features on this grain are rounded, and this may be a result of glacial-fluvial action.

Characteristic glacial textures were universally observed on the glacially derived grains examined. Therefore we believe that these textures can be used as criteria for identifying glacial environments. It will probably be possible, with these techniques, to study surfaces thought to be consolidated glacial deposits in order to determine their precise origin (*3*).

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3. We express our appreciation to Prof. W. B. Crandall of the College of Ceramics at Alfred University and to Bud Kipfer of the Pfaudler Company for their technical advice. We also thank Walter Newman, Miles Silberman, and Aaron Rhodes for their help. This research was partially sponsored by a grant from the Petroleum Research Fund of the American Chemical Society and by the New York State College of Ceramics at Alfred University.
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## Geotropic Stimulation: Effects on Wound Vessel Differentiation

*Abstract.* Wounded excised shoots of *Coleus blumeni* (Benth.) were geotropically stimulated by rotation on a klinostat at 3 rev/min at right angles to the horizontal axis and placed alternately in inverted and upright positions at 24-hour intervals. These treatments resulted in the differentiation of significantly greater numbers of wound vessel members. The pattern of wound-vessel formation was altered by rotation on the klinostat but remained unaffected by 24-hour inversion.

The recent work of Goldsmith and Wilkins (*1*) supports the hypothesis that growth responses of geotropically stimulated shoots result from an asymmetrical distribution of auxin. Experiments by Lyon have indicated that gravity may play a role in auxin transport in the erect stem (*2*). Since Jacobs has shown that auxin is the limiting factor in the differentiation of wound xylem around a severed vascular bundle in stems of species of *Coleus* (*3*), it should be possible to employ wound-xylem strand formation as an indicator of both the pathway and the amount of auxin transported around a wound in geotropically stimulated shoots. The present experiments were undertaken to determine if geotropically stimulated wounded shoots would reveal a change in the number of wound-vessel members differentiated or in the pattern of wound-vessel formation (*4*, *5*).

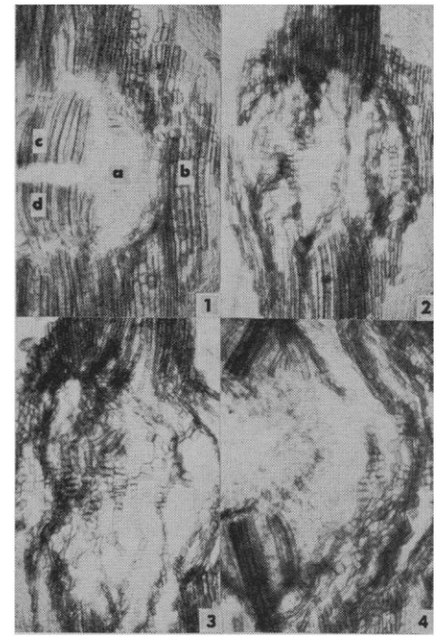
Vegetative single-axis shoots, approximately 10 cm in length, were excised from plants of a single clone of *Coleus blumeni* (Benth.). Leaves and axillary buds below the second internode were excised. The shoots were rinsed in an aqueous solution of calcium hypochlorite, immersed in distilled water for 5 minutes, and positioned with cotton plugs in test tubes containing distilled water. A water-tight seal was made between the stem and test tube with a mixture of beeswax and lanolin. A single lateral incision sufficient to sever a single main vascular bundle was made in the center of the second internode of each excised shoot. The shoots were then placed under one of the following conditions: an erect position for 7 days (controls), rotation for 7 days on a klinostat, rotation for 48 hours followed by 5 days in an erect position, rotation for 24 hours followed by 6 days in an erect position, or inversion once every 24 hours for 7 days. The shoots which were inverted once every 24 hours were inverted immediately after wounding and were rotated 90° once every 24 hours until the end of the 7-day experimental period. The axis of the klinostat was horizontal, and the test tubes containing the shoots were attached to the periphery of the machine at right angles to the axis. The klinostat was rotated at 3 rev/min in all the experiments. Each treatment was replicated three times. The experiments were conducted in the laboratory under continuous fluorescent illumination. The wounded internodes were excised at the end of the experimental period, cleared by a sodium hydroxide-chloral hydrate technique (*4*), and dissected under a stereoscopic microscope to reveal the differentiated wound vessels. The dissected preparations were stained with an aqueous solution of Safranin O and mounted in glycerin jelly. Counts were made of differentiated wound-vessel members from each dissected preparation.

