

Kinetics for Phototropic Curvature by Etiolated Seedlings of *Arabidopsis thaliana*¹

Vladimir Orbović and Kenneth L. Poff*

Michigan State University-Department of Energy Plant Research Laboratory, Michigan State University,
East Lansing, Michigan 48824

ABSTRACT

An infrared-imaging system has been used to study the influence of gravity on the kinetics of first positive phototropism. The development of phototropic curvature of etiolated seedlings of *Arabidopsis thaliana* was measured in the absence of visible radiation. Following a pulse of blue light, stationary seedlings curved to a maximum of approximately 16° about 80 minutes after stimulation. The seedlings then curved upward again or straightened by about 6° during the subsequent 100 minutes. Seedlings rotated on a clinostat reached a similar maximum curvature following photostimulation. These seedlings maintained that curvature for 30 to 40 minutes before subsequently straightening to the same extent as the stationary seedlings. It is concluded that straightening is not a consequence of gravitropism, although gravity has some effect on the phototropism kinetics.

Since the seminal experiments of Darwin (2), it has been recognized that phototropic curvature of a plant shoot toward a unilateral light source results in the shoot receiving an opposing gravitational stimulus. Thus, it would be expected that the shoot would grow upward again or straighten as a result of gravitropism during or following the phototropic curvature.

Because gravitropic curvature may confound phototropic curvature, the kinetics of phototropism have been of interest to a number of investigators during the last 100 years. The kinetics of phototropism have been measured in a variety of experimental systems from the fungus, *Phycomyces* (4), to monocotyledonous plants, such as maize (13) and *Avena* (14, 15), and dicotyledonous plants, such as cress (6). In many of these experiments, seedlings were placed on a clinostat following photostimulation to minimize the effect of gravity on the development of the phototropic response. The generally accepted conclusion of these studies is that the seedling will curve upward as a consequence of negative gravitropism following curvature toward a unilateral light source (13, 15).

Our original intentions were twofold. First, we sought to study the kinetics of the phototropic curvature induced by a pulse of BL² in etiolated seedlings of the dicotyledonous

species, *Arabidopsis thaliana*. Second, these studies were expected to show that the reversal of curvature or straightening of the seedling following phototropic curvature was a consequence of gravitropism. We characterized the kinetics of phototropic curvature of seedlings on and off a clinostat. Our results indicate that gravitropism has little effect on this straightening.

MATERIALS AND METHODS

Growth Conditions

The Estland race of *Arabidopsis thaliana* was used in this study. Seedlings were grown as previously described (16) with some modifications. Strips of microassay wells containing 0.7% (w/v) agar supplemented with 1 mM KNO₃ were sown with two seeds per well. The strips were then placed in transparent plastic boxes sealed with Parafilm and kept for 3 d at 5 ± 1°C in darkness. The boxes with strips were then moved into continuous white light for 19 h at 25 ± 1°C to potentiate germination. The white light treatment was followed by a period of 44 to 48 h of growth in darkness at 25 ± 0.5°C and >90% RH until the experiments were started. Immediately before video recording, the strips with plants were removed from the plastic box and placed on a stationary stand or mounted on a clinostat in complete darkness. All manipulations of the seedlings were performed in complete darkness.

Light Sources

White light (65 μmol m⁻² s⁻¹) used to potentiate germination was provided by General Electric (Cleveland, OH) DeLux Cool-white fluorescent tubes. The actinic light source for phototropism consisted of a projector equipped with a Sylvania (GTE Products, Danvers, MA) 300-W ELH tungsten halogen lamp and a 450-nm interference filter with a half band width of 10 nm (PTR Optics, Waltham, MA). The duration of actinic irradiation was controlled with a Uniblitz shutter (Vincent Associates, Rochester NY). In all experiments, the phototropic response was induced with a BL pulse of 0.9 s at a fluence rate of 0.34 μmol m⁻² s⁻¹ for a fluence of 0.3 μmol m⁻². This fluence is within the range of fluences inducing the peak response in first positive phototropism of etiolated seedlings of *A. thaliana* (12, 16). The IR light source consisted of a Leitz Prado-Universal (Ernst Leitz GmbH, Wetzlar, Germany) projector with a 250-W tungsten halogen lamp and a Kodak Wratten 87c gelatin filter (Eastman Kodak,

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² Abbreviations: BL, blue light; RL, red light.

