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Pollination-Induced Ethylene in Carnation[1]

Role of Stylar Ethylene in Corolla Senescence

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In carnation (*Dianthus caryophyllus* L. cv White Sim) cell to cell communication between the pollen and pistil induces ovary development and corolla senescence. The production of elevated ethylene by the style is the first measurable postpollination response. This is followed by a wave of ethylene production from the other floral organs. To investigate the regulation of ethylene biosynthesis in pollinated flowers we measured ethylene production and the expression of 1-aminocyclopropane-1-carboxylate synthase and 1-aminocyclopropane-1-carboxylate oxidase transcripts in individual floral organs after pollination. Ethylene production by pollinated styles can be defined temporally by three distinct peaks. By pollinating a single style from a multistyle gynoecium, it was determined that the unpollinated style produces ethylene that corresponds to the first and third peaks observed from a pollinated style. Inhibition of ethylene action in the pollinated style by diazocyclopentadiene treatment prevented both pollination-induced corolla senescence and ethylene production from the ovaries and petals. Treatment with diazocyclopentadiene decreased stylar ethylene production during the second peak and completely inhibited the third peak of ethylene in both pollinated and unpollinated styles. This later autocatalytic ethylene in styles is likely responsible for pollination-induced corolla senescence and ovary development.

The plant hormone ethylene plays an important role in interorgan signaling following pollination in many plant species (Stead, 1992; Larsen et al., 1993, 1995; Woltering et al., 1995). Pollination often accelerates ethylene biosynthesis, and this ethylene is involved in postpollination developmental processes, including petal senescence and ovary growth (Stead, 1992; Woodson et al., 1992). The stigmatic surface of the flower is the initial site of pollination perception. In flowers of carnation (*Dianthus caryophyllus* L.) and petunia (*Petunia hybrida* L.), the interaction between the pollen and pistil leads to an increase in ethylene production from the stigma and style. This ethylene is detectable within 1 h of pollination, before any evidence of pollen tube germination or penetration of the stigma (Nichols et al., 1983; Whitehead et al., 1983a; Hoekstra and Weges, 1986; Pech et al., 1987; Larsen et al., 1993, 1995). In petunia both foreign pollen from unrelated species and incompatible petunia pollen elicits this early ethylene production from the pollinated stigma/style (Hoekstra and Weges, 1986; Singh et al., 1992). An incompatible pollination results in only transient ethylene production by the pollinated stigma/style and does not accelerate corolla senescence (Hoekstra and Weges, 1986; Singh et al., 1992; Larsen et al., 1995). Taken together, these results indicate that the distinction between a compatible and an incompatible pollen source occurs beyond the initial increase in ethylene by the stigma and plays a role in postpollination signaling. Furthermore, these results point to the production of a transmissible signal, independent of the original pollen-derived factor that is responsible for interorgan communication.

Gilissen and Hoekstra (1984) provided evidence for the existence of a translocated pollination signal by showing that removal of the pollinated petunia style more than 4 h after pollination is ineffective in preventing accelerated corolla senescence. The source of this translocatable pollination signal is unclear, but auxin, ACC, and ethylene have been implicated (Reid et al., 1984; Hoekstra and Weges 1986; Woltering, 1990; Woltering et al., 1991, 1995; O'Neill et al., 1993). Sequential increases in ACC and ethylene within carnation floral organs following pollination suggest that either may be involved in interorgan signaling in carnation flowers (Nichols, 1977; Nichols et al., 1983; Woodson et al., 1992; Woltering et al., 1995).

Following a compatible pollination, pollinated carnation styles continued to produce elevated ethylene after the first ethylene peak, which could be defined temporally by two additional peaks (Larsen et al., 1995). The second peak was observed approximately 12 h after pollination. Ethylene biosynthesis then decreased by 18 h and peaked again 24 to 36 h after pollination. We are interested in the regulation of ethylene biosynthesis within the individual floral organs of a carnation flower following pollination. In this study we investigate the regulation of ethylene biosynthesis in carnation flowers and the role of stylar ethylene in postpollination signaling.

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Abbreviations: DACP, diazocyclopentadiene; NBD, 2,5-norbornadiene.

MATERIALS AND METHODS

Carnations (*Dianthus caryophyllus* L.) grown under standard greenhouse conditions were used in all of the experiments. Carnation cv White Sim flowers were harvested slightly after anthesis when the styles had fully elongated and were receptive to pollination. Stems were recut to 10 cm, placed in deionized water, and held in the laboratory. Flowers were pollinated by brushing one stigma on each flower with freshly dehisced anthers from cv Starlight carnations. We have previously shown that crosses between cv Starlight and male sterile cv White Sim resulted in the production of viable seeds and induced premature corolla senescence (Larsen et al., 1995).

