 Quantitative Distribution and Metabolism of Auxin Herbicides in Roots

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ABSTRACT

The internal concentrations of four auxin herbicides—2,4-dichlorophenoxyacetic acid, dicamba, picloram, and naphthaleneacetic acid—were measured in the roots of treated pea seedlings. Intact seedlings were immersed in solutions of labeled herbicides at concentrations sufficient to produce toxic symptoms (inhibition of elongation, radial enlargement, and lateral root proliferation). Measurements of volume and herbicide content of segments taken sequentially along the root showed that an aeropetal concentration gradient of each herbicide was established within the root immediately following treatment. Although there was a net loss of herbicide in the following 24 hr, the gradient was maintained. Initially, the concentration of herbicide in the root tips exceeded that in the external medium.

In support of the contention that toxic symptoms due to herbicide treatment are caused by the presence of unmetabolized chemical at the site of action, it was found that metabolism was negligible for all herbicides except naphthaleneacetic acid.

MATERIALS AND METHODS

Pea seeds (Pisum sativum L. var Alaska) were surface sterilized by sequential 10-min treatments with 95% ethanol and 0.5% sodium hypochlorite solution. The sterilized seeds were washed with distilled water and allowed to imbibe a solution containing 3 mM calcium chloride and 2 mM magnesium chloride for 8 hrs. They were then placed in vermiculite moistened with 1 mM potassium dihydrogen phosphate and germinated in a water-jacketed chamber at 27°C for 40 hr. These and all subsequent operations were carried out in the dark or under green safe lights.

The roots of intact 48-hr-old dark-grown seedlings were immersed in aerated, buffered (1 mM potassium dihydrogen phosphate, pH 6.5) solutions of the following herbicides: picloram (4-amino-3,5,6-trichloropicolinic acid-carboxyl-14C, 1.0 mc/mmmole); 2,4-D (2,4-dichlorophenoxyacetic acid-carboxyl-14C, 2.0 mc/mmmole); dicamba 2-methoxy-3,6-dichlorobenzoic acid-carboxyl-14C, 1.89 mc/mmmole); NAA (naphthaleneacetic acid-carboxyl-14C, 50.6 mc/mmmole). Labeled picloram, dicamba, and 2,4-D were products of the New England Nuclear Corporation, naphthaleneacetic acid was obtained from Amersham-Searle. Treatments were accomplished by placing the plants on a stainless steel screen with their roots protruding into the appropriate solution contained in a plastic beaker. After 2 hr the roots were rinsed several times with a solution of nonradioactive herbicide in order to remove any material adsorbed to the root surface. Following a further rinse with distilled water, they were replaced in vermiculite.

Tissue Measurement and Extraction. At appropriate times after treatment, 10 sections, each 3 mm long, were cut from each root starting at the apex. With the aid of a binocular microscope fitted with an ocular micrometer and a base plate etched with 3-mm divisions, section lengths and diameters were measured to the nearest 0.005 mm. After measurement, corresponding sections from each of 10 roots were pooled and homogenized in a small ground glass homogenizer with a minimal volume of 80°C ethanol, and the homogenate was heated gently on a steam table for 10 min. Cellular debris was removed by centrifugation and the supernatant and washings were brought accurately to a volume of 1 ml. Montgomery (unpublished observation) has shown, and we confirmed, that extraction in this manner removes well over 95°C of the herbicide present within the tissue.

Total radioactivity present in the extract was determined by counting an aliquot in a toluene-Triton X-100 counting system (22) using a Packard Tricarb liquid scintillation spectrometer, model 3214. Counting efficiency for each sample was determined by the addition of standardized toluene-14C. Counting times were sufficiently long to ensure a maximal error of ±10°C due to statistical fluctuation.

