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A STUDY OF CELL DIMENSIONS, AMYLOPLAST POSITION AND CERTAIN  
PHYSIOLOGICAL RESPONSES DURING GRAVITROPIC  
BENDING OF DICOT STEMS

by

JULIANNE E. SLIWINSKI

A dissertation submitted in partial fulfillment  
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Plant Science

Approved:

UTAH STATE UNIVERSITY  
Logan, Utah

1982

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Julianne E. Sliwinski

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## ABSTRACT

A Study of Cell Dimensions, Amyloplast Position and Certain  
Physiological Responses During Gravitropic  
Bending of Dicot Stems

by

Julianne E. Sliwinski, Doctor of Philosophy  
Utah State University, 1982

Major Professor: Dr. Frank B. Salisbury  
Department: Plant Science

If a plant is positioned horizontally, the elongating region responds by bending upward within 10 to 12 h until it is vertical, forming a 90° bend with the stem below. If a *Xanthium strumarium* L. (cocklebur) plant is placed horizontally, but restricted to that position for 48 h and then released, the bend to the vertical usually takes place within 10 s, suggesting that bending energy is stored in restricted stems. Some plants that do not bend completely to 90° within 10 s do so within 5 min, and other plants can overshoot the 90° mark by as much as 50°. Microscopic measurements show that cells on the bottom of stems that have been restricted and then released are longer and narrower than cells on the bottom of restricted stems; cells on the top of restricted-and-released stems are shorter and thicker than those on the top of restricted stems. Thus, stems bend upward rapidly after

release in response to changes in cell dimensions, but apparently with conservation of cell volume (i.e., little or no movement of water in or out of cells during the rapid bending). The increased diameter of the cells on the bottom of restricted plants indicates that the cells are taking up water before they are released (apparently accompanied by an increase in cell wall area), while they are not allowed to increase much in length. Any increase in length was accompanied by stretching of cells on top. Thus, energy for bending was stored in stretched upper cells and compressed lower cells that have taken up water.

It was also shown that graviperception takes place in the very tissue that bends, and this perception is not a perception of the tension and compression caused by the weight of a horizontal stem.

Also, amyloplasts were found in a sheath also in the region of bending and were found to settle in the direction of gravity. The location of the sheath between the vascular tissue and the cortex lead to a proposed model of graviperception for green vegetative dicot shoots.

(116 pages)

## INTRODUCTION

### Gravitropic Bending

When a plant is placed on its side, it responds to gravity by bending upward until it is once again vertical. This response does not begin at the tip, but takes place all along the zone of elongation, which in cocklebur (*Xanthium strumarium* L.) is from the tip of the stem to the base of the fifth internode. When the plant dries out, this is the same region that wilts. Below this, the cells have secondary walls and both the primary and secondary wall layers are lignified so that this portion of the stem does not respond to gravity and stays stiff during periods of drought. As the upper portion of stem reaches the vertical, the bend becomes more and more acute until it is localized in the fifth internode. A more general way to state this so as to include tomatoes and castor beans, which were also used in some experiments, would be that the bend became localized in the lowermost internode of the elongation zone. This response was described by Julius Sachs in 1887 and his diagram is shown in Figure 1. He described the initial response as being all along the zone of elongation before it became localized at the base, and he showed that there was some overshoot by the tip in the process of becoming vertical.

### Restricted Gravitropic Bending

It was discovered in our lab that a stem, when laid on its side, but restricted by being placed between two stiff wires and then wrapped

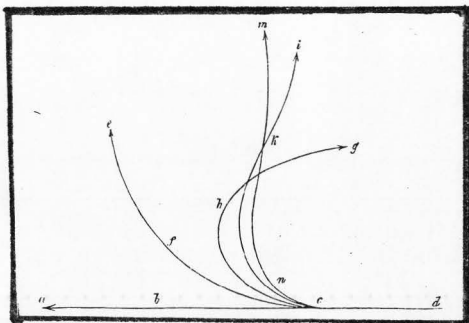


Figure 1. Diagram of gravitropic curvatures gradually made by a shoot a b c d which has been laid horizontally. Bending begins all along the stem and is eventually localized at the base n. Taken from Sachs (1887).



with thread, will spring to a vertical bent position within one to ten seconds after release from restriction (Fig. 2). This phenomenon was apparently unknown to modern plant physiologists but we later found that it was already documented by researchers of the late 1800's. Bateson and Darwin, in 1888, reported that restraining a *Plantago* inflorescence in a horizontal position and then releasing it resulted in a springing upward of the organ. They noted that the phenomenon was "well known".

I noticed that, when the restricted plants were released, the bend was immediately localized to the fifth internode. For this reason, and because the bend in unrestricted horizontal cocklebur plants eventually was also localized in the same internode, I concentrated most of my efforts in that region of the stem.

Measurements of Mueller (1981), using marked (with India ink) cocklebur, tomato (*Lycopersicon esculentum* Mill., var. Bonnie Best), castor bean (*Ricinus communis* L.), and pepper (*Capsicum annuum* L., var. Yolo Wonder) stems, showed that surface growth was either greatly reduced or stopped completely on top of a stem laid on its side, while surface growth on the bottom continued at normal rates or was actually accelerated compared to vertical control plants. When restricted and then released, the top surface actually shrank somewhat, while the bottom surface expanded to account for immediate bending.

In a somewhat similar study, Digby and Firn (1979) studied the free-bending marked stems of *Zea mays* (corn), *Cucumis sativa* (cucumber), and *Helianthus annuus* (sunflower). They measured the rates of growth on the top and bottom surfaces and found that growth on the top was inhibited, while growth on the bottom accelerated or continued

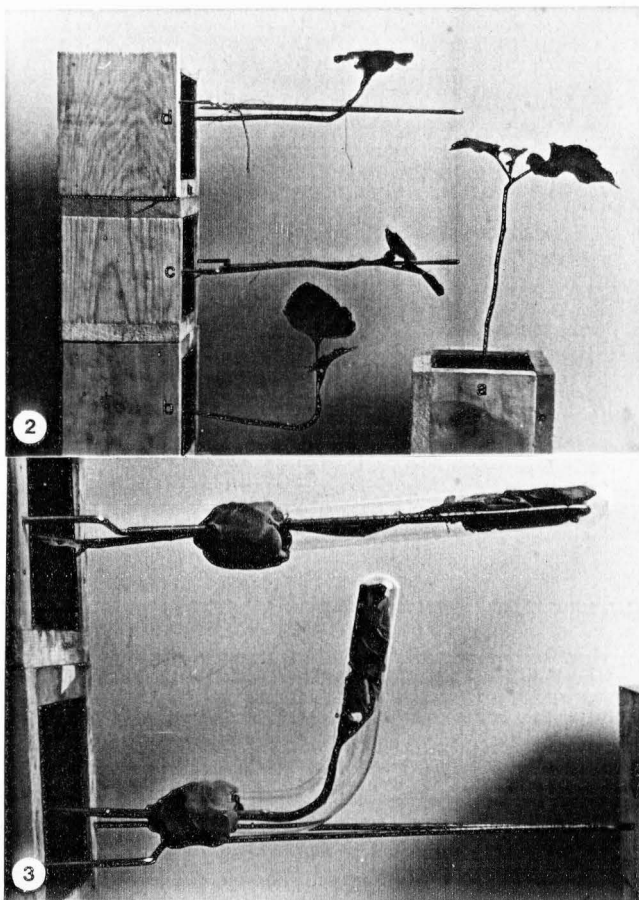


Figure 2. Experimental set up for restricted and non-restricted gravitropic bending in cocklebur. The upright control a, the free bending horizontal shoot b, the shoot restricted to the horizontal by being tied to a wire frame with thread c, and the released horizontal shoot after the string has been cut b.

Figure 3. Cocklebur plants being fixed in place for microscopy. Tubes of Karnovsky fixative were placed over the control and the three treatments and sealed at the base with plasticine modeling clay. The shape of the tube fit the shape of the plant.

at the same rate as in the upright controls. My measurements of cell dimensions confirms these findings at the cellular level.

The phenomenon of fast bending presented some intriguing questions. First, how did the plant store energy for this springing? Second, what happened to cell growth during restriction? Did cells take up water? If they took up water, was it during the fast bending? If water was taken up by the cells but not during bending, then it could have been taken up while stems were still restricted. The difficulty here was that, in the restricted plants, the water would have had to have been taken up against an ever increasing pressure gradient on the bottom side of the horizontal stem. This pressure was enough that it kept the strings, used to restrict the plant, taut. According to the general theory of cell growth, water is taken up when the cell wall is loosened and pressure is lowered, thus lowering the water potential inside the cell. My finding does not contradict this theory but is in contrast to it.

If cell sizes and shapes did change, then the condition of the cell wall must also be important. Some aspects of the cell wall that are involved in cell elongation are elasticity, plasticity, microfibril orientation, and changes in cell wall thickness. These were considered.

#### Perception of Gravity

It was already known, from other experiments in our lab, that a horizontal cocklebur stem responded to gravity by bending upward even when the apical meristem and leaves were removed, prior to the plant being placed in a horizontal position, therefore, these organs were not necessary for perception. In roots, perception takes place in root caps

where the settling of amyloplasts is correlated with gravity but has not been proven to be a part of the perception mechanism.

#### Location of Amyloplasts

If the apical meristem is not necessary for graviperception, then where in the stem does perception take place and are amyloplasts present? If amyloplasts are present, where are they and do they settle in response to gravity?

Since I had already decided to take a microscopic approach to studying the cells, it was logical to look for amyloplasts at the same time.

#### Location of Perception

In looking for a location of gravity perception in the cocklebur stem, a simple and direct approach was to cut off pieces of stem and determine if perception took place in the remaining portion. Perception was detected by observing any bending response. Having considered that cell growth is necessary for bending and auxin is necessary for cell growth (Brauner and Hager, 1957), 1% IAA in a lanolin paste was applied evenly across the surface of the cut stubs of a separate set of plants treated in the same way. It was believed that the amount of applied IAA was great enough to prevent formation of any gradient from the top side to the bottom side of a horizontal plant.

#### Tension/Compression as a Means of Graviperception

In addition to looking for the site of perception and for the existence of amyloplasts, another question was also investigated. That

is, could gravity perception be a perception of the compression on the bottom of a stem held horizontally, combined with the tension on the top of the stem, both created by the weight of the stem? This was tested by reversing the positions of compression and tension by forcibly bending the plant up to  $45^{\circ}$ .

## LITERATURE REVIEW

### Introduction

Gravitropism is the growth of plants toward or away from the force of gravity. When a shoot is placed horizontally, the response is for cells on the bottom to elongate faster than the cells on the top resulting in an upward bending of the plant. When a root is placed horizontally the change in growth of cells is just the opposite, with the cells on the top elongating faster than those on the bottom, resulting in a downward bending. Since gravitropism is a growth phenomenon, it is first essential to understand cell elongation before we can understand gravitropism. Because of the importance of collenchyma cells in the elongating region, a section on collenchyma is also included.

### Cell Elongation

#### Discovery of IAA

An important part of the information on cell growth has been gained through the discovery of the plant hormone indole-3-acetic acid (IAA) and the study of its activity. Several observations led to the discovery of IAA. Darwin (1896) found that the top of a grass coleoptile is essential to the tropistic response of the whole coleoptile; Paal (1919) concluded that a "correlation carrier" is supplied by the tip, and its movement leads to the development of curvature.

Went (1928) actually found such a substance in diffusates from coleoptile tips. He did this by removing several tips from coleoptiles and placing them on a small block of gelatin. Another coleoptile was prepared by removing the tip, waiting a period of time and removing the tip again (because a new "physiological" tip forms). The leaf inside the coleoptile was pulled part way out, and the gelatin block containing the growth substance was placed against the leaf and on the cut surface of the coleoptile. The activity of the substance, which Went called auxin, was detected by bending of the coleoptile caused by enhanced growth on the side to which the agar block was applied.

Using such an assay, indole-3-acetic acid was purified from plant materials by Kogl and Kostermans (1934) and Thimann (1935).

Went also developed an auxin bioassay, the coleoptile curvature test (summarized in Went and Thimann, 1937), in which semi-purified extracts suspected to contain an auxin are incorporated into an agar block of about 10 mm<sup>3</sup> volume and the block placed on one side of a coleoptile stump. The degree of bending is proportional to the auxin concentration within a certain range, and the concentrations as low as 0.05 mg/l (0.2 uM IAA) can be measured.

A less sensitive and less specific but simpler bioassay is the straight-growth test. Five-to-10-mm sections are cut from the third internode of week-old etiolated pea plants and allowed to grow in the buffered solutions that are to be assayed. Most of the auxin-induced growth results in elongation, so the lengths of the sections can be measured after an interval and compared to a standard curve obtained similarly with a known auxin. Potassium ions are necessary in the buffer for growth, and sucrose is needed for continued growth but not

for short-term growth. Coleoptile sections are also often used in a straight-growth test (Went and Thimann, 1937).

Straight growth is also stimulated by gibberellins, but abscissic acid and ethylene are inhibitory, and cytokinins are either inhibitory or have little effect, depending on the species used (Salisbury and Ross, 1978).

#### Cell Wall Extension

Cell wall extension is made up of an elastic and a plastic component. The extension is said to be elastic or reversible if the cells revert back to their former size upon removal of a stress such as turgor pressure, and plastic or irreversible if they do not. Such stress could also be a weight placed on the end of the plant stem by a researcher. Heyn did such an experiment in 1931 (as reported by Bonner and Galston, 1952) in which weights were hung on the ends of secured horizontal coleoptile sections for a measured amount of time during which bending of the sections took place. When the weights were removed, the permanent angle through which the coleoptile sections remained bent was a measure of the plastic bending and the angle through which the sections returned toward their original position was a measure of the elastic bending. When the coleoptile sections were pretreated with IAA, the plastic bending was increased but the elastic bending was not. This and other work on cell wall extension has been reviewed by Cleland (1971) and more recently Cleland (in press).

