

POLLINATION REGULATION OF FLOWER DEVELOPMENT

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ABSTRACT

Pollination regulates a syndrome of developmental responses that contributes to successful sexual reproduction in higher plants. Pollination-regulated developmental events collectively prepare the flower for fertilization and embryogenesis while bringing about the loss of floral organs that have completed their function in pollen dispersal and reception. Components of this process include changes in flower pigmentation, senescence and abscission of floral organs, growth and development of the ovary, and, in certain cases, pollination also triggers ovule and female gametophyte development in anticipation of fertilization. Pollination-regulated development is initiated by the primary pollination event at the stigma surface, but because developmental processes occur in distal floral organs, the activity of interorgan signals that amplify and transmit the primary pollination signal to floral organs is implicated. Interorgan signaling and signal amplification involves the regulation of ethylene biosynthetic gene expression and interorgan transport of hormones and their precursors. The coordination of pollination-regulated flower development including gametophyte, embryo, and ovary development; pollination signaling; the molecular regulation of ethylene biosynthesis; and interorgan communication are presented.

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INTRODUCTION

Pollination regulates a complex syndrome of developmental events in many flowers. Because perianth senescence is the most visible manifestation of this pollination-regulated flower development and because of its horticultural importance, a preponderance of the research focused on this phase of flower development has been on wilting and abscission of the corolla and calyx. However, this review focuses on the broader context of pollination-regulated developmental responses that collectively lead to the shedding of some floral organs that have served their function in pollen dispersal and reception while simultaneously preparing other organs for fertilization, embryogenesis, and fruit development. Thus, pollination delineates prepollination flower development—during which the flower is specialized for pollen dispersal and reception—from postpollination development—whereby the flower becomes specialized to ensure fertilization and nourishment of the developing embryo and seed. Developmental processes associated with this functional transition include senescence of the perianth, pigmentation changes, ovary maturation, ovule differentiation, and female gametophyte development. It has been proposed that perianth senescence and color changes of floral organs serve as signals for insect pollinators to discriminate receptive flowers from those that have advanced to later stages of reproductive development, whereas pollination regulation of ovule maturation serves to coordinate development of the male and female gametophyte after a point where fertilization is all but assured. In addition to the proposed ecological significance of pollination-regulated flower development, processes associated with pollination-regulated flower development are important horticulturally and often result in a reduction of the commercial quality of flowers. Several previous reviews have addressed senescence and postharvest physiology of flowers (13, 50, 51), and pollination-regulated flower development has recently been reviewed from a horticultural perspective (116).

The transition from prepollination to postpollination development can occur in the absence of pollination as part of a temporal program of flower development. However, in many flowers this developmental transition is either strictly regulated by pollination or is accelerated by pollination. Flowers that demonstrate strict pollination regulation have served as excellent model systems to dissect the signals that regulate the developmental transition. A major research goal has been to understand the signals that coordinate diverse developmental responses set in motion as the flower undergoes the transition from a primary role in pollen dispersal and reception to that of fertilization and nurturing the developing embryo and seed. Because these diverse developmental programs occur in distinct floral parts, interorgan signals that coordinate the overall process are implicated. This review focuses on the current state of knowledge of the processes and signals that coordinate pollination-regulated flower development.

POLLINATION REGULATES A SYNDROME OF DEVELOPMENTAL EVENTS

In many flowers, pollination regulates a syndrome of development events that collectively prepare the flower for fertilization and embryogenesis while shedding organs that have completed their function in pollen dispersal and reception. Components of this pollination-regulated syndrome include changes in flower pigmentation, senescence and abscission of floral organs, and growth and development of the ovary. Pollination-regulated developmental events are initiated by a single event—pollination—but because they occur in distinct floral organs the activity of interorgan signals that amplify and transmit the primary signal to distal floral organs is also implicated.

Pollination Regulation of Perianth Senescence

A predominant feature of pollination-regulated development is perianth senescence, although this process is strictly regulated by pollination in only a minority of species. More typically, senescence occurs gradually as part of a temporal program of flower development that may be accelerated by pollination, although there are extreme examples, such as daylily flowers, in which the perianth senesces within 12–18 h after flower opening regardless of their pollination status (79, 85). In flowers that exhibit either pollination-dependent or pollination-accelerated senescence, pollination leads to a rapid increase in ethylene production resembling the climacteric response observed in ripening fruits (16, 53, 117). In these flowers, which include *Petunia*, carnation, cycla-

men, and orchids, perianth senescence occurs in concert with the increase in endogenous ethylene production and can be prevented by treatments that inhibit ethylene production or perception (18, 53, 54, 106, 107, 111, 124, 159).

Very early studies first noted the sensitivity of the perianth of certain flowers to ethylene (26). In these studies, treatment of *Cattleya* flowers with as little as 2 ppb of ethylene for 24 h induced senescence symptoms that were identical to "dried-sepal injury," a physiological disorder that developed in the perianth of orchid flowers. This disorder was inferred to be caused by exogenous ethylene in greenhouses. The association of endogenous ethylene production with perianth senescence was made somewhat later during study of the floral color changes that accompany pollination-regulated development in another orchid species, *Vanda* cv. Miss Agnes Joaquim (2). It was noted that these flowers produced ethylene endogenously, and it was correlated with the floral color changes. Furthermore, the floral color change was accelerated by exogenous ethylene, suggesting that endogenous ethylene production triggered color fading and, by inference, also triggered perianth senescence (2). The effects of exogenous ethylene in this *Vanda* cultivar could be mimicked by emasculation or by pollination, and this further indicated that this initial description of ethylene-regulated flower senescence reflected a pollination-regulated process (SD O'Neill, unpublished observations). It is now well known that ethylene plays an important role in coordinating pollination-regulated perianth senescence in many flower species, and a number of orchid species have been particularly well characterized in this regard. For example, emasculation, pollination, auxin treatment, or wounding of orchid flowers stimulates ethylene production and induces perianth senescence in several orchid genera, including *Arachnis*, *Aranda*, *Cattleya*, *Cymbidium*, *Dendrobium*, *Paphiopedilum*, *Phalaenopsis*, and *Vanda* (2, 7, 20, 26, 27, 43, 58, 102–104, 117, 118, 165, 168, 175). Comprehensive studies have demonstrated large variability in sensitivity to ethylene among different orchid species with *Vanda* cv. Miss Joaquim reported to be the most sensitive to ethylene (43). *Cymbidium*, *Cattleya*, and *Paphiopedilum* were moderately sensitive to ethylene, whereas *Dendrobium* and *Oncidium* were relatively insensitive. Many other flower species, such as carnation, are also similarly sensitive to ethylene, with exogenous propylene (an ethylene analog) promoting a pattern of sustained ethylene production and symptoms of perianth senescence similar to endogenous ethylene production and senescence symptoms induced by pollination (110). These results support the conclusion that ethylene is sufficient to promote the pattern of pollination-regulated perianth senescence that is observed in a number of flowers, including orchid, carnation, and *Petunia*.

