Planta (1999) 209: 180–186

Planta

Auxin redistributes upwards in graviresponding gynophores of the peanut plant

Edgar Moctezuma, Lewis J. Feldman

Department of Plant and Microbial Biology, University of California at Berkeley, 431 Koshland Hall, Berkeley, CA 94720, USA

Received: 25 January 1999 / Accepted: 24 February 1999

Abstract. The peanut (*Arachis hypogaea* L.) produces flowers aerially, but buries the recently fertilized ovules into the soil, where fruit and seed development occur. The young seeds are carried down into the soil at the tip of a specialized organ called the gynophore. Although the gynophore has a typical shoot anatomy, it responds positively to gravity like a root. In this study, we explore the role of the plant growth regulator indole-3-acetic acid (IAA) in the growth and the gravitropic response of the peanut gynophore. With an immunolocalization technique using an IAA monoclonal antibody, we localized IAA within the tissues of vertically oriented and gravistimulated gynophores. We found that in vertically oriented gynophores, IAA labeling occurs in the periphery of the gynophore, in the entire cortex and epidermis. Within 20 min of horizontal reorientation, the IAA signal gradually increases in the upper cortex/epidermis and diminishes in the lower cortex/epidermis. At 1.5 h after gravistimulation, all of the IAA immunolocalization signal is detected in the upper cortex and epidermis – none is detected in the lower side. Growth rate measurements also indicate that after 1–2 h of reorientation, the growth rate maximum on the upper side corresponds temporally and spatially to the growth rate minimum on the lower side. Experiments using radioactively labeled IAA corroborate an upper-side redistribution of this hormone upon horizontal reorientation. These results are analyzed with respect to the current theories of plant gravitropic response, and a model for a possible gravity-induced IAA redistribution from the lower to the upper side of the peanut gynophore is proposed.

Key words: *Arachis* (gravitropism) – Auxin redistribution – Cholodny-Went Hypothesis – Gravitropism – Gynophore – Indole-3-acetic acid

Introduction

The peanut (*Arachis hypogaea* L.) plant has a unique growth habit: it must 'sow' its own seeds in order to complete its life cycle. The peanut is geocarpic, meaning that it produces flowers aerially and buries the recently fertilized ovules into the soil for fruit and seed development to occur underground (Smith 1950; Shushu and Cutter 1990). The young seeds are carried down into the soil at the tip of a specialized organ called the gynophore.

The peanut gynophore has an unusual gravitropic behavior (Hart 1990). Although the gynophore has a typical shoot anatomy, it responds positively to gravity, much in the manner of a root. This positively gravitropic response is essential for the successful completion of the peanut plant life cycle: if the young seeds are not carried and buried into the soil, the fruit and seed will not develop. Its unique gravitropic behavior, and the importance of its graviresponse in the peanut life cycle, have led us to study the mechanisms that govern the gravitropic response of the peanut gynophore.

The plant hormone indole-3-acetic acid (IAA) has a large number of effects in plants, including the stimulation of cell enlargement and cell division, the mediation of tropistic responses of plant organs towards light and gravity, and the induction of growth and setting of fruits in many species (Davies 1995). Previous studies have shown that IAA plays a major role in the growth and development of the peanut gynophore (Jacobs 1951; Zamski and Ziv 1976; Shushu and Cutter 1990; Moctezuma 1999). Jacobs (1951) was the first to demonstrate that IAA was produced in the distal 10 mm of the gynophore, and showed that this plant growth regulator was transported basipetally in a

Abbreviations: IAA = Indole-3-acetic acid; CWH = Cholodny-Went Hypothesis; EDAC = 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride

Correspondence to: E. Moctezuma;
E-mail: edgar@nature.berkeley.edu; Fax: 1 (510) 642 4995

strictly polar manner. Shushu and Cutter (1990) found that IAA, in combination with gibberellic acid, promotes the normal downwards growth of the peanut gynophore.

Indole-3-acetic acid is also known to be a major player in the gravitropic response of plant organs, because it redistributes to different tissues upon gravitropic stimulation (Hart 1990). In coleoptiles and shoots, IAA is known to redistribute to the lower half, thereby increasing the growth on this side and leading to upwards bending (Wilkins 1984; Li et al. 1991). In roots, IAA also accumulates on the lower half, but here it inhibits growth and leads to downward bending (Wilkins 1984; Hart 1990).

