

# **In Search of Eternal Youth: The Delay of Postharvest Senescence in Flowers**

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## **Abstract**

**Higher plants have evolved to produce beautiful flowers for the propagation of species through sexual reproduction. Throughout history, man has sought to produce plants for the beauty of flowers. Today, the floriculture sector of horticulture represents a significant international trade industry. Not surprisingly, the longevity of many flowers is quite short, as their biological function of reproduction is transient in nature. The senescence of floral organs is a highly regulated developmental event, often associated with the end of the useful life of an organ relative to the reproductive process. For example, flower petals' function is to attract pollinators and once pollination has occurred, petals represent an expensive metabolic sink. Removal of petals through senescence and/or abscission could benefit the growth and development of reproductive structures. In the floriculture trade, delaying the onset of senescence is the focus of a great deal of research in an effort to extend the useful life of the product. This paper summarizes our current understanding of the biochemical and molecular processes underlying senescence and describes efforts to delay the process through chemical treatments and biotechnology.**

## **INTRODUCTION**

Petals, typically the most prominent organ of the angiosperm flower, function to attract pollinators. Successful pollination sets in motion a series of biochemical and developmental events that often culminate in the senescence and/or abscission of petals (Stead, 1992). Given the role of the petals as visual cues, senescence has likely evolved as a mechanism to conserve resources by focusing the attention of pollinators on those flowers that are receptive to pollination. A feature of petal senescence that supports this role is the remobilization of cellular constituents from the senescing organ to other structures that continue to develop (Xu and Hanson, 2000). The regulation of developmental events in the corolla following pollination or harvest requires a mechanism for communication among the floral organs. In many species, an increase in the production of the phytohormone ethylene is one of the earliest detectable biochemical events associated with the onset of petal senescence. In addition, increased production of ethylene by the pollinated pistil is an early event often preceding the germination and growth of pollen tubes (Nichols, 1977; Nichols et al., 1983; Hoekstra and Weges, 1986; Pech et al., 1987; Jones and Woodson, 1997; Bui and O'Neill, 1998; Jones and Woodson 1999a; 1999b). In this review, We will focus on the current state of knowledge regarding the regulation of flower senescence.

## **Ethylene and Flower Senescence**

Increased ethylene production has long been known to be associated with the senescence of flower petals (for review see Borochoy and Woodson, 1989). Ethylene is synthesized in plant tissues from the amino acid precursor methionine (Adams and Yang, 1979). The first intermediate in the pathway is *S*-adenosylmethionine (SAM), which is converted to the immediate precursor of ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase (Boller et al., 1979). Finally, ACC synthase is

converted into ethylene by the action of ACC oxidase (Kende, 1993). In many tissues, the application of ACC results in the rapid production of ethylene, suggesting that ACC synthase is a rate limiting step in the *in vivo* synthesis of ethylene (Yang and Hoffman, 1984). Genes encoding ACC synthase have now been isolated from many species (for review see Zarembinski and Theologis, 1994) and have been shown to be a large, divergent multigene family. ACC synthase genes are subject to differential regulation, often in a tissue-specific manner. In striking contrast to ACC synthase, ACC oxidase is constitutively expressed in many tissues and is generally not regarded as rate limiting in the biosynthesis of ethylene (Kende, 1993). However, ACC oxidase transcripts have been shown to increase in response to a number of stimuli such as wounding (Hamilton et al., 1990; Kim and Yang, 1994) and during senescence (Woodson et al., 1992; Tang et al., 1994) and ripening (Hamilton et al., 1990; Balague et al., 1993).

The onset of carnation petal senescence is accompanied by a significant increase in the production of ethylene (for review see Borochof and Woodson, 1989). This ethylene production was shown to be associated with a concomitant increase in the expression of ACC synthase and ACC oxidase mRNA's and enzyme activity, suggesting it was regulated at the levels of both transcription and translation (Woodson et al., 1992). Analogous to ripening in climacteric fruit, ethylene production in senescing carnation petals appears to be subjected to autocatalytic regulation. Exposure of presenescent petals to ethylene induces ethylene production and petal senescence (Nichols, 1968; 1971; Woodson and Lawton, 1988). A number of genes shown to be up-regulated during flower senescence are under the regulation of ethylene (Lawton et al., 1990). These include a thiol protease (Jones et al., 1995), a glutathione-S-transferase (Meyer et al., 1991; Itzhaki et al., 1994; Maxson et al., 1996), and a number of genes of unknown function (Lawton et al., 1989). Treatment of carnation flowers with chemical inhibitors of ethylene synthesis or action prevents the increase in ethylene production, the expression of senescence-related genes, and the premature onset of senescence (Bufler et al., 1980; Reid et al., 1980; Wang and Woodson, 1989; Lawton et al., 1990). These treatments are standard in the floral industry, where delayed senescence is sought to prolong the marketable life of cut carnation flowers. Further evidence for the central role of ethylene in carnation petal senescence comes from experiments in transgenic plants. Savin et al. (1995) expressed an antisense ACC oxidase transcript in transgenic carnations, which led to a reduction in ethylene production and a delay in the onset of petal senescence. This approach promises to improve the longevity of flowers without chemical treatment's currently employed in the industry.

