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# Does Pollination Induce Corolla Abscission of Cyclamen Flowers by Promoting Ethylene Production?

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ABRAHAM H. HALEVY*[1], CHARLES S. WHITEHEAD[2], AND ANTON M. KOFRANEK
*Department of Environmental Horticulture, University of California, Davis, California 95616*

## ABSTRACT

**Very low ethylene production rates were measured in nonpollinated *Cyclamen persicum* Mill flowers, and no change in production was observed during the whole life span of the flower until death. Normal senescence was accompanied by a gradual discoloration and loss of turgor followed by wilting. Pollination induced a dramatic increase in ethylene evolution, culminating in a peak 4 days after pollination, and abscission of the corolla on that day. Silver-thiosulfate, an inhibitor of ethylene action, had no effect on longevity of unpollinated flowers, but completely nullified the effect of pollination on corolla abscission. Exposing unpollinated flowers to very high ethylene concentrations (50 microliters per liter) for 48 hours did not promote corolla abscission or senescence. 1-Aminocyclopropane-1-carboxylic acid, the immediate precursor of ethylene, increased ethylene production by unpollinated flowers more than 100-fold, but did not promote corolla abscission. 1-Aminocyclopropane-1-carboxylic acid did enhance corolla abscission of pollinated flowers. It is concluded that the main effect of pollination in inducing corolla abscission of cyclamen is by rendering the tissue sensitive to ethylene, apart from the promotion of ethylene production.**

It is well established that ethylene can accelerate abscission of leaves, fruits, and flowers (2, 3, 10). Some species, however, are not sensitive to ethylene, and their leaves or flowers will not drop in response to exposure to ethylene (2, 10).

The longevity of flowers is generally much shorter than that of leaves. Flowers may be generally divided into two distinct groups as to the cause for the termination of their life: (a) those showing a gradual change in composition of the corolla with age, loss of turgor, and final wilting; and (b) those in which the life of the flower is terminated by the abscission of the corolla, when it is still fully turgid (10, 15). In both groups ethylene production, and senescence or abscission are accelerated in pollinated flowers. It is, therefore, generally assumed that pollination-induced senescence and abscission are mediated by the increased ethylene production and exposure of the flowers to endogenous ethylene (6, 10–12).

In the present study we examine the role of ethylene and pollination in controlling senescence and abscission of cyclamen flowers, and if corolla abscission is regulated solely by the pollination-induced ethylene production.

## MATERIALS AND METHODS

**Plant Material.** Cyclamen (*Cyclamen persicum* Mill) of a red cultivar bred by Dr. H. C. Kohl (University of California, Davis) were grown from seeds in 15-cm pots using standard greenhouse production methods. Intact flower buds were tagged when they were still in a downward position, and again on the day when at least 4 of the 5 corolla lobes were folded upward. This stage was considered as the opening stage (day 0). In experiments using cut flowers, flowers were harvested on d 3 or 4, since the scape of flowers harvested earlier than this often bent when placed in water (A. H. Halevy, H. C. Kohl and A. M. Kofranek, unpublished data).

**Ethylene Production and Exposure to Ethylene.** On the opening day a group of 60 flowers was pollinated 3 times, while the rest of the flowers were unpollinated. Each day 15 pollinated and 15 unpollinated flowers were harvested and placed in DI.[3] Ethylene production by the flowers was determined by sealing 3 flowers in a 0.5-L Mason jar fitted with a sampling port and measuring the accumulated ethylene after 1 h by GC.

The effect of ethylene and flower age on time to senescence was examined by harvesting pretagged flowers of different ages, placing them for 24 or 48 h in flowing air streams containing 0, 1, 10, and 50 $\mu l \cdot l^{-1}$ ethylene, and evaluating their longevity in DI under conditions described below.

**Determination of ACC Content in Pollen.** Five samples of pollen were collected from flowers in the greenhouse. Each sample was collected from 20 flowers and weighed about 20 mg. Pollen was extracted in hot ethanol (1) and the ACC content of the ethanolic extract was determined according to Bufler *et al.* (5).

**Treatment with STS or ACC.** The anionic $Ag^+$-$S_2O_3^{2-}$ complex was prepared according to Reid *et al.* (14) and applied as a spray to intact flowers.

ACC was applied in three ways: as a spray to intact flowers, as a constant application through the base of the cut flower scapes, and as a dip of the flower heads. Care was taken so that the corolla cup with the stigma was completely filled with the ACC solution for 10 s before draining. Unpollinated and pollinated flowers served as controls. Intact flowers were pollinated three times and were cut 20 h after pollination. ACC spray of intact pollinated flowers was applied 20 h after pollination.

