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Image Analysis of Geo-Induced Inhibition, Compression, and Promotion of Growth in an Inverted *Helianthus annuus* L. Seedling

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ABSTRACT

The growth responses of a sunflower seedling (*Helianthus annuus* L.), subjected to repeated inversion, were characterized by time-lapse recording in conjunction with video image analysis. The investigation revealed a characteristic response pattern and established that the directional movement of the seedling is achieved by both inhibition and stimulation of growth in the normal growing regions. The complex growth changes in contiguous regions of the hypocotyl are such as seem to be inexplicable in terms of an environmentally imposed gradient of a single growth substance.

Plants may have to accomplish massive directional growth movements at various stages of development. To be turned upside down may be a relatively uncommon experience but it can happen, as for example, during ploughing, and seedlings obviously have the ability to cope with such an emergency. Extensive reorientation can also occur during the normal course of development, *e.g.*, the inversion and subsequent straightening of the peduncle during floral development of *Fritillarias* and *Papaver* (6). However, the growth kinetics of reorientation consequent to seedling inversion have not received any detailed study probably because of the technical difficulties encountered. Only recently has the requisite hardware for this type of investigation become available.

Traditional methods of growth measurement are in practice, if not in theory, incapable of handling the unwieldly mass of information comprising sequential readings of consecutive regions of a geo-responding hypocotyl; and a semi-automated device such as a linear displacement transducer, while useful for detecting rapid growth responses (1), is of no assistance in identifying the region in which the response occurs. However, the advent of computer technology has overcome the problem of data processing and, used in conjunction with a cine or video camera, makes possible image analysis of plant growth at either the macro- or micro-structural level (9).

In this paper, we describe an investigation which uses time-lapse recording linked to computer processing to characterize the growth responses of a sunflower seedling subjected to an inversion cycle. The seedling hypocotyl was demarcated on both sides with uniformly spaced resin beads, the subsequent positions of which were followed spatially and temporally as the seedling responded to the geo-stress. Because physical perturbation can itself affect growth (1, 5), it is essential to avoid any extraneous disturbance of the plant, and a specially designed growth chamber in which the seedling could be smoothly rotated was used to minimize disturbance.

Video recording of an experimental sequence has several advantages over cine photography, especially the fact that it is cheaper and also permits on-screen measurements to be made in real time. Moreover, a video camera has the potential for recording under IR radiation of >800 nm. The main advantage of the cine photography lies in the improved quality of photographic reproduction. Several experiments on the growth of inverted seedlings have been filmed with the video camera confirming the general pattern of response of the seedling described here, the growth of which had first been recorded with a cine camera to yield better quality photographs for illustrative purposes.

MATERIALS AND METHODS

Growth measurements of suitably marked seedlings (3) were determined by digitization of analog video signals. The Newvicon video camera (National Panasonic) can be used to transmit signals from an experiment *in situ* as shown in Figure 1 or, as happened in the investigation illustrated in this report (Fig. 2), from a screened projection of a photographically filmed experiment. In either case, the image is transmitted directly to a monitor, or recorded for subsequent display by means of a time-lapse video recorder and time-date generator. An auto-search controller facilitated selection of frames from the recorded sequences (Fig. 1).

Analysis of the image is accomplished by determining the coordinates of the marker beads delimiting discrete regions of the seedling (Fig. 1). Horizontal and vertical cursor lines activated by a Colorado model 321 video analyzer (Colorado Video, Inc., Boulder, CO) can be manipulated by a joystick controller to intersect on each marker bead in turn and the coordinates of its position fed into a CBM model 8032 Pet microcomputer via an analog digital convertor. A program has been written by which the growth rates of sequential zones on both sides of a growing seedling can be computed and a print-out of total growth, and the contribution of each constituent zone to the total growth, obtained at selected time intervals. The only manual and subjective step in the measurement procedure is pin-pointing the individual beads with the intersecting cursors. The accuracy with which this can be done was assessed by carrying out test runs on 1-mm graph paper. These revealed that the length of individual 1-mm zones can be measured with an accuracy of 10%. An accuracy of 1% is achieved in respect of the total length (2 cm)

