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Ethylene Biosynthesis and Accumulation under Drained and Submerged Conditions

A Comparative Study of Two *Rumex* Species

Minke Banga*, E. Judith Slaa, Cornelis W. P. M. Blom, and Laurentius A. C. J. Voesenek

Department of Ecology, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands

A model is presented of the regulation of ethylene biosynthesis in relation to submergence and flooding resistance. It is based on time-course measurements of ethylene production, ethylene accumulation, and concentrations of free and conjugated 1-aminocyclopropane-1-carboxylic acid (ACC) in submerged and drained flooding-resistant *Rumex palustris* Sm. and flooding-sensitive *Rumex acetosella* L. plants. From these data, in vivo reaction rates of the final steps in the ethylene biosynthetic pathway were calculated. According to our model, submergence stimulates ACC formation and inhibits conversion of ACC to ethylene in both *Rumex* species, and as a result, ACC accumulates. This may explain the stimulated ACC conjugation observed in submerged plants. Although submergence inhibited ethylene production, physical entrapment increased endogenous ethylene concentrations in both flooding-resistant *R. palustris* and flooding-sensitive *R. acetosella* plants. However, *R. palustris* plants controlled their internal ethylene levels in the long term by a negative regulation of ACC synthase induced by ethylene. In flooding-sensitive *R. acetosella* plants, absence of negative regulation increased internal ethylene levels to more than $20 \mu\text{L L}^{-1}$ after 6 d of submergence. This may accelerate the process of senescence and contribute to their low level of flooding resistance.

River forelands are flooded frequently during the growth season, and terrestrial plants that live in these habitats are able to survive these extreme conditions because of morphological and physiological adaptations (Blom et al., 1994; Voesenek and van der Veen, 1994). The adaptive trait-enhanced shoot elongation enables completely submerged plants to restore leaf-air contact and to relieve the severe stress of oxygen deprivation and impaired photosynthesis (Ridge, 1987; Armstrong et al., 1994). This phenomenon is found in a wide variety of amphibious plant species but has been studied most extensively in *Ranunculus sceleratus* (Musgrave and Walters, 1973), *Oryza sativa* (Métraux and Kende, 1983), and *Rumex palustris* (Voesenek and Blom, 1989). In addition, within a few hours of submergence, *R. palustris* plants raise their leaves to a more vertical position (Voesenek and Blom, 1989).

Submergence-induced shoot elongation and the reorientation of leaves are initiated by high levels of ethylene (Ku

et al., 1970; Musgrave et al., 1972; Samarakoon and Horton, 1981; Métraux and Kende, 1983; Ridge, 1987; Voesenek and Blom, 1989). Ethylene is synthesized from Met via AdoMet and ACC, and the reaction from AdoMet to ACC is catalyzed by ACC synthase. ACC can either be converted to ethylene by ACC oxidase or be conjugated by *N*-malonyltransferase to MACC (Yang and Hoffman, 1984; Kende, 1993; Zarembinski and Theologis, 1994) or by γ -glutamyltranspeptidase to GACC, as was recently discovered in tomato fruit (Martin et al., 1995). It is generally believed that conjugation is irreversible (Hoffman et al., 1983). Under well-aerated (drained) conditions, ACC formation is usually the rate-limiting step in ethylene biosynthesis, and the amount of ACC synthase available determines the ethylene production rate. ACC oxidase is thought to be constitutive and excessive (Yang and Hoffman, 1984). However, ACC oxidase was recently found to be rate limiting in the shoots of waterlogged tomato plants (English et al., 1995).

In completely submerged plants, gas exchange between intercellular air spaces and the atmosphere is severely impeded. Physical entrapment of ethylene leads to high concentrations in plant tissues (Musgrave et al., 1972; Musgrave and Walters, 1973; Stünzi and Kende, 1989; Voesenek et al., 1993). Moreover, inhibited gas exchange causes deficiency of oxygen and carbon dioxide in shoots (Armstrong et al., 1994; Madsen and Breinholt, 1995). The changed concentrations of these three gases in submerged plants are likely to influence the regulation of ethylene biosynthesis and therefore ethylene production (Yang and Hoffman, 1984).

The objective of this study was to develop a model of the regulation of ethylene biosynthesis in relation to submergence and flooding resistance. The regulation of biosynthesis is accomplished by variation of both the availability (mol enzyme per g plant dry weight) and the specific activity (reaction rate per enzyme molecule) in vivo of the enzymes involved. To investigate the net result of these two parameters, we chose to determine in vivo reaction rates. This was accomplished by determining the time courses of ethylene production, ethylene accumulation,

Abbreviations: AdoMet, S-adenosyl-L-methionine; GACC, 1-(γ -glutamylamino)cyclopropane-1-carboxylic acid; MACC, 1-(malonylamino)cyclopropane-1-carboxylic acid.

* Corresponding author; e-mail minkeb@sci.kun.nl; fax 31-24-3652409.

and concentrations of free and conjugated ACC in submerged and drained plants of two *Rumex* species with contrasting flooding resistances. From the data, the in vivo reaction rates of the three final steps in the ethylene biosynthetic pathway, i.e. ACC formation, ACC conjugation, and conversion of ACC to ethylene, were calculated.

In this paper we report the simultaneous effects of submergence on the in vivo reaction rates of three steps in the ethylene biosynthetic pathway. In addition, a comparison of detailed balance sheets for ethylene biosynthesis of a flooding-resistant (*R. palustris*) and a closely related flooding-sensitive (*Rumex acetosella*) plant species is presented. The data obtained were related to the literature on the regulation of ethylene biosynthesis by oxygen, carbon dioxide, and ethylene. The model shows that in both *Rumex* species, ethylene production is changed in a similar way during the first hours of submergence. However, in the long term, flooding-resistant *R. palustris* plants are able to keep their internal ethylene concentration relatively low, whereas it increases strongly in flooding-sensitive *R. acetosella* plants.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Seeds of *Rumex palustris* Sm. and *Rumex acetosella* L. were collected from field populations around Nijmegen, The Netherlands. *R. palustris* Sm. is a well-adapted flooding-resistant species from frequently flooded field sites, whereas *R. acetosella* L. grows on dry, sandy soils that are never flooded under natural conditions. This flooding-sensitive species does not elongate its petioles upon submergence (Banga et al., 1995). The seeds were germinated on moist filter paper in Petri dishes under a 12-h day/12-h night regime at 25/10°C for 7 d (*R. palustris*) or 27/10°C for 10 d (*R. acetosella*) and a PPFD of 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (over the waveband 400–700 nm; Philips TL 8W/33). Seedlings were transplanted to 200-mL free-draining plastic pots filled with a sand:potting compost mixture (1:1, v/v) and grown for 16 to 26 d in a growth chamber (16 h day/8 h night; PPFD of 180 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Philips TLD 58W/84 and 400-W SON-T sodium lamps; 20°C; 40–70% RH). Plants were sprayed daily with approximately 10 mL of tap water.

