Plant Physiol. (1991) 96, 1360–1367
0032-0889/91/96/1360/08/$01.00/0

Received for publication December 4, 1990
Accepted April 4, 1991

Transport and Metabolism of 1-Aminocyclopropane-1-carboxylic Acid in Sunflower (*Helianthus annuus* L.) Seedlings[1]

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ABSTRACT

Transport and metabolism of [2,3-^{14}C]1-aminocyclopropane-1-carboxylic acid (ACC) from roots to shoots in 4-day-old sunflower (*Helianthus annuus* L.) seedlings were studied. [^{14}C]ACC was detected in, and $^{14}C_2H_4$ was evolved from, shoots 0.5 hours after [^{14}C]ACC was supplied to roots. Ethylene emanation from the shoots returned to normal levels after 6 hours. The roots showed a similar pattern, although at 24 hours ethylene emanation was still slightly higher than in those plants that did not receive ACC. [^{14}C]*N*-malonyl-ACC (MACC) was detected in both tissues at all times sampled. [^{14}C]MACC levels surpassed [^{14}C]ACC levels in the shoot at 2 hours, whereas [^{14}C]MACC levels in the root remained below [^{14}C]ACC levels until 6 hours, after which they were higher. Thin-layer chromatography analysis identified [^{14}C]ACC in 1-hour shoot extracts, and [^{14}C]MACC was identified in root tissues at 1 and 12 hours after treatment. [^{14}C]ACC and [^{14}C]MACC in the xylem sap of treated seedlings were identified by thin-layer chromatography. Xylem transport of [^{14}C]ACC in treated seedlings, and transport of ACC in untreated seedlings, was confirmed by gas chromatography-mass spectrometry. Some evidence for the presence of [^{14}C]MACC in xylem sap in [^{14}C]ACC-treated seedlings is presented. A substantial amount of radioactivity in both ACC and MACC fractions was detected leaking from the roots over 24 hours. A second radiolabeled volatile compound was trapped in a CO_2-trapping solution but not in mercuric perchlorate. Levels of this compound were highest after the peak of ACC levels and before peak MACC levels in both tissues, suggesting that an alternate pathway of ACC metabolism was operating in this system.

Transport of phytohormones from one plant part to another is one method of communication among plant tissues and organs. Such a system may be useful when a signal is perceived by one organ and a response is required by another distant from the site of perception. Before we can fully understand the physiology of a phytohormone and how it might be involved in the integration of activities within the whole plant, we must understand the patterns of transport of the hormone, its precursors and conjugates, and the metabolic fate of these compounds.

Ethylene differs from the other plant hormones in that it is a gas that easily diffuses out of the plant. Although transport of ethylene does occur (17, 32), the directed longitudinal movement of this hormone is limited (33). The immediate ethylene precursor, ACC[2] (2), can be transported in the vascular system (6, 7). ACC is present in relatively low amounts; however, exposure to some types of stress can increase ACC and ethylene synthesis (6, 7, 14).

ACC may be transported among flower parts signaling the initiation of flower senescence following pollination in *Cymbidium* and carnation (21, 25, 32). Increased ACC levels in organs distal to leaves treated with ethephon were found in *Cucurbita pepo* (16), prompting the proposal that ACC is a mode of interorgan transport of an ethylene signal in this species, although the transport of ACC between plant organs was not measured directly. ACC synthesis increases in flooded roots; this ACC is transported in the xylem to the shoots where it causes an increase in ethylene production in leaves and petioles, which then exhibit a number of changes in growth and development (6, 7). Basipetal transport of ACC in decapitated pea plants has also been demonstrated (10).

ACC can be conjugated to form MACC (12, 13). Because large amounts of MACC are synthesized under conditions that induce ACC synthesis, the formation of MACC may be a means of removing ACC, thus controlling ethylene formation (4, 14). Although MACC is a poor ethylene precursor compared to ACC, treatment of plant tissues with MACC does increase ethylene production (14, 26), possibly through enzymatic hydrolysis of MACC to ACC (19). However, either when tissues are treated with MACC or during *in vivo* synthesis, the majority of MACC is transported into the vacuole (5), where it remains (4, 14). Because conjugates of other phytohormones can be a source of active hormone after deconjugation, the metabolism and transport of MACC must also be considered in investigations of ethylene precursor transport. Transport of MACC from shoot to root in peas has been shown by Fuhrer and Fuhrer-Fries (10), whereas MACC is not transported out of tobacco leaves (31). Efflux of MACC from *Acer* protoplasts preloaded with MACC was shown by Bouzayen *et al.* (4), although the majority of MACC fed to these protoplasts was recovered from the vacuole.

Although ACC has been shown to be transported in the few systems mentioned above, the translocation of this eth-

[1] Supported by a National Sciences and Engineering Research Council of Canada grant to D.M.R.

[2] Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; MACC, *N*-malonyl-ACC.

ylene precursor within a whole, unstressed plant has been studied rarely. Compared to the transport of auxins, gibberellins, cytokinins, and ABA, there are few data concerning the transport of ACC. This is surprising considering the large body of data showing the involvement of ethylene in many aspects of plant growth and development. In this paper we report our study of ACC translocation from roots to shoots in young sunflower plants. We also carried out a limited investigation of the metabolic fate of ACC in these two organs.

