Gravitropism in Higher Plant Shoots[1]

IV. FURTHER STUDIES ON PARTICIPATION OF ETHYLENE

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ABSTRACT

Ethylene at 1.0 and 10.0 cubic centimeters per cubic meter decreased the rate of gravitropic bending in stems of cocklebur (*Xanthium strumarium* L.) and tomato (*Lycopersicon esculentum* Mill), but 0.1 cubic centimeter per cubic meter ethylene had little effect. Treating cocklebur plants with 1.0 millimolar aminoethoxyvinylglycine (AVG) (ethylene synthesis inhibitor) delayed stem bending compared with controls, but adding 0.1 cubic centimeter per cubic meter ethylene in the surrounding atmosphere (or applying 0.1% ethephon solution) partially restored the rate of bending of AVG-treated plants. Ethylene increases in bending stems, and AVG inhibits this. Virtually all newly synthesized ethylene appeared in bottom halves of horizontal stems, where ethylene concentrations were as much as 100 times those in upright stems or in top halves of horizontal stems. This was especially true when horizontal stems were physically restrained from bending. Ethylene might promote cell elongation in bottom tissues of a horizontal stem or indicate other factors there (*e.g.* a large amount of 'functioning' auxin). Or top and bottom tissues may become differentially sensitive to ethylene. Auxin applied to one side of a vertical stem caused extreme bending away from that side; gibberellic acid, kinetin, and abscisic acid were without effect. Acidic ethephon solutions applied to one side of young seedlings of cocklebur, tomato, sunflower (*Helianthus annuus* L.), and soybean (*Glycine max* [L.] Merr.) caused bending away from that side, but neutral ethephon solutions did not cause bending. Buffered or unbuffered acid (HCl) caused similar bending. Neutral ethephon solutions produced typical ethylene symptoms (*i.e.* epinasty, inhibition of stem elongation). HCl or acidic ethephon applied to the top of horizontal stems caused downward bending, but these substances applied to the bottom of such stems inhibited growth and upward bending—an unexpected result.

Several evidences suggest that ethylene participates in a positive way in the gravitropic bending of various organs, including dicot stems. Most evidences are indirect, and the positive participation of ethylene in gravitropism remains to be clearly demonstrated, but consider the following:

(a) Ethylene induced diagravitropic (horizontal) growth in peas (classical studies of Neljubow, discussed by Abeles [1]. (b) In pea

and lima-bean roots, 5 and 10% CO_2 inhibited gravitropic bending (5); it was suggested that ethylene was essential for such bending. (c) Ethylene promoted upright stem growth of decumbent varieties of *Lycopersicon esculentum* (30), *Trifolium fragiferum* (9), and *Arachis hypogaea* (29). (d) Four different ethylene antagonists slowed the rate of gravitropic bending (our previous tests [25]). (e) Ethylene evolution increased during clinostating (11, 14, 21), and our results suggest that this was a response to gravity compensation (22). (f) Gravistimulation of dicot stems stimulated production of ethylene in bottom halves of dandelion peduncles (6) and grass leaf-sheath pulvini (26), although inhibitors of ethylene synthesis in those cases did not affect bending.

Many gaps remain. Our studies of the possible roles of ethylene were motivated by the following hypotheses, although each has alternatives, as noted in the discussion section:

(a) If ethylene participates in gravitropic bending, the response might be influenced by exogenous ethylene. (b) If endogenous ethylene plays a positive (even if not essential) role in gravitropic bending, then the slowed bending observed when plants are treated with inhibitors of ethylene synthesis or action might be reversed in the presence of exogenous ethylene. (c) If ethylene participates in gravitropic bending, endogenous levels of ethylene might change during bending; an asymmetry might develop. (d) If an internal gradient in ethylene concentration contributes to gravitropic bending, establishing such a gradient might lead to bending.

To test these hypotheses, we performed the following experiments:

(a) We monitored gravitropic bending in the presence of various concentrations of ethylene; (b) observed gravitropic bending of plants treated with AVG[4] (an effective inhibitor of ethylene synthesis) in the presence or absence of various concentrations of ethylene; (c) measured ethylene in gravitroping stems, separating upper and lower stem halves; (d) and applied ethephon (2-chloroethyl phosphonic acid, a compound that spontaneously breaks down at pH 3.5 or above to produce ethylene, chloride, and phosphate) to one side of various dicot species to see if bending could be induced; for comparison's sake, other growth regulators were also applied. (e) Finally, we studied effects of changing the pH of the ethephon solutions or of applying acids without ethephon.

MATERIALS AND METHODS

Plant Material. In the experiments of Figures 1 to 9 (those of R. M. W.), plants varied from 25 to 90 d old (30–40 d for most experiments). Plants were vegetative cocklebur, *Xanthium strumarium* L. (Chicago strain) and tomato, *Lycopersicon esculentum* Mill. (cv Rutgers). Seeds were sown in flats containing sand

or vermiculite, and seedlings were transplanted into 10-cm square plastic pots following expansion of cotyledons. All plants were grown in a loam-sand mixture (3:1 v/v) and fertilized after transplanting and at regular intervals with a time-release fertilizer (Osmocote, 14:6.2:11.6, N:P:K). Plants were grown in a corrugated-fiberglass greenhouse (diffuse light) with supplemental fluorescent lighting in the morning and evening to provide an 18-h photoperiod, which keeps cockleburs in a vegetative state. Greenhouse temperatures were usually between 24 and 27°C (could range from 20–35°C). RH was 30 to 75%. Older leaves were routinely removed from cocklebur plants so only four fully expanded leaves remained on the plants.

Prior to bending experiments, all leaves larger than the half- to three-quarters-expanded leaf were removed. (Bending was similar whether plants were pruned or not, but pruning facilitated measurements and prevented twisting to one side when stems were horizontal). Bending experiments were conducted in the dark, and measurements were taken either in dim green light or under incandescent room light. Short periods of light had little or no effect on bending, which was only slightly faster in the dark than in the light.

