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Ethylene and flower senescence

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Abstract

The end of the relatively short life of carnations held in air is associated with climacteric rises in ethylene production and respiration, and coordinate rises in activity of the enzymes of the ethylene biosynthetic pathway. Carnation sensescence is associated with derepression of specific genes, increased polyribosome activity, and major changes in patterns of protein synthesis. Isotopic competition assays indicate the presence in carnation petals of ethylene binding activity with the expected characteristics of the physiological ethylene receptor. Inhibition of ethylene production and/or ethylene binding (whether in selected varieties, or by treatment with chemicals) results in longer-lived carnations. Examination of other flowers shows that the carnation is not a universal paradigm for flower senescence. The response to ethylene varies widely, and in many species petal wilting occurs without any apparent involvement of ethylene.

1. Introduction

Sensescence can broadly be defined as the combination of events that leads to the death of cells, tissues, or organs. Such a definition, applied to cut flowers, might include adverse water relations and floret abscission, but we will consider here only the events occurring during ageing and death of floral tissues. It is a common conviction in the floral trade that flowers are very sensitive to ethylene. Amongst plant physiologists, too, the importance of ethylene in the sequence of events we call flower senescence is considered well established. These assumptions are based on a substantial body of research [15] but are not universally true. The hormonal role of ethylene in flower senescence was not established until the development of analytical methods sufficiently sensitive to measure the minute quantities produced by the minimal tissues of a flower. Using gas chromatography, researchers have shown that, depending on the species, ethylene may be involved in several phases of flowering, from induction through growth of floral tissues to petal senescence.

In recent exhaustive survey, Woltering and van Doorn [30] studied the role of ethylene in petal senescence of flowers of 93 species representing 23 families. Except for a few families (all tested members of the Campanulaceae, Caryophyllaceae and Malvaceae, and most Orchidaceae), most of those flowers in which wilting was the primary senescence symptom were not sensitive to ethylene (Table 1) [29, 30]. Where petal abscission was the initial symptom of senescence the flowers were mostly sensitive to exogenous ethylene (Geraniaceae, Labiatae, Ranunculaceae, Rosaceae, Scrophulariaceae). Flowers that respond to low exogenous concentrations of ethylene are probably those in which ethylene is naturally involved in senescence.

We briefly review here what is known of the role of this hormone in flower senescence.

2. Ethylene and flowers

2.1 Effects of applied ethylene

For many cut flowers, the importance of ethylene in senescence was first examined by testing the effects of exogenous ethylene.

In the 1930s, researchers at the Boyce Thompson Institute treated many different plant tissues, including flowers, with relatively high concentrations of ethylene gas, and recorded the responses [41]. The reactions varied, and included abscission

Table 1. Ethylene and senescence of flowers from different families (from the data of Woltering and van Doorn [30]).

Family	Symptoms	Sensitivity[a]
Monocotyledonae		
Amaryllidaceae	Wilting	0–2
Iridaceae	Wilting	0–1
Liliaceae	Wilting	0–1
Orchidaceae	Wilting	3–4
Dicotyledonae		
Campanulaceae	Wilting	3–4
Caryophyllaceae	Wilting	4
Compositae	Wilting	0–1
Dipsacaceae	Wilting	2–4
Euphorbiaceae	Wilting	1
Geraniaceae	Abscission	4
Labiatae	Abscission	4
Malvaceae	Wilting	4
Primulaceae	Abscission	3–4
Ranunculaceae	Abscission	3–4
Rosaceae	Abscission	3–4
Scrophulariaceae	Abscission	2–4
Umbelliferae	Wilting	0

[a] Sensitivity from 0 (insensitive) to 4 (very sensitive).

of petals, failure to open, and senescence of the open flowers. More recent studies, such as those of Woltering and van Doorn [30] have already been referred to.

