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Enhancement of Ethylene Release from Leaf Tissue during Glycolate Decarboxylation[1]

A POSSIBLE ROLE FOR PHOTORESPIRATION

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ABSTRACT

When leaf discs of *Xanthium strumarium* L. and *Salvia splendens* L. are incubated in sealed flasks in the light, more C_2H_4 gas is released in the presence of added CO_2 (30–200 millimolar $NaHCO_3$) than without CO_2. In *Salvia*, the maximum rate of C_2H_4 release occurs when sufficient CO_2 (above 125 millimolar $NaHCO_3$) is added to saturate photosynthesis confirming previous studies. The maximum rate of C_2H_4 release from illuminated discs is similar to the rate in the dark with or without CO_2 in both species. Glycolate enhances a CO_2-dependent C_2H_4 evolution from illuminated leaf discs. However, the maximum rate of C_2H_4 release with glycolate is the same as that observed with saturating CO_2. When photosynthesis is inhibited by darkness or by 3-(3,4-dichlorophenyl)-1,1-dimethylurea, glycolate has no effect.

Studies with [2,3-^{14}C]-1-aminocyclopropane-1-carboxylic acid (ACC) show that the pattern of C_2H_4 release and the specific activity of the $^{14}C_2H_4$ in the presence and absence of glycolate is similar to that described above, indicating that glycolate does not alter uptake of the exogenously supplied precursor (ACC) or stimulate C_2H_4 release from an endogenous source at appreciable rates. Glycolate oxidase *in vitro* generates H_2O_2 which stimulates a slow breakdown of ACC to C_2H_4, but since exogenous glycolate is oxidized to CO_2 in both the light and the dark it is argued that the glycolate-dependent increase in C_2H_4 release from illuminated leaf discs is not mediated directly by the action of enzymes of glycolate catabolism. The effects of glycolate and CO_2 are not easily explained by changes in stomatal resistance. The data support the view that glycolate decarboxylation at subsaturating levels of CO_2 in the light stimulates C_2H_4 release by raising the CO_2 level in the tissue.

Recent studies from our laboratory with both C_3 and C_4 species indicate that the amount of carbon released in the light in the form of C_2H_4 gas is minute compared with either the rates of photosynthetic CO_2 assimilation or CO_2 losses from dark respiration or during photorespiration (8–10). However, these investigations have shown that the amount of C_2H_4 generated by photosynthetic tissue can be controlled by the availability of CO_2. The relationship between CO_2 levels and C_2H_4 production is an important consideration when leaf tissue is placed in a sealed flask in order to allow for the production and accumulation of sufficient C_2H_4 in the headspace of the flask for accurate assay by current gas chromatographic techniques. In all of the C_3 and C_4 leaf tissues which we have studied, C_2H_4 release by the tissue is lowest in the light when the tissue is allowed to deplete the CO_2 level from 330 ppm (ambient) to the compensation point. In C_3 plants, light and dark rates of C_2H_4 release are similar when CO_2 is available for photosynthesis. In C_4 plants when CO_2 is available, C_2H_4 release is higher in the light than in the dark (8–10). In C_4 plants, the internal CO_2 level may be raised 'naturally' in the light as a result of internal decarboxylation reactions. In our view (8, 10), failure to maintain CO_2 levels around photosynthetic tissue has resulted in several erroneous reports that light is an inhibitor of C_2H_4 production.

A sealed reaction flask creates an environment in which gas exchange is restricted. During the time period of an experiment (30–120 min), CO_2 levels tend to approach the CO_2 compensation point. Although CO_2 can clearly become a limiting factor in C_2H_4 production (8–10, 14) it must also be realized that at low CO_2 concentrations glycolate metabolism (i.e. photorespiration) becomes a more important reaction sequence relative to net photosynthesis (20, 23, 26). Nothing is known about the way glycolate metabolism itself might affect C_2H_4. Considering that the lowest rates of C_2H_4 release from illuminated tissue are observed near the compensation point where photosynthesis is balanced by photorespiration, a study was undertaken to explore the relationship between ACC[2]-dependent C_2H_4 production and glycolate metabolism.

MATERIALS AND METHODS

Plant Material. Seeds of *Salvia splendens* L. cv St. John's Fire purchased from Stokes Seed, St. Catharines, Ontario, Canada were germinated in soil. Seedlings were transferred to 15-cm standard pots and grown in a greenhouse. Mature recently expanded leaves were used. Fully expanded leaves of *Xanthium strumarium* L., which were grown as described previously (8), were also used.