Experimental Conditions

Plants that started the development of their fifth leaf were transferred to the laboratory (16 h day/8 h night; PPFD 180 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Philips TLD 26W/84; 22°C; 40–60% RH). Whole plants were used in all experiments.

Ethylene release and ethylene concentrations were measured by laser-driven photoacoustic spectroscopy (Voeselek et al., 1992). Prior to these measurements, the soil was gently removed from the roots and the plants were placed in 100-mL glass vials filled with ballotini (diameter 5.6 mm) and approximately 15 mL of tap water according to the procedure of Horton (1992). Only the lower parts of the root system were flooded. Removal of soil prevented interference of ethylene production and/or absorption by

soil microorganisms (Arshad and Frankenberger, 1990). Under submerged conditions, this procedure has no significant effects on shoot elongation or biomass production during a 4-d period (data not shown). Vials with plants were placed individually in glass cuvettes, and a cuvette without plant material was used as a zero-ethylene reference. To avoid interference of stress ethylene production, submergence was delayed for 16 h. Handling of the plants and cleaning of the root system causes a peak of ethylene production, which lasts for 8 to 10 h and is maximal after about 2 h (Voeselek and van der Veen, 1994).

Ethylene Release

Vials containing one *R. palustris* or two *R. acetosella* plants were placed in 1800-mL glass cuvettes filled with 1100 mL of tap water. Plants were kept above the water level by placing the vials on a stainless steel platform on top of a rod that was put through a water-tight seal at the bottom of the cuvette. By moving the rod downward, we could submerge the plant(s) without opening the cuvette. The cuvettes were connected to a continuous flow of ethylene-free air (flow rate 1 L h⁻¹) in line with the ultrasensitive photoacoustic detection system for ethylene. Measurement of ethylene release rates (nL h⁻¹) continued for 4 d, and submergence started after approximately 16 h. Since ethylene given off by submerged plants was measured in the air above the water surface, no ethylene could be detected during the early times following the start of the flooding treatment. During this period, part of the ethylene released by the plants dissolves in the water rather than being transported to the air phase above it. After approximately 17 h, a steady state is reached, during which one molecule of ethylene is given off to the air for every molecule that escapes from the plant into the water (Voeselek et al., 1993). Gas bubbles released by plants also transport small amounts of ethylene at a much faster rate from the plant to the air phase. An estimation of the masked underwater evolution rate of the plant during the first hours of submergence is included in Figure 2B. It was made by linear interpolation between the ethylene release rates just before the start of submergence and 17 h later when the above-mentioned steady state was reached.

After ethylene was measured, plants were oven dried (20 h, 105°C) and biomass was determined. Initial dry weights had been estimated from a comparable set of three plants. Since dry weight did not change much during the experiments, a linear interpolation was made between the two dry weight values. Ethylene release data were divided by this line to obtain ethylene release rates on a dry weight basis (nL h⁻¹ g⁻¹ dry weight). Measurements were replicated five times per species and treatment.

Ethylene Concentrations in Submerged Plants

Vials containing one *R. palustris* or two *R. acetosella* plants were placed in 600-mL glass cuvettes filled with 400 mL of tap water. Plants were kept above the water level as described above. Cuvettes were left open and were not yet connected to the flow-through system and the photoacous-

tic ethylene detection system. The next day, all plants were submerged for various durations from 1 to 144 h. Just before removal from submerged conditions, the cuvette was closed (Fig. 1) and connected to the flow-through system, which was then flushed with pure nitrogen (flow rate 4 L h^{-1}). During the next 10 min, the cuvette was wrapped in aluminum foil. Thereafter, the flow rate was decreased to 1 L h^{-1} , gas outlet "b" was closed, and water outlet "c" was opened. As soon as the water level was lowered from level I to level II (approximately 80 mL left), outlet "c" was closed, outlet "b" was opened, and the measurement was started. In this way plants were removed from submerged conditions in an atmosphere without oxygen. Under these conditions, ethylene production was completely prevented because oxygen is necessary for the conversion of ACC to ethylene by ACC oxidase (Adams and Yang, 1979). The ethylene that had accumulated in the plants during submergence was released within minutes and could be quantified without interference of ethylene production (Voeselek et al., 1993). Since the roots remained submerged, it is possible that not all accumulated ethylene was released from them. However, because the root system contains about 5% of the total internal gas volume of a plant, this can only slightly affect the results.

A linear relation had been determined between injections of known amounts (nL) of ethylene and the height of the

ethylene release peak obtained (nL h^{-1}). Using this relationship, we calculated the amount of entrapped ethylene, which escaped from plants after removal from submergence, from the height of the peak in ethylene release that was measured. Thereafter, the internal gas volume of the same plant(s) was determined by means of a modified pycnometer method (Jensen et al., 1969; Banga et al., 1995). By dividing the amount of entrapped ethylene (nL) by the internal gas volume (mL), we calculated the ethylene concentration ($\mu\text{L L}^{-1}$) in the intercellular gas spaces of a plant just before removal from submergence. All treatments were replicated three times.

Determination of Free and Conjugated ACC

After the plants were transferred to the laboratory, they were left untreated for 1 d to acclimatize them. The next day, one-half of the plants were completely submerged in tap water. Submerged plants were harvested 4, 8, 16, 24, and 48 h later; however, drained plants were harvested at only time 0 and time 48 h, since previous experiments (with harvests at 0, 0.5, 1, 2, 4, and 8 h) had demonstrated that their levels of free and conjugated ACC were constant in time. The soil was removed, the shoot and root systems were separated, and fresh weights were determined. Plant parts were wrapped in aluminum foil, frozen in liquid nitrogen, and stored at -20°C . The harvesting procedure took less than 10 min. A sample consisted of one (*R. palustris*) or two (*R. acetosella*) shoots or root systems, and three to six replicates were used per species, treatment, and plant part.

