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Short Communication

Burst of Ethylene upon Horizontal Placement of Tomato Seedlings[1]

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ABSTRACT

Seedlings of *Lycopersicon esculentum* Mill. cv Rutgers emit a pulse of ethylene during the first 2 to 4 minutes following horizontal placement. Because this burst appears too rapid and brief to be mediated by increase in net activity of 1-aminocyclopropane-1-carboxylic acid synthase, it might result from accelerated transformation of vacuolar 1-aminocyclopropane-1-carboxylic acid to ethylene.

A variety of stimuli can increase ethylene production with lags usually ranging from 15 to 20 min (3; see also 7) upward. These stimuli include displacement of a plant from its position of equilibrium in the gravitational field (1, 2, 9). The purpose of this paper is to demonstrate an increase of ethylene production beginning immediately upon such displacement and subsiding within 2 to 4 min.

MATERIALS AND METHODS

Plants. For each assembly of intact plants, approximately 500 seeds of tomato (*Lycopersicon esculentum* Mill. cv Rutgers) were soaked in 0.5% NaOCl (10% commercial bleach) for 15 min, washed thoroughly in distilled H_2O, and spread onto four layers of cotton gauze mounted in a frame and dipped into a solution containing half-strength Hoagland macronutrients (2.5 mM $Ca(NO_3)_2$, 2.5 mM KNO_3, 1 mM $MgSO_4$, 0.5 mM KH_2PO_4) and 10 $\mu g\ l^{-1}$ Peter's micronutrients (Robert B. Peters Co., Allentown, PA); the solution was renewed daily. For experiments with excised hypocotyls, seedlings were sown in vermiculite moistened with the nutrient solution. The seedlings were permitted to grow for 7 d in a chamber maintained at 24 ± 0.5°C and providing mixed incandescent and fluorescent light of 8 to 15 w m^{-2} intensity during daily 18-h photoperiods. (The variability in intensity occurred because, over the relatively long period of experimentation, lamps aged and were replaced).

Measurement of Ethylene. Samples of air were injected onto a 600- × 4-mm column containing 60/80 mesh Alumina F (Anspec Co., Ann Arbor, MI) and mounted in a Varian 2100 gas chromatograph equipped with a flame ionization detector. Peaks of ethylene were identified and quantified by comparison with peak parameters of a standard mixture of 1 ml ethylene/l He (Anspec Co.).

Preparation for Experiments. Work was begun 6 to 7 h after the onset of the light period in a 24 ± 0.5°C chamber. Each assembly of intact seedlings was prepared by transferring a frame with gauze and seedlings to a fitted, shallow, transparent, 500-ml Lucite box with a port closed by a rubber septum (serum vial cap), and sealing a lid in place. When sampling needles were plunged through the septum, their apertures came to rest at the edge of the array of plants, about 2 mm from the closest hypocotyls. Each assembly of excised hypocotyls was prepared by placing four discs of wetted gauze at the bottom of a vial of 15 mm inside diameter and 45 mm inside length and then setting 50 hypocotyls about 20 mm long around the inside perimeter. The vial was sealed with a rubber septum. When sampling needles were inserted, their aperture came to rest in the center of the cylinder formed by the hypocotyls. During manipulation as well as after placement of the boxes or vials for vertical or horizontal treatment, the illumination received at the apices of the seedlings was about 1 w m^{-2}. It was checked with intact plants that suddenly dropping the intensity 10-fold did not measurably alter the rate of ethylene production for at least 32 min, twice the duration of the longest horizontal treatment.

Diffusion Estimates. The diffusion coefficient D of ethylene is given by Landolt-Börnstein (8) as 1.85×10^{-5} $m^2\ s^{-1}$. Accordingly, over the 120-s interval between measurements, the root mean square displacement $(2\ D\ t)^{1/2}$ of a diffusing ethylene molecule is roughly 67 mm. Therefore, there can be no significant boundary layer around either the closely spaced intact plants or the excised hypocotyls. A worst-case analysis, assuming no convective mixing prior to reorientation and perfect convective mixing immediately afterward, indicates that such drastic redistribution could elevate the measurements of ethylene production rate by at most 2% for the intact plants and should actually diminish such measurements for the excised hypocotyls.

