

Increased Ethylene Production during Clinostat Experiments May Cause Leaf Epinasty

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ABSTRACT

Ethylene production from tomato (*Lycopersicon esculentum* L. cv. Rutgers) plants based on a clinostat doubled during the first 2 hours of rotation. Carbon dioxide blocked the appearance of leaf epinasty normally associated with plants rotated on a clinostat. These results support the idea that epinasty of clinostated plants was due to increased ethylene production and not to the cancellation of the gravitational pull on auxin transport in the petiole.

Early observations of epinasty are associated with reports on the effect of illuminating gas and cigarette smoke on plants (22). Harvey (11) was the first to show that the active component was ethylene. Crocker *et al.* (6, 7) found that concentrations of ethylene, CO, acetylene, and propylene which would induce epinasty would also reduce seedling growth. Denny (8) reported that CO₂ would interfere with the ability of plant emanations (presumably ethylene) to induce epinasty. Whereas only half of the 113 species of plants examined responded to ethylene (7), the effect was widespread and included monocots which showed a curling and looping of leaves (12). The dose response curve for epinasty was similar to that observed for other ethylene-mediated processes such as abscission and growth reduction. Levels as low as 17 $\mu\text{l/l}$ caused some curvature (6), while concentrations greater than 100 $\mu\text{l/l}$ had no additional effect (15). These results indicate that the site of ethylene action in epinasty is probably the same as that for abscission, growth inhibition, fruit ripening, and other ethylene-mediated phenomena.

Onset of curvature is rapid, and induction times of 1 hr (7), 2 hr (18), 3 hr (23), and 5 hr (8) have been reported. Longer times are required for epinasty of huisache (6 hr) and mesquite (18 hr) (4).

Curvature on the upper side of the petiole is due to expansion or growth of cells (5, 7, 19, 23) and probably involves the action of auxin. No epinasty was observed in plants placed on clinostats when the petioles had been cut off from an auxin supply by deblading. However, they do curve when auxin is reapplied to the cut surfaces (13, 21).

Placing plants on a clinostat is supposed to cancel the effect of the earth's gravitational pull on leaves. Epinastic leaf curvature of plants on a clinostat was thought to indicate that the

absence of a unidirectional gravitational field disrupted normal auxin transport in the petioles. In a series of papers, Lyon (13-15) developed the idea that three types of auxin transport occur in leaf petioles: polar (toward the node), dorsal (toward the upper side of the petiole), and ventral (toward the lower side of the petiole). Dorsal transport is thought to function continuously, whereas the ventral transport system is supposed to be sensitive to changes in gravitational pull and ethylene. Lyon (15) suggested that ethylene induced epinasty because it blocked ventral but not dorsal auxin transport, and the curvature resulted because of an accumulation of auxin on the upper side. The same phenomenon was thought to occur on a clinostat, only here the downward movement of auxin was stopped because a gravitational field was no longer detected by the plant.

A number of earlier observations suggest that this interpretation of the action of clinostats may be incomplete. Crocker *et al.* (7) pointed out that petioles of plants held upside down did not curve and, in fact, failed to curve even when ethylene was added to the gas phase. Weight of the leaves was not a factor since the epinastic force was 15 to 30 g, which was four to eight times the weight of the leaf. The lack of curvature is surprising because both ethylene and the fact that the plant was upside down should have caused curvature, assuming the dorsal auxin transport system was functioning. Secondly, Denny (9) demonstrated that horizontal plant tissue produced more ethylene than vertical controls by using potato leaf epinasty as a bioassay. We (1) confirmed his observations and found that, compared to vertical plants, horizontal *Coleus* plants produced enough additional ethylene to account for the accelerated abscission of debladed petioles. From these observations, we were led to believe that placing plants on a clinostat caused epinasty as a result of an increase in ethylene production.

MATERIALS AND METHODS

Tomato (*Lycopersicon esculentum* L. cv. Rutgers) plants were started in vermiculite-filled flats and transferred to 8-cm plastic pots when they were 3 cm tall. The plants were raised for 6 to 8 weeks (30 cm high) in a greenhouse (temperature: day = 23 ± 2 C; night = 18 ± 2 C).