Perianth senescence is an active process that is accompanied by changes in gene expression (13, 77, 78, 170). Three cDNAs (SR5, SR8, and SR12) were initially isolated from senescing carnation petals. Two were regulated by ethylene and the third by both ethylene and by temporal cues (77, 78). Two of the senescence-related cDNAs are likely to encode β -galactosidase (SR12) and glutathione S-transferase (SR5) (90). The potential role of β -galactosidase in senescing flower petals is likely to be in cell wall disassembly that accompanies most senescence processes. The role of glutathione S-transferase was suggested to be in the detoxification of lipid and DNA hydroperoxides associated with senescence-induced oxidative processes (146). As discussed below, genes encoding key ethylene biosynthetic enzymes are also regulated by pollination and associated pollination signals in several flower species, thus providing the signaling linkage between pollination, ethylene, and the regulation of genes that contribute to perianth senescence (101, 117, 119, 147, 149). Overall, these results indicate that specific biochemical events are regulated at the level of gene expression during perianth senescence and that these genes, like the overall process, are regulated by ethylene.

Pollination Regulation of Floral Pigmentation Changes

It has been reported that over 74 angiosperm families exhibit floral pigment changes in response to pollination or flower aging and proposed that pollinators recognize color changes and preferentially visit previously unpollinated flowers (44, 157). Pollination can induce diverse patterns of pigmentation changes including color fading, enhanced pigmentation, or intensification of pigmentation in discrete spots. In *Cymbidium* orchid flowers, pollination induces anthocyanin formation, and this process has been well studied as the first visible manifestation of pollination-regulated flower development that also leads to perianth senescence (4–9, 166, 167). The change in *Cymbidium* lip coloration can be accelerated by pollination, emasculation, or treatment of the stigma with auxin (7). Subsequent studies in *Cymbidium* also demonstrated that a small incision at the base of the lip prevented emasculation-induced coloration and that treatment of completely excised lips with 1-aminocyclopropane-1-carboxylic acid (ACC) or ethylene promoted anthocyanin accumulation, suggesting that lip coloration resulted from the translocation of ACC to the lip where it was converted to ethylene in situ (167). Pigmentation changes in *Vanda* orchid flowers are also associated with pollination and ethylene production but, in contrast with *Cymbidium*, *Vanda* flowers undergo rapid color fading (2). In lupine flowers, the color of the banner spot changes from yellow to magenta as the flower ages (140). This change in banner spot color

also appears to be regulated by ethylene and is most likely accelerated by pollination. The change in banner spot color precedes flower wilting by several days and may serve to maintain the attractiveness of a large floral display from a distance but still provide a signal for pollinators at close range.

Relatively little is known about the specific biochemical events that underlie the process of floral pigmentation changes, although certain floral organ pigmentation changes have been associated with carotenoid or anthocyanin biosynthesis (95), anthocyanin degradation (126), or changes in tissue pH (10). Because of the diversity in patterns of pigmentation changes that are pollination regulated, it is likely that different biochemical mechanisms contribute to the changes observed in different flowers. It has been suggested that pollination-regulated color changes evolved independently in angiosperms many different times (157), suggesting that the underlying biochemical mechanisms are likely to differ among taxa.

Pollination Regulation of Female Gametophyte Development

The end result of pollination is fertilization, which leads to zygote formation and subsequent embryogenesis, all of which have been reviewed extensively (14, 62, 68, 86, 88, 94, 114, 128, 130). In some species, female gametophyte development is incomplete before pollination, and the pollination event itself regulates ovule and gametophyte initiation, development, and maturation in preparation for subsequent fertilization. In certain orchids such as *Cattleya*, *Sophranitis*, *Epidendron*, *Laelia*, *Phalaenopsis*, *Dendrobium*, and *Doritis*, ovules are completely absent in unpollinated ovaries, and their development is triggered by pollination (29, 30, 65, 133, 173, 174, 176). In many other orchid genera, such as *Cypripedium*, *Paphiopedilum*, *Phragmipedium*, *Herminium*, *Epipactis*, and *Platanthera*, ovule primordia are present before pollination but remain suspended at a premeiotic stage until pollination triggers further ovule development (29, 36, 37, 138). In still other orchid species, immature ovule primordia are present at anthesis that have not yet progressed to the stage of archesporial cell differentiation (38, 76).

