

Temperature-dependent Inhibition of Hypocotyl Elongation in Some Soybean Cultivars: I. Localization of Ethylene Evolution and Role of Cotyledons¹

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Apical 2-cm hypocotyl segments from seedlings of a "short-hypocotyl" cultivar Amsoy 71, like whole seedlings, evolve about twice as much ethylene at 25°C as at 30°C. Segments consisting of two cotyledons and an attached epicotyl evolve ethylene at low rates at both 25°C and 30°C. Hypocotyl segments from seedlings of Cutler 71 also show enhanced ethylene evolution at 25°C. Hypocotyl segments from Corsoy, a "long-hypocotyl" cultivar, however, evolve ethylene at low rates at both 25°C and 30°C. Wounding of Amsoy 71 hypocotyl segments does not increase their ethylene evolution. Ethylene evolution at 25°C is reduced and the short-hypocotyl phenomenon is reversed by partial (50%) removal of Amsoy 71 cotyledons at planting time.

Key words: Cotyledons — Ethylene — Hypocotyl elongation — Temperature sensitivity.

Certain soybean cultivars exhibit poor emergence when planted at a depth of 10 cm and grown at 25°C. The poor emergence is a consequence of reduced hypocotyl elongation (Burris and Fehr 1971, Burris and Knittle 1975, Gilman et al. 1973, Grabe and Metzger 1969, Samimy 1978, Samimy and LaMotte 1976). Such "short-hypocotyl" cultivars show normal hypocotyl elongation at 15, 20 and 30°C; hypocotyls of some other cultivars of "long-hypocotyl" type elongate normally at all four temperatures (Grabe and Metzger 1969). Burris and Knittle (1975) have demonstrated that removal of 50% of the cotyledonary tissue from anomalous cultivars transforms hypocotyl elongation at 25°C to normal. The significance of such a cotyledonary factor also was recognized by Samimy (1978) who demonstrated that more ethylene is evolved when cotyledons are left attached to the apical 1-cm section of the hypocotyl than when cotyledons are detached.

This paper reports on 1) the localization of temperature sensitivity in some anomalous soybean cultivars and 2) the significance of cotyledons in ethylene evolution by hypocotyls of Amsoy 71 seedlings.

Abbreviations: H, apical 2-cm hypocotyl segment; HW, wounded hypocotyl segment; C+E, cotyledons + epicotyl; C+E+H, cotyledons + epicotyl + hypocotyl segment left attached to each other; pt, plant part; PVC, polyvinyl chloride; SD, standard deviation.

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Materials and Methods

Seeds of different cultivars of soybean [*Glycine max* (L.) Merr.] were preconditioned to ca. 21% moisture. They were surface-sterilized with 5% sodium hypochlorite for 30 sec followed by several rinses of distilled water and 30 sec of 0.01 N hydrochloric acid. Seeds were soaked in aerated, running distilled water for 1.5 hr. A rolled towel germination procedure reported by the Association of Official Seed Analysts (1965) and later modified by Burriss and Fehr (1971) and Samimy and LaMotte (1976) was used to produce seedlings. Twenty-five seeds were placed 10 cm from the wet paper's top and oriented in a single row with radicles pointing downward. The rolled towels were placed in a plastic container (18×28×30 cm) and covered with a plastic bag. After 48 hr, 250 ml of water was added to each container.

Seedlings were grown for 4 and 5 days at 30 and 25°C, respectively. These ages were chosen because seedlings had developed to similar extents as judged by observation of primary root lengths. Primary root elongation does not show the anomalous temperature sensitivity (Samimy and LaMotte 1976). All manipulations of plant material were conducted under a dim green light in a dark room (Samimy 1978).

For determination of ethylene evolution by intact seedlings, groups of 25 seedlings, earlier grown in rolled paper towels, were sealed into PVC tubes (7.5 cm i.d. × 50 cm) each containing 20 ml of water. A screw-type head allowed easy access to seedlings. Vacuum grease was used to seal the head. A rubber septum was fitted into the head to permit gas sample withdrawal. After 18 hr incubation, a 1-ml sample was analyzed for ethylene content. Gas analysis was accomplished with a Varian model 3700 gas chromatograph equipped with a flame ionization detector coupled to a Cary model 401 vibrating reed electrometer.

