Plant Physiol. (1981) 68, 831-834
0032-0889/81/68/0831/04/$00.50/0

Effects of Carbon Dioxide on Ethylene Production and Action in Intact Sunflower Plants[1]

Received for publication September 9, 1980 and in revised form February 27, 1981

KIRAN R. DHAWAN[2], PAWAN K. BASSI, AND MARY S. SPENCER[3]
Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada, T6G 2P5

ABSTRACT

A continuous flow system was used to study the interactions between carbon dioxide and ethylene in intact sunflower (*Helianthus annuus* L.) plants. An increase in the concentration of carbon dioxide above the ambient level (0.033%) in the atmosphere surrounding the plants increased the rate of ethylene production, and a decrease in carbon dioxide concentration resulted in a decrease in the rate of ethylene production. The change in the rate of ethylene production was evident within the first 15 minutes of the carbon dioxide treatment. Continuous treatment with carbon dioxide was required to maintain increased rate of ethylene production. The rate of carbon dioxide fixation increased in response to high carbon dioxide treatment up to 1.0%. Further increases in carbon dioxide concentration had no additional effect on carbon dioxide fixation. Carbon dioxide concentrations higher than 0.11% induced hyponasty of the leaves whereas treatment with 1 microliter per liter ethylene induced epinasty of the leaves.

Carbon dioxide has been reported to promote, inhibit, or have no effect on the rates of ethylene production at different stages of plant growth and development. For example, carbon dioxide stimulated ethylene production of the senescing tobacco leaf discs (2). Similarly, when CO_2 was removed from the atmosphere with KOH, ethylene production by infected sweet potato roots decreased (11). CO_2 has been shown to inhibit ethylene production by ripening fruit (15, 18). It is not clear whether this reflects an effect of CO_2 solely on ethylene production. An autostimulatory effect of ethylene on its own evolution has been reported in ripening fruit (14). CO_2 may thus be inhibiting an ethylene effect. Effects of CO_2 on ethylene production and/or action may also be involved in the lack of observable effects of CO_2 on the rate of ethylene production of citrus fruits (4). An antagonistic effect of CO_2 on ethylene action has also been observed in a number of plant responses such as petiole epinasty, fruit ripening, abscission and senescence (5–7). Toole *et al.* (17) have suggested that CO_2 acts synergistically to ethylene in the promotion of germination of peanut seeds. CO_2 has also been shown to mimic the effect of ethylene for the promotion of growth in rice (12) and removal of astringency from persimmons (1).

Though CO_2 no doubt has diverse effects, some of the conflicting results may stem from the techniques employed as well as from differences in the tissues used and their stages of development. Most earlier work was done on excised plant parts in closed systems. Under such conditions, the plant material is exposed to continually changing concentrations of CO_2, O_2, and ethylene as a result of the plant's metabolism. The situation is further complicated by the "wounding effect," an increase in respiration and ethylene production when the tissue is cut or injured. There has been no work done on the effect of CO_2 on ethylene production by intact, vegetatively growing plant tissues.

Recently, techniques have been developed in our laboratory that overcome most of the potential problems associated with the conventionally used methods. These techniques also allow simultaneous measurements of ehtylene production and CO_2 exchange by the intact shoots under controlled environmental conditions (3). The present investigation was done with hydrocarbon-free air (8) in a continuous flow system. Relationships between CO_2 concentration and the rate of ethylene production by the intact plant shoots were studied when all the other environmental factors were kept constant. The changes in the leaf axil angle as affected by different concentrations of CO_2 and ethylene were also measured, again in the intact plants, keeping all the other environmental factors constant.

MATERIALS AND METHODS

Seeds of sunflower (*Helianthus annuus* L.) were sown in vermiculite and placed under continuous light in a growth cabinet maintained at a temperature of 27 ± 1 C and RH of 75%. The intensity of light (400 to 725 nm) was constant at 125 $\mu E/m^2 \cdot s$. After 7 days, the uniform seedlings were transplanted to individual pots containing sand, vermiculite and peat-moss (1:1:1).