The longevity of the cut flowers was evaluated by holding the flowers in DI, under cool white fluorescent light (1.5–2.0 $wm^{-2}$, 12-h d) at 20°C and 60% RH.

Each experiment was conducted two or three times.

[1] Department of Ornamental Horticulture, The Hebrew University of Jerusalem, P. O. Box 12, Rehovot 76-100, Israel.

[2] Present address: Department of Botany, Potchefstroom University for CHE, Potchefstroom, South Africa 2520.

[3] Abbreviations: DI, deionized water, ACC, 1-aminocyclopropane-1-carboxylic acid; EFE, ethylene forming enzymes; STS, anionic silver-thiosulfate complex.

## RESULTS

**Ethylene Production and Senescence of Pollinated and Unpollinated Flowers.** Preliminary experiments showed that unpollinated flowers have a relatively long life span on the plant of about 3 weeks. Corolla senescence is first indicated by color change (bluing) followed by gradual loss of turgor and the termination of flower life by corolla wilting. Pollinated flowers have much shorter longevity, and their life is terminated by corolla abscission, when it is still fully turgid. This is also demonstrated in the data of Figure 1 and Table III.

The ethylene production of cyclamen flowers removed from the plant at intervals after opening is shown in Figure 1. Ethylene evolution by unpollinated flowers was very low (about 0.5 nl/flower·h) and did not change significantly during the entire 23-d life of the flowers from opening to wilting. Pollinated flowers stayed intact for only 5 to 6 d before abscising. Their ethylene production rose dramatically from the first day after pollination to a peak just before abscission. The peak of ethylene production of pollinated flowers was 70-fold the basal level of unpollinated flowers.

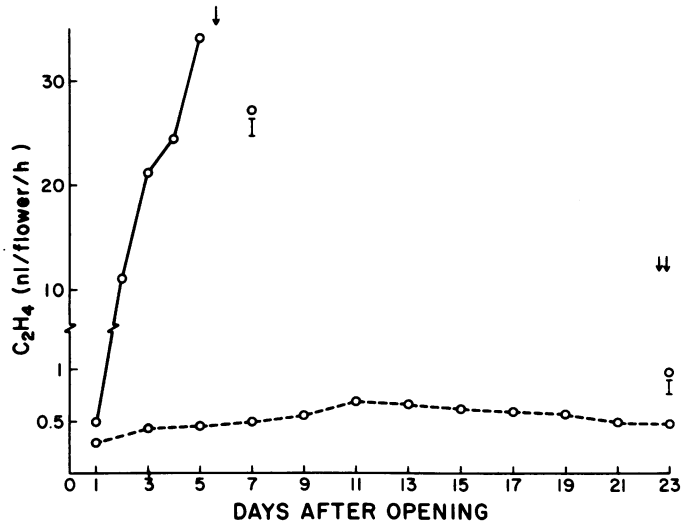


FIG. 1. Ethylene production by pollinated (O——O) and unpollinated (O- - -O) cyclamen flowers of different ages. Intact flowers were left unpollinated or pollinated on opening day. Flowers were harvested daily for ethylene production measurements. Means of five replications. Bars indicate the SE of the means. One arrow indicates the time of corolla abscission and two arrows the time of wilting.

Table I. *Effect of Exposure to Ethylene on Longevity of Cyclamen Flowers*

Flowers were cut at various ages and exposed to various concentrations of ethylene for 24 or 48 h before longevity evaluation. The data of exposure for 24 and 48 h were combined.

| Time (d) after Opening, when Cut | Longevity (d) after Ethylene Treatment | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 10 | 50 | Mean |
| | | | *µl/l* | | |
| 3–6 | 13.2 | 11.4 | 10.9 | 12.4 | 12.0 a[a] |
| 7–10 | 10.4 | 11.1 | 10.0 | 10.6 | 10.5 b |
| 11–14 | 8.1 | 7.5 | 8.3 | 7.4 | 7.8 c |
| 15–18 | 6.0 | 5.6 | 6.7 | 5.8 | 6.0 d |
| 19–22 | 4.3 | 4.7 | 3.9 | 4.0 | 4.2 e |
| Mean | 8.4 | 8.1 | 7.9 | 8.0 | |

[a] Values followed by different letters are statistically different at 5% level by Duncan's multiple range test.