Pollination-regulated ovule initiation and development has been characterized in detail in *Phalaenopsis* (176). Within two days after pollination and before pollen tube germination, cell proliferation is initiated along the placental ridges of the ovary. The placental ridges continue to elongate and branch to form thousands of finger-like ovule primordia by approximately 40 days after pollination, a time still well in advance of fertilization. At this stage, the inner integument appears as a collarlike growth near the tip of the primordia, and the outer integument is initiated shortly thereafter. The archesporial cell enlarges

further to form the megasporocyte that, following meiosis, gives rise to the expanding megaspore. Subsequent mitotic divisions result in the development of a *Polygonum*-type embryo sac in this orchid species (14, 163, 176). The elapsed time between pollination and maturation of the ovule and final fertilization is approximately 80 days in *Phalaenopsis*. The progression of pollination-regulated ovule development in *Phalaenopsis* is illustrated in Figure 1. The initial stages of ovule differentiation, namely cell proliferation in the placental ridge, occurs before pollen tube germination, suggesting that the initial signals for ovule initiation arise from the pollination event and are not dependent on pollen germination or tube growth. The strict regulation of ovule development by pollination results in the synchronous development of thousands of ovules in the *Phalaenopsis* ovary (176). The synchronous ovules have provided the basis to dissect the process of ovule development at the molecular level and to isolate a number of genes whose expression is correlated with discrete stages of ovule differentiation (100).

The unique regulation of megagametophyte development by pollination in a number of orchid species has been related to their reproductive ecology. A generally held view is that orchid ovule development is not initiated before pollination because of the low probability of pollination by highly specific

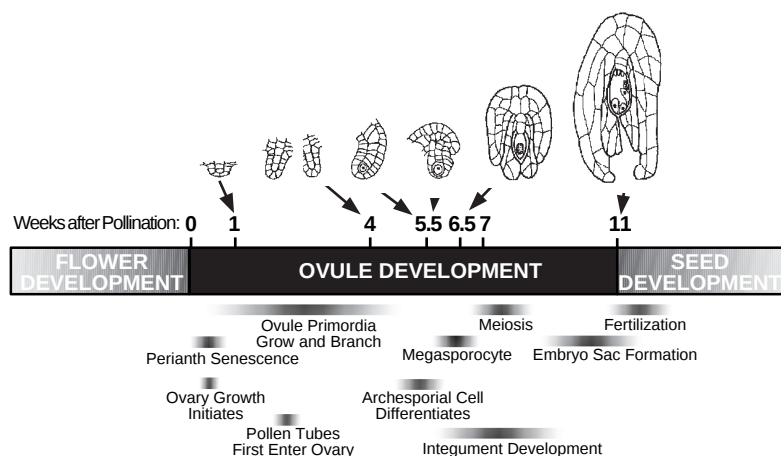


Figure 1 Pollination regulation of ovule development in *Phalaenopsis*. The timeline of ovule development beginning with the event of pollination and ending with fertilization is presented. The span of time during which various developmental changes occur is indicated below the timeline. Anatomical diagrams across the top depict the developmental stage of the ovule primordia, immature ovule, and finally, the mature ovule containing the female gametophyte (modified from Reference 100).

pollinators, compounded by the large investment required for megagametophyte and ovary maturation. The orchid reproductive strategy only invests in female reproductive development after pollination, when fertilization is all but assured. The variation observed in the stage of ovule development at the time of anthesis in different orchid taxa has also been proposed to relate to the environment in which they grow (144). For example, epiphytic orchids that grow in areas where the growing season is long can afford the extended time necessary for complete postpollination ovule development, whereas terrestrial species growing in temperate regions with a short growing season must achieve partial female development before anthesis to complete the reproductive cycle within the season.

Pollination regulation of megagametophyte development is not confined to orchids but is a feature of many higher plant reproductive systems. In many species megagametophyte development is almost complete before pollination, and the final differentiation of cells of the megagametophyte is triggered by pollination. For example, in *Prunus dulcis* (almond), ovule development is arrested at the megasporocyte stage before anthesis, with further development and enlargement of the megagametophyte occurring only after compatible pollination (123). Studies of ovule development in other *Prunus* species in which the megagametophyte is immature at anthesis indicates that the polar nuclei do not fuse before pollination and that this event is pollination regulated (31, 32, 123). Experiments with cotton ovule culture have shown that the addition of 5.0 μ M IAA to the culture media can induce polar nuclei fusion, suggesting that auxin supplied by the pollen may be a natural signal inducing polar nuclei fusion and perhaps other biochemical events in preparation for fertilization (66).

Recently, and unexpectedly, it was shown that the egg cell of most ovules in *Zea mays* is not morphologically mature at the time of pollination (96). The final events of egg cell maturation are completed in the majority of ovules after pollination but before fertilization, suggesting that pollen signals are responsible for inducing the final stages of megagametophyte development in this species as well.

In barley, pollination triggers synergid degeneration before the pollen tubes reached the ovule, which allows the pollen tube to penetrate the megagametophyte during fertilization (93). It has also been shown that calcium accumulates in the synergids in response to pollination. It has been suggested that this plays a role in establishing a chemotropic gradient to attract the pollen tube and/or in causing the pollen tube to burst after it enters the synergid to release the sperm cells (22, 23, 56). It has also been suggested that a signal from the pollen tube is communicated over a short range to the ovule to promote

synergid degeneration in *Nicotiana* (63). A possible candidate for the pollen tube-derived signal in this case was suggested by experiments using cotton ovule culture in which 0.5 μM gibberellin induced synergid degeneration in a manner similar to that after pollination (66), although other hormones associated with the pollen tube may also contribute to this signaling function (89).

Pollination Regulation of Embryo Development

Several studies have demonstrated that apomictic embryo development in some species is dependent on pollination. For example, in the orchid *Zygopetalum*, pollen from an incompatible species, *Oncidium*, that was ineffective in fertilization, nevertheless triggered apomictic seed production (143). This requirement for pollination to induce apomictic embryo development was confirmed in another orchid species, *Orchis* (49). A series of these and related observations led to an early interpretation that pollen-borne chemical substances induced ovary growth that then indirectly promoted embryo development even in the absence of fertilization (48). Other examples of apomictic embryo development that are dependent on pollination include the apomictic grass *Pennisetum setaceum*, which increases the set of apomictic embryos when pollinated with *P. ciliare* pollen, even though this pollen does not result in fertilization (12, 136). Gamma-irradiated pollen that is inefficient at fertilization induces a low percentage of haploid embryos to develop in apple that are presumed to be apomictic (177). Overall, these observations indicate that while pollination is not sufficient to induce embryogenesis in all species, it is required for apomictic embryo development in several plant species that are predisposed to produce apomictic embryos.

