

Interorgan Translocation of 1-Aminocyclopropane-1-Carboxylic Acid and Ethylene Coordinates Senescence in Emasculated *Cymbidium* Flowers

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ABSTRACT

In *Cymbidium* flowers, emasculation by removal of the pollinia and the anther cap leads within 24 hours to red coloration of the labellum (lip). Lip coloration, being the first sign of senescence in these flowers, has been ascribed to the action of ethylene in the lip. When a small incision in the base of the lip is made prior to emasculation, or when the lip is excised and placed in water within 10 to 15 hours after emasculation, coloration is considerably delayed. This indicates that a coloration-associated factor is moving in or out of the lip. Measurements of ethylene production of excised flower parts, isolated at different times after emasculation, showed an increase only in the central column; the other flower parts, including the lip, did not show a measurable change. In contrast, *in situ* measurements of the ethylene production of the central column and the remaining portion of the flower revealed a simultaneous increase in all the flower parts following emasculation. Similarly, application of radiolabeled 1-aminocyclopropane-1-carboxylic acid (ACC) to the top of the central column *in situ* leads to the production of radiolabeled ethylene by all the flower parts. In addition, the ethylene production of isolated lips, measured immediately after excision, was initially high but ceased within 10 to 15 minutes. Treatment of the central column *in situ* with ethylene or ethephon did not stimulate ACC production but did stimulate lip coloration and this was accompanied by an increased internal ethylene concentration in the lip. The data indicate that endogenously produced as well as applied ACC is rapidly translocated from the site of production or application to all the other flower parts where it is immediately converted into ethylene. By excision of a flower organ, the influx of ACC is prevented, causing a rapid decrease in ethylene production. In addition, it was found that ethylene may also be translocated in physiologically significant amounts within the flower. The roles of ACC and ethylene as mobile senescence or wilting factors in emasculation- and pollination-induced senescence is discussed.

Interorgan translocation of ACC¹ was first described by Bradford and Yang (4) for the root-to-shoot transport in tomato. In this species, this xylem transport of ACC was also suggested to be involved in the pathogenic symptom expression upon root-knot nematode infection (6). In addition, Amrhein *et al.* (1) demonstrated that, also in tomato plants, application of labeled ACC to a single leaflet leads to the

¹ Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; AVG, aminoethoxyvinylglycine; NBD, 2,5-norbornadiene.

accumulation of radioactivity in other leaves and in the roots, indicating that ACC may also be transported through the phloem.

ACC may also be translocated between the different organs that compose a flower. Nichols *et al.* (14) observed increased ACC levels in all the different flower parts after pollination of carnation and hypothesized that this may be due, at least partly, to translocated ACC. Stigma wounding in *Petunia* causes an increase in ACC first in the stigma and next in the corolla (15) indicating ACC translocation. Although direct proof was lacking, Hoekstra and Weges (9) also concluded that ACC may be the transported wilting stimulus in pollinated *Petunia* flowers. In isolated carnation petals, ACC was found to be synthesized in the lower portion and was suggested to be transported to the upper portion during the course of senescence (13). Reid *et al.* (17) provided direct proof for the translocation of ACC in carnation flowers by detecting the production of radiolabeled ethylene by the petals from stigma-applied, radiolabeled ACC. The high amount of ACC used in this study, however, made it difficult to judge the significance of this finding for the natural situation.

Besides ACC it has been suggested that other mobile wilting factors may also be translocated in pollinated flowers (8). Eluates from pollinated *Petunia* styles have been shown to possess wilt-inducing properties while these eluates did not contain any detectable ACC or ethylene (5). In fact, only recently two possible candidates, *i.e.* decanoic acid and octanoic acid, were isolated from eluates of pollinated *Petunia* styles (19). In *Cyclamen* flowers, pollination-induced corolla abscission was ascribed to the action of a pollination-induced ethylene-sensitivity factor as abscission in pollinated flowers could be prevented by silver thiosulphate whereas it could not be induced by ethylene or ACC in nonpollinated flowers (7). Similarly, such a mobile ethylene-sensitivity factor was suggested to be produced in the lower portion of the carnation petal (13).

In *Cymbidium* flowers, senescence can be advanced by emasculation, *i.e.* removal of the pollinia and the anther cap. Within a few hours after emasculation, the ethylene production of the flower shows a small peak that lasts about 24 h (21). This emasculation-induced ethylene production was found to result from desiccation of the rostellum, originally covered by the anther cap and the pollinia, and was associated with an increased synthesis of ACC (23). Approximately 24 h after emasculation, the labellum (lip), an organ several cm away from the site of desiccation, shows a slightly pink

coloration which develops into dark-red within the following days (23). Some days later the perianth shows signs of wilting. Coloration of the lip, either induced by emasculation or by natural ageing may be regarded as the first visible sign of senescence in these flowers.

Coloration of isolated *Cymbidium* lips can be induced by exogenous ethylene or ACC and therefore it was argued that emasculation-induced lip coloration may also involve the action of ethylene, either through translocation of ethylene or ACC or through translocation of an ethylene-sensitivity factor from the site of desiccation to the lip (22). The emasculated *Cymbidium* flower was therefore chosen as a model to investigate the possible role of ethylene, ACC, and other senescence or wilting factors in interorgan communication during senescence of flowers.