For the experiments of Figures 10 to 12 (those of R. G. W.), seeds of tomato (cv Heinz), cocklebur, castor bean (*Ricinus communis* L.), sunflower (*Helianthus annuus* L.), and soybean (*Glycine max* (L.) Merr. cv A3127, donated by Asgrow Seed Co.) were germinated in flats of soil (1983) or vermiculite (1984) in the greenhouse during May through July. Seedlings were transplanted to the 10-cm square pots containing the same medium as used for germination. Plants in soil were given Osmocote and NH_4NO_3 pellets; those in vermiculite were watered with Peter's nutrient solution for soilless media (Peat-lite Special: 15:16:17, N:P:K; N about 36 mM). Plants were 2 to 3 weeks old when used.

Chemicals and Treatments. AVG was donated by Hoffman La Roche Company. Solutions with 0.1% (v/v) Tween 20 were applied by gently misting entire plant shoots until they were dripping wet. To observe stem bending in ethylene-enriched atmospheres, plants were sealed in 35-L clear Plexiglas cylinders and turned horizontally. Ethylene was injected through septa on the chambers to create the desired internal concentrations. Stem bending was estimated by viewing through the Plexiglas.

In a series of experiments (reported here only in the text), several growth regulators were mixed in lanolin, which was applied externally with wooden applicator sticks to one side of the apical 10 cm of plant stems. Aqueous ethephon (a 39.5% solution of 2-chloroethyl phosphonic acid; 'Florel' from Amchem, Inc.) solutions with wetting agent were applied similarly with cotton swabs (12). Plants treated in this manner were immediately attached to a horizontal clinostat and rotated at 0.25 rpm (to compensate gravitropic response and thus, presumably, to accentuate bending).

In the experiments of Figures 11 to 13, ethephon was made up in distilled H_2O with 0.1% (v/v) Tween 20, 50 mM PIPES buffer, or 25 mM phosphate buffer. Acid solutions were made from HCl or H_3PO_4 in water or buffer. Concentrated NaOH or KOH solution (maximum of a few drops) was added to solutions where necessary to raise them to the desired pH. Solutions were made immediately prior to use, because the ethephon breaks down rapidly at pH 3.5 and above.

The apical 5 to 10 cm of stems was swabbed (rubbed gently up and down to abrade the cuticle slightly) with cotton-tipped applicators (Q-tips) that had been soaked in solution. Control plants (soybean) swabbed with deionized H_2O were slightly inhibited in their elongation, possibly in response to the mechanical effects of swabbing. Stems were swabbed on one side along the length of the elongation zone (the region where bending occurs) immediately before being placed in position (vertical or horizon-

tal) for the experiment. Some treated plants were clinostated at 0.25 rpm.

Measurements. Angles were measured by shaping a pair of drafting dividers to the angle formed by the basal, nonelongating region and the apical 1 cm of the stems, and measuring the displacement from a straight line with a protractor (*i.e.* a straight stem was recorded as 0° instead of 180°). Stem lengths were measured with calipers or a centimeter rule. Sometimes, stems were marked at intervals with carbon black in immersion oil and photographed on 35-mm film before and after bending. Negatives were projected to facilitate measurements between the marks. Gross tissue responses to solutions or abrading were observed with a hand lens or under a dissecting microscope (stem surface or free-hand sections). Location of the bend, epinasty of leaves, and such other ethylene effects as adventitious root formation were also noted.

Ethylene Extraction. Ethylene was extracted in a method similar to that of Beyer and Morgan (3). Prior to gas extraction, all but the smallest leaves were removed, after which all stem tissue in the bending zone (10–15 cm) was excised, split lengthwise, and weighed. Stem sections were wet with 0.1% (v/v) Tween 20 solution to prevent adhesion of air bubbles to surfaces and then cut into smaller pieces (2–3 cm). All the stem tissue from a treatment was then submerged in a saturated $(NH_4)_2SO_4$ solution underneath an inverted funnel, the top of which was sealed with a rubber septum. A vacuum (150 mm Hg, Figs. 6–9; 550 mm Hg, Fig. 10) was drawn on the submerged funnel and tissue for 5 min (Figs. 6–9) or 10 min (Fig. 10), after which all the extracted gas was withdrawn through the septum and analyzed for ethylene in an HP 5080 A (Figs. 6–9) or HP 5830 A (Fig. 10) gas chromatograph with flame ionization detector. Following extraction, tissue was rinsed in distilled H_2O and oven-dried 48 h at 60 to 70°C (Figs. 6–9) or 100°C (Fig. 10). Most experiments reported here were repeated at least three times (some ethylene measurements repeated twice).

RESULTS

Effects of Exogenous Ethylene on Stem Bending. Ethylene at 1.0 and 10 cm^3 m^{-3} (μl/L) caused a decreased rate of bending of cocklebur stems, but 0.1 cm^3 m^{-3} had no significant effect (Fig. 1). Thus, enriching air around plants in subsequent experiments with ethylene at 0.1 cm^3 m^{-3} was considered to be safe in the sense of not being inhibitory to bending. Similar results were observed with tomato stems, although concentrations of 1.0 cm^3 m^{-3} sometimes had no inhibitory effect on bending.

Overcoming AVG-Inhibition of Bending with Ethylene or Ethephon Treatment. Treating cocklebur plants with 1.0 mM AVG just before gravistimulation delayed the time to reach 60° by about 5 h when compared with untreated control plants (Fig. 2). But supplementing the atmosphere around AVG-treated plants with 0.1 cm^3 m^{-3} ethylene reduced this delay by about 2 h; that is, ethylene partially restored the rate of bending of AVG-treated plants (Fig. 2).

When a dilute ethephon solution (0.1% v/v) was swabbed onto either the top or bottom of the apical 10 cm of AVG-treated cocklebur stems just prior to turning them to the horizontal, the time to reach 60° was again decreased by about 2 h (Fig. 3), although bending was still slower than that of controls. Ethephon solutions at 1.0% or greater usually prevented bending.