Pollination Regulation of Ovary Development

Ovary development after pollination depends upon a supply of auxin that is typically derived from developing ovules and seeds (47). In the absence of pollination and embryogenesis, certain solanaceous species such as tomato and *Petunia* could be induced to form mature seedless fruit by treatment of the ovaries with auxin (45). Extracts of pollen could mimic the effect of auxin, leading to the proposal that pollen contained auxin and that contributed to the initiation of ovary growth (46, 72–74). Unlike the role of developing seeds in promoting ovary growth, the role of pollination as a primary event regulating ovary and fruit development is less firmly established. Early studies of pollination-regulated orchid development identified changes in the curvature of the ovary to be one of the earliest pollination-regulated developmental events (29). More recently, pollination regulation of cell division in the placental ridge has been implicated in the formation of hair cells that expand the central ovary

cavity (176). These morphological changes occur approximately three days before pollen germination and clearly demonstrate that pollination itself, rather than fertilization, triggers the initial stages of ovary development. Exogenously applied auxin and inhibitors of ethylene biosynthesis demonstrated that the elaboration of ovary wall hair cells, the earliest morphological change, was dependent on both auxin and ethylene (176), suggesting that pollen-borne auxin can be translocated to the ovary (141). Pollination effects on ovary development are not restricted to orchids and have been reported in many other plant species, including muskmelon ovaries, which doubled in size within 48 h after pollination (80), as well as in *Brodiaea* and carnation, where ovary expansion was promoted by ethylene treatment, which also promoted perianth senescence (55, 108, 109, 112, 113). It is possible that ovary growth responses were directly related to mobilization of carbohydrate from the senescing petals to the ovary and were thus only indirectly related to pollination.

POLLINATION SIGNALS

Pollination is first perceived at the stigmatic surface, and pollination-regulated developmental events are initiated before pollen germination or penetration of the style by the growing pollen tube. This suggests that a physical event closely associated with the pollen-stigma interaction or a pollen-borne substance is responsible for initiating pollination-regulated flower development. In most species, the primary pollination event is accompanied by an increase in ethylene evolution in the stigma and style within hours after pollination and well before pollen germination (39, 40, 42, 82–84, 97, 110). In self-incompatible species, pollination responses differ between compatible and incompatible pollinations, and this has provided some insight to the primary and secondary signals that operate in pollination-regulated flower development. Compatible pollination in *Petunia* results in two distinct phases of ethylene evolution occurring after approximately 3 and 20 h, respectively, whereas self-pollination triggers only the first phase of ethylene production (81, 137). The early phase of ethylene evolution was attributed to direct conversion of pollen-borne 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene and the second phase of ethylene evolution to endogenous synthesis of ACC in floral organs distal to the stigma. These results implicate the involvement of a primary and a secondary signal in the pollination response, the first of which is perceived in the stigma followed by a subsequent secondary signal that transmits and amplifies the primary pollination signal to distal floral organs (115). Although it is likely that the primary and secondary pollination signals are distinct, they are both linked to ethylene evolution.

Primary Pollination Signals

The primary pollination signal has been proposed to result from physical contact between the pollen and stigma, from pollen tube penetration of the stigma (40, 41), or from pollen-borne chemical messengers (60, 137, 159). In *Petunia*, carnation, and orchid flowers, the initial response to pollination is rapid ethylene evolution by the stigma. In each case, pollination-induced ethylene evolution precedes germination of the pollen tube, suggesting that penetration of the stigmatic surface by the pollen tube is not required for the induction of ethylene biosynthesis (20, 42, 54, 59, 110, 117). Furthermore, mock pollination of orchid flowers with latex beads, a pollen surrogate that has been used to study the role of the stylar matrix in pollen tube extension (135), failed to trigger ethylene production or any other pollination responses, indicating that physical contact alone is insufficient to induce any components of the pollination-regulated developmental syndrome (176). The preponderance of results suggests that physical contact between the pollen and stigma, or wounding reactions associated with pollen tube growth, are not the primary pollination signals, and considerable experimental attention has thus focused on the identification of a pollen-borne chemical that may serve as a primary pollination signal. Chemicals that have been identified in pollen include ACC, auxin, pectic oligosaccharides, brassinosteroids, and methyl jasmonate, all of which are known inducers of ethylene biosynthesis, making them attractive candidates for the primary pollen signal molecule (3, 28, 33, 67, 71, 92, 131, 134, 151, 152, 154, 156, 162, 172).

Shortly after the identification of ACC as the immediate biochemical precursor of ethylene (1), several reports identified its presence in pollen and suggested that ACC may be the primary pollen signal (127, 158). The role of pollen-borne ACC in triggering the initial burst of ethylene production and its potential translocation to other floral organs has now been extensively studied, especially in model systems such as *Petunia* and carnation (158–161). In spite of its presence in relatively large quantities in some pollen (127, 137, 158), the role of ACC in supporting the initial ethylene production in the stigma has been controversial. For example, Hoekstra & van Roekel (60) reported that ACC content of pollen from various sources is not well correlated with the level of pollination-induced ethylene production, and several reports indicated that treatment of the stigma with an inhibitor of ACC synthase, aminoethoxyvinylglycine (AVG), before pollination prevented pollination-induced ethylene production (61, 117, 169, 176). Thus, these reports suggested that pollination-induced ethylene production is derived from endogenous production of ACC rather than from exogenous pollen-borne ACC. In contrast, Singh et al

(137) reported that wide variations in pollen-borne ACC content in different *Petunia* genotypes were well correlated with the initial peak of pollination-induced ethylene production and that this early ethylene production was not inhibited by a different inhibitor of ACC synthase, aminooxyacetic acid (AOA). Thus, these data suggested that pollen-borne ACC was the substrate for initial pollination-induced ethylene production in *Petunia* (137). Other reports have suggested that the quantity of pollen-borne ACC would be vastly insufficient to support the amount of ethylene produced in the stigma following pollination (139), and two reports indicate that diffusion of ACC from the pollen is likely to be restricted under conditions that prevail in vivo, which would further restrict its availability to support ethylene production in the stigma (60, 121). Although the conflicting data indicate that pollen-borne ACC cannot account for the initial pollination-induced ethylene production in the stigma of all flowers, it is possible that exogenous pollen-borne ACC may be responsible for initiating ethylene production in the stigma of at least some flowers, and this early ethylene production may be subsequently enhanced by autocatalytic production of ACC in the stigma.

