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Apical Correlative Effects in Leaf Epinasty of Tomato[1]

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ABSTRACT

The influence of the stem apex on leaf curvature was investigated using debudded tomato (*Lycopersicon esculentum* Mill. cv Anahu) plants and petiole explants, consisting of a section of petiole attached to a section of stem.

Decapitation of the main shoot of tomato plants induced hyponasty of petioles in young leaves. Application of auxin in place of the removed apex or fumigation of intact tomato plants with ethylene produced epinastic curvature at the base of the petiole. Simultaneous carbon dioxide treatments prevented the development of petiolar epinasty due to auxin and ethylene treatments. Application of ethylene gas to the decapitated shoot or injection into the stem, induced petiolar epinasty. In a saturating level of ethylene gas, tomato petioles did not respond to indole-3-acetic acid applied to the cut apex. Auxin-induced ethylene production in petiole explants preceded the development of epinasty. Application of indoleacetic acid in lanolin to the entire lower side of the petioles of leaves *in situ* produced petiole epinasty. Petiolar epinasty due to apically applied indoleacetic acid resulted from differential cell elongation.

The auxins indole-3-acetic acid, 2,4-dichlorophenoxyacetic acid, and naphthalene-1-acetic acid induced epinasty when applied apically to decapitated tomato plants, while gibberellic acid, kinetin, abscisic acid, and auxin or gibberellin antagonists had no effect. When such compounds were applied to petiole explants, only indole-3-acetic acid and kinetin caused an increase in ethylene production and the effect of kinetin was relatively weak.

Application of 2,3,5-triiodobenzoic acid around the stem did not change the effect on petiolar epinasty of auxin applied to the decapitated shoot or around the stem. Radioautography showed that the label from ^{14}C-indoleacetic acid applied apically entered the petiole and midrib tissue; however, extraction showed that only a fraction of the label in these tissues was in the form of indoleacetic acid.

Removal of leaflets from leaves induced hyponasty in the midrib region, and application of auxin to the leaflet stubs produced midrib epinasty; carbon dioxide did not block the action of auxin in this type of epinasty. Removal of leaflets from leaves did not alter the effect of apically applied auxin on petiolar epinasty.

The data are consistent with the hypothesis that the oblique orientation of leaves in tomato plants is influenced by two epinastic responses. Petiolar epinasty is controlled by the apical region on the stem and is due to the action of auxin-induced ethylene; and midrib epinasty is due to an action of auxin other than through ethylene.

The orientation of a leaf can be described as the resultant of three processes, each responsive to a different set of factors, and each responsible for the establishment of leaf position within a different frame of reference. The negative geotropism of a leaf is a response to the stimulus of gravity and orients the leaf with reference to the gravitational force. The inherent epinasty of the leaf is a response of its dorsiventrality and orients the leaf with reference to its internal geometry in structure and function. The response of a leaf to the apex of a plant arises from stimuli transmitted from the apex to the leaf and orients the leaf with respect to the plant axis. The three processes may be postulated to interact in arranging the orientation of individual leaves to give an over-all leaf pattern of optimal benefit to the plant.

Studies on mechanisms of leaf orientation have concentrated upon the negative geotropic response and inherent epinastism (4, 5, 10, 12, 15, 17, 20, 21) and have led to the theory that the preferred orientation of a lateral organ is the result of a balance between auxin accumulated on the ventral side in response to gravity and auxin accumulated on the dorsal side in response to inherent epinastism. The present study concentrates upon the relatively neglected role of the stem apex in determining leaf orientation and the function of ethylene in this role is explored. Effects of ethylene have been observed in other recent investigations (9, 16, 22), which have differed in their approach to epinasty from our approach.

The basic observations (17–19) on the effect of the stem apex on leaf orientation are that decapitation of the main shoot induces hyponasty of young leaves and the application of auxin to the cut apex produces epinasty. The mechanism of this auxin effect, as it relates to the effect of the stem apex on leaf orientation, is probed in the present work.

MATERIALS AND METHODS

Uniform tomato seedlings, *Lycopersicon esculentum* Mill cv. Anahu, grown in a glasshouse for 6 weeks when they were 50 to 60 cm tall, were randomly assigned to various treatments. Lateral shoots were removed during the growth period, and no lateral buds remained at the start of the experiments. Twenty-four hr before the initiation of the treatments, plants were transferred to a darkroom (25 ± 2 C), which provided the test conditions. All treatments lasted 24 hr.

Treatments were applied to intact and decapitated whole

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plants. Decapitation of plants consisted of the removal of the apex and adjacent young leaves. Epinastic curvatures of the top four leaves on the stem were observed, and the youngest leaf remaining after decapitation was designated No. 1. Treatments were also applied to petiole explants consisting of 3 or 4 cm of petiole tissue centered on 3 or 4 cm of stem. The three uppermost petioles from each plant were used.

Degree of Curvature. The degree of epinastic curvature in a leaf was measured by tracing manually the outlines of stem and leaf shadows, before and after treatment. Shadows from pot margins, the main shoot, and a wooden stake were used as reference points. For all measurements, the location of light, plant, and tracing paper were constant. The degree of total leaf curvature was obtained from the intersection of tangent lines drawn from the leaf tip before and after treatment; this procedure is that of Lyon (11–13). Total leaf epinasty was analyzed into midrib epinasty, which was the difference in the angle of the intersection of tangent lines drawn at the tip and the base of the midrib region before and after treatment, and petiolar epinasty, which was the difference between the total epinasty and midrib epinasty. Positive curvature means epinasty, and negative curvature indicates hyponasty. The degrees of curvature in petiole explants were measured by tracing the outlines of explants before and after treatment and the degree of epinasty was the change of angle in tangent lines drawn from the end of the petiole. Readings from 12 leaves or 9 petiole explants were averaged to give the degree of epinasty.