Chemicals. ACC, Epps, FMN were purchased from Sigma Chemical Co. The radiolabeled compounds used in these studies included Na-[1-^{14}C]glycolate, Na-[1-^{14}C]glycine, $NaH^{14}CO_3$, and [2,3-^{14}C]ACC, the last being a custom synthesis, and were purchased from New England Nuclear. Unless otherwise specified, the other chemicals were of the highest purity available from Fisher Scientific, Toronto, Ontario, Canada.

Estimation of C_2H_4 Release from Leaf Discs. The release of C_2H_4 was routinely determined using 7-mm discs cut from the interveinal regions of several leaves, washed in distilled H_2O, and distributed randomly between treatments in a manner similar to

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[2] Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; Epps, 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid.

that described previously (8, 10). A typical flask contained 15 discs floated on a 2-ml test incubation medium, containing 200 mM Epps-NaOH buffer (pH 8.2), 50 mM $NaHCO_3$, 0.5 mM ACC, and 10 mM Na-glycolate in a 25-ml Micro-Fernbach flask sealed with a rubber serum cap. The flasks were illuminated (170 μmol m^{-2} s^{-1} [PAR 400–700 nm]) from below a glass-bottomed water bath held at 25°C. Flasks were shaken at a rate of 90 strokes min^{-1}. Ethylene was assayed as described previously (8).

Uptake of [2,3-^{14}C]ACC and Release of $^{14}C_2H_4$. The uptake of exogenously supplied ^{14}C-radiolabeled ACC and its conversion to $^{14}C_2H_4$ in leaf discs were determined in a manner similar to that of Woodrow (24). Leaf discs were incubated as described above except that 1.0 μCi [2,3-^{14}C]ACC (22.90 mCi $mmol^{-1}$) was added to the incubation mixture containing 0.5 mM unlabeled ACC, thus giving a final specific activity of 0.5 μCi μmol^{-1} ACC. The leaf discs were incubated on this solution for 2 h and uptake was stopped by rinsing quickly with two changes of reaction mixture (without [^{14}C]ACC) and extracting twice in 2 ml boiling 80% ethanol. The radioactivity of the ethanol-soluble fraction was determined by liquid scintillation counting in a cocktail consisting of toluene:ethylene glycol monomethyl ether (Eastman Chemical, Toronto) 5:4 (v/v) and 0.6% PPO (w/v). A correction factor for Chl quenching was always determined by counting aliquots of [2,3-^{14}C]ACC in the presence and absence of aliquots of an ethanol extract obtained from unlabeled leaf discs.

The radioactivity in the ethanol-insoluble fraction was determined by counting a suspension of the extracted discs (2 ml) in a toluene:Triton X-100 (2:1, v/v) scintillation mixture containing 0.5% (w/v) PPO. Accumulation of [2,3-^{14}C]ACC in the free space (apoplast) of the leaf discs was also determined so that uptake by the symplast could be evaluated (Fig. 4). In each experiment, four replicates of 15 discs each were incubated on the labeled ACC medium before being transferred to unlabeled media (with [^{12}C]ACC) for a 5-min post-wash. Discs were extracted as outlined above. The difference between the radioactive content of the post-washed tissue and a parallel unwashed tissue was taken as the free space ACC content. The correction factor was applied to all extractions. 'Total C_2H_4' release and the specific activity of the $^{14}C_2H_4$ in the headspace of the flask was determined at the end of the incubation period. A 500-μl sample was withdrawn for analysis in the gas chromatograph and then a further 5-ml sample (equivalent to 26% of the Total C_2H_4 in the incubation flask) was injected into a sealed partially evacuated glass scintillation vial containing 200 μl 0.1 M mercuric acetate in methanol which was incubated at 4°C overnight as described by Abeles and Abeles (2). The vials were then opened and 5 ml scintillation cocktail used for the ethanol solubles was added for determination of $^{14}C_2H_4$. Ethylene absorption by the mercuric acetate was 100%.

Glycolate Decarboxylation in Leaf Discs. The release of $^{14}CO_2$ from [1-^{14}C]glycolate (approximately 1.0 μCi/flask) was determined in the same sealed Micro-Fernbach flasks used for the determination of C_2H_4 release. At the required time interval, after the 500-μl gas samples were taken from the flasks for C_2H_4 estimation, the incubation mixture was acidified by addition of 0.5 ml 2 N HCl to terminate the reaction and release any $^{14}CO_2$ in the incubation mixture which contained 200 mM NaOH-Epps buffer at pH 8.2. The $^{14}CO_2$ was trapped in a 200 μl KOH solution (20% [w/v]) which was injected into a plastic centerwell trap (Kontes Ltd., Toronto) immediately after addition of the HCl. The flasks were not opened during the period of C_2H_4 sampling, acidification, and introduction of the KOH, all of which was accomplished in less than 30 s. The $^{14}CO_2$ released was determined by scintillation counting. The efficiency of trapping $^{14}CO_2$ released was over 99%.