Chemical Treatment

For treatment with ethylene flowers were sealed in a 24-L chamber, and ethylene was injected to yield a final concentration of 10 μL/L. For NBD treatments liquid NBD was injected onto filter paper in a 24-L chamber to yield a concentration of 2500 μL/L after volatilization. Control flowers were held in chambers without added ethylene or NBD. Flowers were removed from NBD at various times after pollination, and floral organs were immediately isolated for analysis of ethylene production. Carnations were removed from ethylene and held in air for 30 min prior to ethylene analysis to allow exogenously applied ethylene to dissipate. To treat individual pollinated styles with DACP, styles were isolated by sealing a 1.5-mL centrifuge tube around the base of the style with petroleum jelly. The tube was capped with a septa, and photoactivated DACP (Sisler and Blankenship, 1993) was injected into the tube to give a final concentration of 5 μL/L. The tubes were removed after 30 min, flowers were exposed to air for 10 min to allow excess DACP to dissipate, and the single treated style on each flower was pollinated. Styles from control pollinated flowers were treated for 30 min with air and then pollinated. Entire flowers were treated with DACP for 30 min by placing them in a 1-L jar and injecting DACP to a final concentration of 5 μL/L.

Ethylene Measurements

The rate of ethylene production by individual styles, ovaries, and petals was measured by enclosing tissue isolated from intact flowers in air-tight vials for 15 min. The headspace in these vials was then sampled and analyzed for ethylene using a gas chromatograph (Varian, Sunnyvale, CA) equipped with an activated alumina column and flame-ionization detector. To determine ethylene production from pollinated styles still attached to the flower, 6-mL tubes were sealed to the base of the styles (enclosing all styles of the gynoecium) with a siliconized acrylic latex caulking (Squeeze N Caulk, Elmer's, Columbus, OH). When capped with a septum, this apparatus proved to be air-tight. To measure ethylene from the pollinated style remaining attached to the flower, this vial was capped for 15 min, and a gas sample was analyzed as described above. Each experiment utilized a replication of at least six flowers per treatment. Graphed values represent the mean ethylene production ± SE for the replications. All experiments presented were conducted a minimum of three times with similar results.

RNA Extraction and Gel-Blot Analysis

Treated carnation flower tissue was frozen in liquid N_2 and stored at −70°C until used for RNA extraction. Total RNA from carnation tissue was extracted as previously described (Lawton et al., 1990) and quantified spectrophotometrically. Ten micrograms of total RNA was separated by electrophoresis through a 1% (w/v) agarose gel containing 2.2 M formaldehyde. The separated RNAs were transferred to Nytran membranes (Schleicher & Schuell) and cross-linked with a controlled (Stratalinker, Stratagene) UV light source. Membranes were prehybridized for 4 h at 42°C and hybridized for 22 h at 42°C with 5×10^5 cpm/mL ^{32}P-labeled cDNA, as described previously (Jones et al., 1995). Membranes were washed at 55°C in 2× SSC and 0.1% SDS for 45 min and exposed to Kodak XAR-5 film using a single intensifying screen at −70°C. Blots were used for multiple hybridizations by stripping in boiling 0.1% SDS. For detection of ACC synthase a 1175-bp PCR clone DCACS2 (Henskens et al., 1994) and a 1250-bp fragment DCACS1 (Park et al., 1992) were utilized. ACC oxidase expression was detected with the carnation cDNA DCACO1 (Wang and Woodson, 1991). Blots using DCACO1 as the probe were exposed to film for 18 to 24 h, whereas blots probed with DCACS1 and DCACS2 were exposed for approximately 3 d.

RESULTS

Postpollination Response of Unpollinated Styles from a Pollinated Gynoecium

The cv White Sim has a syncarpous gynoecium with from two to four styles. This anatomical characteristic allowed us to investigate the role of pollination-induced ethylene production by unpollinated styles from a gynoecium in which only one style had been pollinated. When one style within a multistyle carnation flower was pollinated, elevated ethylene biosynthesis was detected from all styles within the flower (Fig. 1). Immediately following pollination, unpollinated styles from a pollinated gynoecium produced a small peak of ethylene that was equivalent to that detected from the pollinated style (Fig. 1A). Ethylene production from the unpollinated styles then decreased to levels only slightly higher than those measured from styles of unpollinated control flowers (Fig. 1B). At 24 h after pollination, the unpollinated styles within a pollinated flower started to produce elevated ethylene levels similar to that produced by adjacent pollinated styles. By 36 to 48 h after pollination, ethylene production by the unpollinated styles was equivalent to levels synthesized by the pollinated styles.

Unpollinated styles were stained with aniline blue and observed under fluorescence microscopy to ensure that they had not inadvertently been pollinated. No pollen

