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Ethylene: A Natural Regulator of Sex Expression of *Cucumis melo* L.

(cucumbers/gynoecious/monoecious/perfect flowers/muskmelon)

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ABSTRACT **Sex expression in cucumber (*Cucumis sativus* L.) and muskmelon (*C. melo* L.) was correlated with endogenous ethylene production. Plants of gynoecious (all female) sex types of the two species produced more ethylene than monoecius (male–female) plants. *C. melo* plants of a gynoecious sex type that normally produce only pistillate (female) flowers, when grown with hypobaric ventilation to facilitate removal of endogenous gases by diffusion, produced perfect (hermaphroditic) flowers. When either the plant was returned to atmospheric pressure or when the reduced-pressure ventilating stream was supplemented with ethylene, the same plants produced pistillate flowers. Enrichment of the atmosphere at either normal or reduced pressure with CO_2, a competitive inhibitor of ethylene action, also resulted in development of perfect flowers. Foliar application of a benzothiadiazole, a postulated inhibitor of ethylene action, resulted in formation of perfect flowers on gynoecious plants of *C. melo* and of staminate (male) flowers on gynoecious *C. sativus*. Based on these findings, it is proposed that ethylene is an endogenous regulator of sex expression in *C. sativus* and *C. melo*.**

Sex expression in cucurbits is influenced by genetic, environmental, and hormonal factors. Monoecious strains of cucumber (*Cucumis sativus* L.) and muskmelon (*C. melo* L.) bear staminate (male) and pistillate (female) flowers. Gynoecious strains normally produce only pistillate flowers. Other cucumber and muskmelon strains produce staminate or pistillate and, in addition, perfect (hermaphroditic) flowers in various combinations. For example, andromonoecious strains are those that begin with staminate flowers and, eventually, also produce hermaphroditic flowers. Exogenous application of auxin (1, 2) and inhibitors of gibberellin biosynthesis (3) promote monoecious strains to form pistillate flowers, that is, increase femaleness. Application of gibberellin promotes formation of male flowers in monoecious and gynoecious phenotypes of cucumber (4, 5). Sex expression can be modified by daylength and temperature. Generally, short days and cool temperatures favor femaleness, while long days and high temperatures favor maleness, although there are exceptions (6). Determinations of endogenous growth substances indicate that strains with genetically strong female sex expression contain more auxin (7) and less gibberellin-like substances (8) than strains with strong male sex expression. There are certain differences between species; for example, gibberellin application does not cause male flower formation in gynoecious muskmelon (9). However, the results obtained with hormone applications and hormone determinations suggest the hypothesis that sex expression in cucurbits is controlled by an endogenous auxin–gibberellin balance (3, 7, 8, 10).

Ethylene and 2-chloroethylphosphonic acid (ethephon), an ethylene-releasing compound, have recently been shown to promote femaleness in cucurbits (11, 12); thus, the effect of ethylene is similar to that of auxin. Exogenous application of auxin increases ethylene production by cucumber plants (13). Ethylene causes many growth responses in plants (14), and some responses to auxin are now attributed to auxin-induced ethylene synthesis (15). Conclusive proof for a genuine regulatory function of *endogenous* ethylene in plant growth and morphogenesis is, however, very scarce. This scarcity led us to examine the possibility that endogenous ethylene plays a role in sex expression of cucurbits.

Experiments were performed with cucumber and muskmelon and consisted of: measurements of ethylene production in strains of different sex types, studies in which plants were grown at below normal versus normal concentrations of endogenous gases, use of CO_2, a competitive inhibitor of ethylene action (16), and studies with a new chemical, 5-methyl-7-chloro-4-ethoxycarbonylmethoxy-2,1,3-benzothiadiazole, which inhibits certain plant growth responses to ethylene (17, 18). The results show that ethylene is indeed a natural regulator of sex expression in cucurbits.

MATERIALS AND METHODS

Ethylene Production. Cucumber and melon plants of a wide range of sex types were grown in a greenhouse at about 20–25°; supplemental light from fluorescent lamps was provided. When the plants had developed five leaves, the shoot tip was enclosed in a 500-ml erlenmeyer flask for 13–15 hr. The stems were sealed in the flask with silastic rubber (A RTV, Dow Corning), prepared with a nonphytotoxic catalyst (Herter T 1, Wacker Chemie GmbH, Munich, Germany). The ethylene content of the atmosphere within the flask was measured by gas chromatography; a Varian Aerograph model 1700 equipped with an activated alumina column and a flame ionization detector was used. The data presented for *C. sativus* are averages of three experiments consisting of at least five single-plant replicates, while the data for *C. melo* are from four experiments. Ethylene production during germination and early growth of the seedling of gynoecious and monoecious *C. sativus* was determined by analyses of the ethylene that accumulated over seeds enclosed in 50-ml erlenmeyer flasks, the oxygen content of which was maintained at 21%.

