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Ethylene and the Growth of Rice Seedlings[1]

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ABSTRACT

Etiolated whole rice seedlings enclosed in sealed vials produced ethylene at a rate of 0.9 picomole per hour per seedling. When 2-centimeter-long shoots were subdivided into 5-millimeter-long sections, the sections containing the tip of the shoot evolved 37% of the total ethylene with the remaining 63% being produced along a gradient decreasing to the base of the shoot. The tip of the coleoptile also had the highest level of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid and of the ethylene-forming enzyme activity. Ethylene is one of the factors controlling coleoptile elongation. Decapitation of the seedling reduced ethylene evolution to one-third its original level and inhibited coleoptile growth. In short-term experiments, the growth rate of decapitated seedlings was restored to almost that of intact seedlings by application of ethylene at a concentration of 10 microliters per liter. Apart from ethylene, O_2 also participates in the control of coleoptile growth. When rice seedlings were grown in a gas mixture of N_2 and O_2, the length of the coleoptiles reached a maximum at a concentration of 2.5% O_2. Lower and higher concentrations of O_2 reduced coleoptile growth. The effect of exogenous ethylene on coleoptile growth was also O_2 dependent.

A remarkable characteristic of the rice coleoptile is its ability to grow in an environment containing very low levels of O_2 or no detectable amounts of O_2 at all (13, 25). The capacity of this organ to grow under hypoxic conditions has been interpreted as an adaptation of the rice seedling to flooding conditions, allowing it to emerge from the water where the O_2 concentration is much lower than in the air. This adaptive behavior seems to be shared by all cultivated species of the genus *Oryza* (13), including the wild rice, *Zizania aquatica* (2). *Echinochloa crus-galli* (11) and *Peltandra virginica* (3) are also able to germinate and grow under hypoxic conditions. In low O_2, the growth of rice seedlings is restricted to the coleoptile and mesocotyl while root and leaf growth is severely inhibited (22). Coleoptile elongation is stimulated by ethylene (12) and by high levels of CO_2 (12, 18). Low levels of O_2, high levels of CO_2 and ethylene contribute equally to the stimulation of coleoptile growth (18). We report here on further investigations concerning the role of ethylene and low levels of O_2 in the promotion of coleoptile growth and the site where ethylene biosynthesis takes place in the seedling.

MATERIALS AND METHODS

Plant Material. Rice seeds, *Oryza sativa* L. cv M9, were purchased from the Rice Experiment Station, Biggs, CA. Seeds were surface sterilized with 10% commercial bleach (0.5% NaOCl) for 10 min, followed by many rinses with tap and distilled H_2O.

Germination. Seeds were germinated in darkness at 30 ± 0.2°C under two different conditions to vary coleoptile height. In most experiments, 1-cm-long coleoptiles were used which were obtained by germinating seeds in 14-cm Petri dishes containing 30 ml of distilled H_2O for 2 to 3 d. In some experiments, longer coleoptiles (about 2 cm) were needed; these were obtained by germinating seeds in plastic boxes (20 × 10 × 8 cm) covered with 905 Reynolds plastic film. Six-hundred seeds and 20 ml of distilled H_2O were placed in each plastic box, and the seedlings were grown for 6 d. Under these conditions, the coleoptiles grow taller because the duration of coleoptile growth is extended in sealed containers (see *e.g.* Raskin and Kende [18]).

Growth Measurements. Length of coleoptiles, leaves and total height of the seedlings were measured with a ruler. All seedlings were discarded after growth measurements. For continuous measurement of seedling growth, an angular transducer (model R30D, Schaevitz Engineering, Pennsauken, NJ) connected to a recorder was used (see Fig. 4). The whole seedling was placed in a glass chamber containing 1.5 ml of water, which was enough to cover half of the seed.

Growth of Seedlings in Sealed and Flow-Through Containers. Ten 2-d-old seedlings were transferred from Petri dishes to 8-dram shell vials (25 × 95 mm) containing 2 ml of distilled H_2O. The vials were sealed with serum-vial caps. To circulate air or gas mixtures through the vials, a 4-inch, 19-gauge hypodermic needle was inserted through the serum cap as an inlet and a 3.8-cm, 20-gauge hypodermic needle as an outlet. All experiments were carried out in the dark at an ambient temperature of 23 ± 0.5°C.

