Potamogeton pectinatus Is Constitutively Incapable of Synthesizing Ethylene and Lacks 1-Aminocyclopropane-1-Carboxylic Acid Oxidase[1]

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A highly sensitive laser-driven photoacoustic detector responsive to ≤ 2.1 nmol m^{-3} ethylene (50 parts per trillion [v/v]) was used for ethylene analysis. Dark-grown plants of Potamogeton pectinatus L. growing from small tubers made no ethylene. Exposure of shoots to white light, wounding, submergence in water followed by desubmergence, partial oxygen shortage, indole acetic acid, or carbon dioxide failed to induce ethylene production, although clear effects were observed in Pisum sativum L. Some ethylene was released after applying high concentrations of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC; 10 mol m^{-3}) to P. pectinatus, but the amount was trivial compared with that released by P. sativum. More endogenous ACC was found in P. pectinatus than in P. sativum. Considerable ACC oxidase activity was present in tissue extracts of P. sativum. However, no ACC oxidase activity was found in P. pectinatus, indicating that this is where ethylene production is arrested.

Ethylene is one of the major hormones regulating many aspects of plant growth and development. Although its physiological activity at small concentrations was recognized more than 90 years ago (Neljubov, 1901), rigorous identification of ethylene, as a product of metabolism, was not achieved until 1934, when Gane chemically identified the gas as a product of ripening Worcester Pearmain apples. In subsequent years, various methods, including bioassays (Denny and Miller, 1935), GC (Huelin and Kennet, 1959), MS (Turner et al., 1975), and, more recently, photoacoustic spectroscopy (Harren et al., 1990), have shown that all vegetative and reproductive plant parts appear to synthesize ethylene. As far as we can determine from the literature (Yang, 1984; Osborne, 1989; Mattoo and White, 1991; Abeles et al., 1992), no species of higher plant, nor any growing organ or tissue, has been found incapable of synthesizing ethylene, unless oxygen is withdrawn. Thus, ethylene has come to be regarded as an essential component of the hormone compliment needed for normal growth and development by plants. For example, most aquatic and semiaquatic species examined (Musgrave et al., 1972; Jackson, 1985; Kende, 1987; Pearce et al., 1992; Voesenek et al., 1992) produce ethylene in amounts sufficient to accelerate elongation under water. Thus, by means of ethylene action, shoots quickly regain contact with better lit and aerated conditions if they become totally submerged by rising water levels.

In all vascular plants reported to date, a Met-based pathway predominates (Adams and Yang, 1979). Key steps in this pathway include the enzymic conversion of S-adenosyl-L-Met to ACC by ACC synthase and the oxidation of ACC to ethylene by ACC oxidase. However, in some ferns, mosses, lycopods, and microorganisms, other pathways can operate (Osborne, 1989).

Against this background, we were surprised by our preliminary experiments (Summers and Jackson, 1993), the results of which suggested that growing shoots of overwintering tubers of Potamogeton pectinatus, a common monocotyledonous aquatic weed of temperate climates (Devlin and Karczmarczyk, 1975), do not appear to synthesize ethylene. These results were obtained by conventional flame-ionization GC, a technique capable of routinely detecting above approximately 0.4 μmol m^{-3} in 10^{-6} m^3 (0.01 parts per million, v/v). However, as is common practice with this approach, ethylene production was sought after first sealing excised pieces of tissue into small containers for at least 1 h prior to head space analysis. Unfortunately, this method can give erroneous data for a variety of reasons (Morgan et al., 1990; Brailsford et al., 1993), including wound-stimulated ethylene production (Jackson and Campbell, 1976) and separation from metabolism-sustaining plant parts, such as a starchy tuber in the case of P. pectinatus (Summers and Jackson, 1994).

In the present work, we overcame the problems of conventional head space analysis associated with GC by using a more sensitive ethylene detector. This allowed groups of two to six intact plants to be monitored on-line in a slowly flowing stream of moist air. Any ethylene released by the

Abbreviation: ppt, parts per trillion.

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plants into the air stream was quantified using a laser-driven photoacoustic detector that was at least 200 times more sensitive than flame-ionization GC and had a working sensitivity of approximately 2 nmol m^{-3} ethylene (50 ppt, v/v). By this means we demonstrate, with the maximum available rigor, that rapidly growing *P. pectinatus* shoots are unable to produce ethylene in air, even when exposed to treatments that stimulate biosynthesis of the gas in other species.

