

**Short Communication**

# Evaluation of Ethylene as a Mediator of Gravitropism by Tomato Hypocotyls<sup>1</sup>

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## ABSTRACT

Assessments of the participation of ethylene in gravitropism by hypocotyls of tomato (*Lycopersicon esculentum* Mill.) indicate that gravitropism can occur without substantial change in ethylene production. Moreover, lowering or evaluating ethylene over a considerable range, as well as inhibiting ethylene action, fails to influence gravitropic bending. This vitiates the possibility that ethylene is a mediator of the primary, negative gravitropic response of tomato shoots.

On the basis of experiments with stems of tomato and other representative dicots, it has been suggested by Wheeler and Salisbury (16, 17) that there is "a positive and possibly primary role for ethylene in shoot gravitropism" and that "Measurements should be made of ethylene evolution from . . . stems as a function of time after changing the plant's orientation with respect to gravity. Such studies could lead to a thoroughly revised model of how stems respond to gravity" (17). In analyzing experiments on the effect of application of inhibitors of ethylene synthesis and action this view has been reiterated (18), but measurements of ethylene production during gravitropism have not been forthcoming.

The purpose of this paper is to provide not only such measurements of gravitropic ethylene production, but also measurements of the gravitropic effects of raising and lowering ethylene levels and of adding inhibitors of ethylene action. The experiments have been performed with the light-grown tomato hypocotyl. Its gravitropism is simpler than that of tomato epicotyls and leaves, in that it is exclusively orthogravitropic rather than plagiogravitropic. Moreover, we have been unable to find any growth regimes under which it exhibits the gravitropic counterreactions seen under some conditions in many kinds of essentially orthogravitropic rather than plagiogravitropic. Moreover, we have been unable to find any growth regimes under which it exhibits the gravitropic counterreactions seen under some conditions in many kinds of essentially orthogravitropic seedlings (12) and believed in certain kinds of plants to involve ethylene. Therefore, the hypocotyl is an ideal organ with which to investigate whether or not there is a mediational role for ethylene in the primary phase of tomato gravitropism.

## MATERIALS AND METHODS

Plants were grown for 7 d under the same conditions as previously described (7). All experiments were begun about 5 h after the beginning of the daily photoperiod.

The capability of the hypocotyls for gravitropic curvature was greater the higher the ambient humidity during growth as well as during response. The humidity of the experimental chamber was regulated between 70 and 80%, but the growth chamber was less rigorously controlled and its fluctuations appear to be the primary reason for the usually minor differences in mean rate of gravitropism from day to day.<sup>4</sup>

For Figure 2, plants were grown on gauze as previously described (7). For Figures 1 and 3, plants were grown in 2-ml shell vials filled with vermiculite, and plants selected for uniformity were aligned in racks which could be mounted in 650-ml rectangular gas-tight transparent Plexiglas containers with rubber ports for injection and removal of gas; shadowgraphs were made at appropriate times for goniometric measurement.

In general, experimental conditions and equipment as well as the method of measuring ethylene were as previously described (7). Organic chemicals employed were aminoethoxyvinylglycine obtained from Fluka, 1-aminocyclopropane carboxylic acid from Sigma, and bicyclo[2,2,1]hepta-2,5-diene (2,5-norbornadiene) from Aldrich. The method of application of AVG<sup>5</sup> and ACC as well as of various inorganic inhibitors was spraying 5 ml of 0.01% Tween 80 and the specified solution at vertical racks of plants from a distance of 15 cm. Plants treated with AVG or inorganic ions were incubated 4 h before experimentation (9), while those treated with ACC were incubated only 20 min. Ethylene and CO<sub>2</sub> were trapped by placing a wide dish of mercuric perchlorate (1) or saturated KOH solution, respectively, in the gas-tight experimental containers. NBD was added by placing a specified amount on cheesecloth in a vial enclosed in the treatment box (13).

## RESULTS

**Time-Course of Response.** Figure 1 shows typical time courses of gravitropic curvature for two representative individual plants (Fig. 1A) and for a set of 70 plants (Fig. 1B).