Gases. O_2-free N_2 (<5 $\mu l\ l^{-1}$ O_2, manufacturer's specifications), CO_2 and O_2 were purchased from Matheson Gas Products (Joliet, IL). Air was humidified by passing it through water and was filtered through a 0.45 μm Millipore filter (Millipore Corp. Bedford, MA) and connected to a circular gas distributor with outlets. For experiments with O_2-free N_2, a modified version of the above gas distributor was built. Intramedic polyethylene tubing (Clay Adams Inc., Parsippany, NJ) running inside a Tygon flexible plastic tube (0.16 cm wall) was used to make the flexible connections, and glass tubing was employed as much as possible. The level of O_2 was checked at the exit of the gas distributor and was found to be less than 20$\mu l\ l^{-1}$. Traces of ethylene in the cylinders or in the air duct were removed by passing the gases through a column of Purafil (Purafil Inc., Atlanta, GA). Flow rates were monitored with flowmeters (Gilmont Instruments Inc., Great Neck, NY) inserted into the gas lines.

Determination of ACC[2] and Ethylene. For ACC determinations, the plant material was homogenized and extracted with 60 mM Tris-HCl buffer (pH 7.9). After centrifugation at 13,000*g* (Micro-Centrifuge, Fisher) for 15 min, the pellet was washed and centrifuged again. An aliquot of the supernatant was used to

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[2] Abbreviation: ACC, 1-aminocyclopropane-1-carboxylic acid.

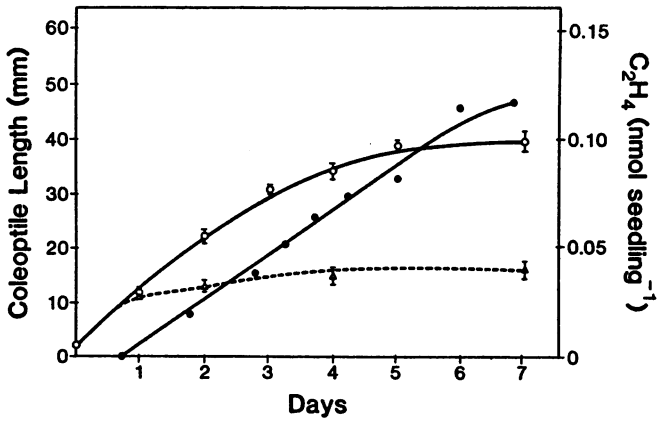

FIG. 1. Thirty 6-ml vials containing ten 2-d-old seedlings and 2 ml of distilled H_2O were tightly closed with serum vial caps and incubated in darkness. One-ml gas samples were taken for ethylene determinations at the specified times. Coleoptile lengths are the average of 30 seedlings ± SE in sealed vials (O) and in open (aerated) vials (Δ); (●), ethylene levels.

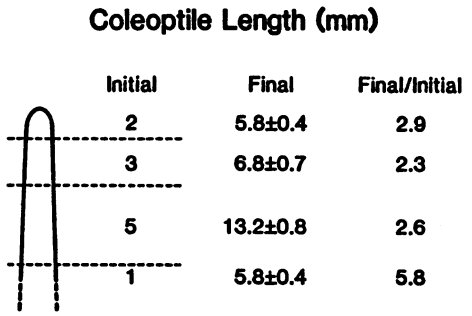

FIG. 2. Pattern of coleoptile and mesocotyl growth. Shoots of intact rice seedlings were marked along the coleoptile and mesocotyl with India ink and incubated for 3 d in tightly closed vials containing initially 4% O_2 and 96% N_2. The first three segments from the tip were located on the coleoptile, the fourth corresponded to the mesocotyl. Initial and final values of coleoptile and mesocotyl segments are the average of 20 seedlings ± SE.

Table I. *Comparison of Ethylene Production Rates from Different Parts of Rice Seedlings*

Shoots, seeds, and roots from 80-h-old seedlings were incubated in 5-ml-vials with or without 0.2 mM ACC in 10 mM Mes buffer (pH 6.0). After 4 h, ethylene evolution was determined. Each number is the average value of three experiments ± SE.