#### Hydrogen Promoted Growth

The growth of coleoptile segments has also been shown to be promoted by hydrogen ions. In 1934, Bonner reported that growth of



coleoptile sections was 8 times greater at pH 4.1 than at 7.2. He also noted that a low pH induced a rather large increase in the extensibility of the cell wall. Because the effect of  $H^+$  on the growth and wall extensibility of coleoptile segments is in many ways similar to the effect of IAA on growth and wall properties, Rayle and Cleland (1970) decided to study this aspect in more detail in hopes that the information would lead to a more general understanding of the cell enlargement process. In their work, the elongation of coleoptiles was measured by the high-resolution continuous recording technique of Evans and Ray (1969). Coleoptile segments were mounted one on top of another to obtain a vertical column of segments within a specially constructed glass chamber. An arc lamp was used to cast a shadow of the uppermost segment onto photographic paper moving horizontally at constant speed behind a vertical slit in a baffle about 1 m from the glass chamber. The rate of extension of the entire column of segments could thus be recorded shadowgraphically. Extension analysis of the cell walls was performed with an Instron TM-S linear extensometer, a procedure that consists of incubation of sections in boiling methanol, deproteinizing with pronase, and then subjecting the sections to force extension analysis (Cleland, 1967). They found that the maximal response occurred at a pH of approximately 3.0. At pH's lower than 2.6, little growth was induced; in fact, shrinkage was observed in some experiments, suggesting that these pH levels damage the cell membranes. The effect of pH on extensibility and growth rate are such that extensibility does not decrease below pH 3, but growth rate does. This indicates that wall loosening is occurring without accompanying growth. In the time course of growth and extensibility at pH 3.0, the growth response is initiated

rapidly (less than 1 min), and a steady rate is achieved that lasts about 30 min. The rate then gradually begins to decline, finally reaching a low rate. In the time course of plastic extension, extensibility increases rapidly but does not reach a maximum until after the growth response has ceased. They also showed that by changing the incubation solution from pH 3.0 to 7.0, the growth response stopped rapidly. That is, low pH does not act like a trigger but must be present continuously for rapid growth to occur.

Several different buffers and several different molarities were used to show that the response was to  $H^+$  and not to the particular type of buffer used.

#### An *in vitro* System that Simulates Plant Cell Extension

Rayle, Houghton, and Cleland (1970) designed an *in vitro* system that simulates plant cell extension. They used frozen-thawed tissues that had lost their biochemical activity, showing that any auxin- or low-pH-induced extension is caused by wall loosening and not cell wall synthesis. Wall synthesis is important in long-term cell elongation, however. The technique also involved applying a constant external force to replace turgor pressure. This enabled them to determine when factors that affect the growth rate were doing so by influencing the wall properties and not by changing the turgor pressure.

In 1934, when Bonner worked with low pH solutions that resulted in the same cell-wall extensibility as did applied auxin, he proposed that the  $H^+$  caused the activation of IAA. This was criticized by Burstrom (1961) because of work done with such inhibitors as KCN and dinitrophenol, which stopped the activity of IAA but not the activity of the  $H^+$ . The *in vitro* system of Rayle, Houghton, and Cleland also

disproved this, because the frozen-thawed tissue was metabolically inactive but still responded to low pH. Therefore, in both cases the  $H^+$  could not be acting by converting IAA into active form.

#### Similarity of Auxin Activity to Low pH Activity

The *in vivo* auxin and low pH response are similar with regard to the maximum rate and duration of extension and the temperature dependence of elongation. This suggests that a single type of wall extension is common to both types of cell elongation.

Certain responses to auxin, such as enhanced synthesis of protein and RNA (Key, 1969), occur after so long a lag phase that they seem not to be associated with the primary action of the plant growth hormone on wall extension. Of more immediate interest are the responses to auxin that occur within 1-10 min, including an alteration in the rate of elongation of stem sections and increased extensibility in stem sections, as already discussed. Kang and Burg (1971), working with tritiated water, found that auxin also immediately changes water flux in etiolated pea-stem sections within 1 min after application of the hormone. This supports their hypothesis that a primary action of auxin is on a membrane system, and it occurs before or coincident with auxin-induced enhancement of growth and cell-wall extensibility.

In other work, Rayle and Cleland (1972) showed that walls of frozen-thawed *Avena* coleoptile tissue that have been deproteinized with pronase or have been treated under conditions that should denature proteins (incubations with urea or sodium lauryl sulfate (SLS) or at 42<sup>o</sup> C) are still capable of extending in response to  $H^+$ . They, therefore, suggest that the action of the  $H^+$  must be to cause the cleavage of some

cell-wall crosslink by directly participating in the hydrolysis of some acid-labile cell-wall bonds.

The possibility that the wall-loosening agent activated by auxin is actually  $H^+$  seemed unlikely at first, in view of the fact that auxin stimulates the elongation of sections incubated in buffered solutions, but it is possible that the buffer does not penetrate the cuticle and thus does not modify the pH of the cell-wall solution (Rayle and Cleland, 1972). Then in 1973, Rayle found that when he peeled the coleoptile sections, the maximum pH for elongation was between 4.8 and 5.0 and not at 3.0. This made the link between auxin and  $H^+$  much more physiologically reasonable, since pH 3.0 eventually caused the death of cells.

#### Auxin Induced the pH of Cell Walls to Drop

It was still necessary to show, however, that applied auxin caused the pH of the cell walls to drop. This was done by Rayle (1973) and by Cleland (1973). They showed independently that when pea stem sections were placed in a  $K^+$  phosphate buffer at pH 6.0, the pH remained at 6.0 (as long as 2 h) until auxin was added. The pH then began to decrease and dropped to 5.0 within 2.5 h. According to Cleland's calculations, the auxin-induced  $H^+$  excretion is sufficient to lower the pH of the cell-wall solution to 5.0 in a matter of minutes. This is much shorter than the time it takes to lower the pH of the bathing solution and fits in well with the time frame of rapid cell elongation. They also showed that the appearance of  $H^+$  is due to excretion and not just leakage, since the auxin-induced efflux of  $H^+$  can be prevented by inhibitors. The agents that interfere with cell elongation also interfere with  $H^+$  excretion. Cleland (1973) used carbonyl cyanide-m-chlorophenyl-

hydrazone, KCN, and cycloheximide, and Rayle (1973) used dinitrophenol (DNP), abscissic acid (ABA), cycloheximide, and valinomycin. They concluded that the immediate activity of auxin is to cause the excretion of  $H^+$ , which in turn causes cell-wall loosening, allowing cell elongation by uptake of water.

The treatment with  $H^+$  mimics only a portion of the auxin-induced growth response, however, auxin itself is necessary to maintain a long-term, steady increase in growth rate (Rayle, 1973) such as cell-wall synthesis and RNA and protein synthesis, as already mentioned.

#### Mechanism of $H^+$ Excretion

In trying to work out the mechanism of  $H^+$  excretion, Marre et al. (1974) found evidence the IAA-stimulated proton excretion is tightly coupled to the active uptake of  $K^+$  in pea internode segments. If segments were partially depleted of the  $K^+$  in the Donnan free space (DFS) by preincubation with either auxin or acidic buffers, and then transferred into fresh medium, the IAA-promoted proton extrusion was strongly stimulated by the presence in the fresh medium of  $K^+$ , while  $Na^+$  appeared much less active. Dicyclohexylcarbodiimide (DCCD), and inhibitor of  $K^+$ -ATPases, inhibits growth and proton extrusion. They suggested that the coupling of these two processes might occur at two levels. One could be a single catalyst such as the plasmalemma  $K^+$ -ATPase. The looser model might consist of two different proteins, closely interdependent in their activity, one mediating  $K^+$  influx and the other the  $H^+$  efflux. They found the ratio between  $K^+$  uptake and  $H^+$  extrusion usually to be close to 1 (about 0.9), which supports the first model. Yet it occasionally varied from 0.5 to 2.0, but the variance could have been due to different experimental conditions.

In dealing with ionic and metabolic relations of  $H^+$  excretion, Haschke and Lüttge (1977) also found a ratio of close to one between IAA-induced net- $K^+$ -influx and net- $H^+$ -efflux in *Avena* coleoptile segments. They also found a stimulating effect of IAA on the fixation of  $^{14}CO_2$  by coleoptile segments and its incorporation into malate. The additional  $CO_2$  fixation observed in the presence of auxin is almost entirely due to malate synthesis. Pulse-chase experiments showed that most of the malate synthesized by  $^{14}CO_2$  dark fixation in the presence of IAA is not subjected to metabolic turnover. Hence malate is accumulated in the tissue, probably in the cell vacuoles. Other metabolic pathways leading to amino acid synthesis or gluconeogenesis are not affected by IAA.

Auxin-induced malate accumulation is stoichiometrically correlated with net- $K^+$  uptake and hence with IAA-dependent  $K^+/H^+$  exchange.

#### A Model of IAA Activated Cell Wall Loosening

To summarize their ideas and ideas of other researchers already given in the paper on auxin-induced proton extrusion, Haschke and Lüttge (1977) hypothesized the following model: The IAA activates some inactive pump in the plasmalemma that excretes  $H^+$  by the use of energy from ATP. This lowers the pH in the cell walls, and some acid-labile bonds are chemically cleaved, causing the walls to loosen. The pumping out of  $H^+$  is stimulated and electrochemically balanced by the uptake of  $K^+$  in stoichiometric amounts. The pumping out of  $H^+$  also raises the pH in the cytoplasm, stimulating PEP carboxylase to incorporate  $CO_2$  into malate. Auxin *in vitro* does not itself influence the activity of PEP carboxylases from *Avena* coleoptiles (Hager, personal communication to

Haschke and Lüttge, 1977). The raised pH acts as an intermediary step. Each newly synthesized malate molecule associates with two  $K^+$  and is stored in the vacuole. This in turn lowers the osmotic potential of the cell so that the osmotic potential is maintained as water moves in in response to the lowered pressure potential ultimately resulting in cell elongation. While the hypothesis remains to be tested, it provides an excellent framework for future experimentation and for speculations about the gravitropic responses of stems.

### Gravitropism

#### Early Work on Gravitropism

Early work on gravitropism was done by Ciesielski in 1871, who demonstrated that the root cap was the site of perception of gravity and the site of control of the response in roots. When he decapitated roots and placed them horizontally, they did not respond gravitropically. When he placed them on their side for a while and then removed the cap, he found that they bent in the correct manner, no matter what position he placed them in. He, therefore, postulated that some influence had already moved out of the root cap that signalled the correct response in the root. In 1873, Sachs disagreed with Ciesielski (as reported by Darwin, 1896), so Darwin repeated the experiments, and in the course of getting the same results, he also noted that if the cap of a vertical root was nicked on one side only, the root grew away from the side that was cut.

#### Statoliths

The immediate response to gravity is the acceleration of mass toward the center of the earth. In trying to decide what it was inside

of plants (root or shoot) that perceived gravity, Berthold (1886) and Noll (1892) were among the first to describe the statolith theory for gravity perception in plants. They speculated that some cell inclusion could be settling in response to gravity, creating an asymmetry in the organ. In 1900, Nemeč and Haberlandt independently identified the fast falling bodies in plant cells as starch-filled amyloplasts.

To be considered a statolith, the cell inclusion must have enough density to move through the cytoplasm within the time frame of gravitropism, and it must move toward the lower side of the cell in response to gravity. Amyloplasts meet this criterion, but dictyosomes, the nucleus, and mitochondria are not dense enough and move too slowly as shown by centrifugation experiments (Bouck, 1963a, b).

Two comprehensive reviews that cover the involvement of amyloplast sedimentation in gravitropism are those of Hoshizaki (1973) and Juniper (1976). There is no proof, at this time, that amyloplast sedimentation plays a causal role in gravitropism; only strong correlative evidence. The following list was taken from Shen-Miller and Hinchmann (1974): (1) There is a strong correlation between amyloplast movement and gravitropic response. (2) There is a quantitative correlation between the presence of amyloplasts and gravitropic sensitivity in organs. (3) There is a quantitative correlation between the rate of sedimentation of amyloplasts and the presentation time in various plant organs. (4) Amyloplasts are the only organelles to show significant sedimentation (under a light microscope) in gravi-stimulated root cap cells of broad beans (*Vicia faba*) and in graviperceptive regions of shoots and grass coleoptiles. (5) There is a rough quantitative correlation between the amount and size of amyloplasts and the degree



of gravitropism (Hertel et al., 1969). (6) There is a direct proportionality of size and asymmetric amyloplast distribution to the auxin differential in shoots of cereal plants (Hertel et al., 1969, Filner et al., 1970). (7) Cress roots (*Lepidium sativum*) devoid of starch did not elicit the gravitropic response (Iversen, 1969).

Others have found some evidence against the starch statolith theory, such as the absence of starch grains in gravisensitive organs of several plant species including the perianths of Clive lily (*Clivia*) and the aerial roots of *Laelia* orchid. In other experiments, gravitropism has been demonstrated in plants whose starch grains have been depleted by chemical or environmental manipulations. Pickard and Thimann (1966) showed that wheat-shoot cells depleted of visible starch grains (light and electron microscope observations) were capable of carrying out the gravitropic curvature response, although the gravity stimulation in the study was exceedingly long (5 h). When Iversen (1974) did the same experiments with the same organism, except at a higher incubation temperature (34 C instead of 30 C), he found that the plants did not respond to gravity. After 20-24 h in the light, however, the plants regenerated starch and, simultaneously, the response to gravity reappeared. As for plants that do not have amyloplasts, it is possible that amyloplasts play a role in gravity sensing in plants where they are present, but that other systems or organelles may perform this function in plants that do not have amyloplasts. In the *Chara* rhizoid, which does not have amyloplasts, barium sulfate crystals have been shown to act as statoliths (Volkman and Seivers, 1979).

Several possible mechanisms for how statoliths influence the gravitropic response are reviewed by Juniper (1976), Volkman and Seivers (1979) and Audus (1979) and are not discussed here.

Other organelles can be involved without acting as statoliths. During gravity stimulation, more dictyosomes appear on the side of apical cells of oat coleoptiles before the curvature response without a decrease in numbers of dictyosomes in the upper halves of cells. This does not indicate sedimentation but rather a formation of new dictyosomes in the lower halves of the cells (Shen-Miller and Hinchman, 1974).

#### The Cholodny-Went Theory

After the initial development of the statolith theory, at the turn of the century, came the discovery of auxin and its involvement in cell elongation. This led to the Cholodny-Went theory of gravitropism (Cholodny, 1926; Went, 1927). The theory states that when a plant is placed horizontally, gravity induces the movement of auxin from the upper to the lower side of an organ, promoting growth in stems but inhibiting growth in roots. This was later supported by Dolk (1936), who demonstrated the development of an auxin gradient in gravitropism. The resulting auxin concentration gradient apparently caused the differential growth that results in gravitropic curvature (Went and Thimann, 1937).

The growth-control mechanisms of gravitropism in roots, coleoptiles, and dicotyledonous shoots and grass nodes have been reviewed by Wilkins (1979), and each of these four plant organs will be discussed.