The contribution of pollen-borne ACC to pollination regulation of flower development has also been tested by exogenous application of ACC to the stigma (61, 117, 127). In each case, ACC promoted an initial burst of ethylene production but did not accelerate wilting or perianth senescence unless extremely high concentrations were used. ACC is not detectable in orchid pollen, yet pollination induces rapid and high levels of ethylene production in the stigma (117). In *Petunia*, compatible pollination elicited two phases of ethylene production and caused rapid perianth senescence, whereas self-pollination only triggered the first phase of ethylene production, and perianth senescence was delayed even though both compatible and incompatible pollen contained ACC (137). Collectively, the data suggest that pollen-borne ACC is not a universal primary pollen signal that is sufficient to elicit the full pollination-regulated developmental response. Nevertheless, even small amounts of ACC may trigger autocatalytic ethylene production in the stigma and thus may play a role in triggering the initial burst of ethylene production in some flowers.

A number of potential primary pollen signals have been tested for their capacity to induce ethylene production and stigma closure, two early pollination-regulated responses in *Phalaenopsis* orchid flowers (JA Nadeau & SD O'Neill, unpublished observations). Pollen-derived proteins, systemin (120), pollen-derived lipids, flavonoids, methyl jasmonate, and jasmonic acid were all eliminated as likely candidates because they failed to elicit either ethylene production and/or stigma closure over a time frame consistent with a role in pollination signaling. Only auxin (both IAA and NAA) was active in trigger-

ing ethylene production and stigma closure in this orchid bioassay (176). This result is consistent with very early reports of a substance, termed pollenhor-
mon, that was present in orchid pollen that caused wilting of the flower and
was later shown to be auxin (34, 35, 99). Even before auxin was identified as a
component of orchid pollen, it was demonstrated that exogenous application of
auxin could mimic pollination and initiate most pollination-regulated develop-
mental events (25, 64). Since then, the capacity of auxins to initiate pollina-
tion-regulated developmental events in orchids has been repeatedly demon-
strated (4, 7–9, 20, 21, 25, 142). Auxin was proposed to serve as the primary
pollination signal by the direct transfer of pollen-borne auxin to the stigma,
from where it diffused to distal floral organs and promoted autocatalytic ethyl-
ene production throughout the flower, leading to perianth senescence (20).
Auxin has been identified as a component of *Nicotiana*, *Antirrhinum*, *Cycla-
men*, *Petunia*, and *Datura* pollen, as well as of germinated pollen of *Pinus
radiata*, suggesting that this putative primary pollination signal is present in a
wide range of higher plant pollen (11, 89, 98, 145). While the role of auxin as
the primary pollen signal has been consistently supported, other reports argue
against the idea that auxin diffuses to the perianth where it directly stimulates
ethylene production, suggesting that a distinct signal transmits the primary
pollination event to distal organs (141).

Although auxin is present in orchid pollen and may act as the primary
pollen signal, the levels of auxin in orchid pollen may be insufficient to be the
sole primary pollen signal. It is possible that pollen contains other forms of
auxin, such as auxin conjugates, or other pollen-borne factors, which may
participate synergistically with auxin to elicit pollination-regulated responses
in orchid. Arditti (4) suggested that the “pollenhormon” described by Fitting
may be a mixture of biologically active molecules. A number of candidate
signal molecules should be examined more intensively in this regard, includ-
ing pectic cell wall fragments that have been shown to elicit ethylene produc-
tion (150) and that are potentially produced by pollen-derived polygalacturo-
nase and pectate lyase (17, 164).

Secondary Pollination Signals

Developmental changes such as ovule initiation and floral color changes are
initiated in floral organs distal to the stigma shortly after pollination, implicat-
ing the role of secondary signals that transduce and amplify the primary
pollination signal. The interorgan regulation of the pollination-regulated re-
sponse suggests that the secondary signal is transmissible and moves from the
stigma, through the style, to other floral organs. Early evidence for such a
secondary pollination signal came from surgical experiments demonstrating

that a mobile wilting factor is transmitted through the style to the corolla of *Petunia* flowers within 6 h after pollination (42). Styler exudates also promote perianth senescence in *Petunia* and carnation, implicating the role of a chemical messenger either produced in the style or translocated through it (42, 132). Because auxin, ethylene, and ACC have been implicated in primary pollen signaling, these same molecules have been extensively evaluated as potential transmissible signals in pollinated flowers.

The model of Burg & Dijkman (20) proposed that the primary pollination signal, auxin, diffused to the labellum where it triggered ethylene biosynthesis. Although early experiments demonstrated that ^{14}C -IAA applied to the stigma was mobilized to the column and labellum, subsequent research indicated that ^{14}C -IAA applied to *Angraecum* and *Cattleya* stigmas was largely immobilized at the point of application with some translocation to the ovary (141). Because the pollination signal spreads to distal floral organs (petals and sepals) much faster than exogenously applied ^{14}C -IAA, it was concluded that auxin is unlikely to be the secondary pollination signal that regulates perianth senescence. However, the data that auxin is translocated primarily to the ovary is consistent with other evidence that auxin translocated from the stigma may specifically contribute to the regulation of the initiation of ovule differentiation in that organ (176).