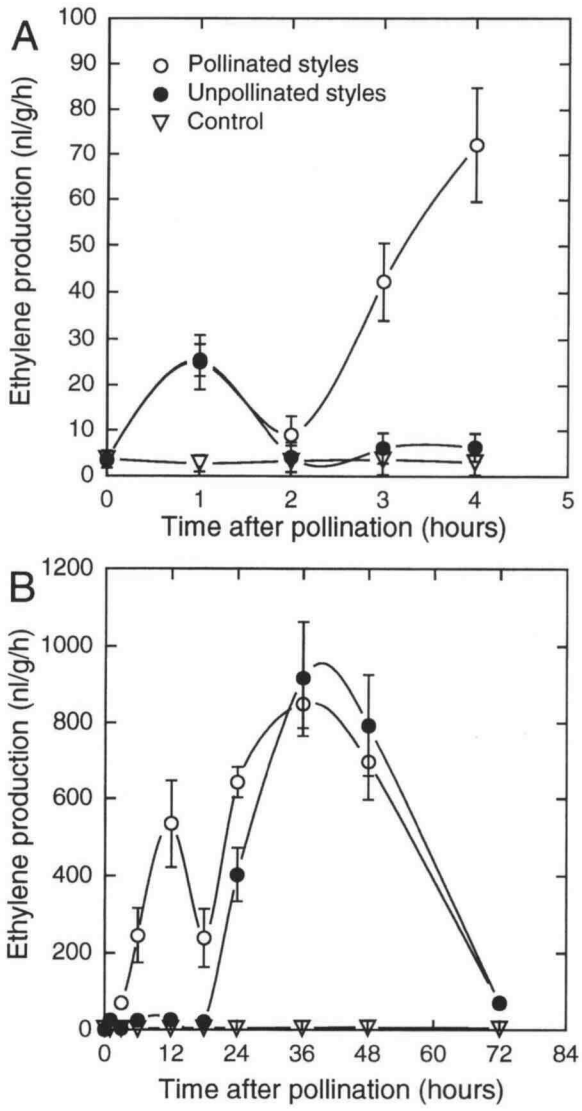

Figure 1. Ethylene production by pollinated and unpollinated styles from a pollinated flower and control styles from an unpollinated flower. Individual styles were isolated from intact flowers and enclosed in a 6-mL vial, and ethylene production was measured after 15 min. Values represent means ± SE of 12 flowers. A, Early ethylene production by styles from 0 to 4 h after pollination. B, Ethylene production by styles from 0 to 72 h after pollination.

tubes were observed in the unpollinated styles (data not shown). Induction of ethylene biosynthetic pathway genes in the unpollinated styles was delayed from that seen in the pollinated styles (Fig. 2). The abundance of ACC synthase mRNAs, as detected by the cDNA DCACS2, increased 1 h after pollination in both pollinated and unpollinated styles from a pollinated carnation flower. Levels of ACC synthase mRNA remained undetectable in control styles from unpollinated flowers throughout the experimental period (data not shown). Levels of ACC synthase mRNA further increased in the pollinated styles 6 h after pollination, but this increase was delayed to 12 h in unpollinated styles from the same flower. In contrast to ACC synthase, ACC oxidase mRNA was present at high constitutive levels in control styles. Whereas the abundance of ACC oxidase mRNA increased following pollination, it was not clear that the pattern differed for pollinated versus unpollinated styles.

Effect of NBD on Pollination-Induced Ethylene

To determine the role of ethylene in the regulation of pollination-induced ethylene biosynthesis and senescence, carnation flowers were placed in an atmosphere of NBD immediately after pollination. Treatment with NBD prevented corolla senescence, and pollinated carnations were visually indistinguishable from unpollinated controls (data not shown). In addition to corolla senescence, pollination leads to a rapid increase in ethylene production by the stigma/style and subsequent waves of increased ethylene in the ovary and the petals. Treatment of pollinated flowers with NBD prevented this increase in ethylene production by both ovaries and petals (Fig. 3). An increase in the abundance of ACC oxidase mRNAs was detected in pollinated ovaries 6 h after pollination and increased further until 18 h, after which time mRNA levels decreased (Fig. 4). The treatment of pollinated flowers with NBD prevented this increased ACC oxidase expression. ACC synthase gene expression was not detected in pollinated ovaries by either the DCACS1 or DCACS2 cDNA probes. Both ACC oxidase (DCACO1) and ACC synthase (DCACS1) transcripts were detected in petals 18 h after pollination and further increased until 48 h after pollination (Fig. 4). Petals from pollinated flowers did not accumulate ACC synthase mRNAs, as detected by DCACS2. The increase in ACC synthase and ACC oxidase mRNAs in petals following pollination was prevented by treatment with NBD.

Both pollinated and unpollinated styles from NBD-treated pollinated flowers produced ethylene immediately following pollination, corresponding to the first ethylene peak detected in pollinated and unpollinated styles from flowers pollinated and held in air (Fig. 5). Twelve hours

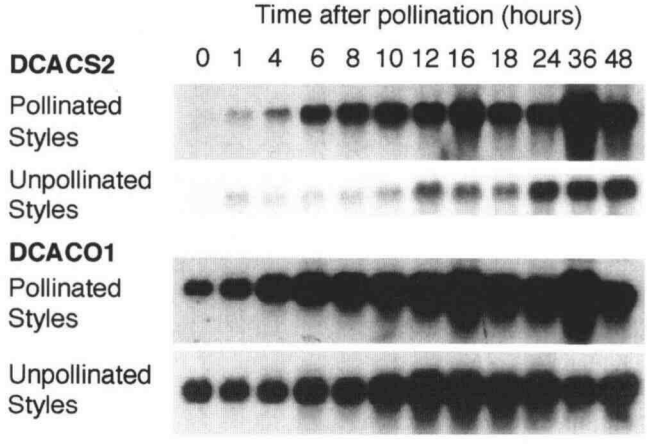

Figure 2. RNA gel-blot analysis of ACC synthase and ACC oxidase mRNAs in pollinated and unpollinated styles from a pollinated flower. Ten micrograms of total RNA isolated from style tissue was separated by electrophoresis through agarose and hybridized with ^{32}P-labeled DCACS2 and DCACO1.