MATERIALS AND METHODS

Plant Material

Tubers of *Potamogeton pectinatus* L., each bearing a prominent apical shoot (Summers and Jackson, 1994), were collected from an established perennial stand in the River Evenlode (Oxfordshire, UK) in September 1993 and stored in the dark for up to 6 months at 2°C in wet, coarse gravel. Before use, tubers were surface sterilized in 2% (v/v) commercial sodium hypochlorite solution and rinsed in distilled water, and two to six individuals (approximately 1 g fresh weight) were placed into 30 × 10^{-6} m^{-3} glass cuvettes and held under water by glass ballotini (English Glass Company, Leicester, UK) at 20°C for approximately 24 h. By this time, shoot elongation had started. Cuvettes were then drained and connected to a flow of moist air (1 × 10^{-3} m^3 h^{-1}) from which ethylene and other hydrocarbons were first removed using a platinized catalyst heated to 400°C (Voesenek et al., 1992) and alkaline potassium permanganate adsorbed onto silica gel (Stayfresh, London, UK). Any chemical or gas treatments were given to the plants at least 15 h after first connecting the cuvette to a laser-driven intracavity photoacoustic detector tuned for maximum ethylene absorbance (Harren et al., 1990). *Pisum sativum* L. cv Eminent seeds were soaked in distilled water overnight and grown in the dark for 5 d in moist vermiculite at 20°C. Two seedlings with approximately 50-mm-long shoots were derooted, transferred to a cuvette, and held down with glass ballotini and sufficient water to cover the seed. The cuvette was then connected without delay to a supply of moist air and the photoacoustic detector.

Ethylene Detection

A cuvette containing elongating shoots of *P. pectinatus*, each attached to their tuber (two to six individuals), and an empty reference cuvette were connected in parallel to the humidified air flow. At 20- to 30-min intervals, the outflows from each of the two cuvettes were switched alternately to the photoacoustic detector (Harren et al., 1990). When continuous measurements on one cuvette were needed, e.g. when output of ethylene from pea plants was changing rapidly, a cuvette containing plant material was sampled every 90 s for up to 4 h. Unless otherwise stated, flow rates were 1 × 10^{-3} m^3 h^{-1}. The experiments with *P. pectinatus* were repeated with *P. sativum* using two 5-d-old, dark-grown, derooted seedlings per cuvette. All experiments were performed at least twice. Plants were handled either in the dark or under dim-green light. Ethylene out-

put data are pmol kg^{-1} fresh weight s^{-1} (1 pmol kg^{-1} s^{-1} = 0.0964 nL g^{-1} h^{-1}).

Chemical Treatments

Fifteen hours after connection to the photoacoustic detector, plants within the cuvettes were completely submerged in 20 × 10^{-6} m^3 of distilled water and 10 mol m^{-3} ACC or 0.1 mol m^{-3} IAA. The solutions were injected into the cuvettes through a needle inserted through a septum. The cuvettes remained closed and connected to the detector, and ethylene released from solution-covered plants continued to be monitored for 4 h. Treatment solutions were then withdrawn through the needle, and ethylene subsequently released from the desubmerged plants was monitored for approximately 20 h.

Gas Treatments

After 15 h the inflow of air through cuvettes was switched either to nitrogen containing 5 kPa oxygen or to air enriched with CO$_2$ to 10 kPa. These treatments lasted 6 h, after which time the inflow was switched back to air.

Wounding

Between 10 and 12 shoots of *P. pectinatus* or *P. sativum* were placed in the cuvettes and allowed to acclimatize for 15 h. Plants were removed from the cuvette and shoots were wounded by lightly crushing with a small roller or scoring longitudinally with a needle. Shoots were then returned to the cuvettes and reconnected to the flow-through system and flushed through with ethylene-free air for 5 min before ethylene measurements were recommenced.

Assay for Endogenous ACC

Concentrations of ACC in *P. pectinatus* subjected to the various submergence or chemical treatments were measured using a method modified from Lizada and Yang (1979). In brief, 0.25 g fresh weight of tissue was frozen in liquid nitrogen, ground dry with sand in a mortar and pestle on ice, reground with 4 × 10^{-6} m^3 of 96% (v/v) ice-cold ethanol, and centrifuged at 1500g for 5 min. The pellet was extracted in 4 × 10^{-6} m^3 of 96% (v/v) ethanol prior to recentrifugation. The supernatants were combined and evaporated to dryness. The residue was taken up in 8 × 10^{-6} m^3 of 50% (v/v) dichloromethane and thoroughly mixed. After the sample had undergone phase separation, 0.6 × 10^{-6} m^3 aliquots of the water layer were placed in two 5 × 10^{-6} m^3 Erlenmeyer flasks, to which 0.1 × 10^{-6} m^3 of 100 mol m^{-3} mercuric chloride was added with either 10 × 10^{-9} m^3 of 78 mmol m^{-3} ACC as a conversion standard or 10 × 10^{-9} m^3 of water. Flasks were sealed with Suba-seal rubber puncture caps (W. Freeman and Co., Bradford, UK) and 0.2 × 10^{-6} m^{-3} of a mixture comprising saturated sodium hydroxide, a 12.5% (v/v) solution of commercial bleach (sodium hypochlorite—5% available chlorine) and water (4:8:3, respectively) was injected. The reaction mixture was shaken and kept at room temperature for 9 to 15

