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Light Stimulation of Ethylene Release from Leaves of *Gomphrena globosa* L.[1]

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ABSTRACT

The effect of light and CO_2 on both the endogenous and 1-aminocyclopropane-1-carboxylic acid (ACC)-dependent ethylene evolution from metabolically active detached leaves and leaf discs of *Gomphrena globosa* L. is reported. Treatment with varying concentrations of ACC did not appear to inhibit photosynthesis, respiration, or stomatal behavior. In all treatments, more ethylene was released into a closed flask from ACC-treated tissue, but the pattern of ethylene release with respect to light/dark/CO_2 treatments was the same.

Leaf tissue in the light with a source of CO_2 sufficient to maintain photosynthesis always generates 3 to 4 times more ethylene than tissue in the dark. Conversely, the lowest rate of ethylene release occurs when leaf tissue is illuminated and photosynthetic activity depletes the CO_2 to the compensation point. Ethylene release in the dark is also stimulated by CO_2 either added to the flask as bicarbonate or generated by dark respiration. Ethylene release increases dramatically and in parallel with photosynthesis at increasing light intensities in this C_4 plant. Ethylene release appears dependent on CO_2 both in the light and in the dark. Therefore, it is suggested that the important factor regulating the evolution of ethylene gas from leaves of *Gomphrena* may be CO_2 metabolism rather than light *per se*.

Recently, there has been a growing interest in ethylene metabolism in leaf tissue (4, 7, 8, 11, 13–17, 24). However, it appears that whereas there have been attempts to relate ethylene metabolism and action to the metabolism of the leaf in the light, the primary role of photosynthesis itself in regulating carbon flow within the tissue is often ignored. Ethylene is viewed in the literature as a key growth regulator (1), but it must always be remembered (14) that the amount of carbon flowing through ethylene pathways in leaves is very small (pmol carbon mg^{-1} Chl h^{-1}) compared with the flux of carbon through photosynthesis, photorespiration, and dark respiration pathways (μmol carbon mg^{-1} Chl h^{-1}). Several groups suggest that light inhibits ethylene production in leaves because the rate of ethylene release is less in the light than in the dark (7, 11, 24). We have shown that the conclusion that light is an inhibitor of ethylene production is inconsistent with data obtained when leaf tissue is illuminated and an attempt is made to maintain CO_2 levels within the experimental system above the compensation point during the time in which the ethylene determinations are made (14, 15). It is our contention that the major inhibitory effect on ethylene metabolism in leaves attributed to light is a low CO_2 effect mediated by the balance of photosynthetic and respiratory activity within the tissue (14). Effects of CO_2 on ethylene metabolism in plant tissues other than leaves are well documented (1, 6, 25). We are unaware of any data which show that in leaves it is only ethylene biosynthesis from precursors such as ACC[3] (25) which is markedly affected by light and/or CO_2 *in vivo*. Therefore, we argue that at this stage of our understanding of ethylene metabolism a broader working hypothesis of possible control mechanisms of CO_2 (and light) on ethylene metabolism and action in leaf tissue has relevance (14). The availability of CO_2 within the tissue might influence both the processes leading to the synthesis of ethylene and the processes of retention, metabolism, and further action of ethylene (14, 15).

Over the last 30 years, plant physiologists have come to recognize the existence of several pathways by which CO_2 is assimilated in leaves. The major photosynthetic groups (C_3, C_4, and CAM plants) represent, in our view, an unusual opportunity for studying the interactions of light and CO_2 on ethylene metabolism without the use of exogenously applied inhibitors whose specificity *in vivo* must always be an issue of concern (18). One current theory relating to C_4 metabolism suggests that the internal decarboxylation of C_4 acids generated in the light maintain high internal levels of CO_2, a process which contributes to the operation of ribulosebisphosphate carboxylase-oxygenase as the carboxylase (18, 26). In all the studies in which it was concluded that light inhibits ethylene production, only C_3 plants were examined (7, 8, 11, 24). The present study on *Gomphrena globosa* was undertaken to show how light and CO_2 interact to control the amount of ethylene released from photosynthetically active leaves and leaf discs of a dicotyledonous C_4 plant (21). A preliminary report of these studies was presented recently (15).

MATERIALS AND METHODS

Plant Material. Seeds of Globe Amaranth, *Gomphrena globosa* L., purchased from Stokes Seeds, St. Catharines, Ontario, were germinated in soil. Seedlings were transferred to 20-cm standard pots and grown under 18-h photoperiods in a greenhouse. Mature, recently expanded leaves were used as the starting material in all experiments.