When AVG-treated tomato plants were placed in an atmosphere containing 0.1 cm^3 m^{-3} ethylene, the time to reach 60° was shortened almost 8 h (Fig. 4), but again the response was not restored to that of controls. Applying 0.1% ethephon solutions to the apical 10 cm of tomato stems prior to gravistimulation also shortened the time to reach 60° by 5 to 8 h in one experiment (Fig. 5) but only by 1 to 2 h in another experiment (not shown). Application of ethephon to the top of tomato stems
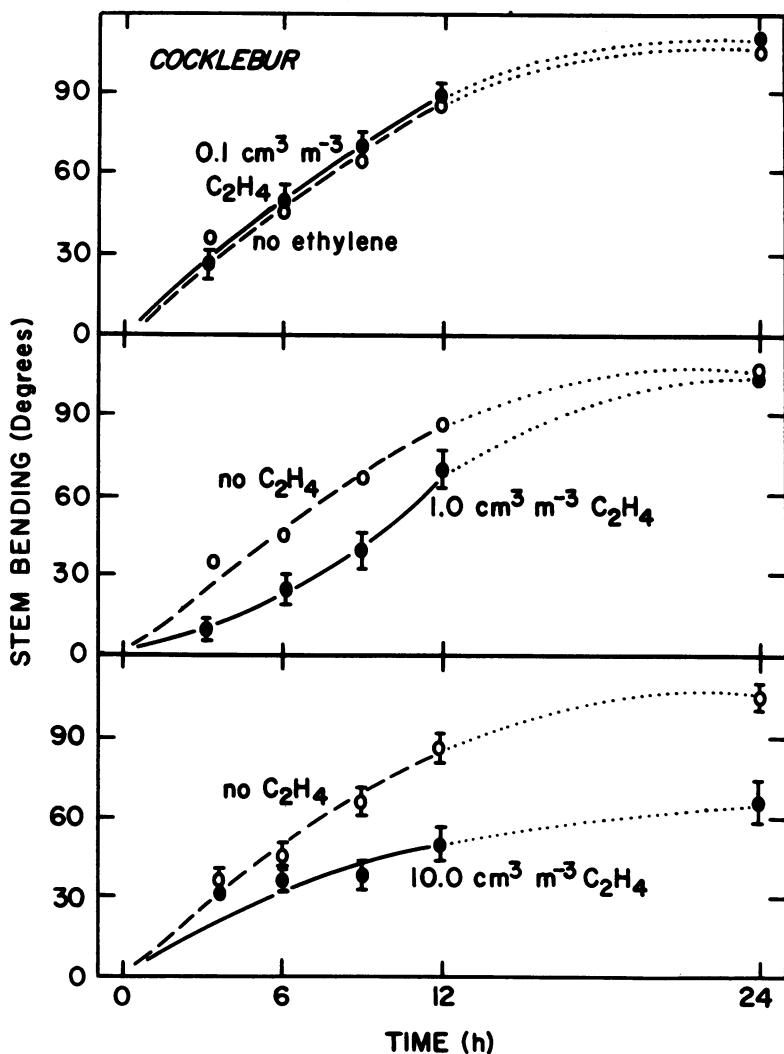
FIG. 1. Gravitropic stem bending of *Xanthium* (cocklebur) plants enclosed in Plexiglas cylinders containing 0.1, 1.0, 10.0 cm^3 m^{-3} (μl/L) or nearly zero ethylene. Concentrations of 1.0 and 10.0 cm^3 m^{-3} delayed the upward bending of horizontal cocklebur stems, while 0.1 cm^3 m^3 had no detectable effect when compared with control plants enclosed in cylinders with no ethylene supplement (open circles on each graph). Bars indicate standard error of the mean.
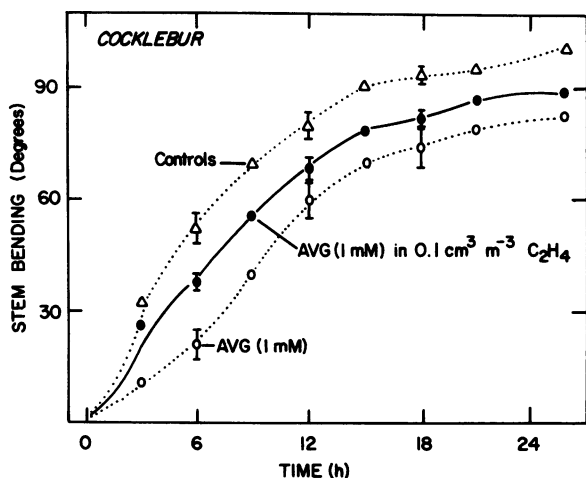

FIG. 2. Effect of 1.0 mM AVG or 1 mM AVG and 0.1 cm^3 m^{-3} ethylene on gravitropic bending of horizontal cocklebur stems. AVG-treated plants were delayed in their upward stem bending compared with untreated controls, but enclosing AVG-treated plants in cylinders enriched to 0.1 cm^3 m^{-3} ethylene speeded bending somewhat. Addition of ethylene did not fully restore bending to that of controls, however. Standard errors of means shown.
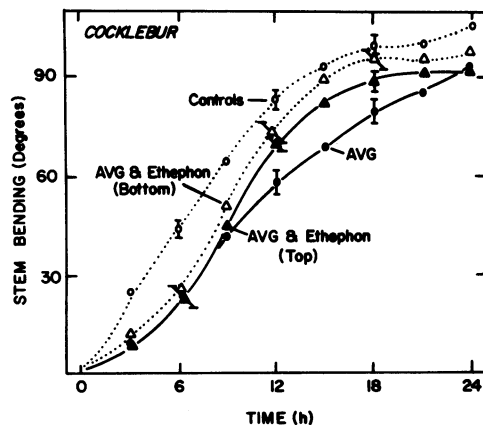

FIG. 3. Effect of swabbing 0.1% (v/v) ethephon (2-chloroethyl phosphonic acid) solution onto the top or bottom of the apical 10 cm of horizontal cocklebur stems treated with 1.0 mM AVG. AVG treatment as usual delayed upward stem bending compared to untreated controls, but addition of ethephon solution to the bending region of AVG-treated stems partially reversed this effect. Both bottom or top applications of ethephon were effective, but neither restored bending to that of controls. Standard errors of means shown.
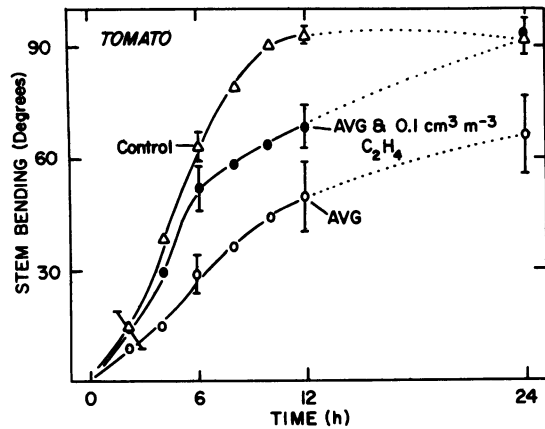
FIG. 4. Effect of 1.0 mM AVG or 1.0 mM AVG plus 0.1 cm³ m⁻³ ethylene on gravitropic bending of horizontal tomato stems. AVG treatment caused a large delay in the upward bending, but enclosing AVG-treated plants in an ethylene-enriched atmosphere restored some of the bending rate. Standard errors of the mean shown.

