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Light-stimulated Ethylene Production by Germinating Cucumber Seeds[1]

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Additional index words. vegetable breeding, sexual phenotype, *Cucumis sativus*

Abstract. **The degree to which white light stimulated ethylene production in germinating seeds of cucumber (*Cucumis sativus* L.) was influenced by the length of time the seed had been germinated in the dark before being exposed to light. Maximum stimulation occurred when 24 hour old dark-grown seedlings were exposed to 40 μE m^{-2} sec^{-1} light for 24 hours. Ethylene production increased with the duration and intensity of light exposure at all seedling ages. Neither the light or dark rates of ethylene, or carbon dioxide production, nor their ratios, were highly correlated with the sexual phenotype of the 9 cultivars examined.**

Ethylene has been reported to influence the sexual phenotype of cucumber plants (2, 14). Reducing the physiologically active concentration of ethylene with inhibitors of its synthesis or action, or with hypobaric ventilation caused gynoecious plants of the closely related species *Cucumis melo* L. to exhibit more maleness; i.e. to produce perfect flowers as well as pistillate flowers (2). In contrast, elevating the endogenous concentration of ethylene by the application of (2-chloroethyl)phosphonic acid promoted more femaleness in monoecious lines of cucumber plants *(C. sativus)* (11, 18). The natural rate of ethylene production has been shown to be higher in gynoecious than in monoecious cucumber plants (2, 12, 14). Androecious cucumber plants produced less ethylene than monoecious plants (16). These phenotypic differences in the rate of ethylene production were present in 1- to 2-day-old seedlings and generally persisted in the adult plant.

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Cucumber breeding would be easier if there were criteria by which the sexual phenotype of the adult plant could be determined in 1- to 2-day-old seedlings of a segregating population. If ethylene production rates by single seedlings were correlated with the sexual phenotype of the adult plant, then screening for specific rates of ethylene production by germinating seeds could serve this purpose. Rudich et al. (15) found that the rate of ethylene production in the seedling stage was not always correlated with the sexual phenotype. In their study, ethylene production rates by seeds germinated in the light were measured in the light. No explanation was given as to why this condition was chosen.

The rate of ethylene production following exposure to light has been observed either to increase, as in cranberries (3), etiolated sorghum seedlings (4, 5), rose tissue cultures (10), and oat seedlings (13), or to decrease, as in rice coleoptiles (8), bean hooks (9), and etiolated pea seedlings (6, 17). The phytochrome system has been shown to be responsible for some of these light-induced changes in the rate of ethylene production (4, 6, 8, 9). For example, a few minutes exposure to red light reduced ethylene production from etiolated peas and rice coleoptiles within 5 hr (6, 8). However, red light may also cause increased ethylene production, as in lettuce, by stimulating seed germination rather than by actually stimulating ethylene synthesis (1).

In our study the effect of light on ethylene production by germinating cucumber seed was investigated to determine if it was related to the sexual phenotype of the cultivar.

Materials and Methods

Cucumber seeds of the gynoecious breeding lines Gy-2, Gy-3, Gy-14, and cultivars 'Femcap', and 'Explorer'; of the monoecious 'Addis', 'Chipper', and 'Model'; and of the androecious breeding line MSU 5802A were used in this study. The seeds were placed on filter paper moistened with distilled water and germinated at $23^{\circ} \pm 1^{\circ}$C in the dark or under Cool White fluorescent light for 24, 36, or 48 hr. Seedling age will always refer to the time from the start of imbibition. Uniformly germinated seeds were selected and transferred under dim green light (<0.1 μE m^{-2} sec^{-1}) to either 10-ml glass syringes or 10-ml flow-through tubes containing moistened filter paper. Some containers were covered with aluminum foil to exclude light. Light intensity was adjusted by varying the distance between the containers and the Cool White fluorescent lights, or by placing layers of nylon netting between the lights and the containers. A model LI-185 Li-Cor Quantum Meter with a LI-190S quantum sensor was used to measure light intensity. The containers were maintained at a uniform temperature of $23^{\circ} \pm 0.5^{\circ}$ in all experiments. The syringes were set to 4 or 10 ml, depending on whether they contained 1 or 5 seedlings, and capped with rubber serum stoppers. One-ml samples were taken after 1 hr and analyzed for ethylene and carbon dioxide as previously described (17). Periodic measurements were made by ventilating the syringes with humidified, ethylene-free air between 1-hr accumulation periods. Longer experiments were conducted using flow-through tubes which were constructed from glass tubes capped at each end with rubber serum stoppers. Humidified, ethylene-free air was supplied from a manifold to each chamber through a thin tube terminating in a hypodermic needle which was inserted in a serum stopper at one end of the container. Gas escaped through another hypodermic needle inserted in the serum stopper at the other end of the container. Removal of both hypodermic needles isolated the flow-through container during the 1 hr accumulation period. The needles were reinserted, and a flow of ca. 5 ml min^{-1} was maintained between accumulation periods.