The classic Cholodny-Went Hypothesis (CWH) for gravitropic response of stems states that the gravity stimulus triggers a redistribution of auxin to the lower half of a horizontally oriented stem, leading to an increase in the growth rate on the lower surface of the stem that is coupled to an equal magnitude decrease in the growth rate on the upper surface (reviewed by Wilkins 1984; Hart 1990). Some workers, however, question the role of IAA in the gravitropic response of plant shoots (Firn and Digby 1990), and they pose serious doubts about the validity of the CWH for dicotyledonous shoot systems (Digby and Firn 1979). As Wilkins (1984), Hart (1990) and others emphasize, there is a strong need to further investigate the mechanisms of gravitropic response in a wide range of dicotyledonous shoot systems. The work presented in this paper on the gravitropism of the peanut gynophore may help to elucidate different aspects of the gravitropic response mechanisms in plant dicotyledonous shoots, especially the possible gravitropically induced redistribution of IAA in horizontally oriented gynophores. Some of the questions that will be addressed in this study are: How is IAA localized in vertically growing gynophores? How do the patterns of IAA localization change in a horizontally oriented gynophore as compared to a vertically growing one? How are these patterns related to the changes in the growth rates observed in gravistimulated gynophores (Moctezuma and Feldman 1998)? What are the possible mechanisms for these gravitropically induced IAA changes (Parker 1991)?

With the advancement of plant hormone immunoassay techniques (Leverone et al. 1991; Shi et al. 1993; Caruso et al. 1995) and with the use of "classical" radioactively labeled IAA experiments, we attempt to answer these questions about IAA in the peanut gynophore – as a first step towards elucidating the role that IAA plays in its growth and development. In this study, we: (i) report for the first time the successful immunolocalization of endogenous IAA patterns in the tissues of vertically growing vs. horizontally oriented peanut gynophores; (ii) correlate these immunolocalization patterns with growth rate measurements of gravistimulated gynophores; and (iii) perform IAA transport experiments in order to explore possible mechanisms of IAA redistribution in graviresponding gynophores.

Materials and methods

Plant material, fixation and sectioning. Peanut (*Arachis hypogaea*, L. cv. Virginia 93B) plants were grown as previously described in Moctezuma and Feldman (1998). Young gynophores (20–30 mm long) were excised from the plant after various periods of time in either a vertical or horizontal orientation. The tissues were immediately pre-fixed in freshly-prepared 3% aqueous 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride (EDAC) (Sigma), and post-fixed in a formalin-acetic acid-alcohol (FAA) solution as described in Moctezuma (1999). The EDAC pre-fixation serves to crosslink IAA to structural proteins within the tissues of the gynophore, and it also preserves the antigenicity of the IAA to this particular monoclonal IAA antibody (Caruso et al. 1995; Moctezuma 1999). In both the pre-fixation and fixation steps, the orientation of the gynophores was maintained in the vials by the use of metal pins that immobilized the tissue samples. The fixed tissues were then dehydrated, infiltrated with paraffin and sectioned at 10–12 μm.

Procedure for IAA immunolocalization. The IAA immunolocalization procedure was done as described in detail in Moctezuma (1999). Briefly, the sections were blocked, incubated first in the primary IAA antibody (kindly provided by Dr. J. Caruso, University of Cincinnati, Ohio, USA), and subsequently incubated with a secondary antibody (Anti-mouse IgG AP Conjugate; Promega, Madison, Wis., USA). After several rinses, the sections were developed with ready-to-use Western Blue stabilized substrate for alkaline phosphatase (Promega), which turned blue/purple as it reacted with the antibody. Sections were mounted and photographed. The results for each experiment were repeated at least 3 times, and 5–10 tissue samples of each treatment were used.

Controls for IAA immunolocalization technique. Because it was critical to document the specificity and affinity of the monoclonal IAA antibody for the IAA within the tissues, several negative and positive controls were performed (Moctezuma 1999). First, EDAC pre-fixation was omitted, and tissues were fixed only in FAA. We also deleted the primary IAA antibody and the secondary antibody from the procedure. Another control involved the substitution of the primary IAA monoclonal antibody with a control monoclonal antibody (P3X63), having no known antigen (Leverone et al. 1991). Finally, a competition-assay control was done: the IAA primary antibody was incubated overnight with the antigen (a 1×10^{-3} M solution of IAA) for saturation of the binding sites of the antibody (Kerk and Feldman 1995). The primary antibody-IAA mixture was applied to the specimen. This control was performed to determine the specificity of the primary antibody for the IAA.

The positive controls were as follows: approximately 0.5 mm of the tip was excised in two sets of gynophores. In the first set, an agar block with 1×10^{-3} M IAA was applied to the tip; whereas an agar block without IAA was placed on the second set. After approximately 1 h of this treatment, the tissues were fixed and processed for IAA immunolocalization as above. This control allowed us to corroborate that the antibody is indeed detecting IAA.

Growth rate measurements. The growth rates of graviresponding gynophores were determined as previously reported (Moctezuma and Feldman 1998). Briefly, nine gynophores 20–30 mm long were marked in planta with black ink at 0.4- to 0.6-mm intervals, reoriented horizontally, recorded with a videocamera using a time-lapse videocassette recorder, and the displacement of the marks away from the tip was calculated for each mark from the recorded video images. The Relative Elemental Growth Rates (REGR) were calculated for each point along the gynophore using Erickson's 5-point formula for numerical differentiation (Erickson 1976). The data were analyzed and plotted as percent growth vs. distance from the tip in order to detect zones of maximum elongation growth in the upper and lower surfaces (Silk 1984; Bret-Harte and Silk 1991).

