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Shoot Inversion-Induced Ethylene Production: A General Phenomenon?

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Abstract. Shoot inversion induction of ethylene production was found in inverted shoots of corn, peas, soybean, sunflower, tomato, and *Pharbitis nil*. The increases in ethylene production were found to range from two- to threefold in soybean to eightfold in corn and sunflower. The occurrence of peaks of ethylene production ranged from 16 h following shoot inversion in corn to 72 h in *Pharbitis*. That the enhanced ethylene production was due to activation of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase is supported by the finding of increased ACC content in inverted shoots of all species tested. Shoot inversion inhibition of elongation was found in inverted shoots of pea, soybean, sunflower, tomato, and *Pharbitis nil*. This inhibition is thought to be mediated via increased ethylene production in the inverted shoots. That shoot inversion induction of ethylene is not a persistent effect is supported by the finding that ethylene synthesis could be terminated by reorientation of shoots to the upright position and could be reinitiated by the subsequent inversion of the shoots. The effects of shoot inversion on the enhancement of ethylene production and on the inhibition of elongation of the inverted shoot appear to be general phenomena.

Horizontal orientation of plants causes enhanced ethylene production (Abeles and Gahagan 1968, Clifford et al. 1983, Denny 1936, Harrison and Kaufman 1982, Osborne 1974, Prasad and Cline 1985a, Wheeler et al. 1986, Wright et al. 1978). There is also increased ethylene evolution 4 h after inversion of detached sunflower leaves (Palmer 1973). Ethylene-mediated epinasty has been observed in leaves of *Xanthium* plants that have been inverted periodically for 4 h (Salisbury and Wheeler 1981). We have recently shown in *Pharbitis nil* that

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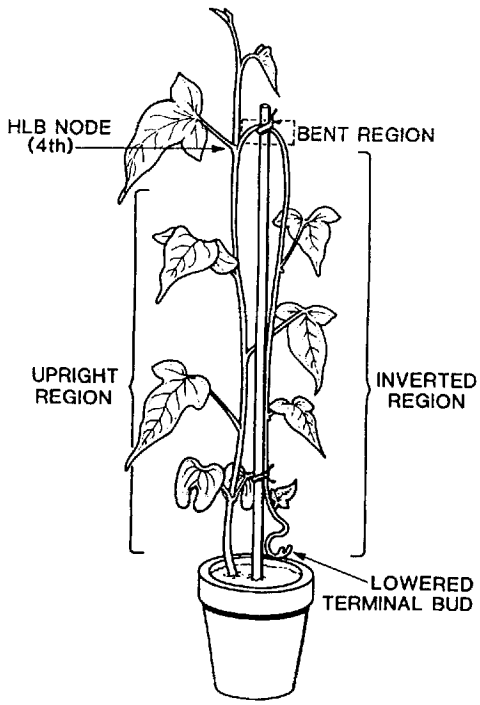

Fig. 1. Shoot inversion results in the enhancement of ethylene production and in the retardation of elongation in the lower inverted growth region (the terminal 10 cm) of the *Pharbitis nil* shoot.

shoot inversion results in enhanced ethylene production and in the inhibition of elongation of the inverted shoot (Prasad and Cline 1985a, 1987b). This inversion of the upper shoot is accomplished by the gentle bending down of the stem at the fourth node from the cotyledons and the securing of the lowered inverted shoot apex to a stake with a string (Fig. 1).

An important question arises as to the generality of this *Pharbitis* shoot inversion response. Is shoot inversion-induced ethylene production a widespread response in the plant kingdom? Is the subsequent retardation of growth in the inverted stem a common occurrence in other species? In the present paper we report the existence of shoot inversion-induced ethylene production and inhibition of elongation in five dicot species and one monocot species.

Materials and Methods

Seeds of corn (Golden Cross Bantam), sunflower (Mammoth), peas (tall, early Alaska), soybeans (Amcor), and tomato were presoaked in water for 4–5 h and planted in sandy loam soil. The seeds of *Pharbitis nil* (L.) [also known as *Ipomoea nil* (L.) Roth] were scarified for 30 min in H_2SO_4 and pregerminated in Petri dishes before planting in sandy loam soil. All plants were grown under continuous light (cool-white fluorescent and incandescent lamps; General Electric; 54–126 μmol m^{-2} s^{-1}, a range of photosynthetic radiation from the minimum to the maximum plant height as measured with a Li-Cor 185B Radiometer).

Pharbitis, sunflower, pea, and soybean plants were used for experimental studies at the age of 20–25 days, and corn and tomato plants were used at the age of 45–50 days. Fertilizer treatment was given to *Pharbitis* and tomato plants on days 10 and 30, respectively. All experimentation with *Pharbitis* plants was reported previously (Prasad and Cline 1986b, 1987a). These data are included here with comparative purposes.