For each experiment, one 16-day-old plant (6-leaf stage) was sealed around the stem in a specially designed glass cuvette described earlier (3). After sealing the plant in the cuvette, the system was allowed to equilibrate overnight in an air stream to avoid any problems related to abnormal ethylene production from mechanical stimulation during sealing. By the next morning, CO_2 levels and ethylene production were constant in two sequential determinations. Air containing different concentrations of CO_2 (0 to 5.0%, v/v), obtained from Matheson Company Inc., was passed through a stainless steel tube (2.54 cm diameter) packed with 5% platinized asbestos. The temperature of the catalyst was maintained at 700 C in order to oxidize all the hydrocarbon contaminants (8). The purified air was then directed through the cuvette at a flow rate of 200 ml/min metered through a needle valve. Continuous light (400 to 725 nm, 125 $\mu E/m^2 \cdot s$) was provided with six incandescent lamps, 150 w each. This light beam was passed through a Plexiglas filter containing a 12 mm $CuSO_4 \cdot 5\ H_2O$ (2% w/v) solution with a cut-off point at 730 nm. The light intensity and its spectrum were measured with a spectroradiometer (Techtum-QSM-2500, Mandel Scientific Co.). Temperature inside the cuvette was maintained at 27 ± 1 C by circulating water, from a constant temperature water bath, in the jacket around the cuvette. The air and leaf temperatures were measured with copper-con-

[1] Financial Assistance from the Natural Sciences and Engineering Research Council of Canada in the form of Grant A-1451 to M. S. S. is gratefully acknowledged.

[2] Deceased.

[3] To whom reprint requests should be addressed.

stantan thermocouples. RH inside the cuvette was measured with a dew point hygrometer.

Ethylene in the air coming out of the cuvette was collected over silica gel (0.5 g, 60–80 mesh) kept at a temperature of −86 C in a Dry Ice-acetone bath. The details of this method have been described earlier (8). The air stream was bubbled through a saturated solution of KOH contained in a 75 ml gas washing bottle, in order to remove CO_2 and water vapor, before directing it to the ethylene collection trap. Quantitative determinations of ethylene were done on a gas chromatograph (Hewlett Packard 5830A) equipped with Porapak Q column (80 to 100 mesh) and a flame ionization detector. CO_2 concentrations were determined either with an IR gas analyzer (Beckman Model 865) or with the thermal conductivity detector of the gas chromatograph. After each experiment, the plant shoot was dried in a vacuum oven at 60 C and a vacuum of 711 mm Hg for determination of dry weight. For each experiment, two measurements on the rate of ethylene production were made at normal CO_2 (0.033%) before changing the CO_2 concentration. The time for equilibration of the cuvette with the changed CO_2 concentration was determined by measuring the concentration of CO_2 coming out of the cuvette. The rate of CO_2 fixation was calculated by the method of Gaastra (9). All other environmental parameters were kept the same as in the ethylene production experiments. To study the change in axil angle, 16-day-old plants were treated with different concentrations of CO_2 or ethylene. Hyponasty of the leaves was assessed by measuring the angle between the stem and petiole before and after 11 h of treatment. The first and the second leaf pairs in the text refer to the oldest and next oldest leaf pair, respectively. Each experiment was repeated several times and the data from a typical experiment are presented for each treatment.

RESULTS AND DISCUSSION

Figure 1 shows the rate of ethylene production as affected by different CO_2 concentrations. Different plant shoots produced ethylene at rates ranging from 5 to 48 nl/g dry weight·h when the CO_2 concentration in the cuvette was maintained at 0.033%. Because of the large variability in the rates of ethylene production by different plants, the first ethylene measurement made at normal CO_2 for each experiment was adjusted to one for comparative purposes. A decrease in the concentration of CO_2 entering the cuvette decreased the rate of ethylene production (Fig. 1). (Even