Rochester, NY) transmitting light >800 nm (transmission $<1.5\%$ at wavelengths lower than 800 nm, measured using a Perkin-Elmer Lambda 7 spectrophotometer). RL (at $0.6 \mu\text{mol m}^{-2} \text{s}^{-1}$) used for preirradiation was obtained from 2 gold fluorescent tubes (GTE, Sylvania) wrapped with red cellophane (Highland Supply Corp., Highland, IL). This source provides radiation from 560 to 720 nm with maximum output at 620 nm. Fluence rates were measured with a Li-Cor (Lincoln, NE) Li-190 SA quantum sensor in combination with a Li 1000 Data Logger.

IR-Imaging System

An IR-imaging system (Fig. 1) was used to monitor the seedlings under radiation to which the seedlings are physiologically blind (*i.e.* in "physiological darkness"). This system consisted of two spatially separated stations, one for recording and one for monitoring. The recording station, which was situated in a dark room, consisted of an IR-sensitive Cohu solid state camera 4815-2000 (Cohu, San Diego, CA) and IR light source. The camera was equipped with an extension tube and an 85-mm, f2 lens (Minolta Co., Osaka, Japan) to magnify the seedling images. Equipment in the monitoring station included a Panasonic AG 6300 video cassette recorder (Matsushita Corp., NJ) and Electrohome V-6 type (Electrohome Ltd., Kitchener, Ontario, Canada) monitor. Images of the seedlings were recorded throughout an experiment while being monitored in "real time." The camera was focused on a particular plane. The strip containing the seedlings was subsequently positioned in darkness on a stand such that the seedlings were in focus for the camera.

Following an experiment, the angles of curvature were measured while playing back the recording. The video cassette recorder was connected to an IBM AT computer with a Java video analysis program (Jandel Scientific, Corte Madera, CA). Recorded images of seedlings were displayed on a high resolution Electrohome ECM 1312U monitor.

Angles of curvature were measured and recorded using the Java software. In this procedure, one straight line was made along the lower part of the image of the seedling frozen on the screen. A second line was then made as a tangent to the curved portion of the hypocotyl below the hook. The angle of curvature was the angle, theta, between these intersecting lines (Fig. 2). The initial curvature of each seedling was measured from recordings made approximately 5 min before the actinic BL irradiation. Subsequent curvatures for stationary seedlings (*i.e.* not placed on the clinostat) were then measured at 10-min intervals for 180 min following photostimulation. Seedlings mounted on the clinostat were oriented with their longitudinal axes perpendicular to the axis of rotation (0.48 rpm). For these, curvatures were measured at approximately 15-min intervals following photostimulation. (Images of the seedlings were recorded when they entered the field of view of the camera, so the intervals were 15 ± 3 min.) If the seedling was curved at the beginning of the experiment, that curvature was subtracted from the curvatures measured subsequently. Thus, all curvatures are degrees of curvature during the course of the experiment.

The repeatability of the angle measurement was assessed by measuring the same curved seedling 15 times. The repeatability error ranged from $\pm 2^\circ$ for a 5° angle to $\pm 5^\circ$ for a 55°