MATERIALS AND METHODS

Chemicals

AVG and [¹²C]ACC were purchased from Sigma (St. Louis); NBD from Aldrich-Chemie (Steinheim, West-Germany); [2,3-¹⁴C]ACC from Commissariat à l'Énergie Atomique (Gif-sur-Yvette, France), and Ethrel (480 g/L ethephon) from Luxan (Elst, The Netherlands). The radiolabeled ACC solution was supplied to us in HCl and was buffered to pH 7 with a phosphate buffer solution from Merck (Darmstadt, West-Germany) prior to use. A mixture of ethylene in air was made by combining a known amount of ethylene (UCAR Specialty Gases Ltd., Oevel, Belgium) with compressed air (Hoekloos, Veenendaal, The Netherlands).

Plant Material and Vase Life Studies

Cymbidium flower spikes were obtained from a commercial grower and transported dry to the laboratory. The experiments were carried out with individual flowers, cut from the middle region of the spike. Generally, after excision, the flowers were placed in water under controlled environmental conditions of 12 h white light from fluorescent tubes (15 $\mu\text{mol}/\text{m}^2 \cdot \text{s}$), 12 h darkness at 20°C and 60% RH. Most experiments were performed with the cultivar Jacobi; in addition, cv King Arthur and cv Ivy Fung Sultan were also used in some experiments.

The incidence of lip coloration was visually evaluated each day. Lip coloration was classified on a scale from 0 to 4 using color prints as follows: 0 = original color, 1 = slightly pink coloration, 2 = pink coloration, 3 = red coloration, and 4 = dark red coloration (22). Unless otherwise indicated, time to coloration represents the average number of days to red coloration of the lip (color score ≥ 3).

Localization and Blockage of Vessels in the Lip

To study the distribution of vessels in the lip, excised lips were placed for a few hours with their cut bases in an aqueous solution of acid fuchsin. Due to this procedure, the vascular bundles became visible as red stripes. From this experiment, a rough estimation could be made where to make incisions in order to block a certain amount of vessels.

After the incisions were made *in situ*, a small piece of PVC-film was inserted to ensure that no contact between the two parts of the lip could occur.

Treatment of the Central Column with Chemicals

Treatment of the column with AVG was done immediately after emasculation. An aqueous solution (2–10 μL , depending on the experiment) containing a known amount of AVG was pipeted onto the rostellum and allowed to dry. When the rostellum was also treated with ACC or ethephon, a known amount of these chemicals (aqueous solution) was pipeted onto the rostellum approximately 20 h after the AVG treatment.

Treatments with ethylene, NBD, or a combination of both were carried out by flushing the gases with a flow rate of 0.6 L/h through a 7 mm wide tube that was placed over the upper half of the central column. NBD vapor of approximately 4000 $\mu\text{L}/\text{L}$ was made by flushing compressed air over liquid NBD heated to about 30°C. To prevent condensation, the manifolds and tubing were also heated. The concentration was calculated by weighing of the NBD solution at intervals. To prevent diffusion of ethylene or NBD from the outlet of the tube to the lip or petals, a ventilator was placed in front of the flowers.

Treatment of the Lips with Ethylene

Lips, while attached to the flower, were treated for 20 h with ethylene by enclosing them in 15 mm wide tubes that were flushed with ethylene as described above. In this case, a comparison was made between the effect of ethylene on coloration of lips in nonemasculated flowers and in flowers that were emasculated and immediately treated with AVG in a manner as described above.

Alternatively, lips excised from nonemasculated flowers and from flowers that were emasculated and treated with AVG at about 15 h prior to excision, were placed in water and treated for 20 h with different concentrations of ethylene. The ethylene treatments were carried out by placing the isolated lips in 70 L stainless steel chambers in which a known amount of ethylene was injected. During the treatments, the carbon dioxide concentration was kept low (<0.05%; v/v) and the oxygen concentration was maintained at ambient level (20). After the ethylene treatment the development of red coloration was evaluated.

Ethylene Production of Isolated Flower Parts

At different times, flowers were dissected and the ethylene production of the different flower parts was measured by enclosing them in 30 mL glass vials (3 parts per vial), allowing ethylene to accumulate. Prior to closure, the vials were flushed with ethylene-free air. Isolated columns and ovaries showed a wound-induced increase in ethylene production starting 1.5 to 2 h after incubation and, therefore, ethylene measurements were done within 1 h. The isolated lips, petals, and sepals did not show a measurable wound response and were incubated for 3 to 4 h. When isolated lips were treated with ACC they were regularly incubated for about 2 h in 270 mL glass containers (10 lips each).

Samples of the headspace were analyzed for ethylene by GC. The GC was equipped with a stainless steel column (2.5 m; i.d. 4 mm) filled with Alumina-GC (Chrompack, Middelburg, The Netherlands) and a flame ionization detector. The minimum detection limit for ethylene was about 2 nL/L.

Determination of Internal Ethylene and ACC Concentrations

Internal ethylene concentrations were analyzed using the method described by Beyer and Morgan (3). For each measurement 10 lips were exposed for approximately 2 to 3 min to 20 mbar pressure. Thereafter, a 0.3 mL sample from the extracted gas was analyzed by GC.

ACC in the central column was estimated approximately 20 h after emasculature or treatment with ethylene. The tissue was frozen in liquid nitrogen, dried *in vacuo*, and extracted with 80% methanol. ACC was analyzed by the method of Lizada and Yang (11) with internal standardization.