**Effect of Ethylene on Cyclamen Flowers.** Flowers cut at different ages showed gradual decline in vase life with age (Table I). Table I does not include data of young flowers (d 0–2) since the scape of these flowers tended to bend when placed in water. Exposing flowers to ethylene even at the very high concentration of 50 µl/l had no effect on flower longevity. The values of Table I are the combined data of exposure to ethylene for 24 and 48 h, since no difference was found between these two treatments.

**ACC Content of Pollen and the Effect of Applied ACC.** Since pollination induced a rise in ethylene production and corolla drop, it was of interest to compare the ACC content of cyclamen flowers with that of other flowers (18). Cyclamen pollen was found to contain a low concentration of ACC, 5.08 ± 0.16 nmol/g. The average amount of pollen applied to the stigma was 0.6 mg. The amount of ACC applied with pollination was therefore approximately $3 \times 10^{-3}$ nmol, which could liberate about 0.075 nl of ethylene.

Table II shows that ACC both as base application and as corolla dip treatments to cut flowers greatly stimulated ethylene production. Base treatment with 10 mM ACC stimulated ethylene production more than 100-fold that of unpollinated flowers and 4 to 10 times that of pollinated ones. In flowers dipped in ACC, ethylene production declined in the second day to approximately one-tenth the rates in the first day, indicating the depleting ACC supply.

In spite of the very high ethylene production by the ACC-treated cut flowers there was no effect on senescence or on

Table II. *Effect of Pollination and of ACC Applied to Unpollinated Flowers through the Cut Scape Base or as a Corolla Dip, on Corolla Abscission, Longevity, and Ethylene Production 24 and 60 Hours after Pollination or the Start of ACC Treatment*

Means of six replications (each of two flowers) ± SE.

| Treatment | Ethylene Production | | Corolla Abscission (out of 12 flowers) | Longevity |
|---|---|---|---|---|
| | After 24 h | After 60 h | | |
| | *nl/flower·h* | | | *d* |
| Pollinated control | 8.1 ± 1.2 | 24.3 ± 2.3 | 11 | 3.4 ± 1.0 |
| Unpollinated control | 0.4 ± 0.1 | 0.6 ± 0.1 | 0 | 9.2 ± 1.8 |
| Corolla dip | | | | |
| DI | 0.4 ± 0.1 | 0.5 ± 0.2 | 0 | 10.4 ± 1.6 |
| ACC | | | | |
| 10 mM | 53.3 ± 4.6 | 6.4 ± 1.3 | 0 | 8.1 ± 2.0 |
| 2 mM | 28.2 ± 3.3 | 3.1 ± 1.2 | 0 | 8.6 ± 1.7 |
| 1 mM | 10.0 ± 2.4 | 1.3 ± 0.5 | 0 | 8.9 ± 1.8 |
| Continuous base application | | | | |
| ACC | | | | |
| 10 mM | 92.2 ± 6.4 | 104.3 ± 7.7 | 0 | 7.6 ± 1.8 |
| 2 mM | 23.4 ± 2.8 | 46.2 ± 3.6 | 0 | 8.7 ± 1.7 |
| 1 mM | 6.0 ± 1.1 | 11.8 ± 2.4 | 0 | 8.8 ± 1.9 |

Table III. *Effect of ACC Spray to Intact Pollinated Cyclamen Flowers on Ethylene Production 16 Hours after ACC Application, and on the Times of Corolla Abscission*

Means of 16 flowers ± SE.

| Treatment | Time of Abscission | Ethylene Production |
|---|---|---|
| | *h* | *nl/flower·h* |
| Unpollinated | | 0.5 ± 0.1 |
| Pollinated—no ACC | 108 ± 4.3 | 6.4 ± 1.1 |
| Pollinated—ACC 1 mM | 92 ± 3.8 | 7.8 ± 1.8 |
| Pollinated—ACC 2 mM | 77 ± 2.6 | 23.2 ± 2.3 |
| Pollinated—ACC 10 mM | 62 ± 2.4 | 42.0 ± 3.4 |

Table IV. *Effect of Spraying Intact Pollinated and Unpollinated Cyclamen Flowers with STS, on Flower's Longevity*

Means of 24 flowers.

| STS | Longevity | | Corolla Abscission (out of 24 flowers) | |
|---|---|---|---|---|
| | Pollinated | Unpollinated | Pollinated | Unpollinated |
| *mM* | *d* | | | |
| 0 | 5.4 b[a] | 21.5 a | 23 | 1 |
| 1 | 20.3 a | 22.6 a | 1 | 0 |

[a] Figures followed by different letters are statistically different at 1% level.

corolla drop. While pollination induced corolla abscission in 11 out of 12 flowers within 3 to 4 d, none of the ACC-treated cut flowers abscised, and flowers senesced by bluing and wilting at a date not significantly different from that of unpollinated controls.

