Geotropism in Corn Roots: Evidence for Its Mediation by Differential Acid Efflux

Abstract. *The elongation zone in intact growing corn roots secretes acid leading to a reduced* pH *along the surface of the root and in the adjacent medium. This can be detected by placing the root on an agar medium containing the* pH *indicator dye bromocresol purple. When the root is treated with a growth inhibitory concentration of the hormone indole-3-acetic acid, the acid efflux is reversed and growth is greatly retarded. When the root is mounted vertically, acid secretion is uniform along the elongation zone, and the root grows straight downward. When the root is placed horizontally, there is enhanced acid efflux along the upper surface of the elongation zone and reduced acid efflux along the lower surface. An increased rate of elongation of the upper cells relative to the lower cells then results in downward curvature of the root. The correlation between acid efflux patterns and growth patterns indicates that proton efflux is important in the control of root growth.*

There is increasing evidence that the control of plant cell elongation by the hormone indole-3-acetic acid (IAA) (*1*) is mediated, at least in part, by control of hydrogen ion movement from the protoplast to the cell wall. This idea is supported by evidence that (i) exogenously applied acid stimulates the growth of stems and roots, (ii) promotion of growth by IAA in stems and coleoptiles is preceded by enhanced efflux of acid from the cells, and (iii) inhibition of root growth by IAA is preceded by inhibition of hydrogen ion efflux from the cells of the elongation zone (*1*). Nevertheless, the hypothesis that acid controls IAA action has been questioned; because much of the evidence was obtained by use of excised tissue segments from which the surface cells had been removed to facilitate hydrogen ion movement, the possibility of wound-induced alteration of tissue physiology arises.

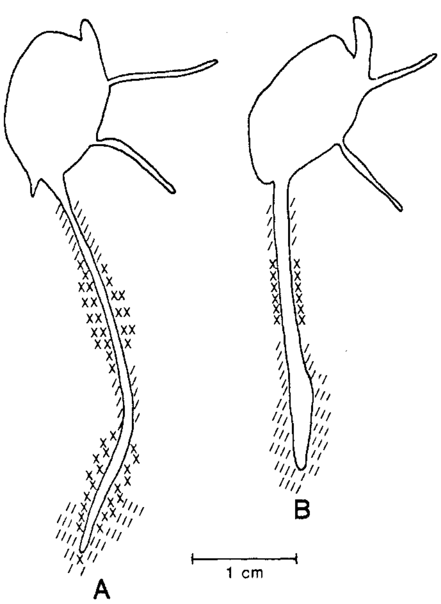

Fig. 1. Surface *p*H patterns on the primary root of corn 2 hours after the root is placed on an agar-indicator dye medium in the (A) absence or (B) presence of a growth inhibitory concentration (2 μM) of IAA. Regions of high *p*H (purple on the original plates) are indicated by slashes. Regions of low *p*H (yellow on the original plates) are indicated by X's. In the absence of IAA, the root grows rapidly, and a bright yellow region develops along the elongation zone, an indication that hydrogen ion efflux is occurring there (A). In the presence of IAA, root growth is strongly inhibited, and a purple region develops along the elongation zone, indicating that hydrogen ion efflux has been reversed and the *p*H is increasing (B). The yellow zone near the base of the root in (A) and the yellow zone remaining after IAA treatment in (B) are associated with the root hair zone (*4*).

By using a *p*H indicator dye to detect surface *p*H patterns (*2*), we can visualize hormonal changes and environmentally induced changes in *p*H patterns on the surface of intact unaltered roots. These changes in *p*H patterns are correlated with changes in growth patterns in a manner consistent with the acid hypothesis of growth regulation.

Surface *p*H patterns on corn roots were visualized by the method of Weisenseel *et al.* (*2*). Three-day-old seedlings of corn (*Zea mays* L. Bear Hybrid WF 9 × 38) are placed on an agar medium containing the *p*H indicator dye bromocresol purple (*3*). The agar medium is adjusted to *p*H 5.0 during preparation. At *p*H 5.0 the agar-dye medium is orange. At *p*H's less than 5.0 the medium becomes yellow, and at *p*H's above 5.0 the medium becomes red to purple. Within 3 to 8 minutes after the seedling is placed on the agar surface, the *p*H pattern along the root surface can be seen (Fig. 1A). A bright yellow zone develops in a position corresponding to the region to cell elongation (*4*), an indication that acid efflux is occurring there. In contrast, a strong purple color develops along the region of cell maturation behind the elongation zone and around the meristematic region at the tip of the root, an indication that the *p*H increases in those zones. Thus the elongation zone appears to secrete acid while the cells on either side of the elongation zone appear to take up acid (*5*). A minor exception to this pattern is frequently observed at the extreme tip of the root, where a small circular yellow zone appears within the purple halo surrounding the root tip. This may be due to secretion of root cap slime, which contains acidic polysaccharides, or it may represent a localized zone of H^+ efflux at the extreme root tip.