MATERIALS AND METHODS

Plant Material

Sunflower (*Helianthus annuus* L. var Dahlgren 131) seeds were germinated aeroponically with a 16-h photoperiod of 300 μE m^{-2} s^{-1} under a 24/18°C day/night temperature regimen. Seedling roots were misted with modified one-quarter strength Hoagland solution (double amount of iron chelate). The aeroponic germination system produced plants with completely undamaged roots. At the 4-d stage, plants with stems 5 to 7 cm tall and roots 8 to 10 cm long were selected. The cotyledons had emerged from the testa but were not fully expanded, and the lateral roots were beginning to emerge.

ACC Treatment

The seedlings were treated by immersing roots of two plants in 5-mL tubes containing 4 mL of 10 μM [2,3-^{14}C]ACC (specific activity 2.96 GBq/mmol; Commissariat á l'Energie Atomique, Gif sur Yvette, France) in one-quarter strength Hoagland solution that was aerated before the experiment. Roots were gently coiled into the tubes, avoiding sharp bends so as to avoid wounding. The plants were treated in this solution for 1 h under Gro-Lux lights (185 μE m^{-2} s^{-1}). After the roots were fed, they were rinsed twice in distilled water, and the plants were transferred to aeroponics chambers (15) or harvested immediately. As controls, an equal number of plants were immersed in pairs in 4 mL of the above nutrient solution without ACC and were handled exactly as the [^{14}C] ACC-treated plants.

Tissue Gas Evolution

At each harvest, the seedlings were cut at the root-shoot junction, and each portion was placed in a separate 10-mL syringe with the plungers adjusted to 6 mL. After 10 min, a 5-mL gas sample was transferred to a second gas-tight syringe through a three-way valve (we find that wound-induced ethylene cannot be detected for at least 30 min). This sample (1 mL) was analyzed for ethylene content using a Photovac 10S10 gas chromatograph with a 3.2-mm × 2.45-m 60/80 Carbopack B column (1.5% XE-60/1% H_3PO_4; Supelco Canada, Oakville, Ontario, Canada) and a photoionization detector. An aliquot (2 mL of the 5-mL sample) was injected through a septum into a scintillation vial containing 1.5 mL of mercuric perchlorate (1). Vials were incubated overnight with shaking. Scint A XF scintillation cocktail (10 mL [Packard Instrument Co., Downers Grove, IL]) was added to each vial, and radioactivity was determined with a Packard Tri-Carb 2200CA liquid scintillation analyzer. To determine whether radiolabeled gases, other than ethylene, were produced in these experiments, a trap of $^{14}CO_2$ cocktail (Carbon 14 Cocktail; R. J. Harvey Instrument Corp., Hillsdale, NJ) was used. This cocktail contains a scintillant and is a highly efficient CO_2 trap. The final 2 mL were injected through a septum into a vial containing 10 mL of $^{14}CO_2$ cocktail and incubated with shaking overnight, after which the amount of trapped radioactivity was determined. The fresh weights of the roots and shoots used for gas analysis were determined immediately after sampling. Each experiment was performed at least twice. Within each experiment, measurements were replicated four times.

Trapping Efficiencies

To determine the efficiency of the $^{14}CO_2$ cocktail for trapping ethylene, 100 pmol of C_2H_4 were injected into vials containing 10 mL of $^{14}CO_2$-trapping cocktail and incubated as above. Ethylene remaining in the headspace was determined using a gas chromatograph equipped with a Porapak R column (2 m × 2 mm i.d., 80 to 100 mesh; Waters Associates Inc., Milford, MA) and a flame ionization detector. The ethylene-trapping efficiency of the mercuric perchlorate was determined in a like manner. Each solution was also tested for trapping efficiency of $^{14}CO_2$, which was derived from acid breakdown of $NaH^{14}CO_3$ (Amersham Corp., Arlington Heights, IL).

Mercuric perchlorate trapped C_2H_4 with 99% efficiency. Efficiency of ethylene trapping by the $^{14}CO_2$ cocktail was found to be 35%. Each trapping solution was tested for efficiency in trapping CO_2, because it was possible that $^{14}CO_2$ was released in these experiments. The $^{14}CO_2$ cocktail trapped $^{14}CO_2$ with 98% efficiency. Mercuric perchlorate did not trap $^{14}CO_2$. These efficiencies were taken into account when determining the amounts of radioactive gases produced.