min before 1×10^{-6} m^{-3} of head space gas was analyzed for ethylene by flame-ionization GC. The reliability of the assay for endogenous ACC was tested by establishing the conversion percentage of ACC to ethylene and demonstrating a 1:1 linearity of conversion to ethylene over a wide range of ACC concentrations added to plant extracts. Conversion percentages for *P. pectinatus* and pea were on the order of 82% and 88%, respectively. Since conversion levels were similar over a wide range of ACC concentrations, only one concentration of standard ACC (0.78 nmol ACC/vial) was routinely used to estimate the efficiency of ACC conversion in each extract.

Because the Lizada and Yang assay for ACC has been criticized for lack of specificity and accuracy under some circumstances (Hall et al., 1989; Shauvaux et al., 1994), we used two mass spectrophotometric methods to confirm the presence and amount of ACC in elongating, dark-grown shoots of *P. pectinatus*. In brief, ACC in extracts of *P. pectinatus* was derivatized to *N*-benzoyl *n*-propyl ACC and purified by HPLC (Hall et al., 1993). Purified samples were evaporated to dryness before being taken up in 100 mm^3 of ethyl acetate. The presence of endogenous ACC was determined by injecting 1×10^{-9} m^3 to 2×10^{-9} m^3 into a Mega gas chromatograph (Carlo Erba, Milan Italy) linked to a MS80RFA mass spectrometer (Kratos, Manchester, UK). The gas chromatograph was fitted with an OV1701 capillary column (31 m $\times$ 0.22 mm); the source temperature was 200°C, and the injector and interface temperatures were 250°C. Splitless injection was used with the split valve opening after 30 s. The instrument was set to scan at 1 s per decade from mass 500 down to 40 in the positive electron-ionization mode. The total analysis time was 30 min. The quantity of endogenous ACC in purified, derivatized extracts of shoots of *P. pectinatus* was determined in one set of extracts using selected ion monitoring with a Hewlett-Packard 5890AGC gas chromatograph coupled to an HP5970 mass-selective detector (Hall et al., 1989). The ions monitored were at *m/z* 159 and 187. These are common to both *N*-benzoyl *n*-propyl ACC contained in derivatized plant extracts and to *N*-benzoyl isobutyl ACC, which was used as an internal standard that chromatographed separately from the *N*-propyl derivative.

ACC Oxidase Activity

ACC oxidase enzyme activity was measured in extracts from elongating shoots of *P. pectinatus* and 5-d-old pea shoots according to the method of Smith and John (1993). All extractions were performed in an anaerobic work station (Forma Scientific, Marietta, OH). Fresh tissue (2.5 g) was frozen in liquid nitrogen, ground with acid-washed sand in 4×10^{-6} m^3 of extraction buffer (100 mol m^{-3} Tricine, pH 8.0, 30% [v/v] glycerol), filtered, and centrifuged at 1500g for 20 min. The supernatant was desalted using a Sephadex (PD 10 G 25 M) column. Aliquots of the desalted supernatant were incubated in 5×10^{-6} m^{-3} Erlenmeyer flasks, at 30°C, with a mixture containing 100 mol m^{-3} Tricine, pH 7.5, 30 mol m^{-3} NaHCO$_3$, 30% (v/v) glycerin, 0.1 mol m^{-3} FeSO$_4$, 30 mol m^{-3} sodium ascorbate, and 1 mol m^{-3} ACC. After 1 h, 1×10^{-6} m^{-3} head space

gas was analyzed for ethylene content by flame-ionization GC. Protein was determined by the method of Bradford (1976) with thyroglobulin as the standard. Boiled extracts were used as blanks.