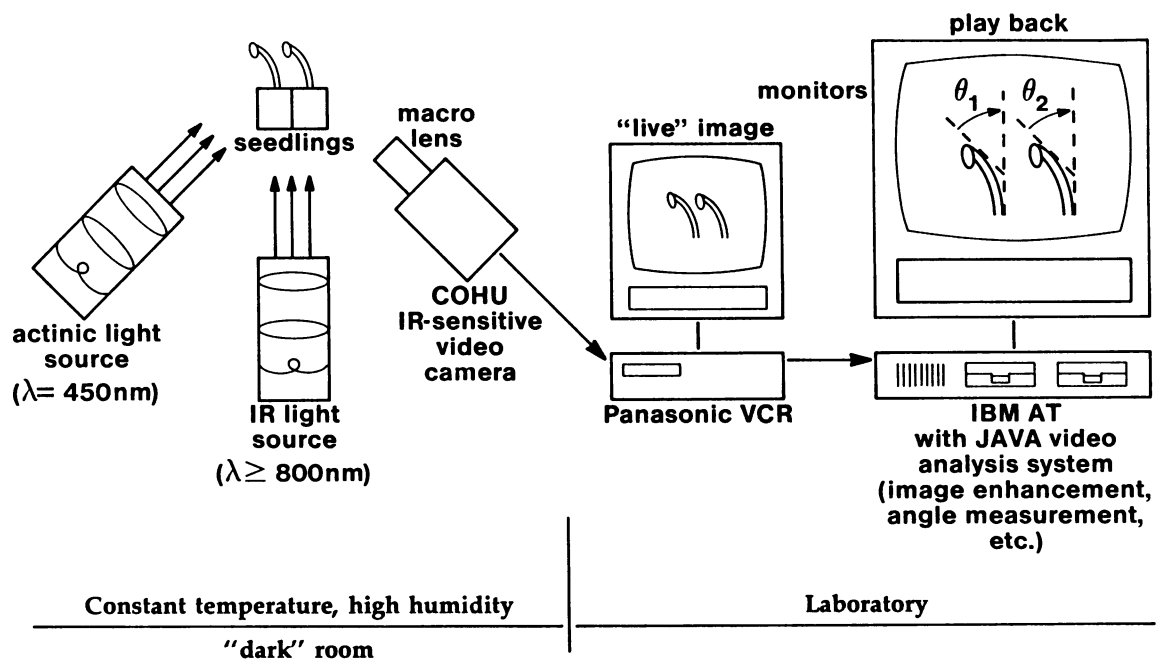


Figure 1. Block diagram of an IR-imaging system. Seedlings are imaged using an IR-sensitive camera with IR radiation at wavelengths longer than 800 nm. The camera is aligned orthogonal to the long axis of the seedlings and orthogonal to the actinic light ($\lambda = 450$ nm). This portion of the system is located in a dark room with controlled temperature and humidity. The camera is connected to a computer station in a separate room. VCR, video cassette recorder.

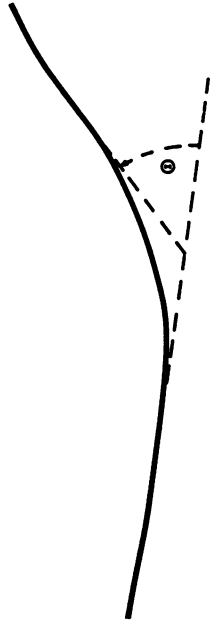


Figure 2. Diagram illustrating the measurement of curvature by hypocotyls of *A. thaliana*. The bold line represents the seedling hypocotyl; the dashed lines represent splines tangent to the curved and uncurved hypocotyl portions. The angle, theta, between the splines is measured as the angle of curvature.

angle. This source of error is largely a consequence of variability in positioning the straight line tangents.

Line tracings of entire representative seedlings were also recorded from the video images using the Java software. By tracing a seedling image at various times, we generated image sequences showing curvature as a function of time.

RESULTS

The time courses for phototropic curvature to a BL pulse by three individual seedlings demonstrate the considerable variability observed in the responses of different seedlings (Fig. 3). An average time course for phototropic curvature can be constructed from the average curvature of a number of seedlings at each time. Such an average time course (Fig. 4) shows a lag time of about 10 to 20 min following the BL pulse during which the seedling shows no measurable curvature. Following this lag period, curvature toward the light source increases with time to a maximum of about 16° at 80 min after photostimulation. Curvature then decreases in a straightening phase, resulting in a loss of about 6° of curvature by 180 min after the BL pulse. Control seedlings, treated in the same manner as the experimental seedlings but lacking the BL pulse, showed no significant curvature (Fig. 4). Some seedlings exhibited no curvature to the unilateral BL (data not shown).

A sequence of line images of a single seedling (Fig. 5) demonstrates the curvature and straightening which are shown graphically in Figure 4 for the average of many seedlings. The seedling shown in Figure 5 was selected as representative of the kinetics of curvature. However, this seedling's

curvature was greater in amplitude than the mean curvature of the population. The image sequence shows that seedling growth continues throughout the experiment. Curvature appears to begin just below the hook region and progresses down the hypocotyl. In addition, straightening is not limited to any single portion of the previously curved hypocotyl.

The average time course for phototropic curvature of seedlings rotated on a clinostat (Fig. 6) is very similar to the average time course for stationary seedlings (Fig. 4). The lag phase of about 15 min, the average maximum curvature of about 17° , and the average final curvature of 10° for the seedlings on the clinostat are similar to the corresponding values for the stationary seedlings. However, a slight difference was observed between stationary seedlings and seedlings on a

