Plant Physiol. (1977) 59, 591-593

Regulation of Senescence in Carnation (*Dianthus caryophyllus*) by Ethylene

MODE OF ACTION[1]

Received for publication April 9, 1976 and in revised form November 16, 1976

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ABSTRACT

Carnation (*Dianthus caryophyllus*) flowers were exposed to 2 μl/l ethylene and examined at intervals to determine the time course of wilting, decrease in water uptake, and increase in ionic leakage in response to ethylene. A rapid decrease in water uptake was observed about 4 hours after initiating treatment with ethylene. This was followed by wilting (in-rolling of petals) about 2 hours later. Carbon dioxide inhibited the decline in water uptake and wilting and this is typical of most ethylene-induced responses. Ethylene did not affect closure of stomates. Ethylene enhanced ionic leakage, as measured by efflux of ^{36}Cl from the vacuole. This was judged to coincide with the decrease in water uptake. Gassing flowers with propylene initiated autocatalytic ethylene production within 2.4 hours. Since the increase in ethylene production by carnations preceded the increase in ionic leakage and the decline in water uptake by several hours, it is apparent that the change in ionic leakage does not lead to the initial increase in ethylene production as reported (Hanson and Kende 1975 Plant Physiol 55:663-669) in morning glory but may explain the autocatalytic phase of ethylene production.

The association of ethylene with senescence of flowers is widely recognized and carnation flowers have been thoroughly examined in this respect (13). As the cut flower approaches senescence, a dramatic rise in the rate of ethylene production occurs followed soon after by wilting of the petals. Senescence can be hastened by as little as 30 nl/l of ethylene (2, 15). The events taking place between the rise in ethylene production, or exposure to ethylene, and the development of visual symptoms remain obscure. Lieberman *et al.* (11) have shown that ethylene accelerates the loss of water from the carnation inflorescence, and Nichols (13) demonstrated that a loss in fresh weight of carnation flowers is correlated with the rise in ethylene production. The influence of ethylene on physiological changes leading to the wilting phenomenon of carnations was investigated to determine the sequence of certain events culminating in flower senescence.

MATERIALS AND METHODS

Effect of Ethylene on Wilting. Carnation flowers *Dianthus caryophyllus*, cv. Improved White Sim) were cut to about 10 cm over-all length, and placed individually in 20-ml vials. Flowers were obtained from a local grower and experiments were begun on the day of harvest. Four flowers in a 10-liter desiccator with a CO_2 scrubber (NaOH solution) comprised a treatment. Flowers were exposed to ethylene at 2 μl/l for 0, 3, 6, 9, or 12 hr, after which they were ventilated with ethylene-free air at a rate of 100 ml/min for the remainder of the experiment. Flowers were observed at 1-hr intervals to determine the onset of wilting symptoms as judged by in-rolling of petals.

Effect of Ethylene on Rate of Water Uptake. Cut flowers were fitted into a specially designed glass sphere in which the flower bud was maintained in the desired gas atmosphere while the stem was connected to a potometer (5) and water uptake was measured at 30-min intervals. The flowers were ventilated continuously with 2 μl/l ethylene in dry air or with ethylene-free at a flow rate of 40 ml/min.

Effect of Ethylene on Stomatal Aperture of Carnation Sepals. Flowers were cut to 10 cm over-all length and treated with ethylene in desiccators as described above. Flowers were removed at 3-hr intervals from each of four desiccators and the sepals were excised, frozen in liquid N_2, and freeze-dried. Sections (4 × 4 mm) were sputter-coated with gold and the surface was observed with a scanning electron microscope to determine degree of opening of the stomates.

Effect of Propylene on Ethylene Production. Individual flowers were fitted into 220-ml conical chambers equipped for aeration and gas sampling. The chamber accommodated an open flower without exerting mechanical pressure on the petals and the protruding flower stem was immersed in deionized H_2O. The funnel chamber was ventilated with ethylene-free air at a flow rate of 40 ml/min. Ethylene production was monitored at 24-hr intervals for 1 day by alternately sealing and ventilating the chamber. On the 2nd day, 38 hr after the experiment was initiated, the chambers were closed and some of the flowers were treated with propylene (260 μl/l) for 8 hr and ethylene production was measured at 2-hr intervals. The chambers were then ventilated for 20 hr after which ethylene production was again monitored at 2-hr intervals as before. Ethylene determinations were made on a Varian 1700 gas chromatograph employing a flame ionization detector and an alumina column.

