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Role of ethylene in flower senescence of *Gypsophila paniculata* L.

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ABSTRACT

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Flowers on *Gypsophila paniculata* L. plants senesced about 10 days after the onset of opening. The first indication of senescence was translucency of the petals, followed by desiccation. In harvested panicles kept in water, the flowers lasted for 4 days prior to petal wilting, 6 days when 200 mg l^{-1} Physan (a disinfectant) was included in the vase solution, and 10 days when both Physan and sucrose (15 g l^{-1}) were included. Petal wilting was preceded by a sharp rise in ethylene production. Treatment directly after harvest with silver thiosulphate (4 m*M*) for 30 min increased flower longevity in each of the foregoing solutions. In cut panicles, young flowers and buds also wilted, and panicle wilting was delayed by two days if stems were held at 90% RH rather than at 60%.

It is concluded that petal senescence in *G. paniculata* is regulated by ethylene, and that early senescence of the flowers on cut panicles is related to ethylene production which is apparently caused by adverse water relations.

Translucency, the first indication of impending senescence, is a result of infiltration of cell sap into the apoplast.

INTRODUCTION

The senescence of flowers of *Gypsophila paniculata* L. has been assessed in experiments using cut panicles which contain numerous flower buds and some open flowers (Farnham et al., 1978; Barendse, 1986; Tandler et al., 1986; Downs et al., 1988). The senescence of individual flowers was not studied in detail in these experiments.

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Abbreviations: DI, deionized; PAR, photosynthetically active radiation; STS, silver thiosulphate.

In carnation (*Dianthus caryophyllus* L.), another member of the Caryophyllaceae, petal senescence is characterized by inrolling of the petals, followed by wilting. Petal inrolling of carnation flowers is apparently under control of ethylene as it is preceded by a peak in ethylene production, and hastened by a treatment with exogenous ethylene (Nichols, 1966; Maxie et al., 1973). Furthermore, a pulse treatment with the anionic silver thiosulphate complex (STS), which inhibits ethylene action, was found to delay the onset of senescence and the autocatalytic production of ethylene (Beyer, 1976; Veen and Van de Geijn, 1978). In carnation flowers, sucrose is also known to delay the onset of the climacteric rise in ethylene production (Mayak and Dilley, 1976). Petal wilting of carnation flowers is accompanied by an increase in leakage of solutes from the symplast to the apoplast (Mayak et al., 1977; Thompson et al., 1982; Sylvestre and Paulin, 1987). The cut flowering shoot of *Gypsophila paniculata* consists of numerous buds, opening flowers, and open flowers of different physiological ages. Previous preliminary experiments have shown that flowers on cut flowering branches of *G. paniculata* also respond to ethylene and that their longevity was extended by STS-treatment (Woltering and Van Doorn, 1988). These data implied endogenous control of senescence by ethylene.

We tested this hypothesis by measuring ethylene production from individual flowers, by determination of the effects of exogenous ethylene, pulsing with STS, and by inclusion of sucrose in the vase solution.

MATERIALS AND METHODS

Plant material. In intact plants of *Gypsophila paniculata* L. 'Perfecta' (a selection of the cultivar Bristol Fairy) grown in a greenhouse some individual flowers were tagged with tie-on tags and the symptoms of petal senescence were observed at intervals. Panicles from plants grown in the field at Santa Barbara, California with approximately 20% open flowers were harvested in the afternoon, pretreated in the field as required, and transported immediately to Davis (8 h at 20°C), and held in deionized (DI) water at 2°C until required. In other experiments (in Holland) panicles of the same maturity were harvested in the morning in a commercial greenhouse, held in water at 2°C, transported in water to Wageningen within 5 h, and used immediately.

Pulse treatment with STS. Immediately after harvest the stems of some panicles were placed in a 4 m*M* solution of STS (silver : thiosulphate = 1 : 6) for 30 min at ambient temperatures, then rinsed with DI water prior to transport. Control flowers were held in DI water under the same conditions.

Evaluation of wilting, opening, and senescence. Stems of replicate panicles (usually six per treatment) were recut under water and placed individually in ca. 100 ml of the required vase solution in a room maintained at 20°C, 60% RH, and with 12 h illumination each day from daylight fluorescent lamps (PAR at plant height ca. 15 μmol m^{-2} s^{-1}). Longevity of individual flowers was measured in panicles in which the newly opened (8–10 mm diameter) flowers were tagged with colored tape.