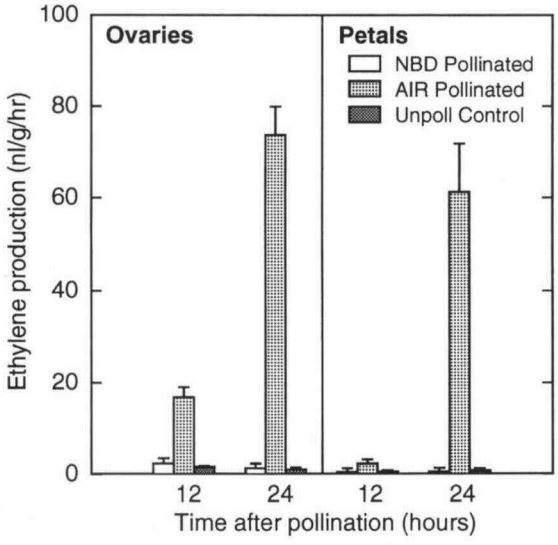

Figure 3. Ethylene production by ovaries and petals from pollinated flowers treated with air or 2500 μL/L NBD and control ovaries and petals from unpollinated (Unpoll) flowers treated with air. Individual ovaries and petals were isolated at various times after pollination and enclosed in a 6-mL vial and ethylene production was measured after 15 min. Values represent means ± SE of six flowers.

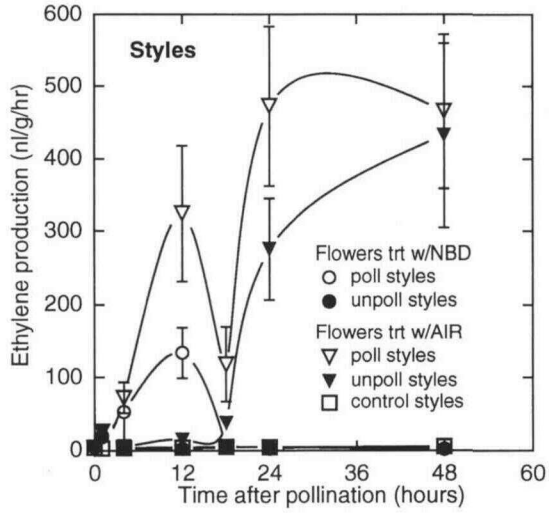

Figure 5. Ethylene production by pollinated (poll) and unpollinated (unpoll) styles from pollinated flowers treated (trt) with air or 2500 μL/L NBD and control styles from unpollinated flowers treated with air. Ethylene production was determined at various times after pollination by isolating styles from the intact flower and enclosing them in a 6-mL vial for ethylene measurements. Values represent means ± SE of six flowers.

after pollination the ethylene produced by pollinated styles from NBD-treated flowers was only one-half that of pollinated styles from flowers in air. After 18 h, little ethylene could be detected from the NBD-treated, pollinated styles. The unpollinated styles from the NBD-treated flowers also failed to produce the third peak of ethylene. NBD treatment prevented the increase in ACC oxidase mRNAs following pollination in both the pollinated and unpollinated styles but did not prevent the constitutive expression of ACC oxidase (Fig. 6). The level of ACC synthase mRNA increased 1 h after pollination in both styles, but further increases were inhibited by NBD. In both pollinated and

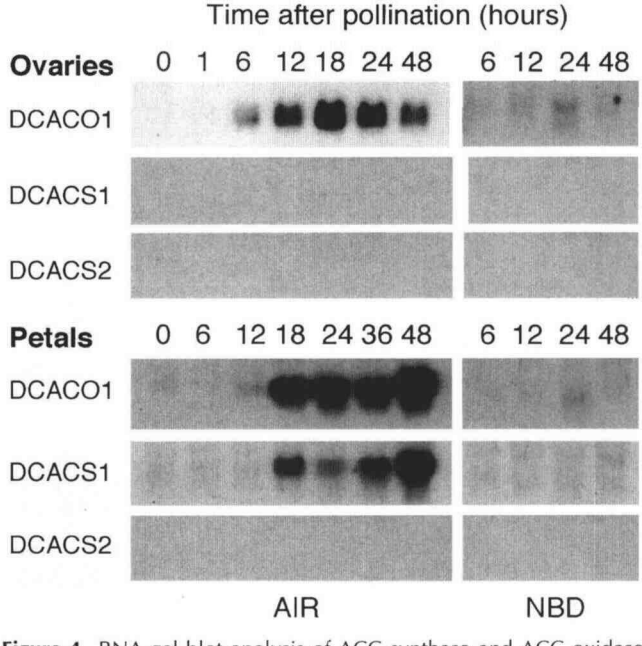

Figure 4. RNA gel-blot analysis of ACC synthase and ACC oxidase mRNAs in ovaries and petals from pollinated flowers treated with air or 2500 μL/L NBD. Ten micrograms of total RNA isolated from ovary and petal tissue was separated by electrophoresis through agarose and hybridized with ^{32}P-labeled DCACS1, DCACS2, and DCACO1.