Location of Growth Zone. India ink markers, 2 mm apart, were placed along the upper and lower sides of the leaf petiole and midrib. Changes in the distances between the markers were recorded after 24 hr of treatment. For the study of cell growth, a 2-cm zone of the upper and lower sides of the petiole base from the two top leaves of tomato plants was marked. After treatment, marked sections were removed, fixed, embedded in paraplast, sectioned 12 μm thick, and stained with hematoxylin. Epidermal cells in the upper and lower sides of the sections were counted.

Application of Chemical Treatments. To prepare aqueous solutions, IAA, GA, Kn,[4] ABA, NAA, 2,4-D, PCPIB, TIBA, or AMO 1618 were wetted with a few drops of absolute ethanol, and this mixture was dissolved in warm deionized water. An aqueous solution was applied to the cut surface of a decapitated plant in glass tubing, using a rubber-tubing connector sealed to the stem with anhydrous lanolin.

To prepare a lanolin paste, a solution of a chemical in a few drops of ethanol was stirred into anhydrous lanolin. Lanolin pastes were applied to plants in layers, 3 mm thick and 1 cm long.

For ethylene treatments of whole plants, measured amounts of ethylene were syringed into 40-liter glass jars containing the plants. To inject ethylene into the stem, a syringe needle was inserted into the stem tissue at the internode between leaves 2 and 3, and the area of contact between the stem tissue and needle was sealed with modeling clay. The cavity of the syringe needle was sealed with a vaccine cap through which 0.5 cc of ethylene was introduced. Ethylene was applied to the decapitated shoots of plants by injecting 1 cc of ethylene into rubber tubing, sealed over the cut stem and blocked at the other end. In control plants, air was similarly administered.

To treat a plant with CO_2, a measured amount of CO_2 in a balloon was placed along with potted plants in 40-liter glass jars. The balloon was punctured through the jar lid to release the CO_2. This procedure permitted the introduction of a large volume of CO_2 (10% of air inside the jar) into the air surrounding a plant, simultaneously with other treatments to the plant. Gas chromatographic measurements of the CO_2 content of the air in the jar showed it to average about 10%. In control treatments, equivalent amounts of air were released into the jars.

Explant Treatment and Ethylene Production. Petiole explants were floated on 8 cc of chemical solution in 140-cc glass jars, with vaccine caps as covers. The solutions on which petiole explants floated were prepared in 10^{-3} M phosphate buffer solution, pH 6.8. In all treatments, the final concentration of the chemicals was 10^{-4} M. Ethylene production was measured by sampling 1 cc of air from each of three or four jars per treatment and injecting it into a flame ionizing gas chromatograph. Following ethylene determinations, explants were weighed.

In a time course study of auxin-induced ethylene production and the development of epinastic curvature in petiole explants, the following procedure was used. Petiole explants were placed upright in 50 cc of moist vermiculite in 140-cc glass jars. After the application of 2% IAA in lanolin or plain lanolin, to the upper cut end of the stem, the jars were sealed. At designated time intervals, the ethylene content of the air inside the jar was measured and the explants were removed to determine the degree of curvature, then the explants were weighed and discarded. The ethylene determinations recorded are the averages from four jars and determinations for epinasty are the averages from 12 petiole explants.

Radioautography. The usual method of supplying aqueous solutions to the decapitated apex was used to apply radioactive ^{14}C-IAA and ^{14}C-glucose (as control) to plants. The IAA was carboxyl labeled with a purity of 98% and a specific radioactivity of 57 mCi/mmole. Radioactive IAA was applied to the decapitated shoot dissolved in 10^{-4} M ^{12}C-IAA solution. The glucose molecule was labeled at all 6 carbon atoms. During a 24-hr treatment in the dark, a total of 9 μCi ^{14}C-IAA and 45 μCi of glucose were taken up. Then the top three leaves and the stems (split in half) were dried and exposed to x-ray film: ^{14}C-IAA-treated plants for 40 days and ^{14}C-glucose-treated plants for 15 days.

^{14}C-IAA Extraction. During a 24-hr treatment period, 17.5 μCi of ^{14}C-IAA in 10^{-4} M IAA was taken up by the decapitated shoot of a tomato plant. The petiole and midrib tissues of the four top leaves were extracted three times with hot 80% ethanol. The extract was reduced in volume and along with a sample of ^{14}C-IAA and ^{12}C-IAA was partitioned by paper chromatography with a mixture of isopropanol-ammonia-water (8:1:1) as a solvent system. The relative activity of ^{14}C-IAA was measured by means of a GM tube.

RESULTS

Ethylene and Auxin in Petiolar Epinasty. The dose-response curve for the effect of ethylene on the leaves of the intact tomato plants used in the present work (Fig. 1A) is similar to that obtained by others. For most leaves the development of epinasty is restricted to the petiolar region but, at 10 ppm and higher, some leaves also show curvature in the midrib and in the leaflet petioles. At 100 and 1000 ppm of ethylene, abscission of older leaves is hastened. Carbon dioxide reverses the effect of 10 ppm of ethylene on the epinasty of tomato petioles (Table I).

Decapitation of the main shoot of tomato plants induces a slight hyponasty of petioles in young leaves, and application

[4] Abbreviations: Kn: kinetin; NAA: naphthalene-1-acetic acid; PCPIB: *p*-chlorophenoxyisobutyric acid; TIBA: 2,3,5-triiodobenzoic acid; AMO 1618: 2′-isopropyl-4′-(trimethylammonium chloride)-5′-methylphenylpiperidine carboxylate.