Chemicals. ACC, Hepes, Tris, Bicine, Taps, and triethanolamine were purchased from the Sigma Chemical Co. Other chemicals obtained from Fisher Scientific were of the highest purity available.

Experimental. In experiments using leaf discs, 7-mm discs were cut from several leaves, washed in distilled H_2O, and distributed randomly between treatments. Unless otherwise specified, 20 discs

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[3] Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; Taps, Tris(hydroxymethyl)methylaminopropane sulfonic acid.

were maintained on a 2-ml test incubation medium in 25-ml Erlenmeyer flasks sealed with a rubber injection cap. The flasks were maintained at 25°C in a glass-bottomed waterbath, shaken at a rate of 90 strokes/min. For light treatments, flasks were illuminated from below by a bank of General Electric 30-w reflector incandescent lamps at a photon fluence of 170 μmol m^{-2} s^{-1} (400–700 nm) as measured with a Li-Cor LI 185 light meter with a quantum response probe held at tissue level within the flask. Flasks containing the dark control were covered in opaque plastic tape and wrapped in aluminum foil. When the effects of varying light levels were tested, neutral plastic screening was taped to the outside of the flask.

Estimation of Ethylene Release by Leaf Tissue. Ethylene levels in the flasks were measured as described previously (14). At the required time intervals, 500-μl gas samples were taken from the flasks and injected onto a 183- × 0.3-cm stainless steel column containing Alumina F (80–100 mesh) in a Varian 3700 gas chromatograph at 110°C with a flame ionization detector. Injections of known ethylene standards were used to calibrate retention times and peak heights for the gas samples. In these experiments, careful monitoring revealed no spurious ethylene generation by any components of the experimental system. Ethylene release from entire leaves of *Gomphrena* was determined in 25-ml Erlenmeyer flasks sealed with rubber injection caps. Three leaves, with their petioles in 2 ml of a 0.5 mM ACC solution were sealed in each flask. In order to assess the effects of wounding on ethylene release, blades of intact leaves were lacerated at approximately 1-mm intervals between the main veins. The ethylene release from these lacerated leaves was measured as described above.

Estimation of Net Photosynthesis, Respiration, and Transpiration. The effect of ACC on photosynthesis and respiration in *Gomphrena* leaf tissue was measured using two different methods for estimating gas exchange. In experiments on 7-mm leaf discs, bicarbonate-dependent O_2-production (*i.e.* net photosynthesis) was measured manometrically in a Gilson Differential Respirometer. Groups of 20 distilled H_2O-washed leaf discs were incubated at 25°C in 2 ml Warburg buffer (200 mM Na_2CO_3-$NaHCO_3$; pH 9.1) for 1 to 2 h in the light after an initial 20 min preincubation in darkness. Dark respiration rates were measured in the same buffer.

In experiments with detached whole leaves, photosynthesis, respiration, and transpiration rates were measured using an open circuit air-flow system described in detail elsewhere (19). Typically, a maximum of 4.7 L min^{-1} of humidified air at a slight positive pressure was supplied to a temperature-controlled (25°C) leaf chamber. The leaf was held horizontally in the chamber with the lamina surface uniformly exposed to the light source using a grid of fine nylon threads. The petiole was immersed in water containing various concentrations of ACC. The leaf chamber was illuminated by a 400-w high-pressure sodium discharge Lucolux lamp mounted in a commercial ballast reflector. The light intensity was monitored by Li-Cor LI 190S quantum sensor placed inside the leaf chamber and recorded with a Li-Cor LI 185 light meter. Measurements were made at 0, 170, and 1,020 μmol m^{-2} s^{-1}. The CO_2 exchange was measured using a differential IR gas analyzer (Beckman model 865). The dewpoint of the simple air stream was recorded by a digital dewpoint hygrometer (DM, EG, and G model 911) before entering the IR gas analyzer. The CO_2 concentrations, leaf temperature, and dewpoints were recorded on a strip chart. Leaf area was determined using an area meter (model LI 300, Lambda Instruments Co.) fitted with a LI 3050A transparent belt accessory and calibrated to 0.01 cm^2 by the manufacturer.

Chl content of the leaf tissue was determined by the method of Arnon (3).