Observations were made on five flowers on each of three similar panicles. Flowers and buds were considered wilted when the pedicels lost turgor, and open flowers were scored as senescent when the outer petals desiccated.

Vase solutions. Flowers were placed in vase solutions of DI water alone, DI water containing 200 mg l^{-1} Physan-20 (1 g l^{-1} of commercial 20% concentrate), or 200 mg l^{-1} Physan with 15 g l^{-1} sucrose (Physan-20 + sucrose). Physan-20 (Consan Pacific Inc., Whittier, CA) is a commercial mixture consisting of 50% *n*-alkyl (C_{12}–C_{18}) dimethylbenzylammonium chlorides (5% C_{12}, 60% C_{14}, 30% C_{16}, 5% C_{18}), and 50% *n*-alkyl (C_{12}–C_{14}) dimethylethylbenzylammonium chlorides (68% C_{12}, 32% C_{14}). The flower stems were placed in the solutions in sterile plastic bags in hard plastic holders, or in glass pots that were washed between uses.

Determination of ethylene production. Ethylene production by detached flowers was determined by sealing them in a 5 ml vial for 3 h, withdrawing a sample of the vial atmosphere and determining its ethylene content by gas chromatography. In some experiments, flowers at various development stages (five replications per stage) were placed individually in vials with their pedicels in DI water. In other experiments, individual flowers (five replications) with their pedicels placed in 1 ml of 200 ppm Physan-20 + 1.5% sucrose were kept in unsealed vials throughout development and senescence. The vials were sealed at intervals for determination of ethylene production.

Effects of ethylene. To determine the effects of exogenous ethylene, branchlets from variously treated panicles were placed in DI water in glass tanks ventilated (ca. 30 l h^{-1}) with air, or air containing ethylene (0.3 or 3.0 $\mu l\ l^{-1}$) at 20°C. The treatment concentration was continually monitored by automatic photoionization gas chromatography (Photovac 10S30). Alternatively, panicle stems were placed in water in stainless steel tanks maintaining 0.3 and 3.0 $\mu l\ l^{-1}$ ethylene for 24 h in the dark at 20°C. Relative humidity in the tank was held at 90%. Panicles were then placed in DI water at 20°C in the climatized room.

RESULTS

Symptoms of petal senescence. Flowers of intact flowering plants of *Gypsophila paniculata* L. senesced about 10 days after anthesis. Petals became translucent, then showed inrolling, wilting, and desiccation. Similar symptoms were observed in flowers on cut panicles. Senescence of individual flowers occurred about 10 days after anthesis in panicles held in a solution containing 200 mg l^{-1} Physan-20 and 1.5% g l^{-1} sucrose (Fig. 1). Senescence occurred earlier in panicles held in a solution containing Physan-20 or DI water only (Table 1).

Cross sections of mature petals held under water and observed under a microscope showed gas in the intercellular space. In translucent and wilted petals the shiny appearance of the cross sections was lost.

TABLE 1

Longevity (days to petal wilting) of individual flowers of *Gypsophila paniculata*, on panicles held in DI water, 200 mg l^{-1} Physan-20 or in 200 mg l^{-1} Physan-20 and 15 g l^{-1} sucrose

	Longevity (days)
DI water	3.8 ± 0.4a
Physan	5.9 ± 1.6b
Physan + sucrose	8.8 ± 0.6c
STS – DI water	5.1 ± 1.0b
STS – Physan	10.7 ± 2.2c
STS – Physan + sucrose	16.5 ± 2.5d

Flowers were 8–9 mm diameter at the onset of determination of longevity. The panicles were pretreated with DI water or STS (4 m*M*) for 30 min. Data are means of fifteen flowers ± SD. Significant differences ($P < 0.05$) are indicated by a different letter.

Effects of STS. A short pulse with STS (4 m*M*, 30 min.) directly after harvest delayed the onset of petal senescence in individual flowers. Flower longevity was increased regardless of the vase solution used (Table 1).

Production of ethylene. The rate of ethylene production by individual flowers held in Physan-20 + sucrose was low, but rose to a sharp peak prior to the translucent appearance of the petals, then fell to low levels as petals desiccated (Fig. 1). A similar pattern was observed when ethylene evolution was measured in flowers at different development stages, taken from panicles held in DI water for two days.