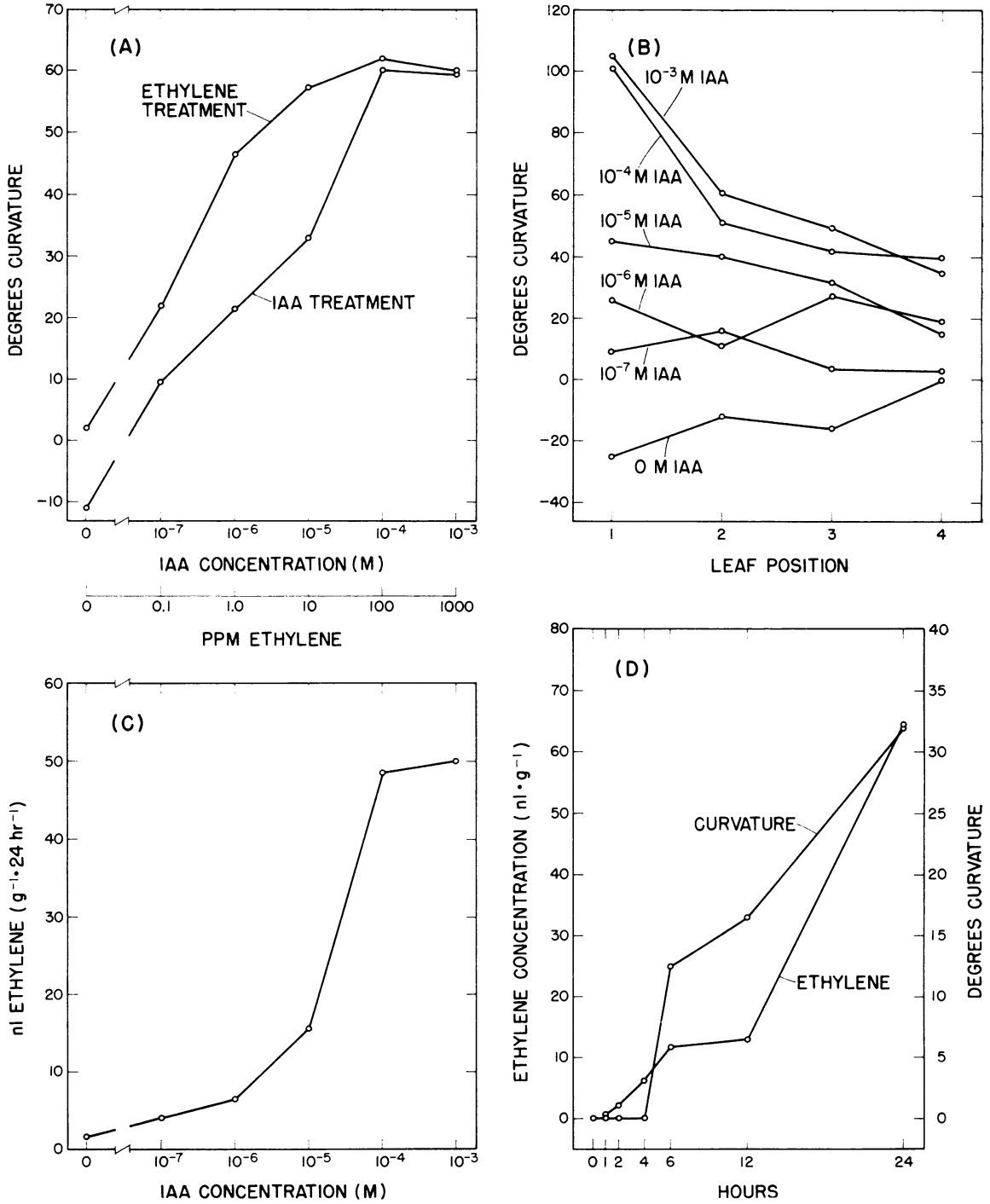

FIG. 1. A: Dose-response curves for the epinastic curvature of petioles of tomato leaves on intact plants exposed to ethylene in a closed chamber and on decapitated plants treated apically with IAA. Each point represents average readings from 16 leaves on ethylene and 24 leaves on IAA-treated plants. B: Effect of apically applied IAA at different concentrations on the petiolar epinasty of decapitated plants with respect to leaf position. Leaf 1 is the closest to IAA source. Each point represents readings from six leaves. C: Dose-response curve for the effect of IAA concentrations on the production of ethylene from the tomato petiole explants. D: Time course of ethylene production and the development of epinastic curvature in petiole explants, treated with 2% IAA in lanolin on the upper cut surface of the stem. Each point is the difference between treatment and a lanolin control.

of auxin to the decapitated shoot induces petiolar epinasty (Fig. 1A). Generally, application of increasing concentrations of auxin to the cut apex yields corresponding increases in epinasty. IAA concentrations up to 10^{-4} M, produce leaf epinasty, which is mainly due to petiolar epinasty (Table II); however 10^{-3} M IAA, which causes senescence of the cut apex, produces curvature in the midrib region in addition to the petiolar epinasty. The degree of epinastic curvature induced in the petioles of leaves by IAA varies with leaf age and distance from the source of IAA (Fig. 1B). Generally, the younger leaves which are also those closest to the auxin source, yield the greatest epinastic curvature. The effect of apically applied IAA on the development of petiolar epinasty did not occur when the atmosphere surrounding IAA (10^{-4} M) treated plants

contained 10% CO_2 (Table I). Application of ethylene gas at the saturating level of 100 ppm, prevents tomato petioles from responding to a full range of IAA concentrations applied to the cut apex (Table III).