The effect of ethylene and CO₂ on epinasty was measured by inserting petiole explants (3 cm petiole tissue centered on 4 cm of stem tissue) in agar-filled Petri plates and by incubating the tissue in 10-liter desiccators. Ethylene and CO₂ were added to the gas phase by injecting predetermined amounts through a sidearm on the cover sleeve. The amount of curva-

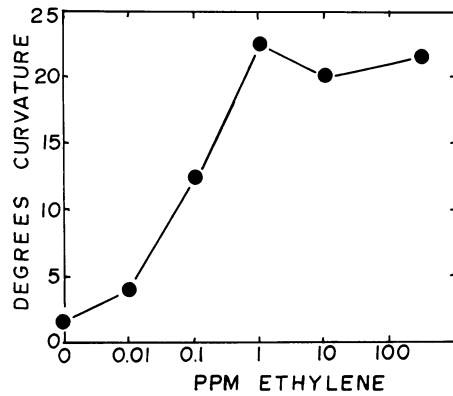


FIG. 1. Dose response curve for the effect of ethylene on the induction of epinasty in tomato petiole explants. Data shown are the difference between the initial axil angle and the angle after 24 hr of treatment with ethylene.

Table I. *Effect of Ethylene and CO₂ on Tomato Leaf Epinasty*

Petiole explants consisting of 3 cm of petiole tissue centered on 4 cm of stem tissue were placed upright in Petri plates filled with 1.5% agar and incubated for 24 hr in 10-liter desiccators filled with 10 μ l/l ethylene and/or 10% CO₂. Curvature is the difference in axil angle before and after the incubation period.

Treatment	Curvature after 24 Hr
	<i>degrees \pm SD</i>
Control	2 \pm 1
Ethylene	11 \pm 2
CO ₂	0 \pm 0
Ethylene + CO ₂	4 \pm 1

ture was calculated as the difference between petiole axil angles before and after a 24-hr incubation.

Plants were rotated horizontally at 12 revolutions per hour by tying the pots to a Model 150 multipurpose rotor¹ (Scientific Instrument Co., Springfield, Mass.). At the end of various rotation intervals, petiole explants (1 cm petiole tissue centered on 2 cm of stem tissue) were excised and placed in 45-ml gas collection bottles fitted with rubber vaccine stoppers and lined with moistened filter paper. Accumulated ethylene was measured after a 2-hr incubation period by sampling the gas phase with a syringe, and by injecting a 2-ml sample into a flame ionization gas chromatograph fitted with a 60 cm \times 6.5 mm (1/4 in.) copper column filled with alumina.

Reversal of clinostat-induced epinasty by CO₂ was performed by enclosing the clinostats in 22.6-liter plastic boxes. CO₂ was added by enclosing a preweighed block of Dry Ice in the box before sealing it for the 24-hr rotation period. All experimental data represents experiments repeated on two or more occasions.

RESULTS

A dose response curve shows that epinasty is similar to a number of other ethylene-mediated processes, since 0.1 μ l/l causes a half maximal response (Fig. 1), and 10 μ l/l is a

saturation level. Also characteristic of ethylene response is the fact that CO₂ reversed the action of ethylene (Table I).

When tomato plants were fastened to a clinostat and rotated, the rate of ethylene production doubled for a 2-hr period, following the start of the experiment, and then returned to the initial rate of gas production (Fig. 2). The data in Table II shows that the clinostat treatment caused epinasty in air but failed to do so in a 10% CO₂ gas phase. The appear-

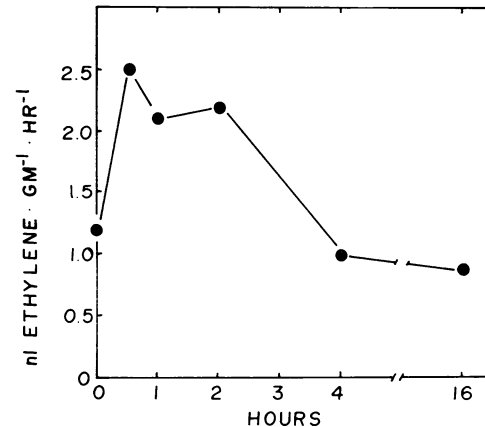


FIG. 2. Increase in the rate of ethylene production following rotation of tomato plants on a 12-revolution per hour clinostat. After the indicated periods of rotation, sections of tissue including the base of the petiole and stem tissue were excised. Ethylene production is based on a 2-hr incubation period of petiole explants in gas collection bottles.