Metabolic Studies. The remainder of the ethanolic extract was applied to Whatman No. 1 paper and subjected to descending paper chromatography in butanol-ammonia-water (1:butanol, saturated with 1.5 m ammonium hydroxide). After development

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for 30 cm, the chromatograms were dried and scanned for the presence of radioactive metabolites with a Vanguard Autoscan Strip Scanner, model 880.

In order to determine whether any significant amount of the herbicide was decarboxylated subsequent to the treatment period, sets of 10 treated seedlings were sealed in 125-ml Erlenmeyer flasks in the dark. Each flask was equipped with a center well containing 1 M KOH to absorb CO₂ and filter paper strips moistened with 3 mM potassium dihydrogen phosphate solution to maintain the humidity. At the end of the experiment, CO₂ absorbed in the center well was precipitated as barium carbonate, weighed and assayed for radioactivity by scintillation counting.

Where desired, the total soluble protein in a segment was measured by homogenizing in buffer, centrifuging to remove cell debris and precipitating with trichloroacetic acid. The protein in the washed precipitate was estimated by the procedure of Lowry et al. (19).

**RESULTS**

Identification and quantitation of morphological changes are necessary prerequisites to measurement of internal herbicide concentrations. Initially, therefore, we identified the morphological changes in pea roots which occurred after herbicide treatment, and we measured the accompanying changes in volume of root sections.

Gross morphological changes were induced in peas as a consequence of treatment with herbicidal levels (5 × 10⁻³ M) of 2,4-D (Fig. 1). Three phases could be distinguished: inhibition of root elongation; rapid radial swelling adjacent to the root tip; and, later, massive proliferation of lateral roots. Appropriate concentrations of other herbicides were found to give identical responses. Thus the changes of Figure 1 could be induced when plants were treated for 2 hr with picloram, 10⁻³ M; dicamba, 10⁻³ M; or NAA, 10⁻⁴ M. In the case of NAA the treatment was only partially effective since primary root meristems were inhibited for 12 hr, but grew out of the inhibition by 24 hr.

Temporale changes in the volume of sections taken along the root after herbicide treatment are listed in Table I. In the tip region (0-3 mm) a small over-all increase in volume was induced within 24 hr by 2,4-D, picloram, or dicamba. Tips from plants treated with NAA were much larger owing to the outgrowth of the primary meristem by 24 hr. Radial swelling of the region immediately behind the tip (3-5 mm) was also evident. During the course of the experiment, this region increased in volume by factors of 4.5, 3.0, 1.7, and 2.5 for NAA, picloram, dicamba, and 2,4-D, respectively. The remaining portion of the root, from 6 mm back to the cotyledonary node, showed no significant volume changes in the first 24 hr although extensive lateral proliferation occurred 48 to 72 hr later.

From a knowledge of the volume of each root segment, the amount of radioactivity present (dpm/segment) and the specific radioactivity of the herbicide used, it is possible to calculate the apparent internal herbicide concentrations within the root, based on the assumption that the herbicide is isotropically distributed throughout a given segment volume and that no accumulation has occurred in any particular area of the tissue or

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**Fig. 1.** Alteration of normal seedling development by 2,4-D. Seedlings were germinated, treated between 48 to 50 hr with 5 × 10⁻³ M 2,4-D (controls were treated with buffer), and observed after treatment.

**Table I.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc</th>
<th>Time after Treatment</th>
<th>Segment Volume at the Following Distances from Root Tip</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HR</td>
<td>0-3 mm</td>
</tr>
<tr>
<td>NAA</td>
<td>100</td>
<td>0</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>3.70</td>
</tr>
<tr>
<td>Picloram</td>
<td>10</td>
<td>0</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>1.62</td>
</tr>
<tr>
<td>2,4-D</td>
<td>50</td>
<td>0</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>1.33</td>
</tr>
<tr>
<td>Dicamba</td>
<td>10</td>
<td>0</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>1.51</td>
</tr>
</tbody>
</table>

¹ Volumes were calculated from measurements of length and diameter of freshly excised root segments. The tip sections were assumed to be conical; subsequent sections, cylindrical. Each value is the average of 10 determinations.
Although isotropic distribution is highly unlikely, one can by this means set a lower limit to the herbicide concentration which must be present at some point within the tissue.