The persistence of light-stimulated ethylene production was studied as follows. Gynoecious cucumber seeds (Gy-3) were germinated in the dark for 24 hr and then either left in the dark or exposed to 100 μE m^{-2} sec^{-1} light for an additional 24 hr. Uniformly germinated seedlings were selected and transferred to glass flow-through tubes. After transfer, the tubes were kept in the dark and flushed with humidified, ethylene-free air for 3 hr. The containers were then either kept in the dark, exposed to 8 μE m^{-2} sec^{-1}, or 100 μE m^{-2} sec^{-1} light. One-half hr ethylene accumulations were measured at the start of these light treatments and after 1 and 5 hr.

All experiments were repeated with from 3 to 20 replicates per treatment. Data were subjected to standard analysis of variance procedures.

Results and Discussion

The rate of ethylene production by germinating Gy-3 cucumber seeds followed the same general pattern whether the seedlings were kept in the dark or exposed to light after imbibition for 24 or 36 hr (Fig. 1). The maximum rate of ethylene production from dark controls and from 24 and 36 hr old seedlings exposed to light occurred at about 48 hr after imbibition. Ethylene production then slowly decreased. Seeds imbibed for 48 hr produced less ethylene when exposed to light than seeds imbibed for 24 or 36 hr.

Subsequent experiments used seedlings which had been imbibed for 24 hr in the dark before being exposed to light for an additional 24 hr. Under our experimental conditions, the radicle emerged from the seed 24 hr after imbibition. By 48 hr, the radicles of the dark controls had grown to around 4.0 cm in length, while the radicles of the seeds exposed to 100 μE m^{-2} sec^{-1} light had only grown to 1.5 cm. In either case, the epicotyl hook and peg structure (19) were visible by 48 hr. An increase in ethylene production might have been expected prior to radicle emergence since physical restraint of growth has been shown to stimulate ethylene production (7). However, this was not the case. The similar trend in ethylene production by seedlings kept in the dark, or exposed to light at different ages, suggests that a specific developmental stage may be associated with the maximum rate of ethylene production which occurred about 48 hr after imbibition.

The rate of ethylene production by Gy-3 cucumber seeds germinated in the dark for 24 hr and subsequently exposed to light intensities of 0, 6, 24, 40, 80, and 170 μE m^{-2} sec^{-1} gradually increased during exposure to light (Fig. 2). Maximum ethylene production occurred after 24 hr exposure to light, or dark, and then decreased regardless of light intensity. Ethylene production was stimulated by as little as 4 hr exposure to 6 μE m^{-2} sec^{-1}, and was saturated at 4 hr, by exposure to between 40 and 80 μE m^{-2} sec^{-1} light (Fig. 2). The maximum rate

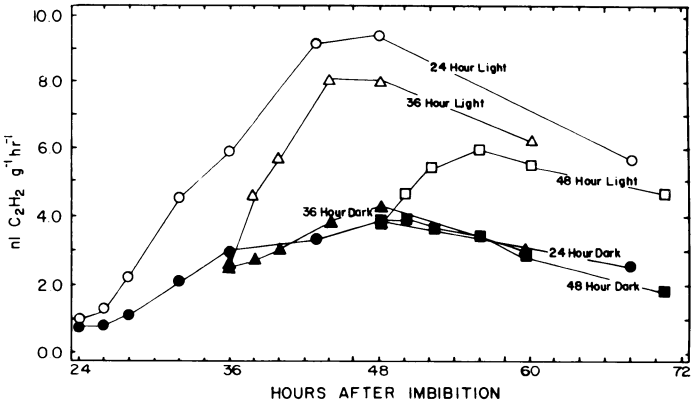

Fig. 1. Ethylene production from different aged Gy-3 seedlings kept in the dark, or exposed to 100 μE m^{-2} sec^{-1} light. Each data point is the mean of 3 replications (flow-through-tube) each containing 5 seedlings.