Effect of Glycolate and ACC on Photosynthetic Activity in Leaf Discs. The photosynthetic rate of leaf discs treated with glycolate was determined during the period of C_2H_4 estimation by measuring $^{14}CO_2$ incorporation. Typically 1.0 μCi of $NaH^{14}CO_3$ (55.5 mCi $mmol^{-1}$) was added to each flask containing 50 mM unlabeled $NaHCO_3$. At the end of the reaction period, the discs were rapidly removed, washed with fresh incubation mixture (containing unlabeled bicarbonate), and placed into 1 ml boiling 80% (v/v) ethanol. The radioactivity in the ethanol-soluble fractions (i.e. five consecutive washes) and the ethanol-insoluble fractions was determined by scintillation counting. The radioactivity in the pooled ethanol extracts was determined by acidifying 0.1-ml aliquots with 0.1 ml 2 N HCl and counting in toluene:methanol (85:15 [v/v]) containing 0.5% (w/v) PPO. The radioactivity in the ethanol-insoluble fraction was determined by counting a gel suspension of the extracted discs in a cocktail containing 0.5% PPO (w/v) in toluene:Triton X-100 (2:1, v/v).

Estimation of Stomatal Behavior. Leaf discs which had been used in the C_2H_4 studies (see above) were removed from the test flasks and, within 15 s, impressions of the leaf surfaces were made using cellulose acetate strips. Extreme care was taken to keep the discs over the lights during the entire procedure. Each treatment was performed in triplicate and six impressions per treatment were made which meant that approximately 10^3 stomates per treatment were scored for 'open' or 'closed' using an ocular micrometer.

RESULTS AND DISCUSSION

Glycolate Enhancement of C_2H_4 Release. When the key photorespiratory intermediate, glycolate, was added to the reaction mixture, the illuminated leaf discs of both *Xanthium strumarium* L. (Fig. 1) and *Salvia splendens* L. (Table I) released more C_2H_4. Several conditions appear to be necessary for this enhancement to be observed. Briefly, these are as follows: (a) the tissue must be illuminated (Table I; Fig. 2); (b) the tissue must be photosynthetically active (Fig. 2, Tables I and II); and (c) a source of CO_2 (*i.e.* bicarbonate) must be present (Fig. 1; Tables I and II), although the level of CO_2 cannot be so high as to saturate C_2H_4 release prior to glycolate addition (Fig. 2).

Effect of Light on the Glycolate Enhancement of C_2H_4 Release. The stimulatory effect of glycolate on C_2H_4 release is light-dependent as well as CO_2-dependent (Table I: Fig. 2). There was no effect of glycolate in the dark even though it was taken up and metabolized in both light- and dark-treated discs (Table I). In the light the maximum rate of C_2H_4 release with glycolate was never greater than that observed with saturating CO_2 (without glycolate). This maximum rate was similar to those in all dark treatments (Table I; Fig. 2).

Glycolate Enhancement of C_2H_4 Release from Photosynthetically Active Tissue. Glycolate catabolism in *Salvia* and *Xanthium* was not blocked by 10 μM DCMU (data not shown), although photosynthesis and refixation of CO_2 derived from glycolate was inhibited by DCMU in the same manner as reported in other photosynthetic systems (12, 26). When leaf discs were incubated in the light with the photosynthetic inhibitor, DCMU, glycolate did not stimulate C_2H_4 release above the rate attributable to DCMU alone (Table II). In the presence of DCMU (plus or minus added $NaHCO_3$ and plus or minus glycolate), C_2H_4 release from illuminated discs increased (Table II) to the level of the dark controls (data not shown) in the manner described previously (8). Thus, the glycolate-enhanced C_2H_4 release cannot be detected when photosynthesis is blocked either 'naturally' by darkness (Table I; Fig. 2) or 'artificially' by the addition of DCMU (Table II).