Table II. *Effect of CO₂ on Clinostat-induced Leaf Epinasty*

Clinostats bearing 30 cm tall tomato plants were placed in 22.6-liter plastic boxes filled with air or air + 10% CO₂. Curvature is the difference in axil angle before and after 24-hr rotation period.

Treatment	Curvature after 24 Hr
	<i>degrees \pm SD</i>
Control atmosphere	18 \pm 6
10% CO ₂	-12 \pm 3

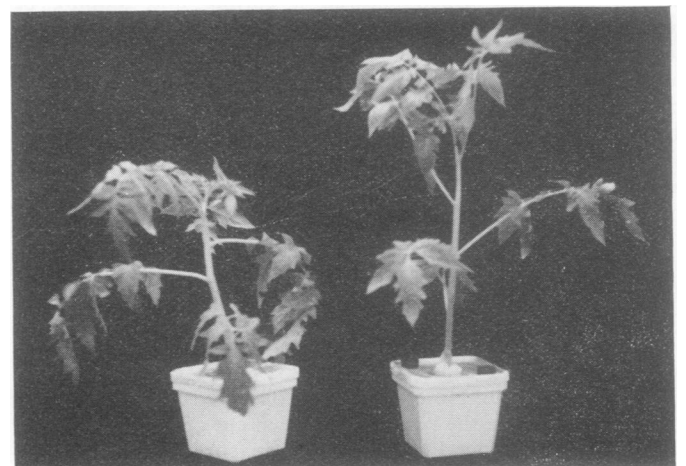


FIG. 3. CO₂ reversal of epinasty of tomato plants rotated on a 12-revolution per hour clinostat. Plant on the left was rotated for 24 hr in air, and the plant on the right was rotated for 24 hr in the presence of 10% CO₂.

¹ Mention of a trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

ance of these plants after rotation in air and CO₂ is shown in Figure 3. The negative value shown for the CO₂ treatment indicated that the leaves actually bent upward during the course of the rotation.

The effect of short periods of ethylene treatment on epinasty is shown in Table III. The data indicate that one or more hours of ethylene treatment were sufficient to cause epinasty. The curvature of petioles was the same as that shown when they were re-examined 24 hr later.

Greater curvature was observed when explants were treated with ethylene in the presence of auxin. Figure 4 shows that a 24-hr exposure to 10 μl/l ethylene increased petiole curvature by 20°. Application of IAA to various parts of the explant resulted in even greater curvature and the maximum effect was observed when the auxin was applied so that it would reach the upper side of the petiole.

DISCUSSION

The data in Figure 2 show that ethylene production doubles following rotation on a clinostat. It is likely that the real increase would be larger if there were a way to subtract the contribution of wound ethylene to the data shown. It is also possible that the increase in ethylene production occurred in a critical area of the petiole. However, while these are not unreasonable postulates, there is no data to support either of them.

The question of whether a 2-hr increase in ethylene production was sufficient to cause the epinasty observed some time later is partially answered in Table III. We observed that one or more hours of ethylene treatment was sufficient to cause epinasty measured 4 hr later. As others have reported earlier (7), the response to ethylene was rapid. Petiole curvature in tomatoes took place 1 hr after they were treated with ethylene.

Table I presents data showing that CO₂ was able to reverse the ability of ethylene to cause epinasty. The epinasty induced by the clinostat was also reversed by CO₂ (Table II and Fig. 3). These observations support the idea that horizontal rotation on a clinostat causes epinasty by increasing ethylene production. However, a question arises concerning the specificity of CO₂ action during epinasty. Is the effect of CO₂ limited to blocking ethylene action or does it interfere with the growth processes associated with curvature? We applied 1 mM auxin to the petiole portion of explants and observed that 10% CO₂ had no effect on the resulting epinasty. This observation indicates that CO₂ does not have any effect on growth of the upper side of the petiole.