### Root Tips

The recent elaborate and detailed work done with root tips, beginning in 1966, has led to the understanding that separate systems are working in roots than in shoots and that their fundamental hormonal basis is probably different. Studies with *Zea mays*, in which removal of the cap abolished the gravitropic response in primary roots (Juniper et al., 1966), initiated the most recent phase of work on the gravitropic response mechanism in roots. By removing one half of the cap of the primary root of *Z. mays* seedlings, Gibbons and Wilkins (1970) showed that the root always developed a large curvature toward the side upon which the remaining half cap was located, regardless of the orientation of the root with respect to gravity. They said the cap must, therefore, be the source of a net growth-inhibiting influence, which they referred to as a growth inhibitor, although they recognized that more than one substance could be involved. Further work by Pilet (1973) and Shaw and Wilkins (1973) showed this conclusively and characterized the activity in more detail. Among several other growth inhibiting substances, abscisic acid (ABA) has been shown to be synthesized by the cap. Abscisic acid can cause bending of decapitated roots when applied asymmetrically. Also, Wilkins and Wain (1974) demonstrated that in darkness, ABA is not present in the root caps of the light-requiring variety of *Z. mays*, LG11. After exposure of the caps to light, however, ABA is present. When the light-requiring roots were kept wholly in darkness, those that were intact developed a positive gravitropic response on immersion in  $10^{-9}$  M solution of ABA, whereas those that had been decapped did not (Wilkins and Wain, 1975). This evidence supports the view that ABA is the growth inhibitor that is

present in the root cap, and upon which the gravitropic response of the *Z. mays* root depends.

### Coleoptiles

Working with coleoptiles, Dolk (1936) measured lateral movement of auxin toward the lower side of a horizontally placed coleoptile by cutting off tips and placing them in contact with agar blocks separated from each other by a thin piece of mica to prevent mixing of the auxin that diffused into the agar from the upper and lower coleoptile portions. Auxin in each agar block was bioassayed with the coleoptile curvature test. Seventy percent of the auxin moved to the lower half and 30% moved to the upper half. Dolk attributed the difference in growth promoting activity to lateral transport, but Wilkins (1979) criticized the technique because it could equally well have arisen from the upward transport of an inhibitor or even from different rates of synthesis, release, longitudinal transport, metabolism, immobilization, or secretion of growth hormone by the upper and lower halves of the horizontal organ.

The downward lateral transport of IAA in horizontal shoot tissue was then demonstrated unequivocally by Goldsmith and Wilkins (1964). Donor blocks containing  $^{14}\text{C}$ -IAA were supplied asymmetrically to the apical ends of *Avena* coleoptile segments, and the relative distribution of total radioactivity in each half was determined. It was found that approximately twice as much IAA moved to the lower half of a horizontal segment supplied with an upper donor as moved across in a vertical segment. The radioactive IAA found in the non-donated half of the coleoptile therefore must have come from the half in contact with the donor block. The Cholodny-Went hypothesis can therefore be regarded as

valid for *Zea* and *Avena* coleoptiles to the extent that apically synthesized IAA can be laterally transported downward within the horizontal coleoptile (Goldsmith and Wilkins, 1964), but this did not show that the transported auxin accounts for the bending.

There is some evidence for growth hormones other than IAA becoming asymmetrically distributed when the gravity-sensing mechanism is stimulated. Railton and Phillips (1973) collected more gibberellin-like activity in agar blocks in contact with the lower half of the apex of a horizontally placed *Zea* coleoptile than in blocks in contact with the upper half in a ratio of 4:1. They also found significant growth-inhibiting activity in the upper receiver block and not in the lower block.

It is not known how the asymmetric distribution of the GA takes place, because Wilkins and Nash (1974) found that externally applied  $^3\text{H}$ -GA<sub>3</sub> was not transported laterally downward, although this could have been due to a gibberellin other than GA<sub>3</sub> being present in the plant.

#### Dicotyledonous Shoots

Dicotyledonous shoots, both etiolated and green, are also used in gravitropism experiments. Working with etiolated hypocotyl tissue of gravitropically stimulated *Phaseolus vulgaris* and *Helianthus annuus*. Wilkins (1979) found a small but significant lateral movement of asymmetrically applied  $^{14}\text{C}$ -IAA or  $^3\text{H}$ -IAA. The lateral movement was much less than in *Zea* coleoptiles (Wilkins, 1979), but Goldsmith and Wilkins (1964) suggest that lateral transport in a segment is probably much less than in an intact organ.

In horizontal, green, first-internode segments of *Helianthus*, no asymmetric distribution of lateral transport of either  $^{14}\text{C}$ -IAA or  $^{14}\text{C}$ -GA<sub>1</sub> could be detected by Phillips and Hartung (1976).

Removal of the apical bud strongly suppressed internode elongation in light grown seedlings of *H. annuus* and *Phaseolus multiflorus*. Application of GA<sub>3</sub> to the cut shoot restored elongation to that observed in the control; but IAA was without effect. Simultaneous application of GA<sub>3</sub> and IAA did not lead to restoration of the normal growth; the IAA obviously antagonised the effectiveness of the GA<sub>3</sub> (Phillips and Hartung, 1976).

In other work, Phillips (1972) has shown that in excised apical buds of light-grown *H. annuus* seedlings, an asymmetric distribution of gibberellin-like activity developed in basally applied receiver blocks following gravitropic stimulation. The ratio of growth activity between the lower and upper blocks was, on the average, about 10:1. Furthermore, the total yield of GA<sub>3</sub> equivalents, on a per-bud basis, was approximately three times as great in horizontally oriented buds as in vertical ones; so, upon gravistimulation, new GA is synthesized. So far, it has not been shown that the asymmetry in gibberellin-like activity is causally related to the gravitropic response (Wilkins, 1979).

Movement of molecules other than hormones takes place in gravitropically stimulated shoots. During gravity stimulation, more CA<sup>++</sup> was found in the upper halves of *H. annuus* and more P and K<sup>+</sup> were found in the lower halves (Arslan-Cerin, 1966; Goswami and Audus, 1976).

### Grass Nodes

In work done with grass nodes, when the mature, non-elongating flowering stalks of certain grasses, e.g. *Echinochloa colomum* and *Triticum aestivum* are placed on their sides, elongation of cells on the lower side of the leaf-sheath bases is initiated so that bending takes place in the nodal regions until the vertical position is restored (Sachs, 1865). When a leaf sheath base was excised from the plant, a small amount of growth was initiated while still vertical, indicating a release from inhibitory activity of the leaf sheath or internode. The amount of growth was inversely proportional to the amount of tissue remaining attached to the leaf-sheath base after excision. When the leaf sheath base was placed on its side, the upper side elongated even less but the lower side elongated more resulting in upward curvature. When another excised leaf sheath base was split longitudinally and placed horizontally, the segment with its epidermis up elongated about 2% and the segment with its epidermis down elongated about 45% over a 24 h period, compared to intact controls, that, in the vertical position, were not growing at all. If the segments were then reversed, their activity was also reversed (Wright and Osborne, 1977). When excised and longitudinally split leaf-sheath bases elongated in the vertical and responded gravitropically in the horizontal, this indicated that neither lateral redistribution of materials from upper to lower sides of nodes nor their transport from adjoining tissue was necessary to mediate the growth (Bridges and Wilkins, 1973a).

### A Mechanism Other than a Gradient in IAA

Some experiments done by Ganot and Reinhold (1970) also suggest some mechanism other than the direct participation of the lateral

distribution of IAA in gravicurvature; they concerned the involvement of  $H^+$  in the gravitropic response. Etiolated and light-grown seedlings were decapitated and kept for four days in the dark in a saturated atmosphere to starve the tissue of IAA. When segments of hypocotyl were excised and given a gravistimulation, they did not respond gravitropically until they were immersed in an acid buffer, at which time they responded by bending in the correct direction. When hypocotyls, treated in the same manner, were immersed in an auxin solution, curvature was restored to the light-grown hypocotyls but only at supra-optimal concentrations of IAA (10 mg/l).

Ganot and Reinhold (1970) suggested that gravitropic stimulation may have brought about a physiological asymmetry in the tissue, apart from the asymmetrical distribution of auxin, since no auxin was present, and this physiological asymmetry caused a differential response to acid buffer. According to the "acid-growth hypothesis" auxin must be present, *in vivo*, for the pumping out of  $H^+$  from the cells into the cell walls, and, during gravistimulation, there may be a differential response of the walls to the  $H^+$ .

Digby and Firn (1976) summarized evidence, found in other people's work, against a direct participation of the lateral distribution of auxin in shoot gravitropism. They have criticized the lack of correlation between the magnitude of lateral auxin transport and the observed growth rates in *Avena* coleoptiles. They reported that Navez and Robinson (1933) found that, at the onset of curvature, the lower side of a horizontal *Avena* coleoptile was growing 12 times faster than the upper side. The relationship of IAA concentration to growth is log-linear (Cleland, 1972), and therefore the growth observed by Navez and



Robinson would require that endogenous auxin concentrations in the upper and lower halves should differ by a factor in excess of 100, but the difference is only a factor of two (recall the familiar 30%-70% distribution; Dolk, 1936; Goldsmith and Wilkins, 1964). They concluded that there was not enough IAA present in the lower half of the coleoptile to cause the observed increase in the rate of growth. Also, upon examining the results of Filner et al. (1970) and Hild and Hertel (1972), both of whom worked with *Zea* coleoptiles, Digby and Firm (1976) concluded that there was no unequivocal evidence that gravity-induced auxin redistribution takes place soon enough to be the cause of the bending.

#### Changes in Growth Rates of Horizontal Stems

Firm, Digby, and Riley (1978), working with etiolated sunflower, and Digby and Firm (1979), working with the light-grown sunflower hypocotyl, etiolated *Zea* coleoptile, and light-grown cucumber hypocotyl, carried this work further when they analyzed the changes in growth rate occurring during the initial stages of gravicurvature in shoots. They measured the changes in growth rates of the upper and lower sides of shoots by placing a series of black dots on both sides and photographing them at intervals of time, first in the vertical and then in the horizontal positions. They then measured the changes in distances between the dots to get growth rates prior to and during gravistimulation. In all cases, the upper sides of the shoots ceased to grow. Growth of the lower side of the shoots varied according to species as follows: For etiolated sunflower hypocotyl the growth rate on the lower side increased to eight times compared with the growth rate prior to

stimulation; light-grown sunflower hypocotyl increased to 4.0 to 4.4; etiolated *Zea* coleoptile + mesocotyl increased to 1.5 to 2.5, and light-grown cucumber hypocotyl did not increase its growth rate on the lower side at all. For the etiolated and light-grown sunflower hypocotyl, the increase in rate of growth was too great to be accounted for by the two-fold increase in auxin. In the etiolated *Zea* coleoptile + mesocotyl, where there was a slight increase in growth on the lower side, and especially in the light-grown cucumber hypocotyl where there was no increase in rate of growth on the lower side, the redistribution of auxin was not necessary, the curvature being a result of cessation of growth on top.

Another point brought out by the measurement of the distance between the dots during gravistimulation was that in all cases curvature was initiated at all points along the shoot (Digby and Firn, 1979) and did not start at the apex and move to the base as previously thought (Navez and Robinson, 1933).

In all the species of plants used, growth ceased on the upper side during gravistimulation, but only in the light-grown cucumber hypocotyl is the importance of this inhibition of growth, in causing curvature, emphasized. A direct effect of gravity seems to be inhibition of growth on the upper side. This work and others are reviewed by Firn and Digby (1980).

## Collenchyma

### Morphology of Collenchyma

Collenchyma is a living tissue composed of long narrow cells with primary non-lignified walls thickened at the junctions with adjoining

cells. The walls thickened at the junctions have an angular appearance. The structure of the thickened primary walls of collenchyma cells of several species has been investigated by a number of workers using polarizing and bright-field microscopy and x-ray diffraction (Majumbar, 1941; Majumbar and Preston, 1941; Preston and Duckworth, 1946; and Chafe, 1970). The primary walls of collenchyma of celery petioles (*Apium graveolens* L.) have been studied in depth by Esau (1936), Spurr (1957), and Beer and Setterfield (1958). The structure and arrangement indicates that the primary function of the tissue is support, and this is always in conjunction with turgor pressure. Collenchyma characteristically occurs in a peripheral position in stems just below the epidermis or separated from it by a few layers of parenchyma. In the subepidermal position, it occurs in a continuous or sometimes discontinuous cylinder or in discrete strands.

In monocots this tissue is referred to as collenchymatous because, although it serves the same function, it is not identical in morphology to that of dicots. The collenchymatous tissue often occurs as bundle caps (Esau, 1965).

The overall effect of the similarity of these arrangements to a cylinder gives young tissue support by the same mechanical principle that a steel cylinder is more difficult to bend than a solid steel rod made of a similar amount of metal (Cutler, 1977).

The thickened, primary, non-lignified walls are also very well adapted for young tissue because of their great ability to elongate. The walls are highly hydrated, and the layers high in cellulose and low in pectin alternating with layers high in pectin and low in cellulose lend plasticity. The only lignification of tissue in the regions of

elongation occurs where annular and helical secondary thickening of xylem allows the cells to elongate and at the same time prevents them from collapsing (Esau, 1965).

### The Secondary Wall

When collenchyma has finished elongating and is completely differentiated, a secondary lignified wall is formed (Wardrop, 1969; Calvin and Null, 1977). The secondary lignified wall is evenly thickened and round in cross section and surrounded by the collenchyma wall, which remains more or less non-lignified (Calvin and Null, 1977). These cells are then referred to as sclerenchyma (Esau, 1965).

### Mechanical Stress

In other studies, it has been found that mechanical stress caused by wind motion results in thicker collenchyma cell walls of *A. graveolens* L. and an increase in the number of cells, leading to a greater cross-sectional area (Venning, 1949; Walker, 1957; and Walker, 1960).

### Extensibility Studies

Pilet and Roland (1974) isolated collenchyma cells from petioles of *A. graveolens* L. for use in studies of extensibility (elasticity and plasticity). They reported that naphthaleneacetic acid (NAA) acts on the cell wall by lowering the pH and increasing extensibility. Their system was used in further investigations. Extensibility of young and old collenchyma cells was studied in greater detail (Jaccard and Pilet, 1975). The extensibility of the collenchyma cells is increased at low pH's of 3 and 5, but this response decreased with increasing

differentiation. They also looked at the effects of fusicoccin and found that it enhanced extensibility (Jaccard and Pilet, 1979).

#### Hormonal Control of Bending

In dicots, the young internodes are elongating, and the plant has the ability to respond gravitropically if it is placed on its side. Cells on the bottom grow faster than those on the top, which are inhibited, resulting in an upward bend. A mature grass stem at the time of anthesis, is no longer elongating but retains the ability to respond gravitropically in two different regions of tissue just above the nodes. These regions are called pulvini; they contain non-elongated cells. The pulvinus is a highly specialized organ with radial symmetry and is made up of epidermal, vascular, parenchymatous, and collenchymatous tissue, along with the collenchymatous cells that occur as bundle caps (Dayanandan, et al., 1976). The pulvinus at the leaf sheath base is a feature common in most members of the sub-family Festucoideae (wheat, barley, oats, rye). Many other grasses, primarily members of the sub-family Panicoideae, either lack leaf sheath pulvini or possess poorly developed ones, although they have another specialized region at the bases of internodes that is sensitive to gravitropic stimulation (Brown et al., 1959). When these grasses are placed on their sides, a negative gravitropic bending is displayed by the pulvini until the flowering stem is again vertical. This curvature is caused by an initiation by gravity of cell elongation on the lower sides of the pulvini, either the leaf sheath base pulvinus or the internode base pulvinus (Maeda, 1958; Arslan and Bennett-Clark, 1960; Bridges and Wilkins, 1973a; Wright and Osborne, 1977; and Dayanandan et al., 1976). These researchers have also found that exogenously applied IAA (indole-3-acetic acid) can

stimulate growth in upright nodes. Bridges and Wilkins (1973b) induced growth in upright nodes of *T. aestivum* (wheat) with buffer solutions of pH 3. Wright, Mousdale and Osborne (1978) carried this further by determining that endogenous IAA increases in lower halves of gravitropically stimulated *E. colinum* pulvini and decreases in the upper halves; the opposite then occurs when the positions of the pulvini are reversed.