ACC and MACC Extraction and Identification

During each harvest, four [14C]ACC-treated plants were cut at the root-shoot junction. Each root and shoot was weighed separately, frozen in liquid N_2, and stored at −70°C. The extraction of ACC and MACC was based on previously described methods (22, 29). The tissue was powdered in liquid N_2 and extracted three times in 80% ethanol at 70°C for 15 min, each followed by filtration through a Whatman No. 1 filter. The combined extract was reduced to dryness *in vacuo* at 40°C and redissolved in 5 mL of water plus 1.5 mL of $CHCl_3$. The $H_2O/CHCl_3$ extract was centrifuged at 2500*g* for 15 min to minimize emulsion formation. The aqueous phase was passed in tandem through columns containing polyvinylpolypyrrolidone (4.5 × 0.8 cm i.d., 80 mesh; Sigma Chemical Co., St. Louis, MO) and cation exchange resin (AG 50W X8, H^+ form, 4.5 × 0.8 cm i.d., 100 to 200 mesh; Bio-Rad Laboratories, Richmond, CA). The columns were washed with water; this wash contained MACC and was collected. ACC was eluted from the cation exchange resin with 2 N NH_4OH. Radioactivity in each fraction was determined. ACC and MACC fractions from tissues harvested at 1 and 12 h were reduced *in vacuo* at 40°C to approximately 1 mL, and

an aliquot of this was subjected to two-dimensional TLC on either microcellulose (250 μm) or silica gel (250 μm) plates (Whatman Inc., Clifton, NJ). These were developed in the first dimension in 1-butanol:acetic acid:water (12:3:5, v/v/v) and in the second dimension in 1-propanol:NH_4OH (7:3, v/v). The plates were autoradiographed and compared with authentic [^{14}C]ACC and [^{14}C]MACC standards for identification purposes. Standard [^{14}C]MACC was prepared using the protocol of Satoh and Esashi (27), except that the quantity of ACC precursor used was substantially lower (40.9 μg, 32.4 μCi), and instead of recrystalization, purification of the [^{14}C]MACC was done by preparative TLC on 1-mm silica gel plates (Whatman) in the first dimension solvent described above. The [^{14}C]MACC was located by autoradiography, scraped, and eluted from the silica gel. Confirmation of [^{14}C]MACC identity was accomplished by GC-MS as described below.

ACC and MACC Leakage

Estimates of radioactivity leakage from the roots were determined at 1, 6, 12, and 24 h after the commencement of treatment by placing the root of one [^{14}C]ACC-treated plant in 15 mL of one-quarter strength Hoagland solution for 15 min. The radioactivity in this solution was then determined. Three replications were made at each sample time. In a separate experiment, 2 h after commencement of root treatment with [^{14}C]ACC, the Hoagland solution collecting the root leakage was fractionated into ACC and MACC components using cation exchange resin, and radioactivity in these fractions was determined.

Analysis of Xylem Sap

Collection of Sap

Thirty-three seedlings were fed [^{14}C]ACC as described above and were transferred to the aeroponics chambers. The plants were decapitated with a razor blade approximately 1 cm below the cotyledons 2 h after terminating treatment, and xylem exudate was collected during a 4-h period. The sap was fractionated on cation exchange resin into ACC and MACC fractions of which aliquots were counted for radioactivity. These samples were analyzed by silica gel and cellulose TLC and autoradiography as described above. To determine whether ACC and MACC were naturally present in xylem sap, 11-d-old seedlings (germinated and grown aeroponically) were decapitated approximately 1 cm below the cotyledons, and xylem exudate was collected during a 4-h period. Two samples pooled from 40 (30 mL) and 60 plants (55 mL) were collected.

Ion Exchange and HPLC

The purification and analysis of ACC was based on the method of McGaw *et al.* (20). The xylem sap was dried *in vacuo* at 40°C, redissolved in 0.1 N acetic acid and applied to a column of AG 50W X8 resin (H^+ form, 4.5 × 1.3 cm i.d.) that was washed with 0.1 N acetic acid. The washings contained MACC. The ACC was eluted with 2 N NH_4OH, dried *in vacuo* at 40°C, redissolved in 0.1 N NH_4OH, and loaded onto an anion exchange column (AG 1 X8, CH_3COO^- form, 4.5 × 1.3 cm i.d., 200 to 400 mesh; Bio-Rad) that was then washed with 0.1 N NH_4OH. The ACC was eluted with 0.1 N acetic acid, which was reduced to approximately 2 mL *in vacuo* at 40°C. This sample was transferred to a vial and freeze dried. Two milliliters of 30 mM phthalic anhydride (once resublimed) in glacial acetic acid were added to the vial, which was sealed and heated to 100°C for 1.5 h. After the addition of 2 mL of water, the sample was partitioned against 5 mL of ether. The ether phase was dried *in vacuo* at 40°C. The residue was dissolved in 300 μl of methanol, after which 700 μl of 0.1 N acetic acid were added. The sample was chromatographed on a reverse-phase C_{18} Partisil ODS_2 HPLC column (110 × 4.7 mm i.d., 5-μm particle size; Whatman) in 30 to 45% methanol in a 0.1 N acetic acid gradient for 10-min. The fraction containing phthalidimo-ACC was collected and dried *in vacuo* at 40°C and methylated three times with freshly prepared diazomethane. The methylated sample was dissolved in 32% aqueous CH_3CN and chromatographed on the Partisil HPLC column isocratically in 32% aqueous CH_3CN. The fraction containing phthalidimo-ACC-methyl ester was collected and dried *in vacuo* at 40°C.