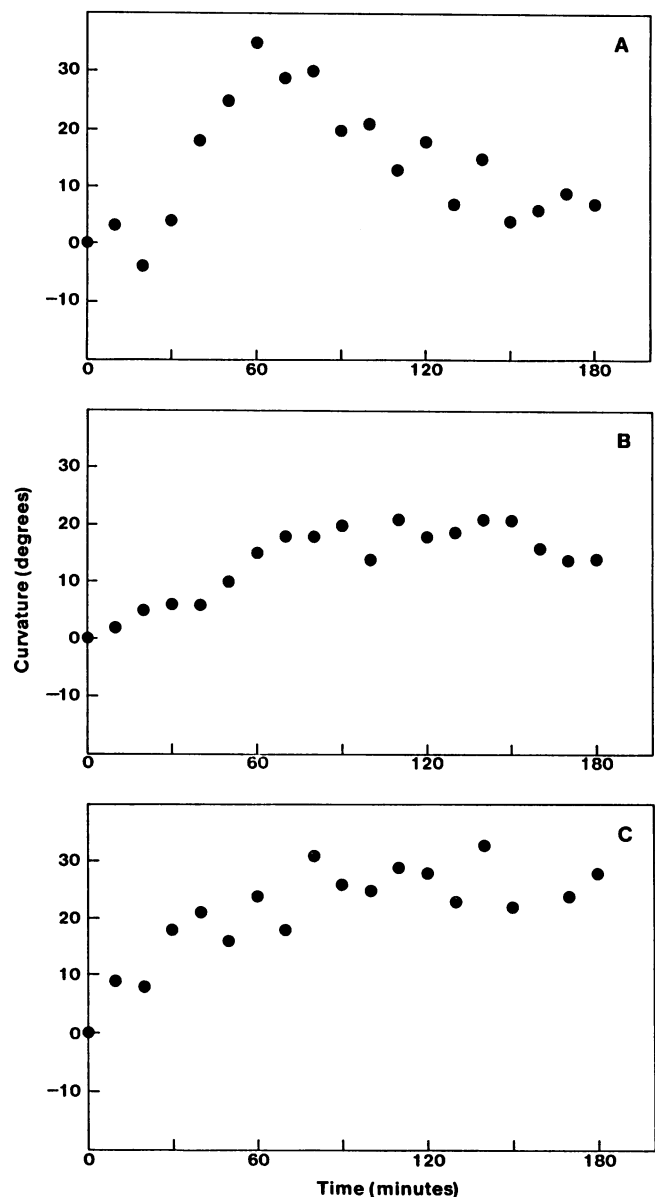


Figure 3. Time course for development of curvature by three individual seedlings (A, B, and C).

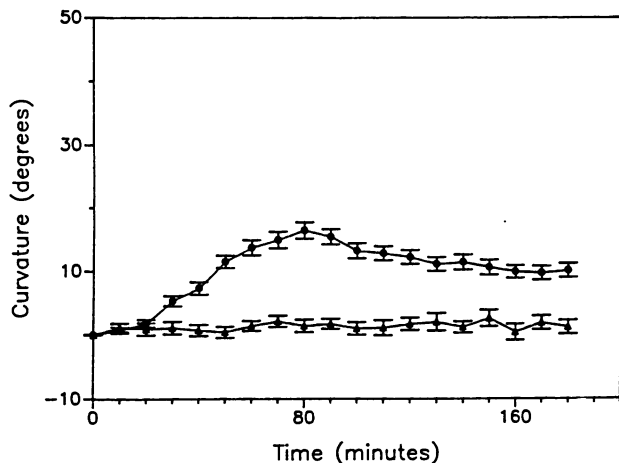


Figure 4. Time course for development of average curvature to a BL pulse by stationary seedlings: ●, stimulated, $n = 55$; ▲, nonstimulated, $n = 18$; vertical bars, ± 1 SE.

clinostat. Control seedlings, which were rotated on the clinostat but had not been photostimulated, showed no significant curvature (Fig. 6).

The effect of RL on the curvature and straightening was examined by measuring the time course for phototropic curvature of seedlings that had been preirradiated from above with 60 min of RL immediately before the BL pulse. The results (Fig. 7) show a curve shape that does not appear to be different from that for seedlings not RL preirradiated (Fig. 4). The lag phase, time to maximum curvature, and straightening are all similar. However, the amplitude of the curvature is

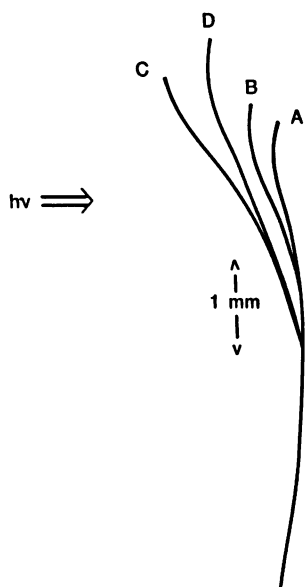


Figure 5. Line images of an *A. thaliana* seedling during phototropic curvature and straightening. Each line represents the seedling at the indicated times following the BL stimulus: A, 20 min; B, 40 min; C, 80 min; D, 130 min.