Preliminary studies with several of the glycolate pathway enzymes (26) including glycolate oxidase (EC 1.1.1.3), catalase (EC 1.11.1.6), serine-glyoxylate aminotransferase (EC 2.6.1.45), and mitochondrial glycine decarboxylase indicate that small amounts

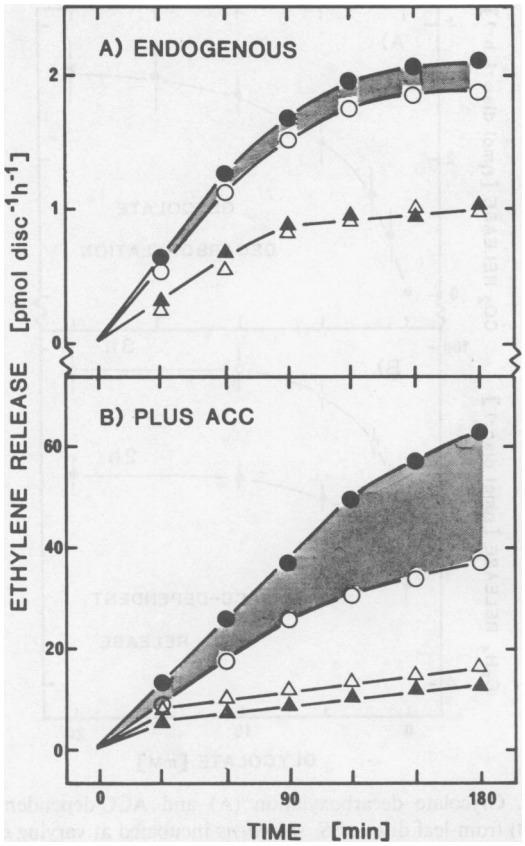

FIG. 1. Time course of ethylene release from leaf discs of *X. strumarium* with no ACC added to the incubation (A) and with 0.5 mM ACC (B). Fifteen leaf discs were incubated in 200 mM Na-Epps buffer (pH 8.2) containing 50 mM $NaHCO_3$ in the presence (●) and absence (○) of 10 mM Na-glycolate as outlined in "Materials and Methods." Discs were also incubated without bicarbonate in the presence (▲) and absence (△) of glycolate. The shaded areas represent the glycolate-dependent C_2H_4 release from the discs.

of peroxide generated by glycolate breakdown can stimulate C_2H_4 production from ACC at low rates (Grodzinski and Boesel. Proc Can Soc Plant Physiol Eastern Meet, Ottawa, 1982, abstract 11). Even though these processes could partially explain enhanced C_2H_4 production from ACC (*i.e.* pmol C_2H_4 mg^{-1} Chl h^{-1}) in glycolate-treated leaf tissue, it seems that the rapid oxidation of glycolate in the dark and in DCMU-treated discs (where glycolate did not enhance C_2H_4 release) argues that the action of the enzymes themselves cannot explain our data.

Effect of CO_2 Availability on Glycolate Enhancement of C_2H_4 Release. When leaf discs of *Xanthium* (Fig. 1) and *Salvia* (Table I) were incubated in sealed flasks in the light with 50 mM $NaHCO_3$, a limiting amount of CO_2, a net photosynthetic rate of approximately half the maximum rate in these discs (120–140 μmol O_2 released mg^{-1}Chl h^{-1}) was maintained. Both endogenous and ACC-dependent C_2H_4 release were markedly enhanced under this condition when compared to the rate of C_2H_4 release without added $NaHCO_3$. Without added CO_2, the tissue predictably depleted the CO_2 level in the headspace of the flask to the CO_2 compensation point (approximately 50–60 ppm) for these two C_3 plants. In *Salvia*, much lower rates of C_2H_4 release are observed in the light compared to those in the dark when CO_2 is not sufficient to saturate photosynthesis (Fig. 2) confirming observations with *Xanthium* (8). In the light with increasing CO_2 availability (*i.e.* added $NaHCO_3$), C_2H_4 release rose and appeared to be saturated at about 125 mM $NaHCO_3$ (Fig. 2). This C_2H_4 release value (about 20.5 pmol $disc^{-1}$ h^{-1}) closely approximates the rate observed in the dark (21–24 pmol $disc^{-1}$ h^{-1}) over the entire range of bicarbonate concentrations tested.

