Geotrophic Response of Coleoptiles under Anaerobic Conditions

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Summary. Coleoptiles of *Avena sativa* and *Zea mays* do not develop a geotropic response under anaerobic conditions.

It has been reported recently that coleoptiles of *Zea mays* develop a geotropic response under anaerobic conditions (2). The geotropic response of a coleoptile arises from the differential growth of the upper and lower halves of the organ. At least 3 independent investigations have shown that in *Avena sativa* coleoptiles growth is totally arrested in the absence of O₂ (1,6,9). It is therefore difficult to reconcile the inhibition of growth under anaerobic conditions with the reported development of geotropic curvature. The geotropic responses in coleoptiles of *Zea mays* and *Avena sativa* under anaerobic conditions have therefore been re-examined in an attempt to clarify this confused situation.

Materials and Methods

After an initial soaking in distilled water, seeds of *Avena sativa* L. var. Svalof and *Zea mays* L. var. Giant Horse Tooth were germinated and grown in moist vermiculite in darkness at 25°. The coleoptiles were harvested after 4.5 days in the case of *Avena* and after 6 days in the case of *Zea*.

The experiments were conducted on excised coleoptiles from which the primary leaf had been removed. Special precautions were necessary to ensure the removal of O₂ from the coleoptile cavity. This was achieved by mounting the coleoptiles on hollow glass needles which were attached to a plexiglass strip. The basal ends of the coleoptiles made contact with rectangular pieces of moist filter paper surrounding the bases of the needles. Fourteen coleoptiles, each about 1.5 cms long, were mounted in this way and then the plexiglass strip was fixed in a rectangular, gas-tight, plexiglass chamber, which could be orientated in various directions with respect to gravity. The general arrangement of the coleoptiles in the chamber is shown in figure 1. The chamber was connected to the anaerobic evacuation and flushing system that has been used and described by Wilkins and Martin (12).

The experiments were carried out at 20° in normal laboratory conditions of diffuse daylight. The following procedure was adopted. The coleoptiles were harvested and mounted in the chamber in the vertical position. The chamber was then flushed with O₂-free N₂ for 1 hour. During the first 30 minutes of this flushing period the chamber was partially evacuated

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Fig. 1. Longitudinal section through the plant chamber (A) showing the arrangement of the coleoptiles (E) on the hollow glass needles (D) which were fixed to the plexiglass strip (B). Moist filter paper (C) made contact with the base of the coleoptiles. The chamber was evacuated and flushed through tubes F and G.
to 10 cm of mercury and then refushed with \textit{N}_2 on 6 occasions. At the end of the flushing period of 1 hour the chamber was rotated through 90° so that the coleoptiles were brought into the horizontal position. Nitrogen continued to flow through the chamber for as long as anaerobic conditions were required. A control sample of coleoptiles was prepared in a similar way and kept under aerobic conditions.

To transfer the coleoptiles from anaerobic to aerobic conditions the chamber was merely flushed with air. When this change in O\textsubscript{2} tension occurred, a further sample of coleoptiles was prepared and geotropically stimulated in air to act as a control. This control enabled a comparison to be made between the rate of curvature of the coleoptiles that had been subjected to prolonged anaerobic conditions before being transferred to air, and that of freshly harvested coleoptiles.

Curvature of the coleoptiles was recorded by means of shadowgraphs on photographic paper. Each experiment has been carried out on 4 occasions and in each case almost identical results have been obtained. The 2 experiments reported in detail in this paper gave typical results.

Results

Under anaerobic conditions detached, deleafed coleoptiles of \textit{Avena sativa} and \textit{Zea mays} show no geotropic curvature, even after being in the horizontal position for 15 hours (figs 2, 3). During this time coleoptiles kept in air developed a large upward curvature. The coleoptiles were obviously not damaged by being deprived of O\textsubscript{2} for 15 hours because on the readmission of air to the chamber they rapidly developed an upward curvature. In the case of \textit{Avena sativa} this curvature occurred at approximately the same rate as in freshly harvested coleoptiles (fig 2). On the other hand, in all 4 experiments with \textit{Zea mays} coleoptiles the rate of curvature of the coleoptiles previously exposed to anaerobic conditions was slightly greater than that of freshly harvested coleoptiles (fig 3).