Within about 10 min after being set horizontal, about 60 of the set of hypocotyls began to bend downward (Fig. 1B), and continued until they had attained a downward curvature ranging up to 7 to 8° (compare Fig. 1A). The average maximal downward

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<sup>4</sup> In certain preliminary experiments, poor control of humidity gave rise to an erroneous impression (12) that very low levels of ethylene could promote tomato hypocotyl gravitropism slightly. Early indications of inhibition by Ag<sup>+</sup> were similarly founded.

<sup>5</sup> Abbreviations: AVG, aminoethoxyvinylglycine; ACC, 1-aminocyclopropane carboxylic acid; NBD, bicyclo[2,2,1]hepta-2,5-diene (2,5-norbornadiene).

curvature, 5%, was attained by about 15 min. Experiments intended for another publication indicated that the downward phase of bending, often described for seedlings (11, 12), was not primarily sagging, but rather resulted from an active process.

Upward bending was typically underway within about 20 min, and soon stabilized at an essentially constant rate until on the average the plants had almost reached 90°.

Thereafter, there were considerable differences from plant to plant. Sometimes, at one extreme (circles, Fig. 1A), a plant overshot 90° without slowing; then, the direction of bending reversed suddenly and the angle of curvature returned rapidly to 90°. Often, a plant slowed as it attained 90°, overshot gradually, and returned very slowly after a considerable lag. Occasionally, at the other extreme (triangles, Fig. 1A), a plant attained the vertical position without hunting. Because of the cumulative effects of slight variability in rates of main-phase curving and in change of rates in the vicinity of the vertical, plants were out of synchrony by the time they attained 90°; thus, the average values plotted in Figure 1B do not effectively represent the overshooting.

**Ethylene Production.** The rate of ethylene production by intact tomato seedlings is essentially unchanged during gravitational stimulation, both by horizontal placement and by clinostatating at 3 rpm. This is illustrated by plots of net ethylene production in Figure 2A and plots of the same data expressed as rate differences from controls in Figure 2B; these plots represent 12 experiments

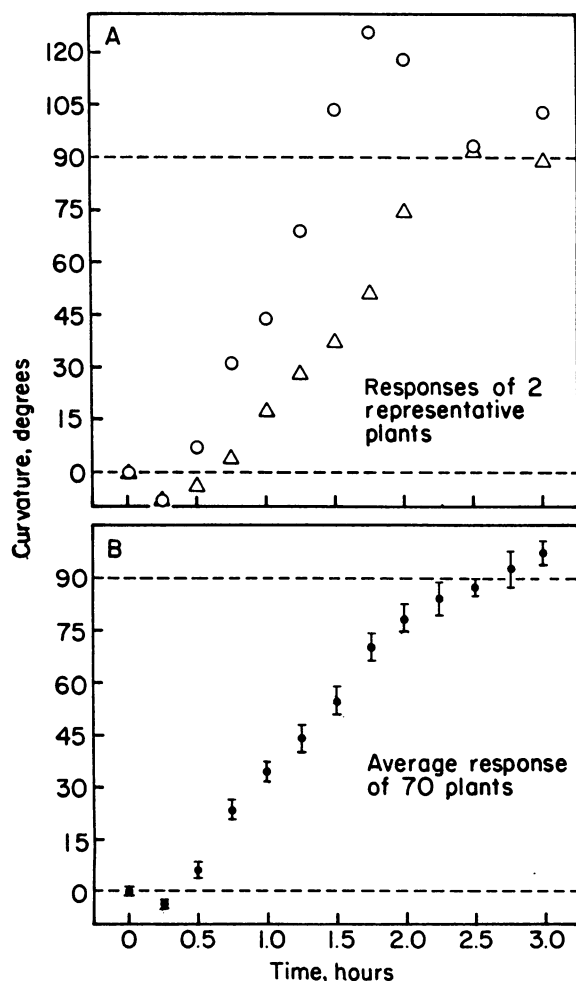


FIG. 1. Representative individual time courses of gravitropism (A) and the average time course for 70 plants (B). The experiments depicted were performed in the dark (with dim red worklight), but similar experiments in diffuse white fluorescent light of about  $1 \text{ W m}^{-2}$  intensity showed indistinguishable patterns and magnitudes of curvature.

