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Carbon Dioxide Enhances the Development of the Ethylene Forming Enzyme in Tobacco Leaf Discs[1]

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ABSTRACT

Since CO_2 is known to stimulate ethylene production by promoting the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene, the effect of CO_2 on the activity and the development of the ethylene forming enzyme (EFE) was studied in tobacco (*Nicotiana tabacum* L. cv Havana 425 and Xanthi) leaf discs. In addition to previous observations that EFE activity is dependent on CO_2 concentration and is saturable with 2% CO_2, present data show two saturation curves at 2% and 10% CO_2. Promotion of EFE development was dependent also on CO_2 concentration (saturated at 2% CO_2) and duration (maximum at 24 in the dark), and was abolished by 20 micromolar cycloheximide. Application of exogenous ethylene (20 microliters per liter) or light treatment further increased the CO_2-enhanced development of EFE, implying that these two factors can also affect EFE development via interaction with CO_2. The results suggest that CO_2 exerts its stimulatory effect on the conversion of ACC to ethylene by enhancing not only the activity but also the synthesis of EFE in leaf discs.

CO_2, which is known as an antagonist of ethylene action and retards ethylene-mediated responses (17), has been shown to promote ethylene production in a variety of plant systems. In photosynthetic tissues, the promotive effect of CO_2 was observed with isolated protoplasts (20, 26), with excised leaf segments in closed systems (1, 2, 8, 10, 12–14, 20–22) and with intact plants (3, 5, 7). CO_2 stimulated both basal (1–3, 7) and ACC[2] dependent (5, 8, 12–14, 20–22, 26) ethylene production, but hardly affected ACC production (14), suggesting that CO_2 exerts its promotive effect on the step of ACC conversion to ethylene. The effect is obtained when CO_2 is supplied either directly as a gas or indirectly as bicarbonate (12, 20). Many investigators have observed that light markedly inhibited ACC-dependent ethylene production of excised leaf segments enclosed in flasks (4, 6, 9, 11, 14, 22, 27) and several mechanisms have been proposed to account for this inhibition (6, 9, 27). However, with intact plants in an open system the effect of light on ethylene production is marginal (4). Recently, it was established that the inhibitory effect of light observed with excised segments reflects partial depletion of endogenous CO_2 pools by photosynthetic fixation. When sufficient CO_2 is provided, light becomes a stimulating factor, suggesting that the amount of ethylene generated by photosynthetic tissues is controlled directly by the availability of CO_2 (11, 12, 14, 28).

Since the CO_2 effect on ethylene evolution from leaves does not seem to be a direct result of stomatal control (13), the controversy remained to determine whether CO_2 exerts its effect by modulating the enzymes involved in ethylene biosynthesis as proposed by Kao and Yang (14), or at the level of ethylene retention and/or metabolism, as suggested by Grodzinski *et al.* (11). Our present study provides further evidence that CO_2 affects the *in vivo* enzyme involved in the last step of ethylene biosynthesis. Since the stimulation of ethylene production by CO_2 is rapid and readily reversible in short incubations, Kao and Yang (14) have proposed that CO_2 exerts its effect by activating the *in vivo* conversion of ACC to ethylene (EFE). In an effort to distinguish between enzymic activity and synthesis by employing short and long incubations, respectively, we demonstrate that CO_2 exerts its promotive effect also by enhancing the *in vivo* development of EFE.

MATERIALS AND METHODS

Plant Materials and Incubation. Tobacco plants (*Nicotiana tabacum* L. cv Havana 425 and Xanthi) were grown in a greenhouse under natural light at temperatures between 20 and 30°C. Fully expanded mature leaves (from 11- to 12-week-old plants) were washed under running tap water, surface sterilized by soaking for 30 s in 0.5% (w/v) NaOCl, and rinsed several times with distilled H_2O. Discs, 1 cm in diameter, were excised and treated as described previously (1, 2, 21). Samples of eight leaf discs, weighing about 0.1 g, were usually incubated on filter paper in 50-ml flasks containing 1 ml of 10 mM Mes buffer (pH 6.1), except for one experiment where discs were floated on 1 ml of water. Rice (*Oryza sativa* L. cv Taichung Native 1) seeds, obtained from Taiwan, were sterilized, soaked, and germinated for 9 d at 30°C as described previously (14). Samples of ten rice leaf segments (the apical 3 cm of the third leaves), weighing about 0.05 g were floated on 5 ml of water in 50-ml flasks. Oat (*Avena sativa* var. Garry) seeds, obtained from Stanford Co. New York, were soaked for 8 h, sown in vermiculite moistened with tap water, and grown under continuous fluorescent white light at 25°C for 9 d, as described before (22). Samples of six oat leaf segments (the apical 3 cm of the first leaves), weighing about 0.12 g were floated on 1 ml of water in 50-ml flasks. All the flasks were flushed with air, sealed with rubber serum stoppers, and incubated at 30°C either under light (540 $\mu E \cdot m^{-2} \cdot s^{-1}$), provided by a mixture of cool-white and Grolux tubes, or in darkness. Where indicated, 5 mM ACC (Cal Biochem), 20 μM CHI (Sigma), 20 μl/L ethylene or various CO_2 concentrations were included. To obtain desired ethylene or CO_2 concentrations, known amounts of ethylene or CO_2 were injected into the sealed