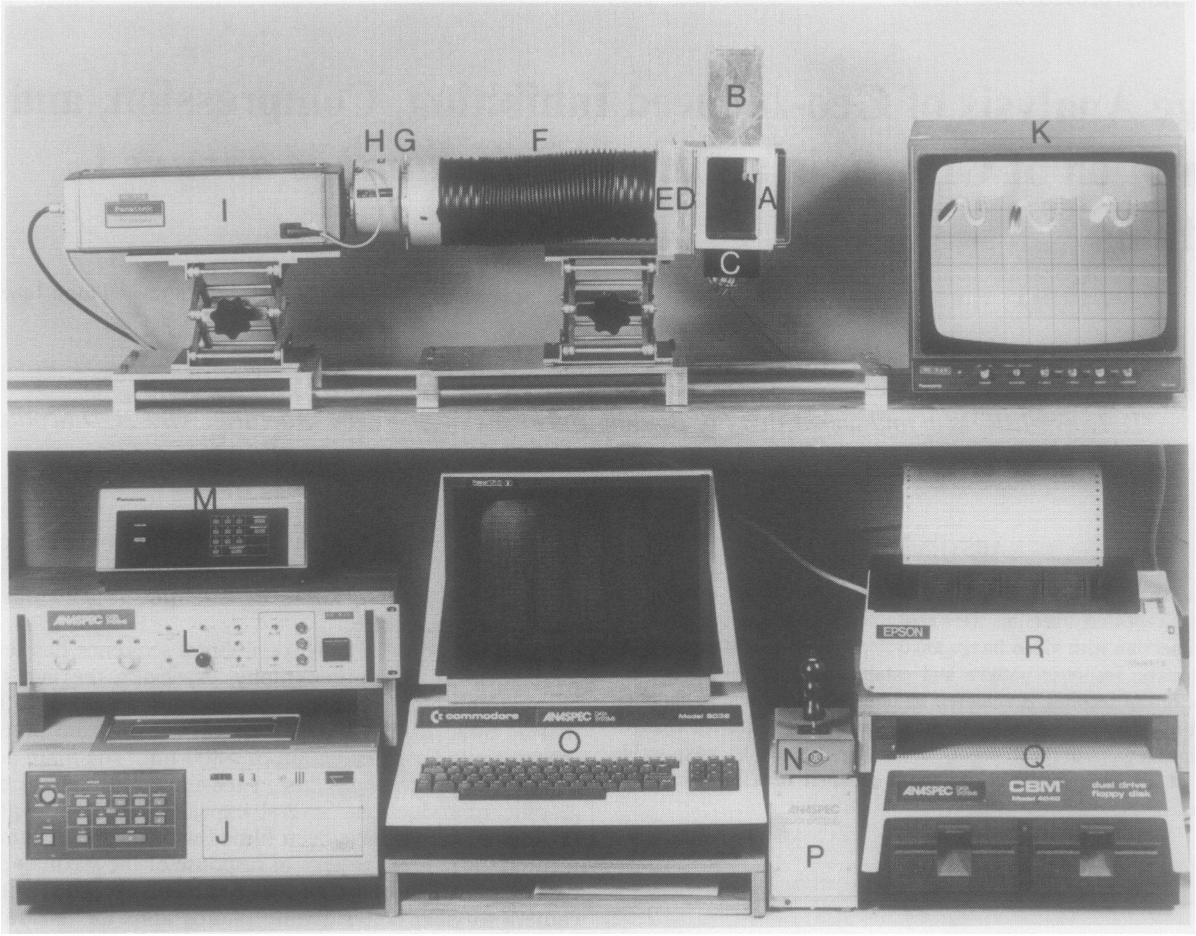

FIG. 1. General view of the video image analysis equipment showing inverted sunflower seedlings in the growth chamber (A) with a clear Perspex side panel inserted to reveal the location of the seedlings. The growth chamber as shown has been inverted and the seedlings are attached to the platform of a minature jack (B), by the racking of which the seedlings can be kept in view. At the opposite end from the jack a bank of IR light-emitting diodes can be seen (C). The growth chamber dovetails into a terminal block (D) which can be rotated on the terminal block (E) of the trunking (F) linking the growth chamber to the focusing ring (G) of the camera lens (H). The image being recorded by the video camera (I) in conjunction with the time lapse video recorder and time date generator (J) appears on the monitor (K). Also shown are the video analyzer (L), the auto-search controller (M), the joystick controller (N) for operating the cursor lines visible on the monitor, the Pet microcomputer (O), the analog/digital convertor (P), the floppy disk unit (Q), and the printer (R).

calculated from the sum of 20 individually measured 1-mm zones. Screen linearity was checked and adjusted using potentiometers on the video analyzer and camera.