Discussion

Our experiments clearly show that coleoptiles of \textit{Zea mays} and \textit{Avena sativa} do not respond to geotropic stimulation under anaerobic conditions. This result would have been anticipated on the findings by Ball and Dyke (1), Harrison (6) and Wilkins and Warren (9), that growth is totally inhibited in coleoptiles deprived of O\textsubscript{2} for more than 1 hour.

The results presented in this paper are in direct conflict with those reported by Dedolph, Breen, and Gordon, who found that under anaerobic conditions a curvature of approximately 17° developed after 4 hours geotropic stimulation (2). It is somewhat difficult to explain why these 2 investigations should have yielded different results. Dedolph et al. (2)
used intact seedlings which had been grown in red light. The coleoptile cavity would therefore contain a green leaf capable of releasing O\textsubscript{2} when exposed to photosynthetically active light. Perhaps the most likely explanation for the curvature developed in the experiments of Dedolph et al. (2) is that the O\textsubscript{2} concentration in the coleoptile cavity was somewhat higher than in the surrounding atmosphere. The higher concentration in the cavity could have arisen by photosynthesis occurring in the primary leaf while the seedlings were being grown, and manipulated during the course of the experiment, when they were exposed to a radiant flux of 1.6 $\times$ 10\textsuperscript{4} ergs cm\textsuperscript{-2} sec\textsuperscript{-1} in the red region of the spectrum. Although our experiments were carried out in normal laboratory illumination in which some photosynthesis might have been expected, the leaf had been removed from the coleoptile cavity. This complication had therefore been eliminated in our investigation, and, in any event, no curvature was ever observed under anaerobic conditions.

Dedolph et al. (2) found in their experiments that the development of geotropic curvature under anaerobic conditions was not accompanied by the development of the geoelectric effect. The lack of geoelectric effect under anaerobic conditions has also been found by Grahm and Hertz (4) and Woodcock and Wilkins (unpublished). If the results of Dedolph et al. (2) for the geotropic response can be ascribed to the presence of O\textsubscript{2} in the cavity of the coleoptile, they raise the interesting possibility that either the geotropic and geoelectric responses are differentially sensitive to oxygen tension or that the geoelectric response is more closely associated with the outer epidermal cells of the coleoptile than with the other cells of the organ.

Bearing in mind the consistency between the results in this paper and those of Ball and Dyke (1), Harrison (6) and Wilkins and Warren (9), the fact that we cannot detect a geotropic response in coleoptiles under anaerobic conditions makes it impossible for us to accept the views of Dedolph et al. (2) on the lack of a direct relationship between the geoelectric and geotropic responses. There is a large amount of evidence which shows that both phenomena occur as the result of a lateral gradient in auxin concentration being set up in the organs (3, 4, 5, 8, 11). The finding of Grahm and Hertz (5) that the geoelectric effect develops in Zea coleoptiles after a single decapitation is not evidence for the occurrence of the geoelectric effect in the absence of auxin. A physiological tip would have developed in the 5-hour rest period between decapitation and the beginning of electrical measurements (8). Grahm (3) has indeed shown that the capacity to develop a geoelectric effect is lost in freshly decapitated coleoptiles, but returns after a few hours.

The development of a geoelectric effect but not a geotropic response in the experiments of Grahm and Hertz (5) is most probably due to the different rates at which the 2 phenomena develop. In non-decapitated coleoptiles the geoelectric effect develops rapidly, reaching a maximum value after approximately 30 minutes (2, Woodcock and Wilkins, unpublished). Geotropic curvature, on the other hand, develops much more slowly, especially in coleoptiles grown in red light (7, 10), as were those used by Grahm and Hertz (4, 5). The difference between the rates of development of the geoelectric effect and the geotropic response could well be accentuated in decapitated coleoptiles which had developed a physiological tip.

Literature Cited