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[2] Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; CHI, cycloheximide; EFE, ethylene forming enzyme.

flasks. To achieve a CO_2-free or ethylene-free atmosphere the CO_2 and ethylene evolved were absorbed, respectively, by 0.2 ml of 20% KOH and 0.25 M $Hg(ClO_4)_2$ solutions, applied each in a plastic center well hung in the flask, as detailed previously (14, 21). After each ethylene determination, the flasks were flushed with fresh or CO_2-free air as indicated and when required, CO_2 was reintroduced.

Determination of Ethylene. A 1-ml gas sample was withdrawn from each flask with a hypodermic syringe at the indicated periods, and ethylene concentration in the sample was determined by a gas chromatograph equipped with an activated alumina column and a flame ionization detector.

Determination of CO_2. A 0.5-ml gas sample was withdrawn from each flask with a hypodermic syringe before and after the indicated incubation periods. Thus, the CO_2 values presented are the average of these two measurements. Fluctuations in CO_2 concentration in the course of 1 h incubation were insignificant. Concentrations of CO_2 in the flask were determined with an IR gas analyzer. The CO_2 concentration in the CO_2-free treatment was below 0.01%.

Determination of ACC. At the indicated periods, sample of eight tobacco leaf discs were extracted twice with 5 ml of boiling 80% ethanol. The ethanol was evaporated under vacuum at 55°C, the residues were dissolved in 2 ml water, and pigments were removed by addition of 0.5 ml chloroform. ACC content in 0.25 ml aliquots of the aqueous solution was assayed according to the method of Lizada and Yang (18).

Assay of EFE. Since EFE has not been yet isolated free from intact cellular material, EFE is routinely measured in the tissues (29). *In vivo* EFE activity was determined by measuring the capability of leaf segments to produce ethylene in the presence of a saturating concentration of ACC (19). ACC was applied either by preloading the leaf segments with ACC or by injecting ACC (5 mM final concentration) to the medium in the sealed flasks. For preloading, segments were floated on a 3 or 5 mM ACC solution for 2 or 3 h in the light, blotted dry on a filter paper and then enclosed in flasks with water medium for measuring their ethylene production in the light or in the dark as indicated. In this study we have determined that feeding the tissue with 3 mM or more ACC for 2 h (with or without CO_2) yielded maximal ethylene production rates (data not shown), indicating that the internal ACC levels have reached saturation under such feeding conditions. Thus, the ethylene produced during the following 20 min or 1 h incubation periods under light or dark after the ACC preloading can be taken as a measure of *in vivo* EFE activity (14). For EFE development, assay discs were preincubated with various CO_2 concentrations for 24 h, followed by a 2-h incubation with 5 mM ACC. The flasks were then flushed with fresh air, sealed and 10% CO_2 was introduced. The ethylene produced under 10% CO_2 in the following 30 min of dark incubation was taken as an index of EFE development.