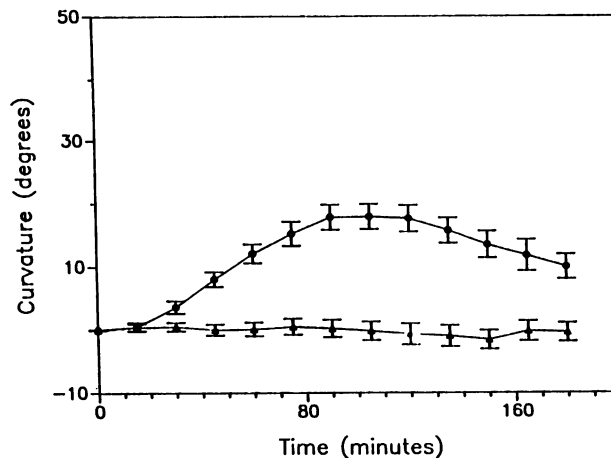


Figure 6. Time course for development of average curvature to a BL pulse by seedlings rotated on clinostat: ●, stimulated, $n = 32$; ▲, nonstimulated, $n = 25$; vertical bars, ± 1 SE.

greater for the RL-preirradiated seedlings than for the non-preirradiated controls.

DISCUSSION

It has been noted that kinetic measurements are important for an understanding of sensory responses (5, 7). However, to obtain such data for the phototropism of higher plants, it is necessary to measure curvature in physiological darkness. For this reason, an IR system has been used, such that the response of the seedlings lacks the confounding effect of visible light. This is of particular importance because no wavelength in the visible region of the spectrum can be considered "safe," blue and green inducing phototropism (17) and RL causing the enhancement of phototropism (10). For these reasons, we agree with Iino and Carr (8) that sensory responses of plants to light should be observed under IR radiation.

The time courses for phototropic curvature by different

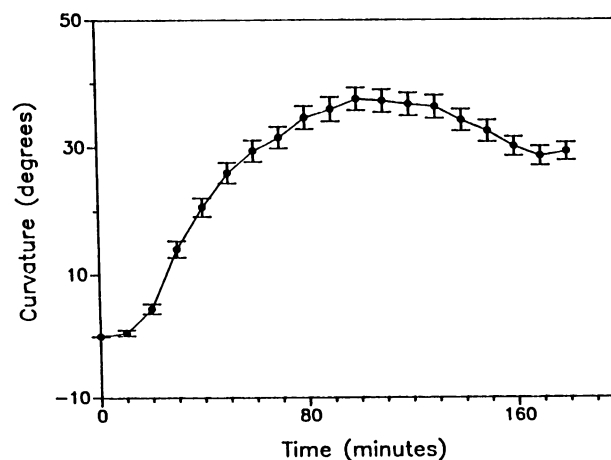


Figure 7. Time course for development of average curvature to a BL pulse by seedlings preirradiated with RL. $n = 32$; vertical bars, ± 1 SE.

seedlings showed significant differences in lag time, the amplitude of the response, and the time required for maximum response. These variations would result in considerable noise for the curvature of a number of seedlings measured at a particular time. At least part of that variation results from variable position of the seedling with respect to the light source. It has been reported that phototropic curvature is low if the side of the hook with the cotyledons attached is positioned toward the source of the light (11). It is possible that much of the variability in the kinetics is a consequence of this dependence of curvature on hook position. However, considerably more data would be necessary to document any effect of the hook position on the extent of the lag phase or on the amplitude of the final response. We believe that the oscillations in curvature of an individual seedling are a consequence of nutation of that seedling. This is based on observations of seedlings from above (data not shown).

If the variability is minimized by averaging the response at each time for a number of seedlings, several general conclusions become possible. First, the maximum curvature induced by a pulse of BL is transient for both stationary seedlings and seedlings rotated on a clinostat (Figs. 3, 4, and 6). Second, the time course for phototropism of stationary seedlings is quite similar to that of seedlings rotated on a clinostat. In particular, the lag phase and the final curvature are the same (Figs. 4 and 6). Third, stationary seedlings exhibit straightening immediately after reaching their maximum curvature, whereas seedlings on the clinostat maintained their maximum curvature for about 30 min before exhibiting straightening.