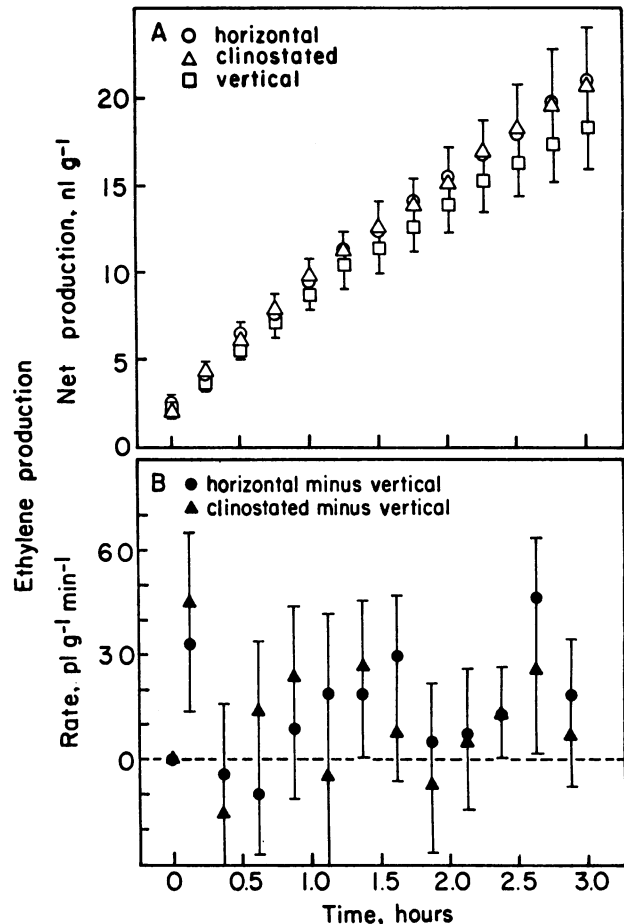


FIG. 2. Ethylene production by plants set horizontal or rotated on a 3-rpm clinostat compared with vertical controls. A, Unprocessed data; B, calculated rate differences. The experiments depicted were performed in diffuse  $1 \text{ W m}^{-2}$  white light, but a similar 1.5-h experiment with vertical plants in the dark (with dim red worklight) yielded indistinguishable rates of ethylene production.

each involving about 500 seedlings in a 500 ml chamber. It has been reported in a previous paper (7) that a small but significant transient elevation in rate begins at once and is completed during the first 3 to 4 min after plants are set horizontal or after they are placed on a clinostat: this doubtless is the reason that in Figure 2A the first measured points (at 15 min) for horizontal and for clinostated plants are displaced upward slightly from the control. Except for this hint of a small but well verified initial shift for stimulated plants, the three plots of Figure 2A maintain essentially similar behavior. (In Fig. 2B a single difference between horizontal and vertical plants, between 150 and 165 min, has an error bar which does not touch the baseline; however, for this point a *t* test yielded  $p \sim 0.1$ , which for one among a set of 24 points otherwise characterized by large *p* values probably indicates only normal stochastic fluctuation. In any case, Fig. 1 shows that plants have already reached the vertical by this time.)

On the basis of the essentially unchanging production of ethylene evidenced in Figure 2, then, this hormone is an unlikely candidate for either a primary or secondary mediator of gravitropic curvature in tomato hypocotyls. However, there remains the remote possibility that subtle asymmetry of production could occur. Asymmetry of production has, in fact, been documented in some organs (2, 6), and asymmetry of production or transport has been suggested (17) as a mechanism whereby ethylene might mediate curvature. It is unlikely that such a mechanism could be tested directly with the tiny young hypocotyls, but there is a

reliable indirect way to assess its validity.

**Influence of Raising and Lowering Levels of Ethylene.** If it is hypothesized that gravitropic asymmetry of ethylene leads to asymmetry of growth, and hence gravitropic curvature, it must be predicted that the rate of gravitropic curvature would be influenced by changing the ambient level of ethylene. Indeed, if ethylene plays any mediational role whatsoever, variation of ethylene level would be expected to affect gravitropic curvature. Figure 3 shows that, on the contrary, gravitropism of young tomato hypocotyls is insensitive to varying ethylene levels within a wide range above and below the physiological level, and even well above it.