The bicarbonate concentration in the medium is a critical factor since CO_2 availability is dependent on the HCO_3^-/CO_2 equilibrium (see Ref. 5). The glycolate enhancement was observed over a very restricted range of bicarbonate concentrations (*i.e.* 30–100 mM; Fig. 2). At 50 mM $NaHCO_3$, the addition of 10 mM Na-glycolate routinely doubled C_2H_4 release in the light. Although bicarbonate concentrations greater than 100 mM were required to saturate C_2H_4 release without glycolate, in the presence of 10 mM glycolate bicarbonate concentrations from 50 to 100 mM were sufficient to elicit maximal C_2H_4 release rates (Fig. 2). Enhancement of C_2H_4 release attributable to glycolate was maximal with the addition of 10 mM glycolate (Fig. 3B), a concentration at which $^{14}CO_2$ from[1-^{14}C]glycolate was also highest (Fig. 3A). The data reported above are consistent with the concept that CO_2 generated internally from glycolate breakdown is an important factor contributing to the observation that glycolate enhances C_2H_4 release in the light. This explanation is consistent with earlier suggestions that CO_2 generated from (photo)respiratory decarboxylation reactions can increase C_2H_4 release (8–10); however, several other possible explanations merit scrutiny.

Reduced Stomatal Resistance in the Presence of Glycolate and Bicarbonate. Many factors, including stomatal resistance, could regulate exchange of C_2H_4. Stomatal function does not appear to directly involve the glycolate pathway (19, 21); however, when epidermal strips or leaf discs are floated on solutions containing glycolate pathway intermediates, stomatal behavior can be al-

Table I. *Effect of ACC on Net C_2H_4 Release and $^{14}CO_2$ Release from [1-^{14}C]Glycolate in Leaf Discs of S. splendens*

In each flask, 15 leaf discs were incubated as described in "Materials and Methods" for 2 h at 25°C in the presence of 10 mM Na-[1-^{14}C]glycolate and 200 mM Na-Epps buffer (pH 8.2). The data represent results from a single experiment in which each value is the mean of four replicates of each treatment. Standard errors of the mean are indicated. The experiment was repeated three times with similar results.

Additions	Light		Dark	
	C_2H_4 Release	$^{14}CO_2$ Release	C_2H_4 Release	$^{14}CO_2$ Release
	pmol flask⁻¹ h⁻¹	*nmol flask⁻¹ h⁻¹*	*pmol flask⁻¹ h⁻¹*	*nmol flask⁻¹ h⁻¹*
Control (−ACC)	2.5 ± 0.0	8.1 ± 1.5	7.8 ± 2.5	52.9 ± 5.0
Control (−ACC) + $NaHCO_3$	6.6 ± 1.2	23.7 ± 1.1	6.0 ± 0.8	47.0 ± 5.3
0.5 mM ACC	90 ± 10.2	10.0 ± 1.2	294 ± 21	52.4 ± 4.9
0.5 mM ACC + $NaHCO_3$	344 ± 36	27.1 ± 3.3	328 ± 31	46.1 ± 8.1

Table II. *Net Release of C_2H_4 from Illuminated Leaf Discs of Salvia in the Presence of DCMU and Glycolate*

Leaf discs were preincubated with DCMU for 20 min before the addition of 0.5 mM ACC to the incubation buffer (*i.e.* Epps [pH 8.2]). Parallel experiments showed that photosynthesis was inhibited by over 90% by addition of 10 μM DCMU. C_2H_4 release was estimated as described in "Materials and Methods" (see also Table I).

Additions to Incubation Buffer	C_2H_4 Release	
	− DCMU	+ 10 μM DCMU
	pmol disc^{-1} h^{-1}	
Control	6.0 ± 0.2	19.5 ± 2.6
10 mM glycolate	4.9 ± 0.6	20.6 ± 2.1
50 mM bicarbonate	12.5 ± 1.8	21.5 ± 3.2
50 mM bicarbonate + 10 mM glycolate	25.9 ± 2.1	23.2 ± 2.8