Radiolabeled-IAA experiments. For IAA-transport experiments, the agar-block method for applying radiolabeled IAA developed by Tsurumi and Ohwaki (1978) was followed. Gynophores samples 20–30 mm long were treated directly on the plant with the agar blocks. To facilitate the uptake of the radiolabeled IAA into the gynophore, less than 0.3 mm of the tip was excised in all samples without affecting the gravitropic response (Moctezuma and Feldman 1998). For radiolabel loading, plastic tubes (Phenix Research Products, USA) were filled with agar and applied to the gynophore tip. Each tube contained 25 μL of 1% agar, 1×10^{-6} M IAA, and approximately 74 kB_q (2 μCi) per ml of [^{3}H]IAA (15–30 Ci per mmol; Amersham).

Gravistimulation experiments. The [^{3}H]IAA was applied to the tips of gynophores still attached to the plant ($n = 11$). After 1 h loading of radioactive IAA, and with the agar block still attached to the sample, the gynophore was positioned vertically (tip downwards) for an additional 2 h. The gynophores were excised and bisected longitudinally into two equal halves (called "left" and "right" halves), and the radioactivity was measured in each of the two segments. Similarly, the radiolabeled IAA was applied to the tips of vertically oriented, intact gynophores still attached to the plant ($n = 10$). After 1 h application of radioactive IAA, the gynophores were reoriented horizontally for 2 h, with the radioactive block still attached to the sample. Samples were then excised and bisected longitudinally into two halves: upper and lower. Radioactivity was measured in each of the two segments.

In order to obtain information on the pathways of IAA movement and redistribution within the gynophore during gravistimulation, barriers to prevent IAA transport were used by inserting a small piece of aluminum foil through an incision made with a scalpel. The barriers were placed in either the lateral cortex (at the elongation zone) ($n = 6$) or in the upper half cortex (proximal to the seed region) of horizontally oriented gynophores ($n = 8$).

Following the gravistimulation treatments and the loading of [^{3}H]IAA, the excised and bisected tissues were dissolved with 0.5 ml of tissue solubilizer (Amersham) for 15 h at room temperature. A 3 ml solution of scintillation fluid (ScintiVerse BD; Fisher Scientific Co., Rochester, N.Y., USA) was added to each vial, and the samples were analyzed in a scintillation counter (Beckman). The units of radioactivity from the scintillation counter were in counts per minute (cpm).

Results

Controls. All controls showed little or no signal. No purple/blue staining is detected if the samples are not prefixed with EDAC. Tissue sections that were incubated without the primary IAA antibody (Fig. 1B), without the secondary antibody or with substitute antibody P3X63 also did not show any staining. The sections of the control competition experiment had very little, almost negligible amounts of blue staining. The positive controls showed a signal in the gynophore tips that were provided with an agar block containing IAA, whereas no signal was observed with a plain agar block without IAA (data not shown).

Immunolocalization of IAA during vertical growth and during gravistimulation. In vertically growing gynophores, a "halo" of IAA signal was observed surrounding the seeds (Fig. 2A,D), in an area corresponding to the intercalary meristem region of the gynophore (Shushu and Cutter 1990; Moctezuma 1999). In addition, the IAA signal was located completely around the epidermal and cortical tissues of the vertically growing gynophore. In transverse sections of vertically growing

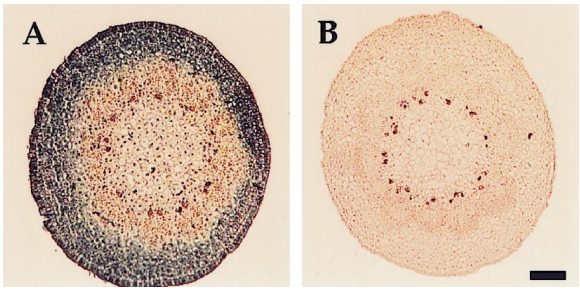

Fig. 1A,B. Controls for the IAA immunolocalization technique. Cross-section of a young gynophore with the complete IAA immunolocalization treatment (**A**), and without primary antibody (**B**). Sections of the following controls also resulted in no signal, similar to **B**: without EDAC pre-fixation, without secondary-antibody incubation, and incubation with substitute antibody P3X63 (which has no known antigen). The competition-assay control (see *Materials and methods*) also resulted in a very light, almost negligible signal. Bar = 0.25 mm