The MACC fraction was dried *in vacuo* at 40°C, dissolved in 0.1 N NH_4OH, and applied to a column of AG 1 X8 ($HCOO^-$ form, 4.5 × 1.3 cm i.d., 200 to 400 mesh; Bio-Rad). The resin was washed with 0.1 N NH_4OH, and the MACC was eluted with 4 N HCOOH. The sample was dried *in vacuo* at 40°C, and the residue was dissolved in a minimum amount of methanol, three drops of HCOOH, and approximately 5 mL of ether. The resulting precipitate was removed by filtration (GF/C glass fiber filter) and the extract dried under N_2. This precipitation was repeated, and the sample was methylated three times. Dimethyl MACC was dissolved in H_2O and purified using the Partisil HPLC column in a gradient of 0 to 15% CH_3CN in H_2O for 15 min. The fraction containing dimethyl MACC was collected and dried *in vacuo* at 40°C. In addition, ACC derived from MACC after hydrolysis in 6 N HCl for 1.5 h (12) was derivatized and analyzed as above.

GC-MS

A Hewlett-Packard HP-5980 series II gas chromatograph equipped with a DB5–15N capillary column (15 m × 0.25 mm i.d, 0.25-μm film thickness; J. & W. Scientific, Folson, CA) coupled to a HP 5970A mass selective detector was used. The phthalidimo-ACC-methyl ester was dissolved in ethyl acetate, injected on-column, and run on a temperature program of 60 to 160°C (40°C min^{-1}) and then to 206°C (6°C min^{-1}). Injections of dimethyl MACC were made in ethyl acetate at an initial temperature of 60°C and temperature programming of 60 to 80°C (40°C min^{-1}), followed by 80 to 250°C (12°C min^{-1}). In both analyses, the interface temperature was maintained at 280°C and helium linear gas flow at 40 cm min^{-1}.

RESULTS

Gas Evolution by the Tissues

We supplied radioactive ACC to roots for 1 h and examined ethylene evolution during a 3-h period. As expected, much

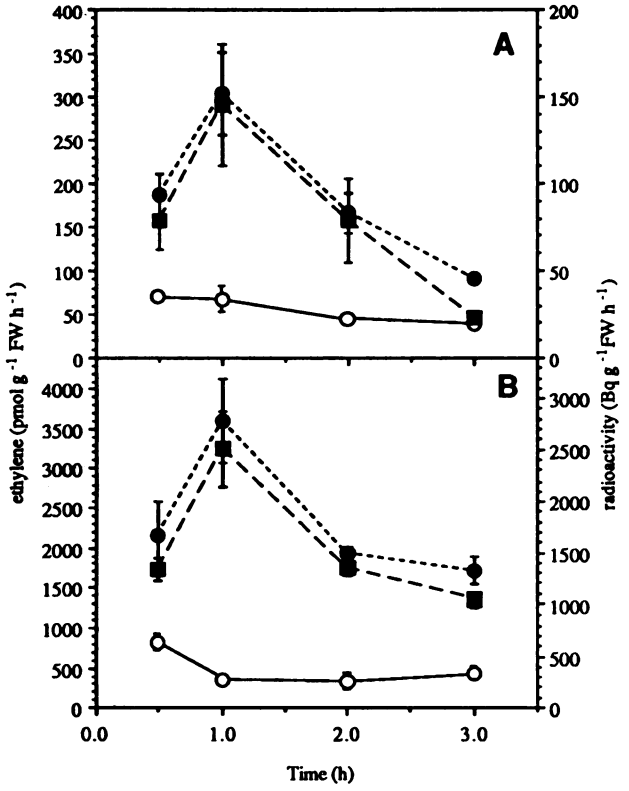

Figure 1. Ethylene release during 3 h from (A) shoots and (B) roots of 4-d-old sunflower seedlings. Nonradioactive C_2H_4 was estimated by GLC. Radioactivity associated with $^{14}C_2H_4$ was trapped in mercuric perchlorate and estimated by scintillation counting. O, control; ●, C_2H_4 from ACC treatment estimated by GLC, ■, radioactive C_2H_4. Error bars, SEM.

more ethylene was produced by both shoots and roots of ACC-treated plants than by plants that did not receive additional ACC. In shoots (Fig. 1A) of the ACC-treated plants, ethylene production peaked at 1 h and then declined to near control levels by 3 h. The release of radioactive ethylene was also monitored by use of a mercuric perchlorate trap (1). Radioactive ethylene was produced in the shoots from [^{14}C] ACC within 0.5 h of the application and increased to a maximum at 1 h. Thereafter, radioactivity declined to lower, but measurable, levels at 3 h (Fig. 1A). The pattern of radioactivity in ethylene trapped from shoots was similar to the pattern of ethylene evolution from the ACC-treated plants as measured by GLC. When the quantities of ethylene measured by GLC were plotted against the radioactivity, there was a strong positive correlation ($r^2 = 0.986$). Therefore, much of this latter ethylene was produced from the [^{14}C]ACC that had been fed to roots.

Ethylene evolution by roots showed a similar picture except that overall ethylene production was approximately 10 times higher than in shoots, and C_2H_4 emanation from the ACC-treated roots at 3 h was approximately 3 times control levels (Fig. 1B). As seen in the shoots, the pattern of radioactivity in ethylene (measured by GLC) trapped from roots was very similar to the quantities of radioactive ethylene trapped in mercuric perchlorate ($r^2 = 0.984$ [Fig. 1B]).