ACC spraying of unpollinated intact flowers did not promote corolla abscission. However, ACC spray of pollinated flowers advanced their abscission (Table III).

**Effect of STS.** Spraying intact unpollinated flowers with STS had no effect on the longevity of the flowers. Pollination considerably reduced longevity and induced corolla abscission in 23 out of 24 flowers (Table IV). STS completely counteracted the pollination effect, preventing corolla abscission and restoring flower longevity to that of unpollinated flowers. STS-treated pollinated flowers terminated their life by discoloration and wilting as did unpollinated flowers.

## DISCUSSION

Ethylene production by flowers and their sensitivity to ethylene varies greatly in different plant species (10). In the present study we have found that cyclamen flowers undergo an entirely different senescence pathway depending upon whether they have been pollinated or not. Unpollinated flowers produce very little ethylene (Fig. 1) and do not respond to external ethylene even when exposed to very high concentrations of the gas (Table I), or when treated with massive amounts of ACC which increased the endogenous ethylene production more than 100 times (Table II). Generally the sensitivity of flowers to ethylene increases with age (10). For example, flower buds of *Ecballium* respond to ethylene by abscising only after they have reached a critical stage in their development (21). In cyclamen, however, no increase in sensitivity with age was found and even aged flowers did not abscise in response to ethylene (Table I). Unpollinated flowers terminated their life by gradual loss of turgor and final wilting. Pollinated flowers terminated their life by abscission of the fully turgid corolla. Pollination caused a rapid rise in ethylene production by the flowers (Fig. 1), and silver applied as STS, which inhibits ethylene action (4, 10, 14), completely nullified the pollination-induced corolla abscission (Table IV). This presumably indicates that the promotion of corolla drop in pollinated cyclamen flowers is mediated by ethylene.

The increase of ethylene production is apparently by stimulation of ACC-synthase as was found in carnation (13) and petunia (19) flowers. The low ethylene production of unpollinated flowers cannot be due to low EFE activity, since application of ACC to the flowers greatly increased their ethylene production (Table II) as was found with other plant tissues (7, 22). The ACC content in the pollen is much lower than that found in petunia, carnation, and sweet pea flowers (18), and cannot account for the increase in ethylene production. This increase may have been triggered by the wound inflicted to the style by the germinating pollen tubes (9, 15, 19).

In many flowers pollination accelerates senescence and abscission (6, 10, 11, 13, 15, 16, 19). It is generally assumed that pollination-induced corolla senescence is mediated by the increase in ethylene production, presumably via promotion of ACC synthase (10, 11, 13, 15, 19). This view is based on three main findings: (a) pollination promotes ethylene production (6, 11, 13, 15, 19); (b) exposure to ethylene promotes corolla abscission (10, 15); and (c) treatment with inhibitors of ethylene synthesis or action reduce or prevent abscission (8, 10, 17, 20).

In cyclamen flowers pollination, indeed, greatly promoted ethylene production, and treatment with STS counteracted the effect of pollination on corolla abscission. However, exposure of unpollinated flowers to very high concentrations of ethylene for up to 48 h, or supplying them constantly with ACC that promoted ethylene production by the flowers more than 5 times that of pollinated flowers, did not induce corolla drop. This indicates that the promotive effect of pollination on corolla abscission cannot be caused merely by stimulation of ethylene production. It is obvious that apart from the promotion of ethylene evolution, pollination also renders the tissue *sensitive* to ethylene. Indeed, unlike the case with unpollinated flowers (Table II), ACC enhanced abscission by pollinated flowers (Table III). This means that ethylene is necessary but not sufficient to induce corolla abscission.

It seems that pollination-induced corolla abscission in cyclamen involves at least two signals, one promoting ethylene production (apparently via ACC synthase) and the other increasing the sensitivity of the tissues at the abscission zone to ethylene. It was suggested that auxin is the pollination senescence signal (6). It is unlikely, however, that this unknown 'sensitivity' factor is auxin. Although auxin is known to be present in pollen (6), its movement in the style is very slow (16). Also, application of IAA to the stigma of digitalis did not cause rapid corolla abscission as is the case with pollination (15).

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