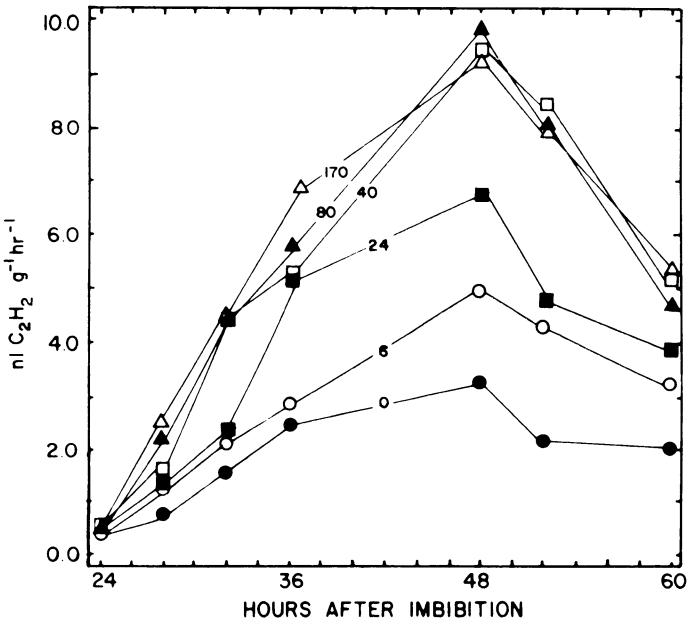

Fig. 2. Ethylene production from 24 hr old Gy-3 seedlings exposed to 0(●—●), 6(○—○), 24(■—■), 40(□—□), 80(▲—▲), or 170 (△—△) μE m^{-2} sec^{-1} light. Each data point is the mean of 3 replications (flow-through-tube) each containing 5 seedlings.

of ethylene production occurred 48 hr after imbibition for all light intensities, and increased with increasing light intensity until the rate of light stimulated ethylene production at 48 hr was saturated at 40 μE m^{-2} sec^{-1} light. A similar peak in carbon dioxide production was not observed, but rather, for the 68 hr duration of the experiment its rate of production increased in a near linear fashion after an initial 6 hr lag period. Light intensity had no effect on the rate of carbon dioxide production during this period (data not shown).

Table 1. Persistence of light-stimulated ethylene production. See Materials and Methods section for experimental procedure.[y]

Light treatments[z]			nl ethylene g^{-1} hr^{-1}		
0 – 24 hr	24 – 48 hr	48 – 54 hr	48 hr	49 hr	53 hr
Dark	Dark	Dark	2.1	2.0	2.2
Dark	Light	Dark	9.5	7.5	5.4
Dark	Light	Dim	9.9	7.9	5.4
Dark	Light	Light	10.8	8.5	6.6
		LSD 5%	2.0	2.2	1.3

[z]The 24 hr old seedlings were exposed to 24 hr of 100 μE m^{-2} sec^{-1} light (Light), or 8 μE m^{-2} sec^{-1} light (Dim). Gynoecious (Gy-3) cucumber seeds were used.

[y]Data are means of 3 replications (flow-through-tubes) each containing 5 seedlings.

Table 3. Ethylene production from germinating cucumber seeds of different phenotypes. Rates of production were measured after 24 hr of germination in the dark, and again after 24 hr exposure to 100 μE m^{-2} sec^{-1} light.[z]

Line or cultivar	Phenotype	nl g^{-1} hr^{-1}		Light/dark Ratio
		Dark	Light	
Gy-2	Gynoecious	2.9	4.3	1.5
Gy-3	Gynoecious	2.5	6.3	2.5
Gy-14	Gynoecious	3.4	4.4	1.3
Femcap	Gynoecious	1.6	4.4	2.7
Explorer	Gynoecious	2.2	3.6	1.7
Chipper	Monoecious	1.2	2.9	2.5
Model	Monoecious	2.6	4.6	1.8
Addis	Monoecious	2.5	5.7	2.3
MSU 5802A	Androecious	2.3	3.5	1.5
	LSD 5%	1.4	1.6	1.0

[z]Data are means of 20 replications (Syringe set to 4 ml) each containing 1 seedling.