To investigate basipetal transport of apically-applied IAA as a factor in petiolar epinasty in the leaves, the effect of TIBA on auxin responses was studied. Application of 2% TIBA in lanolin as a ring 2 to 3 cm below the apically-applied IAA (10^{-4} M) solution had no significant effect on the development of petiolar epinasty. Application of 10^{-4} M IAA solution to the decapitated shoot produced an average petiolar epinasty of 62 degrees, while apically applied IAA, with a TIBA ring on the stem, caused an average petiolar curvature of 60°. Control (water- and lanoline-treated) plants averaged −16° of epinasty. Data in Table IV show that 2% TIBA in lanolin, applied as a ring 1 cm above or below a ring of 1% IAA in lanolin applied to the internode between leaves 2 and 3, resulted in a reduced degree of epinastic curvature, when TIBA was applied below the IAA source. However, analysis of variance indicated that TIBA has no significant effect on the development of auxin-induced petiolar epinasty.

Table I. *CO_2 Reversal of Ethylene and Auxin-induced Petiolar Epinasty*

Experiment I: 10 ppm ethylene, 10% CO_2, or both, were applied to intact plants. Experiment II: 10^{-4} M IAA solution or deionized water were applied to the decapitated shoots of tomato plants, in the presence or absence of 10% CO_2. Means within columns, with different letters, are significantly different at 0.05 *P* level.

Experiment I		Experiment II	
Treatment	Curvature	Treatment	Curvature
	degrees		*degrees*
Ethylene	77c	IAA	59a
CO_2	2d	CO_2	3b
Ethylene + CO_2	17d	IAA + CO_2	−12b

Table II. *Analysis into Petiolar and Midrib Epinasty of Leaf Curvature Due to Apically Applied IAA*

Mean degrees of curvature developed in the midrib and petiolar portions of the leaf following the application of IAA, at the concentrations shown, to the decapitated apex. Means with different letters are significantly different at 0.05 *P* level.

Location of Curvature in	IAA Concn	
	10^{-3} M	10^{-4} M
Petiole	54a	56a
Midrib	76a	1b
Total	130c	57a

Table III. *Effect of a Saturating Level of Ethylene on the Response of Petioles of Tomato Leaves to Various Concentrations of IAA*

Varying concentrations of IAA were supplied to the decapitated shoots of tomato plants which were enclosed within 40-liter glass jars with 100 ppm ethylene. Values shown are degrees of curvature and are the average readings from eight leaves.

	IAA Concn (M)				
	0	10^{-6}	10^{-5}	10^{-4}	10^{-3}
	degrees				
Curvature	51	44	49	48	49

Table IV. *Effect of TIBA on the Development of Petiolar Epinasty Due to IAA Applied to the Stem Surface*

Two per cent TIBA in lanolin was applied as a ring 1 cm above or below lanolin rings containing 1% IAA. Both chemicals were applied to the uninjured surfaces of the internode between leaves 2 and 3. Each number is the average of readings from 10 leaves. Means in columns with different letters are significantly different at 0.05 *P* level.

Relative Position of IAA and TIBA on the Stem	Mean of Degrees of Curvature in Petioles of Leaves	
	Below the site of chemicals	Above the site of chemicals
IAA alone	35a	45c
TIBA alone	−4b	−4d
IAA above TIBA	25a	43c
IAA below TIBA	30a	50c
Lanolin control	−3b	−3d

Table V. *Effect of IAA Applied to Different Parts of the Stem and Petioles on Epinasty*

Degrees of petiolar epinasty developed following application of 1% IAA in lanolin to the entire upper side of petiole (treatment A), or the entire lower side of the petiole (B), or as a ring on the petiole 4 cm from the stem (C) or as a patch on the stem opposite to the petiole (D). Values are the differences between the treatments and the respective controls. The decapitated shoots of all plants were supplied with deionized water.

	Treatment			
	A	B	C	D
Mean of degrees of curvature compared to controls	55	56	69	63

Application of Auxin and Ethylene to Petiole and Stem. Effects of the asymmetric application of auxin to the upper and lower sides of the petiole were studied by applying 1% IAA in lanolin to the entire upper or lower sides of each petiole, or as a ring on each petiole 4 cm from the stem, or as a patch on the stem opposite to the petiole. Plain lanolin treatments were applied as controls. All four auxin treatments produced a significant degree of petiolar epinasty over the respective controls (Table V).

Ethylene was administered directly to locations where it might be anticipated to be induced when auxin treatments were applied at the apex or to the stem; thus ethylene or air (control) were introduced to the decapitated shoot or injected into the internode between second and third leaf of tomato plants. Both means of ethylene treatment produced petiolar epinasty, significantly different from the controls at 0.01 *P* level (Table VI). When ethylene was injected into the stem, the curvatures of petioles above and below the injection point were 54 and 30 degrees, respectively.

Auxin and Ethylene Production from Petiole Explants. Application to petiolar explants of concentrations of IAA from 10^{-6} to 10^{-4} M induces increasing ethylene production (Fig. 1C), and application of 100 ppm of ethylene gas to petiole explants not treated with IAA induces a significant increase in petiolar epinasty (33°) over the air controls (2°). To investigate the rate of production of auxin-induced ethylene relative to the

rate of development of epinastic curvature in petiole explants, the upper stem portions of explants were treated with 2% IAA in lanolin or lanolin alone, then the explants were held upright in jars. Ethylene increased in the atmosphere around the petiole explants about 3 hr before epinastic curvature could be detected (Fig. 1D).