Table II contains the results of such calculations. The first effect noted was the ability of the root tips to accumulate three of the herbicides in excess of the external solution concentration. Immediately after treatment in 50 μM 2,4-D, the tips had accumulated a concentration of 89 μM. Dicamba and picloram showed similar initial accumulation but NAA did not. The internal concentrations of 2,4-D, NAA, and dicamba decreased with distance away from the root tip and also as a function of time after exposure. The only exception to this generalization was the concentration in the 3- to 6-mm section, which in some instances was lower than that in more basal sections. This, however, may be explained by the very extensive radial swelling which occurred exclusively in this region 12 to 24 hr after treatment (see Table I).

Piloram differed from the other herbicides in that it appeared to be considerably more mobile. It was accumulated by the tip to about the same concentration as that in the external solution, but in the succeeding 12-hr period almost all of the herbicide in this region was lost. There was a correspondingly rapid decline in other regions of the root. Measurement of the total amount of radioactivity present within the root (Table II, last column) showed that while there was net loss of all herbicides from the roots during the experimental period, the loss was greatest for picloram. Measurements showed that significant amounts of radioactivity were located in the cotyledons and shoots, and that these levels increased with time.

Although the determination of radioactivity gives a measure of the amount of herbicide which has entered a tissue, it does not guarantee that what is being measured is still the original compound. In order to determine the amount of metabolism of each chemical, a portion of the ethanolic extract prepared 24 hr after treatment was subjected to paper chromatography and examined for the presence of metabolites. Extracts from tissues treated with dicamba, 2,4-D, or picloram contained only the parent compounds which in butanol-ammonia-water had Rf's of 0.60, 0.58, and 0.28, respectively. No traces of metabolites were evident in these extracts. By contrast, NAA was rapidly and extensively converted into other compounds. As shown in Figure 2, three compounds were present immediately after treatment. Peak P had an Rf of 0.88 identical to that of the parent compound. Peaks A and B with Rf's of 0.57 and 0.07 were metabolites. As illustrated in Table III, these substances were formed very rapidly in all tissue regions and constituted the major radioactive species within the tissue shortly after treatment. After 6 hr, metabolite B was the major radioactive species present. The NAA-14C used in this experiment was homogeneous upon chromatography and showed no traces of either peaks A or B.

Because the label was located in the carboxyl group, separate experiments were carried out to determine the extent of decarboxylation in the 24 hr after treatment. Measurements were made of the evolution of both radioactive and total CO2 since the herbicides could conceivably have an effect on the rate of respiration of the tissue. Table IV summarizes this data. As measured by CO2 evolution, respiration was not altered significantly. The amount of radioactive CO2 arising concurrently from herbicide decarboxylation was extremely low. Maximal evolution of CO2 was observed in the case of NAA but the amount of decarboxylation expressed as a percentage of the total herbicide within the root tissue (Table

![Fig. 2. Chromatographic distribution of radioactivity from an extract of pea roots treated with NAA-14C. Extract was prepared from root sections (10-13 mm from the tip) immediately following 2-hr treatment with NAA. Metabolite A: Rf = 0.57; metabolite B: Rf = 0.07; P: (parent compound): Rf = 0.88.](https://plantphysiol.org/)

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Table III. Distribution of Metabolites of NAA after Treatment

Seedlings were treated with NAA-14C for 2 hr and examined for metabolites of NAA at suitable times thereafter as described in the text. A chromatogram of the NAA-14C before use showed only one radioactive peak corresponding in mobility to authentic NAA.