The fate of the labeled ACC was then evaluated during 24 h. Ethylene evolution was high at 1 h from both organs and decreased with time (Fig. 2). Ethylene production by shoots decreased to control levels by 6 h (Fig. 2A); however, root ethylene production did not decrease to control values until 12 h after treatment and was slightly higher than controls at 24 h after treatment (Fig. 2B). As before, root ethylene evolution was approximately 10-fold higher than that from the shoot. There was again a strong positive correlation between the quantity of trapped radioactive ethylene and ethylene measured by GLC from roots ($r^2 = 0.989$); however, an anomalous 6-h radioactive ethylene level from shoots gave a poorer correlation coefficient for this graph ($r^2 = 0.568$).

Radioactivity released from the plant as $^{14}C_2H_4$ and a second gas (probably $^{14}CO_2$) trapped in the $^{14}CO_2$ cocktail from plants treated with [^{14}C]ACC is shown in Figure 3. In the shoots (Fig. 3A), except for the 1-h time, radioactivity associated with ethylene was lower than radioactivity associated with the second volatile compound. At its peak at 6 h, production of this second gas was substantially greater than with $^{14}C_2H_4$ evolution. At the termination of the experiment, radioactivity associated with the second gas remained relatively high (136 Bq g^{-1} fresh weight h^{-1}), whereas radioactive ethylene levels were low (3 Bq g^{-1} fresh weight h^{-1}). Labeled ethylene and the unidentified second compound were also released from the roots of treated plants (Fig. 3B). Levels of

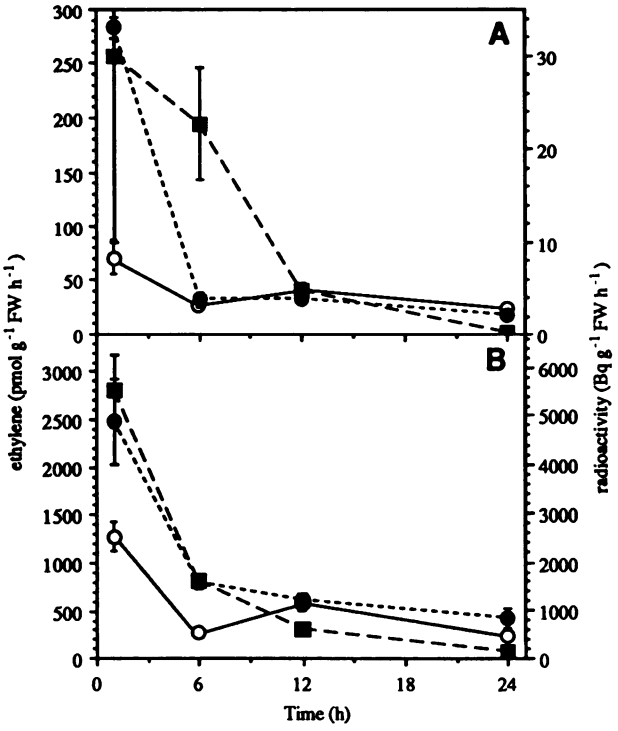

Figure 2. Ethylene release during 24 h from (A) shoots and (B) roots of 4-d-old sunflower seedlings. Nonradioactive C_2H_4 was estimated by GLC. Radioactivity associated with $^{14}C_2H_4$ was trapped in mercuric perchlorate and estimated by scintillation counting. O, control; ●, C_2H_4 from ACC treatment estimated by GLC, ■, radioactive C_2H_4. Error bars, SEM.

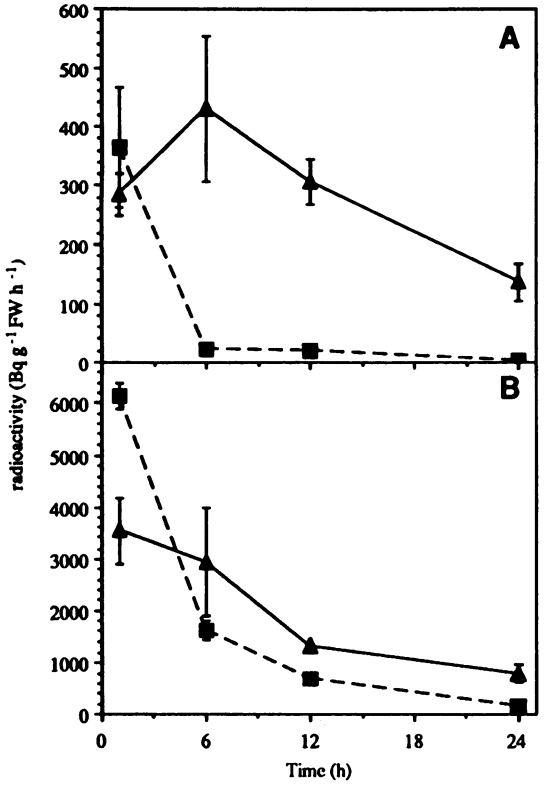

Figure 3. Release of gaseous radioactivity from (A) shoots and (B) roots of 4-d-old sunflower seedlings, after root treatment with 10 μM [2,3-^{14}C]ACC. ■, radioactive C_2H_4 ; ▲, radioactivity trapped by CO_2 cocktail.

both ethylene and the second gas were highest at 1 h (6127 and 3541 Bq g^{-1}fresh weight h^{-1}, respectively) and then steadily declined. Radioactive ethylene levels decreased rapidly from 1 to 6 h, whereas radioactivity associated with the second gas declined more slowly.