	Ethylene Evolution	
	−ACC	+ACC
	nmol g fresh wt^{-1} h^{-1}	
Shoots	0.057 ± 0.006	0.812 ± 0.141
Seeds	0.005 ± 0.001	0.013 ± 0.005
Roots	0.011 ± 0.003	0.183 ± 0.056

assay ACC according to Lizada and Yang (14). Ethylene determinations were carried out by first injecting 1 ml of air or N_2 into the flask with a tuberculin syringe and then withdrawing 1 ml for analysis by GC (10).

RESULTS

The coleoptile of seedlings growing in open air remain short while growth of the first emerging leaf is rapid. In contrast,

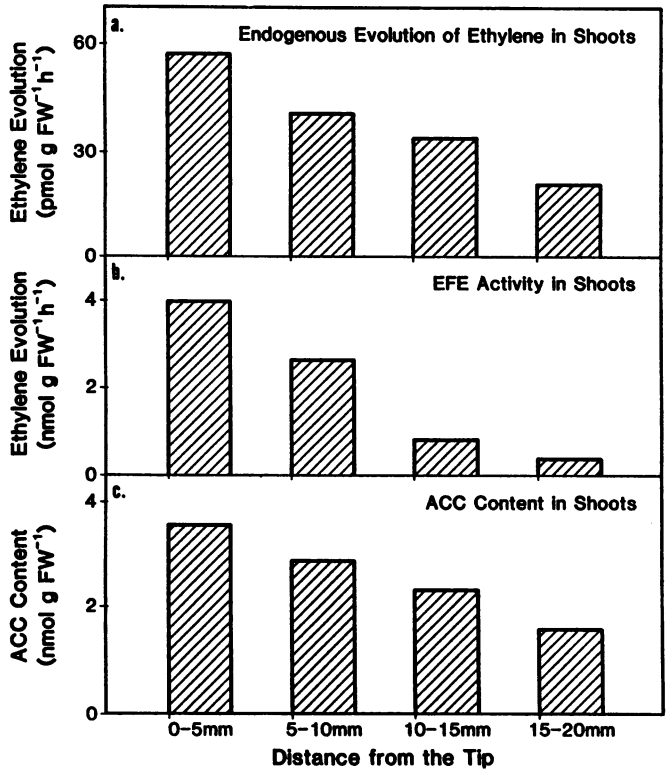

FIG. 3. A, Histogram of endogenous ethylene released from 5-mm shoot sections. Two-cm shoots from 3.5-d-old seedlings were cut into 5-mm sections with a razor blade and incubated in 5-ml vials containing 0.5 ml 10 mM Mes buffer (pH 6.0). After incubation for 30 h, ethylene was determined as before. Data are the average of two separate experiments. B, *In-vivo* distribution of the C_2H_4-forming enzyme (EFE activity). Ethylene evolved from 4-d-old shoots cut in 5-mm sections and incubated in a solution of 0.2 mM ACC. Ethylene production was measured 5 h after the vials were closed. C, ACC content in freshly excised 5-mm shoot sections. Four-d-old rice seedlings were harvested, cut, and immediately extracted for ACC determination.

Table II. *Effect of Ethylene on Intact and Decapitated Coleoptiles*

Ten 3-d-old seedlings with or without tips were incubated for 3 d in tightly closed, 36-ml vials. Coleoptiles were decapitated by excising the top 1 mm of tip. (A) Ethylene was removed from the vials with 0.5 ml of 0.25 M mercuric perchlorate placed in a center well. (B) Ethylene was injected into the vials to a final concentration of 6 $\mu l\ l^{-1}$. (C) In presence of endogenously produced ethylene. Levels of endogenously produced ethylene at the end of the experiment are shown in the last column. Values are averages of 3 vials ± SE.

	Coleoptile Length			Ethylene
	A	B	C	
	mm			*μl/l*
Intact	25.4 ± 0.7	39.8 ± 0.9	35.6 ± 1.0	0.6
Decapitated	12.1 ± 0.9	19.3 ± 0.8	11.3 ± 0.5	0.2

coleoptiles growing in sealed vials showed marked morphological alterations. In a closed vial, coleoptiles grew for 5 d. After 7 d, coleoptiles of seedlings in closed vials were more than twice as long as those of seedlings kept in air (Fig. 1). The level of ethylene also increased markedly between day 1 and 3, reaching a final concentration of 0.9 $\mu l\ l^{-1}$ at the end of the experiment.