RESULTS

Basal Ethylene Production

For 10 h after plants were placed in the cuvettes, peas produced a large but declining amount of ethylene (Fig. 1A). This was probably wound-induced ethylene associated with initial handling and root excision. This handling effect has also been observed by laser analysis of ethylene production by roots of intact *Zea mays* seedlings (Brailsford et al., 1993). After 10 h, a steady production of approximately 0.6 fmol s^{-1} per seedling was established (Fig. 1A). In contrast, *P. pectinatus* failed to evolve any detectable ethylene during the first 10 h after connection to the detector or during the following 14 h (Fig. 1B). This was also the case in 33 other similar experiments with *P. pectinatus* plants. When the sensitivity of the system was enhanced 2-fold by halving the air flow rate to 0.5×10^{-3} m^3 h^{-1}, ethylene production by *P. pectinatus* remained undetectable. In some instances, shoots of *P. pectinatus* were exposed to white light for 75 h within the cuvettes. During this time they became green, but the output of ethylene remained undetectable (result not shown).

Submergence in Water, IAA, or ACC

Four-hour-long submergence treatments in water or in solutions of ACC or IAA started approximately 15 h after plants were first placed in cuvettes, i.e. at least 5 h after steady-state basal production was achieved in pea. Figure 2, A, C, and E, shows characteristic ethylene output curves for pea before, during, and after treatment. In pea, ethylene release decreased during 4 h of submergence in water (time between the two arrows in Fig. 2A). This was a result of water entrapping ethylene within the submerged shoot and slowing its escape into the cuvette head space and
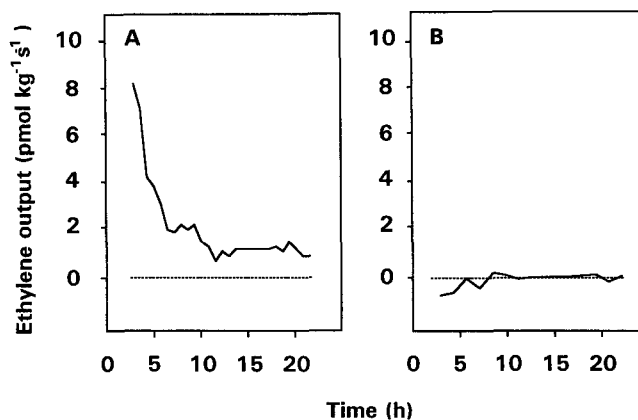

Figure 1. Ethylene production during the initial 25 h after connection of cuvettes to the photoacoustic detector system. A, *P. sativum*; B, *P. pectinatus*. The traces connect measurements made every 20 min.
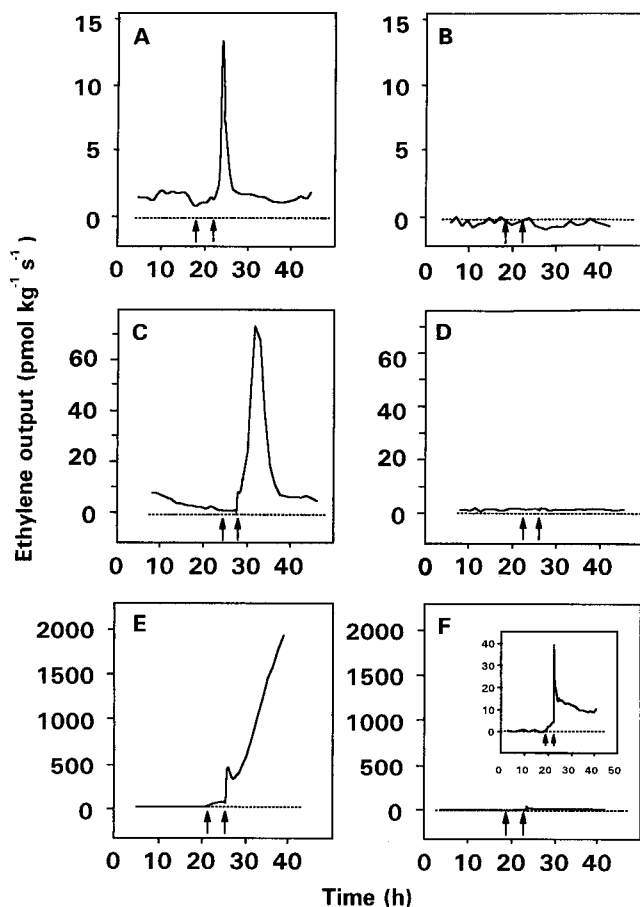
Figure 2. Effect of 4-h submergence treatment in water (A and B), 0.1 mol m^{-3} IAA (C and D), or 10 mol m^{-3} ACC (E and F) on ethylene release from growing shoots of *P. sativum* (A, C, and E) and *P. pectinatus* (B, D, and F). The inset in F shows the response to exogenous ACC when the scale for ethylene output is expanded by a factor of 25. The period of submergence treatment is shown by the arrows. Plots are of individual cuvettes. The traces connect measurements made every 20 min.

