCl) and saturated NaOH was injected. The assay tubes were held in an ice bath and after 3 min a gas sample was withdrawn for measurement of ethylene content by gas chromatography. The efficiency of ACC conversion to ethylene in each sample was determined by adding a known amount of ACC as an internal standard to a replicate assay tube (at least three times the anticipated ACC content of the tissue sample). The identity of ACC in carnation tissue was verified by cochromatography with authentic ACC on Whatman No. 3 paper developed with butanol-acetic acid-water (4:1:5, v/v) as described by Lizada and Yang (1979).

The whole experiment was repeated twice, three flowers were taken at each sample time, and extracts from each sample were assayed at least twice.

Results

1. Changes in ACC Content, and Ethylene and CO_2 Production During Senescence of Carnation Flowers. The change of ACC content in petals and in the rest of the flower was compared with the pattern of ethylene production and respiration (Fig. 1a). Although there was a considerable change in the fresh weight of the petals as the flowers senesced, the pattern of the change was very similar, regardless of whether the change was expressed on a fresh weight or per flower basis. The climacteric rise in respiration and ethylene production was accompanied by visible wilting of the flowers. The increase in ACC content of the petals coincided closely with the increase in ethylene production by the flowers; each increased rapidly after 4 d from harvest. The ACC content of the petals increased 30-fold over the next two days, while the ethylene production rate increased 1,000-fold. In the remaining portion of the flower, the ACC content showed a transitory increase 1 d after harvest, and remained at a low level until the onset of senescence. The increase in ACC content of this portion of the flower appeared to precede the rapid increase in ACC content of the petals, and reached a peak while the ACC content of the petals was still rising.

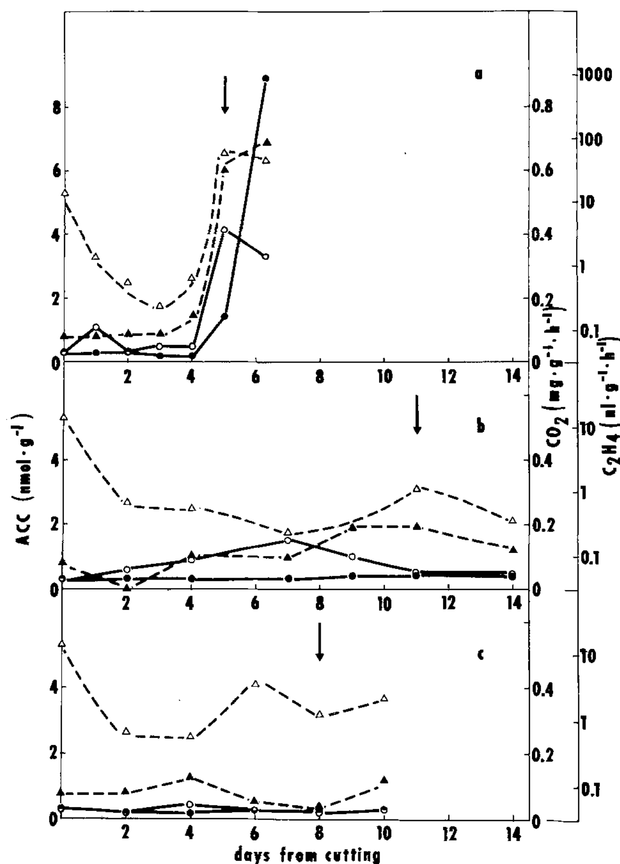

Fig. 1a–c. Changes in ACC content and ethylene and CO_2 production during senescence of cut carnation flowers. Ethylene and CO_2 production were measured in intact flowers. ACC content in the petals and the remaining parts of these flowers was estimated separately. Each value represents the mean of three flowers. **a** Control flowers; **b** flowers pre-treated with 4 mM STS for 10 min; **c** flowers held in vase solutions containing 2 mM AOA. △-----△ CO_2 production by whole flowers, ▲-----▲ C_2H_4 production by whole flowers (graphed against a logarithmic scale), ●—● ACC content of petals, ○—○ ACC content of the remaining portion of the flower. The arrow indicates the first visible signs of senescence

2. Ethylene Production and ACC Content of Carnation Flowers at Different Stages of Senescence. The relationship of the various stages of senescence of cut carnation flowers to their ethylene production and ACC content is shown in Fig. 2. Since rapid loss of fresh weight occurred during wilting of the flowers the results were expressed on a per flower basis. The production of ethylene fell as the petals became severely wilted and dried. ACC content of the remaining portion of the flower followed the same pattern. In contrast, the ACC content of the petals remained high even when they were completely desiccated.