RESULTS AND DISCUSSION

Effects of Light and Dark Periods on Ethylene Release. The effect of light and darkness on the release of ethylene from ACC by leaf discs of *G. globosa*, a dicotyledonous C_4 plant (21), is shown in Figure 1. More ethylene is released into a sealed flask during the first 90-min light period when 200 mM sodium bicarbonate is added as an exogenous source of CO_2. This stimulation of ethylene release by CO_2 and light is similar to that reported previously (13, 14) in closed systems with tissue from *Ranunculus sceleratus* and *Xanthium strumarium* (both dicotyledonous C_3 plants) and *Zea mays* (a monocotyledonous C_4 plant) and for a flow-through system containing intact plants of *Helianthus* (a dicotyledonous C_3 plant) where raising the CO_2 levels from 1% in an airstream also resulted in a marked increase in ethylene release (8). The data in Figure 1 also reveal differences between the amounts of ethylene detected during illumination compared to the amount observed when the tissue is in the dark. The patterns of ethylene release observed during the light-dark-light transients are clearly dependent on the presence or absence of bicarbonate.

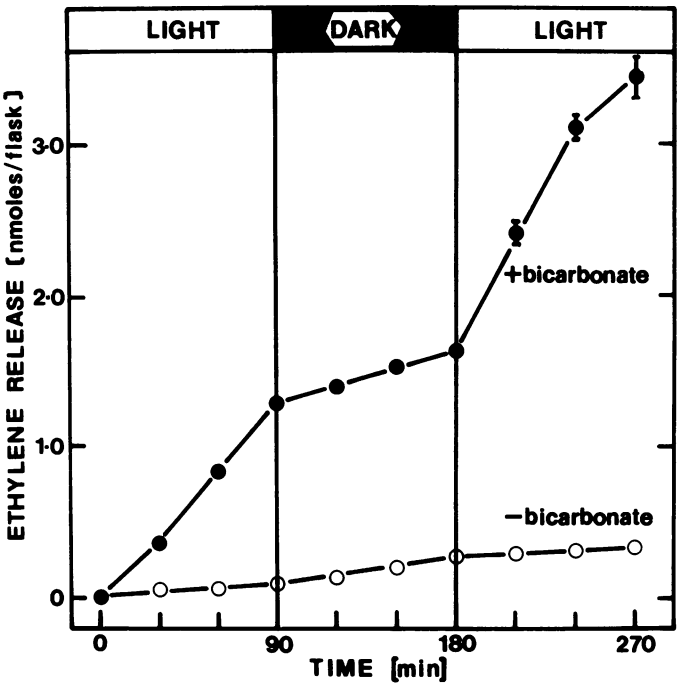

FIG. 1. Time course of ethylene release during subsequent light and dark periods from leaf discs of *Gomphrena* incubated in 0.5 mM ACC in the presence (●) and absence (○) of 200 mM $NaHCO_3$. The photon fluence (PAR) during the light periods is 170 μmol m^{-2} s^{-1}. Each point represents the mean of duplicated ethylene samples from triplicated biological samples with the range indicated. The data presented is from a single experiment which was repeated several times with similar results.

Without added bicarbonate, photosynthesis in the *Gomphrena* leaf tissue illuminated at 170 μmol m^{-2} s^{-1} rapidly depleted the CO_2 levels in the sealed flasks to approximately 10 μl/l (the CO_2 compensation point). At this low CO_2 level, the rate of ethylene release during both the initial and final 90-min light periods was lower than that during the interposed 90-min dark period (Fig. 1). However, in the presence of added bicarbonate, when photosynthesis does not deplete the CO_2 level to the compensation point, the rate of ethylene release from illuminated tissue was 3 to 4 times greater than that occurring in the dark (Fig. 1).

Effect of pH and Buffers on ACC-Dependent Ethylene Release from Leaf Discs. In the experiments reported in Figure 1, an incubation mixture which was not artificially buffered was used. The incubation mixture, without bicarbonate, had a final pH of 8.3 ± 0.2, whereas in the presence of bicarbonate the pH was 9.1 ± 0.2. Because of the possible significance of the effects of pH of the incubation medium on (a) the amount of available carbon (*i.e.* CO_2 or HCO_3^-), (b) the uptake and conversion of ACC to

Table I. *Effect of Using Different Buffers of Varying pH on ACC-Dependent Ethylene Release (0.5 mM ACC) from Leaf Discs*

The leaf discs were incubated in the light or in the dark for 2 h at 25°C in the presence or absence of 200 mM $NaHCO_3$

