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Abstract. A procedure for the study of the negative geotropic response in the *Coleus* stem and *Avena* coleoptile is presented. Time-lapse photography, employing a motordriven 35mm camera and specially designed chambers, is utilized in recording plant responses. Data from 3″ x 7″ photographic prints are recorded on computer punch cards, a line of best fit is determined and the rate of geotropic curvature at forty-five degrees calculated. This unique technique of reporting curvature rates emphasizes the dynamic character of the geotropic response and eliminates variation involved in the plant's perceptive mechanism.

In reviewing literature concerned with negative geotropism it is disconcerting that almost all of the work in this area has been done with coleoptiles of seedlings, investigations with mature plants being neglected.

Many methods have been devised to measure geotropic response in immature tissue, especially that of coleoptiles. Some approaches accepted by current investigators are described in a review by Rufelt (1961), who explains that even for these small and relatively undeveloped plants quite elaborate and complex methods are required. Methods involving mature plants have also been used but only to study the plagiogeotropic response in leaves and branches; no acceptable method has been proposed for their use in the investigation of the orthogeotropic response.

THE PROBLEM

It was the objective of this investigation to determine the response pattern of mature dicotyledonous plant stems experiencing a 24 hour period of geotropic stimulus. A method of obtaining data was devised that would accurately and dynamically examine the movement of the plant organ.

In using time-lapse photography to record plant responses, exposure of the subject to light is an inherent necessity. It has been noted that in immature tissue, such as grass coleoptiles, white light inhibits growth, decreases sensitivity and affects the reactivity of the organ (Bremekamp, 1921). In mature plants this interference appears to be absent, or present only at extremely low levels. The phototropic response, however, is present in both coleoptiles and mature stems. It has been found that wavelengths between 520 and 560 mu as well as 620 mu and above elicit little or no phototropic response (Schrank, 1950), and are therefore appropriate wavelengths for safe lights used in observing geotropic responses.

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Materials and Methods

Experimental Plants

Coleus blumei Benth. (cultivars, Red Rainbow and Scarlet Rainbow), tropical members of the large mint family, Labiatae, were used as experimental plants. *Coleus* was chosen as an example of a mature dicotyledon because it is easily propagated vegetatively, has opposite leaves, is easily oriented because of its square stem and responds relatively rapidly to the stimulus of gravity.

Clones of both cultivars were started at the University of Iowa by taking repeated cuttings from a single stock plant of each cultivar. Thus all experimental plants of the same cultivar were assumed to possess the same genotype.

The cuttings were placed in sterilized sand to root. After approximately four weeks they were transferred to four and one-half inch clay pots with a sterilized general mix soil preparation. The plants were grown under normal greenhouse conditions until they reached a height of 20 to 25 cm above the pot rim. Lateral buds were removed as they appeared so that the plans had a single stem.

Plants found to be growing approximately 0.2 cm per 24 hours were selected for normalcy and straightness and then subjected to experimentation.

Avena sativa L. (Cultivar, Victory) coleoptiles were used as a standard for the geotropic response so that the results from the mature plants might be related to previous literature. The oats were prepared according to the procedure outlined by Ferguson (1970).

Apparatus

The experimental chamber was a light-tight box with a 35 mm motor driven camera mounted at one end. At the opposite end, facing the camera, the experimental plants were mounted horizontally against a flat black background (Fig. 1). A clock, elapsed time meter and thermometer were mounted in the field of focus to record the time and temperature simultaneously with plant curvature. The data, experiment number, type of treatment and a white vertical line were also recorded in each photographic frame.

Lighting was accomplished by six rheostatically controlled 75-watt reflector lamps evenly spaced on the two sides of the chamber. The light quality was limited to maximum intensity at 525 mu by six green filters as prescribed by Ernest (1965). At every exposure 0.881 uw cm^{-2} of green, non-phototropic light was received for one second by the plants.

A photographic record was made every 30 minutes by a device utilizing tandem microswitches and cams driven by synchronous