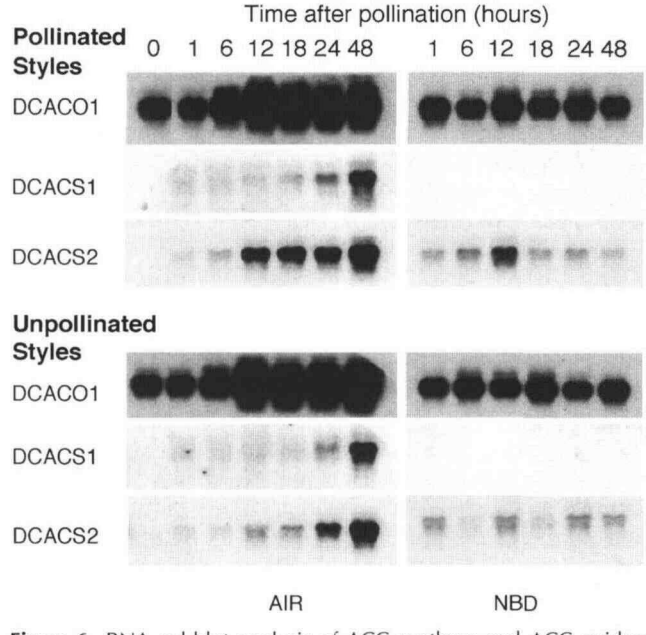

Figure 6. RNA gel-blot analysis of ACC synthase and ACC oxidase mRNAs in pollinated styles and unpollinated styles from a pollinated flower treated with air or 2500 μL/L NBD. Ten micrograms of total RNA isolated from style tissue was separated by electrophoresis through agarose and hybridized with ^{32}P-labeled DCACS1, DCACS2, and DCACO1.

unpollinated styles a low level of ACC synthase (DCACS1) mRNA was detected 48 h after pollination. This was not detected in either style when flowers were treated with NBD.

The Effect of DACP on Pollination-Induced Ethylene

In an attempt to localize the effects of the inhibition of ethylene action on flower senescence, individual styles still attached to the flowers were treated with DACP. Preirradiated DACP is an inhibitor of ethylene action, which has been shown to inactivate ethylene binding and block physiological activity irreversibly (Sisler and Blankenship, 1993). Inhibiting ethylene action in only the pollinated style of a carnation flower effectively prevented petal senescence (data not shown). These pollinated flowers did not exhibit premature corolla inrolling or elevated ethylene biosynthesis from the ovaries or petals (Fig. 7). Treatment of the pollinated style with DACP prevented the increase in abundance of ACC oxidase mRNA in the pollinated ovaries and petals and prevented the increase in abundance of ACC synthase mRNA in petals (Fig. 8). The early increase in ethylene production from pollinated and unpollinated styles was unaffected by DACP treatment, whereas ethylene biosynthesis from the DACP-treated pollinated style 12 h after pollination was less than one-half of the ethylene synthesized by pollinated styles treated with only air (Fig. 9).

Subsequent ethylene production was completely inhibited in both the pollinated and unpollinated styles by treatment of the pollinated style with DACP. RNA gel-blot analysis revealed a limited increase in ACC synthase mRNA 12 h after pollination in pollinated styles treated with DACP, with decreased levels detected by 24 h after pollination (Fig. 10). The levels of ACC synthase mRNA were less than the levels detected in pollinated styles treated with air. The unpollinated styles from the DACP-treated flowers exhibited only a slight increase in ACC synthase mRNAs 24 h after pollination. The abundance of ACC oxidase mRNA increased slightly at 12 h in the DACP-treated pollinated styles, with only constitutive levels detected in the corresponding unpollinated styles.

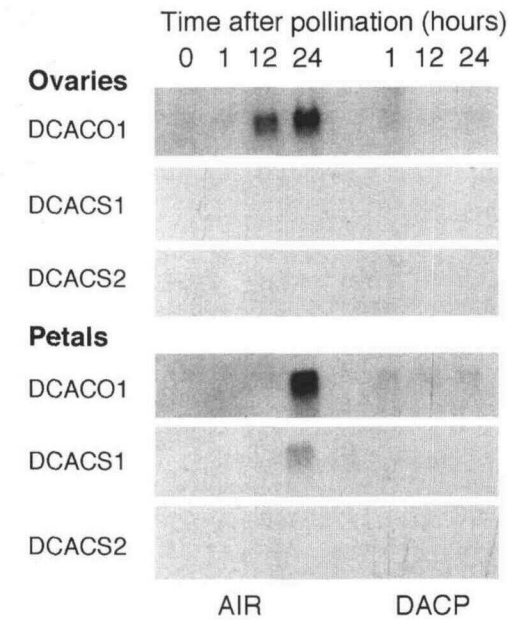

Figure 8. RNA gel-blot analysis of ACC synthase and ACC oxidase mRNAs in ovaries and petals from flowers with the pollinated styles treated with air or 5 μL/L DACP for 30 min. Ten micrograms of total RNA isolated from ovary and petal tissue was separated by electrophoresis through agarose and hybridized with ^{32}P-labeled DCACS1, DCACS2, and DCACO1.