Plant material was homogenized and extracted twice in the cold with 4.5 mL of 96% (v/v) ethanol. After the sample was centrifuged, the supernatant was evaporated to dryness in a vacuum at 55°C (Speed-Vac [type RC10.10], Jouan, Saint Nazaire, France) and dissolved in 4 mL of dichloromethane. Free and conjugated ACC were extracted with 4 mL of distilled water. To quantify the concentration of conjugated ACC (i.e. MACC plus GACC), a portion of the aqueous extract was hydrolyzed in 1.84 N HCl at 100°C for 3 h and consequently neutralized with NaOH according to the method of Hoffman et al. (1983), who originally developed this method for determination of MACC concentrations. Martin et al. (1995) showed that GACC also is hydrolyzed by this treatment. ACC was determined using the method described by Lizada and Yang (1979) with internal standardization (standard ACC was from Sigma; standard MACC was a gift from Dr. G.H.L. Nefkens, Department of Organic Chemistry, University of Nijmegen, The Netherlands). ACC was chemically converted into ethylene, which was determined on a model 438A gas chromatograph with a Porapak Q column (length 100 cm; Chrompack International, Middelburg, The Netherlands) at 60°C , packed to 0.34 g cm^{-3} . The difference in ACC content before and after hydrolysis was taken as the concentration of conjugated ACC. Results with recovery percentages less than 70% for ACC, less than 60% for ACC after the hydrolysis procedure, and/or less than 45% for MACC after hydrolysis were discarded.

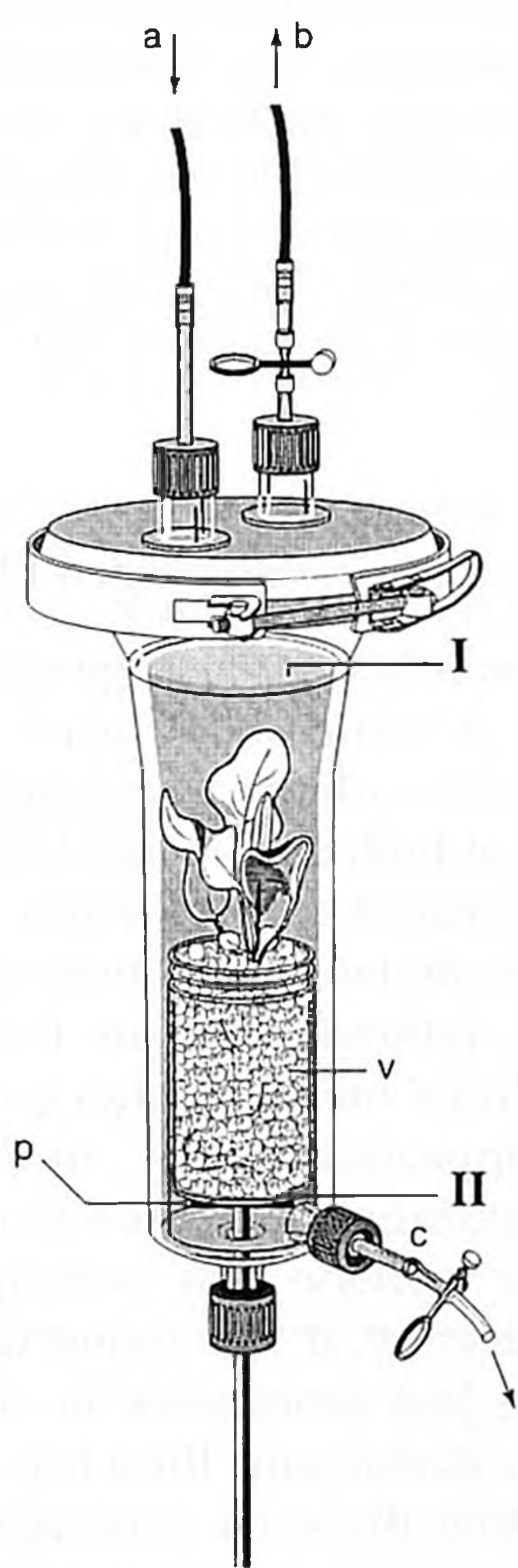


Figure 1. Cuvette (containing an *R. palustris* plant) used for the determination of endogenous ethylene concentrations. Inlet (a) and outlet (b) of the continuous gas (N_2) flow system. c, Outlet for water. p, Stainless steel platform connected to a rod that is put through a water-tight seal at the underside of the cuvette. v, Vial filled with ballotini and one *R. palustris* or two *R. acetosella* plants. I and II, Water levels.

From an extra set of comparable plants, shoot and root dry weights were determined after 0 and 48 h of control growth and after 24 and 48 h of submergence. After linear interpolation, these data were used to calculate concentrations on a dry weight basis. The experiment was repeated twice.

Calculations

Ethylene release data (including the estimation; $n = 4-5$), endogenous ethylene concentrations ($n = 3$), and levels of free ($n = 4-6$) and conjugated ($n = 3-6$) ACC of whole plants were combined to calculate in vivo reaction rates for ACC formation, ACC conjugation, and ethylene formation over known intervals of time, as follows:

AdoMet \rightarrow ACC, accumulation rate of (ACC + conjugated ACC + ethylene) + ethylene release rate; ACC \rightarrow GACC + MACC, accumulation rate of conjugated ACC; ACC \rightarrow ethylene, accumulation rate of ethylene + ethylene release rate.

RESULTS AND DISCUSSION

Ethylene Release

The use of photoacoustic spectroscopy made it possible to measure ethylene release of whole plants in relatively large cuvettes connected to a continuous flow system. This enabled us to monitor kinetics of ethylene release for a period of 4 d under drained and submerged conditions without artifacts due to excision of plant parts and the use of sealed vials.

Figure 2 shows representative examples of ethylene release from both *Rumex* species. Submergence inhibited ethylene release in plants of both species. *R. acetosella* plants, especially, showed extremely slow ethylene release rates during submergence. These results differ from those of Kende and co-workers, who found that partial submergence stimulated ethylene production in stem sections of rice (Métraux and Kende, 1983; Raskin and Kende, 1984). However, under partially submerged conditions, ACC may be transported from the anaerobic root system to well-aerated parts of the shoot, where it can be readily converted to ethylene (Bradford and Yang, 1980). This is not possible in our completely submerged plants.

During light periods, ethylene release was reduced, especially in drained *R. acetosella* and submerged *R. palustris* plants. It has been reported in the literature that light (Horton, 1985) and, more precisely, photosynthesis (Grodzinski et al., 1982) inhibit ethylene release. This can be explained by a limiting level of carbon dioxide in plants under light, because of photosynthetic carbon dioxide fixation. Carbon dioxide stimulates the specific activity of ACC oxidase but does not change the availability of this enzyme (Kao and Yang, 1982; Smith and John, 1993). In vivo ethylene production is saturated at carbon dioxide concentrations greater than 10 mL L^{-1} (Kao and Yang, 1982).