Incubation Medium	Ethylene Release[a]				
	Light		Dark		
	+ $NaHCO_3$	− $NaHCO_3$	+ $NaHCO_3$	− $NaHCO_3$	− $NaHCO_3$ (+KOH)
	% of control				
H_2O	100	10.2	35.2	14.9	11.2
200 mM Warburg buffer[b]	46.2	—	18.2	—	—
200 mM Taps					
pH 9.0	21.2	7.2	8.5	8.9	7.1
pH 8.5	25.9	5.7	11.1	7.5	6.5
pH 8.2	26.5	7.3	8.4	8.4	7.1
200 mM Hepes					
pH 8.2	30.0	7.4	10.3	6.2	5.2
pH 7.2	22.8	8.9	8.9	5.9	—
200 mM K_2HPO_4-K_2HPO_4					
pH 8.2	12.9	1.7	1.7	1.9	1.6
pH 7.2	14.8	4.1	2.9	3.9	3.6
200 mM Triethanolamine, pH 8.5	19.6	7.0	4.0	2.5	—
200 mM Tris, pH 8.5	23.2	5.8	13.6	9.4	7.3
200 mM Bicine, pH 8.5	18.9	4.9	10.0	8.0	7.1

[a] Ethylene release is expressed as per cent of the ethylene release from the H_2O control.
[b] Warburg buffer, 200 mM $NaHCO_3$-Na_2CO_3, pH 9.1.

ethylene, and (c) the retention and further metabolism of ethylene gas, a series of experiments employing a variety of buffers was carried out using *Gomphrena* leaf discs (Table I). Incubation of leaf discs in buffers of sufficient strength to maintain pH at values between 7 and 9 caused inhibition of measured ethylene release (Table I). This effect was seen when buffers were employed in the light or dark, and in the presence and absence of added bicarbonate. However, it is important to note that despite the apparent inhibition of ethylene release by buffers, the pattern of ethylene release in relation to light, dark, and CO_2 availability shown in Figure 1 was still evident. Ethylene release in the presence of added CO_2 was higher in the light than in the dark, but all dark treatments except those in phosphate buffer (pH 8.2) and triethanolamine (pH 8.5) (Table I), resulted in higher levels of ethylene release than those occurring in the light at low CO_2 levels. Phosphate and other buffers inhibited the endogenous release (data not shown) of ethylene from *Gomphrena* leaf tissue, as well as the ACC-dependent ethylene release (Table I). The reason behind the general inhibition of ethylene from *Gomphrena* leaf discs by the test buffers is not clear. These observations are, however, consistent with those of Fuchs *et al.* (10) who reported that phosphate inhibits ACC-dependent ethylene production in a variety of fruit and vegetative tissues.

Although we have been unable to define fully an ideal buffer system for these experiments, the fact that the light/CO_2 control of ethylene release is evident (a) at a range of pH values in a variety of buffers (Table I; Fig. 3), (b) in intact leaves of *Gomphrena* (Table III) and *Xanthium* (14), and (c) in intact plants of *Helianthus* maintained in airstreams enriched with CO_2 (4, 8), strongly suggests that we are not simply dealing with an artifact of the experimental system. The level of available CO_2 appears to be a critical factor regulating ethylene release both in the light and in the dark. In the experiments reported in Figure 1 and Table I, the overall CO_2 level in the tissue is obviously coupled to the CO_2 availability from added bicarbonate. However, CO_2 will also be controlled by the metabolic activity of the tissue. In the light, photosynthetic depletion of CO_2 might regulate the release of ethylene. At least three treatment conditions which alter the CO_2 level in the dark need to be considered as well (Table I). Discs incubated in the dark without added bicarbonate released approximately 30% less ethylene than discs incubated with bicarbonate (Table I; Fig. 1). Furthermore, when discs were incubated in the dark, without bicarbonate, with a center well containing 20% KOH to trap any CO_2 evolved from respiratory processes, even less ethylene was evolved. The observation that the removal of respiratory CO_2 in the dark resulted in a further decline in ethylene release is consistent with the view that it is CO_2 metabolism rather than light *per se* which regulates ethylene release from ACC in *Gomphrena* leaf discs.