In panicles held in DI water or Physan-20, individual senescence of open flowers was accompanied by complete drying of the entire panicles (including opening flowers and buds). This early, relatively synchronous desiccation was

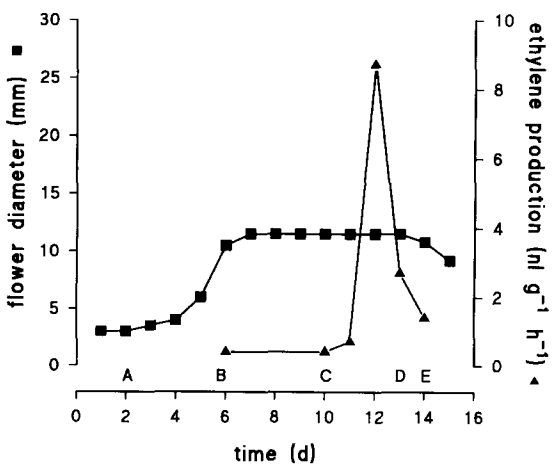

Fig. 1. Flower diameter (■) and ethylene production (▲), in individual flowers of *Gypsophila paniculata* L. Flower stages, as defined in this study, are given by letters. A, flower bud; B, opening flower; C, open flowers, close to senescence; D, translucent petals, E, wilted petals. Flower peduncles were held in an aqueous solution containing 200 mg l^{-1} Physan-20 and 15 g l^{-1} sucrose.

TABLE 2

Ethylene production of individual opening flowers (8–9 mm diameter) on cut panicles of *Gypsophila paniculata*

	Ethylene production ($nl\ g^{-1}\ h^{-1}$)
Day 3	0.8 ± 0.3a
Day 4	
– no wilting symptoms	3.8 ± 1.0c
– wilting symptoms	1.5 ± 0.2b

Panicles were held in DI water for 3 days (panicles not wilting) or 4 days (panicles wilting). From the latter panicles some flowers were cut which did not yet show wilting symptoms and some other flowers which had already wilted. Flowers were placed in vials containing ethylene free air and no solution. Data are means of five replications ± SD. Significant differences ($P < 0.05$) are indicated by a different letter.

apparently not related to natural senescence. The early desiccation of the whole panicle was preceded by an increase in ethylene production by the flowers (Table 2).

Effects of exogenous ethylene on petal senescence. When the panicles were continuously held in ethylene (0.3 or 3 $\mu l\ l^{-1}$) at 20°C and 90% RH no effects of ethylene were observed for up to 3 days of exposure, but after 4 days nearly all open flowers on panicles treated with either 0.3 or 3 $\mu l\ l^{-1}$ ethylene were senescent (Fig. 2). Effects of ethylene were observed about 2 days earlier when the RH was held at 60%. The same effect of RH on senescence was found in control panicles held in water (results not shown).

A 24 h treatment with 0.3 or 3 $\mu l\ l^{-1}$ ethylene at 20°C reduced the time to senescence of the open flowers from about 5 days in controls to 2.5 days in treated

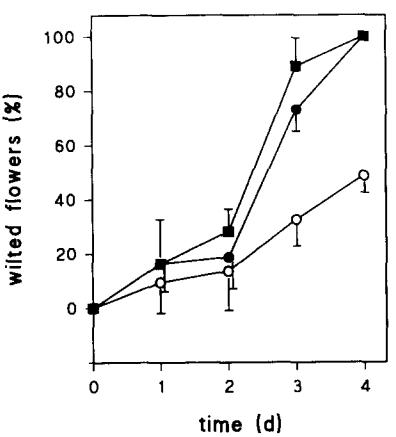

Fig. 2. Effect of exogenous ethylene on the number of wilted flowers of *Gypsophila paniculata*. Panicles were held in DI water in air (○) or ethylene at 0.3 $\mu l\ l^{-1}$ (●) and 3 $\mu l\ l^{-1}$ (■), at about 90% RH. The bars represent SD values.

panicles. The 24 h ethylene treatment at relatively low concentrations (0.3 $\mu l\ l^{-1}$) did not result in senescence of half open flowers, indicating lower sensitivity than in open flowers. Upon prolonged exposure to ethylene desiccation was observed in flower buds, half open flowers, and in open flowers, prior to these symptoms in control panicles (results not shown).