ACC and MACC Levels in Treated Seedlings

[^{14}C]ACC was present in both roots and shoots 0.5 h after the roots had been supplied with [^{14}C]ACC, and levels peaked at 1 h (Fig. 4). In shoots, [^{14}C]ACC levels decreased from 1 to 2 h and then remained constant until 3 h, and levels in roots decreased from 1 to 2 h and then increased again slightly until 3 h. MACC levels increased steadily in both organs during the 3-h period. Shoot [^{14}C]MACC levels surpassed peak [^{14}C] ACC levels at 2 h and continued to increase until 3 h (Fig. 4A), whereas root [^{14}C]MACC levels remained low relative to [^{14}C]ACC levels (Fig. 4B).

Subsequent 24-h experiments again showed the maximum amount of radioactivity in the ACC fraction from shoots 1 h after commencing treatment (Fig. 5A). Radioactivity in this fraction then declined rapidly from 1 to 6 h, thereafter slowly decreasing. Radioactivity in the MACC fraction increased fourfold from 1 to 12 h. At 12 h there was about 10 times as much radioactivity in the MACC fraction than there was in the ACC fraction. At the termination of the experiment, MACC levels decreased from the high at 12 h but were still 2.7 times higher than at the first sampling time of 1 h. Extracts from the roots showed a similar pattern except that they had higher levels of radioactivity (Fig. 5B). Radioactivity in the ACC fraction of roots was highest at 1 h and declined until 24 h. Radioactivity in the MACC fraction increased from low values at 1 h to a peak at 12 h and decreased slightly from 12 to 24 h.

Samples of ACC and MACC fractions from organs harvested at 1 and 12 h were subjected to TLC and autoradiography to determine the number and identity of labeled compounds present in each fraction. The two major radioactive zones co-chromatographed with either standard [^{14}C]ACC or [^{14}C]MACC, and, with only one exception, we found that the results were in full agreement with the studies shown in Figures 4 and 5. The exception was that there was a strongly labeled compound on cellulose TLC of the 1-h shoot ACC fraction that did not co-chromatograph with [^{14}C]ACC or [^{14}C]MACC. In all autoradiographs, other compounds were detected in minor amounts, some of which co-chromatographed with minor impurities in the [^{14}C]ACC and [^{14}C] MACC standards.

ACC and MACC Leakage from Roots

Leakage of radiolabeled compounds into 15 mL of one-quarter strength Hoagland solution was high at 1 h (194 ± 53 Bq g^{-1} fresh weight h^{-1}), low at 6 h (40 ± 15 Bq g^{-1} fresh weight h^{-1}), and stable from 12 to 24 h (93 ± 15 and 95 ± 36

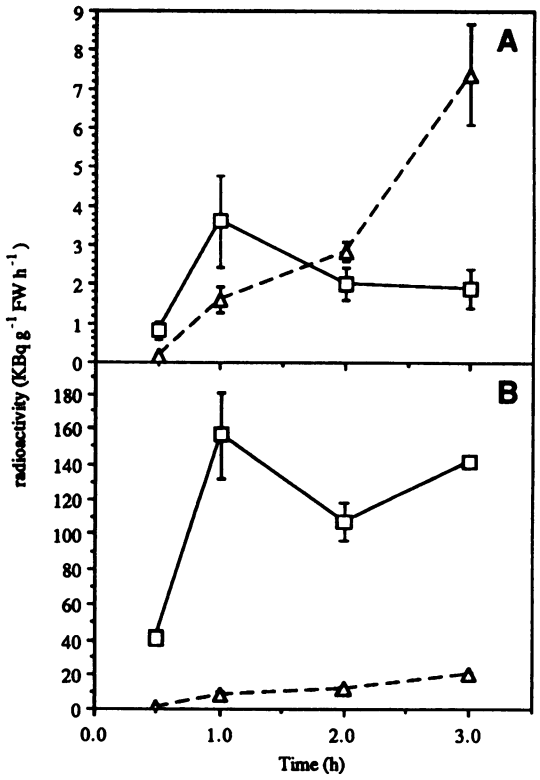

Figure 4. Radioactivity in ACC and MACC fractions from (A) shoots and (B) roots of 4-d-old sunflower seedlings after root treatment with 10 μM [2,3-^{14}C]ACC, measured during 3 h. □, ACC; △, MACC.

