BRIEF PAPERS

EFFECT OF 2,4-DICHLOROPHENOXACETIC ACID AND OTHER GROWTH-REGULATORS ON THE FORMATION OF A RED PIGMENT IN JERUSALEM ARTICHOKE TUBER TISSUE.1

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In the course of investigations concerning cellular growth in Helianthus tuberosus (Jerusalem artichoke) tuber discs, it was noted that 2,4-dichlorophenoxyacetic acid (2,4-D) induced the formation of a red pigment which was clearly evident only when the discs and the ambient culture solution were brought into prolonged contact with a cellulose substrate such as filter paper or cheese cloth. The 2,4-D acid used was triply recrystallized to minimize contamination by 2,4-dichlorophenol. Attempts to produce the pigment in greater quantity resulted in finding that tuber slices approximately 250 microns thick, incubated on filter paper impregnated with 2,4-D for 24 to 48 hours, gave a maximum pigmentation. Although the pigment was visible directly in the tissue, most of the red color appeared on the filter paper, which acted as a reservoir on which the pigment was strongly adsorbed.

Localization of pigment formation was evident when slices cut normal to the tuber axis were incubated with 10 mg/l 2,4-D on filter paper. Maximum pigment production was coincident with the occurrence of the vascular bundles. Microscopic examination of the sections showed strong red pigment coloration in the phloem and xylem elements suggesting a relationship with the phenolphenolase system which is localized in the peripheral and vascular tissue of Jerusalem artichoke according to Belval and Legrand (1).

Pigment formation is obligately aerobic as indicated by the complete lack of red color when tuber slices were incubated with 2,4-D in an atmosphere of purified nitrogen. Experiments with dark-prepared 2,4-D-treated tuber slices exposed to red, far-red, and unfiltered radiation as well as continuous darkness showed equal pigment formation with all treatments, indicating no photoperiodic response.

The pigment was only very slightly water-soluble and rapidly turned brown when so removed from the stabilizing influence of the filter paper. Moderate solubility was obtained with methanol, and greatest solubility was attained with pyridine and dioxane. Reflectance measurements of the red impregnated filter paper using the G. E. Hardy spectrophotometer showed absorption maxima at 540, 510, and 460 m\(\mu\). Optical density data from methanol and pyridine extracts of the pigment were obtained with a Beckman DU quartz spectrophotometer. In methanol, absorption maxima were at 518, 485, 455 (weak) and 340 m\(\mu\), and in pyridine the maxima were 526, 490, 455 (weak), and 340 m\(\mu\). Browning of the extracts occurred if water was not excluded, with a loss in absorption at the peak near 520 and an increase at 340 m\(\mu\). Heating methanol or pyridine extracts of the pigment with zinc caused a reduction to a light amber color which could be oxidized back to red with shaking in air. No acid-base color reversal could be obtained.

One of us (C. E. H.) has earlier noted a red color in the vascular tissue of a number of plant species and an unknown fungus treated with 2,4-D. Because extraction of this color has not been successful it cannot be compared with the present pigment.

A pigment which was stimulated by copper, coumarin, and 2,4-D was extracted from filter paper upon which Big Boston and Grand Rapids lettuce seeds were germinated. Pigmentation was most intense in the vascular tissue of the seedlings, and expression of the pigment was greatest in weakly germinating seeds. A pyridine solution of the pigment showed an absorption maximum at 525 m\(\mu\). The data indicate that the red pigments of Jerusalem artichoke tubers and germinating lettuce seeds are the same, and that their formation is similarly responsive to chemical stimuli. Further evidence of the pigment has been found in germinating Taraxacum officinale and Helianthus annus but not in Lamium amplexicaule, Lepidium virginicum, Barbarea verna, or Agropyron tenerum when treated with copper or 2,4-D.

Transverse sections of the etiolated stems of Helianthus tuberosus treated with 2,4-D showed strongly stained phloem and xylem elements, the former being more intensely stained than the latter.

The Effect of Several Plant Growth-regulating Compounds on the Formation of Red Pigment in Jerusalem Artichoke Tuber Tissue

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>Color Intensity</th>
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</thead>
<tbody>
<tr>
<td>Indole-3-acetic acid</td>
<td>...............</td>
<td>10 ++</td>
</tr>
<tr>
<td>2,4-Dichlorophenoxyacetic acid</td>
<td>...............</td>
<td>5 +++</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>...............</td>
<td>10 ++++</td>
</tr>
<tr>
<td>2,4-D (tissue-crushed)</td>
<td>...............</td>
<td>10 0</td>
</tr>
<tr>
<td>2-Methyl-4-chlorophenoxyacetic acid</td>
<td>...............</td>
<td>10 +++</td>
</tr>
<tr>
<td>2-Methyl-4-chlorophenoxyacetic acid</td>
<td>...............</td>
<td>10 +++</td>
</tr>
<tr>
<td>2,4,5-Trichlorophenoxyacetic acid</td>
<td>...............</td>
<td>10 +++</td>
</tr>
<tr>
<td>Distilled water</td>
<td>...............</td>
<td>+</td>
</tr>
</tbody>
</table>

* 0—no pigment.
+—slight red color.
++—light red color.
+++—moderate red color.
++++—intense red color.

