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1-Aminocyclopropane-1-Carboxylic Acid (ACC)—The Transmitted Stimulus in Pollinated Flowers?

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Abstract. Ethylene production and senescence of petals of pollinated carnation flowers were not prevented by removal of the ethylene produced by the gynoecium, suggesting that these events are a response to movement from the gynoecium of some stimulus other than ethylene gas. Application of 1-aminocyclopropane-1-carboxylic acid (ACC) to the stigmas caused an initial increase in gynoecium and petal ethylene production similar to that reported for pollinated flowers. This response was not seen in flowers whose stigmas were treated with indoleacetic acid (IAA). When [2-^{14}C]ACC was applied to the stigmas of carnation flowers, radioactive ethylene was produced both by the gynoecia and by the petals. The possibility that ACC, transported from the stigmas to the petals, is responsible for the postpollination changes in carnation flowers is discussed.

In a number of flowers it is known that pollination results in stimulated ethylene production and early senescence (Hall and Forsyth 1967, Nichols 1977). These events have been suggested to be a response to IAA present in the pollen (Burg and Dijkman, 1967) or to mechanical wounding of the tissues by the elongating pollen tube (Gilissen 1977). Pollination of carnation flowers causes a sequential increase in ethylene production by their stigmas, ovaries, receptacles, and petals (Nichols et al. 1983). These authors also examined changes in ACC (the immediate precursor of ethylene production in higher plants [Adams and Yang 1979]) in various organs of pollinated carnation flowers (Nichols et al. 1983) and found a sequence that suggested that ACC could be the transmitted stimulus. Subsequent investigations showed the pres-

ence of substantial concentrations of ACC on the exine of pollen from many species (Whitehead et al. 1983a) and suggested that production of this ethylene precursor was stimulated by growth of the pollen tubes (Whitehead et al. 1983b). It therefore seemed quite likely that ACC might be a chemical stimulus, transported to the corolla of the flower following pollination, and causing the changes that lead to hastened senescence of the petals. We report here the results of experiments investigating this hypothesis.

Materials and Methods

Materials

Standard and miniature carnation flowers (*Dianthus caryophyllus* L. cvs. White Sim and Exquisite) were grown in the greenhouse at Davis (day/night, 18/10°C) using normal commercial practices or were acquired from a commercial nursery. ACC, IAA, Tween-20, and Tween-80 were obtained from Sigma and 2-chloroethylphosphonic acid was obtained from Union Carbide. Porapak S, 80–100 mesh, was obtained from Waters Assoc. and S-adenosyl [3,4-^{14}C]methionine was purchased from Research Products International. [2-^{14}C]ACC was prepared enzymatically from S-adenosyl[3,4-^{14}C]methionine (1.8×10^6 Bq/μmole) by reacting with ACC synthase prepared from ripe tomato fruits according to the procedures described by Boller et al. (1979) and Yu et al. (1979). After 12 h, the protein was precipitated by adding perchloric acid (final concentration 5% w/v) to the enzyme solution. After 2 h at 0°C, the solution was centrifuged at 10,000*g* for 10 min; the supernatant was then decanted, neutralized with solid K_2CO_3, and again centrifuged at 10,000*g* for 10 min. The [2-^{14}C]ACC in the supernatant was purified to radioactive homogeneity by ion-exchange chromatography, paper chromatography, and paper electrophoresis as described by Adams and Yang (1979). The specific activity of the ACC was diluted tenfold by adding the appropriate amount of ACC. The ACC solution was evaporated to dryness under N_2 and resuspended in 50 mM potassium phosphate (pH 6.8) plus 0.5% Tween-80.

Pollen was obtained from miniature carnations and applied to the stigmas of standard carnations as previously described (Nichols et al. 1983).