In a study of other changes during gravitropism, Bridges and Wilkins (1974) found an increase in reducing sugar content in the cells on the lower side of *T. aestivum* nodes, but they decided that it did not occur early enough to be essential for initiation of gravity-induced growth. Nonetheless, the finding strongly suggests the direct linkage of a mechanism controlling the activity, synthesis or release of an enzyme to the gravity perception system.

#### Morphological Changes During Bending

Some morphological properties of collenchymatous tissue, and the pulvinus in general, before and after gravitational stimulation, have also been determined. In a scanning electron microscopic study, Dayanandan et al. (1976) showed that, externally, the epidermis over the pulvinus in many grasses is easily distinguishable from that of the internode below and the leaf sheath above by a lack of trichomes, cork silica cells, and stomata. The extent of silicification of the entire surface of the pulvinus is remarkably low compared with the internode below and the leaf sheath above. No silicification occurs during or up to four days after elongation of these epidermal cells in response to gravitational stimulus.

They also used a microscope equipped with crossed polarizers and a first order red plate to study microfibril orientation. The collenchymatous bundle cap was strongly birefringent, and the cellulose microfibrils seemed to have a more transverse orientation in non-elongated than in elongating cells. After bending, when the cells elongated, the microfibrils seemed to be parallel to the length of the cell. Also, after elongation, the uniform birefringence change to a pattern of birefringent areas alternating with areas that were low in birefringence, possibly indicating uneven stretching of the walls. Or this appearance could have been caused by the position of pit pairs (Parker, 1979b).

In another study Dayanandan et al. (1977) surveyed lignification in 23 species of grasses. At the bases of the leaf sheaths and internodes, the gravity-sensitive pulvini showed very low levels of lignification in all plants examined. Immediately above and below the pulvinus region where elongation has been completed during normal upright growth, the bundle cap cells are lignified and appear as typical lignified fibers and are no longer referred to as collenchymatous.

Parker (1979a) described some general morphological features of the collenchymatous cells isolated from gravitropically stimulated and unstimulated pulvini of *E. colonum* and reported some ultrastructural changes that took place in the walls of these cells during the development of gravity induced bends. She found that nonelongating collenchymatous cells in pulvini of upright *E. colonum* stems accumulated electron-dense wall material in infoldings of the plasmalemma. During gravitropism, elongating cells became thin walled, unincorporated wall material disappeared, and a narrow, electron-dense layer of wall formed.

When the gravitational stimulus was removed, growth ceased, the wall rethickened, and the electron dense layer disappeared.

Parker (1979b) also investigated the ultrastructure of the statocytes (amyloplast containing cells) of the leaf sheath pulvinus of *E. colonum*. She observed some lignification in bundle cap cells of the leaf sheath pulvinal material from nodes that had bent for 5 days and in leaf sheath pulvini harvested more than 6 or 7 days after maximum expansion of the inflorescence.



## MATERIALS AND METHODS

## Species and Growing Conditions

All experiments were repeated at least twice with vegetative green cocklebur plants, *Xanthium strumarium* L. (Chicago strain). Plants varied from 30 to 60 days old. Some experiments were also repeated twice with tomatoes (*Lycopersicon esculentum* Mill., var. Bonny Best) and castor beans (*Ricinus communis* L., var. Yolo Wonder).

Seeds (fruits of cockleburs) were planted in flats of sand and transplanted into 10 cm, square plastic pots when about 3 to 5 cm high (1 to 2 weeks old). Tomato and castor bean seeds were germinated in trays of vermiculite. Greenhouse soil was a mixture of dark loam : sand : peat moss (3:1:1, v/v). The soil used was a silty clay loam, pH 7.9 and  $EC_e$  0.61. Soil phosphorus and potassium ratings were 8.3 and 146 ppm, respectively. Approximately 0.5 g of Osmocote 14-14-14 slow-release fertilizer and 0.5 g of phosphorus 0-20-0 were mixed into the soil of each pot prior to transplanting seedlings. Periodically (ca. every 2 or 3 weeks) about 5 to 10 pellets (ca. 0.1 g) of ammonium nitrate 34-0-0 were added to each pot. Waterings were generally required every day. Plants were grown in a corrugated-fiberglass greenhouse (diffuse light) with supplemental lighting from Sylvania 'cool white' fluorescent lights, providing an 18 h photoperiod to maintain cocklebur plants in a vegetative condition. Older leaves were usually removed from cocklebur plants so that only one to three fully expanded leaves remained on the plant. Temperatures in the greenhouse were

usually maintained between 24 and 27°C, although temperatures sometimes dropped as low as 18°C at night in winter and went above 27°C on summer days (Salisbury and Wheeler, 1981).

#### Restricted and Free Bending Gravitropic Experiments

Ten-cm square (inside) wooden boxes were built to hold the plastic pots, and stiff wire frames were shaped to fit into the boxes and to hold the plants tied in position. The plants to be studied for the restricted gravitropic effect were placed between two wires of the frame and wrapped with thread. All the plants, still upright, were placed in the greenhouse for a day to recover from being handled. All plants were then placed in a temperature controlled dark room at 25°C for 48 h. The restricted and unrestricted plants were placed horizontally, and the controls were left upright. The growth chamber was dark to avoid any complications of phototropism. The plants were chosen to be approximately equal in size and vigor.

After 48 h, half of the restricted plants were released and then all the plants were fixed in place for 8 h. This was done by first swabbing the stems briefly with an ether:ethanol solution (3:1) to remove cuticular wax and placing tubes of Karnovsky fixative (Karnovsky, 1965) over the plants and sealing the base of the tubes with plasticine modeling clay. Fixing in place was necessary because the plants to be studied in the restricted condition had to be fixed before the strings were cut, and also, the amyloplasts had to be fixed while they were settled. All the plants were treated in a similar fashion for consistency. The control and three treatments can be seen in Figure 2 and in Figure 3, the tubes of fixative have been placed over the plants.

Tubes were then removed and 1-mm sections were cut from upper and lower sides of the fifth internode. They were marked for orientation by cutting with a razorblade and placed in separate labelled vials so that the top piece from one plant could be compared with the exactly opposite bottom piece from the same plant. There were seven treatments, including the upright control; top free bending, bottom free bending; top restricted, bottom restricted; and top released, bottom released. In each experiment, five pieces of tissue were taken from each treatment, so there were 35 vials. The experiment was repeated four times, twice for longitudinal sections and twice for cross sections.

The sections, once in the vials, were post-fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer pH 6.8 for one h under vacuum, then rinsed twice in 0.1 M cacodylate buffer pH 6.8, 15 min in each rinse, and then placed in 2% OsO<sub>4</sub> in 0.1 M cacodylate buffer pH 6.8 for an additional hour. The following schedule of dehydration, infiltration and embedding was then followed:

10 min 30% ETOH (ethanol)

10 min 50% ETOH

10 min 70% ETOH

10 min 95% ETOH

10 min 100% ETOH

10 min 100% ETOH

5 min 50% ETOH 50% propylene oxide

5 min 100% propylene oxide

30 min 3 parts propylene oxide 1 part epon-araldite epoxy resin  
Mollenhauer (Mollenhauer, 1964)

1 h 2 parts propylene oxide 2 parts epon-araldite

2 h 1 part propylene oxide 3 parts epon-araldite

8 h 100% epon araldite

Specimens were placed in fresh 100% epon araldite in individual embedding flats in proper orientation and placed in a 35°C oven for 8-12 h and then transferred to a 60°C oven for an additional 8-12 h. They were allowed to cure at room temperature for 24 h.

Blocks were trimmed and sections were cut to 1µm thick on a microtome. The block holder on the microtome was adjusted 2° at a time to get good median longitudinal sections. Sections were placed on microscope slides, heated on a slide warmer at 60°C until dry, stained with 2% Toluidine Blue O in 1% CaBorate, heated for two min at 60°C, then rinsed and dried. Slides were made permanent by adding a drop of permount and putting a cover slip in place.

The specimens were viewed with a microscope and pictures were taken on plus-X 4147 professional film. A green filter was added to increase contrast and, for some pictures, phase contrast optics were also used. Pictures were printed on Kodabromide F5 and Agfa Gevaert #6 paper. The 4 in by 5 in format made it possible to take measurements right from the negatives. These included both lengths and diameters of collenchyma cells. Collenchyma cells were measured because they were at the outer edge of the stem where the greatest changes were expected. The sections were taken deep enough into the tissue, from the epidermis to the vascular tissue, to look for amyloplasts in both the cross sections and the median longitudinal sections.

The scanning electron micrographs are of cross-sections of tissue taken from the fifth internode of plants fixed in place and treated the

same until dehydration in 100% ETOH. The schedule used after that was as follows:

10 min	90% ETOH	10% Freon 113
10 min	70% ETOH	30% Freon 113
10 min	50% ETOH	50% Freon 113
10 min	30% ETOH	70% Freon 113
10 min	10% ETOH	90% Freon 113
10 min		100% Freon 113
10 min		100% Freon 113

The tissue was then dried in a critical point drier in the presence of Freon 13. It was then placed on a stub, coated with gold paladium in a vacuum sputter coater, and viewed with a scanning electron microscope.

Free hand cross sections were also taken from cockleburrs, tomatoes, and castor beans and stained with  $I_2KI$  for amyloplast starch and Toluidine Blue 0 to stain the collenchyma cells and other cells to show stem anatomy.

#### Location of Perception Experiments

To determine where in the stem perception of gravity occurs, a series of cockleburrs had successive internodes removed beginning at the tip and moving down the stem until the plants in the last treatment had all tissue above the fifth internode removed. The plants were then placed horizontally. Another series of plants was treated in the same way, but in addition to this, a layer of 1% IAA mixed in lanolin paste was added to the cut stubs. The controls included upright and horizontal plants with apical meristems intact and also upright plants

with the apical meristems replaced with the 1% IAA lanolin. Each treatment, both with and without IAA, contained three plants, and the experiments were done twice. Measurements were made of the angles formed by the entire bending portion of the stems and the angle formed only at the fifth internodes of all plants.

#### Tension/Compression Experiments

Cockleburs, tomatoes, and castor beans were used in these experiments to reverse the position of tension and compression in a horizontal stem. Half the plants were placed horizontally as free bending controls and the other half were also placed horizontally but were fastened to bent wire frames in boxes. The wire frames were bent upward gradually to  $45^{\circ}$  along the portion of frame that corresponded to the growing region of the plant. The plants were placed so that the region from the fifth internode to the apical meristem bent gradually to  $45^{\circ}$  and only the stem below the fifth internode was actually fastened so that any further bending in the growing region was allowed.

## RESULTS

### Anatomy of the Cocklebur Stem

The scanning electron micrograph in Figure 4 is a cross section through the fifth internode of cocklebur, and it shows the overall shape of the stem and the distribution of tissue within the stem. The perimeter of the stem is not circular, and parts even dip in slightly, when viewed in cross section. Epidermal hairs are present. To the inside of the epidermis are five or six layers of collenchyma cells with unevenly thickened walls. To the inside of this are the large thin-walled parenchyma cells. Together, the collenchyma and parenchyma make up the cortex. Next is a typical eustele of closely spaced vascular bundles, and to the inside of this is a very large central pith also made up of parenchyma cells.

### Changes in Cell Dimensions

Figure 5 is a light micrograph of an upright control for one of the experiments with horizontal plants in which cross sectional areas of collenchyma cells were measured. It is an enlargement of a region from the epidermis to the vascular bundles similar to that seen in Figure 4. Figure 6 is a comparison of the top side of a free bending horizontal cocklebur stem to the cells in the opposite portion of stem on the bottom side. The collenchyma cells on the bottom side have a slightly larger cross sectional area than those on the top. In the restricted stems, the plants were placed horizontally but not allowed to bend. In





Figure 4. Scanning electron micrograph of entire cross section of the fifth internode of cocklebur. Just beneath the epidermis E are the layers of thick walled collenchyma cells C, the thin walled parenchyma cells Pa, and next is the continuous cylinder of vascular bundles VB. To the inside of the vascular bundles is the pith Pi.

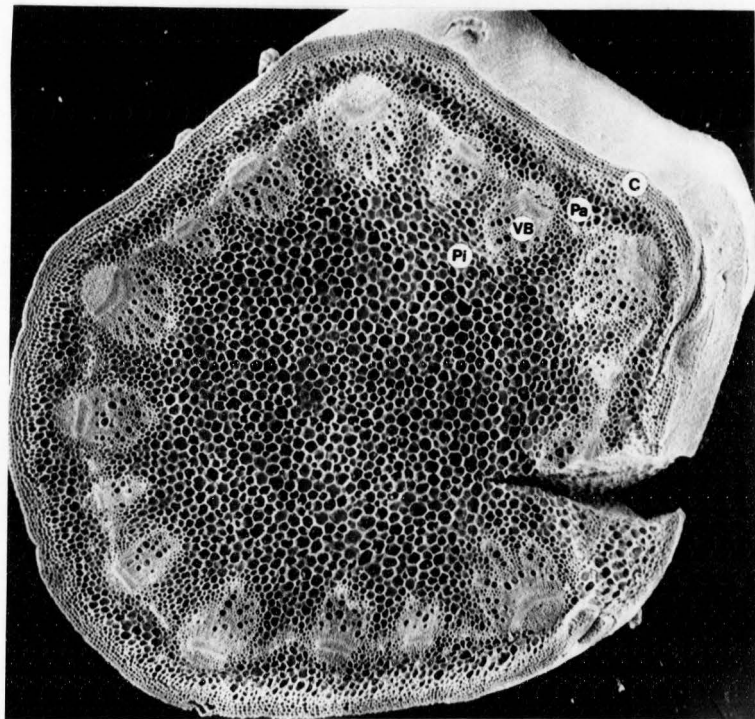
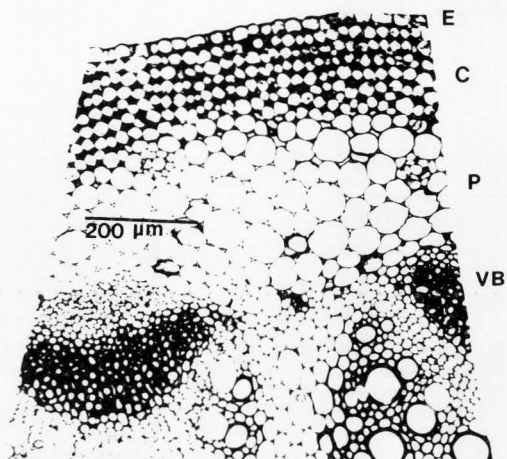




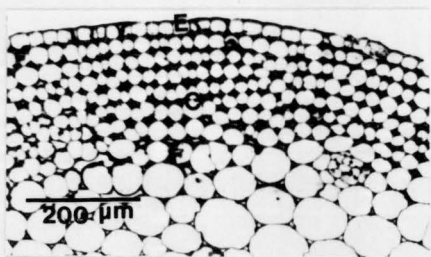
Figure 5. Cross section taken from the fifth internode of the upright cocklebur control. Epidermis E, collenchyma C, parenchyma P, and vascular bundles VB.