In Situ Measurement of Ethylene Production

Ethylene produced by the column and the remaining portion of the flower was determined using a flow-through system in line with a laser photoacoustic detector. The minimum detection limit for ethylene was 0.03 nL/L (21). Inside the cuvette the air was directed into two streams (ratio 1:1), one leaving the cuvette via the normal outlet, the other directed through a small tube that was placed over the central column. In this way, the *in situ* ethylene production of the column and the remaining portion could be calculated. The data were corrected for back diffusion (approximately 5%) that was tested by inserting a small amount of ethephon into the column compartment.

Similarly, the production of ethylene in the column and the remaining portion of AVG-treated flowers was measured after addition of 2 nmol ACC upon the rostellum.

Experiments with Labeled ACC

The experimental setup described above was also used for determination of the translocation of [2,3-¹⁴C]ACC. A small amount (5 nmol) with a specific activity of 3×10^6 Bq/ μ mol was applied to the rostellum of a flower previously treated with AVG.

In this case, the air leaving the cuvette was directed through a solution of 0.25 M mercuric perchlorate (each line two vials in series with 10 mL each) to trap the ethylene. After approximately 20 h of incubation, the radioactivity in the mercuric perchlorate solutions was determined with a Packard Tricarb scintillation spectrometer. The flower was divided into different parts that were ground under liquid nitrogen for determination of the radioactivity in methanolic extracts. In addition, a sample of the extract of the top of the central column was run on cellulose TLC (Merck, Darmstadt, West Germany; solvent *n*-propanol-NH₄OH/7:3). Radioactivity on the plates was determined with Berthold TLC-scanning equipment.

All experiments were repeated at least once; representative data are shown.

RESULTS

Translocation of a Wilting Factor

When lips were excised and placed in water within 10 h after emasculature, coloration was completely prevented in the cultivars under study. However, lips isolated at approximately 20 h after emasculature, at a time they did not yet show any visible sign of coloration, generally developed coloration comparable to lips attached to the emasculated flower (Table I).

To block the presumed translocation of a wilting factor from the column to the lip, prior to emasculature small incisions were made at the base of the lip. This blocked about 30 or 60% of the vessels entering the lip. The lip did not show any visible sign of turgor loss due to this treatment and control (nonemasculated) flowers were, at least during the first 5 d after the incision was made, not visibly affected. However, emasculature-induced coloration of the lip was significantly inhibited in both treatments (Fig. 1).

Table I. Coloration of Lips Isolated at Different Times after Emasculature, for Three Different *Cymbidium* Cultivars ($n = 10$)

Treatment	Time to Coloration		
	cv Ivy Fung Sultan	cv Jacobi	cv King Arthur
Not isolated	2	1.5	1.5
Isolated			
at 0 h	11	20	25
at 5 h	10	21	28
at 10 h	10	19	24
at 15 h	6	4	17
at 20 h	3	2	2
at 25 h	2	1.5	2
at 30 h	2	1.5	1.5

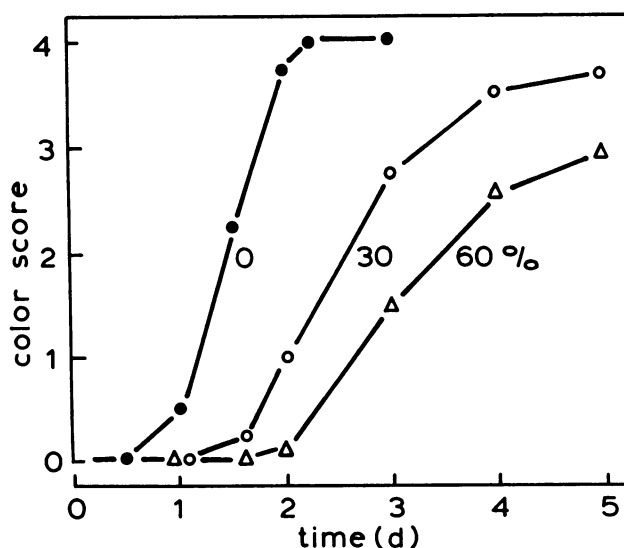


Figure 1. Development of red coloration in *Cymbidium* lips following emasculature when 0% (●), about 30% (○), and about 60% (△) of the vessels entering the lip were blocked ($n=10$).

Inhibitor Studies

After treatment of the column of nonemasculated flowers for 24 h with 4 $\mu\text{L/L}$ ethylene the lip showed rapid coloration (Table II). When NBD was applied simultaneously with ethylene, the ethylene-induced coloration of the lip was completely inhibited. Application of NBD alone was without effect in nonemasculated flowers (Table II).

Lip coloration in emasculated flowers was completely blocked by treatment of the rostellum with AVG. Coloration was restored by an additional treatment of the AVG-treated flowers with ACC, ethylene, or ethephon. Coloration of the lip was not affected by treatment of the emasculated column with air or NBD (Table II).

AVG was shown to effectively block the emasculatation-induced accumulation of ACC in the column and this was not affected by an additional treatment with ethylene or ethephon. Similarly, ethylene did not induce accumulation of ACC in columns of nonemasculated flowers (Table II).

In emasculated flowers and in flowers in which the column was treated with ethylene, ethephon, or a mixture of ethylene and NBD, a higher concentration of ethylene was found than in untreated intact flowers. Treatment of the emasculated flower with AVG prevented the rise in internal ethylene (Table II).

Ethylene Production of Isolated Flower Parts

The pattern of emasculatation-induced ethylene production in different flower parts is shown in Figure 2. During the experimental period a significant change in ethylene production occurred in the central column but hardly any change was observed in the other flower parts (ovaries, lips, petals, and sepals). From this experiment it could be calculated that approximately 90 to 95% of the total amount of emasculatation-induced ethylene was derived from the central column.