Effect of Ethylene on Ionic Fluxes. The change in efflux of ions in the petal tissue was determined in relation to the onset of wilting of carnation flowers preloaded with $H^{36}Cl$ (about 4×10^5 cpm/flower). The ion efflux procedure of Keck and Hodges (8) and Hanson and Kende (7) was used with slight modifications. Half of the flowers were gassed with ethylene at 2 μl/l while the others were ventilated with ethylene-free air. Flowers were removed at 2-hr intervals for efflux measurements. Those which received 8-hr exposure to ethylene exhibited wilting symptoms.

[1] Michigan Agricultural Experiment Station Journal Article No. 7658. Supported in part by the Gillette Company.

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RESULTS AND DISCUSSION

Ethylene-induced Wilting. The first visual symptom of carnations treated with ethylene was an "in-rolling" or wilting of the petal margin as described by Nichols (13). Wilting was first observed at 6 hr for flowers receiving ethylene at 2 μl/l and at 60 hr for those ventilated with air continuously. The number of flowers exhibiting wilting increased with the duration of exposure to ethylene. Nine- or 12-hr treatment with ethylene was sufficient to cause most of the flowers to wilt in 2 days while less than a third of the flowers receiving 3- or 6-hr exposure displayed the wilt symptom in this interval. This demonstrates the variability with respect to sensitivity to ethylene that exists within a given population of presumably uniform flowers.

Water Uptake. The rate of water uptake declined sharply approximately 4 hr after gassing the inflorescence with 2 or 20 μl/l ethylene (Fig. 1) and stabilized at about 70% of the control value. Wilting was observed between 6 and 7 hr after ethylene treatment. Flowers gassed with 0.2 μl/l ethylene showed a decline in water uptake beginning in 12 hr but these did not wilt. Carbon dioxide inhibited the ethylene-induced decline in water uptake and wilting (Fig. 2). Water uptake was slightly lower in the presence of 4% CO_2 which may be attributed to closure of stomates on the flower sepals. The fact that CO_2 negates the decline in water uptake induced by ethylene places this ethylene response in the same category with a long list of other ethylene-induced responses found to be reversibly inhibited by CO_2 (4). It has long been recognized that CO_2 delays wilting of carnations (14).

Scanning electron microscopy of the sepals did not reveal differences in stomate opening, before the onset of wilting, between ethylene-treated and untreated flowers. Thus, the decline in water uptake (Figs. 1 and 2) cannot be attributed to a direct effect of ethylene on stomate closure.

It is obvious but important to note that ethylene caused the decline in water uptake before the visual symptom of wilt. This is indirect evidence that ethylene causes a change in water and solute distribution within the cell perhaps because of a change in membrane permeability and hence a drop in turgor pressure accounting for the decline in water uptake (10). Direct evidence supporting this hypothesis was obtained by ion efflux compartmentation analysis.

Ion Efflux. Ethylene increased leakage of Cl from carnation petals indicated by a greater rate of efflux of ^{36}Cl from ethylene-treated flowers (Fig.3). In this experiment, the flowers were loaded with ^{36}Cl and gassed with ethylene for 5.5 hr since it was established earlier that the decline in water uptake began 4 to 6 hr after ethylene treatment followed by wilt symptoms about 2 hr later. The data, interpreted according to suggested guidelines (8, 12), indicates two phases of ^{36}Cl efflux: a rapidly decreasing initial phase attributed to Cl diffusing from a readily accessible compartment which may include the cell wall and some exchange through the plasmalemma, from which the rate of efflux is similar regardless of ethylene treatment; and a slow linear phase attributed to release of Cl from the vacuole through the tonoplast which occurs at a faster rate with flowers pretreated with ethylene. We conclude that this is due to an increase in tonoplast permeability induced by ethylene. Extrapolation of the linear portion to zero time from equations obtained by the least squares method indicated that 83 and 80% of the ^{36}Cl resided in the vacuoles of ethylene-treated and nontreated flowers, respectively. The rate constant for ^{36}Cl efflux across the tonoplast was calculated from the slope and found to be higher for ethylene-treated flowers (0.69×10^{-3} min^{-1}) than for the control (0.46×10^{-3} min^{-1}). No clear cut effect of ethylene on plasmalemma permeability could be established by the procedures employed.

The increase in chloride ion efflux from the vacuole caused by ethylene was found to coincide with the decrease in water uptake and was discernible about 2.5 hr before wilting was observed. The decline in water uptake began about 2 hr prior to visual wilting.