FIG. 6. Ethylene content of bending-zone (apical 10-15 cm) stem tissue during gravitropic bending of untreated controls and tomato plants treated with 0.5 mM AVG. Solid lines trace the progression of stem bending and dashed lines follow course of ethylene production. Ethylene content in AVG-treated stems was undetectable except at 4 h. Standard error of the mean bars are shown for the bending curves; standard errors are not shown for ethylene measurements, because stems are pooled to provide a single sample for each point.
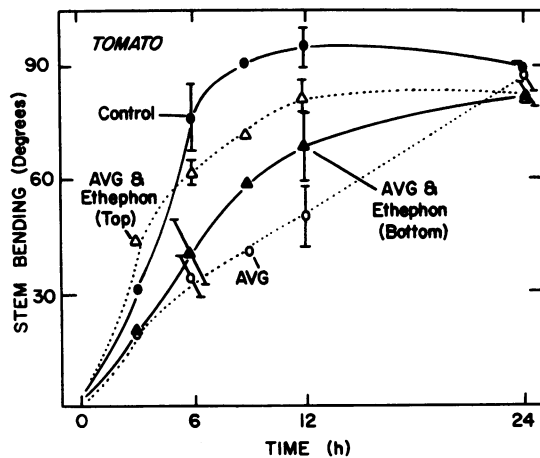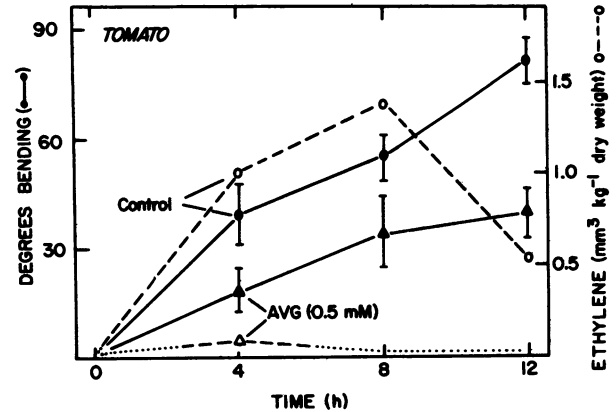

FIG. 5. Effect of swabbing 0.1% ethephon solution onto the top or bottom apical 10 cm of horizontal tomato stems treated with 1.0 mM AVG. AVG treatment as usual delayed the upward bending response and, as with cocklebur, addition of ethephon to AVG-treated plants partially reversed this delay. Top-side applications of ethephon appeared slightly more effective than bottom, but as with cocklebur, full bending was not restored. Standard errors of means are shown.
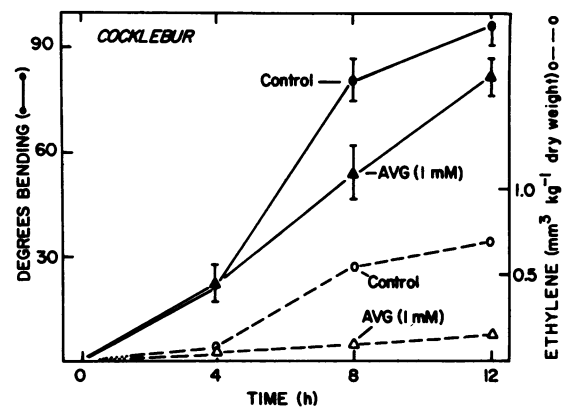

FIG. 7. Ethylene content of bending-zone stem tissue during gravitropic bending of untreated controls and cocklebur plants treated with 1.0 mM AVG. Solid lines follow the bending response and broken lines the ethylene production. As with tomato, AVG both slowed upward bending and reduced ethylene production of the stems. Standard errors of the means are shown.

was generally more effective in restoring bending than application to the bottom.

Ethylene Production during Stem Bending. During the upward bending of horizontal tomato stems, ethylene evolution rose abruptly during the first few hours, peaked around 8 h when stems were bent 50 to 60°, and then diminished sharply by 12 h (representative experiment shown in Fig. 6). Ethylene in tomato stems treated with AVG (0.5 mM) just before gravistimulation was barely detectable, and bending was inhibited compared with controls, as usual. Turning cocklebur stems to the horizontal also promoted ethylene production, but ethylene continued to increase and did not diminish as with tomatoes (Fig. 7). AVG again inhibited both ethylene production and bending.

When stems of gravitroping tomato plants were split longitudinally prior to gas extraction, it became clear that the bottom half of the bending stem was the source of nearly all the ethylene that appeared during bending (Fig. 8); results were similar with cocklebur stems (Fig. 9). Ethylene was barely detectable in upright stems or in top halves of bending stems of both species. When cocklebur stems were restrained from bending (i.e. held

between two stiff, horizontal wires by wrapping with thread), ethylene evolution was about the same as in free-bending stems, although top surface tissues were sometimes slightly damaged by the threads (Fig. 10a). When tomato stems were restrained in Plexiglas holders, much more ethylene was extracted from bottom tissues than in free-bending stems (Fig. 10b), although the pattern was similar.