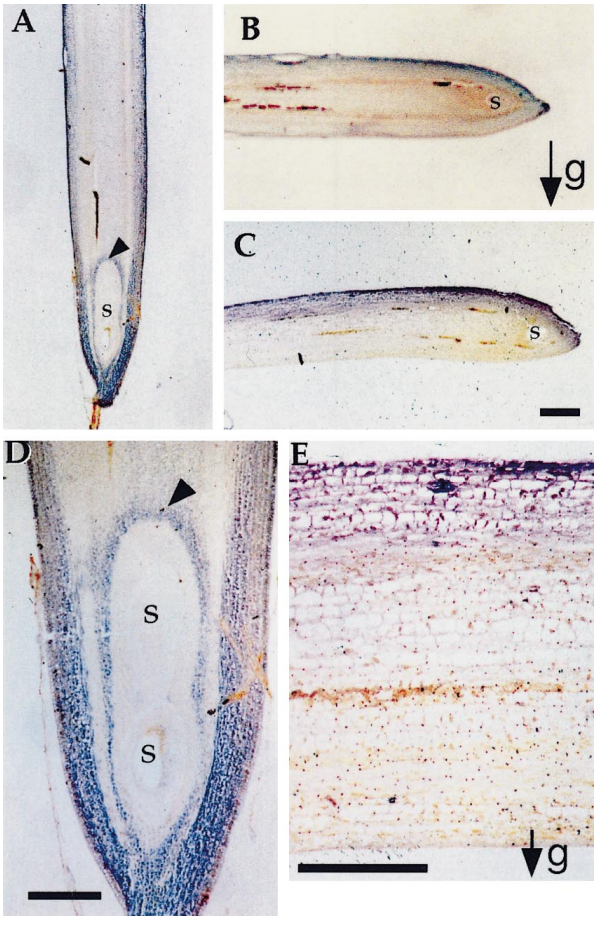

Fig. 2. **A** Positively gravitropic, vertically growing gynophore, 3 cm long (only 7 mm from the tip is shown). Most of the signal occurs in the epidermis and cortex. Also, notice the IAA signal pattern around the seeds (*s*) and in the meristematic region (*arrowhead*). **B** Gynophore reoriented horizontally for 20 min. **C** Gynophore positioned horizontally for 1.5 h. **D** Higher magnification of **A**, showing the seeds (*s*) and the IAA signal in the intercalary meristem (*arrowhead*). **E** Higher magnification of **C**, showing the IAA signal in the upper cortex. Notice that most of the signal in **B**, **C** and **E** is now located in the upper epidermis and cortex, whereas the lower side has no signal. Bar in **C** (for **A–C**) = 0.7 mm; bars in **D** and **E** = 0.35 mm. The *g* and *arrow* in **B** and **E** represent the direction of the gravity vector for all figures

gynophores, a ring of the stain was observed in the epidermal and cortical tissues, surrounding the gynophore (Fig. 1A). Also, Fig. 2A shows an even localization of IAA on the left and the right sides (epidermis and cortex) of the micrograph.

Upon gravistimulation, changes in IAA localization occurred. Figure 2B shows a gynophore reoriented horizontally for 20 min by which time, more staining could be observed in the upper side (Fig. 2B) than the lower side. At 1.5 h after reorientation, the IAA signal was detected only on the upper side (Fig. 2C). Notice that the staining covers a greater depth of cell layers of the cortex of the horizontally oriented than the vertically oriented gynophore (compare Fig. 2D,E). Also note the complete absence of the IAA signal in the lower side, as compared to the strong signal of the upper side, of the horizontally oriented gynophore (Fig. 2C,E).

Growth rate measurements. Figure 3 shows the percent growth of the upper and lower surfaces of horizontally oriented gynophores, at approximately 1.5–2 h of horizontal reorientation. The diagram above the graph better illustrates the spatial distribution of the growth rate maximum of the upper surface as compared to the lower surface. One growth maximum occurred in the upper side, in a location (or distance from the tip) corresponding spatially to that of the growth rate minimum of the lower side.

Transport of radiolabeled IAA. We corroborated that IAA is transported basipetally in a polar fashion as in Jacobs (1951) (data not shown). Results from gravistimulation treatments are shown in Table 1. We found that IAA was distributed evenly in vertically oriented gynophores (Fig. 4A). Upon 2 h of horizontal reorientation, however, there was 2-fold more IAA in the upper half than in the lower half of the gynophore (Fig. 4B). Barrier experiments in which IAA transport in the cortex was impeded also resulted in an uneven IAA redistribution, with 2-fold more IAA in the upper half than in the lower half (Fig. 4C). Barrier experiments that stopped IAA transport from the tip to the upper side resulted in a 1.5:1 distribution of IAA in the upper vs. the lower side (Fig. 4D).