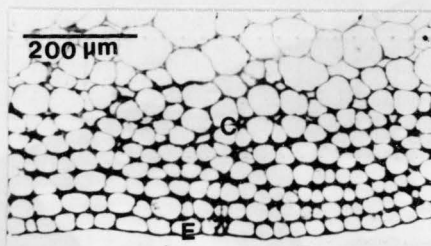
UPRIGHT CONTROL



Figure 6. Cross sections taken from the top and bottom sides of the fifth internode of a free bending, horizontal cocklebur plant. The cross sectional areas of collenchyma cells on the bottom side are slightly larger than those of collenchyma cells on the top.



TOP



BOTTOM

FREE BENDING

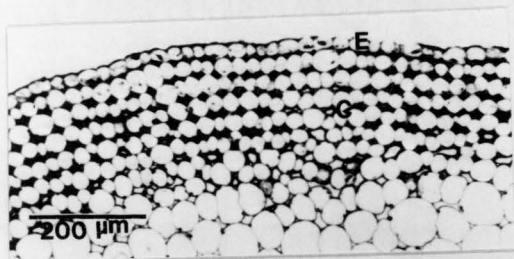


Figure 7, the cells on the bottom side were much wider and had much larger cross-sectional areas than those on the top. The wall thickenings of the collenchyma cells on the bottom are also thinner than those on top. When the restricted stems are released, the cross sectional areas of cells on the bottom (Fig. 8) are no longer much greater than those on top but are about the same size. Also, the walls are no longer thinner. Table I is a summary of these measurements. The first column of data shows the actual measurements, and the second column of data is a comparison of the cross sectional areas ( $\mu\text{m}^2$ ) of cells from each treatment to those of the control with the numbers expressed as percent of control. Because it is difficult to compare cells from one plant to those of another, the measurements of cells on the top side were compared to those of cells on the bottom of the same plant, and conversely, the measurements of cells on the bottom are compared to those on the top of the same plant and both are expressed as relative percent in the third column of data.

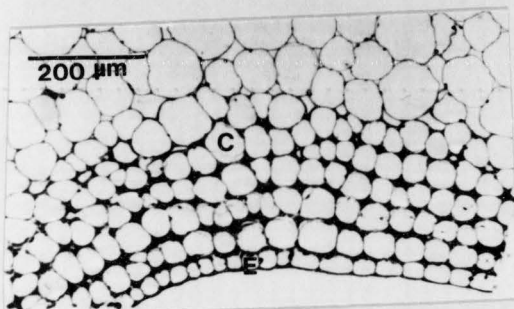
The light micrograph in Figure 9 shows the control for the longitudinal sections in which the lengths of cells were measured. The unevenness of the collenchyma cell wall thickness is represented well. Figure 10 shows that cells on the bottom side of a horizontal free bending plant are longer than those on the top side. When the plants were restricted to the horizontal position (Fig. 11), they could not bend upward, so the cells on the bottom were the same length as those on top. Actually they bent slightly because the tying of stems to the wires was not 100% effective, as can be seen in the picture of the restricted plant in Figure 1. The tying method was used anyway, because it allowed contact with the fixative. The bottom cells were



Figure 7. Cross sections taken from the upper and lower sides of the fifth internode of a cocklebur plant restricted to the horizontal by tying between two stiff wires with thread. The cross sectional areas of cells on the bottom are much greater than those of cells on top. The curve on the bottom is because of the uneven shape of cocklebur stems (refer to Figure 4).



TOP

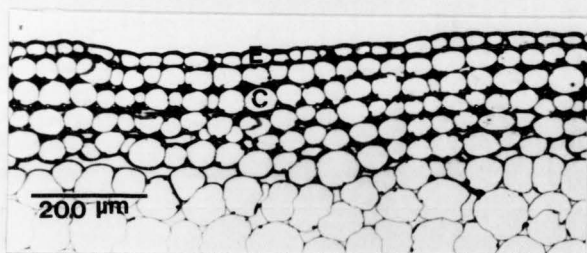


BOTTOM

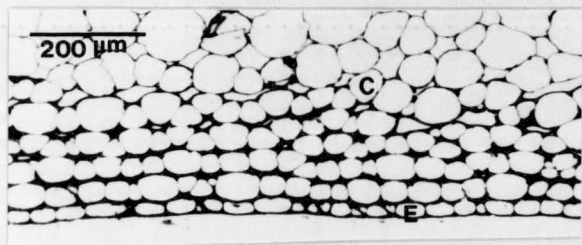
RESTRICTED



Figure 8. Cross sections taken from the upper and lower sides of the fifth internode of a restricted-and-released cocklebur plant. The cross sectional areas of cells on the bottom are no longer greater than those of cells on top.



TOP



BOTTOM

RELEASED

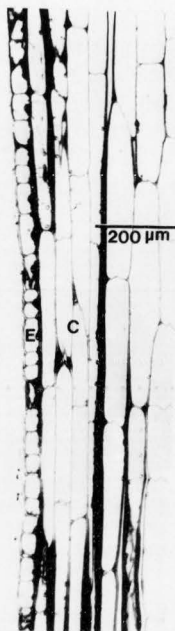
Table 1. Average cross sectional areas of collenchyma cells of cocklebur fifth internode. The first column of data is the average areas  $\pm$  standard errors expressed as  $\mu\text{m}^2$ , the second column of data is each treatment compared to the control and the third column of data is the top cells compared to the bottom cells and the bottom cells compared to the top cells of the same stems expressed as relative percent.

Treatments	Area ( $\mu\text{m}^2$ )	Percent of control	Relative percent of top and bottom cells on same plant
HORIZONTAL PLANTS:			
Free Bending			
Top	596 $\pm$ 23.8	120%	52%
Bottom	1148 $\pm$ 56.8	231%	193%
Restricted			
Top	777 $\pm$ 31.3	156%	36%
Bottom	2146 $\pm$ 91.5	431%	276%
Released			
Top	1582 $\pm$ 68.2	318%	81%
Bottom	1959 $\pm$ 92.5	393%	124%
VERTICAL PLANT: (control)	498 $\pm$ 18.4	100%	--





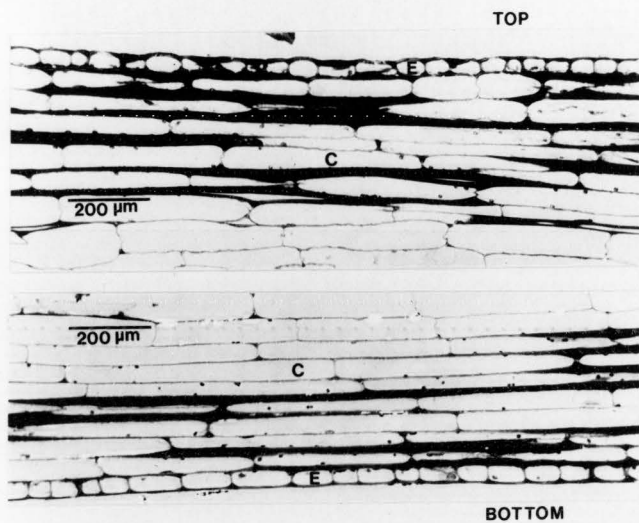
Figure 9. Longitudinal section taken from the fifth internode of an upright cocklebur control. Epidermis E and collenchyma C.



UPRIGHT CONTROL



Figure 10. Longitudinal sections taken from the top and bottom sides of the fifth internode of a free bending cocklebur plant. The average length of collenchyma cells on the bottom is greater than that of collenchyma cells on top.

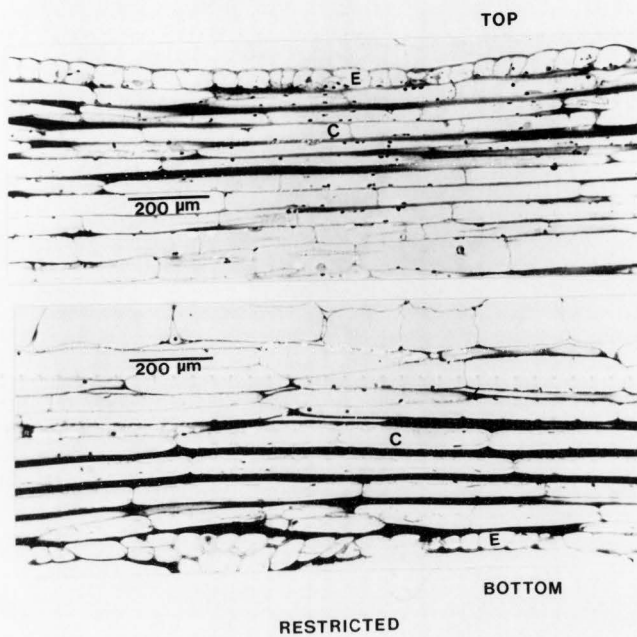


FREE BENDING



Figure 11. Longitudinal sections taken from the top and bottom sides of the fifth internode of a cocklebur plant restricted to the horizontal by tying the shoot between two wires with thread. The average length of collenchyma cells on the bottom is approximately the same as that of collenchyma cells on top.





only slightly longer than those on top, and any lengthening of bottom cells beyond that allowed by the small bending was accompanied by an elastic stretching of cells on the top. Elasticity is the property of recovery of the original size and shape after deformation. After release (Fig. 12), the bottom cells were longer than those on top and also longer than the bottom cells in the restricted stem. Also, the top cells were now shorter than the top cells in the restricted plant. So before release the top cells, which were not being stimulated to grow in the horizontal plant, were stretched elastically; after release, the bottom cells, which were being stimulated to grow, lengthened plastically. Plasticity is the property of becoming permanently deformed when subjected to changes in shape or size. Table 2 is a summary of these measurements. In the second column of data the average lengths in each treatment are compared to the average length in the control, and in the third column of data the average lengths of cells in the tops are compared to those of cells in the bottoms and the average lengths of those bottom cells were also compared to the top cells of the same plants, yielding relative percents.

Part B of Figures 13, 14, and 15 are free-hand cross-sections of cocklebur, tomato and castor bean, respectively, stained with Toluidine Blue O. Part A of each of these three figures is greater magnification of the collenchyma cells. The Toluidine Blue O stain emphasizes the angular, uneven thickenings of the walls. When the tissue was stained with  $I_2KI$  (Part C of each of these figures) a sheath of cells containing amyloplasts, to the outside of and adjacent to the vascular bundles, can be seen.



Figure 12. Longitudinal sections taken from the top and bottom sides of the fifth internode of a restricted-and-released cocklebur plant. The average lengths of collenchyma cells on the bottom is greater than that of collenchyma cells on the top.

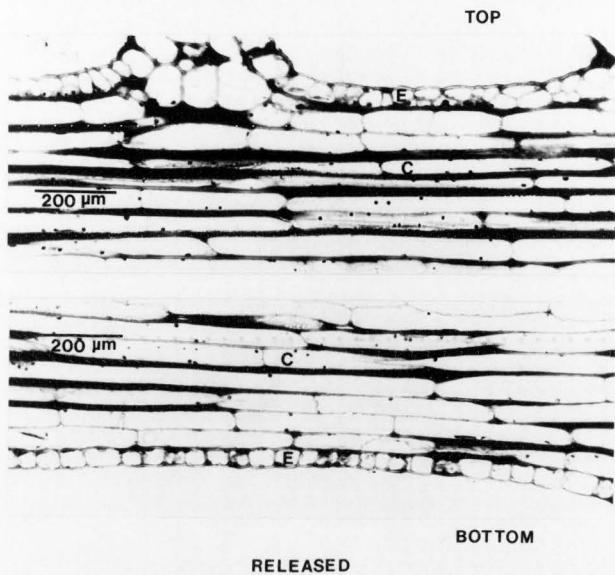
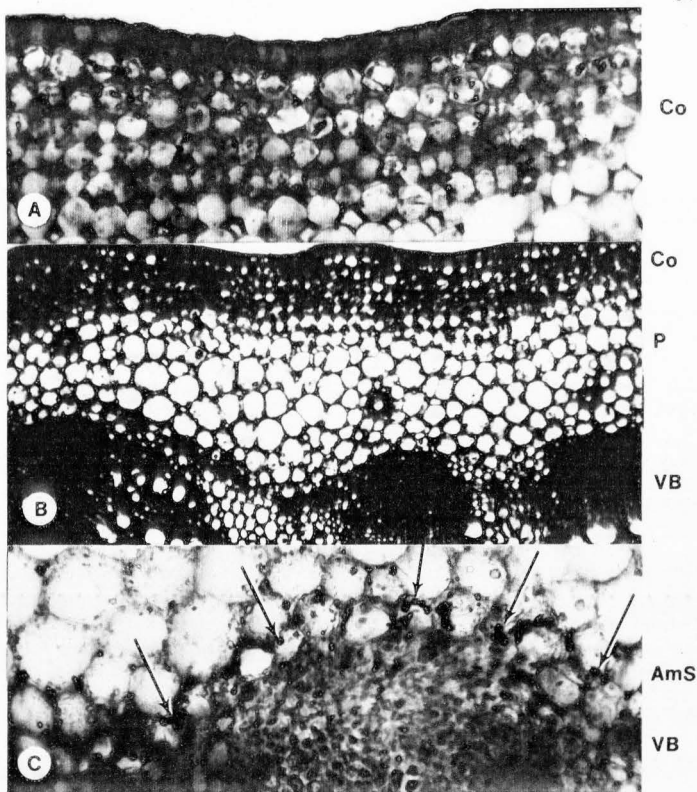


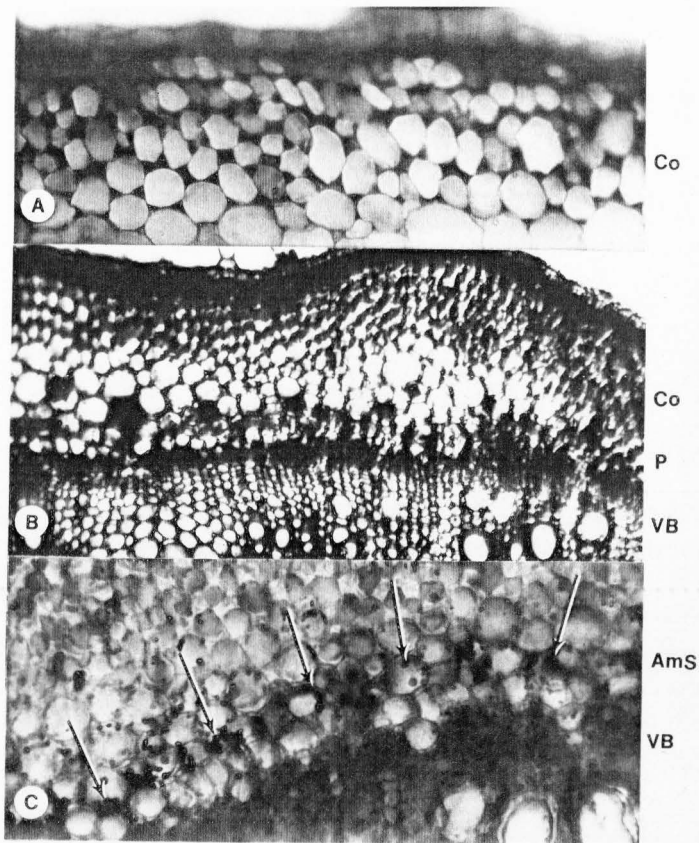
Table 2. Average lengths of collenchyma cells of cocklebur fifth internode. The first column of data is the average lengths  $\pm$  standard errors expressed as  $\mu\text{m}$ , the second column of data is each treatment compared to the control and the third column of data is the top cells compared to the bottom cells and the bottom cells compared to the top cells of the same stems expressed as relative percent.