Etiolated sunflower (*Helianthus annuus* L.) seedlings about 2 cm in height were selected from a batch of seedlings growing in individual rectangular cuvettes (1 × 1 × 2.5 cm) containing 1% agar (7). Resin beads sieved through 25 to 35 mesh (400–500 μm) were lightly coated with lanolin and used to delimit a series of zones, each approximately 1 mm, along the seedling hypocotyl and the cuvettes then attached with adhesive to the basal plinth of the growth chamber (Fig. 1). To keep the growing region of the seedling in view, the position of this plinth can be adjusted by means of a minature jack enclosed in a light-tight box. Rotation of the growth chamber and its carrier assembly, on the terminal block of the extension trunking linking the camera with the growth chamber, allows the seedlings to be smoothly inverted. Growth was filmed using a Beaulieu R 16 cine camera and Schneider Kreuznach Makro-Tele-Zenar F 2.8 lens in conjunction with an intervalometer and autoflash transmitting through a double layer of Rank Strand green Cinemoid film (No. 39). The growth as recorded on film was subsequently analyzed by means of the video equipment.

FIG. 2. Time lapse sequence (with time indicated as on a 24-h clock) of a sunflower seedling before and after inversion and following reinversion. The main regions of growth in the vertical seedling are visible from the increased spacing of the beads at 5.10 and 9.10. At 9.30 the seedling was inverted by rotation of the growth chamber and at 17.50 reinverted by the same means.

The major zones of growth stimulation (····) and growth inhibition or contraction (——), occurring as a consequence of directional movement, are indicated.

The actual growth rates for apical, middle, and basal regions, each comprising a number of zones, are given in Table I. The extremities of the middle region as applicable to either side of the hypocotyl are indicated. Table II gives growth rates for the most active individual zones. At 16.45, four additional beads (arrowed) were added to permit more precise measurements of growth in zones 2 to 4 but the sub-zones thus formed are disregarded in Tables I and II, the information given there referring only to the zones present at the beginning of the experiment. The grid lines are 1 cm apart.