Effect of Propylene on Ethylene Production. Propylene has been found to mimic ethylene action in plants but the concentration required is about 130-fold higher (4). Previous studies have shown that this is true for senescence of carnations in which propylene initiates autocatalytic ethylene production (6). In the present investigation, propylene was administered to carnations for 8 hr at a concentration of 260 μl/l which is equivalent to about 2 μl/l of ethylene in order to determine the time course of its effect on initiating ethylene production. A measurable change in ethylene production was observed at the 6th hr of propylene treatment (Fig. 4). The inset in Figure 4 depicts the time course during the period of propylene treatment. Since autocatalytic ethylene production follows a log-linear course with time it is apparent, by extrapolation, that propylene treatment induced the onset of accelerated ethylene production in 2.4 hr. Evidence

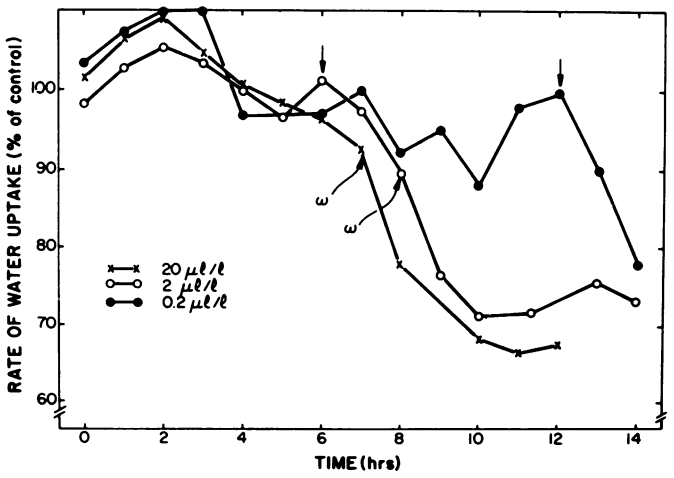

FIG. 1. Effect of ethylene on water uptake by carnation flowers. The flowers, excluding the stem, were ventilated with air with or without ethylene (0.2, 2, or 20 μl/l at 20 C in light at 200 ft-c. Vertical arrows indicate when a decline in water uptake was initiated. W indicates time of initial wilting. Water uptake by control flowers remained steady during the course of the experiment.

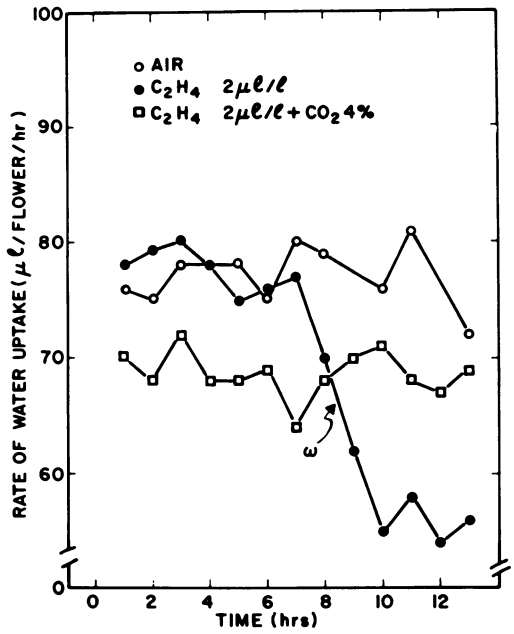

FIG. 2. Effect of ethylene (2 μl/l) with or without 4% CO_2 on the rate of water uptake by carnation flowers. The flowers, excluding the stem, were ventilated with the gas mixtures at 20 C in light at 200 ft-c. W indicated time of initial wilting.