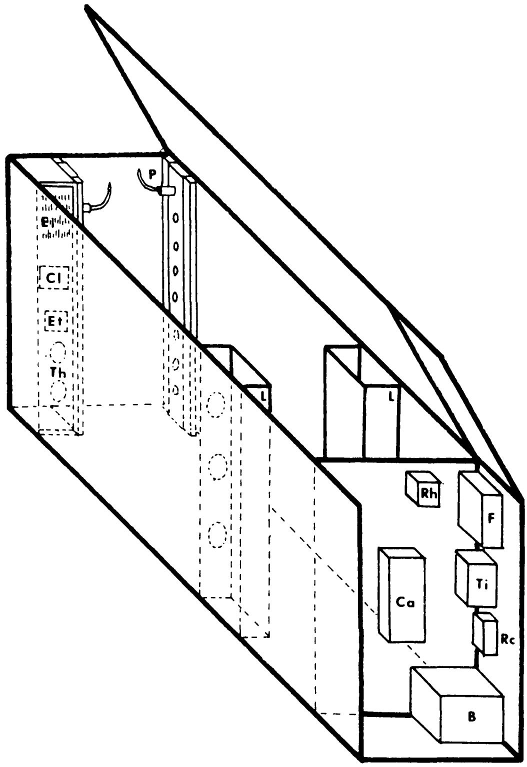

Figure 1. The Experimental Chamber. Legend: Ca, Camera; B, Battery; Cl, Clock, Ei, Experimental Information; Et, Elapsed Time Meter; F, Fuse and Junction Box; Rc, Remote Control Relay; Rh Rheostat; Ti, Timer; L, Light Bank with Filters; Th, Thermometer, Wet and Dry Bulb; P, Plants in Holding Block.

motors. Figure 2 shows a diagram of the timing device.

The timing device activated a remote control relay box that in turn switched the automatic camera on and off. The remote control box can be purchased from a commercial source or built according to Figure 3. A Nikon FTN Motor Drive Model F-250 camera was used. The lens was set at F 4.0 with a shutter speed of 1/15 using Kodak negative Plus-X film (ASA 125) to get adequate exposures utilizing the minimal light described above.

The experimental chamber was placed and leveled in the controlled environment chamber at the University of Iowa where the temperature was maintained at 24°C and the relative humidity was maintained at 85%

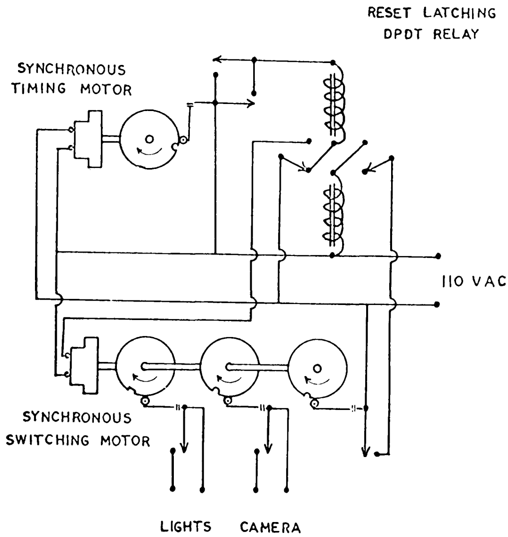

Figure 2. Timing Device for Time-Lapse Photography.

Preparation of Plants

Forty-eight hours prior to experimentation the plants were given continuous light. At the end of the light period the plants were placed in the darkened controlled environmental chamber where they were heavily watered and watched for guttation. Guttation could generally be seen in all plants after two hours. Under these conditions various amputations could be accomplished without admitting air into the vascular system.

In this investigation three types of plant conditions were tested: 1) intact plants rooted in soil, 2) excised stems in vials with leaves and apex, and 3) excised stems in vials without leaves and apex. The leaves and apex were removed while the stem was rooted in soil; petroleum jelly was applied to all cut surfaces to prevent bleeding. Stems were then excised and the cut end immediately placed in distilled water. After a fifteen minute interval they were cut to a total length of 14 cm. While still under water a band of sterile absorbent cotton, ¾″ wide, was wrapped around the base of the stem so that approximately 2 cm of the stem protruded below the cotton. A 25 ml glass vial, 5.8 cm by 2.8 cm, was filled with distilled water and placed under the water containing the stem. The cotton and stem were firmly inserted into the mouth of the vial. A disposable 1½″ hypodermic needle was inserted between the cotton and mouth of the vial to allow for air passage. The cotton and outer portion of the vial mouth were spread evenly

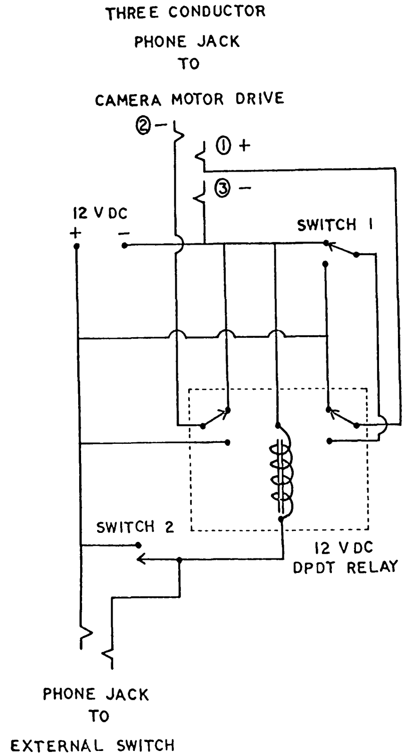

Figure 3. Schematic Diagram for a Remote Control Devise for Camera. Switch 1 selects either a single frame or multi-frame exposure. Switch 2 is a manual switch for camera activation.