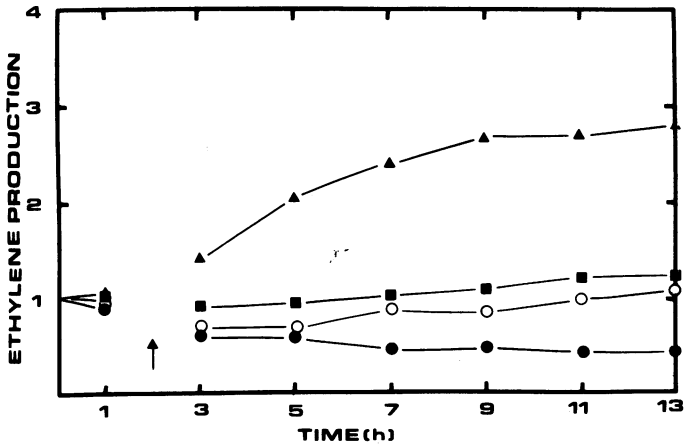

FIG. 1. Effects of various CO_2 concentrations on the rate of ethylene production by the sunflower plant. The plant was allowed to equilibrate overnight at normal CO_2 levels before ethylene measurements were begun. The position of the arrow indicates the time when the concentration of carbon dioxide going in the cuvette was changed. The Y-axis indicates the number of times the rate of ethylene production changed over that of the control. ○, control 0.033%; ●, 0.005%; ■, 0.74%; ▲, 0.11%.

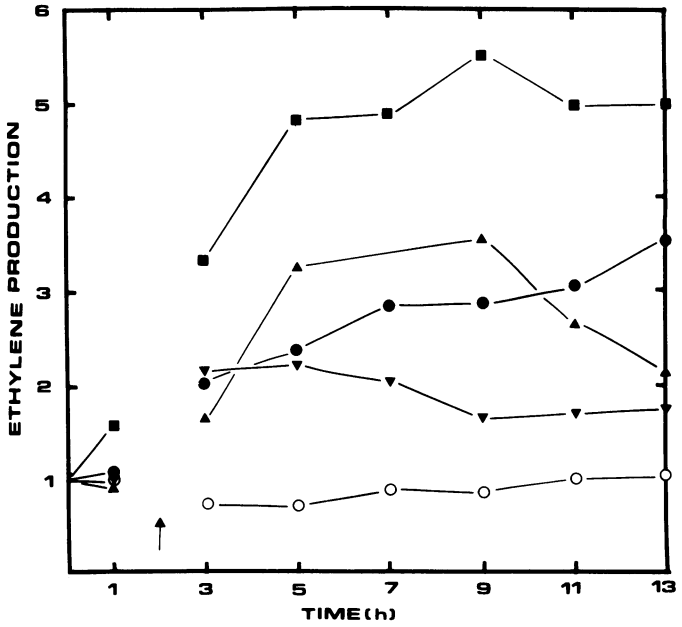

FIG. 2. Effects of above normal CO_2 concentrations on the rate of ethylene production by the sunflower plant. Conditions and Y-axis as in Figure 1. ○, control 0.033%; ●, 0.5%; ■, 1.0%; ▲, 2.5%; ▼, 5.0%.

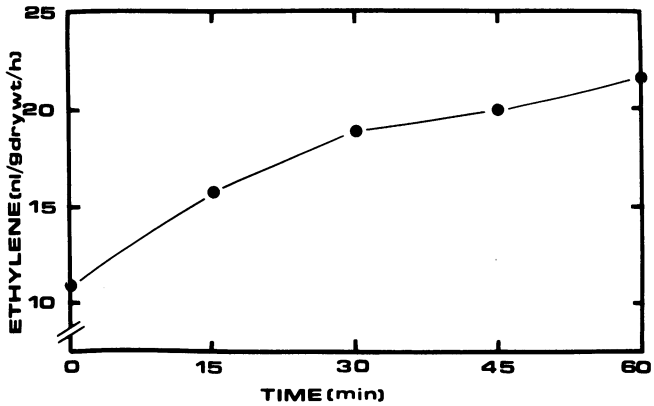

FIG. 3. Effect of 0.5% CO_2 on the rate of ethylene production during the first h of the treatment. CO_2 concentration was increased immediately after the first reading was taken. Each value gives the average rate of ethylene produced over the 15 min interval.

when the cuvette was flushed with CO_2-free air, the CO_2 concentration in the cuvette never fell below 0.005%.) An increase in the concentration of CO_2 from normal to 0.074% had no effect on the rate of ethylene production, whereas 0.11% CO_2 resulted in a marked increase in the rate of ethylene production compared to the control (Fig. 1).