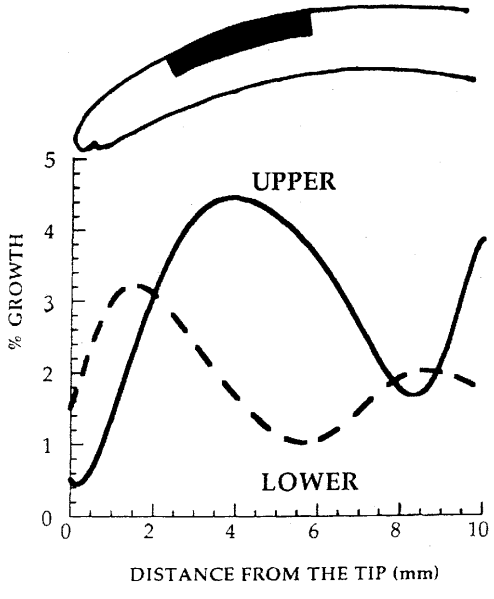

Fig. 3. Diagram of the zone of maximum growth (85% and above the maximum % growth, represented by the black bar) of a gynophore that has been gravistimulated for 2–4 h. The graph below indicates the % growth of the upper (*solid line*) as compared to the lower (*dashed line*) surface of the horizontally oriented gynophore. Spatially, the growth maximum of the upper surface (between 2–5 mm from the tip) correlates with the growth minimum of the lower surface. The graph is a composite measurement of nine gynophores, averaged by computer, with ninth-degree polynomial smoothing in the *y*-axis. Standard errors were typically 0.2–0.5%/h

Discussion

Because of the hypothesized importance of IAA in the gravitropic response of plant organs, we investigated the

Table 1. Transport of [^{3}H]IAA in peanut gynophores (in vivo) in response to different gravistimulation treatments. All values are in counts per minute (cpm), showing a representative sample for each treatment. Values in parenthesis show percent error, with n = 6–11 for the different treatments

Treatment	"Right" or "upper" half	"Left" or "lower" half
Vertically oriented	1553 (71)	1630 (79)
Horizontally oriented		
No barrier	2263 (95)	1090 (59)
Lateral cortex barrier	2082 (104)	1102 (65)
Upper cortex barrier	1844 (73)	1260 (61)

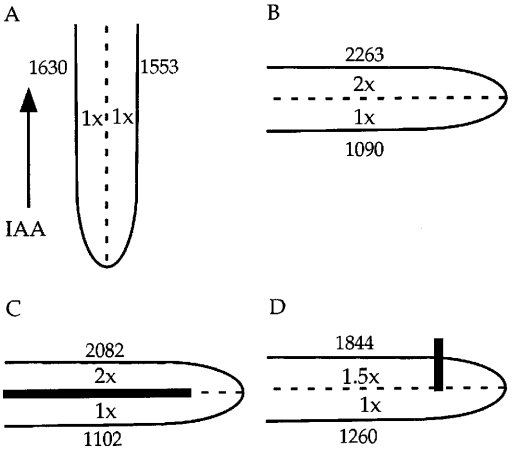

Fig. 4A–D. Diagramatic representation of radiolabeled-IAA transport experiments. **A** Vertical control gynophore, showing both basipetal IAA transport and an even IAA distribution between the two halves. **B** Gynophore oriented horizontally for 2 h, showing 2-fold more IAA in the upper half than in the lower half. **C** Lateral cortex barrier experiments, which still resulted in 2-fold more IAA in the upper half than in the lower half after 2 h of horizontal orientation. **D** Upper cortex barrier experiment, which stopped IAA transport directly from the tip to the upper cortex, resulted in a 1.5 to 1 distribution between the upper and lower halves after 2 h of horizontal reorientation. Figures outside each gynophore represent the amount of radioactively labeled IAA (in cpm) found in each segment (see Table 1)

role of this growth substance in graviresponding peanut gynophores using first an IAA immunolocalization technique. Based on the gynophore's typical dicotyledonous shoot anatomy, its atypical (root-like) gravitropic response, and the supposed redistribution of IAA in horizontally oriented plant organs, we hypothesized that the IAA localization patterns in gravistimulated (horizontally oriented) gynophores would differ from the IAA localization patterns of vertically growing gynophores.

When grown vertically, the gynophore exhibits a uniform IAA localization in the cortex and epidermis (Fig. 2A). Similarly, IAA is distributed uniformly in typical, vertically oriented stems, but they accumulate more IAA in the lower side when they are oriented horizontally, thus increasing growth at the lower surface, which eventually leads to upward bending (Wilkins 1984; Li et al. 1991; Hart 1990). Roots also accumulate higher amounts of IAA in the lower half, but this IAA has an inhibitory effect on the growth of the lower surface, which leads to downwards bending upon gravistimulation (Hart 1990). A conclusion drawn from these studies is that a redistribution of IAA occurs in *intact*, horizontally oriented tissues of stems and roots. Nevertheless, we must also note that experiments with isolated strips of hollow stems of *Reynoutria* (Hejnowics and Sievers 1996) indicate that a stem does not always need the connection between the upper and the lower sides, nor an asymmetric redistribution of IAA between these tissues, in order for a gravitropic bending response to occur.