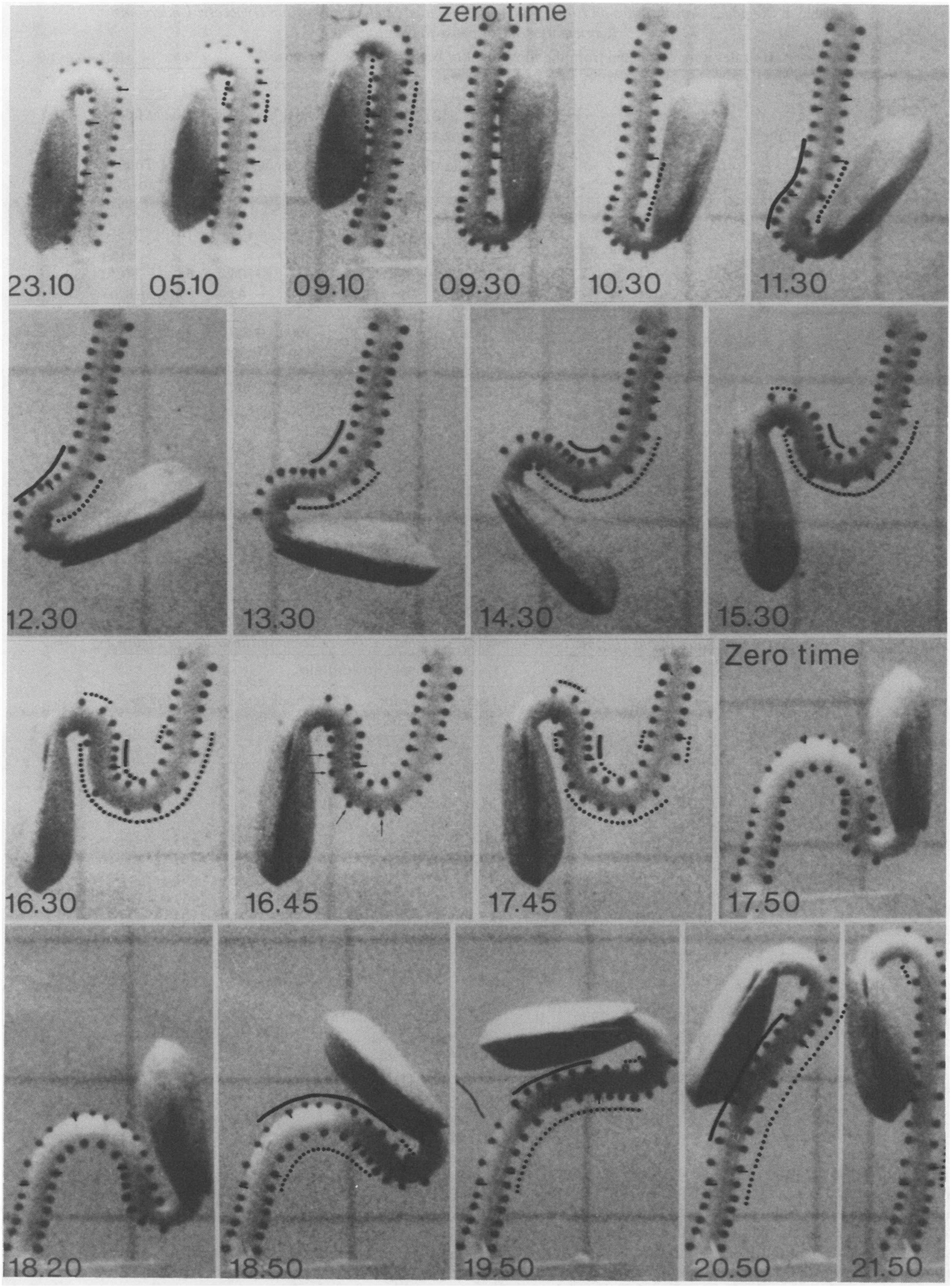
zero time
23.10
05.10
09.10
09.30
10.30
11.30
12.30
13.30
14.30
15.30
16.30
16.45
17.45
Zero time
17.50
18.20
18.50
19.50
20.50
21.50

Table I. *Regional Growth Rates on Either Side of an Etiolated Sunflower Seedling Hypocotyl Undergoing Reorientation in Response to Geostimulus*

The growth rates were calculated from the movement of beads as shown in Figure 2.

Orientation	Time Interval	Regional Growth Rate							
		Inner hook (left) side at following zones				Outer hook (right) side at following zones			
		Apical 1–4	Middle 5–8	Basal 9–13	Total growth	Apical 1–6	Middle 7–12	Basal 13–18	Total growth
		$\mu m\ h^{-1}\ region^{-1}$							
Vertical	05.10–09.10	201	182	16	399	97	199	19	315
Inverted	09.30–10.30	265	97	−158	204	−74	−104	−68	−246
	10.30–11.30	459	48	−9	498	68	−143	−7	−82
	11.30–12.30	547	320	14	881	188	−113	38	113
	12.30–13.30	564	208	−40	732	−190	−159	−22	−371
	13.30–14.30	856	382	32	1270	154	16	34	204
	14.30–15.30	569	320	−10	879	−88	−416	−41	−545
	15.30–16.30	517	283	24	824	271	225	−45	451
	16.45–17.45	366	231	−48	549	36	500	79	615
Vertical	17.50–18.20	405	86	23	514	117	394	1	512
	18.20–18.50	208	−186	−28	−6	305	2127	32	2464
	18.50–19.50	−337	−234	18	−553	170	1252	71	1493
	19.50–20.50	99	−17	131	213	546	1288	142	1976
	20.50–21.50	508	−109	−33	366	367	1139	191	1697
	Approximate total growth over 16 h				7713				8053