2.2 Ethylene production during flower senescence

Maxie et al. [13] demonstrated the close coincidence between the onset of the respiratory climacteric and a burst of ethylene synthesis in cut carnations. A similar relationship has been shown in studies of ephemeral flowers such as those of *Ipomoea* [3, 10, 12] *Tradescantia* [22], and *Hibiscus* [33]. The advent of more sensitive techniques of ethylene analysis such as the photoionization detector for gas chromatography and the laser photoacoustic sytem described by Woltering et al. [32] will permit even more detailed evaluation of the role of ethylene biosynthesis in the natural sensescence of cut flowers. Woltering [31] used his extremely sensitive instrument to examine the role of ethylene in sensescence of emasculated orchid flowers. The reason that removal of pollinia in *Cymbidium* results in senescence of the flower (and blushing of the labellum) is that desiccation of the tissue of the rostrum stimulates the early production of trace amounts of ethylene. These minute quantities of ethylene are, in turn, responsible for initiating general senescence of the flower.

The pathway of ethylene biosynthesis in plants has now been almost completely described [40]. The essential steps of the pathway have been completely identified in cut flowers. ACC (1-aminocyclopropane-1-carboxylic acid) content [5], ACC synthase [18], activity of the ethylene forming enzyme (EFE) [27] and of β-cyanoalanine synthase [11] have all been shown to rise coordinately with the onset of the ethylene climacteric burst in various flower parts. In a study of the control of ethylene biosynthesis during the climacteric rise, Whitehead et al. [27] showed that, unlike some fruit tissues (where EFE activity is always high), the activity of the EFE in senescing carnation petals rises coordinately with the increase in synthesis of ACC. Adam and Mayak [1] prepared an extract from carnation petals which converted ACC to ethylene. Although this preparation had some of the characteristics of *in vivo* EFE activity, its high Km, and lack of stereospecificity (Borochov and Reid, unpublished observation) indicates that it is probably a non-enzyme system, perhaps linked to lipid peroxidation, rather than the *in vivo* enzyme.

The activity of ACC synthase (normally the rate-limiting step) is markedly reduced by inhibitors of pyridoxal phosphate enzymes, such as aminooxyacetic acid (AOA) and aminoethoxyvinylglycine (AVG) [2]. Application of AOA to cut carnations inhibits the rise of ethylene and respiration and extends the vase life of cut carnation flowers [8]. Since AOA only prevents ethylene biosynthesis, it has limited value, offering little protection in ethylene-polluted environments [8, 9].

2.3 Ethylene action and flower senescence

The demonstration by Beyer [4] that Ag^+ was an effective and specific inhibitor of ethylene action provided a powerful tool for examining the action of ethylene in plant growth and development. Using the more mobile silver thiosulphate (STS) complex, Veen [25] demonstrated that inhibition of ethylene action greatly extends the life of cut carnations. This material has become an important commercial treatment for flowers sensitive to ethylene (Figure 1). The cyclic olefin Norbornadiene (NBD), also inhibits ethylene action, presumably by competitively inhibiting the

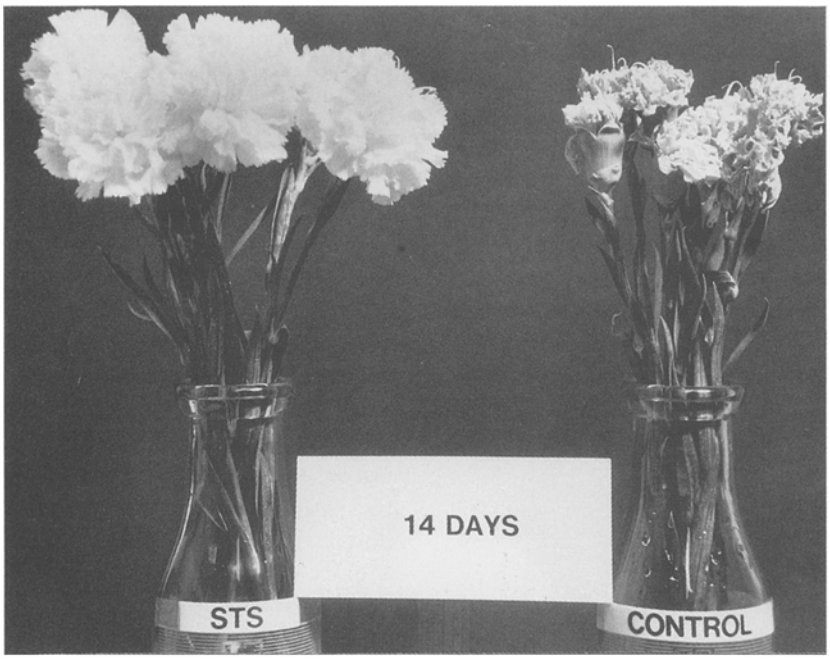

Fig. 1. Effect of STS treatment (1 μmol/stem) on the life of "White Sim" carnations.

ethylene binding site [21]. This material, being volatile, is a useful experimental tool, but its foul odor and carcinogenic properties make it of no practical value.

