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# A Photographic Technique for Measuring Plant Section Growth

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## INTRODUCTION

When comparing the effects of growth regulators on the growth of etiolated plant sections it is desirable to measure not only the final lengths reached after some period of time, but also the rates of growth during that time. This problem has been approached in three ways.

Bonner and Foster (3) have sampled replicates at desired times during the period of growth. The numbers of sections and dishes required is high; individual sections cannot be followed; and, if the variability of growth is at all appreciable, precise determination of growth rates, particularly during short periods of time, is difficult or impossible.

Carroll (4) and Bennet-Clark and Kefford (1) have removed sections from the solutions on which they were floating, placed them on a glass plate, and measured them using red light and an ocular micrometer in a microscope. The number of sections and dishes required is minimal; but, although the growth of individual sections could be followed, this has not been done. The statement is made (1) that removal of sections from solutions for the short periods of time necessary does not affect the final lengths obtained; however such removal and the accompanying handling is not desirable, especially for delicate plant sections whose cells may be crushed easily.

Bentley and Housley (2) follow a procedure similar to the second described except that the plate of sections is placed in a photographic enlarger and the 4X enlarged images produced (with phototropically-inactive light) are measured with a ruler. The preceeding objections apply, although this method does obviate the necessity of straightening any curved sections if measured with a flexible ruler.

The author has been interested in measuring the growth rates of radish hypocotyl sections. Because of the variability of growth, the fragility of the sections and the tendency to curve markedly after 4 to 6 hours (see Figure 1B), especially in water or weak concentrations of regulators, none of the above methods of measuring growth rates seemed practicable and it was decided to try measuring photographs of sections growing in place in the dishes.

Use of red or infrared sensitive film was considered first; but, because of the problem of heat, especially with time exposures,

the cost of flash bulbs, and the success of using a speed-light, no attempts have been made to obtain photographs in this way. This is, however, being contemplated further and will be tried in the near future.

## Methods and Discussion

At the suggestion of Fred Kent of the S.U.I. Photographic Service a speed-light was tried as a light source. Experiments indicate that more flashes than required for growth rate determinations do not significantly alter the final lengths to which either radish hypocotyl or *Avena* coleoptile sections grow (Table 1); therefore use of this light during the growth period should not be objectionable.

The light source is a two-lamp speed-light, Model S 1169, with a 2000 volt D.C. output manufactured by the Electronic Laboratories, Inc., Indianapolis, Indiana. The duration of the flash approximates 0.0002 seconds; the intensity has not been measured, but is high; and the spectral quality is unknown, but the light appears to be quite white.

The menisci at the edges of sections floating in aqueous solutions pose a photographic lighting problem. Photographing dishes of sections on a glass plate over an illuminated piece of white filter paper solved the problem of menisci but was abandoned because of lack of contrast between sections and background. With reflected light low angle lighting is necessary to eliminate bright spots, but acceptable negatives with good contrast and little trouble from menisci are obtained.

**Table 1**

Effect of Light Flashes on Growth of Etiolated Plant Sections

| Expt. No. | Type Sections | No. Sec. L-D* | No. Light Flashes | Indoleacetic Acid | | Indoleaceto-nitrile | |
|---|---|---|---|---|---|---|---|
| | | | | Light† | Dark† | Light† | Dark† |
| 35 | Radish | 60-40 | 15 & 75 | 151 | 150 | 148 | 144 |
| 39 | Radish | 60-60 | 20 | 138 | 138 | | |
| 40 | Radish | 100-100 | 18 | | | 120 | 122 |
| 53 | Radish | 10-10 | 7 | 151 | 146 | 136 | 138 |
| 62 | Radish | 80-40 | 30 | 146 | 148 | 147 | 150 |
| 62 | Avena | 80-40 | 30 | 156 | 154 | 157 | 158 |

*First figure gives number of sections averaged for growth during exposure to light flashes; second, dark.

†Final section lengths in per cent of original length. Neither different experiments nor indoleacetic acid and indoleacetonitrile within any experiment should be compared with each other.

The problems of keeping the sections away from the Petri dish edges and separated from each other were solved by cutting rectangular holes in disks of 1/16 inch polyethylene sheet (see Figure 1). These are chemically inert and float low enough in

the water to be good separators. A standard polythylene section is placed in one of the holes for reference purposes.

Two types of apparatus are presently in use, one for photographing 12 dishes (Figure 2A) and the other, one dish per negative (Figure 2B). Both consist of wood frames with rectangular camera mounts to hold and automatically position the camera over black velveteen covered platforms on which the dishes are placed. The 12 dishes are covered with a plate of glass between photographs; the single dishes, with their regular covers. The camera mount for the close-up photos of single dishes slides over a rectangular opening into four positions, one directly over each of four dishes, which are photographed in succession, thus permitting measurements of four essentially simultaneous trials. The three dishes not being photographed at any one time are covered with black paper to minimize their exposure to light. 3¼ x 4¼ inch Panatomic-X film is used and developed with Microdol. Examples of single dish photographs are shown in Figure 1.