RESULTS AND DISCUSSION

CO_2-Enhanced Ethylene Production. CO_2 (5–15%) was reported previously to enhance endogenous ethylene production in tobacco leaf discs during a prolonged dark incubation for several days (1, 2). Similar results were obtained also with rice leaf segments after 6 h dark incubation or 3 h of light incubation in the presence of 3% CO_2 (14). Nevertheless, this 3% CO_2 treatment did not increase significantly the endogenous level of free ACC (14). When the ACC-dependent ethylene production was examined in tobacco leaf discs during prolonged dark incubation under 10% CO_2, a marked increase in ethylene production was observed (Fig. 1). Similar data were also reported previously (21). The results indicate that CO_2 exerts its promotive effect mainly on the step of ACC conversion to ethylene, presumably by affecting EFE. This may occur by alternating the *in vivo* EFE activity and/or by alternating EFE development (net synthesis and degradation). Since the promotion of ethylene production by CO_2 was rapid (1–3 h) and fully reversible in their system where incubation time was short, Kao and Yang (14) have concluded that 3% CO_2 modulates EFE activity rather than its synthesis. However, in the present system the ACC-dependent ethylene production markedly increased with incubation time up to 24 h under 10% CO_2 (Fig. 1). If CO_2 promoted only EFE activity, the ACC-dependent ethylene production rates, measured under conditions which gave maximal activity, should not change during incubation, unless the enzyme undergoes a rapid turnover in the tissue (29). Hence, our data indicate that under prolonged incubation CO_2 can induce also *in vivo* EFE synthesis in addition to its promotive effect on the *in vivo* EFE activity, thereby resulting in higher ACC-dependent ethylene production rates (Fig. 1).

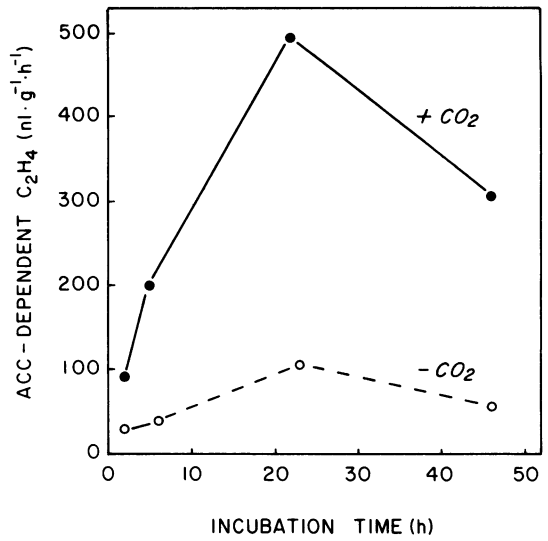

FIG. 1. Effect of CO_2 on the time course of ACC-dependent ethylene production in tobacco leaf discs. After dark preincubation with or without 10% CO_2 for various times as indicated, leaf discs were loaded with 5 mM ACC for 2 h, and ethylene produced during the next hour in 10% CO_2 was determined.

CO_2-Enhanced EFE Activity. Kao and Yang (14) have examined the dependence of EFE activity on CO_2 concentration with rice and tobacco leaf segments, and found that the activity was saturated at 1% CO_2. A similar saturable CO_2 concentration was reported in oat leaf segments (13), Lemna (8), and in intact sunflower plants (3, 7). On the other hand, it was reported previously that both basal (1) and ACC-dependent (21) ethylene production rates in tobacco leaf discs were saturated under 10 to 15% CO_2, during prolonged and dark incubations. Because of this apparent discrepancy, we have reexamined the dependence of EFE activity on CO_2 concentrations during 1 h of light and dark incubation with tobacco leaf discs. Figure 2 shows that EFE activity increased with increasing CO_2 concentrations (0–12%), giving two saturation curves at 2% and 10% CO_2, both in the light and in the dark. These two saturation curves suggest binding of CO_2 to two receptor sites with different affinities. A similar CO_2-concentration dependency curve for EFE activity in the light was found in rice and oat leaf segments (Fig. 3). It is to be noted that EFE was measured in closed flasks containing various concentrations of CO_2. Since CO_2 concentration changed during the incubation, the average of CO_2 injected initially and the CO_2 detected at the end of incubation was employed. It is obvious that when CO_2 concentrations change greatly during the incubation period, such measurements become unreliable. Thus, the discrepancy in the various CO_2-saturable levels obtained by