Nevertheless, it points to the possibility of discovering other organic compounds that may interfere with the ethylene binding site, and hence prevent ethylene action. Such compounds are likely to be of interest in practical control of ethylene responses in flowers long before they can be applied to fruits and vegetables. NBD has been shown to reverse the early stages of ethylene-mediated petal wilting in carnation flowers [26]. An hypothetical model for the action of ethylene in flower senescence is presented in Figure 2. This scheme suggests a membrane-based binding site that is activated, or repressed, by a "sensitivity factor". The ethylene molecule binds to a site where the inhibitors of ethylene action, Ag^+ and NBD, can also bind. When the binding site is sensitized and ethylene binds to it, a second message is generated which interacts with the 5′ (promotor) regions of genes involved in ethylene-regulated senescence, inducing transcription of the genes, and synthesis of the proteins encoded by the genes on polyribosomes. A considerable amount of evidence has accumulated from studies with cut flowers that is consistent with this scheme.

2.3.1 The binding site

Using isotopic competition technique, Sisler et al. [21] demonstrated the presence of a specific ethylene binding site in carnation petals. The application of STS or NBD to the flowers substantially reduced binding activity. Veen [24] suggested that Ag^+ competitively inhibited the ethylene-stimulated growth of carnation styles.

2.3.2 The sensitivity factory

As Trewavas [23] has pointed out, response to a plant hormone may involve increased biosynthesis, increased sensitivity to the hormone, or both. In ageing of some cut flowers there is a clear increase in sensitivity to ethylene [27, 37]. This effect is perhaps most striking in the ephermeral ethylene-sensitive flowers [22, 33] whose sensitivity increases very rapidly as the bud nears the day of opening. Suttle and Kende [22] used pigment efflux as a measure of senescence in *Tradescantia* petals. While ethylene treatment hastened efflux in petals excised from opening flowers, it had no effect on petals taken from buds one or two days before

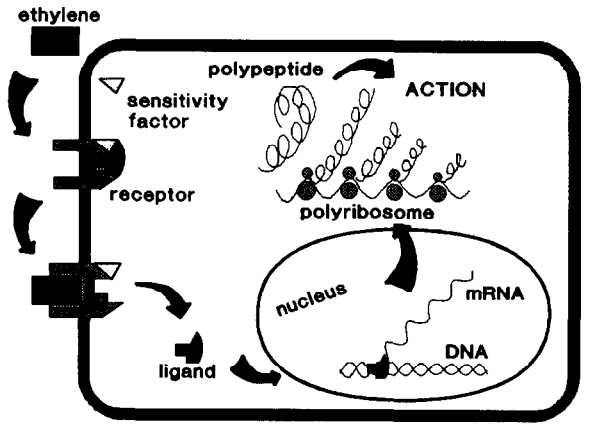

Fig. 2. Hypothetical scheme for the action of ethylene in inducing flower senescence.

opening. The nature of the sensitivity factor and its site of action are unknown, although there is some evidence supporting a short-chain fatty acid as a sensitivity factor in pollinated petunias [28]. The senescence-delaying effects of other growth regulators [14] is consistent with the notion that sensitivity to ethylene is a function of the balance of growth regulators. One means by which sensitivity to ethylene might be regulated is an interaction of the sensitivity factor with the binding site itself, as suggested in Figure 2, but this is only one of a number of possibilities.

The timing of the onset of the ethylene burst and, thus, the timing of senescence seems likely to be controlled by the sensitivity factor. By analogy with the situation in many fruits, the signal would be the endogenously produced ethylene, which would interact with the binding site and induce a response, including a stimulation of ethylene biosynthesis, once the tissue had become sensitive. Some authors have espoused the attractive notion that the timing of senescence is directed by interaction between different floral organs [7]. Mature carnation petals, however, senesce at the same time, whether attached to the flower or held individually in small vials [16]. Furthermore, removal of the gynoecium from the flower does not affect the timing of flower senescence. These data tend to indicate that the timing of petal senescence is related to the petal tissues themselves. There is, however, increasing evidence that the base of the petal plays an important role in timing [17].