The rate of ethylene production continued to increase with increased CO_2 concentrations up to 1.0% (Fig. 2). Further increases in CO_2 concentration to 2.5 and 5.0% led to an initial increase in the rate of ethylene production. However, the magnitude of this increased rate of ethylene production was smaller than for the 1.0% CO_2 treatment. The rate of ethylene production began to decline after 7 h of the treatment in plants exposed to 2.5% CO_2 and after 3 h in plants exposed to 5.0% CO_2. The rate of ethylene production was high compared to the control even after 11 h of treatment.

Earlier workers (2) have shown that in tobacco leaf discs allowed to senesce in darkness in a closed system, high CO_2 (5–10%) stimulated ethylene production during the first 3 days of the senescing period. Imaseki *et al.* (11) observed a decreased rate of

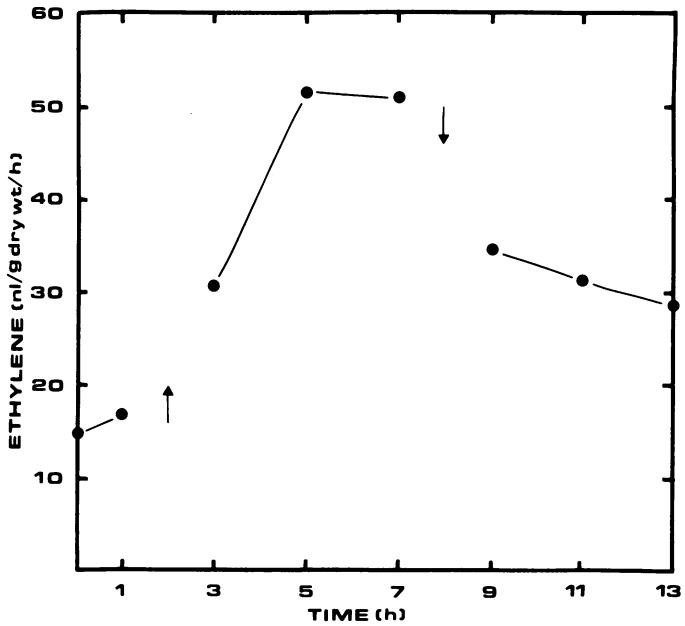

FIG. 4. Effects of change in CO_2 concentration from normal to 0.5% and back to normal, on the rate of ethylene production. The positions of the arrows indicate the points at which the CO_2 concentration was changed. The first ethylene measurement was taken 1 h after the treatment was begun.

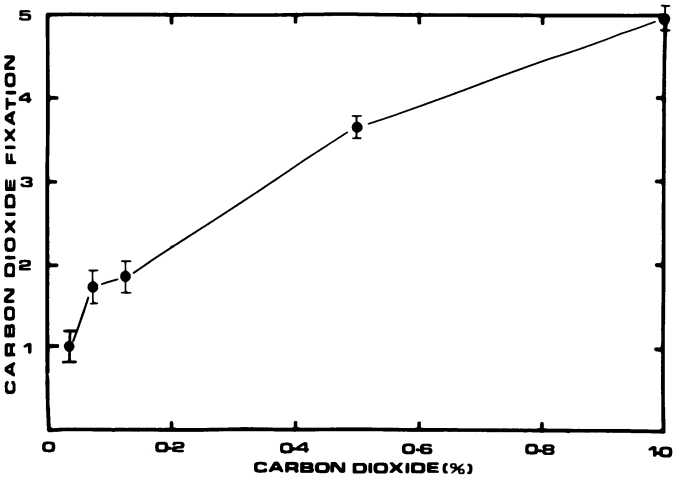

FIG. 5. Effect of CO_2 concentration on the rate of net carbon dioxide fixation. The Y-axis indicates the number of times the rate of CO_2 fixation increased over that of the control CO_2. The CO_2 measurements were made using the thermal conductivity detector of the gas chromatograph.