The wound response from undisturbed shoots is composed of the follow-

Table 1. Summary of the effects of geotropic treatments on the differentiation of wound-vessel members.

| Plants (No.) | Mean o. No. of cells regenerated* | $t$ | $P$ |
|---|---|---|---|
| | *Control* | | |
| 18 | 716 ± 192 | — | — |
| | *Klinostat 7 days* | | |
| 13 | 1171 ± 375 | 3.792 | .005 |
| | *Klinostat 48 hr, 5 days erect* | | |
| 12 | 1046 ± 256 | 4.017 | .001 |
| | *Klinostat 24 hr, 6 days erect* | | |
| 16 | 1280 ± 178 | 9.4 | .001 |
| | *Inverted 24-hr cycles* | | |
| 18 | 905 ± 188 | 2.743 | .05 |

* The mean number of wound-vessel members regenerated is given together with the standard deviation.




Figs. 1–4. Photomicrographs of dissected wounded internodes of shoots of *Coleus*. All of the photomicrographs are oriented with the cicatrix on the left-hand side of the photograph. Fig. 1. Control of wounded undisturbed shoot showing dormant zone (*a*), zone of wound-vessel differentiation (*b*), and severed ends of main vascular bundle (*c*, *d*). The ends of the vascular bundle, severed by the incision, appear to be disengaged from the zone of wound vessel differentiation. This is an artifact arising from the clearing and dissection technique, and microtomed sections prepared in the usual histological manner indicate that differentiation proceeds basally from the end of the severed bundle at the morphologically upper side of the wound. Fig. 2. Rotation 48 hours, 6 days in erect position. Fig. 3. Rotation 24 hours, 5 days in erect position. Note in Figs. 2 and 3 the absence of the dormant zone and the altered distribution of wound vessel strands. Fig. 4. Inverted and placed upright alternately on 24-hour cycles. Note the presence of the dormant zone.

ing regions oriented successively from the surface inward: a cicatrix composed of oxidation products from dead cells, a dormant zone (Fig. 1*a*), a zone of cell division, and a zone of wound vessel differentiation (Fig. 1*b*). The dormant zone is represented by a multilayered region of morphologically unchanged pith parenchyma cells situated between the cicatrix and the zone of cell division (*6*). Previous research has indicated that the extent of the dormant zone can be experimentally modified by treating wounds with chemical inhibitors (*4*). The clearing and dissection technique has eliminated the cicatrix, and the zone of cell division is not visible in the photomicrographs.

Treatment by rotation on the klinostat and inversion resulted in increased numbers of wound-vessel members as compared to the controls (Table 1). Moreover, shoots treated by rotation on the klinostat exhibited differentiated wound-vessel members nearer the cicatrix with a complete absence of the dormant zone (Figs. 2 and 3). However, shoots which were alternately inverted and placed in upright position on a 24-hour cycle contained a dormant zone as in the controls (Fig. 4). The greatest increase in cells differentiated was observed when the cells were treated by rotation for 24 hours followed by 6 days in erect position. As the time of rotation was increased, the wound-vessel pattern and number of cells differentiated became more variable.