Use of Leaf Discs to Study ACC-Dependent Ethylene Release. Leaf discs provide a uniform, metabolically active tissue sample in which to study ethylene metabolism in leaf tissue (2, 7, 11, 14). Leaf discs floated on ACC, the ethylene precursor (25), generate more ethylene than untreated discs (Table II). However, these discs also generate about 10 times more ethylene (per g fresh weight of tissue) than whole detached leaves fed ACC via the

Table II. *Effect of Varying Concentration of Exogenously Supplied ACC on the Pattern of Ethylene Release from Leaf Discs of Gomphrena*

Concentration of ACC	Ethylene Release			
	Light		Dark	
	+ $NaHCO_3$	− $NaHCO_3$	+ $NaHCO_3$	− $NaHCO_3$
M	*pmol flask*$^{-1}$ *h*$^{-1}$			
0	5	0	3	3
5×10^{-6}	36	8	16	15
5×10^{-5}	196	22	56	40
5×10^{-4}	1,278	74	578	98
5×10^{-3}	5,838	217	1,850	156
5×10^{-2}	41,114	593	10,897	203

transpiration stream (data not shown). We have attempted to determine whether the high level of ACC-dependent release in discs was due (a) to wounding which can stimulate ethylene production (27) during the preparation of the discs, or (b) merely reflects enhanced ACC availability to ethylene-generating sites in discs. Detached whole leaves and severely lacerated leaves of *Gomphrena* exhibit a very similar ethylene release pattern and a similar degree of ACC-dependent ethylene release in a varety of light and CO_2 treatments (data not shown) suggesting that wounding the leaf blade by cutting is not a major factor in these experiments. A similar conclusion was drawn from studies with *Xanthium* leaf tissue (14). The rates of ethylene release from whole leaf samples and leaf discs are comparable in the absence of ACC (*i.e.* endogenous ethylene production; Table II). Without added ACC, the pattern of ethylene release from *Gomphrena* is the same as that observed with ACC up to a concentration of 5×10^{-4} M (Table II: the rate in the light plus CO_2 > dark plus CO_2 > dark minus CO_2 > light minus CO_2). More ethylene is released per treatment when more ACC is added. This observation is similar to that in tobacco leaf discs (7) and in discs of apple fruit (2). At concentrations tested above 5×10^{-4} M ACC, the pattern of ethylene is disrupted (perhaps because of high acidity of the medium); but, at all concentrations of ACC, the greatest rate of ethylene release is still observed in the light plus bicarbonate treatment.

Effect of ACC and Ethylene on CO_2 Metabolism in Leaves. The use of exogenous ACC may indirectly alter ethylene production by influencing photosynthetic and respiratory activity of the tissue. Changes in stomatal aperture during light and dark treatments could be important. The data in Figure 2 show the effect of various concentrations of ACC, from 5×10^{-7} to 5×10^{-1} M, on photosynthetic activity in *Gomphrena* leaf discs incubated in 200 mM Warburg buffer (pH 9.1). The rates of bicarbonate-dependent

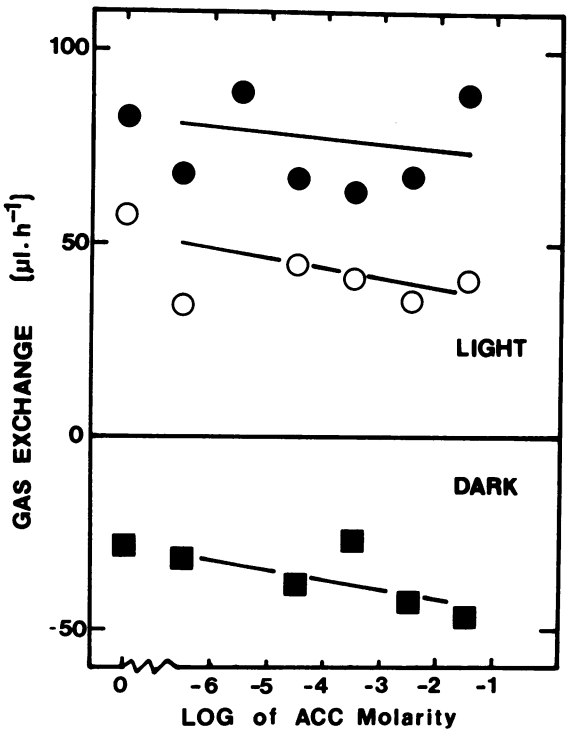

FIG. 2. Effect of varying concentration of exogenously supplied ACC on net photosynthesis and dark respiration of leaf discs of *Gomphrena*. The data represent gas exchange from leaf discs measured manometrically at two light intensities, 170 µmol m^{-2} s^{-1} (●) and 30 µmol m^{-2} s^{-1} (○), and in darkness (■). In all instances, 200 mM Warburg buffer (pH 9.1) was used. Each point represents the mean of duplicate samples from one experiment. The experiment was repeated three times with similar results.

Table III. *Effect of ACC on Photosynthesis, Respiration, Diffusive Resistance, and Transpiration in Whole Detached Leaves of Gomphrena*

Detached whole leaves were treated with ACC at 25°C for 30 min by immersing the petioles in either 0.5 or 5.0 mM ACC solution, pH 7.0.

