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Changes in Ethylene Production and 1-Aminocyclopropane-1-Carboxylic Acid Content of Pollinated Carnation Flowers

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Abstract. Pollination of flowers of standard carnation (*Dianthus caryophyllus* L. cv. White Sim) with pollen from flowers of miniature carnations (*D. caryophyllus* L. cv. Exquisite) caused them to wilt irreversibly within 1 to 2 days. Pollination stimulated a sequential increase in ethylene production by stigmas, ovaries, receptacles, and petals of the flowers. The ACC content of the stigmas increased rapidly in the first few hours after pollination. The possibility that subsequent production of ethylene by other parts of the flower is stimulated by translocated ACC is discussed. Ethylene production and ACC content of other parts of the flower reached their maximum 24 h after pollination. The petal tissues contributed the bulk of the ethylene production *per flower* thereafter. There appears to be a qualitative difference between the enzyme in the stigmas converting ACC to ethylene and that in other parts of the flower.

Pollination causes accelerated wilting and fading of some orchids (Hsiang 1951, Burg and Dijkman 1967, Arditti 1971) and of carnation flowers (Nichols 1977). The morphological changes during pollination-induced carnation senescence are similar to those of normal senescence except that they are condensed into 1 to 2 days, compared with the normal period of about 7 days (at approximately 20°C). Since ethylene treatment of the orchid flower stimulates some of the pollen-induced phenomena (Arditti et al. 1973), it seems probable that this gas

causes some of the symptoms of accelerated senescence. Burg and Dijkman (1967) suggested that pollination of *Vanda* caused transfer of auxin to the stigma, spread of growth hormone to the column and tip, induction of ethylene formation, and diffusion of gas to adjacent tissues where it induced further ethylene production and tissue fading. A similar mechanism could explain the response of the carnation flower to pollination (Nichols 1977). Pollen-induced stimulation of ethylene production from styles has been reported for other species (Hall and Forsyth 1967, Lipe and Morgan 1973, Wallner et al. 1979).

The discovery that 1-aminocyclopropane-1-carboxylic acid (ACC) is the immediate precursor of ethylene in plant tissues (Adams and Yang 1979, Lurssen et al. 1979) suggested to us that it might also be involved in the pollen-induced acceleration of wilting in carnations. Bufler et al. (1980) have shown that the ACC concentration increases 30-fold in carnation petals at the onset of the wilting that is characteristic of normal (unpollinated) senescence.

The study reported here was carried out to determine the changes in ACC content and ethylene production of stigmas, ovaries, receptacles, and petals following pollination of carnations.

Materials and Methods

Pollination

Flowering stems of *Dianthus caryophyllus* L. cv. White Sim were used as the female parent. The stems were cut to 30 cm and placed in deionized water when the outer petals of the inflorescence were reflexed at right angles to the stem. Since this cultivar rarely produces anthers, no precautions were taken to prevent self-pollination.

Flowers of the spray cultivar "Exquisite" were used as the male parent. Undehisced anthers were removed from unopened flowers, placed in an open dish, and left overnight on the laboratory bench. The following day pollen was transferred to the stigma of the "White Sim" flowers by holding the filament of dehisced anthers with forceps and dabbing the pollen lightly on the stigmatic surface. Stigmas of control, unpollinated flowers were touched with sterile forceps to simulate contact of the anther with the stigma. All observations were made on flowers kept in an air-conditioned room at 25 ± 2°C, illuminated continuously by fluorescent lights, irradiance approximately 2 Wm^{-2}, and with a water vapor pressure deficit of about 1.6 kPa (50% RH). Times cited refer to hours from pollination or equivalent for the unpollinated controls.

Sampling and Dissection of Flowers

At approximately 3 and 6.5 h, 6 flowers were selected at random for measurement of ethylene production and ACC content. Petals from these flowers were not visibly wilting. After 24 and 48 h, the petals from most of the pollinated flowers were wilting, and 3 flowers with wilting petals were selected at each time for analysis. Unpollinated flowers were harvested at 0, 24, and 48 h, at which times none of them was wilting.

Flowers were cut from the apex of the stem. Stigmas (2, 3 or occasionally 4 per flower), ovary, and petals were excised. The remaining tissue, minus sepals and bracts, is described as receptacle. All tissues were weighed before measuring their ethylene production and ACC content.