Ethylene itself is a potential transmissible signal to floral parts distal from the stigma, and it has recently been suggested that diffusion of ethylene within the intercellular spaces (interstitial ethylene) may function in interorgan communication (75, 166, 168). Internal ethylene in the *Cymbidium* flower central column was measured at levels up to 15 ppm, and treatment of the column with exogenous ethylene increased ethylene concentration in the perianth, consistent with the proposal that interstitial ethylene from the column is translocated directly to the perianth (75). In contrast, Reid et al (127) demonstrated that aspiration of ethylene produced in the gynoecium did not delay petal senescence in carnation, indicating that volatile ethylene produced because of the primary pollination signal was not the transmissible signal responsible for regulating perianth senescence. Although these results suggest the possibility that interstitial ethylene acts as the secondary transmissible signal between floral organs, this does not appear to be the case in all flowers.

In spite of the suggestions that ethylene, rather than ACC, is translocated between floral organs, there is a large body of research implicating the ethylene precursor, ACC, as an important secondary pollination signal that coordinates interorgan pollination-regulated responses. Translocation of ACC was first demonstrated in waterlogged tomato plants (15). This result illustrated the potential for transport of this water-soluble hormone precursor to effect in-

terorgan regulation of growth responses rather than transport of ethylene itself, which is less amenable to targeted translocation processes because of its gaseous state. Similarly, ACC translocation in pollinated flowers provides a potential mechanism for the targeted translocation of a secondary pollination signal. Following pollination of carnation flowers, ACC levels increased in all flower parts, which was proposed to result from ACC translocation (111). It was subsequently shown that ^{14}C -ACC applied to the stigma of carnation flowers resulted in the production of ^{14}C -labeled ethylene in the gynoecium and perianth, which provided compelling evidence that ACC was translocated from the stigma to the gynoecium and perianth, where it served as a substrate for ethylene biosynthesis (127). Emasculation-induced senescence of *Cymbidium* flowers demonstrated that changes in lip coloration, an early morphological marker of ethylene-regulated senescence, were triggered by ACC translocated from the central column (166). In *Phalaenopsis* orchid flowers, ethylene production by the intact flower significantly exceeded the sum of ethylene production by excised floral organs, which was interpreted to indicate that ethylene production by some floral organs is dependent on ACC import from other floral parts (117). Collectively, there is strong experimental support from several flower systems that ACC is translocated between floral organs, where it serves as a substrate for ethylene biosynthesis. This translocation of ACC also implicates it as a secondary pollination signal in the interorgan coordination of pollination-regulated developmental responses.

A very early response of floral tissues to pollination is increased sensitivity to ethylene (125). Substantial research has focused on the identification of transmissible "sensitivity factors" that render the floral tissue more sensitive to senescence-inducing effects of ethylene. Following pollination of *Phalaenopsis* flowers, there was a significant increase in the endogenous content of short-chain saturated free fatty acids in the column and perianth. Furthermore, exogenous application of these free fatty acids to the stigma increased sensitivity of the flower to ethylene, leading to the suggestion that these short-chain fatty acids may be ethylene sensitivity factors (52). It is likely that elucidation of the molecular components involved in ethylene perception and signal transduction will contribute to better understanding this phenomenon.

REGULATION OF ETHYLENE BIOSYNTHESIS IN POLLINATED FLOWERS

Ethylene is central to the control of pollination-regulated flower development, and the regulation of its endogenous synthesis appears to be an important component of the interorgan coordination of discrete developmental processes.

Examination of the regulation of expression of genes encoding the major biosynthetic steps in ethylene biosynthesis has provided the basis for analyzing the temporal and spatial regulation of ethylene biosynthesis in flowers and in response to both primary and secondary pollination signals. In addition, the cloning of ethylene biosynthetic genes has provided the means to genetically engineer flowers for enhanced longevity (91).

The penultimate enzyme in the ethylene biosynthetic pathway, ACC synthase, is widely regarded as the rate-controlling step. This step is highly regulated at the level of enzyme activity and at the level of gene expression (69, 70). In addition, while considered to be constitutive in many tissues, the final enzyme in ethylene biosynthesis, ACC oxidase, is also regulated at the level of enzyme activity and at the level of gene expression (69, 70). The spatial and temporal expression of both ACC synthase and ACC oxidase in response to pollination have provided significant insight into the interorgan coordination of pollination-regulated flower development.

Pollination Regulation of ACC Synthase

In all species examined to date, ACC synthase is encoded by multiple genes that exhibit differential tissue specificity and/or differential regulation by environmental or hormonal stimuli (70). Many cDNA clones encoding ACC synthase have been isolated from a number of flowers including tomato, carnation, *Petunia*, geranium, and orchid (24, 57, 91, 117, 119, 129). Because of its potential role in the regulation of ethylene production in response to pollination, ACC synthase has been the subject of intense interest regarding pollination-regulated flower development. ACC synthase cDNA clones have been isolated from senescing carnation flowers and from pollinated orchid flowers and their expression at the level of mRNA abundance characterized in detail in relation to pollination and perianth senescence (57, 117, 119, 171). In carnation flowers, ACC synthase mRNA was undetectable in all floral organs immediately after harvest but increased dramatically after 5–6 days in petals and styles coincident with the increase in ethylene production. ACC synthase activity was approximately sixfold higher in senescing styles than in petals, whereas ACC synthase mRNA accumulated to similar levels in both tissues (171). Subsequently, a second divergent ACC synthase mRNA was identified that was predominantly expressed in styles and may account for the apparent discrepancy between stylar ACC synthase mRNA and activity levels (57). Expression of both carnation ACC synthase cDNAs (termed CARACC3 and CARAS1) were themselves ethylene regulated, suggesting that they contribute to autocatalytic ethylene production. Relatively low levels of both ACC syn-

these mRNAs were detected in ovaries, in spite of high levels of ethylene production in this organ, suggesting the existence of a third ACC synthase gene predominantly expressed in the ovary. The discrepancies noted in attributing ACC synthase enzyme levels to the expression of particular ACC synthase illustrate the complexity in understanding the detailed regulation of ethylene production by multiple ethylene biosynthetic genes in floral tissues (57).