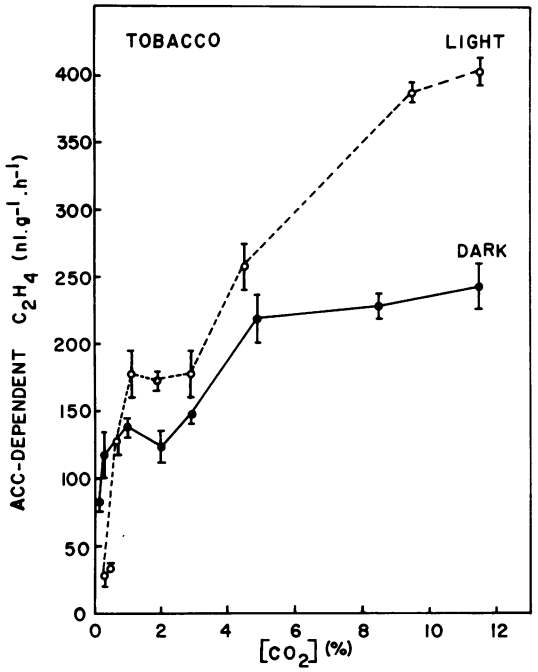

FIG. 2. Effect of CO_2 concentration on ACC-dependent ethylene production (EFE activity) in tobacco leaf discs under light and dark. After leaf discs (Xanthi) were preloaded with 3 mM ACC for 3 h in the light, samples of 8 discs were enclosed in flasks containing 1 ml water, and ethylene produced during the next 1 h incubation under various CO_2 concentrations, either in the light or in the dark, was determined. The indicated CO_2 concentrations represent the average of CO_2 concentrations in the flasks immediately before and after the 1-h incubation.

different investigators may be ascribed, in part, to such changes of CO_2 concentrations in sealed flasks enclosing plant tissues (11, 14, 22). With tobacco discs (Fig. 2) CO_2 levels in the sealed flasks changed little during the 1-h incubation period for ethylene determination. Thus, average values for percent CO_2 ranged as follows: 0.016 ± 0, 0.23 ± 0.007, 0.43 ± 0.0043, and 0.9 ± 0.008 (data not shown). However, with oat and rice segments the light incubation period had to be reduced to 20 min in order to minimize the large decrease in CO_2 levels, due to the photosynthetic CO_2 fixation. During the 20-min light incubation average CO_2 values were similar to those reported above for tobacco discs. The results of Figure 3 are in agreement with the results obtained by Preger and Gepstein (22) with ACC-treated oat leaf segments, although their data show a marked decrease (up to 33-fold) of CO_2 levels in the flasks during 3-h light incubation. Since maximal EFE activity is obtained under 10% CO_2, this concentration was employed in subsequent experiments.

CO_2-Enhanced EFE Development. Ethylene production rates in a tissue depend not only on ACC concentration but also on the activity and development of EFE. When the endogenous ACC levels are at saturating concentration, the ethylene production rates of the tissues should represent the *in vivo* EFE. The enhancement of ACC-dependent ethylene production by CO_2 depicted in Figure I may result from enhancement of EFE activity and/or enhancement of EFE development, since it was assayed under 10% CO_2 (which gives maximal EFE activity) for a prolonged incubation. To measure the effect of CO_2 on EFE development, discs were preincubated for 24 h under various CO_2 concentrations to allow development of EFE, and then 5 mM ACC (final concentration) was introduced for 2 h. EFE activity was assayed by measuring the ethylene production rate under 10% CO_2 during the following 30 min. Figure 4 demonstrates that EFE development was highly dependent on duration of the CO_2 pretreatment. Maximal development was obtained

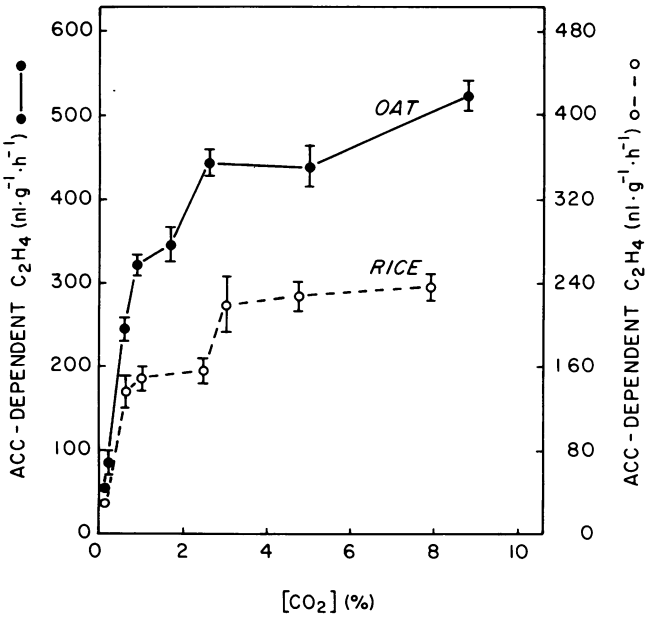

FIG. 3. Effect of CO_2 concentration on ACC-dependent ethylene production (EFE activity) in rice and oat leaf segments under light. Samples of 10 rice leaf segments and 6 oat leaf segments were preloaded with ACC for 3 h and then incubated with various CO_2 concentrations for 20 min in the light, as detailed in Figure 2. Ethylene produced during the 20-min light incubation with CO_2 was determined.