Ethylene Removal. For reduction of the internal ethylene content of the tissue as effectively as possible, the following

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TABLE 1. *Ethylene evolution by germinating seeds of monoecious and gynoecious phenotypes of cucumber*

Sex phenotype	Genetic line	nl of ethylene/g of original dry weight per day				
		1	2	3	4	5
Monoecious	MSU 381M	11	50	100	126	97
Gynoecious	MSU 381G	12	71	159	199	150
Ratio G/M		1.09	1.42	1.59	1.58	1.54

technique was used. Diffusion of gases, including ethylene, from the tissue follows Fick's law (19). Hence, absorbants or ventilation favor diffusion by minimizing the external concentration of ethylene, but these methods, as a rule, do not reduce the internal concentration significantly unless substantial ethylene has accumulated in the ambient atmosphere. A more effective reduction in endogenous gases can be achieved by reduction of the total atmospheric pressure to enhance gas diffusion from the tissue (19). For example, the partial pressure of ethylene at equilibrium in tissues is reduced 5-fold when the atmospheric pressure is reduced to 0.2 atm, even though the rate of ethylene production in the tissue is not changed. Since the partial pressure of O_2 and CO_2 is also reduced 5-fold in this process, it is necessary to use O_2 enriched with 0.15% CO_2, rather than air, to provide normal partial pressures of these gases that are needed for respiration and photosynthesis in the growing plant. Accordingly, gynoecious melon and cucumber plants were grown in peat pots and soil in a greenhouse with supplemental light provided from fluorescent lamps at temperatures of 30° during the day and 18° at night. At the appropriate stage of development, the plants were placed in 250-mm diameter desiccators in each of two growth chambers that were maintained at 18° for a 10-hr night and at 30° during a 14-hr day. The desiccators were continuously evacuated (20). Reduced pressure and flow rate (8–11 liters/hr) were properly regulated by manipulation of the vacuum regulator and a metering valve at the desiccator outlet. Water was administered, intermittently, to the plants, at reduced pressure, through tubing connecting the desiccator to a reservoir at atmospheric pressure.

Inhibitors of Ethylene Action. Carbon dioxide was administered to the plants at the concentrations and total pressures specified in Table 3. Foliar applications of 5-methyl-7-chloro-4-ethoxycarbonylmethoxy-2,1,3-benzothiadiazole (TH6241; Thompson–Hayward Chemical Co., Kansas City, Mo.) solutions were made with a syringe that wetted the leaves thoroughly. Both technical grade and an emulsified formulation of the benzothiadiazole were used; multiple applications were made at various stages of development of the plants. Details are noted in the tables.

TABLE 2. *Rate of ethylene production by intact apical tips of C. sativus and C. melo*

Sex phenotype	Genetic line	Ethylene evolution nl/g per hr
C. sativus		
Gynoecious	MSU 6902G	3.08
Gynoecious	MSU 713-5	2.70
Hermaphroditic	TAMU 950	2.21
Monoecious	MSU 736M	1.86
Andromonoecious	'Crystal Apple'	1.49
C. melo		
Gynoecious	MSU 1G	0.915
Hermaphroditic	MSU 3897	0.485
Monoecious	MSU 3898	0.801
Andromonoecious	'Rocky Ford'	0.999

RESULTS

Germinating seeds of gynoecious cucumbers produced about 50% more ethylene than the monoecious cucumbers; differences in rate began 2 days after the seeds were moistened and continued at least through the fifth day (Table 1). A closely related andromonoecious strain (strong male) produced ethylene at only 10% of the rate of the gynoecious strain. A similar difference was found for shoot apices of the same cucumber lines (Table 2). The sex phenotypes listed in Table 2 are in decreasing order of female tendency. Propensity to produce ethylene follows closely the degree of femaleness in cucumber. This was not true for *C. melo*, although the gynoecious phenotype did produce more ethylene than the monoecious phenotype.