Thus, what type of IAA localization pattern would be observed in a gynophore upon gravistimulation? At 1.5 h after gravistimulation, IAA is detected only in the upper cortex and epidermis of the horizontally oriented gynophore (Fig. 2C). For downwards bending of the peanut gynophore to occur, previous studies showed that the upper side exhibits an increase in growth, whereas the growth rate of the lower side decreases (Moctezuma and Feldman 1998). The classic Cholodny-Went Hypothesis (CWH) states that an asymmetric growth distribution between the upper and lower portion of the responding organ is brought about by an asymmetric redistribution of auxin within the tissues upon gravistimulation (Wilkins 1984; Hart 1990). According to the CWH, more IAA accumulates on the lower side of typical stems, leading to increased growth on the lower surface and an eventual upwards gravitropic bending (Li et al. 1991). In the peanut gynophore, however, a similar but opposite phenomenon occurs: a gradual and rapid increase in the IAA signal is observed on the uppermost side (Fig. 2B), until only the upper epidermis and cortex show the IAA signal – none is observed in the lower side (Fig. 2C). This dramatic and rapid change in the localization of IAA to the upper side correlates temporally and spatially with the increased growth rates observed in the upper surface of the horizontally oriented gynophore (Fig. 3). That is, the observed changes in IAA localization in the gynophore take place before the changes in growth rates of in the upper and lower surfaces. These events, as would be predicted by a "reversed" version of the CWH, lead to the downward gravitropic bending of the gynophore.

The results of the radiolabeled-IAA experiments in this study also suggest new ways of thinking about IAA transport mechanisms during gravistimulation. First, our results strongly suggest that the transport of IAA occurs from tip to base, in a unidirectional polar fashion (data not shown), very likely through the cortex and epidermis of the gynophore, as observed in the IAA immunolocalization experiments (Figs. 1A, 2A). Secondly, in vertically oriented gynophores the radioactivity measurements on the "left" and "right" sides are approximately equal (Table 1, Fig. 4A). However, the radioactive counts per minute on the upper and lower sides are not equal after 2 h of reorientation to the horizontal: the upper side shows approximately twice the counts of the lower side (Table 1, Fig. 4B). The total amount of radioactivity that entered the gynophore tissue was approximately 5% of the total radioactivity in the agar block for all treatments. Thus, the constant total amount of radioactivity found in both the vertical and horizontal gynophores (Table 1) suggests that IAA may be actively transported from the lower to the upper side during gravistimulation.

How do these radiolabeled-IAA results reconcile with the previous IAA immunolocalization results, in which we were unable to visualize any IAA signal in the lower side of gravistimulated gynophores? The radiolabeled-IAA results show that IAA is, potentially, still present – though at a reduced level – in the lower side. Hence, there are two main possibilities: (i) the levels of IAA in the lowermost side may be too low for detection by the IAA immunolocalization technique (see median section of Fig. 5); or (ii) the radioactive signal in the lower portion may not necessarily represent IAA anymore; it is possible that some IAA catabolism, conjugation, degradation, etc. (Parker 1991) may have occurred in the lower half of the gynophore. This issue can be resolved by a detailed gas chromatography-mass spectrometry analysis of the IAA located in the upper vs. the lower halves, in which the amount of conjugated, catabolized, etc. IAA can be compared with the quantity of free, active IAA within the tissues of the graviresponding gynophore. Further analytical studies are needed.

The differences in the IAA between vertical and horizontally oriented gynophores indicate that the IAA originally present in the lower half may have been actively transported to the upper half. Why is a change in IAA transport the most probable mechanism for the observed gravity-induced IAA asymmetric redistribution in the gynophore? Several studies indicate that IAA transport is altered by gravistimulation in plant shoots (Harrison and Pickard 1989; Brock et al. 1991; and Jensen et al. 1998). Basipetal active transport in vertically oriented organs is changed to a lateral redistribution of the IAA in horizontally oriented shoots (Brock et al. 1991). This lateral transport of IAA leads to an asymmetry in this growth substance between the upper and the lower sides, which eventually produces an asymmetric growth response in the gravistimulated plant shoot.

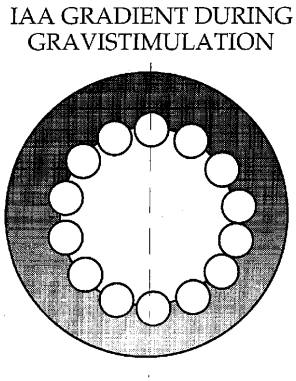

Fig. 5. Model of a cross-section of a peanut gynophore at 3 mm from the tip, showing a gradient of IAA redistribution in the cortex during gravistimulation (darker color = higher IAA concentration). This pattern of IAA localization was observed in an actual cross-section of the gynophore using the immunological technique (data not shown). The small circles represent vascular bundles, and the dashed line represents a median section through the gynophore