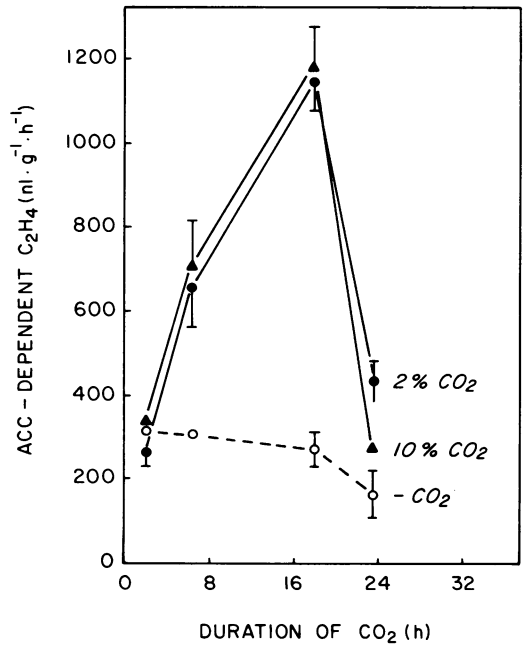

FIG. 4. Effect of duration of CO_2 treatment on development of the capability to convert ACC to ethylene in tobacco leaf discs. Samples of 8 tobacco discs (Havana 425) were preincubated in the dark with 1 ml of 10 mM Mes buffer under 0, 2, or 10% CO_2 for the indicated incubation periods, and then 5 mM ACC was injected into the sealed flasks. After incubation for 2 h with ACC, the flasks were flushed with air, resealed, and 10% CO_2 was reintroduced to all flasks. Ethylene produced during the following 30 min was determined.

after 20 h of incubation; this preincubation period was employed in subsequent experiments. It appears that CO_2 enhances the synthesis of EFE which increases during the initial 20 h of incubation but then decays. Since CO_2 did not affect significantly ACC uptake (data not shown), this decay of the enzyme can be ascribed either to senescence of the tissue or to the dark incubation. These possibilities were examined in subsequent experiments (see Fig. 7). EFE development, tested under optimal conditions in the dark, increased with increasing CO_2 concentrations and was saturated at 2% CO_2 (data not shown). Accordingly, Figure 4 shows the same enhancement of EFE development under 2% and 10% CO_2.

Further confirmation of the data which show that CO_2 enhances EFE development was obtained by employing CHI, a protein synthesis inhibitor. We applied immediately after excision, 20 μM CHI and 5 mM ACC to the discs, which were then incubated in the presence or absence of CO_2. Addition of CHI, as expected, markedly inhibited both the control and the CO_2-enhanced EFE development during the entire course of incubation (Fig. 5). However, similar to the results of Yu *et al.* (29), CHI hardly affected EFE development during the first 3-h incubation period, although the degree of inhibition increased subsequently with time. This indicates, as was proposed by Yu *et al.* (29), that EFE, which was present initially in an adequate quantity, turned over gradually (half-life of about 5 h), and CHI inhibited its replacement by synthesis. Consequently, the ACC-dependent ethylene production decreased with time. When CHI was applied with ACC to CO_2-pretreated discs after 24 h, only a small inhibition could be observed (data not shown), further confirming that *de novo* synthesis of EFE does not occur after the initial 24 h or dark incubation (Fig. 4).

Based on different incubation periods, we have established assay condition under which *in vivo* EFE activity and EFE development can be distinguished. Our data show that EFE activity is more affected by CO_2 than EFE synthesis, giving 9-fold (Figs. 2, 3) and 4-fold enhancement (Figs. 1, 4, 5), respectively. While 10% CO_2 was as effective as 2% CO_2 in enhancing EFE synthesis (Fig. 4), EFE activity tested under 10% CO_2, showed an additional increase over the activity assayed under 2% CO_2, in three different species (Figs. 2, 3).