Epinasty requires both ethylene and auxin for maximum curvature. This is shown by the data in Figure 4. Whereas some epinasty was caused by ethylene alone, additional curvature was observed when IAA was added to the explants. These observations suggest that auxin-induced epinasty cannot be due to increased ethylene production alone. If that idea were true, then the curvature of ethylene and auxin-treated explants would have been the same. Figure 4 shows that the greatest amount of epinasty occurred when auxin was available to the upper side of the petiole. This observation supports Lyon's idea (15) that petiole curvature is a result of the asymmetrical distribution of auxin in the petiole.

The increase in ethylene production observed may be due to an increase in auxin levels in horizontal plants (2, 3, 17, 20). However, Härtling (10) failed to observe an increase in auxin levels in horizontal plants. The reason for higher levels of auxin in horizontal plants is not known, but the findings of Palmer and Halsall (16) suggest that polar auxin transport in sunflowers was inhibited by placing them on a clinostat. Perhaps the reduction of auxin transport causes an accumula-

Table III. Effect of Short Exposures to Ethylene on Epinasty

Petiole base explants were treated with 10 μl/l ethylene for the times indicated, and curvature was measured 4 hr later. Five explants were used for each treatment.

Hr Exposed to Ethylene	Increase in Curvature Compared to Air Controls after 4 Hr
	<i>degrees ± SD</i>
0.5	0.3 ± 6.5
1.0	6.3 ± 5.3
2.5	12.0 ± 7.8
4.0	16.0 ± 7.1

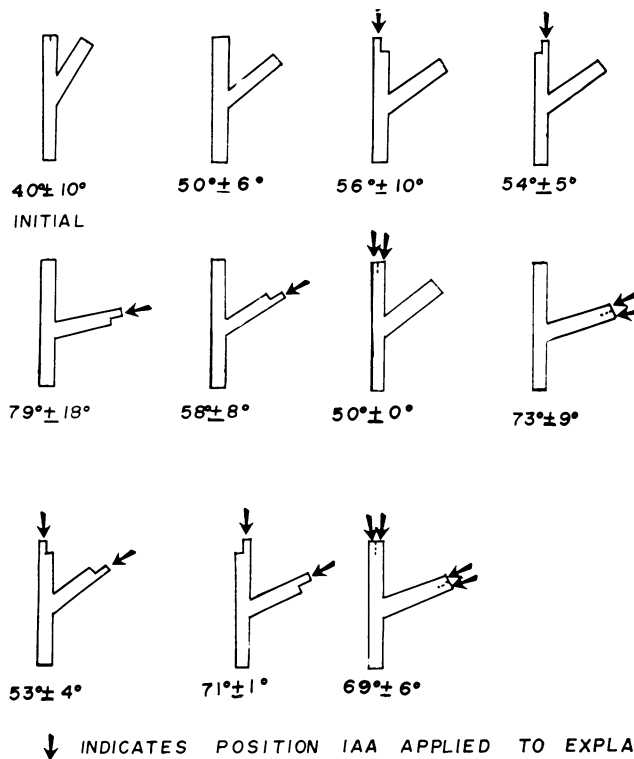


Fig. 4. Effect of simultaneous addition of ethylene and IAA to tomato explants. IAA (1 mM) in agar droplets were applied to the surface of the explants indicated by an arrow. The explants were then exposed to 10 μl/l ethylene for 24 hr and, the petiole curvature was measured. Values shown are the average of six explants.

tion of the hormone in stem and petiole tissue which, in turn, accounts for the rise in ethylene production. However, the opposite explanation may also be true; namely, an increase in ethylene production may block polar transport which, in turn, would cause an accumulation of auxin. Time course studies will be required to distinguish between cause and effect in auxin accumulation in horizontal tissue.

In summary, this and early reports (1, 8) have shown that horizontal plants produce more ethylene than upright ones. The increased abscission of debled petioles, and leaf epinasty associated with horizontal plants, is probably due to this increased ethylene production. It is possible then that altered auxin distribution in petioles is not a direct result of canceling the earth's gravitational field by a clinostat. The data presented inserts ethylene as an intermediate step between a change in orientation and altered auxin transport.

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