<table>
<thead>
<tr>
<th>Section (mm)</th>
<th>NAA 0 hr</th>
<th>Metabolite A 0 hr</th>
<th>Metabolite B 0 hr</th>
<th>NAA 6 hr</th>
<th>Metabolite A 6 hr</th>
<th>Metabolite B 6 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-6</td>
<td>±</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6-9</td>
<td>±</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9-12</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12-15</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Table IV. Decarboxylation of Applied Herbicides

Ten seedlings were treated with radioactive herbicide as described in the text. The 14CO2 evolved during the subsequent 22 hr was collected as BaCO3 and weighed, and the radioactivity was determined.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Radioactive CO2 Produced1</th>
<th>Respiratory CO2</th>
<th>Decarboxylation</th>
<th>Decarboxylation2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA</td>
<td>9900</td>
<td>10.4</td>
<td>85</td>
<td>5.1</td>
</tr>
<tr>
<td>Picloram</td>
<td>60</td>
<td>10.6</td>
<td>27</td>
<td>3.7</td>
</tr>
<tr>
<td>2,4-D</td>
<td>460</td>
<td>10.7</td>
<td>104</td>
<td>1.5</td>
</tr>
<tr>
<td>Dicamba</td>
<td>30</td>
<td>10.7</td>
<td>7.1</td>
<td>0.3</td>
</tr>
<tr>
<td>None</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

1 Data for picloram and dicamba, although low, were significantly greater than background.

2 Expressed as a percentage of the total herbicide present in the root immediately after treatment.

IV, last column) was small in every case. These percentages also represent upper limits to the amount of decarboxylation occurring in the root since the measured evolution of CO2 was from the whole plant.

DISCUSSION

Alteration of root dimensions of dicotyledonous plants by treatment with herbicidal levels of auxin is a well documented phenomenon (4, 11–15, 30) which occurs in three phases: inhibition of the primary meristem, radial expansion of the elongation zone, and lateral root proliferation originating from the pericycle. To date no quantitative measurements of these changes have appeared nor has any correlation been attempted with the internal distribution of the inducing herbicide.

The levels of auxin used in these experiments were sufficiently high to cause complete inhibition of elongation. Valid comparisons of equivalent root zones were thus possible. Untreated roots were elongating extensively at this point and, for this reason, made difficult any comparison with the treated plants.

Quantitative evidence for radial expansion 3 to 5 mm from the root tip is shown by our data (Table I). Torrey (28) pointed out that the region of swelling induced by auxins is that in which normal cell elongation occurs and that only cells capable of rapid expansion are sensitive to auxin-induced reorientation of growth. Cholodny (8) described the same swelling response induced by indoleacetic acid in oat seedling roots.

The absence of other significant volume changes during the first 24 hr after treatment is also consistent with earlier observations. Lateral root induction occurs in the region 8 mm back from the tip to the cotyledonary node but is not evident until later. Pericycle proliferation leading to lateral root formation is initiated in peas as early as 6 to 12 hr after treatment (21) but apparently does not result in any significant volume change during the first 24 hr.

We have shown also that when intact pea seedlings are treated with 0.01 to 0.1 mm solutions of auxinic herbicides, there is established within their roots an acropetal gradient of herbicide whose highest concentration (except in the case of NAA) exceeds that of the external medium. The gradient is established within a very short time after treatment and is maintained over a period of 24 hr, even in the case of NAA which is subject to rapid and extensive degradation. No previous documentation exists for this phenomenon for auxin herbicides although preliminary indications may be seen in the work of Bottrill and Hanson, (5) who showed that the 2,4-D content of treated Zea roots was greater in the tip section than in the other regions for all time periods studied.