To determine the distribution of growth, coleoptiles were marked along their longitudinal axis at different points, and the growth between these points was determined (Fig. 2). No signifi-

Table III. *Effect of 2,5-Norbornadiene on Coleoptile Elongation*

2,5-Norbornadiene was injected as a liquid into a small vial placed inside a 36-ml shell vial to give a final gaseous concentration of 5000 $\mu l\ l^{-1}$. Data are the average of 30 seedlings after 5 d in tightly closed containers ± SE.

	Coleoptile Length	Fresh Wt
	mm	*mg/shoot*
Control	45.9 ± 0.3	24.5 ± 1.7
Norbornadiene	36.1 ± 0.3	29.2 ± 1.9
Ethylene	50.6 ± 2.1	32.3 ± 0.9
Norbornadiene + ethylene	50.1 ± 1.1	29.9 ± 1.8

cant differences were found in the elongation of the three zones that had been marked on the coleoptile. Since cell division takes place at the base of the coleoptile and only during the first 60 h of germination (23), most of the observed growth must have been due to cell elongation.

On a fresh-weight basis, 78% of the ethylene formed by a seedling originated from the shoot (coleoptiles and internal leaves) and only 15% from the roots (Table I). When 2-cm-long shoots were cut in 5-mm-long sections and the evolution of ethylene was measured during a period of 5 h, the section including the tip of the shoot accounted for 37% of the total ethylene evolution. The remaining 63% were produced in a gradient decreasing to the base of the coleoptile (Fig. 3a). Cutting the shoots in small pieces caused wound-ethylene formation (6) as had been shown in other plant systems (*e.g.* Hanson and Kende [5]). Rice shoots cut in four 5-mm sections evolved almost three times more ethylene than did intact shoots. When compared to the controls, ethylene production increased up to 50-fold as a result of treatment with ACC. This result also points to the fact that ethylene evolution in rice seedlings is limited by the availability of endogenous ACC (compare Fig. 3, a and b). Although ACC was available at equal concentrations, not all the parts of the shoot had the same capacity to oxidize ACC to ethylene (Fig. 3b). The ACC content decreased from the tip of the coleoptile towards the base (Fig. 3c).

Ethylene at a concentration of 6 $\mu l\ l^{-1}$ enhanced coleoptile elongation by 57% compared to seedlings which were grown in an atmosphere from which ethylene had been removed with mercuric perchlorate (Table II). Decapitation of 72-h-old rice seedlings reduced the amount of ethylene accumulated during the following 3 d by two-thirds and growth of the coleoptile by 70% (Table II). In such long-term experiments, applied ethylene only partly reversed the inhibition caused by decapitation. Decapitated seedlings in the presence of a saturating concentration of ethylene did not reach the final length of intact seedlings incubated under the same conditions (Table II).

2,5-Norbornadiene counteracts the effect of ethylene on respiration of tobacco leaves and growth of pea epicotyls (19, 20). In the presence of 5000 $\mu l\ l^{-1}$ 2,5-norbornadiene, coleoptile elongation was inhibited by 21% compared to the control (Table III). Ethylene at a concentration of 10 $\mu l\ l^{-1}$ fully reversed the growth inhibition by 2,5-norbornadiene.

The time course of ethylene-induced growth over a period of several hours was investigated using an angular transducer (Fig. 4). Since the seedling was held at the seed, both the mesocotyl and the coleoptile contributed to the total elongation of the shoot. However, in air, mesocotyl growth was negligible. The rate of elongation in response to ethylene did not increase rapidly. Lag time determinations varied greatly from one seedling to another, but in most cases, it was in the range of hours. In short-term experiments such as shown in Figure 4, ethylene reversed the inhibitory effect of decapitation on growth of the seedling.

Little is known about the effect of ethylene on coleoptile growth in anoxia or at low O_2 concentrations. When rice seedlings were grown in gas mixtures of N_2 and O_2, the length of the coleoptile reached a maximum in a gas mixture containing between 2 and 3% O_2 (Fig. 5). Lower and higher concentrations of O_2 reduced coleoptile growth. The effect of exogenous ethylene at different concentrations of O_2 was also plotted in Figure 5. No significant promoting effect of ethylene was observed in the absence of O_2. Stimulation of growth by ethylene increased when the level of O_2 was raised, reaching a saturation when the gas mixture contained about 6% O_2 (difference in the growth response between ethylene-treated and control seedlings, Fig. 5).

