Regulation of Senescence in Carnation (Dianthus caryophyllus)

EFFECT OF ABSCISIC ACID AND CARBON DIOXIDE ON ETHYLENE PRODUCTION

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ABSTRACT

Abscisic acid hastened senescence of carnation flowers and this was preceded by stimulation of accelerated ethylene production. Carbon dioxide delayed the onset of autocatalytic ethylene production in flowers regardless of treatment with abscisic acid. Flowers exhibited a low and transient climacteric of ethylene production without wilting while in 4% carbon dioxide and underwent accelerated ethylene production culminating in wilting when removed from carbon dioxide. Hypobaric ventilation, which lowers ethylene to hyponormal levels within tissues, extended flower longevity and largely negated enhancement of senescence by abscisic acid. Supplemeneningly, the data indicate that abscisic acid hastens senescence of carnations largely as a result of advancing the onset of autocatalytic ethylene production.

Abscisic acid and ethylene (together or separately) promote senescence of flowers (1, 2, 9, 12, 14). The interaction of abscisic acid and ethylene in the control of senescence has been reviewed by Sacher (17). Analysis of the interrelationships between these compounds is made difficult by complex interactions with numerous factors (13). Some (10) believe the effect of ABA to be mediated by ethylene, while others (6) consider it to be unrelated to ethylene. Conversely, ethylene has been demonstrated to promote a rise in the level of ABA (8, 9, 12).

In this study, the effect of exogenous ABA and CO₂ on ethylene production by cut carnation flowers was examined.

MATERIALS AND METHODS

Flowering stems of carnation (Dianthus caryophyllus, cv. White Sim) were obtained from a commercial grower. Flowers were cut in the morning at the commercial stage of development and deployed in the experiments within 3 hr. The flowers were trimmed to 8 cm and placed individually in vials (28 × 50 mm) containing the test solutions. The level of the solutions was maintained 1 cm below the calyx. The flowers were maintained at 20 C under continuous fluorescent light, 200 ft-c. Solutions were changed at least once during each experiment.

Test Solution. Abscisic acid (mixed isomers, Burdick and Jackson Laboratory, Muskegon, Mich.) 50 μM was dissolved by sonication in water. The solution also contained sucrose (5% w/w) or Al₂(SO₄)₃, 18 H₂O (100 mg/l) where noted. Hypobaric Ventilation. Four flowers, in test tubes containing the test solution, were placed in polystyrene trays which was enclosed in a 10-liter desiccator. Calcium sulfate was placed in each chamber to lower the humidity and prevent water condensation. The desiccators were maintained at 0.2 atm with constant ventilation (50 ml/min) with a gas mixture of 99.85% O₂ and 0.15% CO₂ with or without 2.56 μl/l ethylene until termination of the experiment. The gas mixtures were premixed in compressed gas cylinders and the composition was verified by gas chromatography. The chambers were opened daily for a brief period to evaluate flower development and renew the CaSO₄. The experiment was performed twice in a growth chamber at 20 C under continuous fluorescent light and treatments were in quadruplicate. Other details of the hypobaric ventilation equipment are published (5).

Carbon Dioxide Mixtures. Flowers were placed in 10-liter desiccators with CaSO₄. Sodium hydroxide was placed in those chambers designated as the 0% CO₂ mixture. The chambers were maintained at atmospheric pressure with air or a mixture of 4% CO₂ in air at a flow rate of 50 ml/min. The gas concentrations were monitored frequently throughout the experiment.

Ethylene Measurements. Flowers were enclosed in a specially constructed 500-ml glass chamber equipped with three ports. A port on the bottom served as a gas inlet and one on the top as an outlet for ventilation. The third port was fitted with a rubber serum bottle stopper and used for gas sampling with a syringe. One flower, with its stem in a vial of treating solution, was placed in the chamber. During the experiment, the chamber was sealed for 4 hr and duplicate 1-ml gas samples were taken. The chamber was then ventilated with the appropriate gas mixture for 5 hr at a flow rate of 50 ml/min, closed again for 4 hr. sampled, and ventilated for 11 hr. This procedure was repeated for the duration of the experiment. Five flowers were used per treatment and five empty chambers were employed to correct for extraneous ethylene. Approximately 6 ml of ethylene accumulated in 4 hr in chambers without flowers and this was attributed to the rubber stoppers used to seal the glass containers. Ethylene production rate is expressed as nl/flower-hr. The two gas mixtures used for ventilation were air from which CO₂ was scrubbed, and a mixture of 4% CO₂ in air.

Estimation of Senescence. Flowers were observed for the development of petal “in-rolling” symptoms (15) as follows: for those held in the hypobaric pressure, once a day; those used for the C₄H₄ measurements, four times a day; and twice daily for the CO₂ experiments.

RESULTS

Abscisic acid caused an earlier onset of accelerated ethylene production but did not markedly affect the maximum rate achieved when compared to flowers not receiving ABA (Fig. 1). Symptoms of in-rolling of petals generally occur 1 or 2 days after the rise in ethylene production rate. These symptoms, indicated by a W in Figure 1, are the first signs of flower wilting (15). Ventilating the flowers with 4% CO₂ in air delayed the onset of
of accelerated ethylene production. A low transient climacteric of ethylene production was observed, but the flowers did not wilt. Moreover, CO₂ suppressed the magnitude of the accelerated rise in ethylene production, and in-rolling symptoms were not apparent for the duration of the experiment (15 days). Transferring the flowers to air after 10 days caused a second rise in ethylene production followed by wilting. Response of flowers treated with ABA and CO₂ paralleled that of flowers not receiving ABA but rose earlier both before and after CO₂ was removed (Fig. 1).