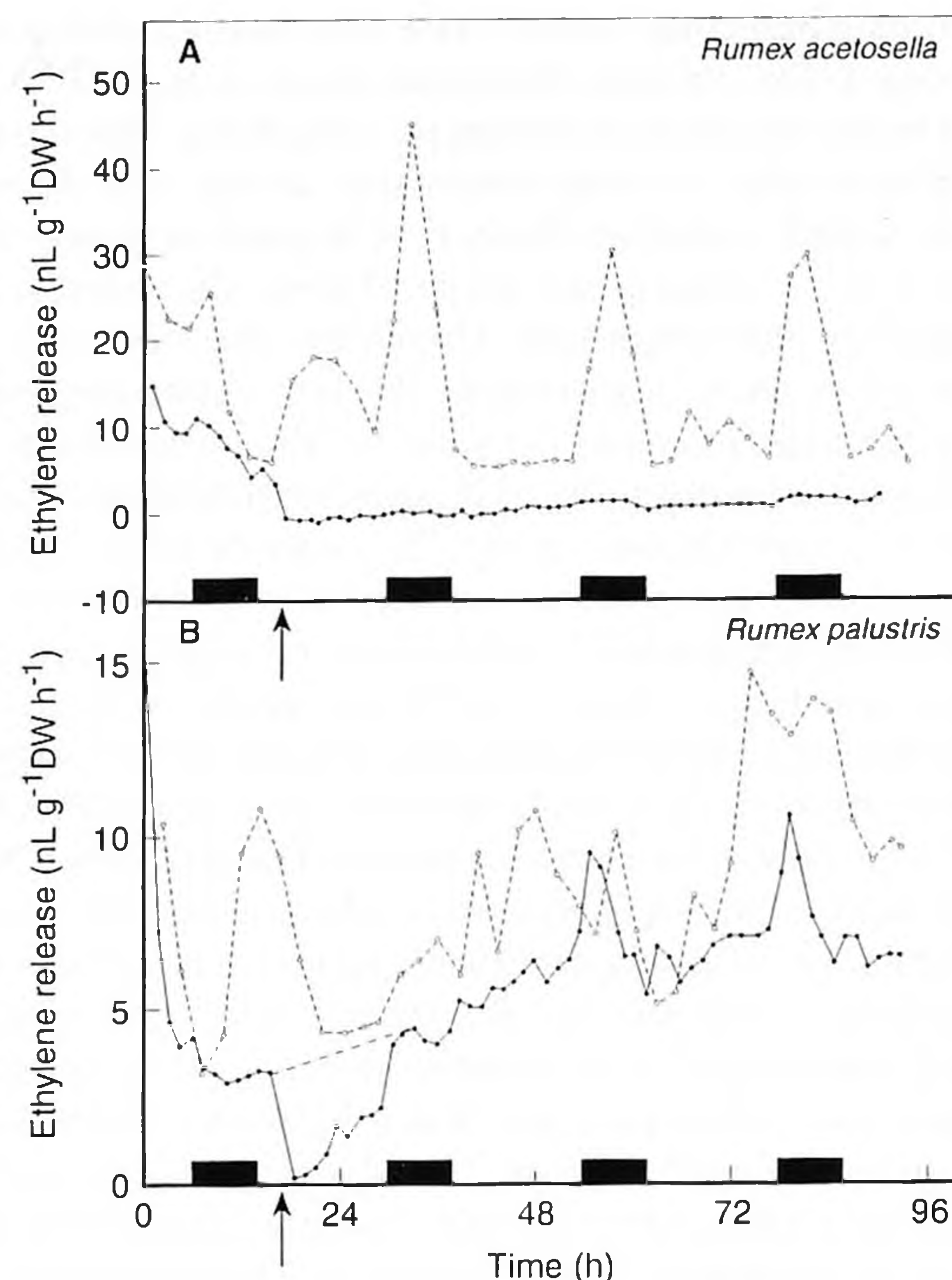


Figure 2. Ethylene release (nL g^{-1} dry weight h^{-1}) measured in the headspace of cuvettes containing drained (○) or submerged (●) plants of *R. acetosella* (A) or *R. palustris* (B). Cuvettes were connected to a continuous flow-through system. Ethylene release was quantified by laser-driven photoacoustic spectroscopy. The estimation (---) indicates the ethylene release of *R. palustris* plants shortly after the start of submergence (see "Materials and Methods"). Representative data (from $n = 5$) are shown. Arrows indicate the start of submergence. Black bars on the x axes indicate dark (night) periods. DW, Dry weight.

Ethylene Concentrations in Submerged Plants

Under drained conditions, endogenous ethylene levels are extremely low. A value of $0.05 \mu\text{L L}^{-1}$ has been found in drained *R. palustris* plants (Voeselek et al., 1993). In submerged plants of both *Rumex* species, ethylene concentrations rapidly increased to more than $1 \mu\text{L L}^{-1}$ within 1 h (Fig. 3). After 16 h, ethylene levels were about $8 \mu\text{L L}^{-1}$. In *R. palustris* plants, internal ethylene levels gradually decreased from the 2nd d onward. After 6 d of submergence, these plants still appeared healthy. In *R. acetosella* plants, the ethylene concentration increased strongly after 4 d of submergence. This increase was accompanied by visible leaf senescence. Recently, it was found that ethylene regulates the timing of leaf senescence in *Arabidopsis thaliana* and tomato plants (Grbić and Bleecker, 1995; John et al., 1995). It is likely that the early senescence of *R. acetosella* plants, when compared to *R. palustris* plants, is related to their extremely high ethylene levels during submergence. Because the process of senescence may not have been completely synchronized between individual *R. acetosella* plants, a high variability was observed among mean ethylene concentrations at this time. The endogenous ethylene levels reported here are in agreement with the concentra-