3. Effect of Silver-Thiosulfate Pretreatment on ACC Content, Ethylene Production and Respiration of Car-

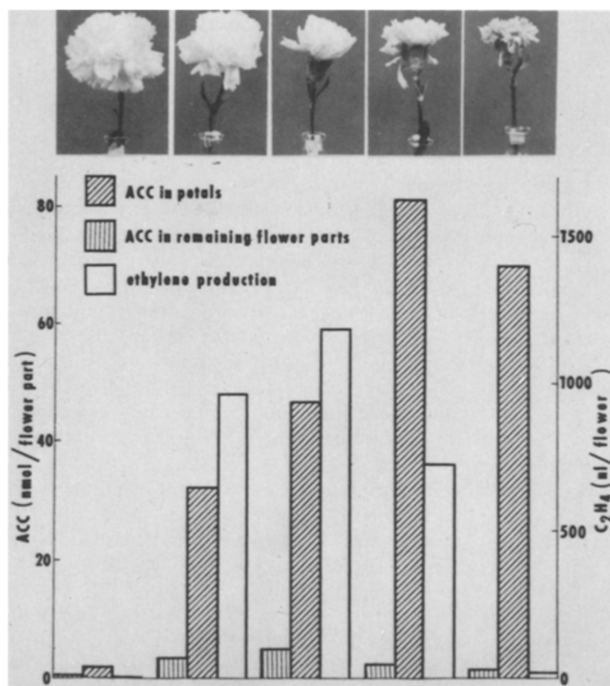

Fig. 2. Ethylene production and ACC content of cut carnation flowers at various stages of senescence. Flowers at different stages of senescence were photographed and their ethylene production was measured before estimation of ACC content of the petals and the remainder of the flower. The stages of senescence, (with the measured ethylene production rates and ACC contents shown beneath) are from left to right: Fresh cut flower, during the climacteric rise, at the climacteric peak, post climacteric, completely desiccated

nations. Changes in the ethylene production and respiration of carnation flowers pretreated with STS are compared with changes in their ACC content in Fig. 1 b. Silver thiosulfate pretreatment extended the vase life of the flowers; the latter did not wilt until 11 d after the start of the experiment, 6 d later than the untreated control flowers. The pretreatment did not reduce the initial levels of ACC or ethylene production, but prevented the climacteric rise in respiration and ethylene production by the flower, and the rise in ACC content of the petals. In the remaining parts of the flower, the ACC content (standard error for both portions of the flower, 0.14 nmol/g) rose steadily after harvest for 7 d, then fell rapidly back to the initial level. The maximum ACC content in the remaining portion of the flower pretreated with STS was less than half that of comparable control flowers.

4. Effect of Aminoxyacetic Acid on ACC Content, Ethylene Production and Respiration of Carnation Flowers. Changes in ethylene production and respiration of carnations held in a solution containing 2 mM AOA are shown in Fig. 1 c. Aminoxyacetic acid prevented the rise in ethylene production normally associated with flower senescence, and delayed wilting of the flowers by 3 d. The transient rise in respiration 6 d after harvest was much less than that seen in control flowers. Determination of the ACC content of senescing AOA-treated flowers required a purification of the aqueous extract using paper chromatography prior to assay of ACC, to avoid an artifact in these extracts which yields ethylene upon reaction with the NaOCl reagent in the normal ACC assay. Separation of ACC was carried out by paper chromatography using the solvent system described in Material and Methods. The ACC content of both the petals and the remaining parts of AOA-treated flowers never exceeded that of freshly-cut flowers throughout the period of the experiment.

Discussion

The changes in ACC content and ethylene production in senescing carnation flowers reported here are similar to those shown by Hoffman and Yang (1980) in ripening fruits. In freshly harvested carnation flowers, ACC content and ethylene production rates were very low. With the onset of senescence, and the accompanying "autocatalytic" rise in ethylene production, there was a rapid increase in the ACC content of the petal tissues. This association between the ethylene production and ACC content of senescing carnation flowers is consistent with the view that ACC is an intermediate in the biosynthesis of ethylene throughout their life. Contrary to a suggestion by Veen (1979), the biosynthetic pathway does not appear to change during senescence. Since the increase in ethylene production during the climacteric rise is much greater than the increase in ACC content of the tissue, it would appear likely that the onset of senescence is associated with an increase in the activity of the enzyme converting ACC to ethylene, as well as in the enzymes involved in the synthesis of ACC.

As senescence progressed, ethylene production fell, but the ACC content of the tissue remained high. This could be the result of a more rapid fall in the rate of ACC conversion to ethylene than in the rate of ACC synthesis. This might be expected since the enzyme system which converts ACC to ethylene is associated with membranes, whereas the enzyme which is responsible for the conversion of S-adenosyl-L-methionine to ACC is located in the cytosol (see Yang 1980).

In parts of the flower other than the petals ("rest"), the pattern of changes in ACC was different from that found in the petals. This tissue does not

senesce with the petals, but becomes the developing fruit. The presence of ACC in this portion of the flower may be related to the onset of growth of the gynoecium, which is associated with the start of flower senescence (Nichols 1971). High rates of ethylene production have been noted during the period of cell division in young fruits (Pratt 1975). Since the rise in ACC in the remaining parts of the flower appeared to precede the rise in the petals, it may also be that ACC synthesized there is translocated into the petals and then converted into ethylene. Nichols (1977) showed that the styles are a major source of ethylene in senescing carnations. The changes in ACC observed in the "rest" fraction may also, therefore, reflect changes in the stylar tissue alone.

Pretreatment of carnation flowers with STS completely prevented the climacteric rise in ethylene production, without affecting basal ethylene production by the flowers, or the basal level of ACC in the petals. These data are consistent with the view that the silver ion inhibits the autocatalytic rise in ethylene production induced by ethylene in normal flower senescence (see review by Lieberman 1979), but not ethylene biosynthesis per se. In contrast to the petal tissue, STS did not completely prevent the increase in ACC content in the "rest" fraction of the flower. It may be that part of this increase is induced by some factor other than ethylene, so that the presence of silver ion does not completely inhibit it. No detectable increase in ethylene production by the whole flower was observed when there was an increase in ACC in the "rest" fraction. This tissue represents only 10% of the total weight of the flower, and an increase in its ethylene production would not have contributed greatly to the total ethylene production.

Since the presence of AOA in the solution increased the life of the flowers, it would appear that the normal senescence of carnation flowers depends on endogenous production of ethylene. AOA-treated flowers did not show normal "sleepiness" (inrolling of the petals) but senesced by desiccation and necrosis of the petals.

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