Analysis of the Nastic Growth Response. The location of the zones of differential growth responsible for the epinasty resulting from auxin or ethylene treatments were sought. Following the treatment of decapitated shoots with 10^{-4} M IAA solution or deionized water (as control), differential growth was restricted to the base of the petiole (Table VII). Growth was greatest on the upper side of the base of the petiole and it decreased gradually to zero at about 15 to 20 mm from the stem. There was no significant growth in the midrib region or in the lower sides of the petioles. A similar response was observed in leaves of intact plants that were fumigated with ethylene gas (Table VII), and again differential growth was the greatest at the upper side of the base of the petiole and extended 15 to 20 mm along the stem. Younger leaves responded the most to both IAA and ethylene treatments (Table VII).

To investigate the roles of cell division and cell elongation in petiolar epinasty, zones, initially 2 cm long and located at the bases of the petioles of plants that were apically treated with 10^{-4} M IAA solution or water (as control), were sectioned, stained, and the number of epidermal cells in the upper and lower sides were counted. After 24 hr of treatment with IAA, the average lengths of the initial 2-cm zones on the upper side of the petioles had increased to 2.35 cm, but there were no significant changes observed in the lower side of petioles. Also, no changes occurred in the upper and lower sides of petioles of control plants. The counts of epidermal cells were: 472 on the upper side and 480 on the lower side of IAA-treated sections, and 465 on the upper side and 449 on the lower side of control sections, but the differences were not statistically significant. Thus, there is no evidence of an increase in cell number when apically applied auxin produces petiolar epinasty.

Measurements of the average lengths of epidermal cells in the upper side of the petiole indicated that cell elongation was greatest about 1 mm from the stem, and gradually decreased to zero at 2 cm from the stem (Table VIII). Data in Table VIII also indicate that the average length of cells in the lower side of the petioles of control plants is greater than those in IAA-treated plants. Thus, apically applied IAA not only induces elongation of cells in the upper side of the petiole, but it also inhibits the elongation of cells in the lower side.

The over-all rate of elongation of a young leaf (third leaf) over 10 days was affected by decapitation and apically applied IAA as follows: the average increase in the length for leaves on intact plants was 12.9 cm, while on decapitated plants (lanolin was applied to the cut apex) was 13.5 cm and on plants with 2% IAA in lanolin in place of the removed apex was 10.5 cm. The differences between these mean increases were not significant at 0.05 *P* level.

Regulators other than Auxin and Ethylene. While application of IAA at concentrations of 10^{-4} and 10^{-5} M to decapitated shoots of tomato plants induced petiolar epinasty, addition of GA or Kn, or both, to the treatment solution had no significant effect on petiole curvature (Table IX). Nor did solutions of GA, Kn, or other nonauxin regulators affect leaf curvature when applied alone to decapitated plants (Tables IX and X).

Table VI. *Effect of Ethylene or Air Introduced to the Cut Apex or Injected Into the Stem on the Development of Petiolar Epinasty*

Degrees of curvature produced when 1.0 and 0.5 cc ethylene or air were applied to decapitated shoots or injected to the internode between leaves 2 and 3, respectively. Each number represents readings from 12 leaves. Means within rows, with different letters, are significantly different at 0.01 *P* level.

Mode of Application of Gas	Gas Applied	
	Air	Ethylene
To cut apex	−24b	68a
Into stem	−7d	42c

Table VII. *Effect of Apically Applied IAA and of Ethylene on Differential Elongation in the Petiole*

Average increase (mm), during 24 hr, in the lengths of a 30 mm zone marked on the upper side of the base of petioles due to IAA and ethylene treatments: IAA (10^{-4} M) treatments or water controls were applied to decapitated shoots and ethylene (100 ppm) or air controls, were applied to intact plants. There was no significant change in the length of the zones marked on the bottom of petioles, and those on midrib region. The values shown in the table are the differences between the lengths of upper zone due to IAA or water and ethylene or air. Each number represents readings from three leaves. Leaf no. 1 is the youngest.

Treatment	Leaf No.			
	1	2	3	4
IAA	4.17	3.00	2.33	2.00
Ethylene	4.00	3.30	2.83	2.50

Table VIII. *Mean Lengths of Cells Along Upper and Lower Sides of Petiolar Sections*

Mean lengths of cells (μm) in four zones of sections, initially 2 cm, of petiole bases following application of 10^{-4} M IAA or water to the decapitated apices of tomato plants. The zones chosen for measurements were 1 to 2 and 4 to 5 mm from the petiole bases and 0 to 1 and 10 to 11 mm from the other ends of the sections.

Location of Cells through Section	Treatment	Location of Cells Along Section			
		mm from base		mm from end	
		1	4	10	0
Upper side	IAA	42.0	48.9	50.8	49.8
Upper side	Water	31.0	38.5	47.0	49.9
Upper side	Difference	11.0	10.4	3.8	−0.1
Lower side	IAA	37.5	38.8	49.1	50.0
Lower side	Water	42.0	45.8	53.0	50.2
Lower side	Difference	−4.5	−7.0	−3.9	−0.2

Table IX. *Effect of Apically Applied IAA, GA, and Kn in the Development of Petiolar Epinasty*

Values shown are degrees of curvature and are the average readings from eight leaves. Means within rows, with different letters, are significantly different at 0.05 *P* level.

