EXPANSION OF *CHENOPODIUM ALBUM* LEAF DISKS
AS AFFECTED BY COUMARIN

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(WITH ONE FIGURE)

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Although leaf expansion is obviously a fundamental process in the development of plants, its physiological mechanism is little understood, and has been but sparingly investigated.

It is known that auxins affect the elongation of the leaf veins (1) but do not seem to influence the expansion of the laminar leaf tissues. Bonner et al. (3) observed an increase in expansion of radish leaf disks floated on solutions of adenine. Bonner (2) also found potassium nitrate to be effective in promoting expansion and (private communication) believes the salt to have both a potassium and a nitrate effect. de Ropp found several substances, including adenine, to be without effect on elongation of first leaves of isolated rye stem tips (4), and several substances to be without effect on the final dry and wet weights of cabbage leaf disks (5). Various extracts and solutions injected into the hollow petioles of squash plants did not increase the expansion of the leaves in the dark (7).

In an effort to achieve a better understanding of the physiology of leaf expansion, a number of growth inhibitors have been tested. Coumarin is one of these compounds. Coumarin affects Avena root elongation (6), elongation of Avena coleoptile sections, and curvature of split pea stem internodes (8). In the papers cited, emphasis has been placed on growth inhibition by the compound. However, in the latter work, increases in elongation of Avena coleoptile sections were obtained at concentrations lower than $10^{-4}$ M ($10^{-4}$ M is approximately equal to 15 p.p.m.). In the work reported here, coumarin in concentrations ranging from 1 to 200 p.p.m. has been found to increase expansion of leaf disks taken from *Chenopodium album* plants growing outdoors during July, August, and September, 1950.

Disks, 5.0 mm. in diameter, were cut with a cork borer from immature leaves having areas of approximately 250 sq. mm. The largest leaves on the plants were about eight times this size. Two disks were cut from each leaf, one from the basal section on each side of the midvein. A segment of a main lateral vein constituted a diameter in each disk. Sixteen disks with lower epidermis up were floated on 10 ml. of the various solutions in Petri dishes. The dishes were placed in the dark and kept at $25 \pm 1^\circ$ C. The check medium contained 4% d-glucose by weight and KNO$_3$ at 0.2 M. In preliminary experiments, these concentrations were found to be optimal. The pH of each solution was 5.6. At the end of the test period, the disk diameters, perpendicular to the segments of lateral veins, were remeasured.

1 Papers from the Department of Botany, The Ohio State University, no. 524.

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The effect of coumarin at various concentrations is shown graphically in figure 1. Similar curves have been obtained in several experiments.

The increase in diameter was less at 200 than at 100 p.p.m. Probably expansion would be inhibited at higher concentrations. However, even at 200 p.p.m. "toxic" effects were noticeable, the tissues becoming soft and brown. For this reason the effects of higher concentrations were not investigated.

The expansion of the leaf disks was accelerated by coumarin at concentrations (15-200 p.p.m.) at which elongation of Avena roots (6), elongation of Avena coleoptile sections, and curvature of split pea stem sections (8) were reduced. This difference may not be significant, however, since the experimental conditions of the various workers were not identical. Also, the difference may be partially explained by the low wettability of the leaf disks. Thus the amount of coumarin entering the disks may have been rather small. This low wettability apparently made the disks less susceptible to attack by microorganisms and therefore more desirable as test objects than disks cut from other kinds of leaves.

A coumarin effect, although small, was found when either d-glucose or KNO₃ was omitted from the medium (table I). Apparently the action of coumarin is not to increase the permeability to either glucose or KNO₃ alone. The effect was greatest when both the salt and sugar were present. The results in table I are for one experiment only, but similar results have been obtained in two other experiments.

The d-glucose could be replaced by sucrose. KNO₃ could be at least partially replaced by KCl, NaNO₃, or Mg(NO₃)₂ but not by Ca(NO₃)₂.

At equivalent molar concentrations, KNO₃ was more effective than either...
TABLE I

RELATIVE INCREASES IN DIAMETER OF Chenopodium album LEAF DISKS
FLOATED ON SOLUTIONS OF VARIOUS COMBINATIONS OF 4% D-GLUCOSE, 0.2 M KNO₃, AND COUMARIN (50 P.P.M.)

<table>
<thead>
<tr>
<th>Solution</th>
<th>Relative increase in diameter after two days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>100 ± 2.6*</td>
</tr>
<tr>
<td>Coumarin</td>
<td>105 ± 3.9</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>117 ± 3.5</td>
</tr>
<tr>
<td>D-Glucose + coumarin</td>
<td>146 ± 4.4</td>
</tr>
<tr>
<td>KNO₃</td>
<td>146 ± 3.5</td>
</tr>
<tr>
<td>KNO₃ + coumarin</td>
<td>170 ± 4.9</td>
</tr>
<tr>
<td>KNO₃ + D-glucose</td>
<td>200 ± 6.1</td>
</tr>
<tr>
<td>KNO₃ + D-glucose + coumarin</td>
<td>319 ± 4.4</td>
</tr>
</tbody>
</table>

*Standard error.

KCl or NaNO₃. Thus Bonner's separation of the potassium and nitrate effects seems confirmed.

Summary

Coumarin (1–200 p.p.m.) increased the expansion of Chenopodium album leaf disks floating on a basic solution of d-glucose and KNO₃. A coumarin effect, although small, was found when either d-glucose or KNO₃ was omitted from the medium. The glucose could be replaced by sucrose. The KNO₃ could be at least partially replaced by KCl, NaNO₃, or Mg(NO₃)₂ but not by Ca(NO₃)₂. KNO₃ was more effective than either KCl or NaNO₃ in increasing the amount of expansion of the leaf disks.

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LITERATURE CITED