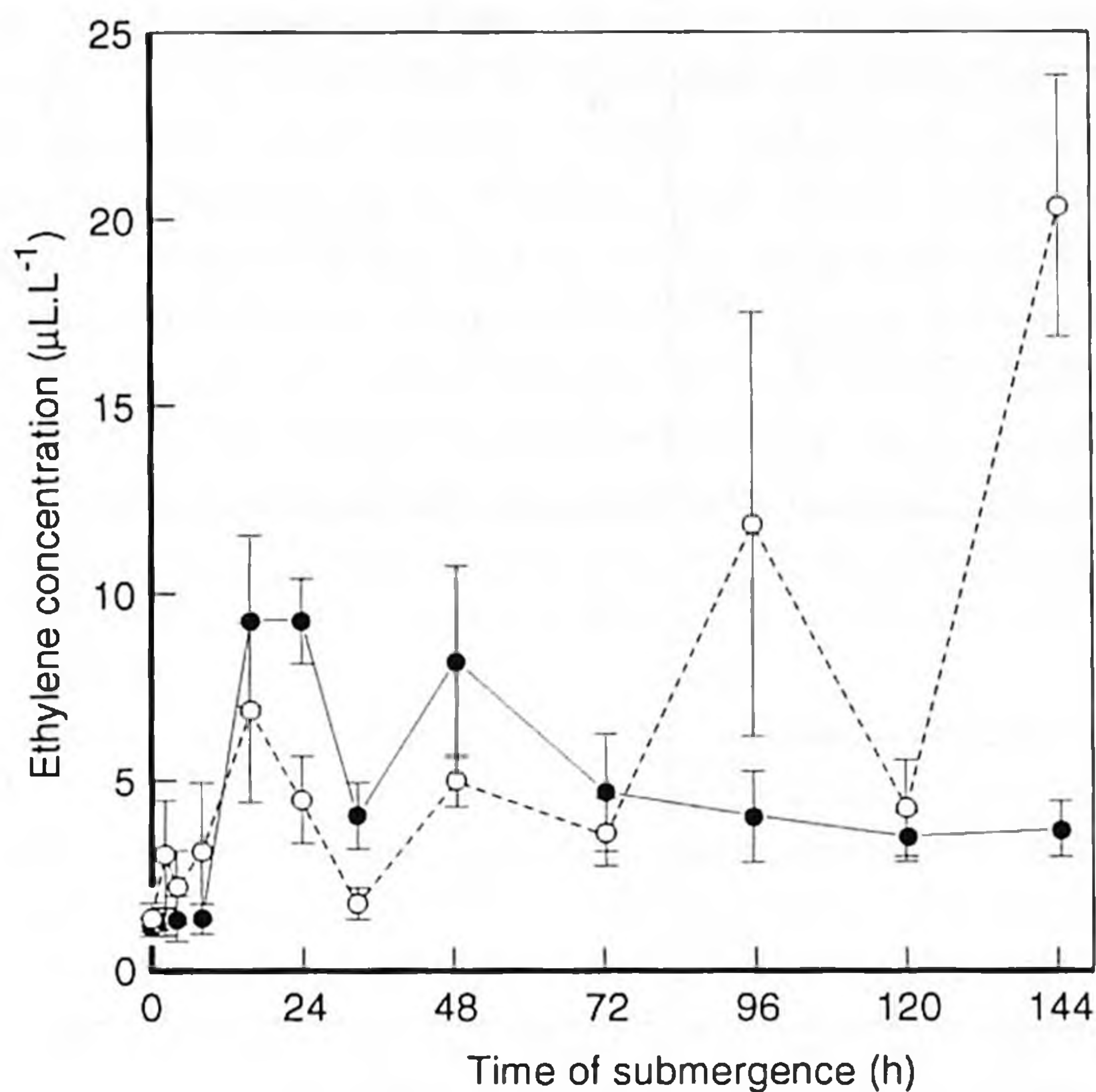


Figure 3. Mean (\pm SE; $n = 3$) endogenous ethylene concentrations ($\mu\text{L L}^{-1}$) of submerged *R. acetosella* (○) and *R. palustris* plants (●). The amount of accumulated ethylene (nL), which escaped from plants after removal from submerged conditions, was quantified by laser-driven photoacoustic spectroscopy and divided by the volume of intercellular gas spaces (mL). For further details, see "Materials and Methods."

tions measured in submerged plants of *Ranunculus sceleratus* ($7 \mu\text{L L}^{-1}$; Musgrave and Walters, 1973) and *Regnellidium diphyllum* ($3.1 \mu\text{L L}^{-1}$; Musgrave and Walters, 1974) and somewhat higher than the levels in submerged *Calitriche platycarpa* ($1.2 \mu\text{L L}^{-1}$; Musgrave et al., 1972) and partially submerged deepwater rice ($0.6\text{--}1 \mu\text{L L}^{-1}$; Métraux and Kende, 1983; Stünzi and Kende, 1989) plants.

Concentrations of Free and Conjugated ACC

In drained *Rumex* plants, ACC levels were rather constant. Submergence led to accumulation of ACC in *Rumex*

plants (Fig. 4). In shoots of both *Rumex* species, high ACC concentrations (5.5 and 11.0 nmol g^{-1} dry weight for *R. palustris* and *R. acetosella*, respectively) were found after 4 h of submergence. Thereafter, shoot ACC content decreased in *R. palustris*, whereas it increased greatly in *R. acetosella* plants during the 2nd d of submergence. ACC accumulation (to 11.4 nmol g^{-1} dry weight) in roots was observed in only submerged *R. palustris* plants. Again, variation between plants in the timing of senescence may explain the large SEs of ACC (and conjugated ACC; Fig. 5) concentrations of *R. acetosella* plants after 48 h of submergence. Comparable ACC accumulation was found in internodes of partially submerged rice plants (Métraux and Kende, 1983). In *R. sceleratus* leaves, submergence caused ACC to accumulate to even higher levels (almost 3 nmol g^{-1} fresh weight after 24 h of submergence; Samarakoon and Horton, 1984).

In *Rumex* plants, the level of conjugated ACC was much higher than that of free ACC and increased with the duration of submergence (Fig. 5). This is expected, since it is generally believed that conjugation of ACC is an irreversible reaction and that conjugated ACC is a stable end product (Hoffman et al., 1983). In shoots and roots of *R. palustris*, conjugation of ACC occurred mainly between 4 and 8 h of submergence; however, in *R. acetosella* plants, ACC conjugation continued, especially in the roots.

Balance Sheets for Ethylene Biosynthesis

All data were combined to generate an overview of ethylene biosynthesis in the two *Rumex* species, contrasting in flooding resistance, under drained and submerged conditions. Similar balance sheets of ethylene synthesis were formerly constructed by Pearce et al. (1992) for deepwater rice and *Echinochloa oryzoides* seedlings exposed to various oxygen concentrations.