Table II. *Growth Rates in the Most Active Zones of the Etiolated Sunflower Seedling Illustrated in Figure 2*

Orientation	Time Interval	Zonal Growth Rates									
		Inner hook (left) side at following zones					Outer hook (right) side at following zones				
		1	2	3	4	5	1	6	7	8	9
		$\mu m\ h^{-1}\ zone^{-1}$									
Vertical	05.10–09.10	0	116	52	33	52	40	0	11	27	43
Inverted	09.30–10.30	0	124	90	51	135	0	−35	48	−83	−42
	10.30–11.30	−34	186	210	97	65	0	−13	−59	−57	−43
	11.30–12.30	1	241	183	122	159	21	46	5	−91	−5
	12.30–13.30	−15	385	154	40	130	44	−76	−28	67	−109
	13.30–14.30	68	232	282	274	174	0	−64	9	−103	38
	14.30–15.30	58	230	152	129	29	40	73	−59	−84	−121
	15.30–16.30	90	159	120	148	140	62	−18	123	42	35
	16.45–17.45	112	142	78	34	33	0	27	172	248	−11
Vertical	17.50–18.20	299	0	96	10	−4	151	8	53	106	108
	18.20–18.50	175	−9	29	13	61	104	74	301	444	553
	18.50–19.50	142	−38	−229	−212	−123	−54	62	258	258	264
	19.50–20.50	238	−20	−204	85	−105	61	221	131	342	337
	20.50–21.50	244	55	25	184	−68	65	18	144	307	163

RESULTS

The corrective behavior of a sunflower seedling after inversion is illustrated in Figure 2. The major regions of hypocotyl expansion and contraction (which processes determine the behavior pattern) are indicated in Table I and Figure 2. The seedling illustrated was demarcated into 13 zones on the inner hook (left) side and 18 zones on the outer hook (right) side. The numbering of the zones as referred to in Tables I and II is solely for the purpose of identification: corresponding numbers do not imply corresponding zones on either side of the hypocotyl. Growth rates have been computed for each zone separately but, for the sake of concise presentation, the zones have been grouped in Table I into three composite regions—apical, middle, and basal—with growth values reported on a regional basis. Again, no anatomical or physiological equivalence is implied. The location of the middle region at each time interval is indicated in Figure 2, the apical and basal regions being the remaining extremities of the seedling.

The growth pattern of the etiolated sunflower seedling growing vertically in darkness (23.10–9.10) is similar to that described previously for etiolated cress (8). On the inner side of the hook, the bulk of the growth occurs in the apical/middle zones, and on the outer side, in the middle zone. That is, very little growth occurs in the actual hook itself, upward growth being mainly sustained by extension in the shank or basal leg of the hook. Upon inversion (9.30), a marked change occurs in this pattern of activity. Within the 1st h (9.30–10.30), the zones on the inner side of the hook that were growing have their growth stimulated, while substantial inhibition of growth occurs in the previously growing zones of the outer side. This new pattern, induced while the organ was vertical (although inverted), is maintained throughout the subsequent development of corrective curvature (9.30–15.30).

The overall growth that brings about the reorientation of the hypocotyl is, of course, compounded of the growth of individual zones, but the major growth events are such as can be described on a 'regional' basis (Table I) and the greater detail that is possible from 'zonal' measurements, does not add significantly to an understanding of the seedling's response. However, growth rates of the most active zones are given in Table II. This serves to highlight the fact that as the inverted seedling returns to the vertical (15.30–17.45), there is a significant deceleration of growth in zones 2 to 5 of the inner hook (left) side and a corresponding acceleration of growth in zones 7 to 9 of the outer hook (right) side, preliminary to equality of growth being achieved on either side.

It should also be noted from Table II that the extent of the growth stimulation in zone 2 on the inner hook (left) side between 12.30 and 15.30 is such as results in compression of the hypocotyl between zones 5 and 9 on the outer hook (right) side. It must be emphasized that this is not an artefact due to the beads on the concave curving surface coming closer together. On curving surfaces, measurements were made, not from the center of one bead to the center of the adjoining one, but from the point of contact of the bead with the surface of the hypocotyl, the measurements therefore being along the line of the hypocotyl.