Methods

Measurement of Ethylene Production

To examine the separate ethylene production of petals and gynoecia of flowers, we utilized the apparatus shown in Fig. 1. Ethylene-free air was passed at a known rate through a 0.5-l chamber containing the flower. Air from this chamber was aspirated through two connected halves of a 3-ml syringe barrel covering the gynoecium at half the rate at which air was entering the chamber. We calculated this rate to be sufficient to prevent significant back diffusion of ethylene from the gynoecium into the chamber. To verify this calculation,

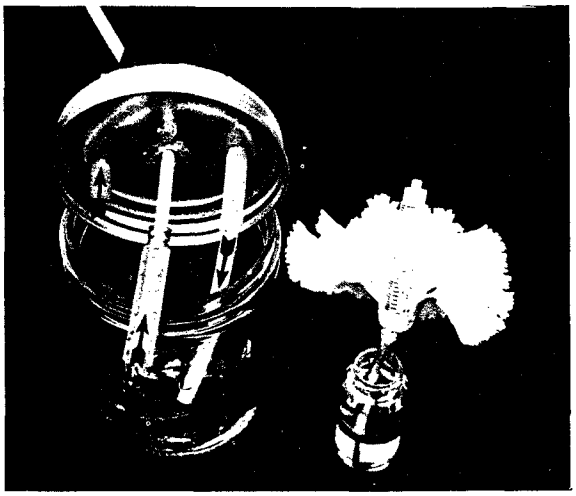

Fig. 1. Apparatus used to measure ethylene production of gynoecia and petals of intact carnation flowers. Arrows indicate the direction of air flow.

stigmas were encircled with filter paper and the flowers were sealed in the chamber. High concentrations of ethylene were then generated within the syringe barrel in the vicinity of the stigmas by applying 10 μl of 2-chloroethylphosphonic acid (20,000 ppm, pH 8) to the filter paper. Ethylene concentrations in the air leaving the chamber or aspirated past the gynoecium were determined by gas chromatography (Bufler et al. 1980).

Exogenous Application of ACC and IAA

Ethylene production by carnation flowers was investigated after applying aqueous 1-mM solutions of IAA and ACC containing 0.01% Tween-20 to the stigmas with a small paintbrush.

Movement of [2-^{14}C]ACC

To examine the movement of ACC from the stigmas to the gynoecium and petals, we applied to the stigmas, using a micropipette, 2 and 20 nmole of a solution containing [2-^{14}C]ACC (2×10^2 Bq/nmole), 0.5% Tween-80, and 50 mM potassium phosphate (pH 6.8). It was necessary to dilute the specific activity of the ACC solution to prevent the stigmas from converting all the label which entered the tissue to ethylene. To prevent cross contamination of the petals this application was made with the lower half of the syringe barrel in place over the gynoecium (Fig. 1). The two syringe halves were then connected and the flower was enclosed in the jar.

[^{14}C]Ethylene was collected from the air lines leading from the chamber by a modification of the procedure of De Greef et al. (1976). The lines were connected to $CaCl_2$ drying tubes which led, through a three-way valve, to glass U tubes (6 mm ID × 11 cm) packed with approximately 0.5 g of Porapak S and held in a dry ice–acetone bath. The other side of the U tube either was

open (main chamber) or was connected to the aspiration line (gynoecium chamber). After ethylene had been absorbed from the air streams for 2 h the three-way valves were used to seal the inlet side of the U tubes while the exit sides were connected to a stopcock on an evacuated 250-ml sidearm flask containing a filter wick (2 × 2.5 cm). The trapped ethylene was transferred to the evacuated flask by opening the stopcock and the three-way valve and allowing a slow stream of air to flow through the U tube and into the flask while the U tube was heated first to 30°C and then to 80°C by immersion in heated water baths. When the vacuum was fully released the flask was sealed and disconnected from the U tube. Ethylene content of the air in the flask was measured by gas chromatography. To measure radioactive ethylene, 100 μl of 0.25 M $Hg(ClO_4)_2$ was injected onto the wick and was allowed to absorb ethylene for 16 h at 4°C. The wick was then placed into 10 ml of scintillation solution and counted in a Beckman Liquid Scintillation counter.