### **Pollination-induced Ethylene and Petal Senescence**

The onset of petal senescence is one of many developmental responses associated with pollination (Stead, 1992; O'Neill, 1997; O'Neill and Nadeau, 1997). Other developmental processes that appear to be coordinated by pollination include changes in pigmentation and development of the female gametophyte. Collectively, these developmental events are thought to facilitate reproduction by preparing the female gametophyte for fertilization and removing organs that have fulfilled their function in the attraction of pollinators.

In many species, an increase in the production of ethylene is one of the earliest detectable biochemical events in the pollinated pistil. This often occurs within the first few minutes or hours following pollination (Nichols, 1977; Hoekstra and Weges, 1986; Pech et al., 1987; O'Neill et al., 1993; Larsen et al., 1995; Jones and Woodson, 1999b). In petunia (Hoekstra and Weges, 1986; Singh et al., 1992; Tang and Woodson, 1996) and carnation (Nichols et al., 1983; Larsen et al., 1995; Jones and Woodson, 1997), increased ethylene biosynthesis by the style is detected within the first 30 minutes following pollination. This increased ethylene precedes the germination and growth of the pollen tube, suggesting that penetration of the stigmatic surface is not required for the induction of ethylene biosynthesis. Pollination-induced ethylene in the stigma is often followed by increased ethylene in other attached organs (O'Neill et al., 1993; Larsen et al., 1995;

Jones and Woodson, 1999a; 1999b). This induction of ethylene in floral organs occurs before fertilization of the ovules, and in the case of orchid triggers the further development of the ovules making them competent to be fertilized (Zhang and O'Neill, 1993).

In carnation, the wave of ethylene production that initiates in the pollinated style is associated with a concomitant increase in ACC content, suggesting the synthesis of ACC is critical to the regulation of ethylene production (Nichols et al., 1983; Jones and Woodson, 1999b). This increase in ACC content was shown to be associated with increased ACC synthase activity in styles and petals (Jones and Woodson, 1999b). In striking contrast, the ovary, which was shown to produce significant amounts of ethylene post-pollination, and to contain increased amounts of ACC, did not exhibit significant ACC synthase activity (Jones and Woodson, 1999b). ACC synthase is encoded by a multi-gene family in all plants studied to date. In carnation, three unique ACC synthase genes have been identified and characterized (Park et al., 1992; ten Have and Woltering, 1997; Jones and Woodson, 1999a). DC-ACS1 was shown to be expressed primarily in senescing petals and styles during the final wave of increased ethylene that follows pollination (Woodson et al., 1992; Jones and Woodson, 1997; Jones and Woodson, 1999a). The expression of DC-ACS1 was shown to be under the regulation of ethylene, as it was blocked by inhibitors of ethylene action such as 2,5-norbornadiene (Jones and Woodson, 1999a). In striking contrast, DC-ACS2 and DC-ACS3 were shown to be expressed in pollinated styles at 1 and 6 hours after pollination, respectively (Jones and Woodson, 1999a). The expression of DC-ACS3 is independent of ethylene and appears to be related to primary signals associated with the interaction of pollen with the pistil. Consistent with this observation, treatment with ethylene action inhibitors fails to prevent the early increase in ethylene by pollinated styles, but prevents the final wave of ethylene produced by both styles and petals (Jones and Woodson, 1997). This final wave of ethylene is primarily associated with the expression of DC-ACS1, which is inhibited by 2,5-norbornadiene. DC-ACS1 appears to play a role in amplifying the ethylene signal by increasing the capacity to synthesize ACC in response to early pollination-induced ethylene.