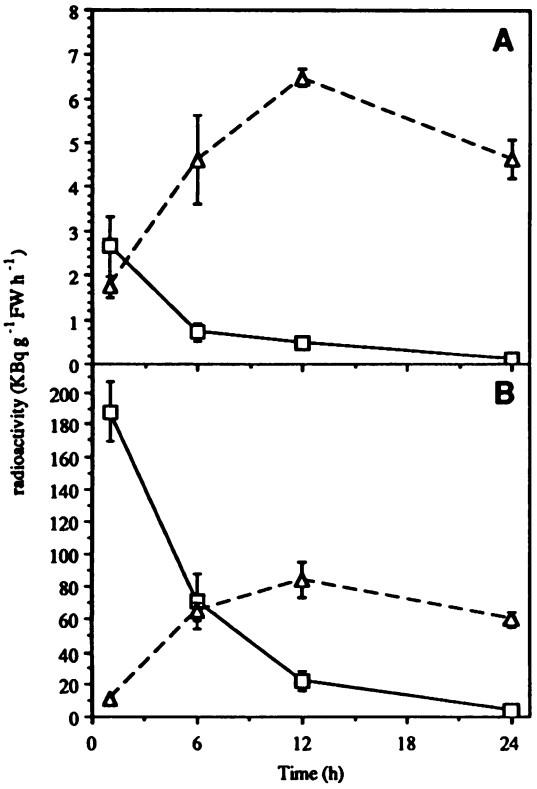

Figure 5. Radioactivity in ACC and MACC fractions from (A) shoots and (B) roots of 4-d-old sunflower seedlings after root treatment with 10 μM [2,3-^{14}C]ACC, measured during 24 h. □, ACC; △, MACC.

Bq g^{-1} fresh weight h^{-1}, respectively). Fractionation on cation exchange resin of a sample collected 2 h after the start of treatment showed 45% of the radioactivity in the ACC fraction and 55% in the MACC fraction.

Analysis of Xylem Sap

Xylem sap collected from seedlings treated with [^{14}C]ACC had large amounts of radioactivity in both the ACC and MACC fractions. Xylem sap collections during a 2- to 6-h period after feeding showed 1917 Bq mL^{-1} in the ACC fraction and 527 Bq mL^{-1} in the MACC fraction. TLC and autoradiographic analysis of this xylem exudate showed six compounds. The two major components co-chromatographed with ACC and MACC standards; 64% of the radioactivity was associated with [^{14}C]ACC and 23% with [^{14}C]MACC. GC-MS analysis positively identified the presence of ACC in xylem sap from [^{14}C]ACC-treated plants.

Xylem exudate obtained from untreated 11-d-old sunflower seedlings was found to contain ACC as shown by GC-MS (Fig. 6). MACC was not found in the xylem sap collected from these seedlings, nor was ACC detected in the MACC fraction after acid hydrolysis of this fraction.

DISCUSSION

Our experiments show that young sunflower seedlings readily take up ACC from a nutrient medium supplied to roots. In other ongoing studies, we find that the concentration of ACC fed to 4-d-old sunflower plants in these experiments (10 μM) is sufficient to cause a statistically significant 65% inhibition of root elongation (data not shown). Other characteristic ethylene effects such as epinasty and hypertrophy of shoots are not seen. The levels of ACC used in these experiments, therefore, represent physiological amounts and not pharmacological quantities.

It is well established that ACC is the direct ethylene precursor (2). We found substantial amounts of ethylene from roots 0.5 h after commencement of treatment with 10 μM [^{14}C] ACC, and ethylene evolution peaks at 1 h (Fig. 1B). After the peak of ethylene synthesis, ethylene production decreases in concert with declining ACC levels (Figs. 1B, 4B, and 5B) and increased ACC conjugation to MACC (Figs. 4B and 5B). This work agrees with previous reports (8, 14) in that the plants show a large capacity to malonylate ACC within a relatively short time (Figs. 4 and 5). Conjugation of ACC has previously been shown to reduce ethylene synthesis (24). Ethylene and MACC synthesis in the root, and transport of these compounds out of the root, reduced root ACC to low levels 12 h after treatment (Figs. 2B and 5B).

Transport of ACC to the shoot occurs rapidly after feeding (Fig. 4A); however, ACC levels in the shoot remain lower than those found in the root (Figs. 4 and 5). Shoot ACC levels are consistently lower than shoot MACC levels (Figs. 4A and 5A). This suggests that either ACC is metabolized immediately into ethylene and MACC upon arrival in the shoot or MACC itself is being exported from the root. TLC and autoradiographic analysis of the xylem sap of treated seedlings showed the presence of both [^{14}C]ACC and [^{14}C]MACC in the xylem sap. The presence of [^{14}C]ACC in the xylem exudate was confirmed by GC-MS analysis; however, [^{14}C]MACC content was not confirmed. We found that large quantities of MACC must be present in a sample before a clear mass spectrum can be obtained, because losses during precipitation of impurities and methylation are substantial. In untreated seedlings, MACC was not found in the xylem sap, nor was it found as

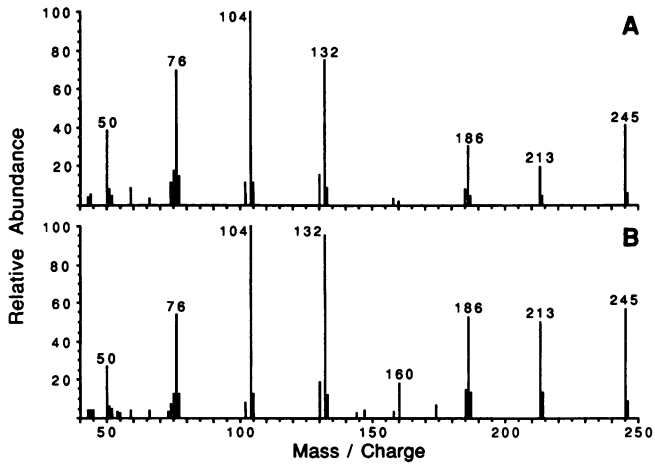

Figure 6. Mass spectra of (A) standard phthalidimo-ACC-methyl ester and (B) phthalidimo-ACC-methyl ester isolated from the xylem sap of 11-d-old untreated sunflower seedlings.