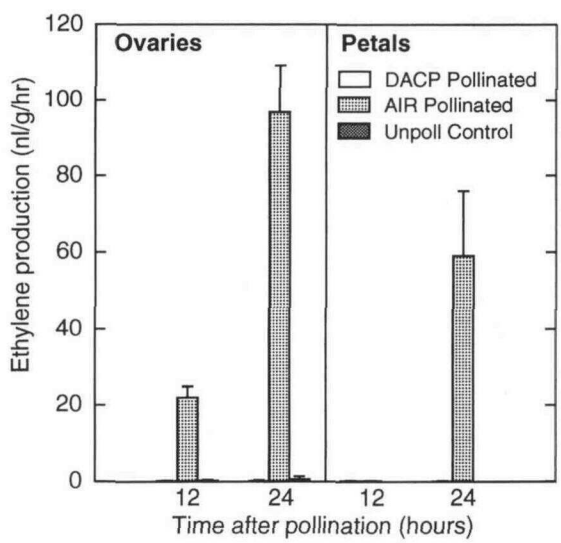

Figure 7. Ethylene production by ovaries and petals from flowers with the pollinated style treated with air or 5 μL/L DACP for 30 min and control ovaries and petals from unpollinated (Unpoll) flowers treated with air. Individual ovaries and petals were isolated from the intact flower 12 and 24 h after pollination for ethylene measurements. Values represent means ± SE of six flowers.

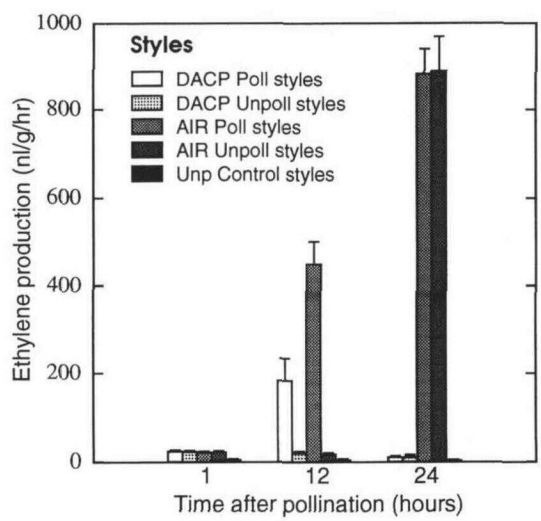

Figure 9. Ethylene production by pollinated (Poll) and unpollinated (Unpoll) styles from flowers with pollinated styles treated with air or 5 μL/L DACP for 30 min and control styles from unpollinated flowers treated with air. Individual styles were isolated from the intact flower 1, 12, and 24 h after pollination for ethylene measurements. Values represent means ± SE of six flowers.

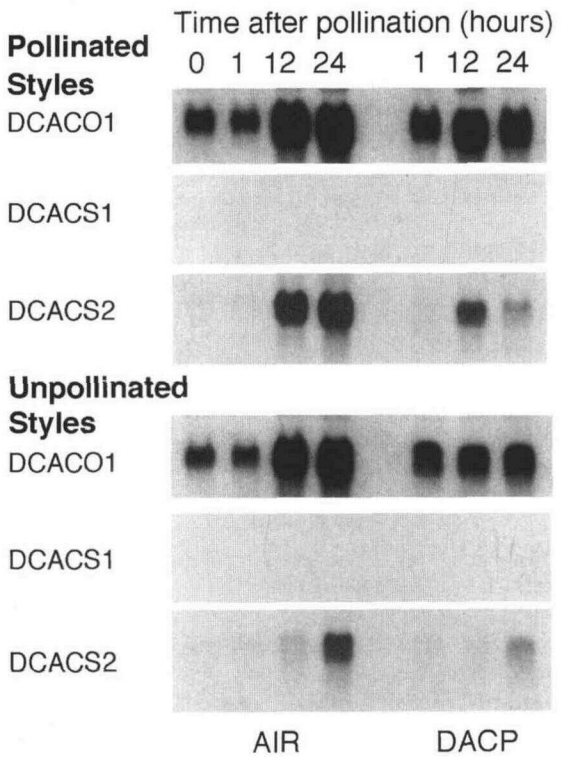

Figure 10. RNA gel-blot analysis of ACC synthase and ACC oxidase mRNAs in pollinated styles and unpollinated styles from flowers with the pollinated style treated with air or 5 μL/L DACP for 30 min. Ten micrograms of total RNA isolated from style tissue was separated by electrophoresis through agarose and hybridized with ^{32}P-labeled DCACS1, DCACS2, and DCACO1.