Considering that coloration of the lip was observed within

24 h after emasculatation, these data indicate that coloration is not the result of an increased ethylene production in the lip.

In Situ Measurement of Ethylene Production

The *in situ* measurements showed that, following emasculatation, both the central column and the remaining portion of the flower produced significant amounts of ethylene. In fact, the remaining portion produced over 80% of the total amount of ethylene (Fig. 3A).

The same type of experiment was carried out with flowers that had been thoroughly treated with AVG (total uptake approximately 160 nmol) immediately after emasculatation (which took place about 20 h before the start of the experiment). In this case, the ethylene production of the central column and the remaining portion was measured after addition of a small amount of ACC (2 nmol) onto the rostellum. Almost immediately after ACC application, the ethylene production of both the column and the remaining portion showed an increase comparable to the situation observed after emasculatation (Fig. 3B). In this experiment, approximately 20% of the applied ACC was recovered as ethylene, the major part (68%) of the ethylene being produced by the portion of the flower other than the column.

Translocation of Labeled ACC

The experimental setup for *in situ* measurements was also used for the determination of the translocation of [^{14}C]ACC. About 55% of the total radioactivity recovered was present in the ethylene traps, the remaining portion producing 58% of the total amount of ethylene (Table III). The extracts, including the holding solution, contained 45% of the activity recovered. Most of this activity (96.7%) was located in the upper part of the central column, *i.e.* at the site of application (Table III).

A sample of the extract of the upper part of the column,

Table II. Effect of Different Chemicals on Lip Coloration, Accumulation of ACC and Internal Ethylene Concentrations in *Cymbidium* Flowers

AVG, ACC, and ethephon were applied onto the rostellum. Air, ethylene, and NBD were applied to the upper half of the central column. AVG was given immediately after emasculatation, thereupon ACC and ethephon were or were not applied. ACC and internal ethylene were analyzed 20 h after the start of the 24 h treatment.

Treatment	Emasculatation	Time to Coloration	ACC Content in Central Column	Internal Ethylene Concentration in the Lip
		<i>d</i>	<i>nmol</i>	$\mu\text{L/L}$
Air	—	9	0.13	0.06
C ₂ H ₄ (4 $\mu\text{L/L}$)	—	1.5	0.14	0.16
NBD (4000 $\mu\text{L/L}$)	—	8	ND ^a	ND
C ₂ H ₄ + NBD	—	9	ND	0.18
Air	+	1.5	1.31	0.25
NBD	+	1.5	ND	0.25
AVG (100 nmol)	+	12	0.09	0.07
AVG + ACC (5 nmol)	+	1.5	ND	ND
AVG + C ₂ H ₄	+	2.0	0.14	0.20
AVG + ethephon (2 μL 1000 ppm)	+	1.5	0.13	0.17
LSD (5%)		3.6	0.08	0.04

^a Not determined.

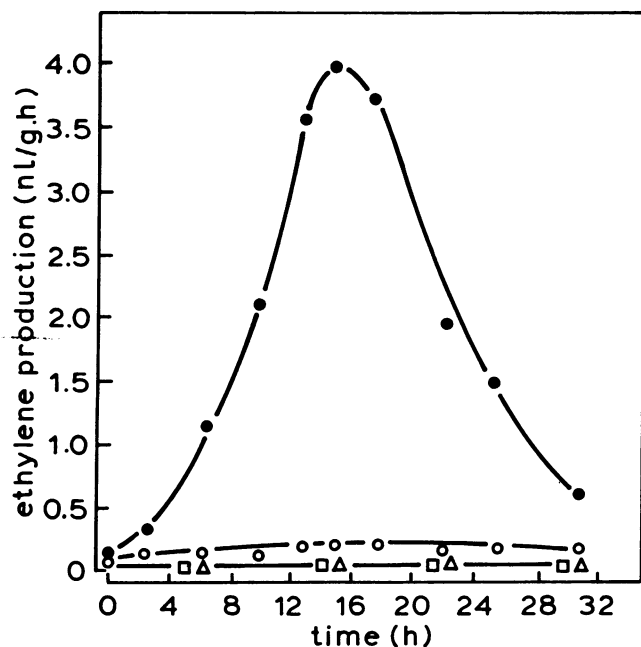


Figure 2. Ethylene production of columns (●), ovaries (○), lips (□), and petals + sepals (Δ) isolated at different times after emasculatation at $t = 0$. Three excised flower parts were incubated per 30 mL glass vial and were flushed with ethylene-free air prior to closure. Lip coloration was apparent within 24 h after emasculatation ($n=2$).

run on cellulose TLC, showed two radioactive positions at R_F -values of 0.23 and 0.48, the latter coinciding with the ACC standard. According to Amrhein *et al.* (1), the other component was *N*-malonyl-ACC. Integration of peak area revealed that about 87% of the activity in the top of the column was present as MACC.

Early Ethylene Production in Isolated Lips

Lips, excised from emasculated and control flowers, were enclosed into small vials within approximately 30 s after

excision. The accumulation of ethylene in the vials was determined by GC.

This experiment showed that the lips excised from emasculated flowers did produce significant amounts of ethylene during the first 5 to 10 min after excision. Thereafter, the production ceased. Lips excised from nonemasculated flowers showed only a very low ethylene production during the experimental period (Fig. 4). From these experiments it could be calculated that the lips, at the time of excision (accumulation during the first minute) produced ethylene at a rate of 1 to 2 nL/g·h.