Measurement of Ethylene Production

Tissues were enclosed in glass vessels fitted with gas sampling ports for about 15 min. Gas samples were withdrawn from the headspace, and the ethylene concentration measured by gas chromatography using an alumina column and a photoionization detector (Bufler et al. 1980). Peak heights and retention times for ethylene were compared with those for ethylene reference standards.

Estimation of ACC

Fresh tissue was frozen in liquid nitrogen, then ground and extracted in 3% v/v perchloric acid (PCA) in a pestle and mortar. The volume of PCA added per unit fresh weight of tissue was 3-, 4-, and 5-fold for petals, receptacles and ovaries, and stigmas respectively. After standing at 2°C overnight, the homogenate was centrifuged at 25,000 g for 15 min. The ACC content of the supernatant was estimated using a method adapted from Bufler et al. (1980) and Lizada and Yang (1979). The supernatant (0.1 ml), mercuric chloride (0.1 ml of a 10 mM solution), and 0.6 ml of water were mixed in a test tube. The tube was sealed with a serum cap and 0.2 ml of an ice-cold mixture (2:1 v/v) of commercial bleach (5.25% NaOCl) and saturated NaOH was injected. The tube was then vortexed for 15 sec, placed in an ice bath, and after 3 min vortexed again for 15 sec. A 3-ml gas sample was then withdrawn for measurement of ethylene. The efficiency of ACC conversion to ethylene was determined by adding at least 3 times the anticipated ACC content as an internal standard to a replicate assay tube.

Statistical Analyses

Owing to the substantial increase in means and variances of the data as a result of the pollination treatment, data were compared by analysis of variance after logarithmic transformation.

Results

Changes in Ethylene Production

At the start of the experiment, the production of ethylene by stigmas was more than 10 times that of the petals and receptacles and more than 6 times that of the ovaries (Fig. 1). Within 3 h, ethylene production by the pollinated stigmas had risen another 10-fold, and production by ovaries and receptacles had more

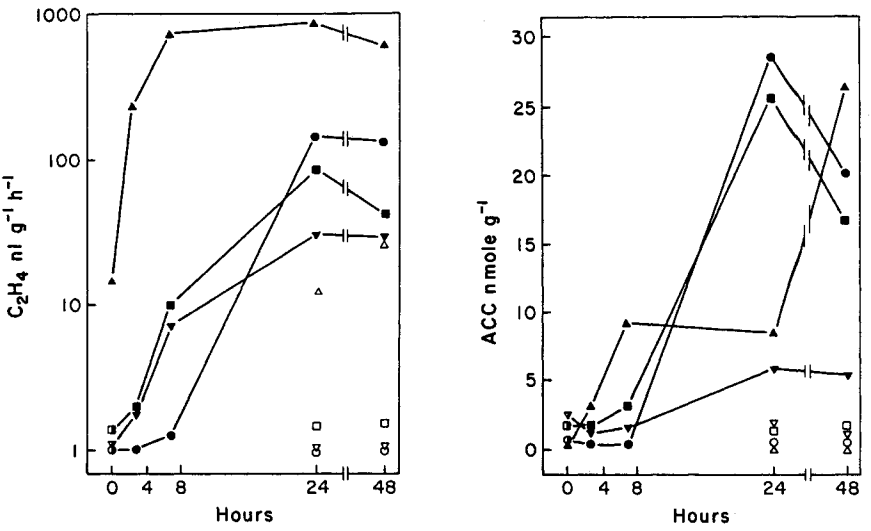

Fig. 1. Ethylene production per unit fresh weight by parts of pollinated carnation flowers. ▲stigmas ■ovaries ▼receptacle ●petals, closed symbols—pollinated flowers, open symbols—unpollinated flowers.
Fig. 2. ACC content per unit fresh weight of parts of pollinated carnation flowers. ▲stigmas ■ovaries ▼receptacle ●petals, closed symbols—pollinated flowers, open symbols—unpollinated flowers.

than doubled. At 6.5 h, production by these 3 tissues had increased even further, but petal tissues had not started to produce substantial quantities of ethylene.

Twenty-four and 48 h after pollination, ethylene production by all tissues from pollinated flowers was very high. Ethylene production by corresponding samples from unpollinated flowers did not change significantly during the period of the experiment.