In an attempt to confirm that the DACP treatment was limited to the style, flowers with DACP-treated styles were treated with ethylene and assessed for ethylene responsiveness in the petals. When treated with 10 μL/L ethylene for 18 h, flowers with DACP-treated pollinated styles exhibited petal inrolling similar to non-DACP- treated control flowers in ethylene (data not shown). When the entire flower was treated with DACP for 30 min, subsequent ethylene treatment did not cause petals to inroll or produce elevated levels of ethylene (data not shown). Taken together, these data indicate that the effects of DACP were confined to the style.

In Situ Ethylene Production by Pollinated Styles

To ensure that the isolation of styles for ethylene determination provided an accurate assessment of ethylene production by styles after pollination, ethylene production was measured from styles still intact on the flower. This method of measuring stylar ethylene production was also utilized to investigate a phenomenon observed frequently during pollination experiments. In previous experiments, it was observed that within a group of cv White Sim flowers pollinated with cv Starlight pollen as many as one-third of the pollinated flowers did not exhibit pollination-induced corolla senescence. It was possible to determine whether any differences in stylar ethylene production existed among the pollinated flowers that exhibited accelerated corolla senescence and those that did not by measuring ethylene production by intact styles from 0 to 48 h after pollination. Ethylene production by styles that did exhibit premature corolla senescence was similar to the ethylene-production patterns described previously for pollinated styles (Fig. 11). Ethylene production by pollinated styles from flowers that did not exhibit accelerated corolla senescence was less than one-half of the the ethylene production of styles from inrolling flowers. After 18 h postpollination, stylar ethylene production was similar to that of unpollinated control flowers. Pollen tubes were visualized in both pollinated styles from flowers that exhibited pollination-induced petal inrolling and those that did not (data not shown).

DISCUSSION

We have investigated the role of stylar ethylene in pollination-induced corolla senescence. In a multistyle carnation flower we have shown that when only a single style is pollinated the remaining style(s) also synthesizes ethylene. In carnation flowers it has previously been shown that ethylene production can be detected from the pollinated style within 1 h after pollination (Nichols et al., 1983; Whitehead et al., 1983a; Larsen et al., 1993, 1995). We have also detected elevated levels of ethylene biosynthesis from the unpollinated styles 1 h after pollination, indicating that very rapid signaling occurs between styles. When pollinated petunia (Gilissen and Hoekstra, 1984) or carnation (M.L. Jones, unpublished data) styles are detached immediately after pollination and placed back in the flower, they fail to senesce prematurely. These experiments provide evidence for a pollination signal that is translocated through the style rather than one that diffuses through the

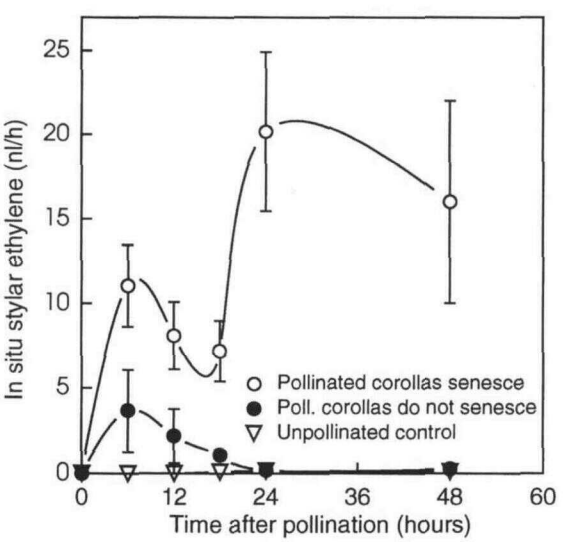

Figure 11. Ethylene biosynthesis from pollinated (Poll) styles remaining attached to the flower. Styles were isolated by sealing a centrifuge tube around the styles. Tubes were capped with a septum, and ethylene was measured after 15 min. Values represent means ± SE of six flowers.

air. The sequential production of ACC and ethylene by styles, ovaries, and petals after pollination indicates that ACC and/or ethylene may be the translocated pollination signal in carnation flowers (Nichols, 1977; Nichols et al., 1983; Woodson et al., 1992; Woltering et al., 1995). The rapid increase in DCACS2 mRNAs in the unpollinated styles supports the premise that a translocated signal induces ethylene production rather than unpollinated styles merely evolving ethylene that was synthesized in the pollinated style. Previous reports have shown that ACC synthase mRNAs accumulate in styles in response to ethylene (ten Have and Woltering, 1997). We have shown that treatment of pollinated flowers with NBD does not inhibit this early increase in ACC synthase mRNAs or ethylene biosynthesis from the unpollinated style, indicating that ethylene is not the signal translocated between styles after pollination. The nature of this rapid signaling between styles remains unclear.