Figure 3A shows controls illustrating that there is no significant difference between gravitropic curvatures in the dark and in diffuse white light of about  $1 \text{ W m}^{-2}$ .

The left section of Figure 3B shows that there is no significant effect of ethylene concentrations which might be considered physiologically relevant—from  $0.01$  to  $1 \mu\text{L}^{-1}$  (20). The right section checks that no diminution is found until ethylene is provided at  $50 \mu\text{L}^{-1}$ , a concentration 10,000 times that found in the room air and well above that a seedling is likely to experience in nature.

Additionally (Fig. 3C), the ethylene precursor ACC was sprayed on the plants and gravitropic curvature was monitored in the open room. It was tested that application of  $10 \mu\text{M}$  ACC did result in increased ethylene synthesis starting about 10 min after application: the rate of ethylene production rose from about  $0.06$  to about  $0.23 \text{ nl g}^{-1} \text{ min}^{-1}$  by the end of the 90-min period allowed for curvature. Nevertheless, the gravitropic response was unchanged by the ACC.

Figure 3D illustrates that lowering endogenous ethylene levels by means of a mercuric perchlorate trap did not inhibit gravitropism. The effectiveness of the trap was confirmed by GC: ethylene was diminished below the sensitivity of the assay. Furthermore, removal of  $\text{CO}_2$  by means of a base trap did not influence curvature; this tends to confirm the conclusion based on the ethylene trap because  $\text{CO}_2$  is thought to act competitively

at ethylene binding sites, thus lowering the apparent effectiveness of whatever ethylene is present (5).

Figure 3E further extends the evidence of Figure 3D by showing that spraying plants with AVG and  $\text{CoCl}_2$ , inhibitors of ethylene synthesis, had no significant gravitropic influence. It was confirmed by direct measurement that the inhibitors indeed lowered ethylene production (90% for  $1 \text{ mM}$  AVG and 80% for  $1 \text{ mM}$   $\text{CoCl}_2$  3 h after spraying).

As shown in Figure 3F, inhibitors of ethylene action were also tested.  $\text{Ag}(\text{S}_2\text{O}_3)_2^{3-}$  (3, 15) had no effect at spray concentrations up to  $1 \text{ mM}$ . NBD (13, 14) had no effect at  $10 \mu\text{L}^{-1}$ , though it did inhibit curvature about 25% at  $50 \mu\text{L}^{-1}$ . Because inhibition by  $100 \mu\text{L}^{-1}$  NBD (about 75%) could not be reversed by supplemental ethylene (Fig. 3F), and because  $500 \mu\text{L}^{-1}$  NBD caused the plants to wilt irreversibly, the effect of NBD must be considered nonspecific for ethylene action.

## CONCLUSION

The above data demonstrate that ethylene cannot be a mediator of gravitropism by hypocotyls of the tomato *Lycopersicon esculentum* Mill. cv Rutgers.

## DISCUSSION

Although these data show that ethylene does not mediate gravitropism of tomato hypocotyls, they do not address the concept elaborated by Zobel (19, 20; see also 4) that a very low level of ethylene is required for the gravitropic response system to function. Internal levels of ethylene are difficult to gauge accurately by measuring ethylene released into the ambient atmosphere, and in particular external ethylene serves as an imperfect index of ethylene bound to specific receptors. Thus, low levels of ethylene doubtless occur in plants sprayed with inhibitors or subject to hypobaric treatment, and these might well be sufficient to saturate whatever system is responsible for tonically enabling the gravitropic response.