When the seedling was reinverted (17.50), further intensification of this growth pattern (deceleration on the inner hook side and acceleration on the outer side) brought about the directional response necessary to achieve a vertical orientation; and doubtless one reason for the more rapid attainment of the vertical from the reinverted position (17.50), as compared with the inverted position (9.30), is that the appropriate growth pattern was already operating, or at least developing, in one case and not in the other. This growth pattern continues through the 1st h (18.50); but, by the 3rd h (20.50–21.50), as the seedling resumes a vertical orientation, growth recommences on the inner hook side and decelerates on the outer hook side. The slightly anomalous behavior of the first zone of the inner hook side following reinversion (17.50) is brought out in Table II. In contrast to the adjoining distal zones on the same side, the first zone maintained a high growth rate throughout the period.

When the reorientation of the seedling was completed (21.50), not only had the hypocotyl adjusted to a configuration identical with the original (9.10), but during all the compensatory changes in growth rate, similar amounts of total growth (Table I, bottom line) had occurred on both sides of the hypocotyl, indicating both the reliability of the method and a high degree of growth coordination in the seedling.

Although the results shown relate only to one seedling, analyses of similar seedlings confirmed that the observed growth pattern is typical of an inverted sunflower seedling. The basically nonspecific placement of the beads together with variations in the vigor of different seedlings, makes the full description of one seedling more meaningful than average values of a 'diagrammatic' seedling.

DISCUSSION

This study has revealed for the first time, the detail of the growth responses along both surfaces of a seedling hypocotyl subjected to an inversion cycle. Some features of the growth pattern are unexpected, notably the fact that the inverted hypocotyl (9.30) resumes upward (negatively-geotropic) growth by curving backwards in a manner which maintains the hook, a response which may be related to a qualitative difference in geosensitivity between the apical and the basal regions of the hypocotyl (7). Reinversion of the contorted seedling (17.50) induces a further negatively geotropic growth response which unwinds the hypocotyl while maintaining the hook. These two features, maintenance of the hook and negatively geotropic growth, would seem to be the overriding objectives of the geo-stressed seedling.

This study has also revealed that reorientation of the hypocotyl is accomplished not by the addition of new material from the apex, nor, as happens with horizontal stems (2, 4), by the development of growth in nongrowing areas, but by the adjustment of cell elongation rates in zones of normal growth. The changes in growth rates involved both growth inhibition and growth stimulation to an extent that causes compression on the concave side. Furthermore, it should be noted that a substantial growth differential between the two sides of the hypocotyl is apparent at the commencement of the reorientation process (9.30–10.30), *i.e.*, at a time when there is no marked gravitational force across the hypocotyl in the region where differential growth is developing (inner hook, zone 2; outer hook, zones 5–8). The hypocotyl, although inverted, is effectively vertical; yet the two sides show markedly different growth rates—a disparity normally associated with an auxin gradient as is thought to arise by a Cholodny-Went mechanism operating in a horizontal stem. To invoke the argument based on Cholodny-Went concepts that the gravitational differential is experienced by the 'horizontal' section of the apical hook and its effects manifested some distance away, does not resolve the difficulty because in this case the upper (normally inhibited) side is that from which the growth stimulation occurs. If the differential growth responsible for the reorientation of the inverted seedling is to be attributed to the redistribution of growth substances, it seems improbable that any such redistribution is accomplished solely in response to a lateral gradient of gravitational influence across the responding tissues. It may be significant that the nongrowing basal region of the inverted hypocotyl shows no resumption of growth in the manner characteristic of the nongrowing basal region of the horizontal hypocotyl (4). This suggests that while directional growth coordination is achieved by the apex, it exercises its control on the basis of information received from more distal regions, from which it may be inferred that the communication of information is not merely unidirectional from the apical end.

However obscure the mechanisms of growth coordination may be, video image analysis as used in this study has established the complex pattern of growth itself, both as regards its location and kinetics, within a relatively simple plant structure. Further elucidation of growth mechanisms will depend heavily on analytical approaches similarly capable of characterizing growth, both spatially and temporally.

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