For shoot inversion, the shoots of *Pharbitis* were bent down at the fourth node, which was 20–25 cm behind the shoot apex. The entire corn plants were inverted instead of merely bending down the shoots 10–15 cm behind the apices, as was done with all other species. The inverted shoots were secured to a stake with string to prevent upward gravicurvature. For growth analyses, the shoots were marked with India ink 5 cm behind the tip at 0 h. Beyond this 5-cm mark there was no stem elongation in any of the species measured (data not shown) except *Pharbitis*. Growth was not measured for corn. Total growth was measured between the tip and the ink mark every 24 h for 7 days in both upright and inverted shoots. Six to 10 plants were used for each treatment.

Ethylene and ACC Determinations

Ethylene determinations were made by enclosing 12 stem segments [consisting of two 2.5- to 3.0-cm sections taken from the 5- to 6-cm region immediately (0.2–0.5 cm) behind the apex of the shoot of six plants] in 10-ml vials sealed with rubber serum caps under the same conditions as the plants were grown. At the end of 1 h, a 1-ml sample of air was taken from the vial and injected into the Hewlett-Packard gas chromatograph fitted with a flame ionization detector for ethylene analysis.

The 1-aminocyclopropane-1-carboxylic acid (ACC) content was estimated according to the method of Miller and Pengelly (1984), which is a modified method of Lizada and Yang (1979). The stem tissue taken from the 5-cm region 0.2–0.5 cm behind the tip from six plants (500–800 mg) was homogenized in 2 ml of 5% (w/v) 5-sulfosalicylic acid (pH 1.8), and the homogenate was centrifuged at 30,000*g* for 30 min at 4°C. To 0.2 ml supernatant were added 0.6 ml of 5% (w/v) 5-sulfosalicylic acid and 0.1 ml of 0.5 mM $HgCl_2$. The vials were sealed and then injected with 0.1 ml of NaOCl reagent (Clorox and 10 N NaOH at 1:1 ratio). The vials were briefly vortexed and incubated for 15 min on ice, and 1-ml gaseous samples were drawn and assayed for ethylene. The efficiency of ACC oxidation, which averaged 57–62%, was estimated by analyzing replicate samples having ACC internal standards.

All the experiments were repeated at least twice with essentially the same results.

Results and Discussion

Shoot inversion was found to enhance ethylene production in stems of corn, peas, soybean, sunflower, tomato, and *Pharbitis nil* (Fig. 2). Our previous *Pharbitis* results (Prasad and Cline 1986b) are included here for comparative purposes. The increases in ethylene evolution ranged from two- to threefold in

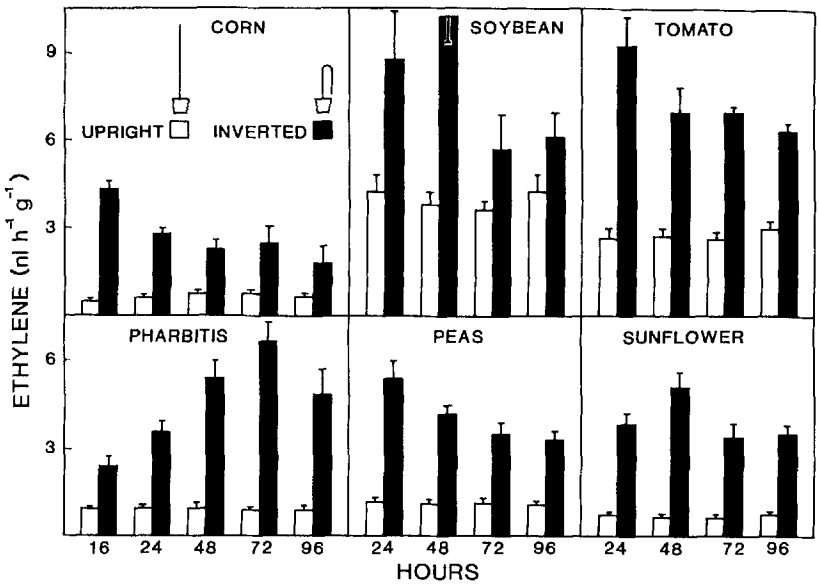

Fig. 2. Shoot inversion enhancement of ethylene production in the inverted stems. The shoots of *Pharbitis nil* were bent down 20–25 cm behind the apices, and the shoots of sunflower, peas, soybeans, and tomato were bent down 10–15 cm behind the apices. In the case of corn, the entire plants were inverted. Ethylene production was measured from segments taken from the 5- to 6-cm growth region 0.2–0.5 cm behind the shoot apex. Vertical lines = SD.

soybean to eightfold in corn and sunflower. Peaks in ethylene production were observed in corn after inversion for 16 h, in peas and tomato at 24 h, in soybean and sunflower at 24 h, in soybean and sunflower at 48 h, and in *Pharbitis* at 72 h. Following these peaks, the rates of ethylene production more or less gradually declined.