The fact that ethylene is not an effector of the gravitropic

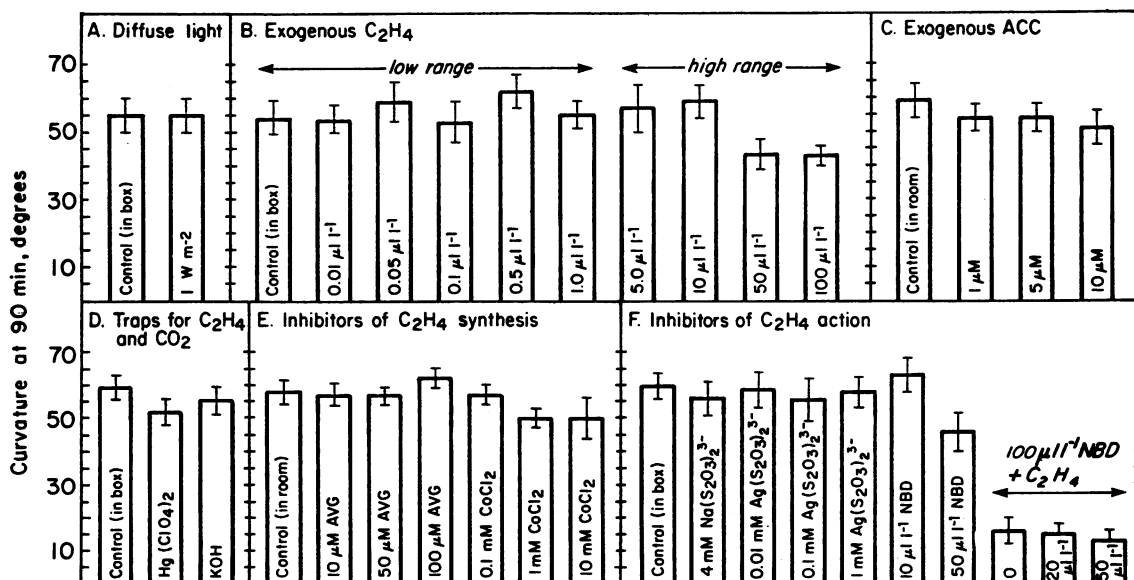


FIG. 3. Lack of gravitropic influence of exogenous ethylene and of factors commonly influencing ethylene production and action. About half of each set of experiments was performed in diffuse  $1 \text{ W m}^{-2}$  white light, and about half in the dark (with dim red worklight). Because no differences were observed under the two conditions, and because the light and dark controls depicted in A were indistinguishable, the data from the two conditions were averaged together for B to D. For A, B, D, and F, which were performed in boxes, it was checked that the contribution by the plants to the ethylene level was negligible: after 2 h, a concentration of about  $3 \text{ nl l}^{-1}$  had built up above the  $5$  to  $7 \text{ nl l}^{-1}$  level in the ambient air. This was consistent with ethylene evolution by the (more crowded) seedlings of Figure 2.

response of tomato hypocotyls strongly suggests that it is not an effector of the primary gravitropic response of main stems, branches, and petioles of the mature plant. This contradicts the suggestion of Wheeler and Salisbury (17) that "ethylene plays an essential role in [mediation of] the gravitropic response of shoots." However, as partially analyzed in a review (12), they worked with limited numbers of plants and their data showed considerable variability. Moreover, much of their argument was based on diminished gravitropic responsiveness following application of various putatively specific inhibitors; yet, no controls were provided to test for the absence of nonspecific effects. Moreover, the inhibitors of action might possibly have blocked the tonic function of ethylene described by Zobel (19, 20).

It is well known that the position of plagiogravitropic equilibrium of lateral branches and petiole-rachises of tomato can be controlled by ethylene (1; see also 8). Indeed, at continuously high levels of ethylene plagiogravitropic organs tend not to attain equilibrium, but may continue to bend until growth ceases. Furthermore, there are reports (10) that ethylene production by stems and petioles of tomato changes in result of clinostating or after repositioning in the gravitational field. Evidently, then, ethylene regulates reactions that counter the primary negative response to gravity.

Most work on plagiogravitropism has been of a preliminary character. Some experiments were confounded by wound ethylene, and most were carried out before GC was developed to its present sensitivity. Because of the importance of plagiogravitropism, investigation should be renewed. The extrapolation from the present study on hypocotyls that ethylene is not involved in the negative response of epicotyls, branches, and petioles should facilitate the delineation of the counterreactive role of ethylene in plagiogravitropism.

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