RESULTS AND DISCUSSION

Intact Plants. Two sets of paired boxes of plants were closed and the confined air was sampled every 4 min for 16 min to check for regularity and comparability of ethylene production. As shown for seven averaged sets in Figure 1, the level of ethylene rose steadily at essentially the same rate in the paired boxes. Then, one box of each pair was turned on its side and sampling was continued every 2 min for the next 16 min. Almost uniformly (six of seven pairs), the initial 2-min air samples from horizontal plants contained more ethylene than did controls. Averaging from two seven-member sets of values and performing a t test, the difference was significant with $P < 0.01$. At 4 min, it is statistically uncertain whether the horizontal boxes showed a continued gain over controls. Certainly, allowing for statistical scatter, beyond this point the two sets of plants produced ethylene

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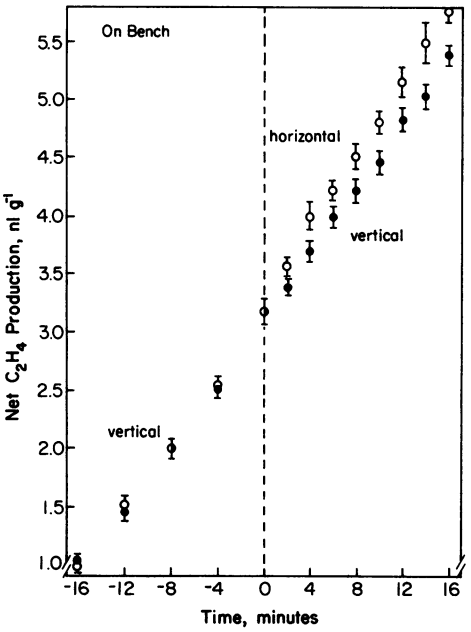

FIG. 1. Immediate, transient increase in ethylene production by seedlings turned horizontal on the bench top. Values were expressed as nl/g fresh weight and averaged from seven pairs of measurements. (Absolute values of the slopes varied not because of differences between bench and clinostat treatments but because of slight differences in intensity of the illumination under which the seedlings were raised.) Error bars represent SE.

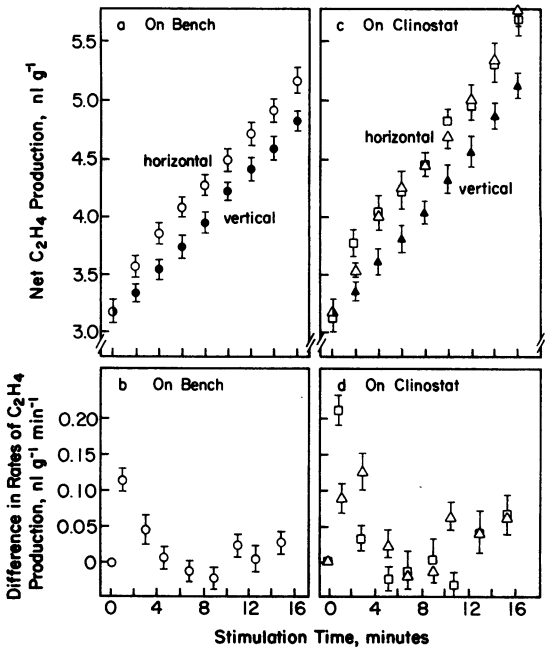

FIG. 2. Immediate, transient increase in ethylene production by seedlings turned horizontal on the bench top (a, b; 14 pairs of measurements) and on the clinostat (c, d; 13 pairs of measurements for rotation with plant axes perpendicular to clinostate axis [Δ] plus eight measurements with plant axes parallel to clinostat axis [□]. a and c show average net production of ethylene as a function of stimulation time for horizontal plants and controls, whereas b and d show average differences between rates of ethylene production by paired sets of horizontal plants and controls or, for the squares of d, differences between averages for experimental plants and controls. The zero values for b and d are interpretational. Error bars represent SE.

at closely similar rates.

For confirmation, the experiment was repeated with 7 more sets of paired boxes, but starting to sample contained air only when experimental plants were swiveled to the horizontal position. Uniformly, the 2-min air samples from stimulated plants contained more ethylene than did the matched controls, and a t test showed that on average the gain was significant with $P < 0.001$. Again, beyond 4 min the production of ethylene by horizontal and control plants was essentially the same.

Combining values for the two experiments (Fig. 2a), rates of ethylene production were evaluated for each measurement interval and the average difference between horizontal and vertical plants is plotted in Figure 2b. This plot emphasizes the brevity of the pulse of ethylene which the plants release upon displacement.