Treatments	Length ( $\mu\text{m}$ )	Percent of control	Relative percent of top and bottom cells on same plant
HORIZONTAL PLANTS:			
Free Bending			
Top	200 $\pm$ 5.1	91%	74%
Bottom	270 $\pm$ 10.0	123%	135%
Restricted			
Top	260 $\pm$ 10.2	118%	90%
Bottom	290 $\pm$ 10.8	132%	112%
Released			
Top	190 $\pm$ 7.4	86%	59%
Bottom	320 $\pm$ 13.1	145%	168%
VERTICAL PLANT: (control)	220 $\pm$ 7.6	100%	--



### COCKLEBUR

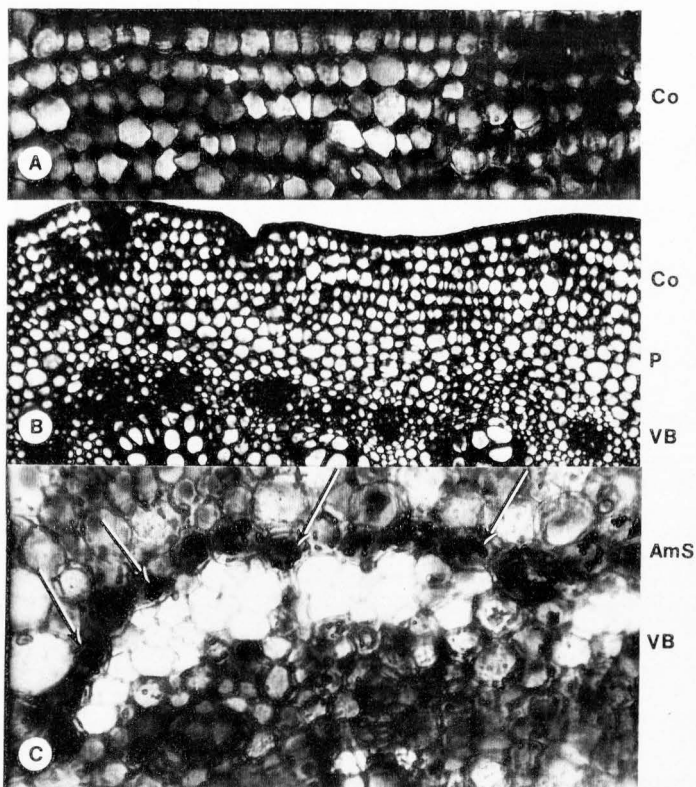
Figure 13. Free hand cross sections of cocklebur stem stained with Toluidine Blue O and  $I_2KI$ . Part B is an overall cross section (mag.=17.6) stained with Toluidine Blue O, showing collenchyma Co, parenchyma P, and vascular bundles VB. Part A is a greater magnification (mag.=44X) of the thickened, angular walled collenchyma cells. Part C (mag.=44X) is stained with  $I_2KI$ , which enhances the starch containing amyloplasts in a sheath of cells to the outside of and adjacent to the vascular bundles. Amyloplast sheath AmS.



## TOMATO

Figure 14. Free hand cross sections of tomato stem stained with Toluidine Blue O and  $I_2KI$ . Part B is an overall cross section (mag.=17.6X) stained with Toluidine Blue O, showing collenchyma Co, parenchyma P, and vascular bundles VB. Part A is a greater magnification (mag.=44X) of the thickened, angular walled collenchyma cells. Part C (mag.=44X) is stained with  $I_2KI$ , which enhances the starch containing amyloplasts in a single layer sheath of cells to the outside of and adjacent to the vascular bundles. Amyloplast sheath AmS.





### CASTOR BEAN

Figure 15. Free hand cross sections of castor bean stem stained with Toluidine Blue O and  $I_2KI$ . Part B is an overall cross section (mag.=17.6X) stained with Toluidine Blue O, showing collenchyma Co, parenchyma P, and vascular bundles VB. Part A is a greater magnification (mag.=44X) of the thickened, angular walled collenchyma cells. Part C (mag.=44X) is stained with  $I_2KI$ , which enhances the starch containing amyloplasts in a sheath of cells to the outside of and adjacent to the vascular bundles. Amyloplast sheath AmS.

Most of the experiments were done with cocklebur, but the similarity in kind and distribution of cells in the stems of these three plants showed that it was logical to get similar results in other experiments where all three species were used. Figures 16 and 17 show, not only that amyloplasts are present in the region of the stem that responds to gravity, but that they also settle in the direction of the gravitational field. Figure 16 is a scanning electron micrograph of a cross section of tissue taken from the upper side of the fifth internode of a horizontal cocklebur and Figure 17 is a light micrograph of a cross section of tissue taken from the same region of another cocklebur plant. In both figures, the amyloplasts can be seen settled against the vascular bundles and away from the cortex.

Figures 18, 19, and 20 are light micrographs of median longitudinal sections taken from the fifth internode of a cocklebur stem in the upright, restricted, and restricted-and-released positions. In the control (Fig. 18), the starch-containing sheath is to the outside of a vascular bundle, and the amyloplasts are settled to bottoms of the cells in the sheath. On the top side of a plant restricted to the horizontal position (Fig. 19), the amyloplasts are settled away from the cortex and toward a vascular bundle. On the bottom side of the same stem, the amyloplasts are settled toward the cortex and away from the vascular bundle.

After the stem was released (Fig. 20), the fifth internode did not bend completely vertical even though the tip did.

The amyloplasts are in the corners, and those on top are no longer in contact as much with the vascular bundle and those on the bottom are no longer in contact as much with the cortex. In all cases the



Figure 16. Scanning electron micrograph of a cross section taken from the top side of the fifth internode of a horizontal cocklebur stem. Amyloplasts can be seen settled against the vascular bundle. The large arrow indicates the direction of gravity.

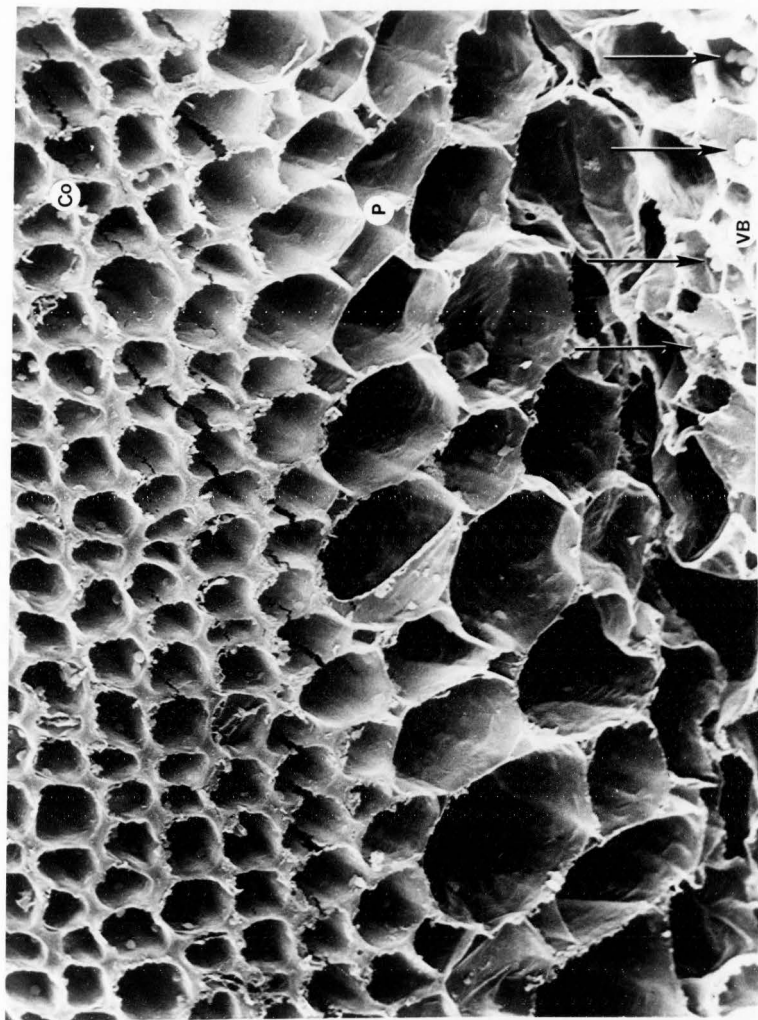




Figure 17. Cross section of the top side of the fifth internode of a horizontal cocklebur stem. The amyloplasts are in a sheath of cells a single cell layer deep just to the outside of and adjacent to the vascular bundle. The amyloplasts can be seen settled against the sides of the cells in contact with the vascular bundles.

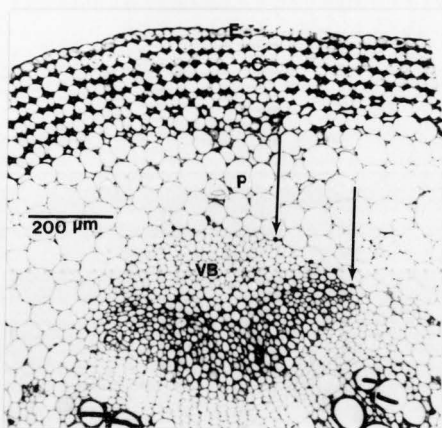






Figure 18. Median longitudinal section from the fifth internode of an upright cocklebur control. The amyloplasts are settled in the direction of gravity indicated by the large arrow. Epidermis E, collenchyma C, parenchyma P, vascular bundle VB, and amyloplasts - small arrows.

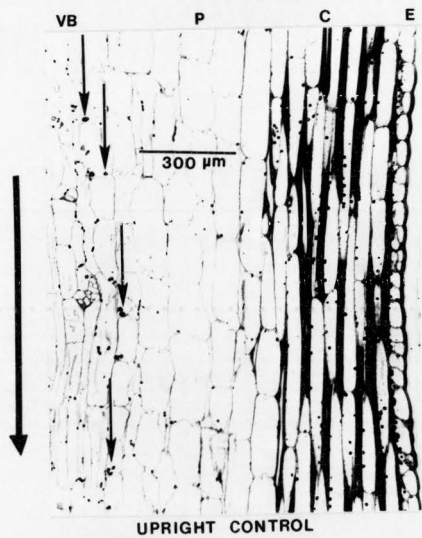




Figure 19. Median longitudinal sections taken from the top and bottom sides of the fifth internode of a cocklebur plant restricted to the horizontal position by tying the stem to a wire frame with thread. In the top section, amyloplasts are settled toward the vascular bundle and away from the parenchyma cells of the cortex and in the bottom, amyloplasts are settled toward the parenchyma cells of the cortex and away from the vascular bundle.

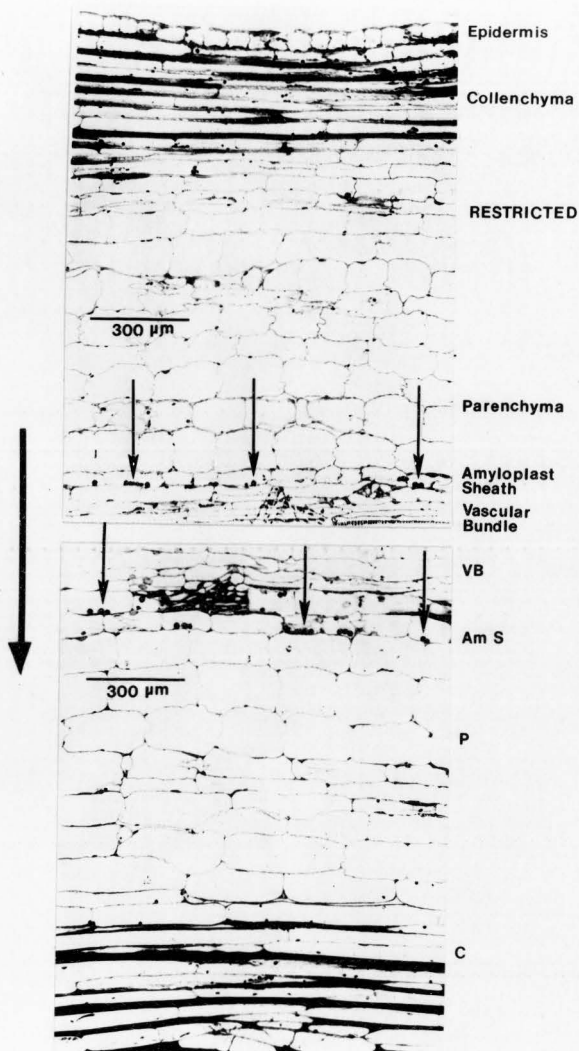
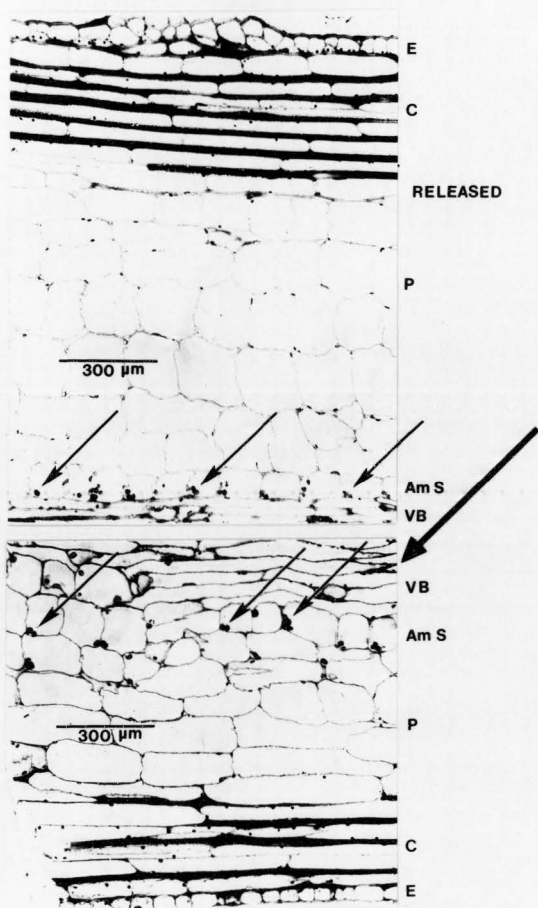




Figure 20. Median longitudinal sections taken from the top and bottom sides of the fifth internode of a restricted-and-released cocklebur stem. The fifth internode was not quite vertical even though the tip was. The amyloplasts are settled in the corners of the cells of the sheath.





settling of amyloplasts correlated with the plant's orientation in the field of gravity, and they were present in the region of bending.