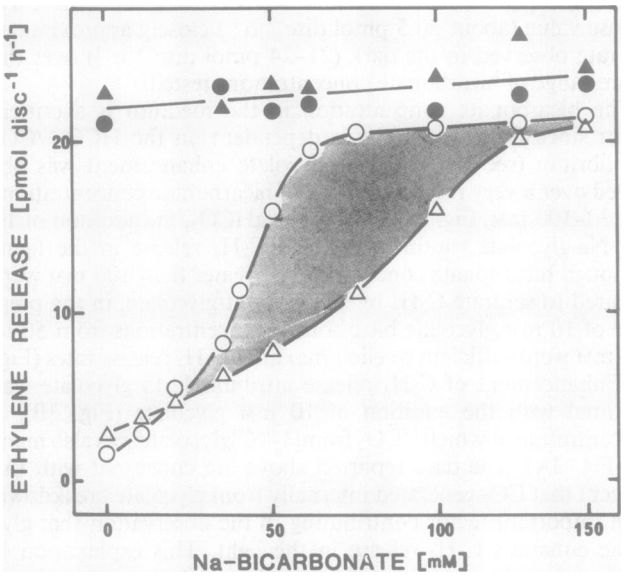

FIG. 2. Release of ethylene from leaf discs of *S. splendens* incubated at varying concentrations of $NaHCO_3$ in 200 mM Na-Epps buffer (pH 8.2) with 10 mM Na-glycolate added in the light (○) and in the dark (●). Control discs were incubated in the light (△) and in the dark (▲) without glycolate. The shaded area represents glycolate-dependent C_2H_4 release from illuminated leaf discs.

tered (25) particularly at low pH (19). When leaf discs of *Xanthium* and *Salvia* were incubated on test solutions containing 50 mM $NaHCO_3$ and 200 mM Epps-NaOH (pH 8.2), stomates closed; but, when 10 mM glycolate was included in the mixture, more stomates were open (Table III). These observations are noteworthy because alone they could be interpreted to indicate that the escape of C_2H_4 gas from leaf tissue may be controlled by stomatal resistance. However, attributing the observed glycolate enhancement of C_2H_4 release solely to a reduction in stomatal resistance is inconsistent with several other observations. The addition of 50 mM KCl, which also prevents stomatal closure with bicarbonate did not stimulate C_2H_4 release (data not shown). Furthermore, both the rate of C_2H_4 release and measured stomatal resistance were lowest from both *Xanthium* and *Salvia* without added bicarbonate (Table III). Although CO_2 causes stomates to close slightly, photosynthesis proceeds because the CO_2 gradient is sufficient to overcome the barrier (5, 25). Thus, even though glycolate promotes stomatal opening in the presence of bicarbonate, glycolate does not affect C_2H_4 release by altering the stomatal resistance.

Glycolate Metabolism in the Presence of ACC. Although

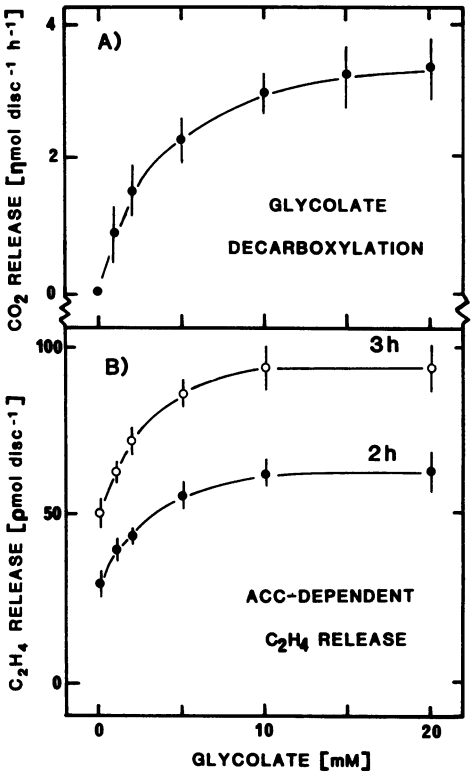

FIG. 3. Glycolate decarboxylation (A) and ACC-dependent C_2H_4 release (B) from leaf discs of *S. splendens* incubated at varying concentrations of glycolate. Glycolate decarboxylation was determined as the amount of $^{14}CO_2$ release from [1-^{14}C]glycolate in 2 h. Ethylene release after 2 h (●) and in a parallel experiment after 3 h (○) are shown.

previous studies indicate that net photosynthesis and dark respiration are not markedly affected by the application of 0.5 mM ACC (8–10), at present there are no published data indicating whether ACC and/or C_2H_4 can modify glycolate metabolism over the duration of such experiments. The data in Table I demonstrate that exogenously supplied [1-^{14}C]glycolate was taken up and metabolized to $^{14}CO_2$ in both the light and the dark by *Salvia* leaf discs as observed previously (11, 12, 26). The addition of 0.5 mM ACC did not alter glycolate decarboxylation in either the light or the dark (Table I). The rate of $^{14}CO_2$ release from[1-^{14}C]glycolate was higher in the dark than in the light because some of the $^{14}CO_2$ produced in the light was refixed. The addition of unlabeled $NaHCO_3$ in the light which stimulated C_2H_4 release (Fig. 1; Table I) also stimulated $^{14}CO_2$ release from [1-^{14}C]glycolate (Table I). Unlabeled $NaHCO_3$ in the incubation mixture diluted the $^{14}CO_2$ generated within the discs during the breakdown of the glycolate; therefore, proportionally less $^{14}CO_2$ was refixed and more escaped. Thus, the flow of carbon through the glycolate pathway does not appear to be affected by the presence of ACC and/or C_2H_4 over this time period.