2.3.3 The second message

Sisler's analysis of the compounds stimulating or inhibiting an ethylene response [20] suggested to him that a trans-effect pi-bonding scheme might be involved. He suggested that the interaction of ethylene with the binding site might release another ligand, which would be an obvious candidate for the second messenger. The nature of this compound is still unknown, but analysis of the structure of the promotor regions of senescence-specific genes in flower petals may provide important clues.

2.3.4 Messenger RNA synthesis

Woodson and his group [34–36] have provided elegant evidence that ageing of carnation flowers and the onset of ethylene biosynthesis are accompanied by synthesis of mRNA species not found in young flowers. They have isolated three cDNA clones from a cDNA library prepared from mRNA isolated from senescing carnation petals. The cDNAs are representative of two classes of mRNAs that increase in abundance during petal senescence. One mRNA (pSR5) is present at low levels during the early stages of development, and begins to accumulate in mature petals prior to the increase in ethylene production. Accumulation of this mRNA is retarded, but not prevented, by inhibiting the synthesis or action of ethylene. The other class of mRNAs are highly regulated by ethylene. They are not detectable (by Northern hybridization analysis) prior to the rise in ethylene production, and increase in abundance concomitantly with the ethylene climacteric. Furthermore, their expression is markedly inhibited by inhibition of ethylene production or action. Analysis of the products of these genes and examination of the control of their expression should provide important information about the way in which ethylene controls senescence.

2.3.5 Polyribosomes

The rapid rise in polyribosome profiles as petal senescence commences in carnations [6] is consistent with the observation that new genes are transcribed during this period. The increase in polyribosome profiles is somewhat unexpected, since total protein showed a concomitant decrease. This is presumably a reflection of the rapid synthesis of catabolic enzymes during senescence.

2.3.6 Proteins

Treatment of flower petals with inhibitors of pro-

tein synthesis substantially delays their senescence [22, 39]. Increased activities in catabolic enzymes such as ribonuclease are associated with decline in macromolecules including DNA, RNA, proteins and membrane lipids [15]. Clearly, the onset of senescence is associated with elaboration of catabolic enzymes that presumably play an important role in remobilizing the cell contents of the petal and the eventual death of the cells. The evidence that has accumulated indicating the general applicability of the scheme depicted in Figure 2 provides substantive support for the view that the senescence of petals is a carefully regulated event, involving the synthesis of specific proteins.

2.4 Ethylene-resistant mutants

In tomatoes, much has been learned about the role of ethylene in fruit ripening by the use of ethylene-resistant slow-ripening mutants [19]. Similar variants have been sought in carnation [38], and two commercial cultivars, Sandra and Chinera, were found to have vase lives about twice that of White Sim [37]. Measurements of ethylene production showed that the senescence of "Sandra" was qualitatively different to that of "White Sim"; these flowers produced no ethylene (Figure 3), and levels of ACC and EFE activity were also low throughout the postharvest period. "Sandra" flowers never showed normal corolla inrolling; their senescence was marked by loss of petal color, and gradual necrosis of the petals. In contrast, "Chinera's" long vase life was due to a marked delay in the onset of the normal symptoms of senescence – petal inrolling, ethylene production and increases in ACC content and EFE activity (Figure 3). "Sandra" proved to be a very interesting model system. Although its flowers produce no ethylene during natural senescence, they are much more sensitive to ethylene than those of "White Sim" (Figure 4). Furthermore, ethylene treatment induces ethylene biosynthesis in "Sandra". The longer vase life of this cultivar is therefore solely due to its failure to initiate autocatalytic ethylene production. "Chinera" was considerably less sensitive to ethylene than "White Sim" (Figure 4). This reduced sensitivity may explain its long vase life.