Changes in ACC Content

At pollination, the ACC content of all the tissues was between 0 and 2.5 n moles/g. ACC content of the stigmas was very low. During the first 6.5 h, there was no significant change in ACC content of any tissue except the stigma, in which the ACC content rose approximately 10-fold (Fig. 2). By 24 h, the pattern had changed dramatically, with a huge accumulation of ACC in petals and ovaries, a substantial increase in ACC in the receptacles, but no further change in ACC content of the stigmas. At 48 h, there was some fall in petal and ovary ACC and a 3-fold increase in stigma ACC levels.

Changes in Total Ethylene Production

Despite the relatively small mass of the stigmas (0.1–0.2 g fresh weight), their ethylene production per flower was 90% of the total ethylene production at 6.5 h (Fig. 3) and was still 12% of the total when the petals (9–10 g) were producing ethylene at their peak rate (24 h).

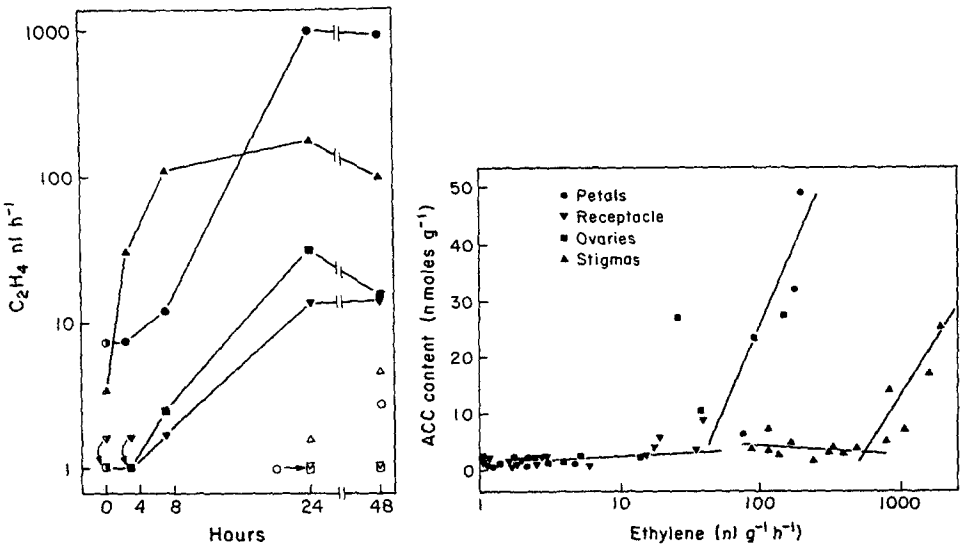

Fig. 3. Ethylene production per tissue by parts of pollinated carnation flowers. ▲stigmas ■ovaries ▼receptacle ●petals, closed symbols—pollinated flowers, open symbols—unpollinated flowers.

Fig. 4. Relationship between ethylene production and ACC content of carnation tissues. Ethylene production and ACC content during the first 24 h after pollination are plotted for each individual sample of each of the tissues studied. ▲stigmas ■ovaries ▼receptacle ●petals.

Relationship Between Ethylene Production and ACC Content of Tissues from Pollinated Flowers

A graph of ethylene production as a function of ACC content for each individual tissue sample (Fig. 4) shows that ethylene production by the tissue increased substantially before there was any significant increase in ACC content. For petals, receptacles, and ovaries, the ACC content started to rise only when the ethylene production rose above 10 nl $g^{-1}h^{-1}$. For stigmas, the ethylene production was over 600 nl $g^{-1}h^{-1}$ before ACC content started to rise.

Stimulation of Ethylene from Stigmas by Pollen

Ethylene production from pollen alone was determined using vials and enclosure times similar to those used for pollinated stigmas. We could not detect ethylene in amounts sufficient to account for the rapid rise in ethylene produced by pollinated stigmas.

Determination of the Time Course of Stigma Ethylene Production

To find out whether there were significant rises in stigma ethylene earlier than 3 h from pollination, ethylene production was measured 1 and 3.5 h from pollination and compared with that from unpollinated flowers. One hour after pollination, ethylene production by the pollinated stigmas was slightly (but not significantly) higher than that of stigmas that had been mechanically stimulated

Table 1. Mean ethylene and ACC production (n = 3 flowers) from stigmas of pollinated (P) and unpollinated (NP) flowers. Values in columns with no subscript letter in common are significantly different (p = 0.05).