with petroleum jelly to prevent leakage. After a one-half hour equilibration period the vials were mounted in holes drilled into a holding block. The holding block could be rigidly mounted in the experimental chamber, putting all of the plants simultaneously at 90° to the vertical line. A sequence of photographs was obtained at 30 minute intervals for 24 hours.

Data Analysis

The 35 mm negative film was printed on 5″ x 7″ Kodak Kodabromide F-4 photographic paper, using a conventional enlarger.

Lines passing through the center of the stem tip and tangent to the radius of curvature were extended to intersect the white vertical line as printed from the negative. The angle of intersection was measured and recorded as the amount of response in degrees at that time.

Four angles, the two just less than 45° and the two just larger than 45°, and their respective times in minutes were punched on computer data cards, along with the specific plant designation, experiment number, and type of treatment. A computer program utilizing an equation for least squares calculated the regression line. The slope of this line is the rate of geotropic curvature at 45°. The average rates of the groups examined were then compared statistically for a difference in means using the t-test (Snedecor, 1946).

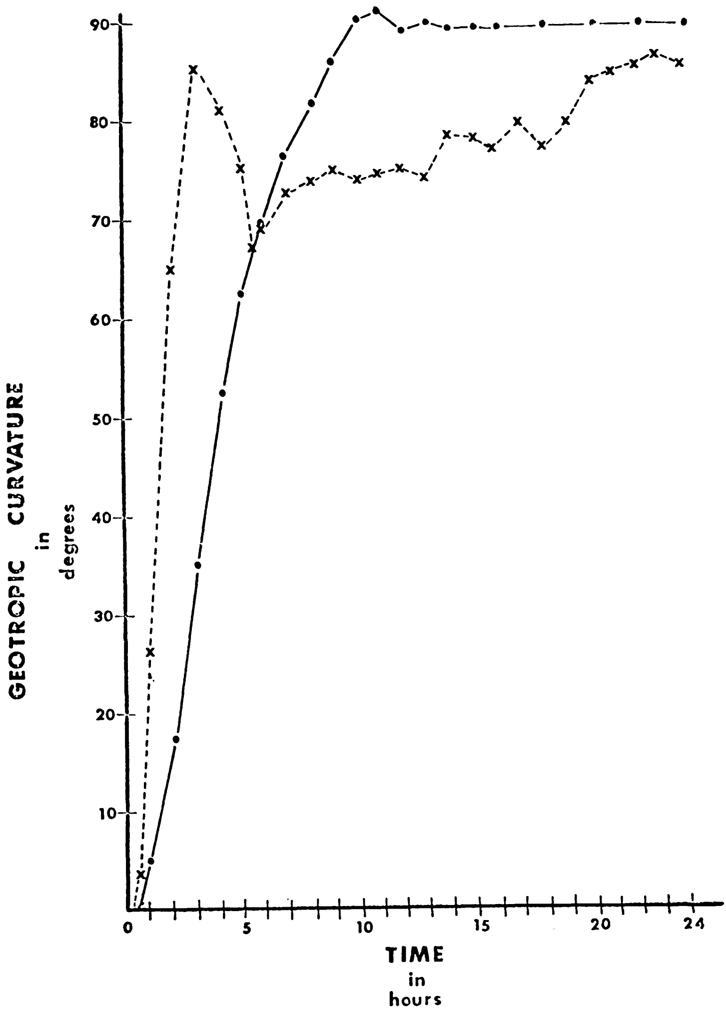

Figure 4. Time Course of the Geotropic Response in *Coleus* and *Avena*. —x—x—x—x represents *Avena sativa* (Victory) Coleoptiles. —— . —— . —— . represents *Coleus bluemi* (Scarlet Rainbow) excised stems with leaves and apex in vials.

Results and Discussion

Figure 4 shows a comparative time course of the geotropic response in *Coleus* and *Avena*. Although time courses extending to 24 hours cannot be found in the literature, the pattern of the *Avena* response from 0 to 2 hours agrees with work by Anker (1954, 1956) and Bara (1957).