Concn	Regulator Treatments							
	Control	IAA	GA	Kn	IAA + GA	IAA + Kn	GA + Kn	IAA + GA + Kn
10^{-4} M	−19b	54a	−15b	−18b	65a	54a	15b	57a
10^{-5} M	−19d	33c	−19d	−15d	27c	27c	−19d	32c

Of a variety of growth regulators applied to petiole explants (Tables X and XI), only the auxins, IAA, 2,4-D, and NAA, and the cytokinin, Kn, induced significant rises in ethylene production. However, the effect of kinetin on ethylene production was small compared to the effect of auxin (Table XI). When IAA and kinetin were applied together to the explants, there was a significant interaction in the rate of the ethylene production. GA alone or in combinations with IAA, kinetin, or both had no significant effect on the rate of production of ethylene. Among the regulators tested, ethylene production from petiole explants is influenced by only kinetin, IAA, and the combinations of the two.

Midrib Epinasty. Removal of leaflets from leaves induces hyponasty, which is mainly due to the uncurling of the midrib region. Twenty-four hr after the removal of leaflets, the average degrees of hyponasty for nine leaves was 65°. Removal of leaflets had no significant effect on the development of petiolar epinasty due to apically applied IAA solution (Table XII). Midrib hyponasty in leaves without leaflets is not reflected in data in Tables XII and XIII since the leaflets were removed 24 hr before the start of the experiment. Application of 0.1% IAA in lanolin to the leaflet stubs induces epinasty, but the development of curvature is mainly restricted to the midrib region; however, some petiolar epinasty also occurs (Table XII). Simultaneous application of 10^{-4} M IAA solution to the decapitated shoot and 0.1% IAA in lanolin to the leaflet stubs produces a significant increase in total leaf epinasty (Table XII). Decapitation of plants, whose leaves are intact or without leaflets, induces leaf hyponasty (Table XII) which is mainly petiolar. The development of midrib epinasty due to the application of 0.1% IAA in lanolin to the leaflet stubs is not blocked by the presence of 10% CO_2 in the atmosphere surrounding the plants (Table XIII); there are no significant differences in the midrib epinasty induced by IAA and IAA + CO_2.

Table X. *Effects of Growth Regulators on the Rate of Production of Ethylene and the Development of Petiolar Epinasty*

Aqueous solutions of growth regulators at 10^{-4} M were supplied to the decapitated shoots of tomato plants, on which curvature was measured, or were the medium for petiolar explants from which ethylene production was measured. Means within columns with different letters from the control are significantly different from the control at 0.05 *P* level.

Treatment	Ethylene Production	Curvature
	nl/g	*degrees*
AMO 1618	1.00b	−16d
ABA	2.65b	−23d
TIBA	3.27b	−14d
PCPIB	2.86b	−11d
2,4-D	39.7a	62c
NAA	72.7a	48c
Control	2.9b	−22d

Table XI. *Effect of IAA, GA, and Kn Alone and in Combinations on the Rate of Ethylene Production from Petiole Explants*

Ethylene produced in 24 hr by petiole explants floated on solutions containing IAA, GA, and kinetin at 10^{-4} M.

	Treatment							
	IAA	GA	Kn	IAA + GA	IAA + Kn	GA + Kn	IAA + GA + Kn	Control
Ethylene production nl/g	142**	15.8	18.9*	115.9	170.5*	20.8	164.9	13.8

** Significant at 0.01 *P* level.
* Significant at 0.05 *P* level.

Table XII. *Analysis into Midrib and Petiolar Epinasty of Leaf Curvature Due to Auxin Applied to the Cut Apex or to Leaflet Stubs*

IAA (10^{-4} M) solution or water were applied to the decapitated shoots of tomato plants. IAA (0.1%) in lanolin or lanolin were applied to leaflet stubs. Leaflets were removed 24 hr before the start of the experiment. Means with different letters are significantly different at 0.05 *P* level.

Treatment		Location of Curvature		
Solution Applied to Apex	Leaf Condition	Petiolar	Midrib	Total
		degrees		
10^{-4} M IAA	Intact leaves	81a	1b	82a
10^{-4} M IAA	Lanolin on leaflet stubs	67a	1b	68a
10^{-4} M IAA	0.1% IAA on leaflet stubs	85a	162de	248f
Water	Intact leaves			−21b
Water	Lanolin on leaflet stubs			−11b
Water	0.1% IAA on leaflet stubs	23b	102ac	125ec

Table XIII. *Effect of CO_2 on the Development of Midrib Epinasty Due to IAA Applied to Leaflet Stubs*

IAA (0.1%) in lanolin or lanolin paste were applied to leaflet stubs on plants held in 10% CO_2 or air. Leaflets were removed 24 hr before the start of the experiment. Values shown are degrees curvature and means with the same letter are not significantly different.

Location of Curvature	Treatment			
	IAA	IAA + CO_2	Lanolin + CO_2	Lanolin
Petiolar	25a	17ae		
Midrib	112b	128bc		
Total	137c	145c	−1d	−4de

^{14}C-IAA Studies. Radioautography studies showed that the label of ^{14}C-IAA, or ^{14}C-glucose (as control), applied to decapitated shoots, was distributed throughout the shoot. Label from ^{14}C-glucose was distributed in the stem, leaf petioles, midribs and leaflets. However, most of the activity from ^{14}C-IAA in the leaf is restricted to the petiole and midrib tissue. Chromatography of 80% ethanol extracts of petiolar and midrib tissues indicated that less than 10% of the radioactivity in both the petiole and midrib tissue was IAA.