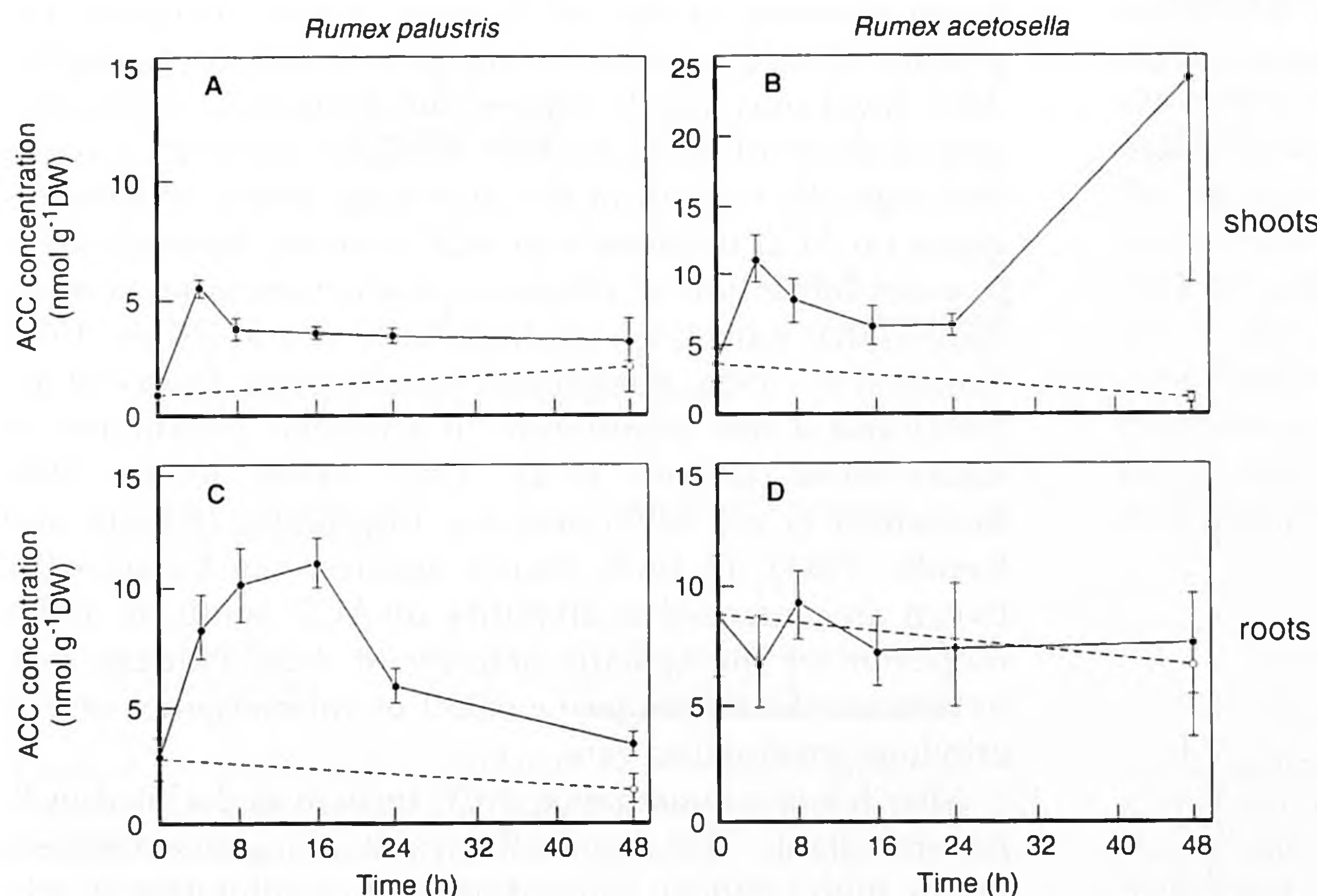
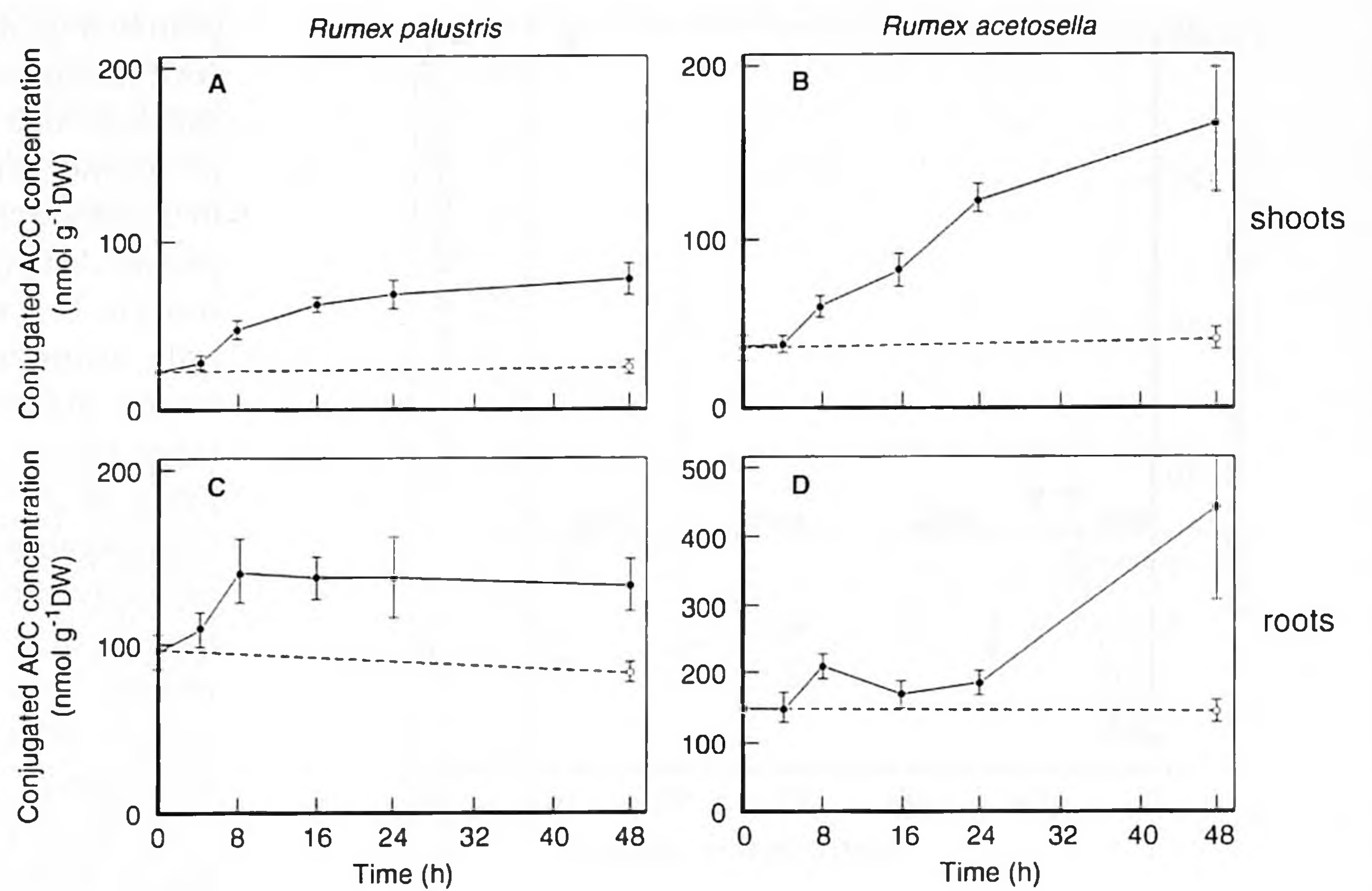


Figure 4. Mean (\pm SE; $n = 4\text{--}6$) ACC concentrations (nmol g^{-1} dry weight) in shoots and roots of *R. palustris* (A and C) and *R. acetosella* (B and D) plants under drained (○) and submerged (●) conditions. Mean recovery \pm SE was $96 \pm 1\%$. Note that the y axis of B has a different scale. DW, Dry weight.