Treatment of Lips with ACC and Ethylene

Isolated lips were placed with their cut base in low concentrations of ACC and the ethylene production and coloration were determined at intervals.

To ethylene production was slightly stimulated by treatment with 5 μ mol/L ACC. This only induced a faint pink coloration. At higher ACC concentrations, the ethylene production was more pronounced and this was accompanied by severe coloration (Fig. 5). From these experiments it may be argued that an increase in ethylene production up to 0.5 to 1 nL/g·h is required to induce a response comparable to the emasculatation-induced coloration.

Both lips in nonemasculated and AVG-treated emasculated flowers showed rapid coloration when treated with ethylene (Table IV). Similarly, isolated lips from nonemasculated and AVG-treated emasculated flowers showed a comparable development of coloration. This indicates that emasculatation itself does not affect the response of lips to ethylene.

DISCUSSION

Emasculatation of a *Cymbidium* flower leads to rapid coloration of the lip, being the first sign of senescence in these flowers. When the lip is excised from the flower within 10 h after emasculatation, coloration is completely prevented (Table I). Blockage of part of the vessels entering the lip considerably delays coloration, indicating that some coloration-associated

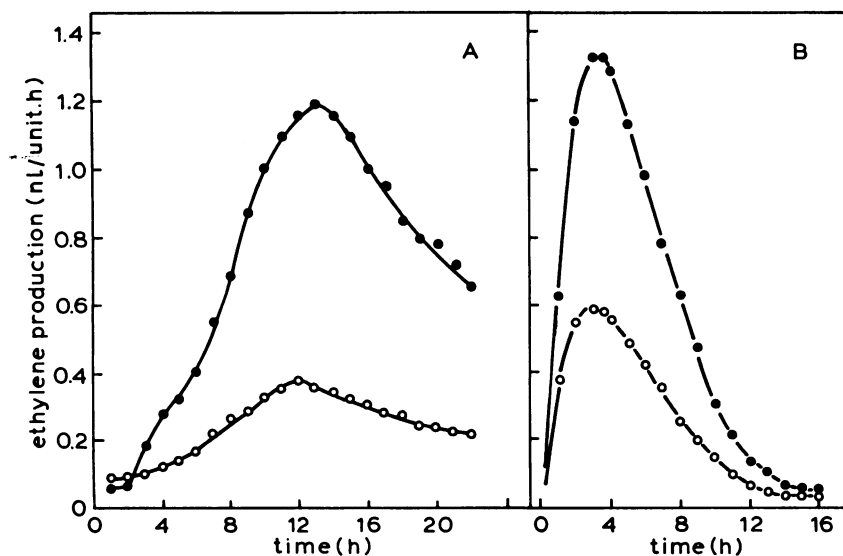


Figure 3. Typical *in situ* measurement of ethylene production in the column (○) and the remaining portion (●) of a *Cymbidium* flower. The measurements were performed in a flow-through system. A, Flower emasculated at $t = 0$; B, after application of 2 nmol ACC to the rostellar surface at $t = 0$. Approximately 20 h before start of the measurements the flower was emasculated and treated with AVG.

Table III. Radioactivity in Ethylene Traps and in Different Flower Parts after Application of Radiolabeled ACC to the Rostellum of an AVG-Treated *Cymbidium* Flower

Data are from an individual flower; duplicate experiments yielded identical results.

Source of Activity	Activity	Total Activity Recovered
	Bq	%
Ethylene traps	6854	55
Extracts (incl. holding solution)	5564	45
		Total Activity in Ethylene Traps
		%
Ethylene from column	2901	42
Ethylene from remaining portion	3953	58
		Total Activity in Extracts and Holding Solution
		%
Extract column top	5380	96.7
Extract column remainder	60.4	1.1
Extract ovary (incl. peduncle)	11.6	0.2
Extract petals	9.9	0.2
Extract sepals	22.2	0.4
Extract lip	0.77	0.01
Holding solution	79.4	1.4

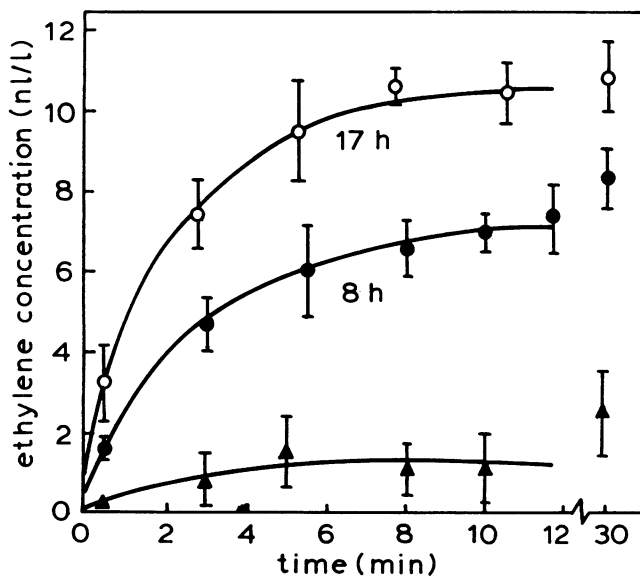


Figure 4. Ethylene accumulation in 30 mL glass vials with six lips each. The lips were excised from control flowers (▲) and from flowers at 8 h (●) and 17 h (○) after emasculatation. Data are means of three vials; vertical bars represent $2 \times$ SD.

factor is moving in or out of the lip. These data are comparable to results in pollinated *Petunia* flowers where it was found that regions distal to small 'windows' cut in the corolla tube show a delayed development of senescence symptoms (12).