Factors Affecting the CO_2-Enhanced EFE Development. The nature of the promotive effect of CO_2 on ethylene production in leaves is still unclear, although various mechanisms have been proposed (11, 13, 14, 20, 22). One possible factor involved in this process is ethylene. Similar to the CO_2-enhanced EFE synthesis which requires prolonged incubations (Fig. 4), the autocatalytic effect of ethylene in citrus leaf discs also involved increased EFE synthesis, which became evident following 24-h of incubation (23). Since CO_2 promotes ethylene production, it is plausible that CO_2 may promote EFE development *via* ethylene. The data presented in Figure 6 show that both CO_2 and ethylene could promote individually EFE development, while simultaneous application of both factors caused a further and significant increase as compared with the effect of CO_2 alone. Although differences between treatments were not large, results were reproducible. Hence, the CO_2-stimulatory effect cannot be entirely attributed to the ethylene effect. A synergistic interaction between ethylene and CO_2 has been reported previously for promotion of seed germination and breaking their dormancy (15, 16, 25).

Another important factor which affects the conversion of ACC to ethylene is light. Light inhibited ethylene production in leaves at the step of ACC conversion to ethylene only when photosynthesis depleted the CO_2 supply in the tissue segments (6, 9, 11, 14, 27), but once sufficient CO_2 was provided, light was not an inhibiting factor (3, 8, 11, 12, 14, 20). This further suggests that

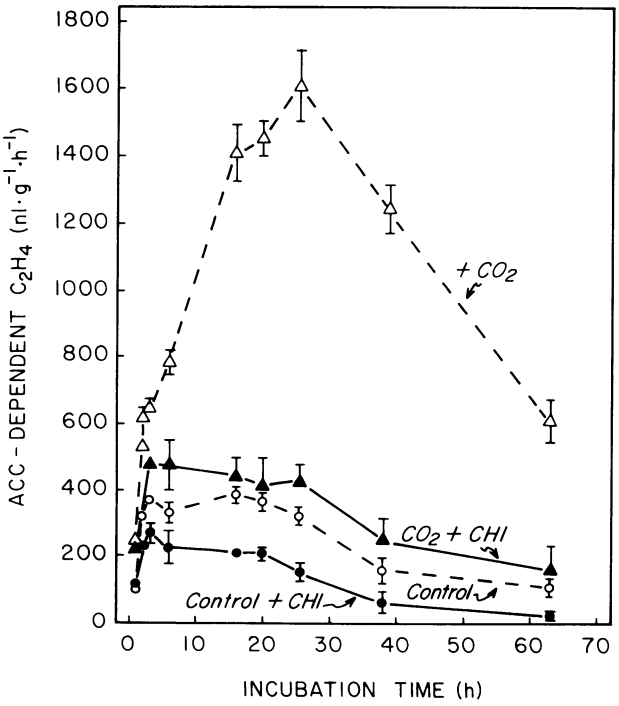

FIG. 5. Effect of CHI on CO_2-enhanced EFE development in tobacco leaf discs. Samples of 8 tobacco discs (Havana 425) were preincubated in the dark with 20 μM CHI where indicated and 5 mM ACC for 2 h. The flasks were then sealed and incubated in the presence or absence of 2% CO_2 for the indicated periods. Ethylene produced during the next 30 min under 10% CO_2 was assayed.

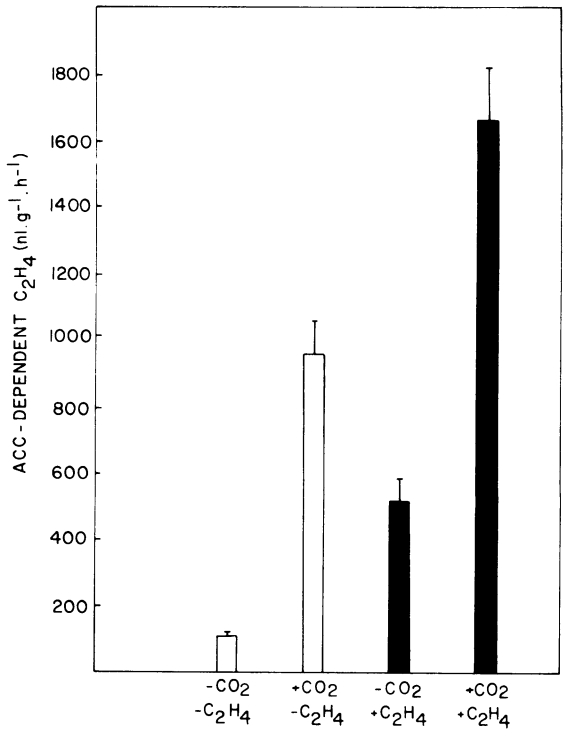

FIG. 6. Effects of ethylene and CO_2 on development of the capability to convert ACC to ethylene in tobacco leaf discs. Tobacco discs (Havana 425) were preincubated in the dark for 24 h with or without 10% CO_2 and with or without 20 μl/L ethylene. At the end of the incubation, 5 mM ACC was applied to all flasks. Ethylene produced during the next 1-h incubation with 10% CO_2 was determined.