Figure 6 shows the time course of coleoptile elongation at 0 and 2.5% O_2, with and without 10 $\mu l\ l^{-1}$ ethylene. While seedlings growing under anoxia continued to elongate during the 5 d of incubation, seedlings growing in a mixture containing 2.5% O_2 ceased growing on about the 3rd d after transfer. The acceleration of the growth rate by ethylene in the presence of O_2 contrasted with the lack of effect of exogenous ethylene under anaerobic conditions (Fig. 6).

DISCUSSION

Growth of the rice coleoptile is enhanced at low atmospheric O_2 and high CO_2 concentrations and by ethylene (7–9, 12, 18, 21). In addition, a fourth, still unknown factor is thought to be involved in the growth of coleoptiles under water (8). The effects

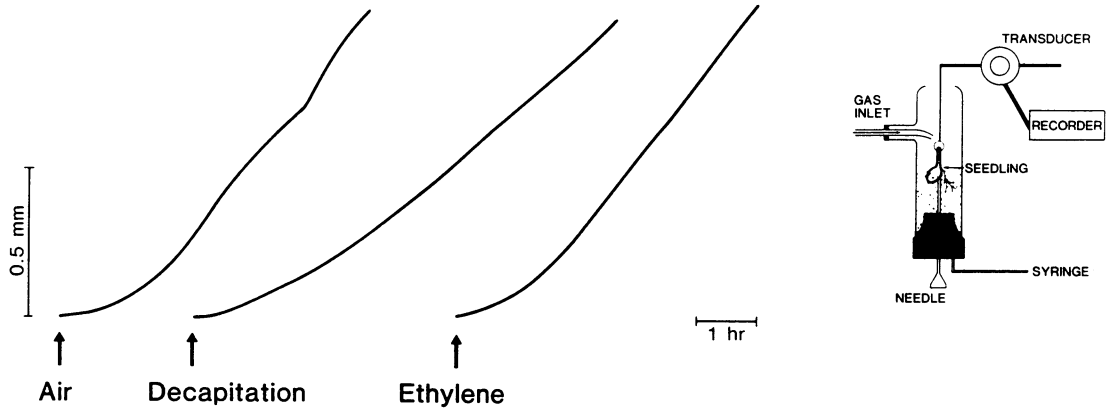

FIG. 4. Effect of ethylene on decapitated rice seedlings. A rice seedling growing in air (first curve), after removal of the top 1 mm of the coleoptile (second curve) and after treatment with 10 $\mu l\ l^{-1}$ ethylene in air (third curve). Air and ethylene were passed through the tube containing the seedling at a flow rate of 35 ml (= 1 volume) min^{-1}. All growth measurements were made with an angular transducer.

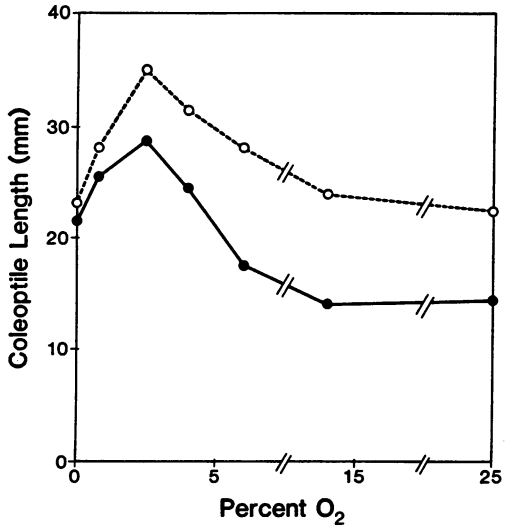

FIG. 5. Effect of different O_2 concentrations on rice coleoptile growth. Two-d-old seedlings were transferred to 36-ml flasks, and a mixture of N_2 and O_2 was passed through these flasks for 3 d at a flow rate of 30 ml min^{-1}. Final coleoptile lengths in the presence of 10 $\mu l\ l^{-1}$ ethylene (O) are plotted along with the control (●). The gas flow was 30 ml min^{-1}.