The inhibition of pollination-induced corolla senescence by continual exposure to NBD has also been reported in petunia flowers (Hoekstra and Weges, 1986), but ethylene production by the other floral organs was not investigated. In carnation styles the initial burst of ethylene production was not inhibited by NBD or treatment of the pollinated style with DACP, both of which inhibit ethylene action. This first peak of stylar ethylene in carnations has been shown to be a general response to pollination, because it can also be induced by foreign and incompatible pollen. Furthermore, in these situations the early increase in ethylene is not always followed by sustained ethylene production by the style and other floral organs (Whitehead et al., 1983b; Larsen et al., 1995).

The sustained production of ethylene from both pollinated and unpollinated styles and ethylene biosynthesis from the ovary and petals is completely inhibited by treatment of flowers with NBD, as well as treatment of only the pollinated style with DACP. The ethylene climacteric observed in senescing carnation petals is known to be under autocatalytic regulation, since exogenous ethylene induces ethylene biosynthesis from petals (Halevy and Mayak, 1981). In senescing carnation petals the ACC synthase transcript detected by the cDNA DCACS1 has been shown to be involved in autocatalytic ethylene production (Park et al., 1992). The ACC oxidase mRNA detected by DCACO1 has also been shown to be induced by exogenous ethylene and to correlate with endogenous ethylene production during natural senescence in ovaries and petals (Woodson et al., 1992; ten Have and Woltering, 1997). We have confirmed the autocatalytic regulation of DCACS1 in pollinated petals and DCACO1 in pollinated ovaries and petals. Expression of the ACC synthase DCACS1 is also detected in styles corresponding to the third peak of ethylene from both pollinated and unpollinated styles, and this induction is completely inhibited by NBD. The absence of detectable levels of ACC synthase mRNA in pollinated ovaries may be indicative of a novel ACC synthase that has not yet been identified, or the substantial ethylene evolved from the ovaries may be the result of ACC translocated from the styles and oxidized to ethylene by endogenous ACC oxidase in the ovary. The autocatalytic burst of ethylene from the petals leads to the senescence of the corolla, which is considered a wasteful structure to maintain postpollination (Lawton et al., 1990). It is reasonable to assume that after pollination the autocatalytic burst of ethylene production by the styles serves a similar purpose.

The inhibition of pollination-induced corolla senescence by inhibiting ethylene action and the resulting autocatalytic synthesis of ethylene in the style suggests that this ethylene plays a vital role in the postpollination phenomenon in carnation flowers. Pollinated flowers that fail to exhibit pollination-induced corolla senescence show a pattern of stylar ethylene production similar to that of those flowers treated with inhibitors of ethylene action. This provides further support for the importance of this latter stylar ethylene in signaling the pollination event to the ovary and petals. In flowers of *Digitalis* sp. (Stead, 1985) and tobacco (Hill et al., 1987), the amount of ethylene produced by the flower correlates with the amount of pollen applied to the stigma. The lower levels of ethylene synthesized by some pollinated styles during the second peak in carnation flowers may also be a function of pollen load. Both of these experiments suggest that ethylene production within the second peak must be over a certain threshold level to induce further pollination events and have suggested the following model for postpollination signaling in carnation flowers. Ethylene produced by pollinated styles corresponding to the second peak is translocated to the ovary, where it induces ACC oxidase, and subsequently ACC, which is translocated from the styles, and oxidized to ethylene. Stylar ethylene or the ethylene produced by the ovaries is then translocated to the petals to induce both DCACS1 and DCACO1.

Although evidence exists for the translocation of ACC in carnations from the stigma to the petals (Reid et al., 1984), the lack of an ACC oxidase message and activity in presenescent carnation petals suggests that translocated ACC would be insufficient to induce ethylene production in the petals without the induction of ACC oxidase (Woodson et al., 1992). Furthermore, it has been shown that DCACS1 and DCACO1 are both induced by ethylene in petals (Woodson et al., 1992). In Phalaenopsis orchids ACC synthase mRNAs are not detectable in the perianth. ACC that is synthesized by the gynoecium is translocated to the perianth, where its conversion to ethylene is catalyzed by endogenous ACC oxidase (O'Neill et al., 1993). Although there is evidence for ACC translocation from the stigma to the perianth in orchids and other flowers, O'Neill et al. (1993) suggested that in Phalaenopsis orchids translocated ACC acts only to actuate a response in the perianth that is induced by ethylene. This conclusion is based on the evidence that ACC oxidase accumulation in the perianth is ethylene-dependent. The translocation of ethylene from the central column to the perianth has been demonstrated in Cymbidium orchids (Woltering, 1990; Woltering et al., 1995).

In this paper we have provided evidence for the importance of ethylene perception in the style on the postpollination response and have provided some explanations for the roles of ethylene produced by both pollinated and unpollinated styles from pollinated flowers. Pollination-

induced senescence of the corolla has likely evolved as a mechanism to deter future visits from pollinators, to avoid predation, and to allow for the remobilization of metabolites from the petals to the developing ovary. With the perception of a successful pollination event, it would also be beneficial to induce the senescence of the styles, another floral tissue no longer needed. Results from our experiments with in situ detection of stylar ethylene may also indicate another mechanism developed by nature to further ensure the preservation of the species, a means by which the flower can prevent premature corolla senescence if the pollen load is insufficient to ensure complete seed set.

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