Unilateral Application of Growth Regulators. As has long been known, auxins applied to one side of a stem cause bending away from the side of application. IAA (1% w/w) in lanolin applied to cocklebur stems caused over 200° of bending away from the side of application after 24 h of clinostat rotation (to eliminate the normal gravitropic response) and up to 450° after 48 h of rotation. IAA (0.05-0.1% in lanolin) applied to the top of a horizontal cocklebur stem caused a downward bending of −80° after 24 h. Tomatoes responded similarly, and concentrations of IAA as low as 0.1 and 0.01% also caused some bending away from the treated side. In some tests, NAA also caused bending away from the treated side, but often NAA-treated stems became contorted with no clear direction of bending. Plants treated with GA₃ (1%) occasionally showed traces of deflected growth away
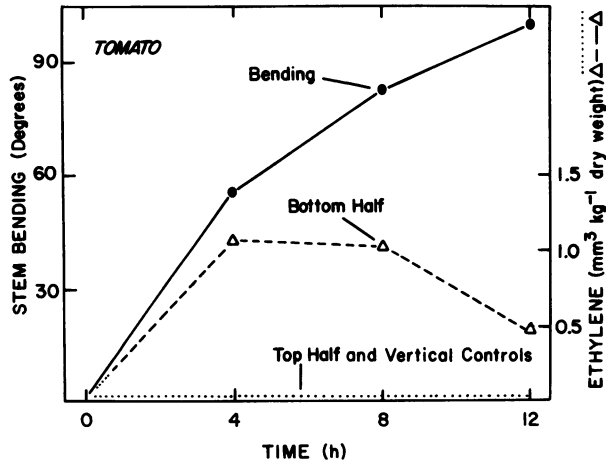
FIG. 8. Ethylene content during upward bending of top and bottom stem tissues of horizontally positioned tomato plants. Stems were split longitudinally, top and bottom, prior to gas extraction. Nearly all the increased ethylene production during gravitropic bending apparently occurs in the bottom half of the stems. Ethylene contents of the top-half tissues and vertical controls were undetectable.
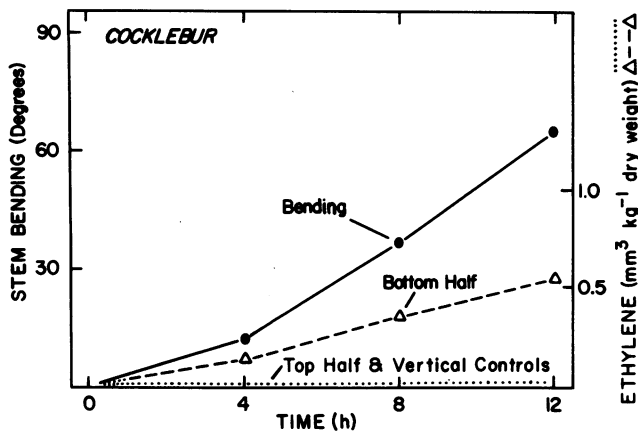

FIG. 9. Ethylene content during upward bending of top and bottom stem tissue of horizontally positioned cocklebur plants. As with tomato (Fig. 8), nearly all the increased ethylene during gravitropic bending was restricted to the lower tissues.

from the side of application, but 1% kinetin and 1% ABA in lanolin were without consistent effect.

In the context of this paper, treatments in which ethephon was applied to one side of stems were most pertinent. In some experiments with tomatoes on the clinostat, stems bent up to 80° toward the side of application, but results were highly variable, and there was never any similar effect with cocklebur stems. Hence, the following experiments were performed.

Unilateral Application of Ethephon and Various Control Solutions to Seedlings: The Participation of Acid Growth in Gravitropic Bending. Figure 11a shows that application of ethephon solutions to one side of vertical young tomato stems caused a bending away from the side of application. Increasing concentrations caused increased bending, and bending reached a maximum 6 to 10 h after treatment, after which stems began to straighten. Response of sunflower stems was similar, but castor bean and cocklebur stems failed to bend or show any response to the ethephon solutions except for inhibition of growth and some tissue damage caused by the strongest solutions.

Ethephon solutions made up in distilled H_2O had very low pH values: pH 1.2 for 3.9% ethephon and pH 1.7 for 1.0% ethephon. So buffered or unbuffered solutions of HCl or H_3PO_4 were

applied unilaterally to young stems. These caused bending of tomato or sunflower (but not castor bean) stems away from the side of application, just as had the acidic ethephon solutions (results with tomatoes shown in Figs. 11 and 12a). (Cocklebur stems were induced to bend with 1.0 M HCl; results not shown.) Increased bending occurred with increasing acidity of either ethephon or other acid solutions, buffered or unbuffered. Significantly, no bending occurred when near-neutral (pH 6.0) solutions of ethephon (regardless of concentration), neutralized acids, or near-neutral buffers were applied unilaterally to the test plants (Fig. 12a). The ethephon solutions, acidic or near neutral, did release physiologically effective ethylene, as indicated by leaf epinasty of all species and adventitious root formation on stems of tomato plants. Furthermore, stem elongation was increasingly inhibited by increasing concentrations of ethephon at pH 6 (Fig. 13).

Solutions of acidic ethephon (pH 2.0), ethephon at pH 6, and HCl (pH 2), as well as distilled H_2O raised to pH 6 (with or without 0.1% Tween 20), were applied to one side of vertical tomato plants (one set treated with acid was clinostated) and to the future top or bottom sides of tomato stems just before turning to the horizontal. Figure 12b shows that HCl at pH 2.0 applied to the top of the stem produced a marked downward bending. Ethephon at pH 2.0 inhibited upward bending in response to gravity but did not cause downward bending. (Note that lower concentrations of acidic ethephon, 0.1%, did not inhibit bending in the experiment of Fig. 5.) Figure 12c shows that acidic solutions of ethephon or HCl applied to the bottom of the stem prevented upward bending (instead of promoting it, as might be expected on the basis of the other results). Stems inhibited from upward bending eventually recovered and bent upwards (after 10 h, Fig. 12; or 5 h, Table I; results not shown).

Measurements of marked intervals (on photographs) of upper and lower surfaces of horizontal stems treated with acid or ethephon showed that failure to bend when stems were treated on the bottom was because growth was inhibited on the bottom rather than being promoted on top (Table I).

DISCUSSION

Effects of Exogenous Ethylene on Stem Bending. If ethylene promotes dicot stem gravitropism, this is not shown by the results of our experiments in which stems were allowed to respond to gravity in the presence of various concentrations of ethylene in the surrounding atmosphere. The lowest concentration was innocuous, and higher concentrations were inhibitory (Fig. 1). There are at least three reasons why these results say little about the possible positive role of ethylene in stem gravitropism: First, the atmospheric ethylene surrounded the stem and might have eliminated internal ethylene gradients acting in gravitropic bending. Second, internal concentrations of ethylene might have already been near optimal, so that any significant addition of ethylene might have raised the internal concentration into an inhibitory range. Third, even lower concentrations than we used might have promoted bending.