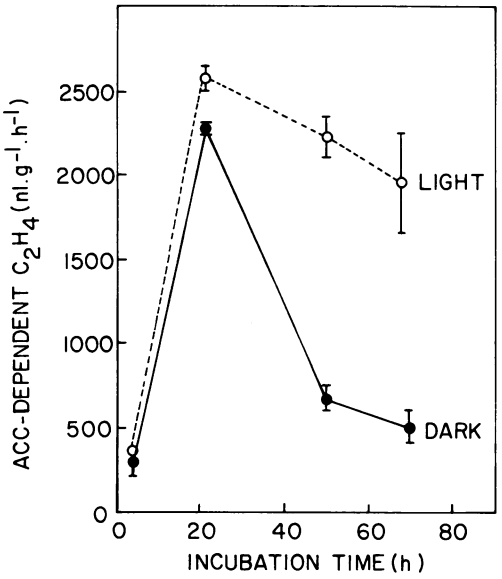

FIG. 7. Effect of light on CO_2-enhanced EFE development in tobacco leaf discs. Tobacco discs (Havana 425) were preincubated for the indicated periods in the light or in darkness under 10% CO_2 and with the continuous presence of ACC (5 mM initial concentration). Ethylene produced during the following 30 min of dark incubation under 10% CO_2 was determined.

CO_2 metabolism, rather than light per se, is the important factor regulating ethylene evolution (11, 28). Unlike most other investigations where the light effect on EFE activity was studied during short incubations (few hours) of excised tissues (6, 9, 12–15, 22), we have examined in our system the possible involvement of light in the CO_2-enhanced EFE development during prolonged incubations (several days). The results depicted in Figure 7 show that light under 10% CO_2 can maintain a fairly high and constant level of EFE throughout the entire course of incubation (70 h), while dark treatment (with 10% CO_2) results in a decay of the enzyme after 24 h incubation, as demonstrated before (Fig. 4). Since this experiment was performed with the continuous presence of ACC which was introduced at a concentration of 5 mM in the beginning of the incubation, it is possible that the decay of EFE in the dark at the later stages of incubation may be due to lack of ACC in the medium. To examine this possibility, fresh ACC was introduced at various incubation periods. Under these conditions a pattern similar to Figure 7 was obtained (data not shown), implying that senescence of the tissue rather than lack of ACC is the contributing factor. Since EFE is a membraneous enzyme (28), it is conceivable that part of the EFE decay may be ascribed to the tissue's senescence, which occurs more rapidly in the dark (2, 10, 24) and cannot be overcome by high CO_2. These results suggest that light can further increase both the level and duration of the CO_2-enhanced EFE synthesis, probably due to its senescence-retarding effect in green tissues (24).

In conclusion, the results presented herein show that in leaf discs CO_2 exerts its effect on ethylene biosynthesis by promoting both the synthesis and the activity of EFE, although the latter is more affected. Additionally, the promotive CO_2 effect on EFE synthesis can be maintained for a longer incubation period under light.