Figure 5. Mean (\pm SE; $n = 3-6$) concentrations of conjugated ACC (nmol g⁻¹ dry weight) in shoots and roots of *R. palustris* (A and C) and *R. acetosella* (B and D) plants under drained (○) and submerged (●) conditions. After hydrolysis, mean recoveries \pm SE were 97 ± 1 and $66 \pm 1\%$ for ACC and MACC, respectively. Note that the y axis of D has a different scale. DW, Dry weight.



Calculated in vivo reaction rates of ACC formation, ACC conjugation, and ethylene formation in *Rumex* plants during 2 d of submergence are presented in Table I. In drained *Rumex* plants, the rates of ACC formation and its conversion to ethylene were about equal and (almost) no conjugation of ACC occurred. In both species, the rate of ACC formation was closely related to the rate of ethylene production under these conditions.

Upon submergence ACC formation was strongly stimulated, whereas the conversion of ACC to ethylene was inhibited in both species. This inhibition was much stronger in *R. acetosella* plants. Under these conditions, this final step in the ethylene biosynthetic pathway determined the ethylene production rate. In completely submerged terres-

trial plants oxygen and carbon dioxide deficiency are the main problems for survival (Armstrong et al., 1994; Madsen and Breinholt, 1995). Both carbon dioxide and oxygen are necessary for the conversion of ACC to ethylene (Kao and Yang, 1982; Yip et al., 1988), although carbon dioxide is not part of this reaction (Yang and Hoffman, 1984). Therefore, carbon dioxide and oxygen deficiency reduce the specific activity of ACC oxidase. However, low oxygen levels have been found to stimulate ACC formation in deepwater rice and *Echinochloa oryzoides* (Cohen and Kende, 1987; Pearce et al., 1992) and to increase the availability of ACC synthase in tomato plants (Wang and Arteca, 1992). Therefore, a stimulation of ACC formation is expected during submergence.

Since ACC and oxygen are co-substrates for ACC oxidase, the affinity of this enzyme for one substrate is increased by high levels of the other (Yip et al., 1988). Thus, an increased availability of ACC synthase can lead to an increase of the ACC level, which increases the affinity of ACC oxidase for oxygen. In this way a higher ACC level may partly relieve the reduction of the specific activity of ACC oxidase brought about by oxygen shortage. As a result of the opposing effects of submergence on ACC synthase and ACC oxidase, hypoxia leads to a net inhibition of ethylene production in most plant species that have been studied (Saltveit and Dilley, 1978; Konze et al., 1980; Raskin and Kende, 1983; Pearce et al., 1992) and a net stimulation of ethylene production in maize roots (Jackson et al., 1985; Atwell et al., 1988; Brailsford et al., 1993) and rice internodes (Raskin and Kende, 1984). In both *Rumex* species, the balance between an increased availability of ACC synthase and a reduction of the specific activity of ACC oxidase may determine the net negative effect of submergence on the ethylene production rate.

After 8 h of submergence, ACC formation declined in *R. palustris* plants. This may be the result of negative feedback by the high ethylene concentration. Autoinhibition of eth-

Table I. In vivo reaction rates of the three final steps of the ethylene biosynthetic pathway under drained and submerged conditions in two *Rumex* species

Species and Treatment	AdoMet → ACC	ACC → GACC + MACC	ACC → C ₂ H ₄
<i>R. acetosella</i>			
Drained 0–48 h	0.4	0.1	0.3
Submerged			
0–4 h	3.5	2.1	0.1
4–8 h	5.5	5.9	0.0
8–16 h	2.2	2.2	0.0
16–24 h	4.7	4.7	0.0
24–48 h	4.0	3.8	0.1
<i>R. palustris</i>			
Drained 0–48 h	0.5	0.0	0.5
Submerged			
0–4 h	3.7	2.3	0.2
4–8 h	3.7	4.0	0.1
8–16 h	1.1	0.9	0.2
16–24 h	1.6	1.4	0.2
24–48 h	0.7	0.4	0.3

ylene production has been found in rice stem sections (Bleecker et al., 1987) and in wounded etiolated pea seedlings (Saltveit and Dilley, 1978). Moreover, ethylene-insensitive mutants of *A. thaliana*, such as *etr1* and *ein2*, are ethylene overproducers (Ecker, 1995). In grapefruit flavedo discs, autoinhibition occurs after exogenous ethylene application ($1 \mu\text{L L}^{-1}$ ethylene for 5 h or more; Riov and Yang, 1982) and results from inhibition of ACC formation and increased availability of conjugating enzymes (Liu et al., 1985a). In *R. palustris* plants, however, the decline in ACC formation was accompanied by a decreased ACC conjugation.

In *Rumex* plants, the factor that causes conjugation of ACC seemed to be a high ACC level rather than a high ethylene concentration. Especially in plants of *R. palustris*, the highest conjugation rate was observed between 4 and 8 h of submergence, which was immediately after the peak in whole-plant ACC content (data not shown). However, the endogenous ethylene level did not increase above $1 \mu\text{L L}^{-1}$ in these plants until they had been submerged for 8 h. Between 8 and 16 h of submergence, the strongest increase of the ethylene concentration occurred, and it remained well over $4 \mu\text{L L}^{-1}$ during the next days (Fig. 3). These results suggest that ACC conjugation was stimulated mainly by high ACC concentrations; the possibility that ethylene stimulates ACC conjugation only if the ACC level is high, as found in preclimacteric tomato fruits (Liu et al., 1985b), cannot be ruled out.