Ethylene production by germinating cucumber seeds, once stimulated by exposure to light, did not decline rapidly upon reduction of light intensity, or upon return to darkness (Table 1). Although seeds maintained in light intensities of 100 μE m^{-2} sec^{-1} always tended to have higher rates of ethylene production during the 48 to 54 hr experimental period than seeds kept in the dark or moved from 100 to 8 μE m^{-2} sec^{-1}, these differences were not statistically significant. This could be attributed to excessive variability in the rate of ethylene production among individual seeds. In uniformly germinated 24 or 36 hr old cucumber seeds which had been exposed to 24 hr of 100 μE m^{-2} sec^{-1} light, the variability was large and similar among phenotypes (Table 2). Expressing the data as pl $seed^{-1}$ hr^{-1} or as nl g^{-1} hr^{-1} had very little effect on the degree of variability.

The rates of ethylene production among the different cultivars was often statistically different (Table 3). However, these differences were not related to the sexual phenotypes of the cultivars. This was also true for measurements of the rates of ethylene production in the dark, or in the light, or measurements of the rates of carbon dioxide production in the dark, or in the light, or combinations of these measurements. Thus, measurements of ethylene production by cucumber seedlings as outlined in this paper, cannot be used to predict the adult sexual phenotype of the plant.

However, germinating cucumber seeds may provide a convenient system with which to study the physiology of light

Table 2. Ethylene production from individual germinating cucumber seeds.[z]

Phenotype	48 hr old seedlings		60 hr old seedlings	
	pl $seed^{-1}$ hr^{-1}	nl g^{-1} hr^{-1}	pl $seed^{-1}$ hr^{-1}	nl g^{-1} hr^{-1}
Gynoecious	531 ± 144(27%)	8.7 ± 2.4(28%)	364 ± 100(27%)	4.2 ± 1.0(24%)
Monoecious	324 ± 78 (24%)	6.4 ± 1.5(23%)	254 ± 73(29%)	3.7 ± 1.0(26%)
Androecious	149 ± 40(27%)	2.8 ± 0.8(28%)	115 ± 41(36%)	2.0 ± 0.7(35%)

[z]The 24 hr or 36 hr old seedlings were exposed to 24 hr of 100 μE m^{-2} sec^{-1} light. Gynoecious (Gy-3), monoecious ('Addis'), and androecious (MSU 5802A) phenotypes were used. Values given are the means of 20 replications (syringes set to 4 ml) each containing 1 seedling, and their standard deviations, and coefficients of variability.

mediated ethylene synthesis. As we have shown, ethylene production by seedlings from genetically different cultivars, responds differently to light exposure. The variable response to light of cucumber cultivars may prove useful in delineating the system responsible for light-stimulated ethylene production.

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Spray Chrysanthemum Production with Controlled-release Fertilizer and Trickle Irrigation[1]

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Additional index words. cut-flowers, water, Osmocote, urea formaldehyde, *Chrysanthemum morifolium*

Abstract. **Various rates, types, and formulations of controlled-release fertilizers were evaluated as potential components in a trickle irrigation production system for spray chrysanthemums (*Chrysanthemum morifolium* Ramat.). Optimum rates of total-N, with 34 kg N/ha as soluble 6-2.6-5 (N-P-K) and the remainder as 14-6.1-11.6 Osmocote, were estimated to be from 489-501 kg per planted hectare in 2 tests. Other formulations or ratios of Osmocote and urea formaldehyde fertilizers at similar rates did not improve production or were not comparable to Osmocote 14-6.1-11.6 or to the commercial practice of weekly overhead liquid fertilization. A water savings of 70-80% was estimated with the controlled-release fertilizer-trickle irrigation system compared to overhead irrigation, while yields (marketable stems and height) were similar to those produced with overhead-liquid fertilization practices.**

Trickle irrigation systems can be used to conserve water, compared to overhead irrigation systems, by confining water to the cropped area. Trickle irrigation systems have occasionally become inoperative in areas with poor quality water. Addition of water soluble fertilizers and the presence of other salts, sulfur, algae and bacteria have further compounded distribution problems and limited the use of trickle irrigation systems (3, 4, 11, 19). Controlled-release fertilizers incorporated in the soil may increase the efficiency of delivery systems by removing an antagonistic component to poor water quality.

Controlled-release fertilizers have been evaluated for production of many horticultural crops (9). Kofranek and Lunt (5) found that one application of coated 25-4.6-4 produced quality potted chrysanthemums and Waters (15) reported that split applications of heavily coated 9-4-5 or 10-4.6-8 produced quality pot-mums. Simpson et al. (13) and Bivins and Kofranek (2) showed that a combination of coated fertilizer and liquid fertilization produced good quality plants. Sharma and Patel

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