	Respiration[a]	Net Photosynthesis[a]	Diffusive Resistance[a]	Transpiration[a]	Ethylene Release[b]
	mg CO_2 m^{-2} s^{-1}		*s cm^{-1}*	*mg cm^{-3} s^{-1}*	*pmol mg^{-1}Chl h^{-1}*
Control	41 ± 6	242 ± 3.5	14.2 ± 1.3	1.16 ± 0.16	23 ± 9
Treated					
0.5 mM ACC	36 ± 8	253 ± 2.6	15.2 ± 2.1	0.92 ± 0.13	142 ± 28
5.0 mM ACC	47 ± 11	223 ± 4.1	17.3 ± 3.4	1.08 ± 0.21	216 ± 31

[a] Measurements of respiration, net photosynthesis, diffusive resistance, and transpiration were made after 30 min in an open gas flow system.

[b] Estimations of ethylene release (in the light, plus bicarbonate) were made from tissues sealed in Erlenmeyer flasks.

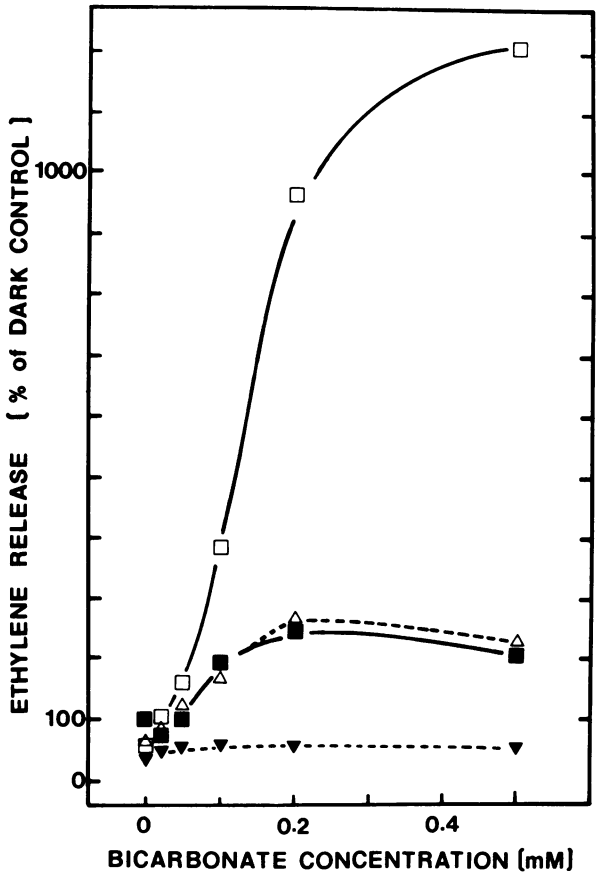

FIG. 3. Effect of different concentrations of added bicarbonate on ethylene release from *Gomphrena* leaf discs in light and darkness. In one set of flasks, discs were incubated in 0.5 mM ACC and no attempt was made to buffer the solution in either the light (□) or dark (■) treatments. In the second set of flasks, discs were incubated in either light (△) or darkness (▼); however, the incubation medium contained 200 mM Hepes (pH 8.2) in addition to the 0.5 mM ACC and the required amount of amounts of $NaHCO_3$.

O_2 evolution were determined at light intensities of 170 µmol m^{-2} s^{-1}, which is saturating for this system, and at 30 µmol m^{-2} s^{-1}, which is a subsaturation light intensity (Fig. 4). There was no significant effect of ACC on photosynthesis at either light intensity (Fig. 2). Although there is some indication that ACC causes enhanced O_2 consumption in both the light and the dark (perhaps