Expression of ACC synthase genes has also been characterized in pollinated orchid flowers (117). In this flower system, three ACC synthase cDNAs have been characterized that collectively account for the observed ACC synthase enzyme activity that has been characterized in the various organs of the flower. mRNAs corresponding to two highly homologous ACC synthase cDNAs (OAS1 and OAS2) accumulated to high levels in the gynoeceum approximately 18 h after pollination, accumulated also in labellum tissue over the same period, but were undetectable in the perianth (excluding the labellum) even at 72 h after pollination (117). These results were consistent with the absence of ACC synthase enzyme activity in the perianth tissues, even though the perianth is the site of substantial ethylene evolution (19, 117).

Because auxin has been implicated as a primary signal in pollination-regulated responses in orchid flowers and because auxin has been reported to induce expression of certain ACC synthase gene family members in several plant species (105, 122, 153), the regulation of the orchid ACC synthase genes by auxin was carefully evaluated (117). Although exogenous application of NAA to the stigma strongly promoted accumulation of ACC synthase mRNA, this induction was completely reversed by pretreatment with AVG, an inhibitor of ethylene biosynthesis. This result indicated that OAS1 and OAS2 mRNAs were regulated by ethylene and, as with carnation ACC synthases, participated in autocatalytic ethylene production. A third ACC synthase cDNA was cloned from pollinated orchid flowers that was divergent from OAS1 and OAS2 and shown to be pollination regulated in the stigma and to be directly regulated by auxin (19). Taken together, in pollinated orchid flowers there appear to be at least two distinct types of ACC synthase genes that differ markedly in their spatial and hormonal regulation. The first type is responsive to a primary pollination signal, auxin; the second type is ethylene regulated and may serve to amplify or sustain the primary pollination signal by regulating autocatalytic ethylene production. None of the ACC synthase genes in pollinated orchid flowers are expressed in the perianth despite high levels of ethylene biosynthesis in those tissues (117). This implies that a source of ACC, other than endogenous production, exists for conversion to ethylene in perianth tissue.

Pollination Regulation of ACC Oxidase

ACC oxidase is the final enzyme in ethylene biosynthesis that converts ACC to ethylene and in most instances is considered to be constitutive (70). However, increases in ACC oxidase have been reported in senescing carnation flower petals (87), and ACC oxidase mRNA levels are pollination induced in *Phalaenopsis* and carnation flowers (101, 155). In carnation, ACC oxidase mRNA was undetectable in presenescent petals, ovaries, and receptacles but was present at significant levels in presenescent styles and increased dramatically in abundance following pollination (171). These results suggest that ACC oxidase may be present in carnation stigmas before pollination. Similarly, it was reported that ACC oxidase levels in *Petunia* flowers were maximal shortly after flower opening and that ACC oxidase enzyme activity was most abundant in the *Petunia* flower stigma before pollination (121). Recently, a family of *Petunia* ACC oxidase genes have been cloned and their expression in *Petunia* flowers studied in detail (147, 148). In agreement with Pech et al (121), each of the three expressed ACC oxidase mRNAs accumulated in the gynoeceum during early flower development, reaching their maximal levels at the time of flower opening (147). In addition, ethylene treatment enhanced the accumulation of all three ACC oxidase mRNAs in the pistil, and ACC oxidase mRNA accumulation was also induced in the floral transmitting tissue by pollination (149). Collectively, these results suggest that ACC oxidase is likely to be present in the stigma of both carnation and *Petunia*, and these stigmas can likely convert pollen-borne ACC to ethylene as a part of the primary perception of pollen by the stigma. In addition, it appears that ACC oxidase genes expressed in flowers are ethylene regulated and so are likely to also participate, with ACC synthase, in autocatalytic ethylene production in pollinated flowers.

ACC oxidase activity and ACC oxidase mRNA accumulation have also been characterized in orchid flowers (101, 117). Unlike *Petunia*, ACC oxidase activity was not detected in mature flowers before pollination but was induced rapidly following pollination (101). Following pollination, orchid ACC oxidase (OAO1) mRNA accumulated to high levels within 48 h in all floral organs, including the perianth (101). This result demonstrated a striking disparity between the accumulation of ACC synthase mRNA, which failed to accumulate in the perianth, and suggested that the sepals and petals developed the capacity to convert ACC to ethylene but could not synthesize ACC endogenously (101, 117). In light of the high level of ethylene evolution from perianth tissues and the absence of ACC synthase enzyme activity or a corresponding mRNA, it has been suggested that ACC must be translocated from

other floral organs to the perianth where it is converted to ethylene by the activity of resident ACC oxidase (117).

MODEL FOR INTERORGAN COORDINATION OF POLLINATION-REGULATED FLOWER DEVELOPMENT

Ethylene with auxin plays important roles in pollination-regulated developmental responses and, in conjunction with ACC, participates in the interorgan coordination of diverse components of pollination-regulated flower development. The model outlined in Figure 2 attempts to summarize data that address the initiation of ethylene biosynthesis by pollination and the interorgan regulation of ethylene biosynthesis in pollinated flowers because these two processes appear to be central to the coordination of pollination-regulated flower development. Much of the model relies on the recent elucidation of the patterns of expression of genes encoding ethylene biosynthetic enzymes in pollinated flowers. In this model, a pollen-associated factor is prominent as the primary stimulus in initiating ethylene biosynthesis as well as the overall process of pollination-regulated flower development. In orchid flowers, an important component of the primary pollen signal is auxin, whereas in other flowers, ACC or other as-yet-unidentified pollen-borne substances may also participate in primary pollen signaling. The earliest response to pollination is the induction of ethylene production in the stigma and style, which may use pollen-borne ACC directly as substrate or, more likely, relies on the rapid induction of ACC synthase in the stigma. In orchid flowers, expression of an auxin-regulated ACC synthase is rapidly induced in the stigma by pollination, and this is likely to account for the rapid transduction of the putative primary pollen signal, auxin, to the initial burst of ethylene production. In other flowers such as *Petunia*, a trace amount of pollen-borne ACC may be converted directly to ethylene, which leads to localized autocatalytic ethylene production in the stigma. The presence of ACC oxidase mRNA in presenescent *Petunia* stigmas and its rapid ethylene-dependent induction following pollination is consistent with this mechanism of initial pollination-induced ethylene production.