The reduction of shoot elongation by inversion after 24 h was 62% in sunflower, 56% in soybean, and 35% in *Pharbitis* (Fig. 3). In soybean and *Pharbitis*, the inhibition increased gradually over a 7-day period. However, in peas, sunflower, and tomato, essentially all inhibition occurred during the first day of shoot inversion.

Shoot inversion enhancement of ethylene production in inverted *Pharbitis* shoots has previously been shown to be mediated by the activation of ACC synthase, the key enzyme in the biosynthetic pathway of ethylene (Prasad and Cline 1987a). That the activation of ACC synthase is also involved in the shoot inversion enhancement of ethylene production in the other five species analyzed in this study is suggested by the observation of a two- to sixfold increase in the content of ACC, the immediate precursor of ethylene, 24 h subsequent to shoot inversion (Table 1).

That the induction of ethylene production following shoot inversion is not a persistent response was demonstrated in all five species (Table 2) as had previously been shown for *Pharbitis* (Prasad and Cline, 1986b). After 24 h of shoot inversion, ethylene synthesis in the growth region of the inverted shoot was

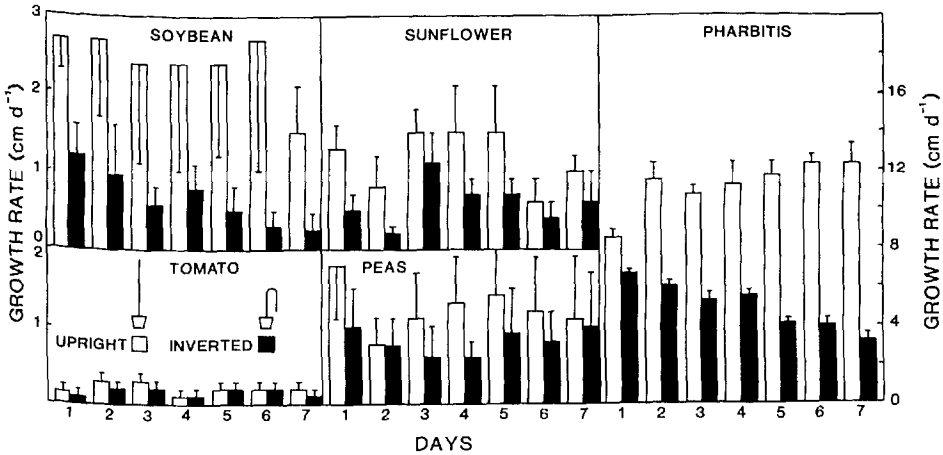

Fig. 3. Shoot inversion retardation of stem elongation of 24-h periods over 7 days. Daily growth was measured from the shoot tip to an India ink mark at zero time 5 or more cm behind apex. Vertical lines = SD.

Table 1. Effect of 24 h of shoot inversion on ACC formation and ethylene production in the apex region of the stem. Mean values of at least two determinations ± SD.

Species	Exp. No.	ACC (nmol/g) Upright (0 h)	ACC (nmol/g) Inverted (24 h)	Ethylene (nl/g/h) Upright (0 h)	Ethylene (nl/g/h) Inverted (24 h)
Corn	I	—	—	0.6 ± 0.1	2.7 ± 0.1
	II	0.3 ± 0	0.8 ± 0.2	0.8 ± 0.1	2.8 ± 0.1
Peas	I	—	—	1.1 ± 0	5.3 ± 0.7
	II	1.7 ± 0.1	4.3 ± 0.1	1.2 ± 0.2	5.8 ± 0.7
Soybean	I	0.6 ± 0.1	2.7 ± 0.4	3.9 ± 0.5	8.8 ± 2.1
	II	0.8 ± 0.1	2.5 ± 0.2	4.2 ± 0.4	8.3 ± 1.3
Sunflower	I	0.2 ± 0	0.9 ± 0.2	0.7 ± 0	3.9 ± 0.9
	II	0.2 ± 0	0.7 ± 0	0.6 ± 0.1	4.2 ± 0.4
Tomato	I	0.3 ± 0.1	1.0 ± 0.1	2.7 ± 0.1	9.1 ± 0
	II	0.2 ± 0	1.0 ± 0.2	2.3 ± 0.2	8.5 ± 0.1
Pharbitis	I	1.2 ± 0.1	2.8 ± 0.4	1.0 ± 0.9	4.0 ± 0.6
	II	1.1 ± 0.1	2.7 ± 0.3	1.0 ± 0.1	3.8 ± 0.6