Overcoming AVG-Inhibition of Bending with Ethylene or Ethephon Treatment. The results of these experiments (Figs. 2–5) are more supportive of a positive role for ethylene in gravitropism. When ethylene synthesis is restricted or inhibited with AVG, stem bending is also inhibited, but when ethylene is added to the tissue (either as the gas or from ethephon), some of the AVG inhibition is overcome. Furthermore, it doesn't seem to matter whether the ethylene is added all around the stem, just to the top (as ethephon), or just to the bottom (Fig. 5). This might suggest that the sensitivity (responsiveness) to ethylene (as measured, perhaps, by available ethylene binding sites) varies, and this could be more important than an internal gradient in ethylene concentration. If ethylene promotes cell elongation in grav-
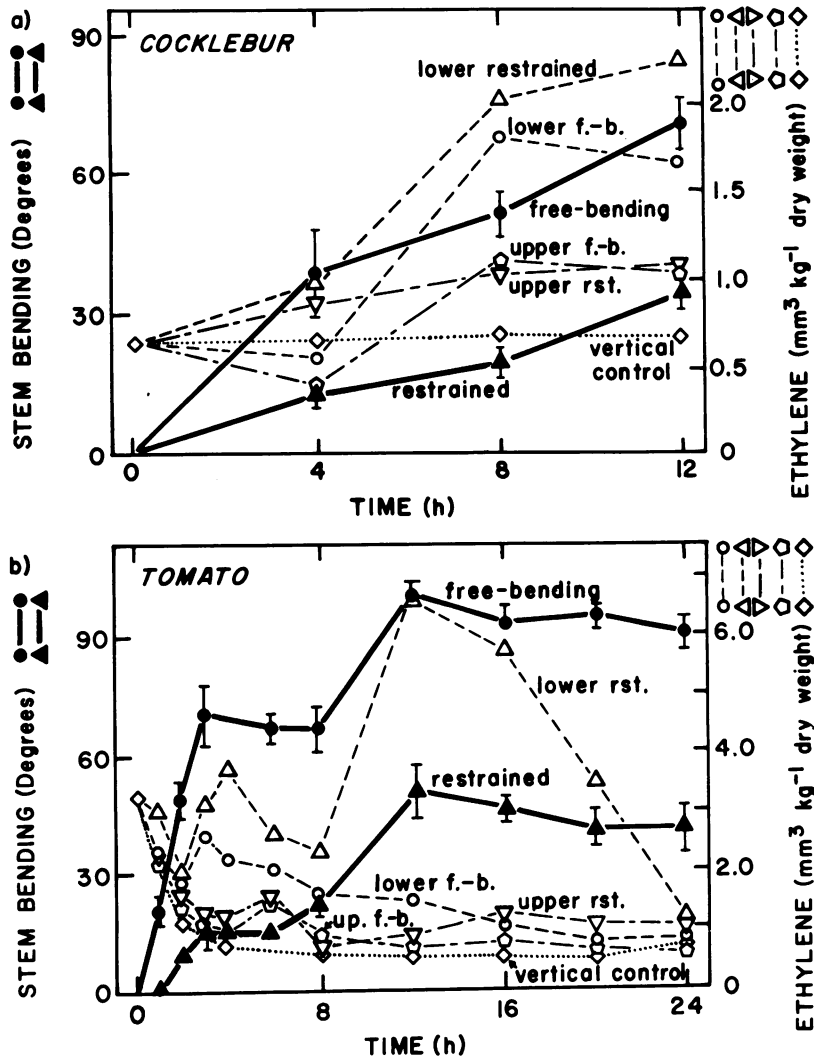
FIG. 10. Ethylene content of top and bottom stem tissue during gravitropic bending of young, free-bending, or restrained cocklebur (a) or tomato (b) plants. Ethylene production from restrained plants was similar to free-bending cocklebur plants, but it was much higher from bottom halves of tomato stems. The high ethylene in vertical tomato controls at the beginning was atypical and may have represented wound ethylene. Standard errors of the mean are shown for bending curves.

itropism, sensitivity to the ethylene might be greatest in the bottom tissues of a horizontal stem; if ethylene inhibits, sensitivity might be greatest in the top.

Tissue Ethylene during Stem Bending. The burst of ethylene during gravitropic bending is striking and probably significant for our understanding of gravitropism (Figs. 6–10). It is apparent that stem bending can begin before ethylene release was detected, as others have noted (6, 10). That ethylene production is confined to the lower half of the stem seems especially significant. Similar results have been reported for grass leaf-sheath pulvini (13, 26) and dandelion peduncles (6). It is especially striking that the ethylene gradient remains in stems restrained in a horizontal position in which the internal gradient in pressure/tension has been reversed. In a free-bending stem, tissue pressures (not necessarily cell turgor pressures [7]) are highest in top tissues (or perhaps about the same as in bottom tissues); in a restrained stem, top tissues are under considerable tension (17). Yet ethylene remains highest in lower tissues (Fig. 10).

In Figures 8 and 9, the magnitude of the ethylene gradient is impressive. Ethylene in upright controls and top halves was sometimes barely detectable on the gas chromatogram (less than 0.01 mm^3 kg^{-1} dry weight), while the bottom halves contained 1.0 to 1.5 mm^3 kg^{-1} of ethylene. This is a gradient of approximately 100-fold. The gradient in Figure 10, where higher vacuum and longer extraction times were used, is somewhat less impressive, but these conditions may have extracted more 'background ethylene' (or wound ethylene?).