In several previous papers (13, 15), plants were reported to develop a stable curvature that was maintained for ≥ 6 h. In contrast, we see a phase of straightening beginning 2 h after photostimulation. These differences observed between the response of *A. thaliana* and the response of *Zea* (13) or *Avena* (15) could be due to their different taxonomic status. However, this difference also could be a consequence of differences in the RL irradiation. RL is known to affect both phototropism (10) and gravitropism (18). It is clear from this study that RL preirradiation increases the amplitude of the response but does not significantly affect the straightening (Fig. 7). However, the RL irradiation protocol used here differed substantially from that used by Nick and Schäfer (13). Thus, there are insufficient data to assess the possibility that the straightening could be eliminated by a continuous RL irradiation. However, it is interesting to note that the time of maximum curvature for RL-preirradiated seedlings seems later than that for the non-preirradiated seedlings. The differences in reported data could also be a consequence of different experimental procedures such as the position of seedlings on the clinostat. Shen-Miller and Gordon (14) reported complete straightening of oat coleoptiles on a clinostat following unilateral BL irradiation. Their clinostat protocol was similar to that which we used, with vertical mounting of the seedlings on a clinostat with horizontal axis of rotation.

It should be noted that the amplitude and kinetics for gravitropism vary from species to species and depend on the other environmental conditions of the plant material (3, 18). Because an earth-based experiment must inexorably confront the ubiquitous 1g force of gravity, any apparent phototropic curvature in seedlings not maintained on a clinostat must be

considered to be an equilibrium between phototropism and gravitropism. Thus, it would not be surprising if the amplitude or kinetics of apparent phototropic curvature varied from species to species.

What is the cause of the straightening? Our results demonstrate that gravity is not the primary cause for the straightening. However, there is a small effect of gravity on the straightening. Because the final curvature developed by stationary seedlings is the same as that developed by seedlings on the clinostat, we can conclude that there is no significant gravitropism during the 180-min duration of the experiment. Moreover, because the maximum curvature developed by stationary seedlings is the same as that developed by seedlings on the clinostat, we can conclude that there is no significant influence of gravity on the development of phototropic curvature for 80 to 100 min. Thus, there appears to be no gravity-dependent interference with the translation of the full amount of photoproduct into curvature. However, gravity does have a relatively subtle effect, causing straightening about 40 min earlier than the straightening observed in seedlings on the clinostat. The growth rate of the seedling may be of importance in evaluating these data. Preliminary measurements of seedling growth rates show that seedlings placed on the clinostat grow slightly faster than comparable stationary seedlings. Such an increase in growth rate has been noted before (1). If a more detailed analysis of growth rates substantiates this measurement, then the subtle effect of gravity on straightening may not be a gravity effect on curvature but, instead, may be a consequence of the altered growth rate.

One can also conclude that straightening is not a consequence of a decay in any component in the signal process leading to curvature. Such a decay would be expected to result in a cessation of the development of curvature, resulting in the maintenance of a stable curvature. In contrast, we observed a decrease in the angle of curvature or curvature in the opposite direction, which we refer to as straightening.

One possible hypothesis to explain the straightening is based on a limited pool of material required for or affecting growth on each side of the seedling. Depletion of this pool would be expected first on the side experiencing the greatest rate of curvature. Given a relatively slow replenishment of the pool, depletion of the pool on one side would result in a lower growth rate on that side and, thus, curvature away from the original stimulus or straightening. The spatial distribution of straightening is consistent with this hypothesis. Straightening occurs throughout the curved region unlike the phototropic curvature itself which is initiated just below the hook region and progresses down the hypocotyl (Fig. 5). The spatial distribution of gravitropism is similar to that of phototropism (data not shown), being initiated below the hook region and progressing down the hypocotyl. The fact that straightening occurs throughout the curved portion of the hypocotyl supports the conclusion that straightening is not a consequence of gravitropism.

Straightening has also been described in the tropistic curvature of maize roots (9). However, interpreting the results of that study is complicated because the stimulus used was gravity and the presentation was continuous. An attractive hypothesis for straightening was suggested by Ishikawa *et al* (9). They base this hypothesis on differences in kinetics on

two sides of the root for a Cholodny-Went hormone redistribution and changes in the sensitivity of the cells to the auxin. At present, there are inadequate data to indicate which hypothesis is correct, if either.

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