enhanced two- to sixfold in all six species. When these inverted shoots were reoriented back to the upright position for 24 h, ethylene emanation was depleted to the original control level. The reinversion of the same shoots for 24 h was then accompanied by a two- to fivefold increase in ethylene production. Hence, shoot inversion may be likened to a reversible switch for ethylene production via the activation of ACC synthase.

Although the gravity stress imposed on the cells in the inverted shoot is seen to function as the primary trigger in this system, the restraining stress incurred

Table 2. Effect of inversion, straightening up, and reinversion of shoot on ethylene production (nl/g/h) in the apex region of the stem over 72 h. Mean values of at least two determinations ± SD.

Species	Exp. No.	Upright (0 h)	Inverted (24 h)	Upright (48 h)	Inverted (72 h)
Corn	I	0.6 ± 0.1	2.7 ± 0.6	0.9 ± 0.1	3.7 ± 0
	II	0.8 ± 0.1	2.8 ± 0.1	0.8 ± 0.1	3.4 ± 0.2
Peas	I	1.1 ± 0	5.3 ± 0.7	1.2 ± 0.1	4.3 ± 0.2
	II	1.2 ± 0.2	5.8 ± 0.7	1.2 ± 0.1	4.6 ± 0.1
Soybean	I	3.9 ± 0.5	8.8 ± 2.1	3.8 ± 0.6	8.1 ± 1.9
	II	4.2 ± 0.4	8.3 ± 1.3	4.2 ± 0.2	7.6 ± 1.3
Sunflower	I	0.7 ± 0	3.9 ± 0.9	0.7 ± 0.1	3.2 ± 0.2
	II	0.6 ± 0.1	4.2 ± 0.4	0.7 ± 0.1	3.4 ± 0.2
Tomato	I	2.7 ± 0.2	9.1 ± 0	2.9 ± 0.6	6.8 ± 0.3
	II	2.3 ± 0.2	8.5 ± 0.2	2.7 ± 0.4	6.8 ± 0.9
Pharbitis	I	0.9 ± 0.3	4.0 ± 0.6	0.8 ± 0.1	4.2 ± 0.5
	II	1.0 ± 0.1	3.8 ± 0.6	1.1 ± 0.1	4.1 ± 0.7

with the tying down of the inverted shoot apex with a string to the stake to prevent gravitropic curvature probably also contributed to the total stress. The precise mechanisms by which gravity and restraining stress may enhance ACC synthase activity to produce ethylene are unknown.

That the ethylene produced by shoot inversion is responsible for the retardation of growth of the inverted shoot seems likely, based on our previous studies with *Pharbitis*, wherein treatments with the ethylene action inhibitor $AgNO_3$ or with a clinostat (which reduces shoot inversion enhanced ethylene production) promoted elongation of the inverted shoot, whereas treatments of upright shoots with Ethrel or mechanical perturbation emitted ethylene and inhibited elongation (Prasad and Cline 1985a,c, 1987a). The region of highest ethylene production in the inverted *Pharbitis* shoot coincided precisely with the elongation region of the stem (Prasad and Cline 1986a). This may also apply to the other species tested here, since the ethylene levels were inversely proportional to growth inhibition in their inverted shoots.

The earliest increase of ethylene production following shoot inversion has been found to be 2.75 h in *Pharbitis* (Prasad and Cline 1986b), which is similar to the 1.5- to 5-h determinations carried out in studies with other plants or plant parts either in inverted (Palmer 1973, Salisbury and Wheeler 1981) or horizontal orientation (Abeles and Gahagan 1968, Wright et al. 1978, Harrison and Kaufman 1982). This 2.75-h latent period of *Pharbitis* coincides closely with the first observed growth inhibition in the inverted shoot (data not shown). As was suggested for *Pharbitis* (Prasad and Cline 1987b), an increased accumulation of hydroxyproline-rich glycoprotein and lignin in the inverted shoot (possibly involving an ethylene signal) may play a role in the shoot inversion inhibition of elongation in all of the species evaluated in this study.

In conclusion, it appears that shoot inversion induction of ethylene synthesis and the subsequent inhibition of elongation in the inverted shoots are general phenomena or are at least common in many species. Furthermore, ethylene enhancement by shoot inversion is not persistent and may be altered by reorientation of the main shoot.

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