

## Bioassay Methods for Geotropically Active Growth Substances

ARTHUR H. WESTING,<sup>1</sup> Purdue University

In conjunction with a study of the hormonal mechanisms involved in the geotropic reactions of conifers, it was felt necessary to develop a means of testing substances for their ability to modify geotropic activity. The tests that are commonly employed for uncovering auxin activity were not considered suitable here since they are designed to test for straight-growth activity.

It was decided to base the geotropic test on the ability of immature oat coleoptiles to begin a rapid geotropic recovery when severed and oriented horizontally, such recovery occurring as a result of a relatively lesser cell elongation on the upper side. The oat coleoptile, of course, has long been a favorite organ of the physiologist. Brighton oats<sup>2</sup> were chosen because they are a hull-less cultivar of comparatively uniform growth.

Coleoptiles for the assay are produced in a "dark" room continuously illuminated with dim red light having wavelengths longer than about 660 m $\mu$ ,<sup>3</sup> a tropistically inactive region of the spectrum. Seeds are soaked for two hours in aerated distilled water, rinsed briefly, and sown on several layers of water-saturated Scotties brand facial tissues. The seeds are then germinated at 22-24°C in moisture-saturated chambers. The germination is carried out under the same continuous dim red light used above which, while tropistically inactive, does suppress the growth of the first internode in favor of the coleoptile. After 70-71 hours, coleoptiles are collected from among vertically straight, 27-30 mm long individuals. The primary leaf is extracted and the apical 20 mm of each coleoptile is retained.

The excised coleoptiles, kept in the "dark" room, are next mounted horizontally (skewered) to a support<sup>4</sup> by their basal (proximal) end so that their vascular bundles are oriented one above the other.

Coleoptiles, prepared as above (and still kept in the "dark" room), are then used in one of three ways. In two of the procedures they are immersed in an aqueous medium with test substances applied via diffusion from the medium, a method suggested by the work of L. Anker (1). In the first procedure the coleoptiles are left intact, in the second a 1 mm decapitation is performed. In the third procedure, coleoptiles are decapitated and enclosed in a moisture-saturated chamber with the test

---

1. The author is indebted to the National Science Foundation for generous financial support (Grant No. 18482) and to Paramajit S. Dhillon for laboratory assistance.

2. *Avena sativa* L. v. Brighton, obtained from T. Rajhathy, 42 Farlane, Ottawa, Canada.

3. Red fluorescent tube, G.E. No. F15T8-R-15W, wrapped with ca. 12 layers of red moistureproof cellophane, DuPont No. 300-MS-C-RED.

4. A vertical plexiglass post (with base) to which are cemented at 1 cm intervals several (usually five) 5.5 mm diameter brass (or stainless steel) pins projecting out horizontally 8 mm. Four such supports can be installed conveniently in a 400 ml beaker.

substances applied to their cut apical end via diffusion from a solidified agar solution, a method suggested by the classical *Avena* curvature auxin assay of F. W. Went.

In any case the coleoptiles soon begin elongating and curving upward, the subsequent response generating an apparently linear function with time for at least two hours. The coleoptiles elongate approximately 5 mm during the first 90 minutes, most of this growth occurring in a zone originally 6 to 10 mm back from the tip. The rate of growth and recovery for the decapitated coleoptiles is very slow, no physiological tip regeneration occurring during this time.

Response usually is measured after a 90 minute incubation period. It is recorded photographically<sup>5</sup> and the vertical angular displacement is measured. The angle, whose vertex is at a horizontal distance of 10 mm from the basal end of the section, is generated from the horizontal by an arm extending an additional 10 mm (angle  $\alpha$  in Figure 1). Measurement

THE MEASURING OF VERTICAL DISPLACEMENT OF 71-HOUR-OLD  
RED-LIGHT-GROWN OAT COLEOPTILES CUT AT 20 MM, MOUNTED  
HORIZONTALLY, AND INCUBATED IN THE DARK FOR 90 MINUTES