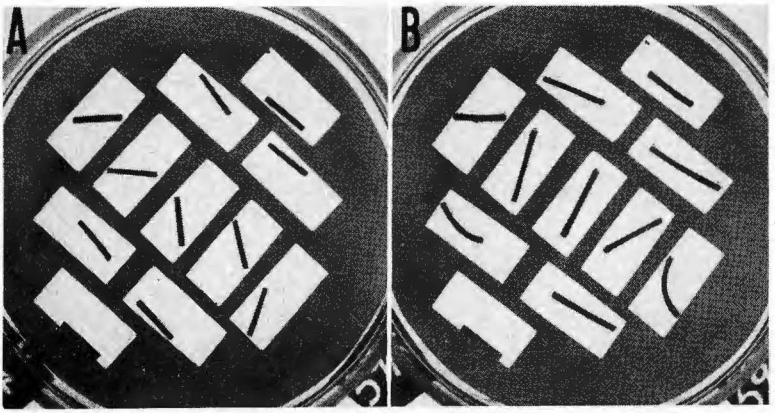


Fig. 1. A. Negative exposed at beginning of an experiment showing one dish per negative. B. Same exposed 12 hours after A.

A standard projector fitted with a 7½ inch anastigmatic projection lens is used to project the approximately 25X enlarged image of the finished negative on a wall. A sheet of paper on piece of masonite is positioned on the wall and the ends of the sections marked with a sharp pencil. If a section shows curvature in the plane of focus, the two marks are connected by a line through the center of the curved image. The distances between marks are measured with a flexible ruler.

Direct measurement of sections with a transparent ruler fitted with an end stop and lateral guide and mounted over an alluminated background gives an error of probably less than 1 per cent; and agreement between such measurements and those obtained from photographs is usually within 2 per cent, except for mark-

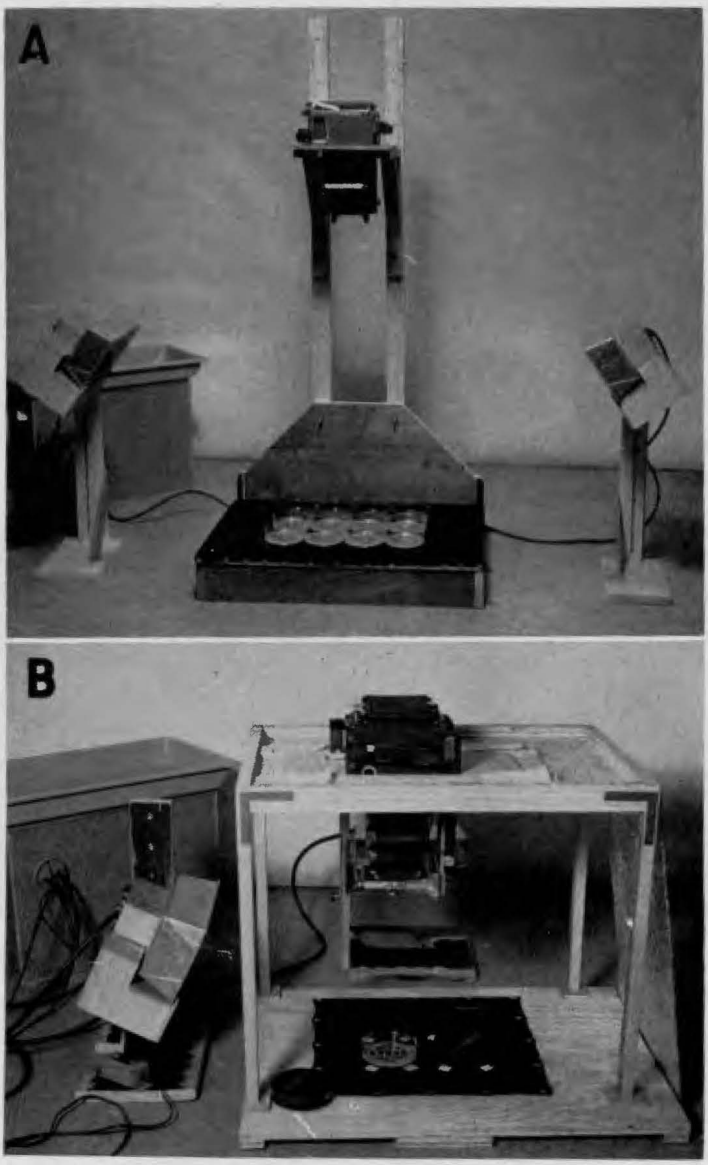


Fig. 2. A. Experimental set-up for photographing 12 dishes per negative. B. Same for one dish per negative.

edly curved sections where differences are as great as 6 per cent. In direct measurement curved sections are straightened against the lateral guide. Such sections often float center down—ends up; consequently accurate measurement photographically is not possible, and this technique should be limited to use with sections that do not become curved or to the early period of elongation before appreciable curvature takes place. That exceptionally small increments of growth can be followed accurately by photographing a single dish per negative is indicated by comparing triplicate measurements of 90 sections. Maximum disagreement was 0.5 per cent; average disagreement, only 0.12 per cent.

## Summary

1. Presently described techniques are inadequate, especially for short time growth rate measurements of etiolated plant sections.

2. A 0.0002 second flash of bright white light does not seem to alter the growth of radish hypocotyl or *Avena* coleoptile sections.

3. From projections of negatives made with the light flash, section length can be determined with sufficient precision to be used for short time growth rate measurements.

4. This technique is limited to use with relatively straight sections.

### Literature Cited

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