ethylene production by infected sweet potato roots when CO_2 was removed from the atmosphere with KOH and suggested that CO_2 stimulates ethylene production. However, these workers did not quantify this effect of CO_2 on the magnitude or duration of the increased ethylene production. From our experiments (Figs. 1 and 2), it seems that there is a direct relationship between the concentrations of CO_2 and the rate of ethylene production by intact sunflower plants.

In our experiments it took from 30 to 45 min for the cuvette atmosphere to be equilibrated with the changed CO_2 concentration. The first ethylene measurement was made 1 h after changing CO_2 concentration and by that time the rate of ethylene production had already increased. Further experiments were conducted where ethylene production was monitored continuously for the 1st h of 0.5% CO_2 treatment, by making four collections of 15 min each

Table I. *The Effects of Carbon Dioxide or Ethylene Treatments on the Axil Angle in Sunflower Leaves*

For each experiment, three 16-day-old plants were treated with different concentrations of CO_2 or ethylene and changes in axil angle were measured. Positive values denote upward and negative values denote downward bending of the leaves after 11 h of the respective treatment.

Treatment	Change in Axil Angle of the Leaves	
	First leaf-pair	Second leaf-pair
	degrees	
Control	3.33 ± 5.16	7.5 ± 4.18[a]
0.11% CO_2	−5.0 ± 6.77	2.5 ± 5.24
1.0% CO_2	6.67 ± 6.06	22.5 ± 8.80
2.5% CO_2	10.83 ± 6.64	21.67 ± 4.08
5.0% CO_2	15.83 ± 5.84	14.17 ± 5.84
1 μl/l C_2H_4	−71.67 ± 9.31	4.17 ± 11.58

[a] Standard deviation.

(Fig. 3). The rate of ethylene production was found to increase even within the first 15 min of the treatment. In other experiments, where the CO_2 concentration was changed back to normal after 3 h of 0.5% CO_2 treatment (Fig. 4), the rate of ethylene production closely followed the changes in CO_2 concentration in the atmosphere surrounding the plant. Thus, continuous treatment with high levels of CO_2 was required to maintain the increased rate of ethylene production.

Inasmuch as photosynthesis is one of the major physiological processes to be affected by high CO_2 levels, the rate of net CO_2 fixation was measured during all CO_2 treatments (Fig. 5). It was found to increase in response to high CO_2 treatments up to 1.0% as analyzed with the thermal conductivity detector of the gas chromatograph. CO_2 concentrations higher than 1.0% led to a poor reproducibility of the CO_2 analysis with this method. However, by the use of the IR gas analyzer, it was shown that further increases in CO_2 concentration above 1.0% had no additional effect on the rate of CO_2 fixation. Besides affecting photosynthesis, CO_2 has also been shown to affect various metabolic pathways via the control of intracellular pH (10) and an anaplerotic synthesis of amino acids (16). The interrelationships among these effects and the effects of CO_2 on the metabolism and the rate of ethylene production has still to be elucidated.

CO_2 concentrations above 0.5% induced hyponasty of the leaves of plants enclosed in the cuvette. The effect appeared to be more pronounced in younger leaves. Table I shows the changes in axil angle as a result of CO_2 and ethylene treatments. The data have been analyzed using analysis of variance and Duncan's multiple range test and the effects discussed below are significant at the 1% level. CO_2, at a concentration of 0.11%, had no effect on the leaf angle of either the first or second pair of the leaves, whereas 1.0 or 2.5% CO_2 treatment resulted in hyponastic curvature of the second pair of leaves. A further increase in CO_2 concentration to 5.0% caused the first pair of the leaves to be hyponastic. When the plants at normal CO_2 levels were treated with 1 μl/l ethylene for 11 h, the first pair of the leaves showed epinastic curvature while the second pair was unaffected. Epinasty is a well known response to ethylene shown by most plants (1), and its reversal by CO_2 has been reported by Leather *et al.* (13). Epinasty is also induced by the compounds that promote ethylene production by the plant, such as IAA, 2,4-D and malformin (1, 7). Higher than normal levels of CO_2 act similarly to these compounds in that they promote ethylene production by sunflower plants. However, these higher levels of CO_2 do not lead to epinasty of the leaves. Instead, the leaves showed hyponasty. The second leaf-pair, which gave the greatest response (hyponastic) to elevated CO_2 levels, did not repond to ethylene treatment (Table I). The first leaf-pair, which was less responsive to high CO_2, did show a typical epinastic