DISCUSSION

The debudded tomato plants used in this investigation exhibited basic nastic responses (Fig. 1, A and B) characteristic of intact plants (18, 19). Decapitation induced hyponasty and application of the auxins, IAA, NAA, and 2,4-D to the decapitated shoot produced petiolar epinasty (Fig. 1A, Table X); thus the role of the shoot apex in inducing petiolar epinasty may be as an auxin source. Fumigation of tomato plants with ethylene induced epinasty (Fig. 1A) as did the administration of ethylene through the cut surface of a decapitated plant (Table VI); so there also exists the possibility that the shoot apex acts as an ethylene source in inducing epinasty. The question of the relationship of the roles of auxin and ethylene in epi-

nasty arises. CO_2 inhibits both ethylene-induced and auxin-induced epinasty (Table I) and CO_2 inhibition is an established means of deciding whether an auxin effect is mediated by ethylene (2). Other indications that ethylene may mediate auxin-induced epinasty, are the stimulation of ethylene production prior to the initiation of epinastic curvature in petiole explants (Fig. 1D), and the parallels between the effects of auxin concentrations on ethylene production (Fig. 1C) and epinasty (Fig. 1A) in explants and decapitated plants respectively. In addition, in a saturating atmosphere of ethylene, tomato leaves gave no additional epinastic response to a full range of auxin concentrations applied apically (Table III).

The present results are concordant with those of Stewart and Freebairn (22), who found no epinasty when auxin was applied apically to tomato plants in which ethylene synthesis was inhibited, while exogenous ethylene did induce epinasty.

The proposal that the effect of apically applied auxin on epinasty is ethylene mediated is contrary to the hypotheses of Kramer (7) and Phillips (18, 19), who propose that petiolar epinasty in tomato and *Helianthus* plants is due to the direct action of auxin from the main shoot. Effects of the auxin-transport inhibitor TIBA (Table IV) suggest that auxin does not need to move into the petiole to induce epinasty. When the IAA treatment, applied to the apex of a decapitated tomato, included ^{14}C-IAA, little of the activity extracted from leaf petioles and midribs was in the form of IAA, which may not be expected in a system reacting directly to IAA moving out of the stem. Ethylene, however, may move from the stem to the petiole because its application through the decapitated apex or by injection into the stem (Table VI) induced petiolar epinasty and ethylene is not known to undergo chemical conversion in plants (1).

From their experience, Lyon (13) and Leather *et al.* (9) favored the hypothesis that ethylene induced leaf epinasty by inhibiting the gravity-stimulated downward movement of auxin in the leaf, resulting in a greater level of auxin in the upper side of the tissue and the development of epinastic curvature in the leaf. If the above theory was correct, application of IAA to the entire lower side of a petiole, should create an asymmetry in auxin level in favor of the lower side, resulting in hyponasty. However, the opposite occurred (Table V). Application of auxin to the lower or upper side of the petiole, as a ring on the petiole or as a patch on the stem opposite of the leaf petiole, all induced a significant degree of petiolar epinasty. These observations are consistent with the theory that auxin applied to tomato tissue induces ethylene production which leads to petiolar epinasty.

The present evidence suggests that auxin-induced ethylene produces petiolar epinasty by acting directly on the growth of petiole tissue and not through lateral distribution of auxin as suggested by Lyon (13) and Leather *et al.* (9). Indeed, the differential sensitivity of cells in the upper and lower sides of the petioles to the auxin-induced ethylene, is a likely basis for the petiolar nastic movements of tomato. An over-all change in leaf growth-rate is not involved because decapitation of tomato plants or application of auxin in place of the removed apex has no significant effect on the rate of elongation of young leaves. Phillips (18, 19) has proposed that auxin from the main shoot of *Helianthus* plants acts directly and that accumulation of auxin from the main shoot into the leaves causes epinasty and inhibition of leaf growth.

The development of petiolar epinasty induced in tomato by auxin or ethylene is due to differential elongation of cells in the upper and lower sides of the petiole (Tables VII and VIII). Palmer (16) has recently reported the acceleration of growth rate in the upper halves of 5-cm sections at the base of *Helianthus* petioles following ethylene treatment. Measurements of the average lengths of epidermal cells on the upper sides of the tomato petiolar sections indicate that the differential elongation of cells in the upper side of the petiole is limited to about 1 cm on the base of the petiole (Table VIII). Although ethylene commonly inhibits growth (see 1), there are cases where ethylene is reported to promote the elongation of cells (8, 14). The average lengths of cells in the lower side of the petioles of control plants, which exhibit hyponasty, is greater than those in plants treated apically with IAA (Table VIII), which is also the case in petioles of ethylene-treated *Helianthus* plants (16). Thus auxin-induced ethylene may not only stimulate the elongation of cells in the upper side of a tomato petiole, it may also inhibit elongation on the lower side. In this case, the petiolar hyponasty in leaves of decapitated plants would be due to differential elongation of cells in the lower side of the petiole. Indirect support for this notion was found by Leather *et al.* (9), who showed that rotation of intact tomato plants on a klinostat induced petiolar epinasty through an increase in endogenous ethylene production. However, when the action of endogenous ethylene was blocked by rotating the plants in an atmosphere with 10% CO_2, only hyponasty of petioles was recorded.

No evidence of the involvement in petiolar epinasty, of classes of regulators other than auxin and ethylene, was obtained. GA and ABA had no effect on leaf curvature or ethylene production, nor did the regulator antagonists AMO 1618, TIBA, or PCPIB (Tables IX, X, and XI). IAA and kinetin induced a significant rise in over-all ethylene production from petiole explants, but the effect of kinetin was small compared with the auxin effect (Table XI). The stimulatory action of kinetin and IAA was additive when the two regulators were combined. Application of kinetin or GA, or both, to the decapitated shoots of plants produced no petiolar epinasty and they did not significantly influence the correlative effects of apically supplied IAA (Table IX). Epinasty may not be detected in kinetin-treated plants because kinetin has little effect on ethylene production in tomato, or because kinetin can affect ethylene production in explants but not in decapitated plants. Fuchs and Liebermann (6) found that kinetin and IAA, alone or combined, induced a significant rise in ethylene production by seedlings.