Abscisic acid shortened flower longevity, and hypobaric ventilation, which enhances diffusion of ethylene and other gases from the tissue, largely negated the effect of ABA (Table I). Flowers receiving ABA began to wilt (senesce) in 3.5 days at atmospheric pressure and this was delayed to 6.8 days with hypobaric ventilation. Carnations without ABA senesced in 5 days while similar blooms persisted for 9.3 days with hypobaric ventilation. Supplementing flowers with ethylene at hypobaric pressure hastened senescence with or without ABA treatment. Hypobaric ventilation apparently did not completely negate the effect of ABA on hastening carnation senescence. Gassing ABA-treated blooms with 3.8% CO₂ in air completely negated the ABA effect (Table II) while 1.2% CO₂ largely overcame the ABA influence.

DISCUSSION

Both abscisic acid and ethylene stimulate senescence of carnation flowers, inducing symptoms typically associated with the natural aging process. The effect of ABA is not surprising, since ABA causes an earlier increase in ethylene production (Fig. 1) which, in turn, induces senescence (7). Moreover, treatment with 4% CO₂ in air also prevents response to ABA, although the flowers senesce on return to normal air.

Evidence that ABA functions in hastening senescence of carnations largely through stimulating ethylene production comes from the hypobaric ventilation experiments. The extension of flower longevity by hypobaric ventilation, which lowers ethylene to hyponormal levels within the tissue, and reversal of this by restoring ethylene to a physiologically active level are evidence of cause and effect (5). It is also clear that exogenous ABA hastens carnation senescence largely as a consequence of enhancing ethylene production, because hypobaric ventilation delayed senescence of ABA-treated flowers while supplemental ethylene overcame this effect.

The observation that hypobaric ventilation did not totally negate the effect of ABA (Table I) can be explained in large measure by the fact that ABA stimulated the onset of autocatalytic ethylene production. Ventilation with O₂ at 0.2 atmosphere would effectively reduce the normal endogenous ethylene level 5-fold. This would be a sufficient reduction to retard senescence, providing ABA did not stimulate ethylene production more than 5-fold. As seen in Figure 1, ABA-treated flowers enter the accelerated phase of ethylene production earlier than control flowers and production rates quickly rise to levels at which ventilation is ineffective. Abscisic acid apparently sets the stage for the onset of accelerated ethylene production since its effect is entirely masked by applying ethylene exogenously under hypobaric conditions (Table I).

Carbon dioxide is a competitive inhibitor of ethylene action (4) and has previously been shown to inhibit ethylene production (20) and delay senescence of carnations (15, 18, 19) and morning glory (11). This is borne out in the present study (Fig. 1) in which 4% CO₂ in air delayed the onset of the normal rise in ethylene, reduced the rate of production, and prevented development of senescence symptoms. The transient climacteric increase in ethylene production by flowers in 4% CO₂, beginning at the 3rd and 5th day by flowers with or without ABA, respectively, was not accompanied by wilting. A substantial increase in ethylene production, culminating in wilting of petals, occurred only after terminating the CO₂ treatment. It appears that the high rates of ethylene production exhibited by the flowers prior

Table I. Effect of ABA on Carnation Senescence in Response to Hypobaric Ventilation With or Without Supplemental Ethylene

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Flower Longevity (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.0</td>
</tr>
<tr>
<td>ABA (50 μM)</td>
<td>3.5</td>
</tr>
<tr>
<td>Control plus hypobaric ventilation¹</td>
<td>9.3</td>
</tr>
<tr>
<td>ABA plus hypobaric ventilation</td>
<td>6.8</td>
</tr>
<tr>
<td>Control plus hypobaric ventilation plus ethylene</td>
<td>1.3</td>
</tr>
<tr>
<td>ABA plus hypobaric ventilation plus ethylene</td>
<td>1.6</td>
</tr>
</tbody>
</table>

S.E. ± 0.29

¹0.2 atm. of 99.85% O₂ + 0.15% CO₂ ± 2.36 µl/l/ethylene.
to wilting are inducible by ethylene and subject to reversal by CO₂ in which case the production rate returns to the normally low steady state. Flowers kept in 4% CO₂ for up to 30 days succumbed to decay without developing typical symptoms associated with ethylene-induced senescence. The presence of CO₂ delays, but does not irreversibly inhibit, developmental processes leading to senescence. ABA-treated flowers senesced earlier than those not receiving ABA when they were removed from CO₂ after 10 days. In this context, ABA functions like the "senescence factor" described by Osborne (16) augmenting the tissues' capability for ethylene production.

Our results suggest that exogenous ABA hastens carnation flower senescence by advancing normal developmental processes which accelerate ethylene production. Tissues senesce directly in response to ethylene since lowering the ethylene concentration by hypobaric ventilation or blocking the site of ethylene action by CO₂ postpones aging of flowers whether or not they received supplemental abscisic acid.

**LITERATURE CITED**