The studies confirm that the amount of carbon involved in C_2H_4 metabolism relative to recycling of glycolate carbon is very small (Table I). Interestingly, Lüttge (16) has shown that under photorespiratory conditions small, in terms of carbon flux during photosynthesis, amounts of carbon monoxide, are generated from C_1 units produced during the breakdown of glycolate (6, 7, 11). Whether glycolate breakdown generates C_1 units which could alter methionine metabolism and therefore C_2H_4 biosynthesis is presently being investigated; but, our studies particularly with ^{14}C-labeled ACC (Fig. 4) support the view that the effect of glycolate is beyond the synthesis of the immediate precursor, ACC (3, 18).

Table III. *Effect of Glycolate on C_2H_4 Release, Stomatal Behavior, and Net Photosynthetic Activity in Leaf Discs of X. strumarium and S. splendens (see "Materials and Methods")*

Additions	ACC-Dependent C_2H_2 Release	Stomates Open	Net Photosynthetic Activity			
			Total	Ethanol-soluble	Ethanol-insoluble	Solubles / Insolubles
	pmol disc^{-1} h^{-1}	%	*μmol ^{14}C incorporated disc^{-1} h^{-1}*			
X. strumarium						
[^{14}C]Bicarbonate	19.1 ± 0.8	47.9 ± 2.1	3.34 ± 0.09	0.38 ± 0.05	2.98 ± 0.04	0.13
[^{14}C]Bicarbonate + glycolate	25.0 ± 1.4	57.6 ± 3.1	3.42 ± 0.15	0.39 ± 0.05	3.03 ± 0.10	0.12
Glycolate	11.0 ± 1.4	66.2 ± 4.1				
S. splendens						
[^{14}C]Bicarbonate	16.4 ± 0.5	21.4 ± 2.6	1.79 ± 0.18	0.29 ± 0.2	1.42 ± 0.16	0.20
[^{14}C]Bicarbonate + glycolate	28.6 ± 1.7	54.2 ± 4.3	1.69 ± 0.13	0.21 ± 0.2	1.48 ± 0.21	0.14
Glycolate	7.6 ± 1.1	62.1 ± 3.2				

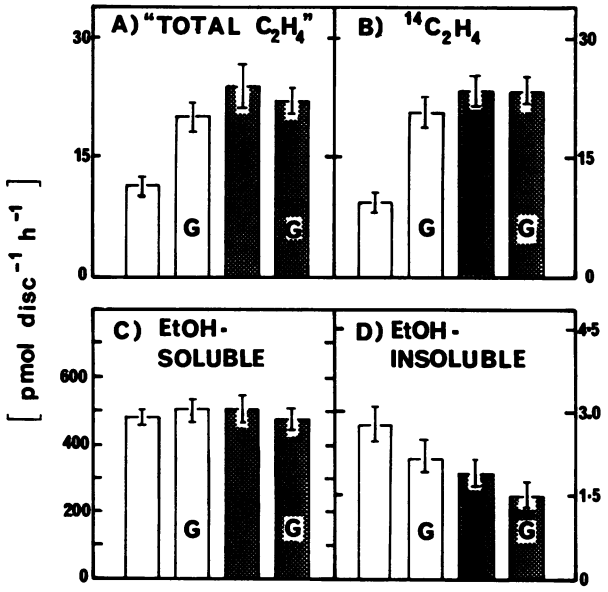

FIG. 4. Effect of glycolate on uptake of [2,3-^{14}C]ACC and release of $^{14}C_2H_4$ from leaf discs of *Salvia*. The 'Total C_2H_4' (A) was determined by conventional GC and the radiolabeled $^{14}C_2H_4$ (B) which was trapped at the end of the experiment (*i.e.* 2 h) was determined by scintillation counting as described in "Materials and Methods." The radioactivity recovered in the leaf discs in the ethanol (EtOH)-soluble and -insoluble fractions are shown in C and D, respectively. Open bars on the histograms represent illuminated leaf discs, whereas shaded bars indicate discs incubated in the dark. In all cases shown in the figure, the incubation medium contained 200 mM Na-Epps buffer (pH 8.2) with 50 mM $NaHCO_3$. The addition of 10 mM glycolate is indicated by the letter 'G' in the bar. Each bar represents the mean of triplicate samples from four treatments with the range indicated.