The regulation of ethylene biosynthesis in pollinated orchid flowers has also been studied extensively. In this flower, three ACC synthase cDNA's have been characterized (O'Neill et al., 1993; Bui and O'Neill, 1998). As in carnation, these genes appear to respond to both primary and secondary signals following pollination in a coordinated manner. One ACC synthase gene (Phal-ACS1) is under the regulation of ethylene and appears to lead to an amplification of the ethylene signal like DC-ACS1 in carnation (Bui and O'Neill, 1998). Two additional ACC synthase genes (Phal-ACS2 and Phal-ACS3) were shown to be expressed primarily in the pistil in response to primary pollination signals. In contrast to carnation, where ACC accumulates in the ovary without the expression of ACC synthase genes or ACC synthase activity, orchid flowers fail to exhibit increased ACC synthase activity or ACC synthase gene expression in petals (Bui and O'Neill, 1998). This is in spite of the fact that petals account for much of the ethylene produced from a pollinated orchid flower.

The final step in the ethylene biosynthetic pathway is catalyzed by ACC oxidase. In contrast to ACC synthase, this enzyme is often constitutive in plant tissues. In carnation, it was shown that petals did not exhibit significant activity of ACC oxidase until the onset of petal senescence (Woodson et al., 1992). This increased ACC oxidase activity was associated with the expression of ACC oxidase mRNA encoded by the DC-ACO1 gene. The expression of ACC oxidase mRNA in carnation petals is under strict regulation by ethylene. Inhibitors of ethylene action prevent the expression of the DC-ACO1 gene in petals and ethylene stimulates expression prior to the onset of senescence. In contrast to petals, styles of mature carnation flowers are capable of converting ACC to ethylene and ACC oxidase mRNA is abundant in this tissue (Woodson et al., 1992; Jones and Woodson, 1997). While pollination stimulates the expression of ACC oxidase, and inhibitors of ethylene action prevents this increase, carnation styles contain constitutive

levels of ACC oxidase mRNA that lead to high levels of ACC oxidase activity independent of pollination. In this species, ACC oxidase expression plays an important role in amplifying the ethylene signal in styles and petals.

The ACC oxidase gene family has been studied extensively in petunia. Four ACC oxidase genes were identified and shown to be arranged in two unlinked clusters of tandemly arranged genes (Tang et al., 1993). Three members of this gene family were shown to be expressed (Tang et al., 1994). As in carnation, petunia petals contain very low levels of ACC Oxidase mRNA. The onset of senescence is associated with a significant increase in ACC oxidase mRNA levels and ACC oxidase enzyme activity (Tang et al., 1994). The PH-ACO1 gene was shown to be responsible for this activity in petals, whereas PH-ACO3 and PH-ACO4 were found to be expressed primarily in pistil tissue. Pistils from immature flowers contained no ACC oxidase mRNA, but the levels increased with flower opening. Localization of ACC oxidase mRNA in pistils revealed much of the expression was in the stigmatic region of the style. Consistent with this, mature styles were shown to be capable of converting ACC to ethylene when applied to the stigma (Pech et al., 1987). Pollination of mature petunia flowers leads to increased ethylene within one hour. In contrast, pollination-induced ethylene in floral buds was delayed by several hours and was associated with increased expression of ACC oxidase (Tang and Woodson 1996). This delay in ethylene production was not associated with a delay in pollen germination or tube growth, suggesting that the low levels of ACC oxidase in pistils from flower buds was rate limiting. The induction of ACC oxidase in pollinated stigmas from flower buds was not prevented by treatment with ethylene action inhibitors, indicating this was a primary pollination response. In mature carnation, petunia and tomato flowers, pollination-induced ethylene in the pistil appears to be under the primary control of ACC synthase.

In contrast to petunia and carnation, the stigma of orchid flowers exhibit limited ACC oxidase activity prior to pollination (Nadeau et al., 1993). This activity increases dramatically following pollination and is associated with increased expression of ACC oxidase mRNA. In this species, ACC oxidase appears to play a significant role in regulating pollination-induced ethylene.