Brain (*7*) has demonstrated that rotation on a klinostat of preparations of *Lupinus albus* resulted in an increase in diffusible auxin. The present experiments lend support to this evidence. Recently Hoshizaki and Hamner (*8*) reported that flowering response of *Xanthium pennsylvanicum* Wallr. is decreased when the plant is rotated around a horizontal axis. Increased diffusible auxin and corresponding increased wound-vessel differentiation resulting from geotropic stimulation may possibly result from increased auxin synthesis, decreased auxin degradation, release of bound auxin, or increased basipetal or acropetal auxin transport. Increased peroxidase activity resulting from geotropic stimulation (*9*) may be involved in stimulating xylem differentiation (*10*). Also the polar transport mechanism of auxin has been shown to be disturbed by geotropic stimulation (*11*). The changed pattern of wound vessel differentiation in plants treated by klinostat rotation may result from an altered bioelectric potential at the wound surface. The experiments of Schrank have demonstrated that geotropic stimulation of coleoptiles of *Avena* altered the bioelectric potential and the pattern of auxin movement (*12*).

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22 October 1962

## The Fungus Beauveria tenella

*Abstract. Beauveria tenella,* an insect pathogen, grows well and sporulates freely in submerged culture. Enzymes that loosened bovine hair that were found in broth cultures were not produced in the presence of chitin, and substitution of peptone broth for peptone-glucose broth did not increase their concentration. Under certain conditions, oxalic acid was the main metabolic product in the peptone medium.

A culture of *Beauveria tenella* (Delacr.) Seim. (*1*) was isolated from laboratory air during a search for depilatory enzymes with unique properties. This organism produces enzymes which loosen the hair on animal hides, but the depilatory action was not particularly strong and did not differ, as far as we could tell, from that of enzymes produced by other fungi. In attempts to increase the production of depilatory enzymes various culture conditions were employed, and some interesting observations were made. MacLeod (*2*) has made an excellent study of the group of insect pathogens to which this fungus belongs.

Figure 1 shows the fruiting habit of the fungus when grown on an agar medium. Growth is very good in shake flasks on common media such as glucose-peptone-mineral salts solution. However, yeast extract and corn steep liquor increase the growth rate. The *p*H of such media decreases with growth and may fall to about 3.5 unless the solution is buffered. The optimum temperature for growth is about 28° to 30°C; growth was noticeably slower at 25°C, and there was no growth at 40°C. Conidia are readily produced in submerged culture. If calcium carbonate is added to the medium after 1 or 2 days' growth in a shake flask, the production of conidia becomes profuse. Newly formed and germinating

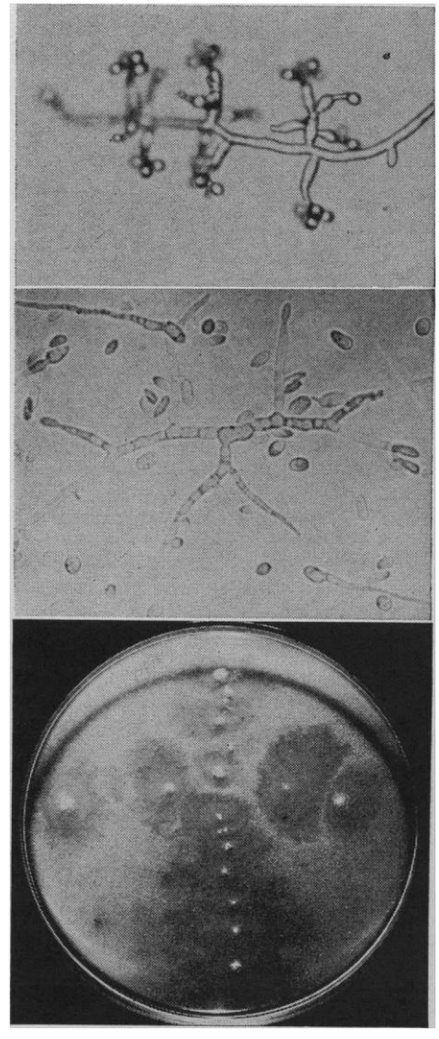

Fig. 1. *Beauveria tenella.* (Top) Conidiophores and conidia formed an agar medium (about × 760). (Middle) Concurrent spore germination and fruiting in submerged culture (about × 343). (Bottom) Chitin decomposition in an agar medium in a petri dish. The fuzzy white dots are mycelia with conidia and the dark areas are regions where the chitin has been digested (about × 0.59).