Workers have argued for decades about the extent and significance of auxin gradients in stems during gravitropic bending. Mertens and Weiler (15), using techniques of immunofluorescence, could detect no IAA gradient in gravitroping stems and roots of dicots, although a small gradient was detected in *Avena* coleoptiles. Wright *et al.* (26) did observe up to a 3- to 4-fold IAA gradient in grass leaf-sheath pulvini, but this was apparently not established by auxin transport but by *de novo* synthesis of auxin (13). Bandurski *et al.* (2) found 56 to 57% of the IAA in corn coleoptiles in the bottom half and the rest in the top half. Such small gradients don't approach the observed gradients in growth rates, where growth on the top of a gravistimulated stem typically stops, nearly stops, or even reverses so that shrinkage occurs (4, 7, 8, 17). The ethylene gradient comes much closer to matching the observed growth gradient than does that of auxin. Yet, most of us remain reluctant to postulate ethylene as the controlling growth regulator in gravitropic bending (*e.g.* 6, 10). One reason is because ethylene inhibitors always fail to eliminate gravitropic bending; yet this could be because the inhibitors are never completely effective in eliminating ethylene production (note Figs. 6 and 7).

Harrison and Pickard (10) concluded that ethylene is not involved in gravitropic bending of tomato hypocotyls—and, by extension, in other stems and branches—because exogenous ethylene did not influence the gravitropic response, they could not detect a burst in ethylene during gravitropism (contrary to their earlier report), and ethylene antagonists did not influence
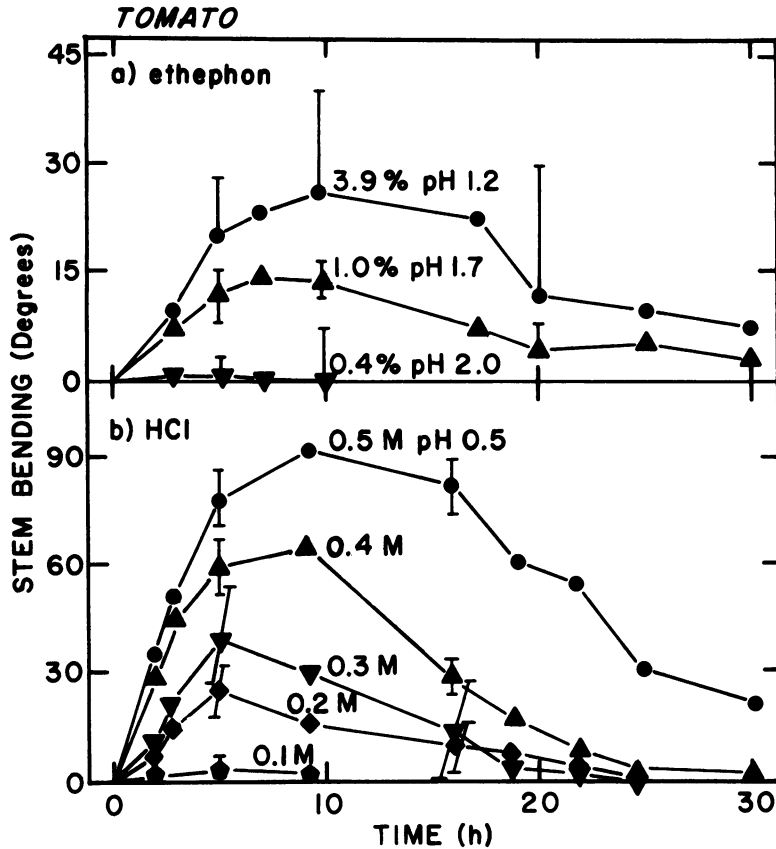
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FIG. 11. Bending of 5-week-old vertical tomato stems away from the side of application following unilateral application of acidic ethephon (a) or of acid (HCl) solutions (b) to the apical 10 cm. Standard errors of the mean are shown for some points.
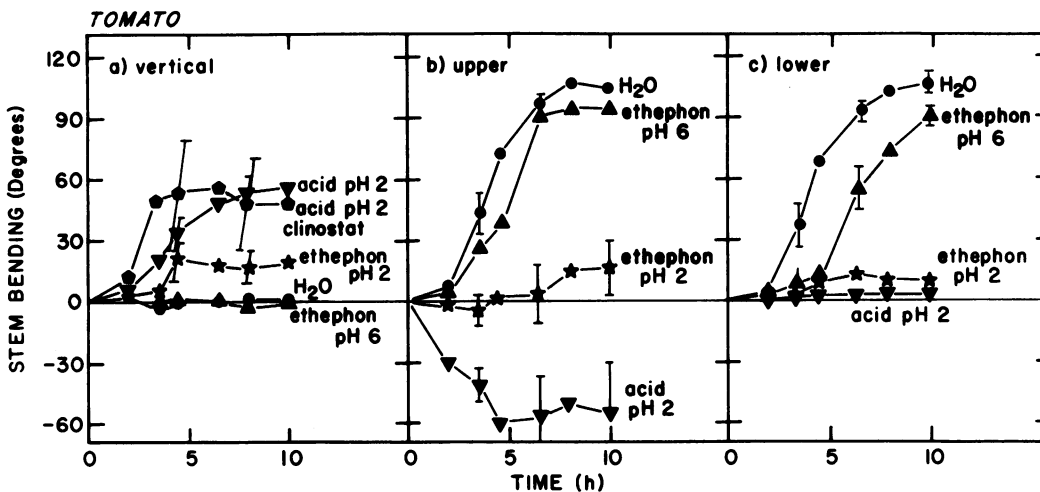

FIG. 12. Effect of unilateral application of 0.3 M HCl, buffered and unbuffered 2.0% ethephon, or distilled H_2O, on bending of vertical (a) or upper (b) or lower (c) surfaces of horizontal tomato stems. Although not shown, all apices oriented to the vertical by 30 h from the time of treatment. Standard errors of the mean are shown for some points.

gravitropic bending. They may be right, but there are problems with their report. It is not clear why exogenous ethylene concentrations that slowed the rate of bending in our experiments apparently did not inhibit bending in theirs, although their tomato hypocotyls might be less sensitive (more resistant to penetration?) than our mature tomato stems (unlikely), and they measured bending only once, when it was nearly complete (this would show no ethylene effect in the middle curves of our Fig. 1). Concentrations higher than ours did inhibit bending in their experiments. Furthermore, their data do show a trend toward increased ethylene production during gravitropic bending (19 out of 25 data points), although only two points are statistically different from controls. They did not separate halves as we and others (6, 13, 26) have done, nor did they extract ethylene from tissues under vacuum. They used AVG only at concentrations

lower than our effective concentration (1.0 mM), and again there seems to be some trend toward inhibition of bending by their inhibitors (especially $CoCl_2$ and NBD).