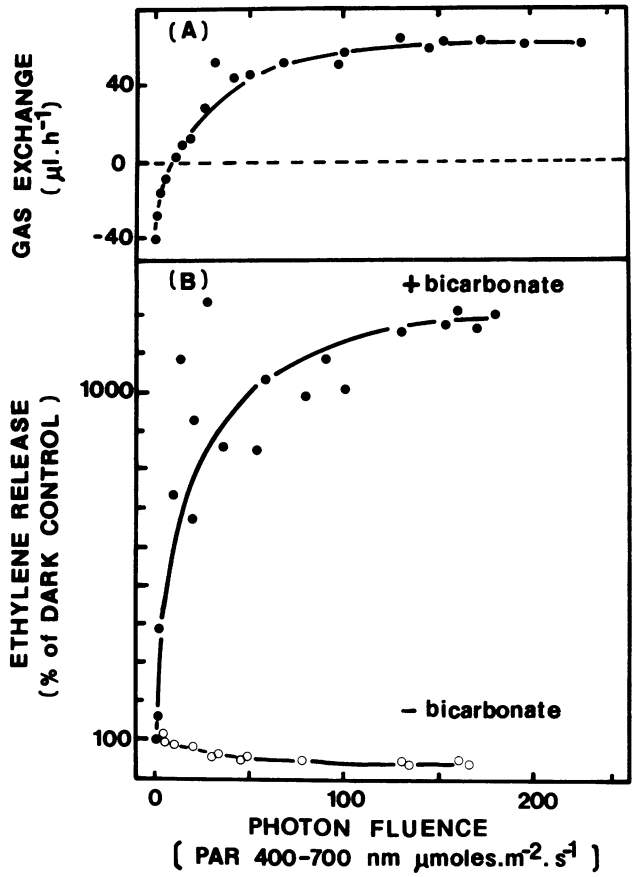

FIG. 4. Photosynthesis and ethylene release from leaf discs of *G. globosa* L. incubated at different light intensities. A, Bicarbonate-dependent O_2 evolution. B, Ethylene release from 0.5 mM ACC in the presence (+ bicarbonate) and absence (– bicarbonate) of an added CO_2 source (200 mM $NaHCO_3$). Each point in A represents the data from a single Warburg flask. Each point in B represents the mean of duplicate ethylene samples from duplicate biological samples performed in 1 d. The experiments outlined in both A and B were repeated several times with very similar results.

Table IV. *Effect of Silver Nitrate on Ethylene Release from Leaf Discs of Gomphrena Incubated in Either the Light or the Dark*

The leaf discs were incubated at 25°C in the presence or absence of 200 mM $NaHCO_3$ with 200 mM Hepes (pH 8.2) and 0.5 M ACC.

Treatment	Ethylene Release			
	Light		Dark	
	+ $NaHCO_3$	– $NaHCO_3$	+ $NaHCO_3$	– $NaHCO_3$
	pmol disc^{-1} *h*$^{-1}$			
Control	44.7 ± 3.4	2.8 ± 0.2	15.6 ± 2.0	3.4 ± 0.2
0.1 mM $AgNO_3$	200.5 ± 26.9	9.5 ± 1.7	30.8 ± 6.8	3.1 ± 0.2

by acting as a respiratory substrate at high concentrations), ACC does not appear to alter dark respiration rates significantly (Fig. 2). In addition, we were unable to find by microscopic examination any significant changes in stomatal aperature in leaf discs incubated under the various light and CO_2 regimes used in these studies. To further assess the effect of ACC treatment on *Gomphrena* leaf tissue, whole detached leaves were placed in open flasks in the light with their petioles immersed in water, 0.5, or 5.0 mM ACC solutions (Table III). The treatment with ACC does not affect the dark respiration rate, the photosynthetic rate, the diffusive resistance, nor the transpiration rate. However, when the same leaves were placed in sealed flasks, it is evident that ACC treatment dramatically promotes ethylene evolution and that the effect is most marked at the higher concentration of ACC (Table III). Whereas there remains a clear need for more information on ethylene flux from leaf tissue in relation to changes in stomatal aperture (17), our studies with both leaves and leaf discs of *G. globosa* provide no evidence of changes in stomatal aperture occurring while ethylene evolution rates are altered. It was previously shown that light and CO_2 can influence ethylene evolution from *Marchantia polymorpha* (23), a liverwort with no functioning stomatal structures.

Effect of Light Intensity and CO_2 Concentration on Ethylene Release. If, as we suggest, CO_2 metabolism is the key factor regulating ethylene release from *Gomphrena* leaf tissue, high levels of available CO_2 in the light should stimulate the release of ethylene. When concentrations of 50 mM sodium bicarbonate and above are added to the incubation medium, the light effect of promotion of ethylene release is clearly demonstrated (Fig. 3). When the incubation medium is buffered at pH 8.2 with Hepes, in an attempt to mitigate any effects of pH shift on the system due to addition of bicarbonate, the buffer severely depresses the rates of ethylene release (Table I). However, the data in Figure 3 demonstrated clearly that the light/dark differences in ethylene release from *Gomphrena* tissue are still evident at bicarbonate concentrations above 50 mM.