Maintenance of the internal gas content in gynoecious *C. melo* at a hyponormal level, by growing the plants under reduced pressure with ventilation, caused perfect flowers to be produced (Table 3) on otherwise normal gynoecious plants. Only pistillate flowers were produced on plants maintained at atmospheric pressure, typical for this particular phenotype. Plants that were subjected to 0.2 atm of pressure, when having developed three leaves, produced perfect flowers at node 5, while plants that were treated when having developed four and five leaves formed perfect flowers at nodes 13 and 17, respectively. This progressive resistance to deviate from the gynoecious character at only slightly later stages of development was also expressed by the decreased number of perfect flowers produced through 25 nodes on the plants that were grown at the reduced pressure. Administration of ethylene at a concentration of 2 ppm in the ventilating stream at reduced pressure maintained the plants in their normal gynoecious sex expression (female flowers only), although leaf expansion and shoot growth were inhibited by about 50%, compared to control plants grown at a pressure of 1 atm. Ventilation with 0.2 ppm of ethylene was partially effective in maintaining the gynoecious character, as expressed by the number of perfect flowers produced. 2 ppm of ethylene introduced into chambers at 0.2 atm pressure is equivalent to 0.4 ppm at 1 atm pressure, which is supraoptimal for most known ethylene effects. Reduction of the pressure of a ventilating stream containing 0.2 ppm ethylene from 1 to 0.2 atm pressure results in a similar 5-fold reduction of the ethylene content to 0.04 ppm, which is close to the threshold for activity. Although the content of other gases produced by

TABLE 3. *Effect of reduced pressure ventilation and CO_2 on sex expression of gynoecious C. melo (MSU 1G) observed through the 25th-node stage*

Pressure (atm)	Gas mixture	Initial age (node)	No. of Trials	No. of Plants	Plants with perfect flowers	Node no. of first perfect flower	No. of perfect flowers
1	Air	3, 4, & 5	8	21	0	None	0
0.2	99.85% O_2 0.15% CO_2	3	1	4	3	5	19
0.2	99.85% O_2 0.15% CO_2	4	1	3	3	13	12
0.2	99.85% O_2 0.15% CO_2	5	1	3	2	17	5
0.2	99.85% O_2 0.15% CO_2 + 2 ppm ethylene	4	2	8	0	None	0
0.2	99.85% O_2 0.15% CO_2 + 0.2 ppm ethylene	4	2	8	6	11	2
0.2	95% O_2 5% CO_2	4	5	18	18	12	8
1	20% O_2 10% CO_2	4	2	8	8	7	8

the plant (excluding O_2 and CO_2) was also reduced by growing plants hypobarically, restoring the normal gynoecious sex expression by adding back ethylene is strong evidence that ethylene is the factor responsible.

Carbon dioxide, a competitive inhibitor of ethylene action (16), caused formation of perfect flowers on *C. melo* plants subjected to both normal and reduced pressure, when the plants were at the fourth leaf stage (Table 3). When 10% CO_2 was included in the gas at 1 atm, perfect flower buds appeared on all plants soon after the treatment began. The foliage appeared normal, but this CO_2 concentration did have an interesting and marked effect: it greatly inhibited the growth of the main shoot and promoted branching. Perfect flower buds formed on both the main and lateral shoots.

The same treatments as those shown in Table 3 were applied to gynoecious *C. sativus* (MSU 713-5), but in this case no modification of sex expression was obtained.

Foliar treatment of both gynoecious *C. melo* and *C. sativus* with 5-methyl-7-chloro-4-ethoxycarbonylmethoxy-2,1,3-benzothiadiazole caused reversion from formation of pistillate to production of perfect and staminate flowers. Gynoecious *C. melo* (MSU 1G) plants treated with the chemical at a concentration of 25 ppm beginning at the third leaf stage produced only perfect flower buds through the 15th node, while those receiving either 0 or 2.5 ppm of the same chemical remained gynoecious (Table 4). Treatment of gynoecious *C. sativus* (MSU 713-5) plants at the second leaf stage caused formation of staminate and some perfect flowers, but plants treated at the fifth leaf stage remained strictly gynoecious (Table 5). The chemical caused some epinasty, growth inhibition, formation of ring fasciations and of tubular and funnel shaped leaves, and petiole swelling. However, these effects may, at least in part, be because applications were made repeatedly and beyond the development stage when sex reversion for the first 25 nodes could be influenced. Similar morphogenetic effects have been described in other plants (17).

TABLE 4. *Effect of 5-methyl-7-chloro-4-ethoxycarbonylmethoxy-2,1,3-benzothiadiazole on the sex expression of gynoecious C. melo (MSU 1G)*

Treatment	Node of first flower bud* Perfect	Node of first flower bud* Pistillate	No. of flower buds to 15th node† Perfect	No. of flower buds to 15th node† Pistillate
Control	0.0	7.0	0.0	7.6
2.5 ppm	0.0	7.6	0.0	7.2
25.0 ppm	7.4	0.0	7.4	0.0

* Each figure is a mean of five plants.

† Applications were begun at the third true leaf stage and continued for nine applications over a period of 2 weeks.