#### Location of Gravity Perception

In elucidating where in the stem perception of gravity takes place, all leaves were removed from cocklebur plants, and, beginning with the apical meristem, portions of the upper stem were removed until all the stem above the fifth internode was removed. The fifth internode was left intact, because it is the internode farthest from the tip that responds gravitropically. This region of gravitropic response is also the zone of elongation. The internodes below this have cells with secondary walls that are lignified. They do not respond gravitropically, and they do not wilt when the plant is allowed to dry out. When the leaves and apical meristem were removed, the stem below still responded; even the fifth internode alone (Fig. 21). The fourth internode did not reach  $90^{\circ}$ , however (it bent to  $60^{\circ}$ ), and the fifth only reached  $30^{\circ}$ .

When the results of the fifth internode alone were compared to those of the fifth internode of the intact horizontal control, it was found that the fifth internode of the control did not bend to  $90^{\circ}$  either (Fig. 22). It did bend to  $60^{\circ}$ , though, which was still further than the fifth internode alone.

It was reported in the literature that the growth hormone IAA is necessary for cell elongation (Brauner and Hager, 1957), so even if an auxin (IAA) gradient is not present or not responsible for gravitropic response (Digby and Firn, 1976), the growth hormone still must be present for elongation to occur in the cells on the bottom side and thus

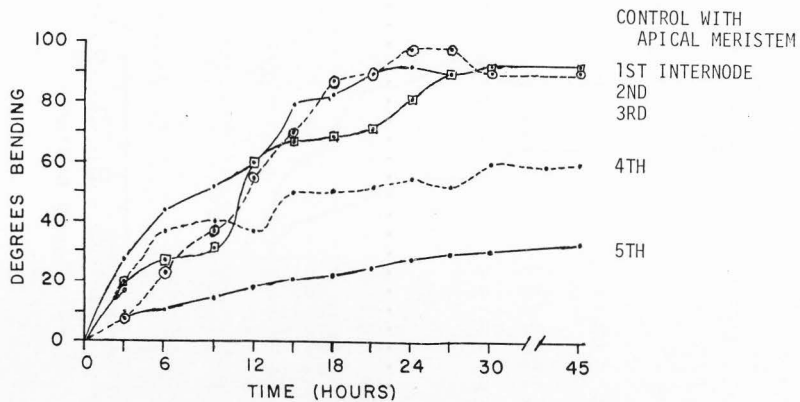


Figure 21. Degrees of bending by horizontal stems that had the apical meristem removed and progressively more internodes removed until only the fifth internode remained. The control was a horizontal plant with the apical meristem intact. Measurements were of the angle formed by the tip of the control or the tip of the cut stub and the nonbending portion of stem below.

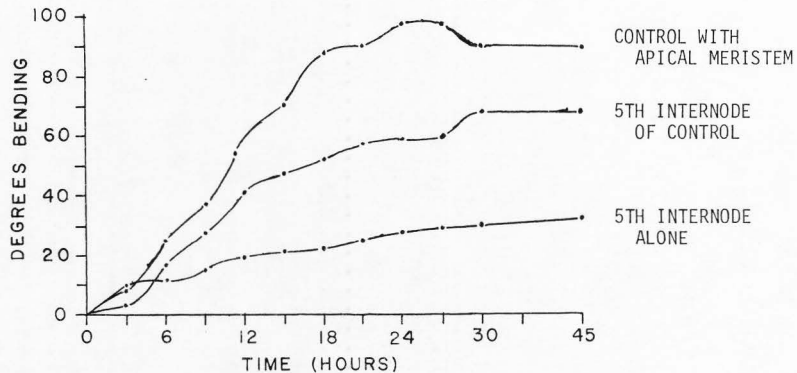


Figure 22. Degrees of bending of the fifth internode of a horizontal cocklebur with all the internodes above the fifth removed compared both to the bending of the fifth internode of the intact control and to the tip of the control. The angle at the cut stub of the fifth internode was not as great as that formed by the fifth internode of the control and both were not as great as the  $90^{\circ}$  angle formed by the tip of the control.

for the bend to take place. When the apical meristem was removed, there was residual IAA in the stem with the least being present in the plants with the most stem removed, such as the fourth and fifth internodes. A separate set of plants were treated identically, except that a lanolin paste containing 1% IAA was added to the cut stubs. Such a high concentration in a thickly applied paste was believed to be overwhelming enough to the plant that a gradient was not formed, although this needs to be tested with  $^{14}\text{C}$ -IAA. The graph in Figure 23 shows that when IAA was applied to the cut end of the fourth internode, the tip of that stub then reached  $90^\circ$ , and when applied to the cut end of the fifth internode, that internode then bent to  $43^\circ$  which was much closer to the bend in the fifth internode of the control ( $60^\circ$ ).

The bar graph in Figure 24 shows the final angles of the fifth internodes of all the plants, each with more stem above removed and with and without added IAA. The added IAA at the cut end of the first internode did not cause any increased bending in the fifth internode of that plant, but it did cause increased bending in the fifth internodes of plants as greater portions of stem were removed. The greatest increase was in the fifth internodes of plants with all above internodes removed.

#### Perception of Tension/Compression as a Perception of Gravity

The graphs in Figure 25 show that the plants of all three species, which had been tied to wire frames previously bent to  $45^\circ$  and laid horizontally, remained at  $45^\circ$  until the free bending horizontal plants also reached  $45^\circ$ , and then both groups bent upward beyond this and reached  $90^\circ$  at the same time.

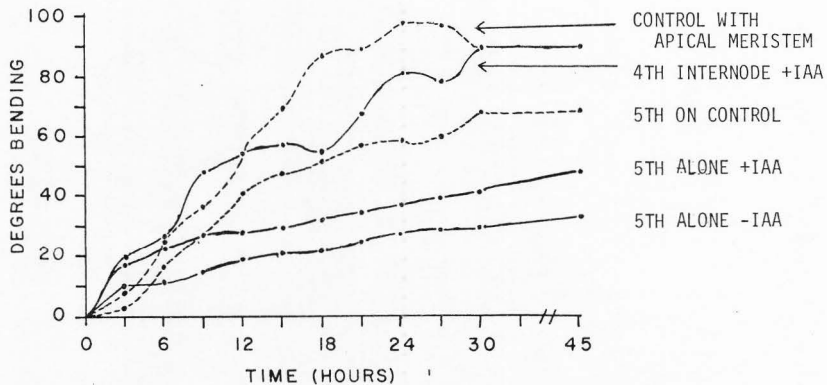


Figure 23. Comparison of bending at the tip of the cut stub of cocklebur plants with and without IAA. When 1% IAA in lanolin is added to the cut stub of the fourth internode, it bends to  $90^{\circ}$  as does the control. When IAA is added to the cut stub of the fifth internode the angle of bending is closer to that of the fifth internode on the control than when IAA is not added.

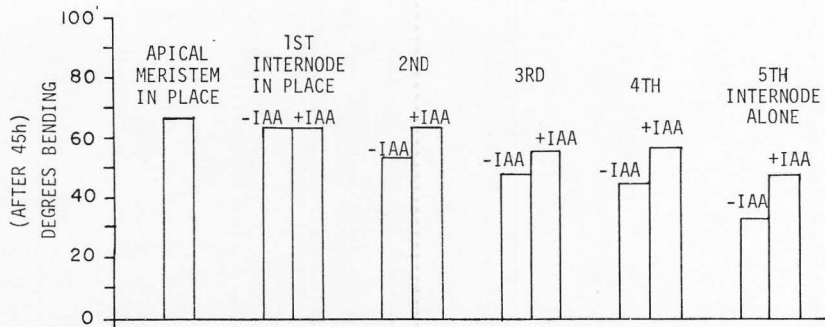


Figure 24. A summary of the angles formed by the fifth internodes of all the horizontal plants including the intact control and each of the plants with more internodes removed until the entire stem above the fifth internode has been removed. When only the apical meristem has been removed and the first internode is in place, the added 1% IAA in lanolin paste does not cause increased bending but as each piece of stem is removed the added IAA causes a greater increase in bending.

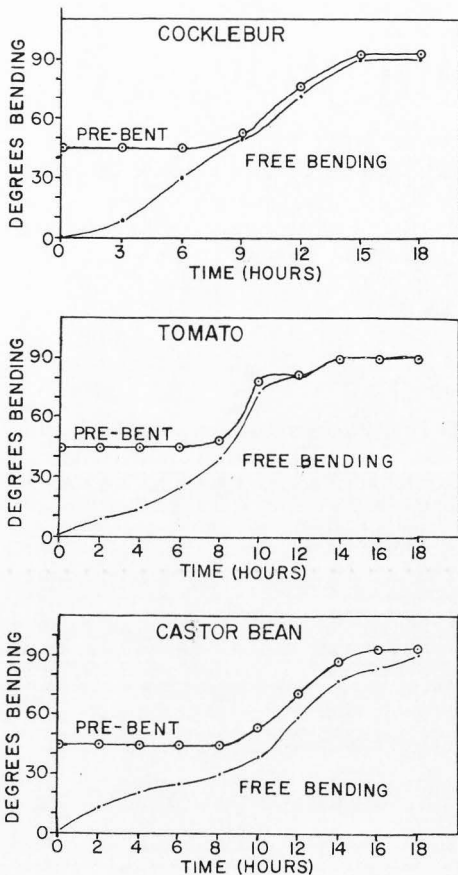


Figure 25. Comparison of bending of horizontal plants pre-bent to  $45^{\circ}$  with horizontal free bending plants. In all three cases the cocklebur, tomato, and castor bean plants pre-bent to  $45^{\circ}$  remained at  $45^{\circ}$  until the free-bending plants also reached  $45^{\circ}$  and then both groups bent at the same rate to  $90^{\circ}$ .



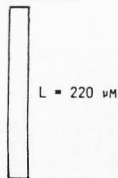
## DISCUSSION

## Changes in Cell Size and Shape

The results of the measurements of cell diameters and lengths indicate that stems bend upward rapidly after release in response to changes in cell dimensions, and they are compatible with the idea that this happens with conservation of cell volume (i.e., little movement of water in or out of cells during the rapid bending). Cells on the bottom elongate but become narrower, conserving volume; cells on top shorten but become thicker, conserving volume. When measurements of cell areas (from Table 1) and cell lengths (from Table 2) were used to draw diagrams of the cells (Fig. 26), their calculated shapes and volumes conformed closely to those expected for a conservation of volume. These are close correlations when considering that the measurements were averages of lengths and diameters of different cells from different plants, before and after bending.

In the plants that were completely restricted in the horizontal position, any elongation had to be approximately equal on both top and bottom (never exactly equal because of the imperfect method of restraint). Elongation of cells on bottom was accompanied by an elastic stretching of cells on top. Elasticity was indicated because they became shorter after release, while the bottom cells got longer plastically. This clearly demonstrated that halting growth on the top of a stem laid on its side is extremely important in gravitropic bending.

VERTICAL CONTROL

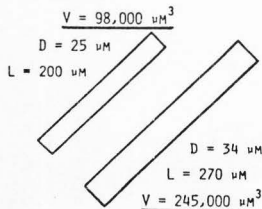


$$D = 22 \mu\text{m}$$

$$V = 83,600 \mu\text{m}^3$$

$$IV = \pi(D/2)^2 L$$

FREE BENDING



$$V = 98,000 \mu\text{m}^3$$

$$D = 25 \mu\text{m}$$

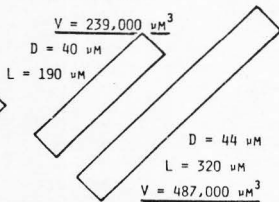
$$L = 200 \mu\text{m}$$

$$D = 34 \mu\text{m}$$

$$L = 270 \mu\text{m}$$

$$V = 245,000 \mu\text{m}^3$$

RESTRAINED  
AND RELEASED



$$V = 239,000 \mu\text{m}^3$$

$$D = 40 \mu\text{m}$$

$$L = 190 \mu\text{m}$$

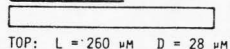
$$D = 44 \mu\text{m}$$

$$L = 320 \mu\text{m}$$

$$V = 487,000 \mu\text{m}^3$$

RESTRAINED

$$V = 160,100 \mu\text{m}^3$$



$$\text{TOP: } L = 260 \mu\text{m} \quad D = 28 \mu\text{m}$$

$$V = 482,000 \mu\text{m}^3$$

$$\text{BOTTOM: } L = 290 \mu\text{m} \quad D = 46 \mu\text{m}$$



Figure 26. Calculated shape and volume of collenchyma cells taken from the top and bottom sides of the fifth internode of free bending, restrained, and restrained and released plants and from the upright control. The volumes of cells on the bottom sides of restrained and restrained and released are the same indicating that water was taken up during restriction and did not move in or out during release. The volumes of cells on the top side of the restrained and released plants are shorter than before release but the volume is greater. This greater volume could possibly be explained by the fact that the same cell could not be measured before and after release and there is variability from plant to plant.

Of particular interest in Figure 26 are the diagrams of the bottom cells while still restricted and after release. The volumes of these cells are very similar and strongly indicates a conservation in volume, with the water having moved in during restriction. The increase in volume of the corresponding top cells could be due to variability from plant to plant or to the wall pressure of the top cells could have been lowered by the shortening of these cells after having been stretched, allowing water to move in during bending.