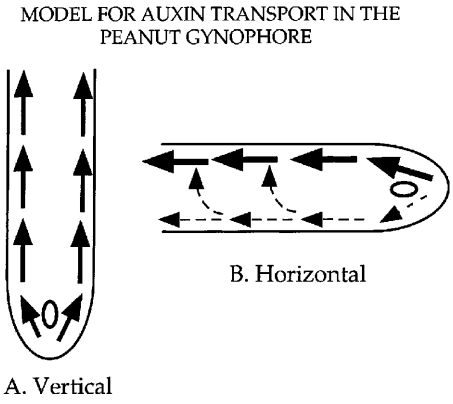

Fig. 6A,B. Model for IAA transport in the gynophore. **A** Vertically oriented gynophore, showing IAA transport (*arrows*) from the tip region to the central elongation zone, primarily through the cortex and epidermis. **B** Probable IAA transport in a horizontally oriented gynophore, showing the predominant source of IAA in the upper side transported directly from the tip (*thick solid arrows*). Very little IAA is transported to the lower side, and some IAA may be actively transported from the lower side to the upper side through the cortex (*thin dashed arrows*). The size and thickness of the arrows are meant to represent the approximate amounts of IAA transported within the tissues

Our results also indicate that there may be two different IAA transport systems (pathways) in the peanut gynophore. Firn and Tamimi (1986) were the first to propose that in plant shoots, the pathways of IAA transport during vertical elongation growth may be different from the IAA transport pathways during the gravitropic response. Recent work by Jensen et al. (1998) also suggests that two different IAA transport systems may be involved in shoots: (i) the basipetal transport of IAA (from the tip to the elongation zones through the epidermis and cortex) for vertical elongation growth; and (ii) the lateral transport of IAA, mainly through the cortex, from the upper to the lower side in gravistimulated plant shoots. The results of Jensen et al. (1998) with the application of 1-naphthylphthalamic acid (NPA), an IAA transport inhibitor, are very similar to the results obtained in previous growth rate studies of the peanut gynophore (Moctezuma and Feldman 1998). In gravistimulated *Arabidopsis* hypocotyls (Jensen et al. 1998), as in horizontally oriented peanut gynophores (Moctezuma and Feldman 1998), NPA does not abolish elongation growth, only differential growth needed for gravitropic bending. This result can be explained by the fact that NPA may be abolishing mainly the gravity-induced lateral transport of IAA, but some basipetal transport of IAA may still be occurring. The barrier experiments (Fig. 4C,D) indicate that IAA is transported both through the cortex (laterally) and directly from the tip to the upper half (Fig. 6), leading to the observed IAA asymmetry in the gravistimulated peanut gynophore (Figs. 2C, 5). Thus, the asymmetric redistribution of IAA may arise as a result of a combination of these two IAA transport pathways: direct basipetal transport and lateral transport through the cortex (Fig. 6B). Although similar to the hypothetical auxin transport model for gravitropic response in roots (Hasenstein and Evans 1988), the gynophore model has several unique innovations: a presumptive source of IAA at the tip of the organ, unidirectional basipetal transport of IAA, possible lateral IAA transport through the cortex and a greater transport of IAA to the *upper* side (not the lower side) of the organ (Fig. 6A,B).

The present study shows, for the first time, a gravity-induced asymmetric localization of endogenous IAA in a gravistimulated organ. The results of this work show that, unlike what the CWH postulates for typical shoots and roots, IAA does not always accumulate in the lower side of gravistimulated plant organs. In the peanut gynophore, IAA is localized (within the levels of detectability of the IAA immunolocalization technique used in this study) at the upper side, against the gravity vector. Furthermore, our results suggest that IAA may be actively accumulated or redistributed upwards to the faster-growing surface of a graviresponding plant shoot. An active mechanism for the creation of this IAA asymmetry through directed transport of this molecule seems to occur in the peanut gynophore. Thus, a major reversal in the direction of IAA redistribution must occur very early in the process of gravitropism in the gynophore, perhaps at the signal transduction stage.

In conclusion, the CWH for shoot gravitropic response has served to stimulate enormous amounts of research in the field of gravitropism (Wilkins 1984; Trewavas 1992). The main foundations of the CWH, although very general in nature, still remain intact: a gravity-induced IAA redistribution and asymmetry is correlated with an asymmetry in the growth rates between the upper and the lower sides upon gravistimulation. However, the results presented in this paper provide novel data that may help explain mechanistically the observed IAA and growth rate asymmetries in gravistimulated plant shoots. The uniqueness of the peanut gynophore (typical dicot shoot anatomy, positively gravitropic response), along with the use of novel and classical techniques for IAA analysis, have helped us to better understand some of the physiological events

that occur during the gravitropic response of plant shoot systems. Still, much work remains to be done to elucidate the cellular, physiological and molecular basis for the gravitropic response in plant shoots.

We thank Prof. Wendy K. Silk, Dr. Syndonia Bret-Harte and Dr. Nancy Kerk for their advice on the growth rates and the IAA immunolocalization techniques; and Dr. Steven Ruzin and Dr. Denise Schichnes from the CNR Biological Imaging Facility at U.C. Berkeley for their expert technical help. We are also indebted to Dr. John L. Caruso (University of Cincinnati) for providing us with his monoclonal IAA antibody, and to an anonymous reviewer for helpful suggestions. This work was supported by a National Aeronautics and Space Administration fellowship to E.M. This work represents two chapters of a dissertation submitted by the first author in partial fulfillment of the requirements for the Ph.D. degree at the University of California at Berkeley.