When the root is placed on an agar-dye medium containing a growth inhibitory concentration (2 μM) of IAA, the growth of the root is severely retarded, and the yellow region adjacent to the elongation zone fails to develop. Instead the indicator dye turns purple along the entire apical portion of the root, indicating that acid uptake is occurring in the elongation zone as well as the root tip and the region of cell maturation (Fig. 1B). Thus inhibition of root growth by IAA is accompanied by an apparent reversal of the flow of H^+ ions from the elongation zone (*6*).

As a further test of the correlation between surface *p*H patterns and elongation, roots growing on agar-dye plates were exposed to a geotropic stimulus, and changes in *p*H patterns were observed before and during geotropic curvature. The plates were mounted verti-

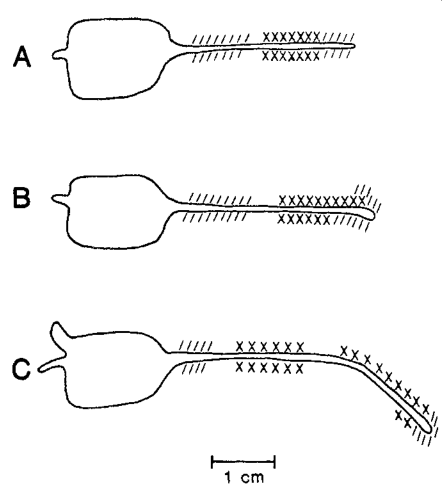

Fig. 2. Sequence of changes in surface *p*H patterns on the primary root of corn exposed to a geotropic stimulus. Regions of high *p*H (purple on the original plates) are indicated by slashes. Regions of low *p*H (yellow on the original plates) are indicated by X's. Times elapsed after the root is placed on the agar-dye in the horizontal position are (A) 2 minutes, (B) 20 minutes, and (C) 120 minutes. When the root is first placed in a horizontal position, the yellow (acid) region develops uniformly along the elongation zone (A). After 10 to 20 minutes, the yellow zone intensifies and extends toward the tip on the upper surface, but contracts and withdraws from the tip on the lower surface (B). This increased acidification on the top and decreased acidification on the bottom is followed by geotropic curvature (C).

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cally, with the root oriented perpendicular to the direction of gravity. The initial pattern of coloration was the same as that in vertically mounted roots, that is, yellow in the elongation zone and purple elsewhere (Fig. 2A). After the root had been held in the horizontal position for about 10 to 20 minutes, this pattern began to change; the yellow zone on the upper surface of the root intensified and extended further toward the tip while, on the lower surface of the root, the purple region of the tip began to encroach on the yellow region of the elongation zone, reducing its length and intensity (Fig. 2B). Shortly thereafter the root began to curve downward, showing typical positive geotropic curvature as the yellow zone on the upper surface increased in intensity (Fig. 2C). Time-lapse movies of these events clearly show the sequence of shifts in color patterns preceding the initiation of curvature.

These data are relevant to the acid efflux hypothesis of growth regulation since they show that (i) the region of acid efflux coincides with the region of cell elongation in an undisturbed intact organ (ii) hormonal inhibition of growth in the elongation zone of roots is correlated with hormonal reversal of H^+ efflux from these cells, and (iii) predictable changes in H^+ efflux patterns accompany geotropically induced shifts in growth patterns. Thus the H^+ efflux patterns are closely tied to the growth of the organ (*7*) rather than to some other physiological function such as ion uptake.