A Model of the Regulation of Ethylene Biosynthesis in Relation to Submergence and Flooding Resistance

A model of ethylene biosynthesis in submerged *Rumex* plants is given in Figure 6. In both flooding-resistant *R. palustris* and flooding-sensitive *R. acetosella* plants, submergence stimulates ACC synthase (availability) and inhibits (the in vivo specific activity of) ACC oxidase. As a result, ACC accumulates (Fig. 4), stimulating ACC con-

jugation (Fig. 5). A high ethylene level may be a prerequisite for this stimulation of ACC conjugation by high ACC concentrations. The net ethylene production rate is inhibited by submergence (Fig. 2), but physical entrapment of ethylene during submergence leads to high endogenous concentrations of this hormone in both *Rumex* species (Fig. 3).

In flooding-resistant *R. palustris* plants, this causes a more vertical orientation of leaves and a greatly enhanced petiole elongation (Voeselek and Blom, 1989; Banga et al., 1995). Furthermore, after 8 h of submergence, high ethylene concentrations negatively regulate ACC synthase in *R. palustris* plants. As a consequence, the ethylene release rate remains at a constant level in the long term (Fig. 2B) and a decline of the internal ethylene concentration is observed from the 4th d of submergence onward. However, in flooding-sensitive *R. acetosella* plants, high ethylene levels do not cause a shoot elongation response (Banga et al., 1995). Moreover, the rate of ACC formation remains high in this species. Consequently, the ACC level again increases greatly during the 2nd d of submergence (Fig. 4B). The resulting extremely high ACC level overcomes the earlier suppression of ACC oxidation to ethylene and leads to an increase of ethylene release in the long term (data not shown) and an increase of the ethylene concentration to very high levels from the 4th d of submergence on (Fig. 3). This may accelerate the onset of leaf senescence.

CONCLUSIONS

Submergence causes the physical entrapment of ethylene, which leads to high ethylene levels in both flooding-resistant *R. palustris* and flooding-sensitive *R. acetosella* plants. This induces a stimulation of shoot elongation only in *R. palustris* plants. Moreover, the ability of this flooding-resistant species to control its internal ethylene level in the long term by negative regulation of ethylene synthesis may form an essential part of its flooding resistance. In this way, the endogenous ethylene level is high enough to saturate petiole elongation but at the same time low enough to avoid the early onset of senescence. Flooding-sensitive *R. acetosella* plants seem to lack the negative regulation of ethylene biosynthesis. The uncontrolled increase of the endogenous ethylene level may be linked to the early senescence and thus contribute to the low flooding resistance of this species. It is conceivable that the mechanism of negative regulation of ethylene synthesis and control of internal ethylene levels, as summarized in the model, is also present in other flooding-resistant plant species.

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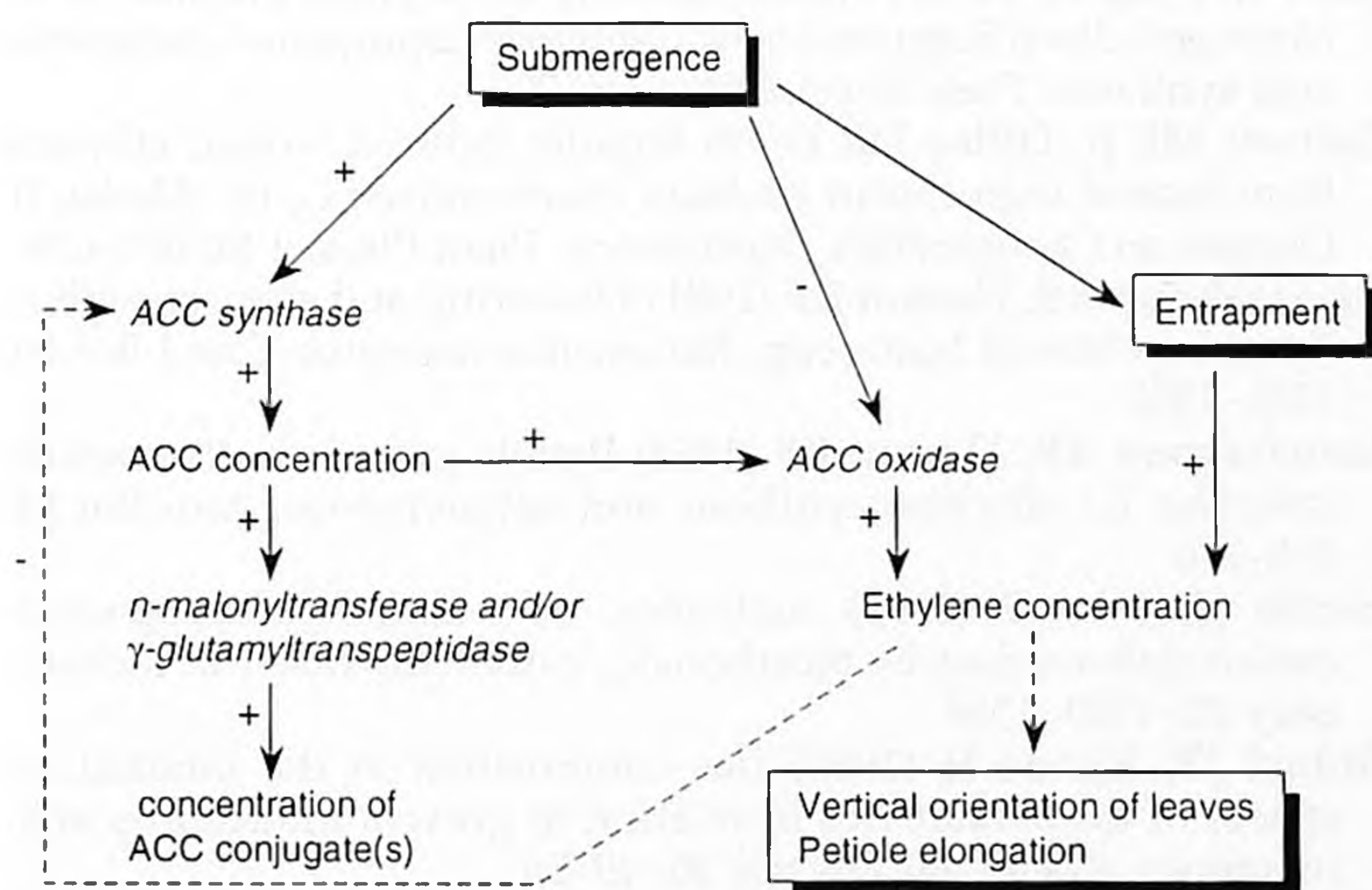


Figure 6. A model of the regulation of ethylene biosynthesis in submerged flooding-resistant *R. palustris* and flooding-sensitive *R. acetosella* plants. -->, Valid for *R. palustris*. -, Inhibition; +, stimulation.

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