The major influence of the shoot apex on leaf curvature is through petiolar epinasty and the correlative agent between the apex and the petiole is proposed to be auxin-induced ethylene. Midrib epinasty is also a component of tomato leaf curvature, but its mechanism of action is different from that of petiolar epinasty. A role for the leaflets in midrib but not petiolar epinasty is indicated by the observations that removal of the leaflets produces midrib hyponasty, while the application of auxin to the leaflet stubs produces epinasty mainly restricted to the midrib (Table XII). When auxin is applied to both the stem apex and the leaflet stubs, an additive effect on total epinasty is exhibited (Table XII). CO_2, which blocks the development of petiolar epinasty, has no such effect on the development of midrib epinasty due to IAA applied to the leaflet stubs (Table XIII). Thus the development of midrib epinasty is probably the result of an auxin action other than through ethylene. This idea is supported by the general observation, of Crocker *et al.* (3) and throughout the present work, that application of ethylene to tomato plants by a variety of means induces leaf epinasty which is mainly restricted to the petiolar region.

No direct evidence of ethylene production by the apical bud of a tomato plant or of the movement of ethylene in the shoot has been presented. However, the accumulated circumstantial

evidence points toward the orientation of a leaf with respect to the shoot axis being regulated by auxin, produced in the stem apex, stimulating the production of ethylene which moves into the petiole base where it induces differential cell expansion in upper and lower tissues. Midrib epinasty, on the other hand, appears to be an auxin effect not mediated by ethylene.

LITERATURE CITED

1. Abeles, F. B. 1973. Ethylene in Plant Biology. Academic Press, New York.
2. Burg, S. P. and E. A. Burg. 1967. Molecular requirements for the biological activity of ethylene. Plant Physiol. 42: 144-152.
3. Crocker, W., A. E. Hitchcock, and P. W. Zimmerman. 1932. Ethylene-induced epinasty of leaves and the relation of gravity to it. Contrib. Boyce Thompson Inst. 4: 177-218.
4. Fischnic, O. 1935. Über den Einfluss von B-Indolylessigsaure auf die Blattbewegung und die Adventivwurzelbildung von Coleus. Planta 24: 552-583.
5. Fretag, H. 1930. Untersuchungen über die Plagiotropie der Blätter von *Tropaeolum minus*. Planta 12: 267-292.
6. Fuchs, Y. and M. Liebermann. 1968. Effects of kinetin, IAA and gibberellin on ethylene production and their interactions in growth of seedlings. Plant Physiol. 43: 2029-2036.
7. Kramer, P. J. 1951. Causes of injury to plants resulting from flooding of the soil. Plant Physiol. 26: 722-736.
8. Ku, H. S., H. Suge, L. Rappaport, and H. K. Pratt. 1970. Stimulation of rice coleoptile growth by ethylene. Planta 90: 333-339.
9. Leather, G. R., L. E. Forrence, and F. B. Abeles. 1972. Increased ethylene production during klinostat experiments may cause leaf epinasty. Plant Physiol. 49: 183-186.
10. Leike, H. and H. Von Guttenberg. 1962. Die Rolle des Auxins bei der epinastischen Krummung platiotroper, Seitensprosse von *Coleus blumei* Benth. Planta 58: 453-470.
11. Lyon, C. J. 1963. Auxin factor in branch epinasty. Plant Physiol. 38: 145-152.
12. Lyon, C. J. 1963. Auxin transport in leaf epinasty. Plant Physiol. 38: 567-574.
13. Lyon, C. J. 1970. Ethylene inhibition of auxin transport by gravity in leaves. Plant Physiol. 45: 644-646.
14. Maxie, E. C. and J. C. Crane. 1968. Effect of ethylene on growth and maturation of the fig, *Ficus carica*. Amer. Soc. Hort. Sci. 92: 255-267.
15. Munch, E. 1938. Untersuchungen über die Harmonie der Baumgestalt. Jahrb. Wiss. Bot. 86: 581-673.
16. Palmer, J. H. 1972. Roles of ethylene and indol-3yl-acetic acid in petiole epinasty in *Helianthus annuus* and the modifying influence of gibberellic acid. J. Exp. Bot. 23: 733-742.
17. Palmer, J. H. and I. D. J. Phillips. 1963. The effect of terminal bud, indoleacetic acid, and nitrogen supply on the growth and orientation of the petiole of *Helianthus annuus*. Physiol. Plant. 16: 572-584.
18. Phillips, I. D. J. 1964. Root-shoot hormone relations. I. The importance of an aerated root system in the regulation of growth hormone levels in the shoots of *Helianthus annuus*. Ann. Bot. (N.S.) 28: 17-35.
19. Phillips, I. D. J. 1964. Root-shoot hormone relations. II. Changes in endogenous auxin concentrations produced by flooding of the root system in *Helianthus annuus*. Ann. Bot. (N.S.) 28: 37-45.
20. Snow, R. 1945. Plagiotropism and correlative inhibition. New Phytol. 44: 110-117.
21. Snow, R. 1947. Further experiments on plagiotropism and correlative inhibition. New Phytol. 46: 254-257.
22. Stewart, E. R. and H. T. Freebairn. 1969. Ethylene, seed germination, and epinasty. Plant Physiol. 44: 955-958.