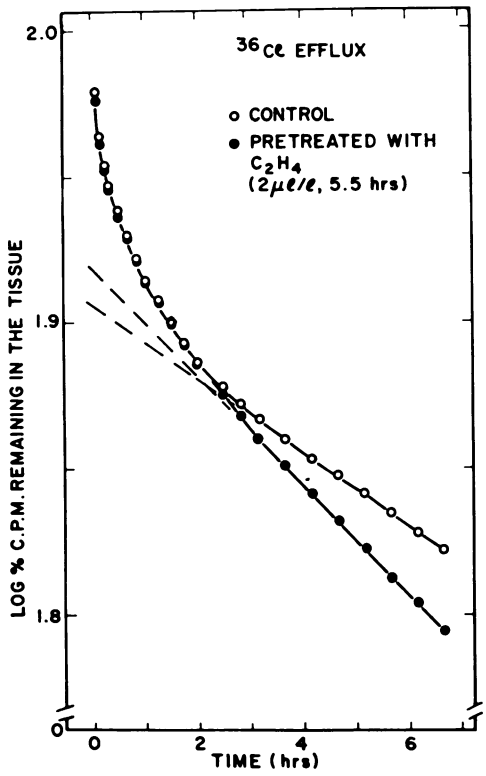

FIG. 3. Effect of ethylene on ^{36}Cl efflux from carnation petals. Dashed lines are interpolation to zero time. Cut flowers were preloaded with ^{36}Cl and treated with ethylene. Results are means of four replicates.

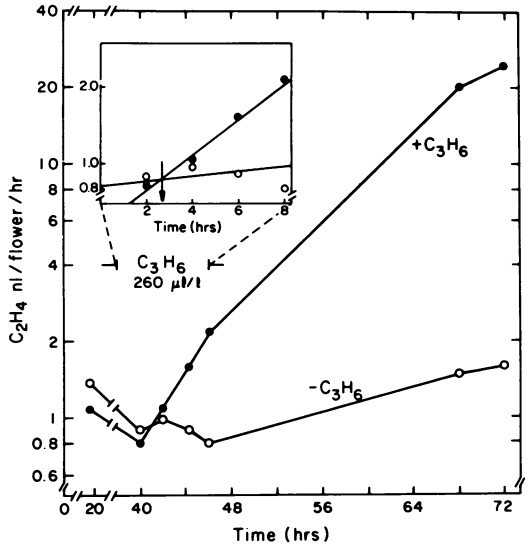

FIG. 4. Effect of propylene on ethylene production by carnation flowers. Propylene (260 μl/l) was administered for 8 hr as indicated. Ethylene production during this period is shown in the inset. Equations describing ethylene production as a function of time are $y = 0.0712x - 0.241$ and $y = 0.0091x - 0.0896$ for propylene treated and control, respectively, with intersection at 2.4 hr.

for autocatalytic ethylene production comes from the fact that ethylene production continues in a log-linear manner even though the flowers were ventilated after only 8 hr of propylene treatment.

The time course for the onset of autocatalytic ethylene production induced by propylene indicates a lag period of about 2.4 hr. It takes 4 to 6 hr for ethylene to cause an increase in tonoplast permeability as measured by the ion efflux experiments, and this coincides with the onset of the decline in water uptake. Since the increase in ethylene production precedes the increase in ion effect and the decline in water uptake by several hours, it is apparent that the change in permeability does not lead to the *initial* increase of ethylene production (7, 9) as it occurs in morning glory. Permeability changes during ripening of banana were also found to follow other ripening parameters in response to ethylene application (3). The change in permeability of carnation petal tissue is perhaps a secondary response of the tissue to the action of an olefin. The experiments with propylene address the question—how is autocatalytic ethylene production brought about? Accordingly, propylene brings about a change in tonoplast permeability which leads to autocatalytic ethylene production. In normal circumstances, an increase in production rate of ethylene, the naturally occurring olefin, initiates the change in tonoplast permeability. The autocatalytic phase of ethylene production may be a consequence of increased substrate availability from within the vacuole as the tonoplast becomes increasingly leaky as proposed by Hanson and Kende (7) for the morning glory.

A partial chronological order of events leading to senescence of carnations may be summarized as follows. An increase in the intercellular ethylene concentration occurs. The intercellular ethylene partial pressure is normally maintained at a low steady-state level which may be viewed as an equilibrium between a low and fairly constant production rate and diffusion from the flower. Factors which cause the internal level of ethylene to increase initiate some event exhibiting a lag phase of about 2 hr which is followed by a log-linear increase in ethylene production. This leads to an increase in tonoplast permeability about 2 hr later. A decline in water uptake by the flower coincides with or probably closely follows the increase in tonoplast permeability. Visual symptoms of petal wilt may appear 2 hr after the decline in water uptake has begun. Wilting is a direct consequence of the loss of semipermeability of the membrane which diminishes the osmotic gradient necessary to maintain turgor. The mechanism by which ethylene increases tonoplast permeability remains to be elucidated but it is apparently indirect rather than direct.

Acknowledgments—The authors thank R. Fleury and S. Masson for capable technical assistance and H. P. Rasmussen for the scanning electron microscope work.

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