The interorgan transmission and amplification of the primary pollen signal requires secondary signals that emanate from the stigma, and an important component of this signal appears to be ACC and its subsequent conversion to ethylene in distal floral organs. In orchid flowers, the role of translocated ACC was implicated by the failure of petals and sepals to accumulate ACC synthase mRNA or enzyme activity, in spite of high levels of ethylene production by these floral organs, suggesting that ethylene production in these organs is entirely dependent on translocated ACC. This situation is less pronounced in

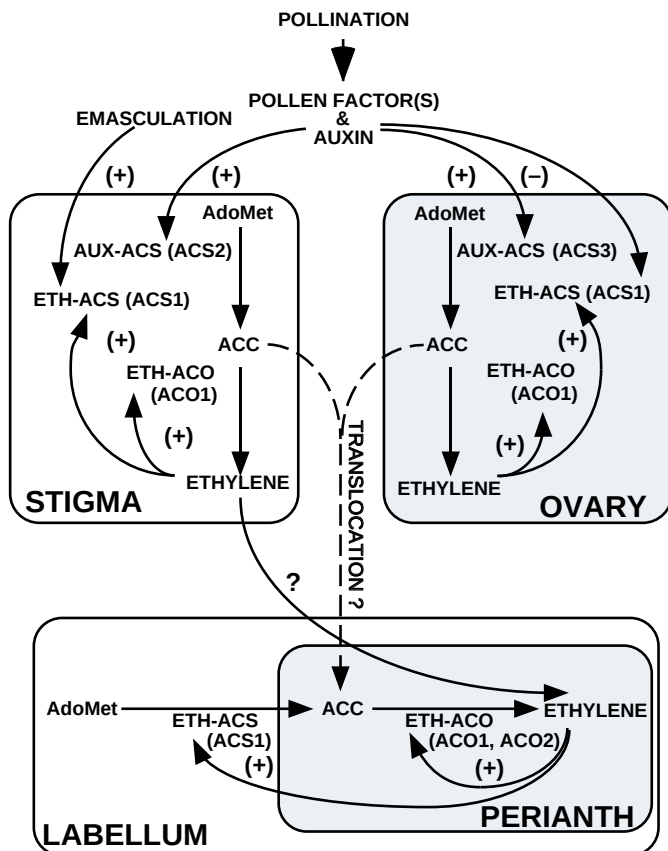


Figure 2 Model for the interorgan regulation of ethylene biosynthetic genes in pollinated flowers, based primarily on data from the *Phalaenopsis* orchid flower, with other flower systems viewed as variations on this basic theme (19, 101, 117). The primary signaling event for pollination-regulated development occurs with the transfer of pollen-borne factors, including auxin, to the stigma surface, the site of initial perception. An auxin-regulated ACC synthase gene (*AUX-ACS*) is induced by the primary pollination signal(s). ACC synthesized in the stigma is converted to ethylene by ACC oxidase, initially present at a low level but induced to high levels soon after pollination to initiate autocatalytic ethylene production in the stigma. Expression of other ethylene-regulated ACC synthase gene(s) (*ETH-ACS*) are also induced by ethylene to further amplify autocatalytic ethylene production. ACC translocated from the stigma to the perianth supports the production of ethylene in that organ, initiating its senescence. This mechanism of interorgan regulation relies on translocation of ACC to distal organs to support ethylene production. In addition to its role as a gaseous signal coordinating ethylene production at the molecular level, ethylene itself may also be translocated within the flower as a translocated signal. Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; ACO, ACC oxidase; ACS, ACC synthase; AdoMet, S-adenosylmethionine; AUX, auxin-regulated; ETH, ethylene-regulated.

flowers such as *Petunia* where ACC synthase is induced in petal tissues. However, the *Petunia* petal ACC synthase is ethylene induced, and the initial production of ethylene in petals required to stimulate autocatalytic ethylene production may be derived from translocated ACC. The model shown in Figure 2 is idealized in that it reflects the situation in *Phalaenopsis* orchid flowers, one of the most extreme examples of pollination-regulated flower development. Data from other species suggest that there are variations on the themes presented in Figure 2.

CONCLUSIONS AND FUTURE DIRECTIONS

Pollination regulates a suite of developmental responses that transform the flower from a structure dedicated to pollen dispersal and reception to one that is dedicated to fertilization, embryogenesis, seed development, and ultimately seed dispersal. One important aspect of this process is the shedding of floral organs, such as the petals and sepals that have completed their service in pollinator attraction. In addition, pollination demands that certain reproductive structures rapidly mature in anticipation of fertilization. Thus, the signals that regulate pollination responses are involved in simultaneously coordinating processes of programmed cell death while initiating the differentiation and/or maturation of important reproductive structures.

Research directed at understanding the nature of the pollination signals has a rich history dating to the beginnings of this century, and it has revealed important elements of the interorgan signaling needed to coordinate the full syndrome of pollination-regulated developmental processes. Although it is clear that auxin, ethylene, and ACC are important actors in this regulatory process, it is likely that this is not the complete cast. More research is needed to more fully elucidate primary pollination signals and how they interact with each other and with the stigma and style to propagate the signal to distal floral organs.

This review focuses on the early events in pollen signaling. However, the growing pollen tube continues to exchange information with the style and ovary, and the nature of these signals also represents a rich area of further research. Much research has characterized a number of model flower systems that are physiologically suited to studying pollination-regulated flower development, but these model systems do not generally overlap with model genetic systems, such as *Arabidopsis thaliana*. Despite the recognized differences between plants in terms of their pollination responses, future progress requires that information from powerful physiological systems be transferred to species

that are well suited to genetic dissection and transgenic manipulation and analysis.

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