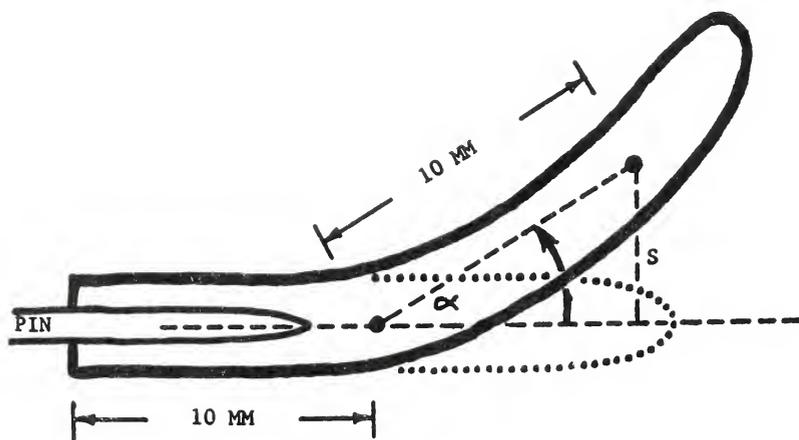


Figure 1

is facilitated by using a protractor with a swinging arm. A typical displacement after 90 minutes of incubation is  $27^\circ$ , and for a decapitated coleoptile,  $10^\circ$ . There is an indication that the response would be more accurately represented by a sine transformation of this angle (line S in Figure 1).

Problems of biological variability besetting the assay are minimized by developing carefully repeated routines of procedure (e.g., beginning each experiment at the same time of day) and by designing each

5. Shadographs are made using a high contrast photographic paper (Kodak Kodabromide A-5).

experiment as a self-contained unit with its own controls. Differences in response between controls and treated coleoptiles are tested for significance by an analysis of variance followed by J. W. Tukey's procedure for multiple comparisons, a conservative test (2). A difference of approximately 5° is usually required for significance at the 1% level.

In the procedures where the coleoptiles are kept immersed during incubation, the substance (or combination of substances) to be tested is dissolved in the ambient medium. While the hydrogen ion concentration of the medium per se seems to have little effect on geotropic recovery, pH can have a profound influence through its control over the state of ionization of substances being tested. Where it is necessary to control pH, it was found that McIlvaine's buffer system (citric acid/ $K_2HPO_4$ ) used 1/10 strength could be employed without side effects. When using the method of total immersion either intact or decapitated coleoptiles are used, the two procedures appearing to detect different classes of compounds. For example, indoleacetic acid in the range of physiological concentrations has little if any effect on the geotropic response of intact coleoptiles, but enhances the response of the decapitated ones (optimum concentration, ca.  $10^{-7}$  M). On the other hand, an unknown substance extracted from white pine stems (*Pinus strobus* L.) appears to enhance the response of intact coleoptiles while being without effect on decapitated ones. In the third method of application, where the substance to be tested diffuses into the apical end of a decapitated coleoptile from a tiny block of agar,<sup>6</sup> indoleacetic acid is most effective in enhancing the geotropic response at a concentration of ca.  $3 \times 10^{-7}$  M.

The agar block technique appears to be suited to disclose activity of the auxin type. However, since a removed tip can be replaced, in effect, only partially by application of indoleacetic acid to the cut stump, this method could conceivably uncover the involvement in geotropism of other tip-produced substances. The method of application via total immersion of the coleoptiles, particularly in the procedure where the coleoptiles are left intact, will perhaps uncover substances capable of modifying geotropic perception or sensitivity.

### Summary

Procedures are presented for performing, analyzing, and evaluating an assay to test substances for their ability to influence geotropism. The technique is based on the ability of immature *Avena sativa* L. coleoptiles to begin a rapid geotropic recovery when excised and oriented horizontally, and for this response to be alterable by applications of test substances.

### Literature Cited

1. ANKER, L. 1958. Influence of the pH on the growth and the geotropism of decapitated *Avena* coleoptiles supplied either with indoleacetic acid or with indoleacetonitrile. *Acta Bot. Neerland.* 7:69-76.
2. STEEL, R. G. D. and J. H. TORRIE. 1960. Principles and procedures of statistics with special reference to the biological sciences. N.Y.: McGraw-Hill, 481 pp.

---

6. A 2 x 2 x 1 mm block of 1.5% Difco bacto-agar buffered at pH 4.9.