For determination of ethylene from seedling parts, ten etiolated seedling parts were placed in each 63-ml, black custom-made Plexiglas chamber. Each chamber contained a filter paper (7-cm diameter) and 7 ml of 50 mM K-phosphate buffer, pH 7, containing 2% sucrose and 0.05% chloramphenicol. The air in the sealed chamber was assayed for ethylene content every 3 hr over a period of 9 hr. After sample withdrawal, the system was flushed with air and sealed for subsequent ethylene determination. Incubation of seedling parts was at the same temperature as that at which the seedlings were grown.

To assess wound-induced ethylene evolution, hypocotyl segments were pricked with a fine needle or were cut into three sections. To assess the role of cotyledons in ethylene evolution half of each cotyledon was excised from imbibed seeds of Amsoy 71 at planting time. The seeds were then planted and grown as indicated earlier.

Results

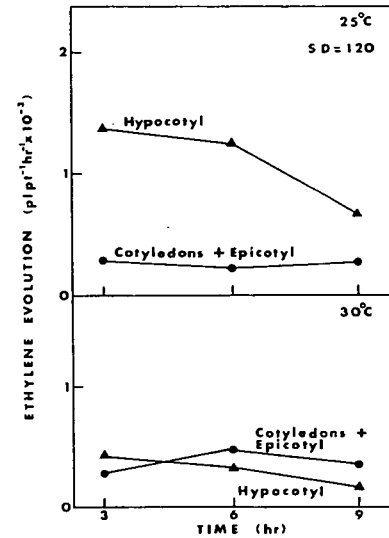
Ethylene evolution by intact seedlings of Amsoy 71 at two temperatures is shown in Table 1. Ethylene evolution is significantly ($p < 0.01$) greater at 25°C than at 30°C.

Table 1 Rate of ethylene evolution by intact Amsoy 71 soybean seedlings

Rate of ethylene evolution (pl seedling ⁻¹ hr ⁻¹)	
25°C	30°C
2,099 ^a	992

^a Significant at 1% level by Student's t test.
n=4, Rate averaged over 18 hr.

Fig. 1 Rate of ethylene evolution during each 3-hr interval as affected by temperature and sampling time in Amsoy 71 soybean seedling parts. SD is standard deviation in picoliters per plant per hr.



To localize the temperature-dependent ethylene evolution in Amsoy 71 seedlings, parts were excised and ethylene evolution was determined. Ethylene evolution (Fig. 1) by C+E is compared with that evolved by H. Results, the average of two replications, show that ethylene evolution by H is significantly greater than that by C+E at 25°C. At 30°C, ethylene evolution by H and C+E is low and similar.

Ethylene evolution by apical 2-cm segments from hypocotyls of three different cultivars is shown in Fig. 2. In segments from seedlings of Amsoy 71 and Cutler 71, two "short-hypocotyl" cultivars, greater rates of ethylene evolution are observed at 25°C than at 30°C. In segments from seedlings of Corsoy, a "long-hypocotyl" cultivar, ethylene evolution is low and similar at 25 and 30°C.

Ethylene evolution by hypocotyl segments from Amsoy 71 seedlings with 50% cotyledon

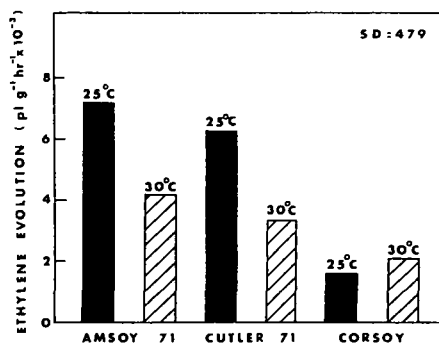


Fig. 2

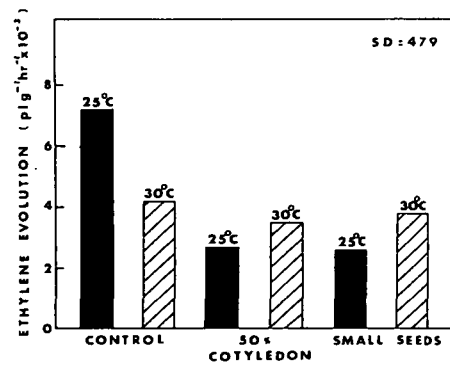


Fig. 3

Fig. 2 Rate of ethylene evolution during each 3-hr interval as affected by temperature in hypocotyl segments from soybean seedlings of three cultivars. SD is standard deviation in picoliters per gram fresh weight per hr.