DISCUSSION

The effects of exogenous ethylene, STS, and sucrose on petal senescence in *Gypsophila paniculata* flowers, and the rise of exogenous ethylene production prior to visible symptoms suggest that petal senescence is regulated by endogenous ethylene. Results with *G. paniculata* are similar to observations made on carnation flowers (Nichols, 1966; Mayak et al., 1977; Veen and Van de Geijn, 1978). Cut carnation flowers held in a solution containing sucrose had a much longer flower longevity than controls (Mayak and Dilley, 1976). In carnations held in a vase solution containing sucrose and adequate antimicrobial compound (Larsen and Frolich, 1969) the longevity of the flowers was extended about as much in flowers pretreated with STS (Veen, 1979). The effects of STS and sucrose on the time to wilting in some carnation cultivars have been found to be about additive (Reid, Farnham and Paul, unpublished). A similar interaction was found in the effects of sucrose and STS on the time to wilting in individual flowers on cut flowering stems of *G. paniculata* (Table 1).

The mechanism of action of sucrose in delaying senescence has not yet been fully elucidated. Sucrose applied to the vase water is taken up in adequate amounts only when the carnation stems were not plugged by micro-organisms (Larsen and Frolich, 1969). Sucrose is then translocated to the flower head partially through the xylem but mainly through the phloem, and is converted to its constituent hexoses in the petals (Ho and Nichols, 1975; Paulin, 1977; Paulin and Droillard, 1982). Feeding with sucrose maintains the pool of respirable substrate in petal cells (Coorts, 1973), although substantial amounts of hexoses were found in senescing petals of carnation not fed with sucrose (Nichols 1973, 1975). Sucrose also protects the petals against ethylene and may therefore delay the onset of the climacteric-like ethylene production and wilting of carnation flowers (Mayak and Dilley, 1976). Sucrose feeding is also known to maintain the otherwise gradually declining osmotic potential. Including mineral salts in the vase water of carnations had a similar, although smaller, effect indicating that sucrose has an effect other than maintaining the osmotic potential (Halevy and Mayak, 1979).

Petal senescence in *G. paniculata* and in carnations also showed differences. Translucency of petals was the first symptom of senescence observed in *G. paniculata*, a symptom not observed in carnation. Premature desiccation of the flower buds was a characteristic observed in *G. paniculata*, not in spray carnations. This desiccation of buds in *G. paniculata* was sensitive to exogenous ethylene. In ethylene treatments (3 $\mu l\ l^{-1}$ at 20°C, various periods of time) with spray carnations (cvs. Silvery Pink, Red Baron and Sunshine) bud desiccation was not observed (Woltering and Harkema, personal communication 1991).

Translucency of the petals may be related to leakage of solutes and water from the symplast into the apoplast. During senescence of petals of *Iris* (Iridaceae), *Gladiolus* (Iridaceae), *Tradescantia reflexa* (Commelinaceae) and *Hemerocallis* (Liliaceae), the apoplast also apparently became infiltrated with a fluid (Bancher, 1938; Horie, 1961, 1962; Reid, unpublished). During senescence in *Tradescantia reflexa* the petal rims become glassy. Under the light microscope, transverse sections of fresh petals looked shiny as they contained air in the intercellular spaces. Senescent petals looked soggy under the microscope as the air was apparently replaced by liquid (Horie, 1961, 1962). The same was now found in the entire petal of *G. paniculata*.

Senescence of individual flowers on cut flowering branches of *G. paniculata* was complicated by early wilting of the whole panicle. Inclusion of Physan-20 in the vase solution delayed this by a few days. The early wilting of cut panicles is related to adverse water relations as increasing the RH increased longevity. Early wilting is apparently due to lack of water uptake from the vase solution (Van Doorn et al., unpublished results). It was associated with a high rate of ethylene production, a common symptom of water stress (Apelbaum and Yang, 1981), even by the opening flowers. The increased production of ethylene in the water-stressed panicles may have stimulated early senescence of the flowers, as pretreatment with STS significantly delayed the onset of wilting of individual open flowers on panicles placed in DI-water.

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