entering the laser detector. Upon desubmergence, this stored ethylene was released as a clear peak with a maximum output of 13.3 pmol kg^{-1} s^{-1}. This desubmergence peak of released ethylene was complete within 3.5 h when the rate of ethylene output returned to that measured before submergence (Fig. 2A). In pea, ethylene output from shoots given 0.1 mol m^{-3} IAA also decreased during the 4-h submergence in the auxin (period between the two arrows in Fig. 2C). Again, this was a consequence of entrapment by the water covering. Upon desubmergence, two peaks in ethylene output from auxin-treated peas were seen. The first represented an initial surge of ethylene produced during the preceding 4-h submergence period and quickly released from the tissues upon desubmergence (small shoulder on the main peak shown in Fig. 2C). Superimposed on this initial ethylene peak was a larger peak (maximum 75 pmol kg^{-1} s^{-1}) representing postsubmergence biosynthesis that was enhanced by IAA treatment. A similar explanation also applies to the two larger peaks

(note the scale) attending desubmergence of peas from a 10 mol m^{-3} ACC solution. The amounts of ethylene generated by applying ACC to peas were so large and rapid that some of the gas even escaped from the water phase during the 4-h submergence treatment (between the two arrows, Fig. 2E).

Results obtained with pea shoots were in marked contrast to those obtained with *P. pectinatus*. At no time before, during, or after the 4-h submergence treatments in water or IAA was there evidence of ethylene evolution from *P. pectinatus*. There were no desubmergence peaks or other evidence of ethylene output (Fig. 2, B and D). Ethylene evolution was observed only when plants of *P. pectinatus* were submerged in 10 mol m^{-3} ACC (Fig. 2F). However, the amount was extremely small and almost imperceptible on the scale needed to accommodate the ACC response in pea (cf. E and F in Fig. 2). Only when the scale was expanded 25-fold was a highly attenuated ethylene-release topography revealed (Fig. 2F, inset). In essence, 10 mol m^{-3} ACC induced rates of ethylene release that barely exceeded those of peas shoots with no external ACC supplied.

Effect of Changes in Gas Composition on Ethylene Production

Ethylene production by peas increased by 2.5 to 3.5 times the base level as soon as the inflowing gas was switched from air to air containing 10 kPa CO_2 (Fig. 3A). On return back to air containing normal levels of CO_2, ethylene production quickly declined to rates similar to those established prior to the switch. In contrast, when the inflowing gas was switched from air to nitrogen containing 5 kPa oxygen, ethylene production by peas decreased rapidly to 0 (Fig. 3C). Production remained imperceptible until 21 kPa oxygen was restored, when production rapidly increased to become comparable with the original base rate. In contrast, ethylene production of *P. pectinatus* remained unaltered by the amount of CO_2 (Fig. 3B) or oxygen (Fig. 3D) in the inflow gas, i.e. it was entirely absent or remained below detectable levels, i.e. 50 ppt (v/v).

Effect of Wounding on Ethylene Production

After pea shoots were wounded by lightly crushing with a small roller and returned to the flow-through system, a small peak in ethylene production was observed 1.5 h later (Fig. 4A). A much larger second peak was measured after a delay of approximately 5 h. In contrast, no wound-induced ethylene production was observed when shoots of *P. pectinatus* were wounded in a similar way (Fig. 4B). Comparable results were obtained when plants were wounded by scoring with a needle (result not shown).

Concentrations of Endogenous ACC

Chromatograms of extracts of dark-grown, elongating shoots of *P. pectinatus* were obtained using a Kratos MS80RFA GC-MS. Interpretation of the spectra was complicated by the presence of relatively large amounts of compounds other than ACC, despite the preceding clean-up steps. Thus, a definitive full-scan spectrum of
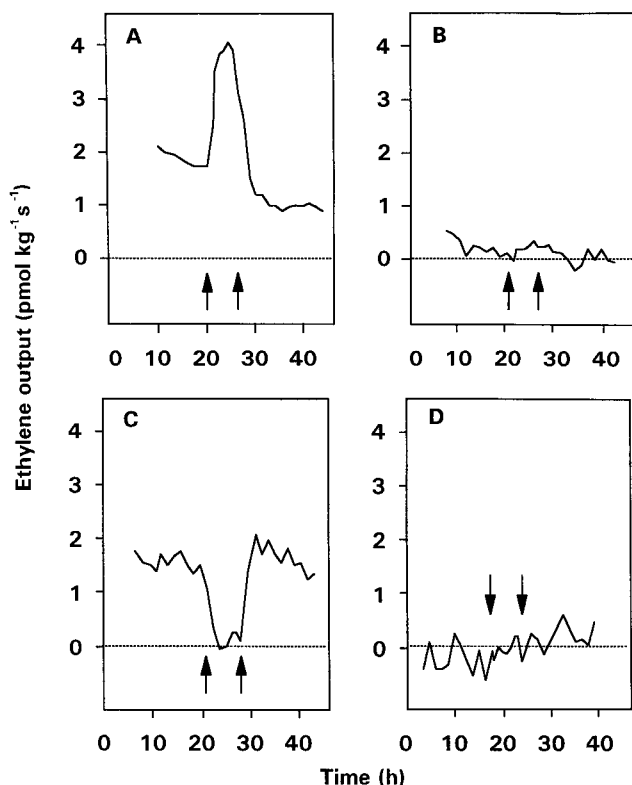
Figure 3. Effect of increasing the partial pressure of CO_2 from ambient (approximately 35 Pa) to 10 kPa (A and B) or decreasing the oxygen partial pressure from ambient (20.8 kPa) to 5 kPa (C and D) on ethylene production by *P. sativum* (A and C) and *P. pectinatus* (B and D). Plots are of individual cuvettes. Arrows indicate the duration of treatment. The traces connect measurements made every 20 min.