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References and Notes

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2. M. H. Weisenseel, A. Dorn, L. F. Jaffe, *Plant Physiol.* **64**, 512 (1979).
3. The medium consists of a 4-mm-thick plate of 0.6 percent agar containing 0.71 m*M* bromocresol purple plus the following inorganic nutrients: 1.5 m*M* $Ca(NO_3)_2$; 1 m*M* each of $MgSO_4$, KH_2PO_4, and KNO_3; 20 μ*M* H_3BO_3; 3.8 μ*M* $ZnCl_2$; 0.18 μ*M* MoO_3; and 0.14 μ*M* $CuCl_2$. The primary root is pressed lightly into the medium so that about half of the cylindrical root is embedded. Under these conditions the roots grow at a normal rate (1.5 to 2.5 mm per hour) during the entire course of the experiment (up to 48 hours).
4. The region of cell elongation is determined by marking the root surface with India ink at 1-mm intervals and observing the displacement of the marks during elongation. This method shows that the elongation zone extends from a point 2 to 3 mm from the tip to about 10 mm from the tip, the most vigorous elongation being in the region extending from about 2 to 7 mm from the tip. As the root grows along the agar-dye medium it leaves a yellow trail, which is caused by acid efflux from the elongation zone. There is also a region of acidity adjacent to the root hair zone. This accounts for the second yellow zone in some of the figures, for example in Figs. 1A and 2C; IAA appears not to affect H^+ efflux from the root hair zone.
5. By this method we measure only *p*H change and cannot distinguish between effects caused by H^+ uptake and OH^+ efflux. However, Weisenseel *et al.* (*2*) measured *p*H shifts and electrical currents in barley roots and concluded that the observed electrical currents were due primarily to flow of H^+; they reported that the direction of H^+ efflux is *into* the elongation zone of barley roots, the opposite of what we report for corn roots. We have examined acidification patterns in barley roots and find the same pattern of coloration as that reported by Weisenseel *et al.*: purple at the tip and yellow farther back. However, we find that the zone of active elongation is in the yellow (most acidic) region, not near the purple (less acidic) tip.
6. Experiments showing apparent acidification and auxin reversal of acidification in the elongation zone of growing roots were done at least 200 times. Experiments showing unilateral acid efflux during geotropism in growing roots were done 80 times.
7. Unilateral hydrogen ion efflux also accompanies positive geotropism in shoots (preferential acid efflux on the lower side of geostimulated corn coleoptiles) as well as positive phototropism (preferential acid efflux on the dark side of unilaterally illuminated sunflower hypocotyls). This indicates that differential hydrogen ion efflux may be generally involved as a mediator of tropistic responses in plant organs.
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Meprobamate Reduces Accuracy of Physiological Detection of Deception

Abstract. *Normal male subjects attempted to deceive an experimenter recording electrodermal, respiratory, and cardiovascular activity. Those who had ingested a placebo or nothing were detected with statistically significant frequency on the basis of their phasic electrodermal responses, which clearly distinguished them from truthful suspects. That was not the case with deceptive subjects who had ingested 400 milligrams of meprobamate, nor did the examiner detect which subjects had received the drug.*

The psychophysiological detection of deception depends upon the subject's having larger physiological reactions to questions associated with deception than to control questions (*1*). Despite both laboratory support for the basic premises underlying the procedure and its widespread use in police investigations and personnel screening, the validity and reliability of the polygraph test have yet to be established and remain a subject of controversy (*2, 3*).

We have investigated the detection of deception both as a practical problem and as a model for studying social stress (*1*). One question that is important for both purposes is whether a tranquilizer selectively reduces the physiological response to social stress—in this instance, the stress of attempting to deceive. Professional polygraphers have assumed that tranquilizers might reduce the physiological response to all test questions as part of a general reduction in tonic arousal levels but that the difference in reactivity to critical and control items would be unaffected (*4, 5*). Clinical and pharmacological views of tranquilizers, however, suggest otherwise (*6*); that is, the effect of a tranquilizer might be precisely to reduce the physiological correlates of fear or anxiety concerning the critical questions.

Empirical evidence for either view is sparse (*7, 8*). Antianxiety drugs have been shown to reduce the electrodermal response (EDR) to some stressful stimuli, such as anticipation of shock, "emotional" words, or riding a Ferris wheel (*9*), but their effects on the EDR to more common and natural social stressors such as interpersonal conflict have not been investigated.

We report here a double-blind test of the effects of a tranquilizer, meprobamate, on polygraph test results (*10*). It was hypothesized that the EDR would accurately discriminate between truthful subjects and deceptive subjects who had not ingested a tranquilizer, as in previous studies (*1*); that deceptive subjects who had taken a placebo would also be accurately discriminated from truthful subjects and would not differ from deceptive subjects who had taken no drug; and that deceptive subjects who had ingested a tranquilizer would not be discriminated from truthful subjects by their EDR's but would be discriminated from no-drug and placebo-treated deceptive subjects. We also tested whether the experimenter could judge which subjects had ingested a tranquilizer, because it has been suggested (*4, 5*) that a tranquilizer would produce overt effects that would be readily discernible to the experienced examiner.

Each subject (*11*) was randomly assigned to either guilty (N = 33) or innocent (N = 11) conditions (*12*). Guilty subjects completed an overlearning task that ensured their sensitization to six common words and were told it would later be their task to convince a polygraph operator that they had not memorized any words. Guilty subjects were randomly assigned on a double-blind basis to one of three groups. Subjects who ingested a pill were told that they were being given a tranquilizer that would

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