Measurement of Radioactivity Within the Tissue

Approximately 3 h after radiolabel was applied, each flower was separated into styles, ovary, and petals. Styles and ovary (approximately 0.1 and 0.2 g) were ground in a mortar containing washed sand and 2 ml of 80% ethanol; petals were ground in 2 ml/g of 80% ethanol. The homogenates were then centrifuged and radioactivity of the extract was determined by liquid scintillation spectrometry. Quenching was determined by re-counting each sample after addition of an internal standard.

Results

Testing for Back Diffusion of Ethylene

At the start of the experiment, ethylene was generated close to the stigma from 2-chloroethylphosphonic acid. After 2 h, the level of ethylene in the air aspirated past the gynoecium was 2 μl/l whereas it was less than 0.02 μl/l in the air leaving the chamber.

Effect of Removal of Ethylene Produced by the Gynoecium on Senescence of Pollinated Carnations

The mean days to wilting of pollinated carnation flowers was not increased when ethylene produced by the gynoecia was selectively removed using the apparatus shown in Fig. 1 (data not shown).

Effect of ACC and IAA on Ethylene Production in Carnations

Application of a solution containing 1 mM ACC to stigmas of cut carnation flowers stimulated ethylene production immediately by the gynoecia and 20

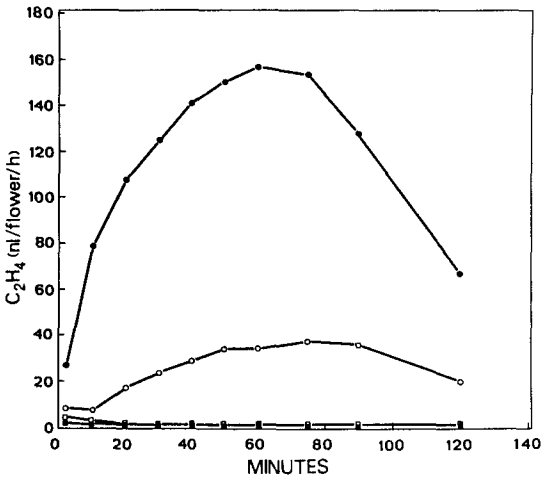

Fig. 2. Ethylene production by carnation gynoecia and petals after treatment of the gynoecia with ACC or IAA. (●——●) Ethylene production by gynoecia after treatment with ACC. (○——○) Ethylene production by petals after treatment of gynoecia with ACC. (□——□) Ethylene production by petals after treatment of gynoecia with IAA. (■——■) Ethylene production by gynoecia after treatment with IAA.

min later by the petals (Fig. 2). A similar application of IAA in 1% ethanol had no effect on ethylene production by either gynoecia or petals during the course of the experiment (Fig. 2).

Movement of [2-^{14}C]ACC Applied to the Stigmas

Two hours after [2-^{14}C]ACC had been applied to the stigmas of cut carnation flowers most of the applied radioactivity was recovered at the point of application (Table 1). Very little radioactivity was recovered in extracts of other parts of the flower. During the 2-h experimental period a substantial amount of the applied [2-^{14}C]ACC was converted to ethylene by the gynoecium, and approximately one-third as much was converted by the petals and calyces. The specific radioactivity of the ethylene produced by the gynoecia was close to that of the applied ACC. However, specific radioactivity of ethylene produced by the petals was half that of the applied ACC. In calculating these specific radioactivities, the ethylene produced by control tissues was subtracted from the ethylene produced by flowers to which ACC had been applied. This allowed us to calculate the specific radioactivity based on ethylene produced in response to exogenous ACC application.

Discussion

The acceleration and coordination of senescence by pollination (the postpollination phenomena) have been suggested to be the result of movement of a message, the pollination "stimulus," from the stigma to the styles, ovary, and petals of the flower (Burg and Dijkman 1967). The observations that pollen contains substantial concentrations of IAA (Curtis, 1943) and that application of IAA to stigmas causes similar responses (in orchids) to those which follow pollination (Burg and Dijkman 1967) have been cited as evidence that the

Table 1. Distribution of radioactivity and production of radioactive ethylene 2 h after a 2 μl application of [2-^{14}C]ACC (200 Bq/nmole) to carnation stigmas. Values are the means from 3 flowers used in 2 separate experiments ± standard error. Average recovery per experiment was 85% ± 2.5%.