benzoyl ACC, such as the one we reported earlier for *Phaseolus* (Hall et al., 1989), was not obtained. Despite this, the expected *m/z* 159, 187 ions, and the 247 molecular ion were present and eluted from the GC column at the same time as those originating from a co-injected standard. Because of this co-chromatography, it is extremely unlikely
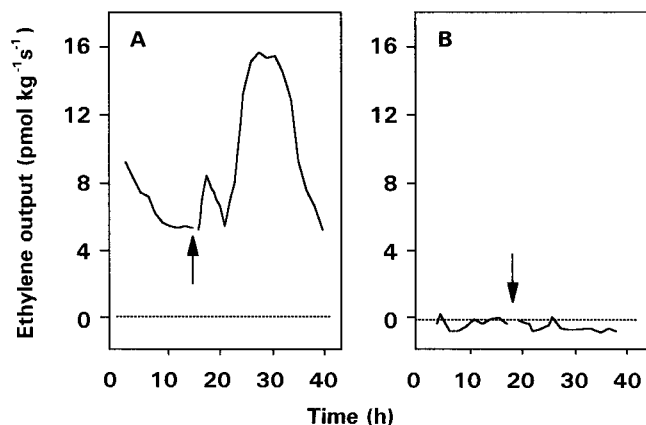

Figure 4. Effect of physical wounding on ethylene production by *P. sativum* (A) and *P. pectinatus* (B). The arrows show the times when shoots were gently crushed with a small roller. Plots are of individual cuvettes with measurements made every 20 min.

that these ions in the extract had a source other than ACC, thus confirming the presence of ACC in extracts of *P. pectinatus*. Endogenous ACC in *P. pectinatus* was also quantified by selected ion monitoring. The quantity of endogenous ACC in elongating shoots of *P. pectinatus* was estimated to be 10.14 ± 1.42 nmol g^{-1} using this method ($n = 5$). Results from this method were statistically comparable ($P = 0.05$) to those obtained by the Lizada and Yang assay, which generated values of 16.36 ± 3.30 nmol g^{-1} ($n = 6$) (Table I).

Using the Lizada and Yang assay, we estimated the internal concentration of ACC in pea shoots to be 5 nmol g^{-1} compared with approximately 16 nmol g^{-1} found in air-grown shoots of *P. pectinatus* (Table I). Similar values were obtained for shoots of *P. pectinatus* after they were submerged in water for 4 h. However, if shoots were submerged in 10 mol m^{-3} ACC solution for 4 h, the internal concentration of ACC was increased 70-fold. Submergence in 0.1 mol m^{-3} IAA for 4 h increased the internal ACC concentrations 5.5-fold in the shoots of *P. pectinatus* (Table I), even though this did not result in detectable ethylene production (Fig. 2B).

ACC Oxidase Activity

The ability of filtered and desalted extracts to generate ethylene from excess exogenous ACC was used as a measure of ACC oxidase activity. Considerable activity was present in extracts of pea, and this increased in proportion to the amount of extract (1.45 ± 0.13 mg protein mL^{-1}) added to the reaction mixture (Fig. 5). In contrast, extracts of *P. pectinatus* (2.01 ± 0.12 mg protein mL^{-1}) produced no ethylene from ACC, regardless of the volume of extract added to the reaction mixture (Fig. 5). Thus, *P. pectinatus* shoots did not appear to contain ACC oxidase.