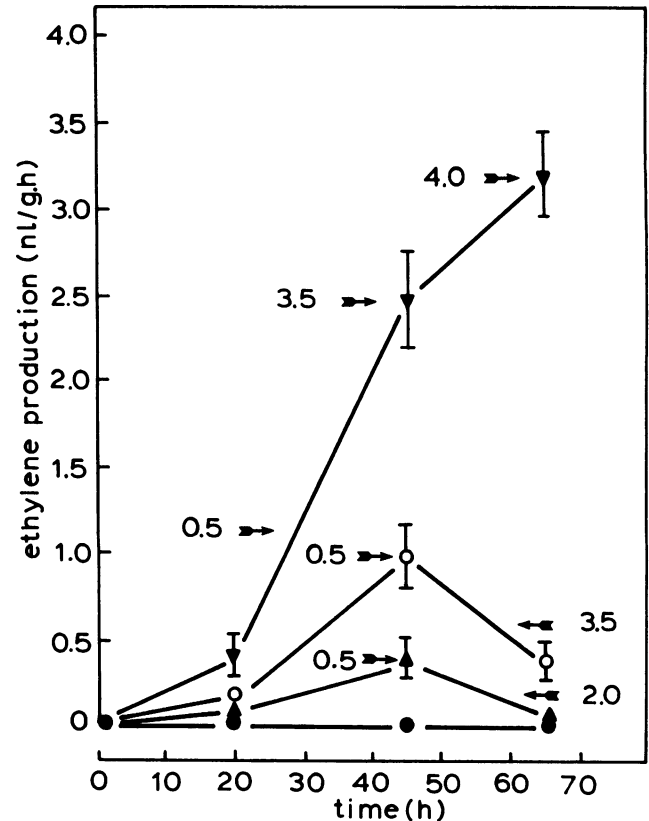


Figure 5. Effect of ACC on ethylene production and coloration in isolated *Cymbidium* lips. After excision, the lips were placed with their cut base in water (●), 0.005 mmol/L ACC (▲), 0.01 mmol/L ACC (○), or 0.02 mmol/L ACC (▼). Numbers indicate color score (see "Materials and Methods"). Ethylene production was measured by enclosing the lips in 270 mL glass containers (12 lips each). Data are means (\pm SD) of two containers.

Coloration of isolated *Cymbidium* lips was shown to be an effect of produced or applied ethylene (22) and, therefore, it may be argued that the transported signal is related to ethylene. This may, for instance, involve the translocation of substances that stimulate the ethylene production in the lip (e.g. ACC, elicitors) or induce an ethylene effect (e.g. ethylene, sensitivity factor[s]).

Determination of the ethylene production in different flower parts, isolated at different times after emasculatation, revealed that only the production of the central column shows a significant increase. The other flower parts, including the lip, have only a very low production throughout the experimental period. It may therefore be concluded that the transported signal is not ACC but, rather, ethylene itself may be translocated or the lip may become more sensitive to ethylene following emasculatation. The latter would meet our expectations as the existence of (mobile) ethylene-sensitivity factor(s) has also been suggested in cyclamen, petunia, and carnation flowers (7, 9, 13, 19).

To further characterize the presumed transported wilting factor, the central column was treated with chemicals (Table II). In emasculated flowers, coloration was effectively inhibited by AVG whereas treatment with air or NBD was without

Table IV. Effect of Exogenous Ethylene on Development of Lip Coloration

Lips from intact (nonemasculated) and AVG-treated emasculated flowers were treated for 20 h with 4 $\mu\text{L/L}$ ethylene while attached to the flower. Alternatively, the lips were excised from intact and AVG-treated emasculated flowers at 15 h after start of the experiment, placed in water and treated for 20 h with different concentrations of ethylene. Color of the lips was evaluated at 24, 48, and 72 h after start of the ethylene treatment. AVG (100 nmol) was applied to the rostellum immediately after emasculatation. Data are means \pm sd of 10 flowers.

Treatment	Ethylene Concentration $\mu\text{L/L}$	Color Score		
		24 h	48 h	72 h
Lips attached to intact flowers	0	0	0	0
	4	1 \pm 0	2.1 \pm 0.2	3.2 \pm 0.4
Lips attached to AVG-treated emasculated flowers	0	0	0	0
	4	1.1 \pm 0.4	2.4 \pm 0.5	3.4 \pm 0.5
Isolated lips excised from intact flowers	0	0	0	0
	0.10	0	0	0
	0.22	0.05 \pm 0.16	0.51 \pm 0.42	0.62 \pm 0.38
	0.41	0.10 \pm 0.12	0.56 \pm 0.60	1.62 \pm 0.52
	0.82	0.38 \pm 0.31	1.71 \pm 1.20	2.25 \pm 1.04
Isolated lips excised from AVG-treated emasculated flowers	0	0	0	0
	0.10	0	0.02 \pm 0.12	0.02 \pm 0.12
	0.22	0.08 \pm 0.21	0.49 \pm 0.40	0.58 \pm 0.48
	0.41	0.10 \pm 0.20	0.75 \pm 0.61	1.60 \pm 0.50
	0.82	0.43 \pm 0.40	1.62 \pm 1.46	2.49 \pm 0.75

effect. Treatment of an AVG-treated flower with ACC restored the normal response. These data indicate that diffusion of ethylene out of the column and subsequently into the lip does not play a role and that column-produced ethylene may also not be involved in the synthesis of the wilting factor. Contrary to what may be concluded from the ethylene production measurements in isolated organs, the effects of AVG strongly suggest that in fact ACC may play a role in signal transduction.