Effect of Glycolate on Uptake of [2,3-^{14}C]ACC and Release of $^{14}C_2H_4$ from Leaf Discs. The uptake, oxidation, and further metabolism of exogenously supplied glycolate are complex processes and could conceivably alter ACC uptake and/or C_2H_4 metabolism in a number of ways. The data in Figure 1 and Table I support previous studies (8–10, 14, 18) which show that C_2H_4 release was enhanced when leaf discs were treated with ACC. Lürssen (17) has demonstrated that a number of L-amino acids can affect the uptake of ACC and subsequent release of C_2H_4 from leaf discs incubated in the dark. In order to determine whether uptake of exogenously supplied ACC is a limiting factor, and whether glycolate alters the uptake of this precursor or enhances C_2H_4 release from an alternate substrate (15), radiolabeled ACC was used. The specific activity of the C_2H_4 released was constant among treatments supporting the view that all of the C_2H_4 released from the leaf discs was the product of fed ACC and did not arise from an unlabeled pool of either ACC or any other substrate in the leaf discs. The pattern of radiolabeled ACC uptake by the discs was not altered by the addition of $NaHCO_3$ in the presence of NaOH-Epps (pH 8.2) (data not shown). The pattern of $^{14}C_2H_4$ release in the light and in the dark in the presence and absence of glycolate was similar to the pattern in Table II. In the dark, glycolate did not alter $^{14}C_2H_4$ release (Fig. 4B). Glycolate stimulated $^{14}C_2H_4$ release only in the light and when bicarbonate was added. When bicarbonate was not added, glycolate caused a slight decrease in $^{14}C_2H_4$ and 'Total C_2H_4' in the light (data not shown) as seen previously (Fig. 2). The data in Figures 4C and 4D indicate that the incorporation of radioactivity from [2,3-^{14}C] ACC into the ethanol-soluble and -insoluble fractions (note scale) was not markedly affected by glycolate treatment. The addition of glycolate in the light primarily stimulated net C_2H_4 release (Fig. 4, A and B) without altering the amount of ACC taken up by the leaf discs; however, conclusions cannot be drawn regarding effects glycolate might have on the availability of ACC at a specific site within the leaf tissue.

CONCLUSIONS

A working hypothesis for dealing with CO_2/C_2H_4 interactions in photosynthetic tissue was originally forwarded (8) to provide an explanation that would encompass a number of observations. The basic premise is that either ACC to C_2H_4 conversion, a yet undefined reaction, is sensitive to varying CO_2 levels (8; also see Ref. 14), and/or the processes controlling escape of C_2H_4 gas are modified by CO_2 availability (8). Several important observations have bearing on this approach. (a) Over the last two decades, CO_2 has been implicated as an antagonist of C_2H_4 action in a variety of plant systems (1, 15). It is difficult to rationalize how CO_2 on the one hand blocks many C_2H_4 related responses (1, 15) while stimulating only C_2H_4 biosynthesis from ACC in the same tissue (14). (b) In both C_3 and C_4 plants, the rate of C_2H_4 release at the compensation point is low relative to dark controls (*e.g.* Fig. 1; Table I). (c) In C_3 plants, there is no significant difference between C_2H_4 release rates in the dark and in the light when CO_2 is not a limiting factor in the light. (d) However, in C_4 plants, which presumably generate CO_2 internally in the light, a stimulation of C_2H_4 release relative to dark controls is observed when the CO_2 supply is not limiting (10).

Our explanation of the glycolate enhancement of C_2H_4 release in two C_3 plants, *Xanthium* and *Salvia*, is that CO_2 produced from glycolate (*i.e.* photorespiration) internally can, under limiting CO_2 conditions (Fig. 2), contribute CO_2 which enhances net C_2H_4 release. A possible interaction between glycolate metabolism and the growth regulators has not received much attention. The suggestion has been made that photorespiration is important because it allows dissipation of excessive reducing equivalents under photooxidative conditions and provides for the recycling of both carbon and nitrogen. Many studies into C_2H_4 related phenomena such as leaf senescence (*i.e.* loss of leaf nitrogen) have been conducted using closed systems in which the CO_2 levels are actually controlled by the balance of photosynthesis, photorespiration, and respiration in the tissue (4, 13, 20, 22, 23). This paper provides the first evidence that glycolate decarboxylation during photorespiration can alter the amount of C_2H_4 produced by leaf tissue.

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