DISCUSSION

Preliminary work using conventional flame-ionization GC failed to detect basal ethylene production in dark-grown shoots of tubers of *P. pectinatus* (Summers and Jackson, 1993). We have now confirmed this result using highly sensitive laser photoacoustics that routinely detects 50 ppt

Table I. *Internal ACC concentrations of shoots of pea and P. pectinatus plants grown in air or submerged for 4 h in water, IAA (0.1 mol m^{-3}), or ACC (10 mol m^{-3})*

ACC was measured using a modification to the method of Lizada and Yang (1979). Means ($n = 6$) with the same letter are not significantly different ($P = 0.05$) based on *t* tests and analysis of variance.

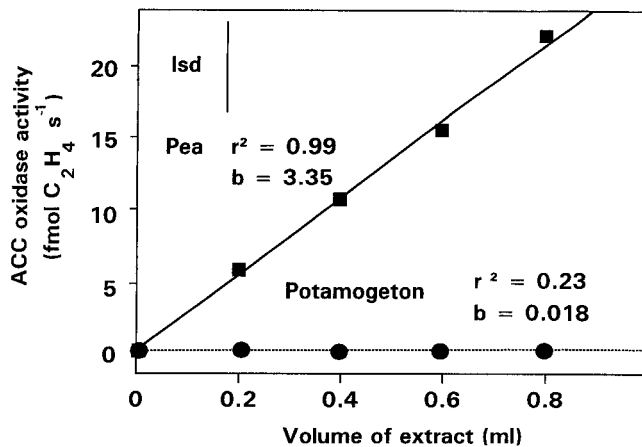| Species and Treatment	Internal ACC Concentration
	nmol g^{-1} fresh wt
P. pectinatus	
Air	16.36a
Water	22.40a
IAA	90.49b
ACC	1188.93c
Pea	
Air	5.20d

Figure 5. Assay of ACC oxidase activity in extracts of shoot tissue of *P. sativum* (-■-) and *P. pectinatus* (···●···). Increasing volumes of extract of similar protein content were added to the reaction mixture, and the response was analyzed by linear regression (means of five replicates). The vertical line is the LSD (LSD at P = 0.05). These results show the assay to give a linear response to increases in enzyme level in *P. sativum* and demonstrate the absence of ACC oxidase activity in extracts of *P. pectinatus*.

(v/v) of ethylene. Even when the chances of detecting ethylene were doubled by halving the rate of air flow through the system, no ethylene signal was obtained. Similarly, when tissue was submerged in water for 4 h to force an accumulation of ethylene within the tissues prior to it being released within a few minutes on desubmergence, no ethylene output was detected. Based on this null response measured by the most sensitive ethylene detector currently available, we conclude that no ethylene is made by growing shoots of *P. pectinatus* tubers. This conclusion was supported by finding that treatments with auxin, CO_2, and physical wounding, which in pea strongly stimulated ethylene production, failed to induce ethylene production in *P. pectinatus*. Only by applying large amounts of ACC was any ethylene production elicited, but in comparison with pea, the yield was insignificant. These results raise questions concerning where in the biosynthetic pathway the production of ethylene is blocked and how underwater elongation is regulated in this aquatic species in the absence of endogenous ethylene. Our findings also question the essentiality of ethylene in plant development, since the ethylene-free shoots of *P. pectinatus* are vigorous and morphologically normal.

Ethylene Biosynthesis

In higher plants, Met, the principal precursor of ethylene (Abeles et al., 1992), is converted to S-adenosyl L-Met, which in turn is converted to ACC by the pyridoxal phosphate-requiring enzyme ACC synthase. Because of the instability of ACC synthase transcripts and enzyme (Nakagawa et al., 1991), this is usually regarded as the rate-limiting step in ethylene biosynthesis. The subsequent and last step in biosynthesis is mediated by ACC oxidase (ACC N-mono-oxygenase), which first binds with oxygen and then with ACC, converting ACC to ethylene in the pres-

ence of CO_2 (Yang et al., 1993). On the basis of the rapid rate at which exogenously applied ACC is normally converted to ethylene, ACC oxidase is usually considered to be in excess of that needed to oxidize the relatively small amounts of endogenous ACC. Thus, under well-aerated conditions, ACC oxidase is often considered unlikely to be a control point for ethylene production. In light of this, it was surprising to find almost no increase of ethylene production when ACC was applied to shoots of *P. pectinatus*, indicating a total block to ethylene production at the last oxidative step. This result explains why treatments that stimulate the preceding ACC synthase step (auxin, physical wounding; Nakagawa et al., 1991) failed to enhance ethylene production in *P. pectinatus*.