Similar conflicting data were reported by Hoekstra and Weges (9). These authors studied the ethylene production of different flower portions of *Petunia*, isolated at different times after pollination. During the early pollination-induced increase in ethylene production the wilting factor was shown to move into the corolla, whereas no extra ethylene could be detected in this organ. In this case, the majority of the ethylene was produced by the gynoeceum. On the contrary, they found that AVG (applied onto the stigma) was a very effective inhibitor of pollination-induced senescence even when the entire style was removed before the AVG could have reached the corolla. This made Hoekstra and Weges (9) conclude that, although direct proof was lacking, ACC may nevertheless be the transported wilting factor in *Petunia*. Pollination-induced corolla abscission in *Digitalis* was also not accompanied by an increased ethylene production in (isolated) corollas (18).

To analyze such conflicting results we used a sensitive laser

photoacoustic detector for measurement of the *in situ* ethylene production of the central column and the remaining portion of the flower after emasculatation and after addition of ACC to an AVG-treated flower. In addition, the transport of radiolabeled ACC was studied.

It was shown that, following emasculatation, a simultaneous increase in ethylene production was observed in the central column and the remaining portion (Fig. 3A). The same was true after addition of ACC to the rostellum of an AVG-treated flower (Fig. 3B). Furthermore, addition of radiolabeled ACC to the column resulted in the production of radiolabeled ethylene by the column as well as the remaining portion (Table III). In all these cases, more than half of the total amount of ethylene was produced by the remaining portion.

Contrary to the results obtained with isolated organs (Fig. 2), these data show that in the intact flower all the different organs may produce significant amounts of ethylene following emasculatation. Furthermore, this ethylene may well be derived from ACC which was shown to be translocated from the site of application to the other parts of the flower (Fig. 3B).

To explain the lack of ethylene production in isolated flower parts it was argued that, if these organs have little ability to synthesize ACC but have the ability to convert ACC to ethylene, the endogenous amount of ACC may rapidly decrease upon excision. This, in turn, should result in a rapid decrease of the ethylene production. This was indeed proven

to be true. During the first minute after excision, the lips produced relatively high amounts of ethylene which ceased within 5 to 10 min (Fig. 4).

From these data it could be calculated that the lip, while attached to the emasculated flower, may produce ethylene at a rate of 1 to 2 nL/g·h, a rate that was found to be sufficient to induce coloration in isolated lips (Fig. 5). It is therefore concluded that translocation of ACC plays an important role in emasculaton-induced coloration of the lip.

The possibility that ethylene itself may be transported was also investigated. When the column of a nonemasculated flower is treated with ethylene, the lip shows rapid coloration. Similarly, application of ethylene or ethephon to the column of an AVG-treated flower also leads to lip coloration (Table II). This already indicates that ethylene may not act through the induction of ACC-synthase and this was verified by analysis of ACC in the columns.

Measurements of ethylene concentrations in the lips revealed that, similarly to concentrations in lips of emasculated flowers, an enhanced ethylene concentration was found in lips from flowers treated with ethylene or ethephon. The same was true for petals and sepals (data not shown).

The transport of ethephon is supposed to be slow (2, 10) and it was verified that lips excised from ethephon-treated flowers, in contrast to the columns, did not show an increased ethylene production (data not shown). This indicates that no ethephon was present in the lip and that ethylene, externally applied or internally derived from ethephon, may indeed be translocated within the flower.

The significance of this finding is evident as we recently found that the ethylene concentrations in columns during senescence of *Cymbidium* flowers may reach levels up to 15 $\mu\text{L/L}$ (our unpublished data). Although presumably not a prerequisite during the early response to emasculaton, the translocation of ethylene may play an additional role in interorgan communication during senescence of these flowers.

To our knowledge, ethylene transport between different flower organs has not yet been reported, and the situation in *Cymbidium* flowers may therefore well be an exception. The relatively thick layer of epicuticular wax in these flowers may form a barrier for gas diffusion. Our results are not in line with data from Pech *et al.* (16). These authors treated the petunia style + stigma for 6 h with 2 $\mu\text{L/L}$ ethylene and did not find any effect on wilting. An explanation may be that the translocation of ethylene is relatively slow and that a much longer application or a higher concentration is necessary to be effective.

To investigate the possibility that, besides ACC and ethylene, an additional factor that renders the lip more sensitive to ethylene, may be synthesized in response to emasculaton, the lips of nonemasculated and AVG-treated emasculated flowers were treated with ethylene. Both in case where the lips were treated while attached to the flower and in case where the lips were treated after excision, the effect of ethylene on development of red coloration was remarkably similar. This indicates that the sensitivity of the lip to ethylene was not changed by emasculaton.

The effect of NBD treatment of the column on ethylene-induced coloration of the lip is difficult to explain. NBD,

when applied simultaneously with ethylene, effectively inhibited coloration, although the accumulation of ethylene in the lip was not affected. It may be concluded that, like ethylene, NBD is transported to the lip. However, if this were true, it should also have been effective in counteracting the effect of emasculaton. But even at concentrations of NBD up to 100,000 $\mu\text{L/L}$ this was not the case (data not shown). An explanation may be that the translocation of ACC is much faster than that of ethylene and NBD. This, however, needs to be ascertained.