Bara (1957) showed an increase in geotropic curvature over a 6 hour time course with no signs of irregularities. Contrary to his findings, however, a reversed reaction to the negative curvature can be seen in the *Avena* coleoptiles after the second hour. The "straightening" reported in observations of roots by Larsen (1957), is preceeded by a basipetal migration of the angle of curvature. In the intact coleoptile curvature is first initiated at a site approximately 2 mm from the tip and then moves completely to the base within a 24 hour period. After the sixth hour the rapid straightening declines and a negative curvature response dominates a slow undulating pattern as the coleoptile approaches the 90° mark.

This pattern indicates that the rapid elongation of the lower cells near the tip may cause a compression of the upper cells and mechanical resistance to a degree that curvature ceases at approximately the 85° point. The effect of the compression would become more pronounced as the apex approaches the vertical. The auxin ratio between the lower and upper cells becomes less, allowing for increased elongation of the upper cells and the production of the straightening phenomenon. Differential distribution of auxin in the lower and upper cells moves basipetally with time and thus produces a basipetal migration of the curvature radius. The secondary curvature is produced by the curvature toward the base of the coleoptile and is responsible for the final attainment of 90°.

The curvature pattern of *Coleus* parallels that of *Avena* but the lag time of *Coleus* is approximately twenty minutes longer and the initial rate of curvature is somewhat less. The intact *Coleus* stem, however, over shoots the 90° point and then goes into a dampening process usually consisting of three oscillations. These undulations are not clearly apparent in Figure 4 due to the shortened time scale, which permitted a complete 24 hour time course to be illustrated in a single figure. According to the explanation above, the undulating pattern of *Coleus* would not be as pronounced as that of *Avena* due to the slower rate of elongation and the more rigid cell wall construction in the stem, which reduces the effect of mechanical pressure.

Table 1 shows a comparison of the curvature rates at 45° in degrees per minute for two cultivars of *Coleus*. It was shown statistically that there was no difference in the rates between the two cultivars nor was there a difference between the response of plants rooted in pots and those who stems were excised and placed in vials of distilled water. However, a notable difference in the standard deviations can be seen, indicating a greater variance in the potted plants. Under the procedure outlined previously, mature plants can be observed with respect to the geotropic response without the root systems and without the variability inherent in soil-root interactions. Therefore the results from excised stems in vials can be generalized to the normal growing state of the plant.

Table 1. Comparative Geotropic Curvature Rate of *Coleus blumei* in Pots and Excised Plants in Vials.

Cultivar	Presented Condition	Number in Sample	Curvature Rate at 45° in deg./min.	Std. Dev.
Scarlet Rainbow	Pot	9	0.2667*	0.0358
Scarlet Rainbow	Vial	25	0.2722*	0.0241
Scarlet Rainbow	Vial Completely Amputated	12	0.0339	0.0182
Red Rainbow	Pot	9	0.2937*	0.0766
Red Rainbow	Vial	15	0.2695*	0.0476
Red Rainbow	Vial Completely Amputated	9	0.0964	0.0274

* No significant difference could be detected between any group at the 0.05 level.

Table 1 also shows the curvature rate that can be obtained from a completely amputated stem (a stem without leaves, axillary buds or an apex). The significantly lower rate could be attributed to the decreased availability of auxin resulting from the removal of auxin contributing members and/or to wounding.

The curvature rate at 45° was selected as the parameter for evaluation of the geotropic phenomenon rather than the degrees of curvature at the end of a given time period because it:

1) emphasizes the dynamic character of geotropism,
2) eliminates the variation of lag time in the plant,
3) can be used to evaluate the physiological contribution of various tissues, compensating for transmission variables,
4) represents a 50% completion of the entire geotropic response,
5) has less variation than parameters involving total response, time for initial response or specific time intervals, and

6) gives an indication of the reaction rate rather than the degree of reaction and might thereby indicate the type of physiological mechanism involved.

Summary

Mature dicotyledon plants can be used, when prepared under the proper conditions, to study the geotropic response. The early pattern of the *Coleus* time course is similar to that of *Avena* but consists of an increased lag time and a slower curvature rate. In *Coleus* the final approach to the 90° equilibrium point is accomplished by a series of undulations, the first being an overshoot of the vertical line. In *Avena* straightening occurs prior to reaching 90°. This reduction in curvature is followed by a somewhat irregular pattern of curvature as the angle increases toward the 90° mark.

Reporting of the geotropic response in terms of curvature rate at 45° is a preferred method as opposed to the usage of curvature attained at the end of specific time intervals.

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