	Bq ± SE	Specific activity Bq/nmole ± SE
Total applied	3,600 ± 100	200
Syringe barrel rinse	526 ± 194	—
Stigma extract	2,298 ± 185	—
Ovary extract	0.5 ± 0.2	—
Petal extract	29 ± 3	—
Gynoecium ethylene	253 ± 80	180 ± 45
Petal ethylene	79 ± 25	108 ± 5

"stimulus" might be IAA or some other auxin. However, radioactively labeled IAA applied to the stigma of orchid flowers is almost immobile (Strauss and Arditti 1982), and ethylene production was not stimulated by application of IAA to carnation stigmas (Fig. 2). These observations indicate that IAA is unlikely to be the pollination stimulus.

It has frequently been shown that pollination stimulates ethylene production by certain flowers (Hall and Forsyth 1967, Nichols 1977) and that the post-pollination phenomena can be stimulated by ethylene treatment (Nichols 1971, Arditti et al. 1973). It seemed possible that the stimulus might be ethylene gas, diffusing from the pollinated stigma to the other tissues of the flower. Experiments using an apparatus designed to isolate ethylene produced by the pollinated gynoecium from the rest of the flower (Fig. 1) demonstrated that removal of ethylene produced by the gynoecium had no effect on the time of petal senescence.

Bradford and Yang (1980) showed that ACC could be transported from the roots to the petioles of waterlogged tomato plants before being converted to ethylene. In a previous study of the changes in ACC concentration and ethylene production of different parts of pollinated carnation flowers, we found a sequential rise in the ACC content of the stigmas, ovaries, receptacles, and petals (Nichols et al. 1983). These data suggested that ACC might be the mobile pollination stimulus. The results reported here are strong additional evidence supporting this hypothesis. Not only does application of ACC to carnation stigmas result in a rapid stimulation of ethylene production by the petals (Fig. 2), but also [^{14}C]ethylene is produced by the petals when [2-^{14}C]ACC is applied to the stigmas.

To be certain that radioactive ethylene measured in the petal chamber was produced by the petals it was necessary to rule out the possibility of back diffusion from the syringe barrel surrounding the gynoecium (Fig. 1). When high concentrations of ethylene were generated in the barrel by degradation of 2-chloroethylphosphonic acid applied to a filter disk surrounding the stigmas, back diffusion was found to be less than 1%.

The specific radioactivity of ethylene produced by the petals was half that of the applied [2-^{14}C]ACC (Table 1). This is probably the result of dilution of

transported radioactive ACC by the unlabeled ACC in the petals. There is approximately 0.3 nmole g^{-1} ACC in the petals (Bufler et al. 1980), and approximately 100 Bq of ACC (0.5 nmole) moved into the petals and was converted to ethylene during our experiment. A 50% reduction in specific activity of the ethylene would result if the transported ACC were distributed over 1–2 g of petal tissue, the approximate weight of the basal portion of the petals where most of the ethylene in carnations is produced (Wulster et al. 1982). It is likely that most of the initial ethylene produced by the petals of ACC-treated flowers arose from ACC translocated from the gynoecium rather than being produced *de novo* by the petal cells.

We have demonstrated that the early production of ethylene by the gynoecium of pollinated flowers may originate from ACC present in the pollen (Whitehead et al. 1983b); the subsequent rise in ethylene production was suggested to be the result of growth of pollen tubes toward the ovary (Whitehead et al. 1983a). We propose that the following sequence of events occurs during the pollen-stimulated senescence of flowers. As soon as the flower is pollinated, ACC in the pollen is oxidized to ethylene by the ethylene-forming enzyme (EFE) present in the stigma. During pollen germination, the growing pollen tubes stimulate ACC synthesis by the stigma and style tissues by physically wounding these tissues (Whitehead et al. 1983). This ACC is not only converted to ethylene by the EFE in the style, but is translocated to the petals, where it is also converted rapidly to ethylene. The ethylene so produced may lead to further autocatalytic ethylene production by the petals.

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