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LITERATURE CITED

1. AHARONI N, JM ANDERSON, M LIEBERMAN 1979 Production and action of ethylene in senescing leaf discs: effect of indoleacetic acid, kinetin, silver ion, and carbon dioxide. Plant Physiol 64: 805–809
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 1982 Effect of carbon dioxide and light on ethylene production in intact sunflower plants. Plant Physiol 69: 1222–1225
4. BASSI PK, MS SPENCER 1983 Does light inhibit ethylene production in leaves? Plant Physiol 73: 758–760
5. BASSI PK, MS SPENCER 1985 Early effects of carbon dioxide on 1-aminocyclopropane-1-carboxylic acid-induced ethylene production in intact tomato plants. Abstract in the 12th International Conference on Plant Growth Substances, Heidelberg, FRG, pp 37
6. DE LAAT AMM, DCC BRANDENBURG, LC VAN LOON 1981 The modulation of the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene by light. Planta 153: 193–200
7. DHAWAN KR, PK BASSI, MS SPENCER 1981 Effects of carbon dioxide on ethylene production and action in intact sunflower plants. Plant Physiol 68: 831–834
8. FUHRER J 1985 Production and release of ethylene from 1-aminocyclopropane-1-carboxylic acid in *Lemna minor* L. in the dark and at different carbon dioxide compensation concentrations. J Plant Physiol 117: 307–317
9. GEPSTEIN S, KV THIMANN 1980 The effect of light on the production of ethylene from 1-aminocyclopropane-1-carboxylic acid by leaves. Planta 149: 196–199
10. GEPSTEIN S, KV THIMANN 1981 The role of ethylene in the senescence of oat leaves. Plant Physiol 68: 349–354
11. GRODZINSKI B, I BOESEL, RF HORTON 1982 Ethylene release from leaves of *Xanthium strumarium* L. and *Zea mays* L. J Exp Bot 33:344–354
12. GRODZINSKI B, I BOESEL, RF HORTON 1983 Light stimulation of ethylene release from leaves of *Gomphrena globosa* L. Plant Physiol 71:588–593
13. HORTON RF, BJ SAVILLE 1984 Carbon dioxide enrichment, transpiration and 1-aminocyclopropane-1-carboxylic acid-dependent ethylene release from oat leaves. Plant Sci Lett 36:131–135
14. KAO CH, SF YANG 1982 Light inhibition of the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene in leaves is mediated through carbon dioxide. Planta 155: 261–266
15. KATOH H, Y ESASHI 1975 Dormancy and impotency of cocklebur seeds. I. CO_2, C_2H_4, and high temperature. Plant Cell Physiol 16: 687–696
16. KEYS RD, OE SMITH, J KUMAMOTO, JL LYON 1975 Effect of gibberellic acid, kinetin, and ethylene plus carbon dioxide on the thermodormancy of lettuce seeds (*Lactuca sativa* L. cv Mesa 659). Plant Physiol 56: 826–829
17. LIEBERMAN M 1979 Biosynthesis and action of ethylene. Annu Rev Plant Physiol 30: 533–591
18. LIZADA MCC, SF YANG 1979 A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal Biochem 100: 140–145
19. MCKEON TA, NE HOFFMAN, SF YANG 1982 The effect of plant hormone pretreatments on ethylene production and synthesis of 1-aminocyclopropane-1-carboxylic acid in water-stressed wheat leaves. Planta 155: 437–443
20. MCRAE DG, JA COKER, RL LEGGE, JE THOMPSON 1983 Biocarbonate/CO_2-facilitated conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene in model systems and intact tissues. Plant Physiol 73: 784–790
21. PHILOSOPH-HADAS S, S MEIR, N AHARONI 1985 Carbohydrates stimulate ethylene production in tobacco leaf discs. II. Sites of stimulation in the ethylene biosynthesis pathway. Plant Physiol 78: 139–143
22. PREGER R, S GEPSTEIN 1984 Carbon dioxide-independent and -dependent components of light inhibition of the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene in oat leaves. Physiol Plant 60: 187–191
23. RIOV J, SF YANG 1982 Effects of exogenous ethylene production in citrus leaf tissue. Plant Physiol 70: 136–141
24. THIMANN KV, RM TETLEY, BM KRIVAK 1977 Metabolism of oat leaves during senescence. V. Senescence in light. Plant Physiol 59: 448–454
25. TOOLE VK, WK BAILEY, EH TOOLE 1964 Factors influencing dormancy of peanut seeds. Plant Physiol 39: 822–831
26. TOPHOF S, N AMRHEIN 1985 Ethylene synthesis in isolated wheat mesophyll protoplasts. Abstract in 12th International Conference on Plant Growth Substances, Heidelberg, FRG, pp 36
27. WRIGHT STC 1981 The effect of light and dark periods on the production of ethylene from water-stressed wheat leaves. Planta 153: 172–180
28. YANG SF, NE HOFFMAN 1984 Ethylene biosynthesis and its regulation in higher plants. Annu Rev Plant Physiol 35: 155–189
29. YU YB, DO ADAMS, SF YANG 1979 Regulation of auxin-induced ethylene production in mungbean hypocotyls. Role of 1-aminocyclopropane-1-carboxylic acid. Plant Physiol 63: 589–590