response to exogenously supplied ethylene. CO_2 may be acting independently to regulate the leaf angle or there may be interaction between CO_2 and ethylene. High CO_2 concentrations may inhibit ethylene action so that the petioles of the treated plants show upward bending as compared to controls.

Thus, CO_2 seems to act antagonistically to ethylene in regulating the axil angle, in the same plant tissue and at the same time as it promotes the rate of ethylene production.

LITERATURE CITED

1. ABELES FB 1973 Ethylene in Plant Biology. Academic Press, New York
2. AHARONI N, M LIEBERMAN 1979 Ethylene as a regulator of senescence in tobacco leaf discs. Plant Physiol 64: 801–804
3. BASSI PK, MS SPENCER 1979 A cuvette design for measurement of ethylene production and carbon dioxide exchange by intact shoots under controlled environmental conditions. Plant Physiol 64: 488–490
4. BEN-YEHOSHUA A, IL EAKS 1970 Ethylene production and abscission of fruits and leaves of orange. Bot Gaz 131: 144–150
5. BURG SP, EA BURG 1967 Molecular requirements for biological activity of ethylene. Plant Physiol 42: 144–152
6. CROCKER LE, FB ABELES 1969 Abscission: role of abscisic acid. Plant Physiol 44: 1144–1149
7. CURTIS RW 1968 Mediation of a plant response to malformin by ethylene. Plant Physiol 43: 76–80
8. EASTWELL KC, PK BASSI, MS SPENCER 1978 Comparison and evaluation of methods for the removal of ethylene and other hydrocarbons from air for biological studies. Plant Physiol 62: 723–726
9. GAASTRA P 1959 Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature and stomatal diffusion resistance. Meded Landbouwhogeschool Wageningen 59: 1–68
10. HASCHKE H, U LÜTTGE 1975 Stoichiometric correlation of malate accumulation with auxin dependent K^+-H^+ exchange and growth in *Avena* coleoptile segments. Plant Physiol 56: 696–698
11. IMASEKI H, T TERANISHI, I URITANI 1968 Production of ethylene by sweet potato roots infected by black rot fungus. Plant Cell Physiol 9: 769–781
12. KU HS, H SUGE, L. RAPPAPORT, HK PRATT 1969 Stimulation of rice coleoptile growth by ethylene. Planta 90: 333–339
13. LEATHER GR, LE FORRENCE, FB ABELES 1972 Increased ethylene production during clinostat experiments may cause leaf epinasty. Plant Physiol 49: 183–186
14. LIEBERMAN M 1979 Biosynthesis and action of ethylene. Annu Rev Plant Physiol 30: 533–591
15. POTTER NA, DG GRIFFITHS 1947 The effects of temperature and gas mixtures on the production of volatile substances by apples during storage. J Pomol Hort Sci 23: 171–177
16. SPLITTSTOESSER WE 1966 Dark CO_2 fixation and its role in the growth of plant tissue. Plant Physiol 41: 755–759
17. TOOLE VK, WK BAILEY, EH TOOLE 1964 Factors influencing dormancy of peanut seeds. Plant Physiol 39: 822–831
18. YOUNG RE, RJ ROMANI, JB BIALE 1962 Carbon dioxide effects on fruit respiration. II. Response of avocados, bananas and lemons. Plant Physiol 37: 416–422