ACC after acid hydrolysis of the MACC fraction. Because GC-MS analysis of ACC in plant samples is fairly sensitive (20), we must conclude that MACC is not a component of xylem sap under the growing conditions used in these experiments. However, when roots are supplied with ACC, MACC is exported in the xylem. It remains to be seen whether MACC is in fact transported in the xylem under conditions that increase root ACC production (*i.e.* drought, flooding, wounding). That MACC can be exported across the plasmalemma (4), is exported from a site of wounding (10), and is found by us in the xylem of [^{14}C]ACC-treated seedlings as [^{14}C]MACC support the speculation that MACC may be a natural component of xylem sap under some conditions. MACC synthesis, long-term vacuolar MACC storage (4, 14), and dilution of ACC and MACC via transport throughout the whole plant may represent a complex system of regulation of ethylene levels in plant tissues.

MACC levels decrease from 12 to 24 h in both roots and shoots (Fig. 5). Possibly some of the MACC is being deconjugated to form ACC (19), although the possibility that MACC is being metabolized via an alternate route cannot be ruled out (28). MACC has been shown to be metabolized to ACC and ethylene in other systems (11, 19); however, the extent of this metabolism in sunflower seedlings has not been determined. In the roots, the leakage of [^{14}C]MACC contributed to the decreasing levels of [^{14}C]MACC found in this organ. The amount of leakage of [^{14}C]ACC and [^{14}C]MACC was substantial. If the leakage was due to residual [^{14}C]ACC in the root apoplasm, it would be expected that washing and time spent in the aeroponic mist would deplete the apoplasm of [^{14}C]ACC. Because large amounts of radioactivity are being released from the root over a long time period (24 h) and a substantial portion can be attributed to MACC (55% 1 h after treatment), this leakage is considered to arise from a symplastic source. Leakage of other compounds from roots in hydroponic and aeroponic environments has been observed (9, 18). Because almost every soluble compound present in plants can be found in root exudates (30), it is not unreasonable to expect ACC and MACC exudation as well.

Treatment of roots with [^{14}C]ACC was found to give rise to a second labeled gas in both roots and shoots. Our working hypothesis is that this compound was $^{14}CO_2$, because the second labeled volatile compound has properties similar to those expected of CO_2: it is absorbed in a cocktail designed to trap CO_2 and it is not absorbed in mercuric perchlorate (and is therefore not an olefin [1]). It is well established that the carboxylic acid group of ACC is released as CO_2 during ACC conversion to ethylene (23). However, the [^{14}C]ACC used in these experiments was labeled on carbon atoms 2 and 3 of the cyclopropane ring. The possibility exists that some of the [^{14}C]ACC was labeled in the carboxylic acid position; however, the release profiles of $^{14}C_2H_4$ and this second labeled substance differ substantially (Fig. 3), indicating that different precursors are involved for these compounds. It is also possible that one or more of the impurities in the [^{14}C]ACC solution (98.5% radiochemical purity) may be precursors of this unidentified gas. However, the amount of this compound released exceeds the amount of impurities supplied to the plants. Although the existence of an as yet uncharacterized pathway of MACC degradation has been proposed (28), it is unlikely that the gas is produced from [^{14}C]MACC, because the levels of [^{14}C]MACC increase in both roots and shoots and peak (Figs. 4 and 5) 6 h after the peak of release of the labeled volatile compound (Fig. 3). $^{14}C_2H_4$ may be metabolized to $^{14}CO_2$ (3); however, the amount of labeled gas produced is in excess of the amount of $^{14}CO_2$ expected from the level of $^{14}C_2H_4$ evolved in these experiments (3). These results suggest that an alternate metabolic pathway for ACC degradation exists. The intermediate steps in the pathway and the significance of production of this second gas from [^{14}C]ACC were not investigated further, because production and sale of [^{14}C]ACC ceased during the course of these experiments.

Transport of [^{14}C]ACC and [^{14}C]MACC from root to shoot occurs rapidly in [^{14}C]ACC-treated plants. ACC is a natural component in the xylem in whole, unstressed sunflower seedlings. This work substantiates that of Bradford and Yang (7) and gives credence to the proposal that ACC is used within the whole plant as a means of interorgan communication. MACC transport occurs under some conditions, although the function of MACC as a signal is uncertain. It was surprising to find that carbon(s) 2 and/or 3 of ACC were metabolized into a gas other than C_2H_4, possibly CO_2.

ACKNOWLEDGMENT

We thank Dr. R. Horgan for his advice concerning the methods of purification and GC-MS identification of ACC and MACC.

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