Fig. 3 Effects of temperature, 50% cotyledon excision at planting time, and small seed size on rate of ethylene evolution during each 3-hr interval in excised hypocotyl segments from Amsoy 71 seedlings. SD is standard deviation in picoliters per gram fresh weight per hr.

Table 2 Rate of ethylene evolution during each 3-hr interval by Amsoy 71 soybean seedling parts as affected by wounding at 25 and 30°C

Sampling interval (hr)	Rate of ethylene evolution (pl pt ⁻¹ hr ⁻¹)			
	25°C		30°C	
	H	HW	H	HW
Exp. I^a				
0-3	1,641	1,146	344	487
3-6	1,484	1,160	265	344
6-9	890	750	289	197
	C+E+H	C+E+HW	C+E+H	C+E+HW
Exp. II^b				
0-3	1,886	1,572	600	658
3-6	3,663	2,256	1,681	1,220
6-9	1,969	1,221	2,083	891

^a In Exp. I, H=apical 2-cm hypocotyl segment; HW=hypocotyl segment pricked 10 times with a fine needle; standard deviation=216 pl pt⁻¹ hr⁻¹. n=2

^b In Exp. II, C+E+H=cotyledons+epicotyl+apical 2-cm hypocotyl segment; C+E+HW=C+E+H having hypocotyl pricked 10 times with a fine needle; standard deviation=237 pl pt⁻¹ hr⁻¹. n=2

excision at planting time is significantly less ($p < 0.01$) than that by excised hypocotyl segments from seedlings with intact cotyledons (Fig. 3). Cotyledon excision does not significantly affect ethylene evolution by hypocotyl segments at 30°C (Fig. 3). The effect of cotyledon removal on ethylene evolution at 25°C can be mimicked (Fig. 3) by selecting for a small seed size (12 g per 100 seeds), each about half the size of a large seed (24.9 g per 100 seeds). Ethylene evolution in these small seeds is low and similar to that of controls at 30°C.

Mean ethylene evolution generally decreased due to wounding, but differences were not statistically significant (Table 2). Similar findings resulted when hypocotyl segments were cut into three sections. At 25°C, the rate of ethylene evolution by H over a 15-min interval (1778 pl pt⁻¹ hr⁻¹) was similar to those measured at first and second sampling times in Fig. 1.

Discussion

Samimy and LaMotte (1976) have shown that 5-day-old etiolated seedlings of Clark, which exhibit an inhibition of hypocotyl elongation, evolve twice as much ethylene at 25°C as do seedlings of Mandarin, which grow normally. Results presented here (Table 1) reveal that Amsoy 71 is similar to Clark in its response to temperature. Moreover, apical 2-cm segments from hypocotyls of Amsoy 71 and Cutler 71 seedlings show a similar temperature dependence (Fig. 2). Segments, each consisting of two cotyledons and an attached epicotyl, evolve ethylene at similar rates at 25 and 30°C (Fig. 1). These findings show that the temperature-dependent enhancement of ethylene evolution is localized in the hypocotyl with a high rate of ethylene evolution immediately below the hypocotyl arch. These findings are consistent with those of other investigators. Goeschl et al. (1967) reported that ethylene evolution by etiolated pea epicotyls was confined to the plumule and plumular hook. Sakai and Imaseki (1971) reported that ethylene production by mung bean was most rapid in the segments immediately below the hypocotyl hook. Ethylene evolution by hypocotyl segments of Corsoy, a "long-hypocotyl" cultivar, is low at both temperatures. We believe that our results could not have been compli-

cated by wound ethylene evolution since early, short term ethylene measurements agreed with those made later.

Burris and Knittle (1975) have demonstrated that partial removal of cotyledons results in normal hypocotyl elongation in Amsoy seedlings grown at 25°C. The present research indicates that 50% cotyledon removal reduces ethylene evolution by ca. 50% at 25°C but not at 30°C.

Results presented here further implicate ethylene in the hypocotyl growth anomaly and indicate that the temperature-dependent enhancement of ethylene evolution is localized in the hypocotyl tissue. Some unknown factor supplied by the cotyledons may regulate ethylene evolution in the hypocotyl at 25°C.

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