References

Bret-Harte MS, Silk WK (1994) Nonvascular, symplasmic diffusion of sucrose cannot satisfy the carbon demands of growth in the primary root tip of *Zea mays* L. Plant Physiol 105: 19–33

Brock TG, Kapen EH, Ghosheh NS, Kaufman PB (1991) Dynamics of auxin movement in the gravistimulated leaf-sheath pulvinus of oat (*Avena sativa*). J Plant Physiol 138: 57–62

Caruso JL, Pence VC, Leverone LA (1995) Immunoassay methods of plant hormone analysis. In: Davies PJ (ed.) Plant hormones: physiology, biochemistry and molecular biology. Kluwer Academic Publishers, Netherlands, pp 433–447

Davies PJ (1995) The plant hormones: their nature, occurrence, and function. In: Davies PJ (ed) Plant hormones: physiology, biochemistry and molecular biology. Kluwer Academic Publishers, Netherlands, pp 1–12

Digby J, Firn RD (1979) An analysis of the changes in growth rate occurring during the inital stages of geocurvature in shoots. Plant Cell Environ 2: 145–148

Erickson RO (1976) Modeling of plant growth. Annu Rev Plant Physiol 27: 407–434

Firn RD, Digby J (1990) New tricks for old dogmas. Nature 347: 718

Firn RD, Tamimi S (1986) Auxin transport and shoot tropisms: the need for precise models. In: Bopp M (ed) Plant growth substances 1985. Springer, Berlin, pp 236–240

Harrison MA, Pickard BG (1989) Auxin asymmetry during gravitropism by tomato hypocotyls. Plant Physiol 89: 652–657

Hart JW (1990) Gravitropism. In: Hart JW (ed) Plant tropisms and other growth movements. Unwin Hyman, London, pp 44–89

Hasenstein KH, Evans ML (1988) Effects of cations on hormone transport in primary roots of *Zea mays*. Plant Physiol 86: 890

Hejnowicz Z, Sievers A (1996) Gravitropic response of the stem of *Reynoutria*. Isolated strips in different positions in relation to the gravity vector. J Plant Physiol 149: 393–399

Jacobs W (1951) Auxin relationships in an intercalary meristem: further studies on the gynophore of *Arachis hypogaea*. Am J Bot 38: 307–310

Jensen PJ, Hangarter RP, Estelle ME (1998) Auxin transport is required for hypocotyl elongation in light-grown but not dark-grown *Arabidopsis*. Plant Physiol 116: 455–462

Kerk NM, Feldman LJ (1995) A biochemical model for the initiation and maintenance of the quiescent center: implications for organization of root meristems. Development 121: 2825–2833

Leverone LA, Kossenjians W, Jayasimihulu K, Caruso JL (1991) Evidence of zein-bound IAA using gas chromatography-selected ion monitoring-mass spectrometry and analysis and immunogold labeling. Plant Physiol 96: 1070–1075

Li Y, Hagen G, Guilfoyle TJ (1991) An auxin-responsive promoter is differentially induced by auxin gradients during tropisms. Plant Cell 3: 1167–1175

Moctezuma E (1999) Changes in auxin patterns in developing gynophores of the peanut plant (*Arachis hypogaea* L.). Ann Bot 83: 235–242

Moctezuma E, Feldman LJ (1998) Growth rates and auxin effects in graviresponding gynophores of the peanut, *Arachis hypogaea* (Fabaceae). Am J Bot 85: 1369–1376

Parker KE (1991) Auxin metabolism and transport during gravitropism. Physiol Plant 82: 477–482

Shi L, Miller R, Moore R (1993) Immunocytochemical localization of indole-3-acetic acid in primary roots of *Zea mays*. Plant Cell Environ 16: 967–973

Shushu DD, Cutter EG (1990) Growth of the gynophore of the peanut *Arachis hypogaea*. 2. Regulation of growth. Can J Bot 68: 965–978

Silk WK (1984) Quantitative descriptions of development. Annu Rev Plant Physiol 35: 479–518

Smith BW (1950) *Arachis hypogaea*. Aerial flower and subterranean fruit. Am J Bot 37: 802–815

Trewavas AJ (1992) What remains of the Cholodny-Went theory? Plant Cell Environ 15: 759–794

Tsurumi S, Ohwaki Y (1978) Transport of ^{14}C-labeled indoleacetic acid in *Vicia* root segments. Plant Cell Physiol 19: 1195–1206

Wilkins MB (1984) Gravitropism. In: Wilkins MB (ed) Advanced plant physiology. Pitman, London, pp 163–185

Zamski E, Ziv M (1976) Pod formation and its geotropic orientation in the peanut, *Arachis hypogaea* L., in relation to light and mechanical stimulus. Ann Bot 40: 631–636