DISCUSSION

Growing gynoecious muskmelon plants at reduced atmospheric pressure, combined with ventilation, which decreases the internal gases in the plant tissues, causes a change of sex expression toward maleness, that is, production of perfect in place of pistillate flowers. The addition of ethylene restores, fully or partially, depending upon the concentration, the original gynoecious sex expression, that is, production of only pistillate flowers, clearly suggesting that ethylene is the factor

TABLE 5. *Effect of 5-methyl-7-chloro-4-ethoxycarbonylmethoxy-2,1,3-benzothiadiazole on the sex expression of a gynoecious line of C. sativus (MSU 713-5)*

Treatment	No. of flower buds to the 15th node* Staminate	No. of flower buds to the 15th node* Pistillate	No. of flower buds to the 15th node† Staminate	No. of flower buds to the 15th node† Pistillate
Control	0.0	15.5	0.0	25.5
25 ppm	4.1	23.0	0.0	24.5

* Two applications were made at the second-leaf stage.

† Two applications were made at the fifth-leaf stage.

responsible for determination of gynoecious sex expression. The fact that germinating seeds and shoot apices of gynoecious *C. sativus* and *C. melo* produce more ethylene than monoecious types supports our contention that femaleness is regulated by endogenous ethylene. Further supporting evidence comes from our experiments in which perfect flowers were formed on gynoecious *C. melo* plants (increased maleness) treated with CO_2, a competitive inhibitor of ethylene action. An associated effect of the CO_2 treatment that was observed, namely promotion of branching, is also of considerable interest since ethylene applied continuously inhibits lateral branching of shoots by inhibition of cell division (21). The removal of this restraint by CO_2 thus implies that endogenous ethylene may control apical dominance by continuously inhibiting cell division in the lateral buds. The observation that 5-methyl-7-chloro-4-ethoxycarbonylmethyl-2,1,3-benzothiadiazole promotes maleness in both *C. sativus* and *C. melo* is a further support for a role of ethylene in the expression of female sex. Our findings offer an explanation to the often observed result that treating monoecious cucurbits with ethylene (11) or compounds that promote ethylene synthesis (12) causes earlier production of pistillate flowers, that is, promotes femaleness.

In contrast to *C. melo*, we were unable to promote maleness in gynoecious *C. sativus* by growing plants with ventilation at hypobaric pressure with or without supplemental CO_2. This failure may be related to the fact that these plants produced more than three times as much ethylene as gynoecious *C. melo*. Reduction of the internal ethylene 5-fold apparently was not sufficient to achieve a below-threshold concentration. Ethylene action is usually a logarithmic function of its concentration. Thus, even a 5-fold reduction in ethylene content from a saturating level may leave enough ethylene remaining to be biologically active. In a similar manner, no amount of CO_2 will prevent ethylene action if the ethylene concentration is at an optimum level. Hence, our failure to modify sex expression in gynoecious cucumber by reducing the internal ethylene concentration does not rule out ethylene as a factor that determines sex expression—particularly the formation of pistillate flowers—in *C. sativus*, since femaleness was correlated with ethylene production and sex modification was obtained with the synthetic inhibitor of ethylene. The benzothiadiazole was effective when applied to gynoecious *C. sativus* at the second leaf stage but not the fifth leaf stage, indicating that sex expression is determined for many subsequent nodes at a very early stage of plant development. The early determination of sex expression is also evident in the *C. melo* experiments with ventilation at reduced pressure (Table 3). Plants ventilated beginning at the third leaf stage produced perfect flowers (maleness) at the fifth node, but those treated beginning only one or two leaves later continued to produce pistillate flowers (femaleness) for at least 10 more nodes.

Our results do not conflict with the existing theory that an auxin–gibberellin balance is the physiological basis of sex determination in cucurbits. The endogenous concentration of auxin may, in fact, determine the endogenous concentration of ethylene. Ethylene would then be an intermediate effector molecule that promotes femaleness. Application of gibberellin promotes maleness in gynoecious *C. sativus* but not in gynoecious *C. melo*. This and the failure to modify sex expression in gynoecious *C. sativus* by reducing the atmospheric pressure suggest that the relative concentration of endogenous gibberellin, endogenous auxin, and endogenous ethylene may be a critical factor in sex expression in different species of cucurbits.

The ability to obtain anther development in gynoecious *C. melo* by chemical means has practical significance. To date, the only method for maintaining a genetically pure gynoecious line has been to graft it onto andromonoecious muskmelon, monoecious pumpkin, squash, and other cucurbit rootstocks (22). The benzothiadiazole could provide a means of producing seed of gynoecious parent lines needed for production of F_1 hybrid muskmelon seed in a manner analogous to the production of hybrid cucumber seed that is dependent on induction of male flowers on gynoecious cucumber lines by gibberellin (5).

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