The relationship between light intensity, photosynthetic activity, and ethylene release in leaf discs of *Gomphrena* is shown in Figure 4. An increase in irradiance causes a marked increase in the rate of photosynthesis, measured as bicarbonate-dependent O_2 evolution (Fig. 4A). Increased light intensity also causes a marked increase in the rate of bicarbonate-dependent ethylene evolution (Fig. 4B), which parallels the increase in photosynthetic activity. Both the photosynthesis and the ethylene release processes are saturated at a photon fluence of about 100 μmol m^{-2} s^{-1}. The results in Figure 4 are consistent with the view that ethylene release in the light is dependent on the photosynthetic activity.

In the absence of added bicarbonate at high light intensities, ethylene release from *Gomphrena* leaf tissue is low (Fig. 4B). When irradiance is sufficient to maintain significant photosynthetic activity (Fig. 4A), the CO_2 level in the flask will eventually be depleted to CO_2 compensation point for this C_4 tissue. This evidently occurs rapidly (about 20 min) when bicarbonate is not added. In the absence of added bicarbonate in the light, O_2 is not rapidly evolved and, in fact, some is consumed during photorespiration (12, 18). Of concern is the knowledge that the conversion of ACC to ethylene in plant tissue is inhibited at low O_2 concentrations (6, 9, 25). Although O_2 may be utilized when ACC is supplied exogenously (Fig. 2), 0.5 mM ACC does not affect photosynthetic O_2 evolution or respiratory O_2 uptake in *Gomphrena* leaf tissue (Table III). Ethylene release from discs in the light without added bicarbonate is lower than that occurring in the dark control (Fig. 4B). It is, however, unlikely that O_2 levels in the flasks are sufficiently depleted (9) to account for the differences in the rates of ethylene release shown in Figure 4. It should be noted that in C_3 plants, such as *Helianthus*, ethylene release into a CO_2-enriched air stream is high in the light (8), and in *Xanthium* (14), O_2 depletion due to dark respiration allows a similar rate of ethylene release to that occurring in the light during photosynthetic O_2-generation.

Use of Inhibitors. A number of compounds have been shown to inhibit ethylene metabolism in plant tissue (1, 2, 6–8, 10, 11, 25). In recent years, some have been ascribed roles at specific sites within the biosynthetic pathway of the gas (1, 2, 7, 10, 11, 25) or at sites of its physiological action (5, 6, 8, 9). Silver ions, which have been shown to be potent antagonists of ethylene action and may inhibit further ethylene metabolism within the tissue (5, 6, 25), cause a dramatic enhancement of the ACC-dependent eth-

ylene release from *Gomphrena* leaf discs in the light in the presence of added CO_2 (Table IV). Although this suggests that possible retention sites are blocked by silver, data of this type should be interpreted with caution. As Lorimer (18) points out, specificity of inhibitors in biological systems is always in doubt. In *Gomphrena* leaf tissue, silver nitrate at these concentrations also inhibits dark respiration by 20% and enhances apparent photosynthesis by 15%. Although these effects appear minor, it is important to note that the flux of carbon through ethylene metabolism is small relative to that through these other pathways (14). Clearly, work on putative inhibitors of ethylene metabolism in leaves can best be interpreted at such time as the demonstrated light/CO_2 controls are also fully understood.

CONCLUSIONS

As pointed out above, a number of investigators have reported that light inhibits ethylene production by leaves (7, 8, 11, 24); however, these observations were apparently made on systems in which no clear attempt was made to maintain the CO_2 levels in the reaction vessels during the course of the experiments. When an external source of CO_2 is available in the light, the rate of ethylene release from C_3 plants, such as *Xanthium* (14), approaches that observed in the dark, either in the presence or absence of added ACC. *Helianthus* leaves studied in a flow-through system show similar results (4, 8). Thus, 'inhibition' of ethylene release in the light is more apparent when photosynthesis is depleting the CO_2 supply. However, we have noted that in *Zea*, a monocotyledonous C_4 plant, ethylene release is stimulated in the light when photosynthetic activity is maintained (14). Most of the earlier observations leading to the view that light is an inhibitor were made using leaf tissue from C_3 plants alone. In the present study, *Gomphrena*, a dicotyledonous C_4 plant, is used; and again, as in corn, light stimulates ethylene release compared to the rate in the dark.

Our view that the metabolism, exchange, and action of ethylene gas in leaves can best be understood in terms of the major fluxes of carbon underscores the view of Trewavas (22) that it is imperative to develop an understanding of the role of any major growth regulator in terms of the total metabolism of the plant.

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