Pollen from a number of plant species has been shown to contain significant levels of the ethylene precursor ACC (Whitehead et al., 1983; Hill et al., 1987). Petunia pollen contains as much as 1,500 nmol ACC/g (Hoekstra and Weges, 1986; Singh et al., 1992). This observation led to the proposal that pollen-borne ACC could account for the ethylene produced by styles immediately following pollination. Singh et al. (1992) reported that the endogenous ACC content of pollen in petunia correlated with the amount of ethylene produced by petunia styles immediately after pollination. They concluded that this early ethylene was due to the conversion of pollen-borne ACC to ethylene. In support of this conclusion, unpollinated petunia stigmas exhibit high levels of ACC oxidase activity and are capable of converting applied ACC to ethylene (Pech et al., 1987; Tang et al., 1994; Tang and Woodson, 1996).

Recently, the synthesis of ACC in developing pollen has been investigated. Lindstrom et al. (1999) reported that the accumulation of ACC in pollen was a rather late developmental event, occurring just as the flower began to open. Furthermore, they showed that this ACC was the product of an ACC synthase gene, PH-ACS2, that was expressed specifically in the male gametophyte late in development. The PH-ACS2 gene and its promoter were analyzed further, and sequences flanking the 5'-region of the gene were shown to direct pollen-specific expression of the GUS reporter gene. A role for ACC or ethylene in pollen development has not been identified to date.

While pollen from petunia contains significant levels of ACC and this ACC could contribute to the ethylene produced following pollination, a number of experiments have brought this into question. The application of AVG, an inhibitor of ACC synthase, to petunia and carnation styles has been shown to effectively block pollination-induced ethylene production by the style, indicating that this early ethylene is dependent on ACC synthase activity (Hoekstra and Weges, 1986; Woltering et al., 1993). Also, the rapid

induction of ACC synthase activity in the styles of petunia following pollination also provides evidence that ACC synthase and the synthesis of ACC in the style rather than pollen-borne ACC is the precursor of this pollination-induced ethylene (Pech et al., 1987). In carnations, pollen-borne ACC is very low (25nmol/g) and would appear insufficient to elicit the level of ethylene produced by pollinated styles immediately following the application of pollen. When taken together with the early induction of ACC synthase genes in the pistil of carnation (Jones and Woodson, 1997) and tomato (Llop-Tous et al., 2000), it would appear that de novo synthesis of ACC is needed for the burst of ethylene immediately following pollination.

### **Ethylene and Interorgan Communication in Flowers**

The sequential nature of increased ethylene by styles, ovary and finally petals following pollination, suggests that ethylene or its precursor ACC may play a role in interorgan communication. In pollinated carnation and orchid flowers, the level of ACC increases in each of the floral organs concomitant with increased ethylene production (Nichols et al., 1983; O'Neill et al., 1993; Larsen et al., 1995; Jones and Woodson, 1997). Also, in a recent study, Jones and Woodson (1999b) reported that ACC content and ethylene production began in the tip of the style (stigma) and proceeded basipetally toward the base of the style following pollination.

As a soluble hormone precursor, it is reasonable to suggest that ACC would be more amenable to targeted translocation within the flower than the gaseous molecule ethylene. Translocation of ACC has been demonstrated to occur in flooded plants, where it is synthesized in roots and translocated to shoots where it is subsequently oxidized to ethylene (Bradford and Yang, 1980). Evidence for movement of ACC in flowers after pollination can be found in both carnations and orchids. In carnations, increased ACC content and ethylene production are detected in the ovary within 12 hours of pollination (Jones and Woodson, 1999b). However, ovaries exhibit no concomitant increases in ACC synthase mRNA (Jones and Woodson, 1997) or enzyme activity (Jones and Woodson, 1999b). Similarly, in orchids (O'Neill et al., 1993; Bui and O'Neill, 1998), ACC and ethylene are detected in petals without evidence for either ACC synthase mRNA or enzyme activity. When petals were removed from the pollinated orchid flower, ethylene evolution decreased, indicating ethylene biosynthesis in petals was dependent on the translocation of ACC from the gynoecium (O'Neill et al., 1993). In summary, these experiments clearly point to ACC as being synthesized in one organ and translocated to another following pollination.

More direct evidence for the translocation of ACC within flowers came from experiments using isotope labeled ACC. Reid et al. (1984) applied  $^{14}\text{C}$ -labeled ACC to the stigma of carnation flowers and detected the production of  $^{14}\text{C}$ -ethylene by the petals. In contrast, Woltering et al. (1995) reported that  $^{14}\text{C}$ -ACC and the ACC analog - aminoisobutyric acid were immobile when applied to the central column or stigma of *Cymbidium* orchid flowers. While it is clear that ACC accumulates in organs that exhibit no apparent capacity to synthesize this compound, the role of ACC translocation in interorgan signaling following pollination remains to be determined.