3. Ethylene-insensitive flowers

The striking effects of ethylene on responsive flowers, the availability of highly sensitive equipment for measuring this gas, and the usefulness of inhibitors of ethylene synthesis and action, have all led postharvest researchers to concentrate on ethylene-sensitive flowers. As a result of this interest, we now have an excellent picture of many of the events occurring during ethylene-induced flower senescence. Commercially, application of inhibitors of ethylene synthesis and action have extended the life of a range of important cut

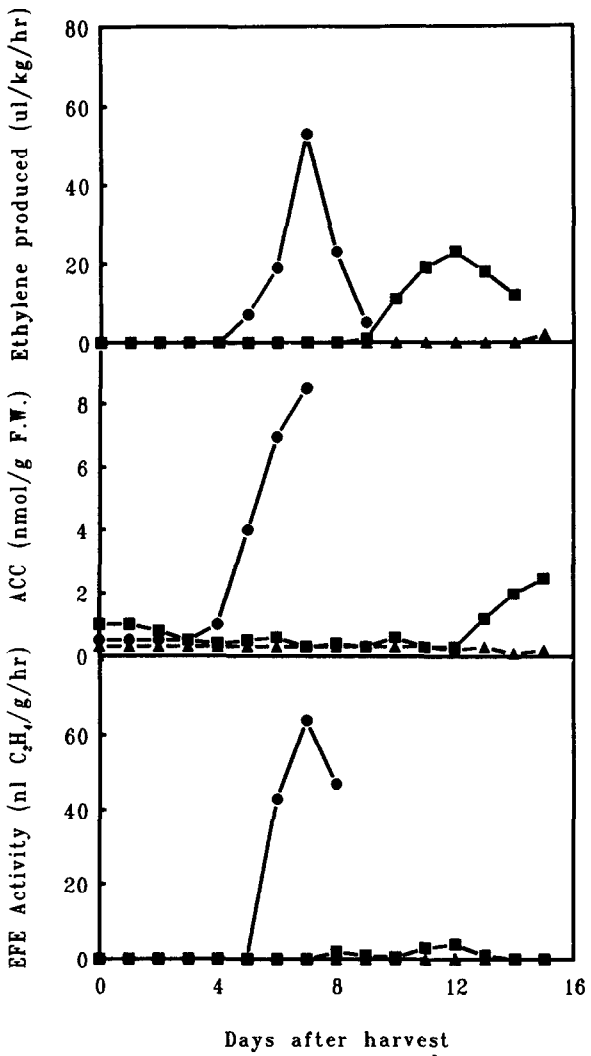

Fig. 3. Ethylene biosynthesis by long-lived carnation cultivars. Ethylene production, ACC content, and EFE activity of cut flowers of the White Sim ● Sandra ▲ and Chinera ■ cultivars of carnation were measured at intervals as previously decribed [38].

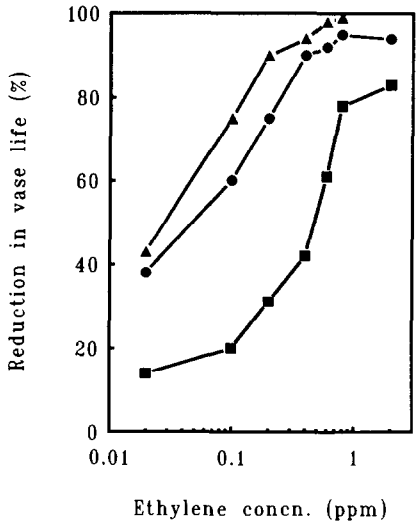

Fig. 4. Sensitivity of long-lived carnations to ethylene. Cut flowers of the White Sim ● Sandra ▲ and Chinera ■ cultivars of carnation were exposed continuously to different concentrations of ethylene in flowing air streams.

flowers. In the near future, we may expect the development of ethylene resistant cultivars, based on existing breeding lines, or on recent discoveries in the molecular biology of ethylene action in plants. As noted above, however, only some of the commercially important cut flowers have an ethylene-sensitive senescence, and these are in only a handful of families. Senescence of the petals of many cut flowers, including most of the important geophytes (such as gladiolus, iris, narcissus, and tulip) appears not to be related to ethylene. Despite the convenience of working with ethylene and its responses, it is clearly time to extend our experiments to other flowers to learn whether the sequence of events taking place in senescing petals of ethylene-sensitive flowers are in any way analogous to those occurring in flowers whose senescence is neither associated with, nor stimulated by ethylene.

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