A marked difference exists between the distribution of 2,4-D and that reported for endogenous IAA. Pilet and Meylan (25) showed that the endogenous IAA concentration of Lens roots rose from a minimum of 10–9 m in the root tip to a maximum of 10–6 m at a distance of 4 to 5 mm from the tip and then declined almost to its original level. In a later study, Pilet and Braun (23) showed that the IAA level of the root cap was less than one-twentieth of that in the meristematic region 200 to 500 μ behind the tip. In contrast, the auxin herbicide content of the tip to be higher than that of the basal regions. Pilet and Galston (24) also demonstrated a gradient of IAA oxidase within the root. The observed IAA profile presumably represents a balance between the establishment of a continuously increasing concentration gradient by the acropetal transport processes demonstrated by Kirk and Jacobs (16) and by Wilkins and Scott (27, 31) and the destruction of IAA by the enzyme. In the case of 2,4-D and the other auxin herbicides no such destruction can occur and one measures the consequences of the transport process only.

The nature of the mathematical function representing the change of herbicide concentration along the root is not clear. If one assumes that a constant acropetal velocity (independent of both distance along the root and herbicide concentration) is balanced by a passive basipetal diffusion process (with a diffusion

![Fig. 3. Change in 2,4-D and protein concentrations within the root. The data for 2,4-D were obtained from Table II. Protein concentration was calculated from the measured protein content per section and the section volume. ○: 2,4-D concentration at 0 hr; △: 2,4-D concentration at 24 hr; □: protein concentration at 0 hr.](https://plantphysiol.org)
coefficient independent of concentration), then it is possible to set up an equation (R. O. Morris and T. H. Lindstrom, unpublished calculations) in which the solution predicts that the logarithm of the herbicide concentration is (within limits) a linear function of the distance from the tip. Figure 3 contains the 2, 4-D concentration data (from Table II) plotted together with the protein concentration in the same regions. Clearly the 2, 4-D concentration increases in a nonlinear fashion. It increases less rapidly, however, than the protein concentration. Apparently neither a simple transport model nor adsorption to tissue protein can account for the observed distribution.

Accumulation of auxins against a concentration gradient has been documented by Andreae and van Ysseltstein (3). They showed that pea seedlings accumulated IAA to concentrations 10 times that of the external medium. Accumulation of auxin herbicides in our experiments was not so marked. However, no attempt was made to establish pH and Ca²⁺ ion optima, both of which are known to play a significant role in the absorption process (2).

Identical morphological consequences result from the accumulation of markedly different levels of each herbicide (Table II). Given the premise that each compound is distributed identically within the tissue, then picloram is the most effective herbicide. It is able to induce morphological change when present at vanishingly small levels. It is also the most mobile of the compounds tested as indicated by its rapid loss from the root as a function of time. Linder et al. (18) have shown that picloram is very readily translocated to other regions of the plant and into the external medium.

The lack of significant metabolism of dicamba, 2, 4-D, and picloram has precedence in the literature. Meikle et al. (20) reported that the major portion of picloram applied to plants could be extracted unchanged. Similarly, although there is some evidence that resistant species can degrade dicamba, susceptible plants are not able to do so (6). The lack of metabolism of 2, 4-D agrees with the findings of Andreae (1) although others have shown (7) that resistant species are able to form a number of hydroxylated derivatives. In contrast, NAA is rapidly and extensively metabolized, and this fact is reflected in the ability of the treated plants to resume growth within 12 hr. Although the nature of metabolites A and B remains to be determined, their mobilities suggest that they are the conjugated and hydroxylated derivatives isolated by Veen (29).

Decarboxylation did not appear to play a significant role in the degradation of any of the four auxins.

We conclude, as have others (1), that alteration of normal root development in pea seedlings is due to the continued presence of unmetabolized chemicals at the site of action. The previously unexplained ability of 2, 4-D to inhibit primary root meristems while allowing lateral root proliferation may also be understood in view of the concentration gradient established within the root.

Finally, it is important to note that isotropic distribution of the herbicides at the cellular level almost certainly does not occur. Two reports have appeared (17, 32) localizing 2, 4-D in the nuclei and nucleoli of treated Vicia root and artichoke tuber cells, respectively. This is in contrast to IAA which has been reported to be localized primarily in cell walls (26). Studies are in progress to describe the intracellular distribution in pea roots.

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LITERATURE CITED


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