Hours from pollination	Treatment	Ethylene nl h^{-1}	nl $g^{-1}h^{-1}$	ACC nmol	nmol g^{-1}
1	NP	3.3_b	21.4_b	—	—
	P	4.5_b	27.1_b	—	—
3.5	NP	1.5_c	11.2_c	0.06_b	0.43_b
	P	11.1_a	64.9_a	0.18_a	1.15_a

(Table 1). At 3.5 h, production of ethylene in mechanically stimulated stigmas had fallen to less than half that of the initial controls, but had increased 3-fold in the pollinated stigmas. This difference was reflected in the ACC content of the tissues.

Discussion

The data reported here demonstrate a dramatic increase in the already substantial ethylene production of carnation stigmas shortly after pollination (Fig. 1). Because the rise in ethylene production probably occurs later than 1 h and earlier than 3 h after pollination, it appears likely to be associated with germination of the pollen rather than fertilization of the ovules. Pollen produced little or no ethylene, so this stimulated production is likely to be the result of an interaction between the pollen and the stigma tissue, probably passage of the pollen tube through the stigma. Gilissen (1977) suggested that in pollinated petunia flowers wilting is coordinated by a signal that could be duplicated by simply injuring the stigma. The observation that the ACC content and ethylene production of carnation stigmas is stimulated by pollination suggests that in carnation and petunia the events following pollination may be a response to penetration of the stigmatic tissue by the growing pollen tubes—a simple wound response (Yang and Pratt 1978).

Production of wound ethylene in *Phaseolus* leaves has been attributed to formation of newly synthesized ACC (Konze and Kwiatkowski 1981). Bradford and Young (1980) have attributed epinasty of petioles of *Lycopersicon* to ethylene formed from ACC generated in the waterlogged roots and transported to the leaves in the transpiration stream, indicating that ACC can act as a transported hormone. The data reported here raise the possibility that the rapid senescence of carnation flowers in response to pollination is a response to ACC translocated from the stigma to the rest of the flower. The sequential rise in ethylene production in the stigma, ovary, receptacle, and lastly petal tissues supports such a hypothesis.

Since ethylene-stimulated ethylene production normally occurs only after a lag of some hours (Riov and Yang 1982), it is unlikely that stigma ethylene can be responsible for the rapid increases in ethylene synthesis seen in the other flower tissues. A solute such as ACC, which is known to cause wilting of carnation petals (Mor and Reid 1981), seems a more appropriate link between

stigmas and petals than ethylene itself. It is possible that other growth regulators present in pollen (Burg and Dijkman 1967) or elicited in the style by pollen (Stanley and Linskens 1974) might be transported stimuli. Auxin does not seem a likely candidate, since labeled IAA does not move in adequate amounts to account for the response (Burg and Dijkman 1967). Certainly, however, synthetic growth regulators and pollination both promote petal wilting, ethylene production, and ovary growth in carnation flowers (Nichols 1977).

In studies on the changing patterns of ethylene production and ACC content of plant organs, it has normally been found that the precursor is present in high amounts only when the tissues are producing substantial quantities of ethylene. This association has been reported in wound-induced ethylene synthesis (Konze and Kwiatkowski 1981, Yu and Yang 1980), in virus-infected tobacco leaves (de Laat et al. 1981), in senescing flowers (Bufler et al. 1980) and in ripening fruits (Hoffman and Yang 1980). In striking contrast to all these systems, freshly cut stigmas from carnation flowers, which were already producing substantial amounts of ethylene, contained less ACC than any other tissues of the flower. The data in Fig. 4 show that in carnation flower tissues the relationship between ACC and the enzyme converting it to ethylene is such that no appreciable increase in ACC content occurs until the tissues are producing more than 10 nl C_2H_4 $g^{-1}h^{-1}$ (600 nl C_2H_4 $g^{-1}h^{-1}$ for stigmas).

Despite the relatively low mass of the stigmas, their ethylene production was a major part of the total ethylene production by the flower even when the petals were at their maximum ethylene production (Fig. 1). The considerable production of ethylene by unpollinated stigmas (Table) and the much higher ethylene production by stigmas before ACC content increases (Fig. 4) suggest that their ethylene production may be qualitatively different from that of other parts of the flower. The 3-fold increase in ACC content of the stigmas between 24 and 48 h was accompanied by a slight decrease in ethylene production, indicating a loss of enzyme activity during that period, possibly associated with stigma necrosis.

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