Ethylene has also been implicated as the translocated signal in pollination-induced senescence. This could occur through the movement of dissolved ethylene or through diffusion of ethylene within intercellular spaces. Internal concentrations of ethylene have been shown to be quite high (Woltering, 1990). The diffusion of gaseous ethylene from styles to petals could offer another explanation for the evolution of  $^{14}\text{C}$ -ethylene from carnation petals when styles were treated with  $^{14}\text{C}$ -ACC (Reid et al., 1984). Ethylene and its analog propylene have been shown to diffuse from the gynoecium to the petals in orchids (Woltering, 1990; Woltering et al., 1995) and carnations (Jones and Woodson, 1999b).

While there is considerable evidence for the translocation of both ACC and ethylene in pollinated flowers, these results do not clearly define either as the translocated pollination signal. It is clear ethylene plays a critical role in pollination-induced petal

senescence, as inhibitors of ethylene action prevent this response (O'Neill et al., 1993; Tang and Woodson, 1996; Jones and Woodson, 1997; Bui and O'Neill, 1998). The expression of ACC oxidase genes in the ovary and petals of carnation is completely dependent on ethylene (Jones and Woodson, 1999a), suggesting that ethylene produced in response to primary pollination signals in the gynoecium is critical to propagate the signal throughout the flower. Similar results were reported in orchid flowers (O'Neill et al., 1993; Nadeau et al., 1993). Consistent with the role of stylar ethylene in propagating the pollination signal, inhibition of ethylene action specifically in the style by treatment with diazocyclopentadiene prevented induction of ethylene in ovaries and petals, preventing pollination-induced senescence (Jones and Woodson, 1997). This points to a role for gynoecium-produced ethylene in the regulation of ethylene production in other floral organs following pollination.

### **Sensitivity to Ethylene**

The capacity to respond to ethylene has been implicated in the regulation of a number of processes including pollination-induced petal senescence. Halevy et al. (1994) demonstrated that pollination-induced abscission of cyclamen petals was prevented by treatment with the ethylene action inhibitor silver thiosulfate. In contrast, ethylene did not induce abscission of petals from unpollinated flowers. This result suggested that pollination resulted in the flowers becoming "sensitive" to ethylene. Pollination-induced ethylene sensitivity increased within a few hours of pollination in the flowers of *Phalaenopsis* orchids (Porat et al., 1995). This increase in ethylene sensitivity was associated with a significant increase in short-chain saturated free fatty acids in the column and perianth of pollinated flowers (Halevy et al., 1996). These compounds had previously been identified in the eluates from pollinated petunia styles and shown to exhibit senescence-inducing properties (Whitehead and Halevy, 1989). Other reports have failed to confirm the effects of these short chain fatty acids on flower senescence in petunia, carnation and orchids (Woltering et al., 1993), calling into question the role of these compounds in post-pollination signaling.

Tomato has become an important genetic and molecular model for studying ethylene biosynthesis and perception in plants. Llop-Tous (2000) reported that pollination of tomato flowers leads to increased ethylene production by the pistil and petals as in other flowers. This ethylene was shown to play an important role in senescence, as the never ripe (Nr) mutant failed to exhibit premature senescence following pollination. The Nr mutant displays ethylene insensitivity (Lanahan et al., 1994) and is defective in a member of the ethylene receptor gene family (Wilkinson et al., 1995). The potential for manipulating flower senescence by engineering ethylene insensitivity into the plant was recently studied. Gubrium et al. (2000) expressed a mutated version of the *Arabidopsis etr1-1* gene in transgenic petunias under the control of the constitutive CaMV35S promoter. The *Etr1* gene has been found to encode an ethylene receptor and the mutated version confers dominant resistance to ethylene (Chang et al., 1993). Petunia plants carrying the mutated *etr1-1* gene from *Arabidopsis* exhibited a delay in the onset of petal senescence following pollination and fruit ripening was also delayed relative to that of the wild-type (Gubrium et al., 2000). Interestingly, other horticultural attributes were affected by the transgene. For example, *etr1-1* plants produced fewer roots on vegetative cuttings. Taken together, these results clearly show the potential for delaying flower senescence through engineering plants to be resistant to ethylene. The development of strategies to express the mutated ethylene receptor in a tissue and/or stage specific manner could overcome some of the limitations to this technology, that could reduce the horticultural performance of the crop.

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