Also, while the plants were restricted, the cross sectional areas of cells on the bottom were much greater than those on top, even though they were approximately the same lengths. They were also greater than cross sectional areas of cells under any of the other treatments. This demonstrated that water was being taken up by the cells on the bottom as they were still being stimulated to elongate as in the usual growth processes. Their cell walls were stretching (they were much thinner than the walls of cells on top) in response to the increased water uptake. After bending, the cells on the bottom were no longer greater in diameter and the walls were no longer thinner than those on top, indicating that these cells had been stretched elastically in their circumference, while their length increased plastically. Therefore, in the restricted condition, active cell elongation ceased on top, but the cells were stretched lengthwise by the continuing growth of the bottom cells. Bending energy was stored in the stretched top cells and the compressed bottom cells that took up water during restriction.

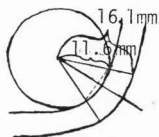
Along with measuring lengths and diameters of cells in the bending region, I did a test to check whether or not the new cellular dimensions were great enough to account for the degree of stem bending that had

taken place. If not, then some other process, such as cell division on the bottom side, could have been taking place. If the new cellular dimensions were indeed great enough to account for bending, then the increase in length of collenchyma cells on the bottom side as compared to those on the top sides should be equivalent to the increase in distance on the surface of the bottom side as compared to that of the top side. The ratio of average lengths of the top and bottom collenchyma cells was compared to the ratio of lengths of stem surfaces in the same part of the bend where the lengths of cells were measured. An easy way of doing this was to make a quick outline of the bent stem, before fixation, and to calculate the ratio of the radii of the circles formed by the upper and lower surfaces of the stems as shown in Figure 27. The radii can be used instead of surface distance because, when one circumference is divided by the other ( $c = 2\pi r/c = 2\pi r$ ), the 2 and  $\pi$  cancel leaving  $r/r$ . So the ratio of radii is equivalent to the ratio of the distances of the surfaces. It is better to use the radii because they remain constant no matter how great, or short, a distance of the circumference is used and because it was easier. In free bending stems, the ratio of radii, 11.3 mm/16.1 mm = 70% from Figure 27, was similar to the ratio of lengths of collenchyma cells, 200  $\mu\text{m}$ / 270  $\mu\text{m}$  = 74% from Table 2. In stems after release, the ratio of radii, 9.6 mm/15.3 mm = 62% from Figure 27, was similar to the ratio of lengths of collenchyma cells, 190  $\mu\text{m}$ /320  $\mu\text{m}$  = 59% from Table 2. Therefore, the increase in lengths of cells was enough to account for bending of stems.

These observations lead to some interesting conclusions and suggestions. First, because the horizontal plants were not being allowed to bend due to the restraint, the water being taken up by the

$$\frac{\text{inner circumference}}{\text{outer circumference}} = \frac{2\pi r}{2\pi R} = \frac{\text{inner } r}{\text{outer } r} \text{ or } r/R$$

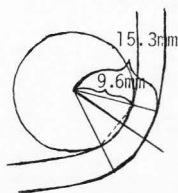
$$r/R = 11.6\text{mm}/16.1\text{mm} = 70\%$$



sections  
taken  
here

FREE BENDING STEM

$$r/R = 9.6\text{mm}/15.3\text{mm} = 62\%$$



sections  
taken  
here

RELEASED STEM

From TABLE 2

ratio of lengths of cells from  
upper and lower sides of  
FREE BENDING stems:

$$200\mu\text{m}/270\mu\text{m} = 74\%$$

ratio of lengths of cells from  
upper and lower sides of  
RELEASED stems:

$$190\mu\text{m}/320\mu\text{m} = 59\%$$

Figure 27. A test whether or not the new cellular dimensions were great enough to account for the degree of stem bending. The upper and lower surfaces in the bending region of the stem determined the circumferences of the circles. The entire circumference of the outer circle was not necessary, only the portion coinciding with the lower surface of bent stem. These circumferences are related to the radii by the formula:  $C=2\pi r/C=2\pi R = r/R$ . Thus the ratio of inner to outer radii equals the ratio of length of upper stem surface to lower stem surface. This is expressed as a percentage. The stem length ratio compares well to the cell length ratio (70% to 74% and 62% to 59%) for both free bending and released stems. Therefore, change in cell length was enough to accommodate bending and no other process such as cell division was taking place.

bottom cells was done so against an ever increasing pressure gradient. This is in contrast with the general theory of cell growth that was established in the 1930's and has been supported by many experiments since. This theory says that plant cell growth occurs as cells take up water osmotically. The osmotic potential within the cells remains virtually constant, however, so that is not the driving force. That is, as cells increase in volume, they also increase in osmotically active solutes at a comparable rate so that osmotic potentials do not change. Rather, the driving force is thought to be a decrease in pressure within the cells caused by a "loosening" of the cell walls. Therefore, the water potential becomes more negative inside the cells as osmotic potentials remain constant but pressures decrease. This is in contrast with my observation of the restricted gravitropic phenomenon where growth was continuing on the bottom of the stems, even though pressures there were increasing (because of the restriction), and growth ceased in the cells on the top of the stem, even though tension (negative pressure) was developing as top cells were being stretched. These pressures are being investigated further by Wesley J. Mueller in collaboration with Dr. P. Thomas Blotter in the Mechanical Engineering Department. Their preliminary results suggest that bending forces cease building up when the pressure caused by bending is about equal to pressures in normal cells (about 5-8 bars). Since more  $K^+$  is found in the bottom halves of *Helianthus annuus* shoots (Arslan-Serin, 1966; Goswami and Audus, 1976), perhaps  $K^+$  is moving into the cells in the lower halves of cocklebur shoots (during restriction to cause an osmotic gradient great enough to overcome the increase in pressure.

Second, the rapid changes in cellular dimensions after restricted stems were released can only be accounted for in terms of the mechanics of the cell wall. The shrinkage of cells on the top of released stems must ultimately be explained by the microfibril arrangements within the cell walls, and the strong tendency for cells on bottom to elongate after release from restriction must also have its explanation in the formation and orientation of cell wall microfibrils during the time of restriction. In her book, Esau (1965) discussed microfibril orientation in primary cell walls. When a layer of microfibrils are laid down, their long axes are parallel to each other but more perpendicular to the long axis of the cell. The angle depends on the kind of cell and kind of tissue. As a consecutive layer is laid down, it is also almost perpendicular but in the opposite direction, creating a criss-cross pattern. As the cell elongates, the microfibrils change from being more perpendicular to being more parallel to the long axis of the cell. A cell whose wall has microfibrils completely parallel to the long axis is no longer capable of elongation.

Albersheim (1974) also reviewed microfibril orientation in primary cell walls, and he discussed a model including chemical changes that must take place for reorientation of microfibrils during elongation growth. In this model, the microfibrils, which are long bundles of parallel chains of cellulose molecules, are connected to each other by several interlinking pectin molecules and chains of xyloglucan, a hemicellulose. The xyloglucan and other molecules are covalently linked to each other but not to the microfibrils. The microfibrils are only connected to the chains of xyloglucan, and this is through a series of hydrogen bonds. Raising the temperature or lowering the pH could loosen

these bonds and allow the xyloglucan chains to slide by cellulose microfibrils by what he described as hydrogen bond "creep", that is, by the xyloglucan moving along the microfibril like an inch worm. The lowering of pH in the cell wall has been studied both by Cleland (1973) and Rayle (1973), and they have proposed that IAA loosens walls by causing  $H^+$  to be pumped out of cells and into the walls, lowering the pH there. This ties in nicely with the proposal that some special orientation of microfibrils is intimately related to the changes that take place during restricted gravitropism, but it is only one of many possibilities of what may be happening.

While thinking about what is happening to the walls of the top and bottom cells, Dr. Frank Salisbury and I have come up with three ideas.

Our first idea involves some kind of active changes in bonds during restriction, such that the new bonds are creating a tendency in the bottom cells to elongate causing increased pressure. This would cause the angles bisected by the circumference to become more obtuse, and the angles bisected by the long axis to become more acute. The microfibrils would have been predisposed, during restriction, to take on these new angles. This is contrary to the traditional idea that the elongating cell pulls the loosened microfibrils to new angles and then the new bonds are formed but rather, the newly formed bonds during restriction are causing the elongation after release.

In studying the thin walls of the cells with the very large cross sectional areas in Figure 7 bottom, I thought of the second idea. The water being taken up by the bottom cells could be elastically stretching the walls circumferentially. That is, the microfibril angles that control diameter are not being broken and reformed, but rather, they



retain the tendency for the cell to go back to its original diameter. Loosening of other microfibril bonds could still be taking place, however, so that, when the stems are released, the cells can elongate plastically to greater lengths that can accommodate the increased volume of water taken up during restriction.

Dr. Salisbury came up with the third idea which is also passive but depends more on the changes taking place in the top cells. While the stem is restricted, the cells taking up water on the bottom are elastically stretching the cells on top. After release, these stretched upper cells, which are going back to their original length, could be pulling the lower cells to elongate.

The bending could easily be a combination of the second and third ideas, that is, upon release, the bottom cells elongate plastically to alleviate the built up pressure, while the top cells shortened elastically.

These ideas could be tested by restricting plants to the horizontal for 48 h and then cutting the stems longitudinally with a razor blade, separating the top half from the bottom half. If the bottom half did not bend upward, the third idea would be supported but if it did bend upward, the first and second ideas would be supported.

#### Location and Movement of Amyloplasts

It was already known from other experiments in our laboratory that cocklebur plants responded gravitropically even when all the leaves and the apical meristem were removed. Therefore, the leaves and apical meristem were not necessary for perception. There are many reports in the literature (e.g., reviews by Hoshizaki, 1973; Shen-Miller and

Hinchman, 1974; Juniper, 1976; Audus, 1979; Volkman and Sievers, 1979) that the movement of amyloplasts is often associated with perception of gravity. The logical next step was to determine where along the stem perception took place and whether or not amyloplasts were present and, if so, did they settle in response to gravity.

Even though I was looking at the changing dimension of only the collenchyma cells, I made my sections deep enough (to the vascular tissue, and in free hand sections, clear to the pith) to enable a search for amyloplasts. As can be seen from Figures 13-20, amyloplasts occur in a sheath, a single cell-layer thick, that is just outside the vascular bundles and to the inside of the cortex. The micrographs in Figure 16-20 all show the amyloplasts settled in the direction of gravity. The position of the sheath and the movement of amyloplasts has led me to a very interesting proposal for a model of graviperception in green dicot shoots of cocklebur, tomato, and castor bean. The scanning electron micrograph in Figure 4 shows the eustele of closely spaced vascular bundles. To the outside, the sheath is always in contact with parenchyma cells of the cortex, but to the inside, it is always in contact with vascular tissue, mostly phloem. When a stem is laid on its side, the amyloplasts in the sheath cells on the bottom of the stem settle against the sides of the sheath cells that are adjacent to cortex cells - cells that could be active in producing, or responding to, auxin or in some way promoting growth on the bottom of the stem. Amyloplasts on top fall away from these cortex cells and settle against the sides of the sheath cells adjacent to vascular tissue - tissue that could be quite inactive in stem growth. Such a model of gravitropic perception suggests that stem growth depends on growing cells being in

contact with cells that have amyloplasts against the sides of cells adjacent to the growing cells, as on the bottom of the stem, and the absence of amyloplasts against the sides of cells adjacent to growing cells on top might account for observed inhibition of growth in cells on the top of a stem laid on its side.

The advantage of this arrangement of amyloplast-containing cells over the arrangement of amyloplast-containing cells in other tissues, such as root tips, is the presence of asymmetry. There is virtually no asymmetry in root tips. In the root tips, the amyloplast-containing cells are clumped together, and it is not known what is different about having the amyloplasts settle against one side as opposed to the other (Juniper, 1976; Volkman and Sievers, 1979). The model present here needs to be tested in the future.

#### Location of Perception

The results from the experiments in which successive internodes were removed, and plants were then placed horizontally, demonstrated that perception can occur in the same tissue that bends. In the initial experiments, very little bending occurred when the fourth internode was removed. This lack of response was probably caused by the lack of auxin for cell growth rather than to any lack of perception. After all, when the first, second, and third internodes were removed (before being placed in the horizontal), the considerable bending that did occur was most likely allowed by the presence of residual auxin in the longer portions of remaining stem. When the experiments were repeated and a second set of plants received 1% IAA in lanolin applied to the cut stub, indeed, more bending occurred in the fifth internode with all the stem

above removed. With such a heavy concentration of supplied IAA, it seems likely that any gradients were minimal and not steep enough to actually account for the bending. This again implies inhibition of growth on the top side of the stem even in the presence of the growth promotive effects of auxin. Also, if a gradient of IAA was indeed prevented by the overwhelming amount of added IAA, the fact that horizontal cut stems bent up even more than those without added IAA, is further evidence against the necessity of an auxin gradient. To prove that no gradients were formed, these experiments need to be repeated using labelled IAA.

#### Perception of Tension/Compression as a Perception of Gravity

I also tested the possibility that gravitropic perception is a perception of the compression on the bottom of a stem held horizontally, combined with the tension on the top of the stem, both caused by the stem's weight. When the positions of tension and compression were reversed by fastening them to the bent frames, stems nevertheless bent upward, allowing this hypothesis to be rejected.

## SUMMARY

1. In a horizontal plant, the cells on the top side stop growing, and elasticity is retained, and cells on the bottom side after release, increase in length plastically but during restraint, their circumferences were stretched elastically.

2. In restricted plants, energy for bending is stored in the stretched upper cells and the compressed lower cells that had taken up water against an increasing pressure gradient.

3. Bending is accompanied by changing cellular dimensions while volume of the cells is conserved, indicating that water does not move in or out during bending. Rapid bending must be allowed by some special orientation or change in bonds of microfibrils during restriction.

4. Perception of gravity takes place in the very tissue that responds but growth hormone must be present for the response to take place. Gravid perception is not the perception of compression/tension caused by the weight of a horizontal stem.

5. Amyloplasts are found in a single layer of sheath cells just to the outside of and adjacent to the eustele of closely spaced vascular bundles. The amyloplasts settle in the direction of gravity (apparently in spite of the very large central vacuole).

6. The location of the sheath of cells containing amyloplasts between the parenchyma cells of the cortex and the vascular bundles suggests an asymmetric model of gravid perception. When a stem is laid horizontally, the amyloplasts in the sheath on the top side settle away from the wall in contact with the parenchyma cells and toward the wall

in contact with the vascular bundles, and the amyloplasts in the sheath on the bottom side settle toward the wall in contact with the parenchyma cells and away from the wall in contact with the vascular bundles. This asymmetry correlates with the inhibition of growth on the top side and stimulation of growth on the bottom side.

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## VITA

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