The presence of considerable amounts of endogenously synthesized ACC that exceeded at least 3-fold those in pea showed that ACC synthase and ACC accumulation were not limiting ethylene biosynthesis in *P. pectinatus*. This reinforces the view that production of the gas is arrested by the absence of ACC oxidation. In addition, IAA treatment substantially increased the internal ACC concentration of *P. pectinatus*, indicating that the enzyme ACC synthase was functional and its activity was capable of regulation, although this did not result in ethylene biosynthesis. Large amounts of exogenous ACC readily penetrated *P. pectinatus* plants given 10 mol m^{-3} ACC, although only trace amounts of ethylene were released. The absence of ACC oxidase activity in tissue extracts confirmed that the absence of this enzyme explains the lack of ethylene production capacity. The small amounts of ethylene discharged from shoot tissues supplied with ACC may therefore have been of chemical or microbiological origin rather than being generated by ACC oxidase action within the plant's cells. ACC oxidase is normally coded for by a small multigene family. In tomato, related genes are differentially expressed in ripening fruits and wounded leaves (Holdsworth et al., 1987). Whether similar genes are absent or mutated in *P. pectinatus* or whether there is a block to transcription or posttranscriptional processes remains unknown. These results help to raise the profile of ACC oxidase as a rate-limiting step to ethylene biosynthesis in certain circumstances. We have recently shown that, in tomato plants stressed by soil flooding, ethylene production by the petioles is limited by the ACC oxidase step (English et al., 1996).

Underwater Shoot Elongation

Most aquatic and semiaquatic species escape submergence in water that contains some oxygen by elongating stems or leaves more quickly in response to entrapped ethylene (Musgrave et al., 1972; Jackson, 1985; Kende, 1987; Voesenek et al., 1993). Shoot elongation in *P. pectinatus* is also promoted by submergence in aerated water (Summers and Jackson, 1994), even though no ethylene is synthesized to mediate in the response. Clearly, there must be some other stimulus to underwater extension growth in this species. We have previously eliminated buoyant tension as the causal factor (J.E. Summers and M.B. Jackson, unpublished data), but there remains the possibility that gaseous

signals other than ethylene are involved. Accumulations of respiratory CO_2 or a partial depletion of oxygen are the most likely candidates, since they are known to promote shoot or leaf extension in certain aquatic or submergence-tolerant species (Ku et al., 1970; Suge and Kusanagi, 1975; Raskin and Kende, 1984a; Pearce et al., 1992), although in some of these cases activity is dependent on ethylene. Our most recent work identifies the acidifying influence of respiratory CO_2 as a contributing factor (Summers and Jackson, 1996).

Ethylene as an Essential Hormone

Although *P. pectinatus* cannot produce ethylene, initial growth and development of these shoots is vigorous and visually normal. Stem growth is also unaffected by exogenously applied ethylene, although leaf extension and outgrowth of root primordia are stimulated (Summers and Jackson, 1993). Thus, endogenous ethylene appears not to be essential for normal development in this species, at least during early growth. Previous work also supports the view that ethylene operates at a more superficial level than some other hormones by regulating preprogramed events rather than helping to establish the basic cellular and organizational framework. For example, normal vegetative growth has been observed under hypobaric pressures at which the concentration of ethylene within tissues is greatly reduced (Reid, 1987). Furthermore, Cameron and Reid (1983) found that, after application of sufficient Ag^+ to strongly inhibit the action of ethylene, there was no perceptible effect on growth development and flowering of various taxa, although the abscission of flower petals was inhibited. The essentially normal phenotype of ethylene-insensitive mutants of *Arabidopsis thaliana* or tomato (Ecker, 1995) also strongly indicates that ethylene is less pivotal to normal growth (i.e. in the absence of marked environmental stress) than are auxins, GAs, and cytokinins. A recent description of ethylene as "... a volatile with a finger in numerous 'developmental pies'" (Roberts and Hooley, 1988) seems appropriate. The secondary role of ethylene compared to GA is highlighted in *Callitriche platycarpa* (Musgrave et al., 1972) and deep-water rice (Raskin and Kende, 1984b), in which the promoting effect of ethylene on stem extension is totally dependent on GA biosynthesis, whereas stimulation of stem extension by GA is independent of ethylene (Bleecker et al., 1987) and our own evidence (Summers and Jackson, 1996) that GA actively promotes stem elongation in *P. pectinatus*, even though there is no endogenous ethylene production.

SUMMARY

Using the most sensitive techniques available, we have demonstrated that young plants of *P. pectinatus* do not produce ethylene. The plants are rich in ACC, but an inherent feature is that conversion of ACC to ethylene is blocked. Thus, *P. pectinatus* appears to be a higher plant species that is incapable of producing ethylene and one of the clearest examples of ACC oxidase limiting ethylene biosynthesis in growing vegetative organs.

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