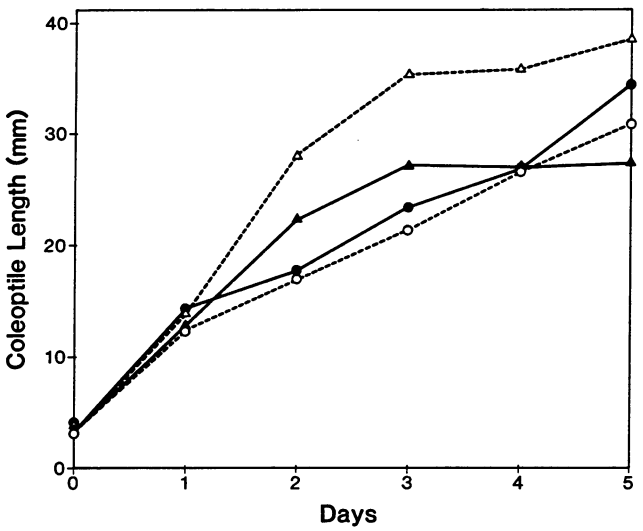

FIG. 6. Effect of exogenous ethylene on rice seedlings growing under anoxia (O, ●) or in a mixture of 2.5% O_2 and 97.5% N_2 (△, ▲). Ten 2-d-old seedlings in 36-ml vials with 2 ml distilled H_2O were grown in a flow-through system (30 ml min^{-1}) for 5 d with (- - -) or without 10 $\mu l\ l^{-1}$ ethylene (——).

of low O_2, high CO_2, and ethylene are additive, and each gas can promote growth independently of the others (18).

Norbornadiene, a chemical reported to counteract the effect of ethylene in tobacco leaves and pea epicotyls (19, 20), inhibits coleoptile elongation by 21% compared to the control. This result agrees with previous ones where ethylene was found to be responsible for about one-third of the total stimulation of coleoptile growth of seedlings in sealed containers (18).

When rice seedlings were grown in vials through which gas was passed continuously, the optimal concentration of O_2 for coleoptile growth was found to be 2.5%. This value is in the same range as those found by previous workers namely, 3.5% (17), 2.5% (16), and 1 to 2% O_2 (4). The same concentration of O_2 was found to be optimal when rice seedlings were grown in the presence of 10 $\mu l\ l^{-1}$ ethylene.

Ethylene promotes coleoptile elongation even at very low levels of O_2 (12). However, ethylene is without any effect under anaerobic conditions. Since ethylene does not stimulate growth under anoxia, the question as to which factor(s) control the growth of the coleoptile under anoxia remains unsolved. However, as was pointed out by Alpi and Beevers (1), only coleoptile elongation is enhanced under N_2; in all the other parameters measured, coleoptiles of seedlings grown in a N_2 atmosphere are markedly deficient when compared to coleoptiles of seedlings grown in air. The greatly increased length of coleoptiles under anoxia compared to the length of those in air is reached because the duration of growth is extended rather than the growth rate accelerated.

More than one-third of the total ethylene produced by the shoot evolves from the first 5 mm of the coleoptile. Ishizawa and Esashi (7) also found that coleoptiles with tips produce twice as much ethylene as did decapitated seedlings. Decapitation of the coleoptile of rice seedlings (24) as well as light (15) produced a marked inhibition of coleoptile elongation which could be partially overcome by the application of ethylene (9). Our results indicate that applied ethylene reverses the growth inhibition in decapitated coleoptiles during experiments lasting several hours but not during experiments lasting several days. Since the apparent K_m of the ACC-dependent ethylene-forming system for O_2 is much lower in coleoptiles than in leaves (S. O. Satler and H. Kende, unpublished data), ethylene formation at low levels of O_2 must occur predominantly in the coleoptile.

Taken together, our experiments indicate that ethylene is indeed one important factor controlling coleoptile growth. Coleoptiles growing in an ethylene-free environment are still able to elongate, but at a slower rate. Our experiments also confirm the notion that factors other than ethylene participate in regulating growth of the rice coleoptile, *e.g.* under conditions of anoxia.

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