In conclusion, it was shown that emasculaton leads to a localized production of ACC and subsequent translocation of ACC and ethylene within the flower. The result is a simultaneous increase in the evolution of ethylene by all the different flower organs. This early ethylene production induces coloration of the lip and may initiate the other senescence phenomena. These early events are very similar to those following pollination of flowers in general. As no indications for the existence of an ethylene-sensitivity factor were found, it may be argued, also, that in pollinated flowers the translocation of stigma-produced ACC or ethylene is responsible for early wilting thereby questioning the validity of the 'sensitivity factor' concept.

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LITERATURE CITED

1. Amrhein N, Breuing F, Eberle J, Skorupka H, Tophof S (1982) The metabolism of 1-aminocyclopropane-1-carboxylic acid. In PF Waering, ed, pp 249-258. Plant Growth Substances, Academic Press, New York, 1982
2. Beaudry RM, Kays SJ (1988) Flux of ethylene from leaves treated with a polar or nonpolar ethylene-releasing compound. J Am Soc Hortic Sci 113: 784-789
3. Beyer EM, Morgan PW (1970) A method for determining the concentration of ethylene in the gas phase of vegetative plant tissues. Plant Physiol 46: 352-354
4. Bradford KJ, Yang SF (1980) Xylem transport of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor, in waterlogged tomato plants. Plant Physiol 65: 322-326
5. Gilissen LJW, Hoekstra FA (1984) Pollination-induced corolla wilting in *Petunia hybrida*. Rapid transfer through the style of a wilting-inducing substance. Plant Physiol 75: 496-498
6. Glazer J, Apelbaum A, Orion D (1984) Reversal of nematode-induced growth retardation in tomato plants by inhibition of ethylene action. J Am Soc Hortic Sci 109: 886-889
7. Halevy AH, Whitehead CS, Kofranek AM (1984) Does pollination induce corolla abscission of cyclamen flowers by promoting ethylene production? Plant Physiol 75: 1090-1093
8. Halevy AH (1986) Pollination induced corolla senescence. Acta Hortic 181: 25-32
9. Hoekstra FA, Weges R (1986) Lack of control by early pistillate ethylene of the accelerated wilting in *Petunia hybrida* flowers. Plant Physiol 80: 403-408
10. Lavee S (1984) Ethephon action in growth and abscission control. In Y Fuchs, E Chalutz, eds, Ethylene: Biochemical, Physiological and Applied Aspects. Martinus Nijhoff/Dr. W. Junk Publishers, The Hague, pp 255-266
11. Lizada MCC, Yang SF (1979) A simple and sensitive assay for

- 1-aminocyclopropane-1-carboxylic acid. *Anal Biochem* **100**: 140–145
12. **Lovell PH, Lovell PJ, Nichols R** (1987) The importance of the stigma in flower senescence in petunia (*Petunia hybrida*). *Ann Bot* **60**: 41–47
 13. **Mor Y, Halevy AH, Spiegelstein H, Mayak S** (1985) The site of 1-aminocyclopropane-1-carboxylic acid synthesis in senescing carnation petals. *Physiol Plant* **65**: 196–202
 14. **Nichols R, Bufler G, Mor Y, Fujino DW, Reid MS** (1983) Changes in ethylene production and 1-aminocyclopropane-1-carboxylic acid content of pollinated carnation flowers. *J Plant Growth Regul* **2**: 1–8
 15. **Nichols R, Frost CE** (1985) Wound induced production of 1-aminocyclopropane-1-carboxylic acid and accelerated senescence of *Petunia* corollas. *Sci Hortic* **26**: 47–55
 16. **Pech JC, Latché A, Larrigandière C, Reid MS** (1987) Control of early ethylene synthesis in pollinated petunia flowers. *Plant Physiol Biochem* **25**: 431–437
 17. **Reid MS, Fujino DW, Hoffman NE, Whitehead CS** (1984) 1-aminocyclopropane-1-carboxylic acid (ACC)—The transmitted stimulus in pollinated flowers. *J Plant Growth Regul* **3**: 189–196
 18. **Stead AD** (1985) The relationship between pollination, ethylene production and flower senescence. In JA Roberts, GA Tucker, eds, *Ethylene and Plant Development*. Butterworths, London, pp 71–81
 19. **Whitehead CS, Halevy AH** (1989) The role of octanoic acid and decanoic acid in ethylene sensitivity during pollination-induced senescence of petunia hybrida flowers. *Acta Hortic* **261**: 151–156
 20. **Woltering EJ, Sterling EP** (1986) Design for studies on ethylene sensitivity and ethylene production of ornamental products. *Acta Hortic* **181**: 483–488
 21. **Woltering EJ, Harren F, Boerrigter HAM** (1988) Use of a laser-driven photoacoustic detection system for measurement of ethylene production in *Cymbidium* flowers. *Plant Physiol* **88**: 506–510
 22. **Woltering EJ** (1989) Lip coloration in *Cymbidium* flowers by emasculation and by lip-produced ethylene. *Acta Hortic* **261**: 145–150
 23. **Woltering EJ, Harren F** (1989) Role of rostellum desiccation in emasculation-induced phenomena in orchid flowers. *J Exp Bot* **40**: 907–912