If ethylene does play a primary role in bending, does it do so by promoting growth on the bottom of the stem or by inhibiting growth on top or both? Most evidence for ethylene action in stems suggests an inhibition of stem-cell elongation (1), which could account for the observed halting of growth on top of gravitroping stems. Yet, the ethylene gradient in gravitroping stems suggests that ethylene might promote stem-cell elongation in the bottom of a bending stem. Promotions of stem growth by ethylene have been reported (16, 20, 24 and references therein). The familiar studies that suggest inhibition of stem growth may have used only concentrations in the inhibitory range.

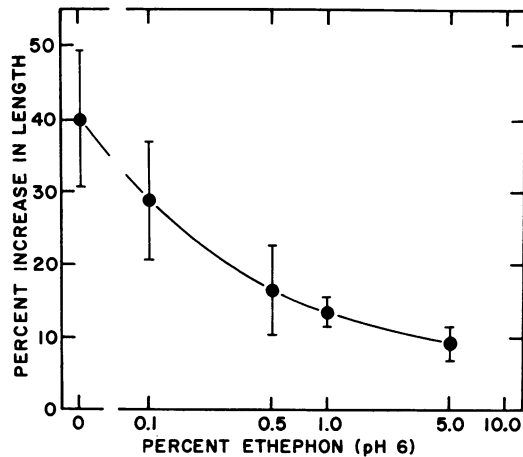Osborne (20) has proposed that auxin accumulates in the

FIG. 13. Percent increase in length of internode 1 of 14-d-old sunflowers during 20 h following unilateral application of ethephon solutions to the apical 10 cm. Standard errors of the mean are shown.

bottom of horizontal grass leaf-sheath pulvini, stimulating the production of ethylene; the ethylene would then diffuse to the top tissues and inhibit their growth. Yet ethylene is quite immobile in many plant tissues (19, 28), although its precursors (especially ACC) move freely. Osborne further suggests that the ethylene would have little effect on the cells where it is produced (bottom half), because the predominant controller of growth in those tissues might be IAA.

Another interpretation of the observed gradient is that the ethylene is an indication of conditions in the stem rather than a cause of these conditions. For example, there might be a gradient in 'functioning auxin' in a stem (i.e. auxin interacting with binding sites) as contrasted with the auxin that is usually extracted and measured. This functioning auxin might cause ethylene release or production.

If ethylene is inhibiting stem-cell elongation on top of gravitroping dicot stems, in spite of the circumstantial evidence favoring promotion on the bottom, the gradient might be explained by strong ethylene binding in top cells. That is, gravitropic stimulation might result in such a large increase in available ethylene binding sites in top cells that no free ethylene is left to measure. This bit of speculation is strained but should be kept in mind.

Unilateral Application of Growth Regulators. We were unable to induce bending by artificially establishing an ethylene gradient in our stems (Fig. 12a). This might be because ethylene (or ACC, induced by the ethylene) diffuses too rapidly across the tissue,

although this is unlikely (19, 28), or because changes in sensitivity to ethylene are more important than ethylene gradients. In any case, failure to induce bending by unilateral applications of GA₃, kinetin, or ABA across the stem, plus the striking and dependable bending caused by unilateral IAA application, are facts worthy of future reference.

Participation of Acid Growth in Gravitropic Bending. Acid was excreted from the most rapidly elongating surfaces of gravitroping roots or shoots, so acid growth might participate in the gravitropic bending of these organs (18, 27). Our experiments show that application of H⁺ to one side of vertical stems or to the top of horizontal stems will cause bending away from the side of application (Figs. 11 and 12). Tomato, sunflower, soybean, and cocklebur are able to respond to the protons by a loosening and extension of their cell walls. Perhaps the protons are unable to penetrate the cuticle of castor bean. The effect is striking when high concentrations of acid are used. Walls become so loose that tissues become flaccid (and sometimes visibly damaged) at the site of acid application. The tension that exists in stems in the cortical tissues, compared with pressure in the pith (23), was released by application of acid, suggesting that the tension is a matter of the 'tightness' of the walls. The tension remaining in the walls of cortical cells on the side of the stem opposite to the site of application apparently pulled the stem into a curved configuration. It is important to note that overall growth was only slightly promoted by acid (Table I and several unpublished experiments).

The most puzzling result of the ethephon experiments was the inhibitory effect of acid solutions (with or without ethephon) applied to the lower surface of horizontal stems. Although acid caused bending away from the side of application of vertical stems (or when applied to the top of horizontal stems), and although the stems would normally bend upward, application of acid to bottoms of horizontal stems prevented growth on the bottom and upward bending (Fig. 12c; Table I). So far, no satisfying explanation for this observation has come to mind. Again, acidic solutions applied to the bottoms of horizontal stems made the tissue rather flaccid, but this was also true for vertical stems, and they bent away from the acid, even at high concentrations (remaining turgid; not just sagging under their own weight). And how do we explain recovery after several hours? In some way, the gravitropic response itself seems to make the difference, and some key to understanding this response remains missing.

Table I. *Effects of Acid on Growth of Upper and Lower Surfaces of Young Tomato Stems*

Increase in length of upper and lower surfaces of horizontal tomato stems was measured after 5 h. At about that time and during subsequent hours, tomatoes treated with acid on lower surface recovered and began to bend upward. Acid was unbuffered 0.1 M HCl. This concentration of acid caused no visible tissue damage (except where a drop accidentally accumulated in some stem hairs).

Treatment	U or L Surface	Length		Increase in Length	Bending at 5 h
		Initial	Final		
		cm		*final/initial*	*deg*
H⁺, lower surface	U	4.67 ± 0.29	4.69 ± 0.29	1.004	22
	L	4.63 ± 0.30	4.68 ± 0.30	1.010	
H⁺, upper surface	U	4.26 ± 0.38	4.29 ± 0.39	1.007	57
	L	4.23 ± 0.38	4.35 ± 0.38	1.028	
Control (no H⁺)	U	4.58 ± 0.25	4.68 ± 0.27	1.022	86
	L	4.58 ± 0.26	5.05 ± 0.32	1.103	

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