Evidence for an early gravity-stimulated burst of ethylene was extended by stimulating on a 3 revolutions/min clinostat. In this experiment, paired vertical and horizontal plants could not be assessed simultaneously, but while the controls (preequilibrated as usual on the bench top) were rotating in upright orientation about a vertical axis, the matched box of plants was equilibrating. As soon as assessment of controls was completed, the clinostat axis was turned horizontal, the experimental plants were placed with axes perpendicular to the clinostat axis, and stimulation was begun. Alternatively, but without temporally paired controls, experimental plants were stimulated with axes parallel to the clinostat axis.

Some early authors have viewed clinostating as a means of avoiding gravitational stimulation; therefore, it is important to note that it is only a means of nulling certain vectorial responses to gravitational stimulation. In keeping with this interpretation, horizontal clinostated plants unambiguously produced more ethylene than controls during the first few min of stimulation (Fig. 2c). Taking into account the differences for all 13 paired treatments in which plant axes were perpendicular to the clinostat

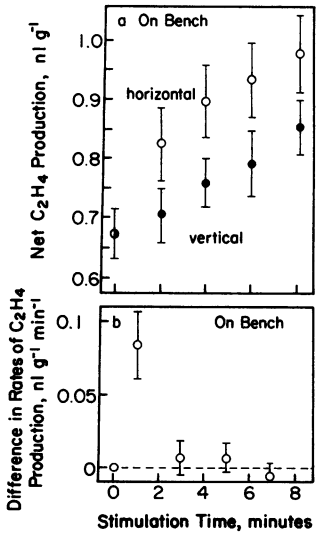

FIG. 3. Immediate, transient increase in ethylene production by excised hypocotyls turned horizontal on the bench top. a shows net ethylene produced as a function of time, and b shows average differences between rate of ethylene production by the 10 paired sets of horizontal plants and controls. The zero value for b is interpretational. Error bars represent SE.

axis, the value of P obtained by the t test for the first 2-min interval was <0.01 and for the second interval was 0.001. Comparing the same 13 controls with eight treatments in which plant axes were parallel to the clinostat axis, the t test for the first 2-min interval yielded $P < 0.001$. In this case, production returned

to the control level for the second 2-min interval, for which $P > 0.5$.

During the following intervals, the increments of ethylene were similar for the horizontally and vertically clinostated plants. Production by the horizontal plants seems possibly to have dropped slightly below that by the vertical plants and then to have risen slightly above. However, this possibility was not further explored.

Figure 2d graphs the data of Figure 2c transformed from net amounts of ethylene produced by horizontal and vertical plants to differences between their rates of ethylene production during each interval; like Figure 2b, Figure 2d emphasizes the immediacy and transiency of the burst.

Excised Hypocotyls. Because of an interest in the geotropic response of tomato stems, we wondered if a momentary burst could be observed in isolated hypocotyls. It was determined that the wound ethylene production initiated by excision nearly subsides within 2 h, so experimentation was begun at that time. Figure 3 shows that isolated hypocotyls indeed produce a momentary burst of ethylene when turned on their sides. (A t test for the 2-min sampling yields $P = 0.01$. This burst, which amounts to about a quadrupling of rate, suggests that hypocotyls contribute to but are not solely responsible for the burst from intact seedlings. However, because of wounding and recovery effects, it is impossible to determine the precise fraction they contribute.

Speculation about Mechanism. Previously observed enhancements of ethylene production usually (3) but not always (7) involve lags of at least 15 min and persist for at least 1 h. They are attributed to synthesis or possibly delayed activation of ACC[4] synthase (6, 10, 11); a substantial amount of the resultant ACC is speedily converted to ethylene. In contrast, the immediate short-lived burst of ethylene observed when tomato seedlings are set horizontal seems unlikely to result from this kind of increase in net ACC synthase activity.

We propose that the burst results from deformation of the tonoplast by amyloplasts settling against and past the central vacuoles of some populations of cells following plant displacement. ACC has been found to accumulate in the vacuoles of some tissue, and the enzyme that transforms ACC to ethylene is also in the vacuoles (4, 5). Enzyme activity of isolated vacuoles depends on tonoplast integrity, suggesting among other possibilities that the enzyme might be situated in the tonoplast (4, 5). If so, tonoplast deformation might transiently increase the capability of the enzyme to attack its vacuolar substrate.

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

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