1 Received February 9, 1956.
2 Present address: Department of Agronomy, South Dakota State College, Brookings, South Dakota.

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From the text, we have the following information:

- **anthus tuberosus, H. annuus, and Xanthium strumarium**, when incubated on filter paper impregnated with 10 mg/l 2,4-D, formed the red pigment in the vascular tissue. Tissue from tobacco stems, sugar beet petioles and roots, and white potato tubers did not produce the pigment. The presence of the pigment is indicated to be extensive in the Compositae.

- The data in table 1 show the effect of optimal concentrations of several plant growth-regulators and herbicides on the red pigment formation. Indole-3-acetic acid (IAA) was active, but less so than 2,4-D. 2-Methyl-4-chlorophenoxyacetic acid, 2-methyl-3-chlorophenoxyacetic acid, and 2,4,5-trichlorophenoxyacetic acid were all approximately equal to 2,4-D in promoting the red pigment formation. Substitution in the 6 position on the ring with chlorine, as in 2,6-dichlorophenoxyacetic acid and 2,4,6-trichlorophenoxyacetic acid, resulted in greatly decreased activity, in agreement with the ortho theory of Muir, Hansch, and Gallup (2).

- Copper ion (10 mg/l) enhanced expression of the pigment as did an equivalent concentration of 2,4-dinitrophenol, but 10 mg/l KCN or sodium diethyl-dithiocarbamate inhibited the pigment. Greater pigment formation was obtained with 10 mg/l 2,4-D than with copper or dinitrophenol at optimal concentrations. Treatment of thin slices or very small dice of the artichoke tubers produced the color, but when the tissue was ground or crushed no red color was induced by 2,4-D. Pigment enhancement by 2,4-D was not inhibited by hypertonie (0.4 M) mannitol but was effectively stopped by 10⁻⁴ M arsenite which may indicate that the production of the pigment is associated with metabolically controlled growth processes.

- The precise chemical nature and physiological properties of the pigment, as well as the mode of action of 2,4-D in its formation, are not known. Its spectrophotometric characteristics and reaction to oxidation-reduction conditions suggest a phenolic or quinonoid structure, and it appears to be an intermediate in a browning reaction.

The authors are grateful to the National Bureau of Standards for aid in obtaining the reflectance data.

**Literature Cited**


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**ABSORPTION OF COBALT⁶⁰ BY LEAVES OF BEAN PLANTS IN THE DARK¹²³**

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In a recent publication (1) the senior author has pointed out that 2-week-old Black Valentine bean plants that had been in the dark from 24 to 48 hours did not translocate any of the Co⁶⁰ applied to the leaves as chloride. Other investigators had previously shown the same for other compounds (2, 3, 4, 5). The treated leaf usually had some radioactivity though never very large. In these experiments there was no way of determining whether the active cobalt was inside the leaf or on the outside. The evidence was against the latter assumption for there was no evidence that the Co⁶⁰Cl₂ had diffused underneath the confining lanolin ring to spread over the leaf, except in rare instances when the count was unusually high, but there always remained some doubt as to the accuracy of this view. Rice (3) makes the statement that bean plants absorbed more 2,4-D in the dark than in the light even though there was no translocation out of the leaf.

To supply unquestionable evidence as to whether there was or was not absorption in the dark, experiments were set up in which the activity of the cobalt chloride solution was increased to 100 µc/ml instead of the usual 30 µc/ml. The solution contained 1 % Tween 20. As in previous experiments 0.05 ml was applied to one of the primary leaves of Black Valentine bean plants 2 weeks old, that had been in a dark room for 48 hours before the beginning of the experiment and that continued to be in the dark until the end of the experiment. The solution was placed between veins, midway between the apex and base. In these experiments the solution was not placed within a lanolin ring because it was feared the lanolin would interfere with the sectioning of the treated area. No difficulty was encountered in confining the solution to a small area.

After 6 hours contact the experiment was terminated and a disk of the treated area plus several millimeters surrounding it was removed with a cork.

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² Paper from the Department of Botany, University of Michigan, No. 1056.
³ This investigation was financially aided by a grant from the Michigan Memorial Phoenix Project. The authors are indebted to Zane Carothers for making the cross sections.

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