Metabolism of organophosphorus insecticides: IV. Translocation and metabolism of $^{32}$P-labelled Dipterex in cotton plant

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The uptake of $^{32}$P-labelled Dipterex by the cotton plant (Gossypium barbadense), has been studied following topical application on the leaf, as well as via root. The insecticide did not penetrate into the leaf cells, when applied topically, but is readily taken up by the root, when immersed in a solution of radioactive insecticide. Also the rate of respiration was found to increase significantly in plants treated with sublethal concentrations of Dipterex.

The metabolic fate of Dipterex within the plant tissues has been also investigated. Dimethylphosphate, monomethylphosphate and inorganic phosphate have been identified as degradation products of the insecticide.

The use of organophosphorus insecticides in the field of plant protection has recently attracted considerable attention. In Egypt, O,O-dimethyl-2,2,2-trichloro-1-hydroxyethyl phosphonate (Dipterex) has been widely used in the last few years for the protection of cotton plant against Prodenia litura F., a serious pest in Egypt.

For this reason, a series of studies seemed desirable to investigate the toxicity of Dipterex to mammals, the insect and cotton plant. The low toxicity of this insecticide to mammals has been demonstrated. Recently Zayed and Hassan studied the distribution and metabolism of Dipterex in the adult larva of the cotton leaf worm.

The present work is concerned with the distribution and metabolism of the insecticide in cotton plant.

Materials

$^{32}$P-labelled Dipterex was prepared from the hemiacetal obtained by interreaction of equivalent amounts of chloral and methanol. Reaction of the hemiacetal with one Mole $^{32}$PCl$_3$ followed by addition of two Moles of methanol gave the $^{32}$P-labelled Dipterex. Purification was achieved as described by Zayed and Hassan.

Cotton seeds (Gossypium barbadense, var. Ashmount) were germinated in pots, 15 cm in diameter, and left to grow in the open under field conditions. Treatments were carried out on 2 leaf-stage plants.

Methods

A. Distribution Studies

1. Leaf application

0.05 ml of an acetone solution containing a definite amount of the labelled insecticide was applied on the upper surface of one leaf of the plant. The treated plants were then allowed to grow normally for different periods of time. The contaminated leaves were collected, washed thoroughly with water to remove the unabsorbed insecticide. The different plant organs: washed leaves, other leaves, stem with terminal bud and root system were weighed, dissolved in concentrated nitric acid and analyzed for their $^{32}$P-activity using an end window counter.


* This method for preparation of Dipterex is under patentation.
2. Uptake through root

Plants were taken out of soil and immediately immersed in a solution of 12 mg labelled Dipterex in 20 ml water. After 1, 3, 5, 24 and 48 hours plants were removed from the solution. The root system was then thoroughly washed in a stream of water, and the different parts of the plant were weighed. Analysis proceeded in the same way as above.

B. Metabolism Studies

For this purpose the plants were left for 72 hours in a solution of 12 mg labelled Dipterex in 20 ml water. The roots were then thoroughly washed in a stream of water and the whole plant was used for the identification of the metabolic products.

1. Determination of inorganic $^{32}$P

To the plant parts, mixed with a small amount of fine sand, 10 ml of 10% cold TCA (trichloroacetic acid) solution were added and the mixture was ground thoroughly and left for 10 minutes. The TCA extract was removed and the process of extraction was repeated twice. To avoid the hydrolysis of unstable compounds, the extract was neutralized with ammonia solution, brought to volume and kept at 5 $^\circ$C. The radioactivity of the total extract was then determined. The inorganic phosphate fraction was quantitatively precipitated as ammonium magnesium phosphate. The clear solution (containing the organic phosphate fraction) was measured for its radioactivity. By subtracting the latter value from that of the total extract, the labelled inorganic P could be determined.

2. Identification and determination of labelled metabolites of Dipterex

The whole plant was extracted 5 times with chloroform (10 ml each) after grinding with fine sand. The combined chloroform extracts were measured for radioactivity, and paperchromatographed in n-butanol/pyridine/water (12 : 8 : 6) (system A). The remaining plant tissues were then extracted 4 times with water (5 ml each); emulsions being broken by centrifugation. The combined water extracts were applied on 40 x 1 cm column of Dowex 1-X8 (anion exchanger Cl$^-$, 100–200 mesh). The column was then washed with distilled water till no more radioactivity was detectable in the eluate. The ionic metabolites (hydrolytic products) were then completely eluted with HCl ($\text{pH}$ 1). Samples from the water washings and the acidic eluates were chromatographed in system A and system B (2-propanol/ammonia/water; 75 : 24 : 1) respectively.

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Results

Distribution Studies

From Table 1 it can be seen that the amount of radioactivity taken up by the leaf is time independent and never exceeds 7% of the applied dose. Furthermore, plant parts other than the contaminated leaf proved to contain no detectable radioactivity.

<table>
<thead>
<tr>
<th>Time following application [days]</th>
<th>Weight of treated leaf [mg]</th>
<th>$^{32}$P. activity per leaf [cpm]</th>
<th>Specific activity [cpm/gram leaf]</th>
<th>% of applied dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>1520</td>
<td>7600</td>
<td>6.6</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>1520</td>
<td>21700</td>
<td>6.6</td>
</tr>
<tr>
<td>5</td>
<td>130</td>
<td>840</td>
<td>6460</td>
<td>3.7</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>1160</td>
<td>11600</td>
<td>5.1</td>
</tr>
<tr>
<td>9</td>
<td>80</td>
<td>1000</td>
<td>12500</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Table 1. $^{32}$P-activity retained within the leaf after elapse of different periods of time following topical application of 1.5 mg $^{32}$P-insecticide per leaf.

Fig. 1 shows that $^{32}$P-activity in both stem and leaves increased with time, reached its maximum after 24 hours and then remained more or less constant. On the other hand, the activity within the root showed a gradual increase.

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Respiration Measurements

The rate of respiration — as determined by oxygen uptake by terminal buds — of plants treated with different concentrations of the insecticide is illustrated in Fig. 2. It is worthy to mention that the concentration usually used in the field is 0.62%; and concentrations as high as 2% and 3% caused

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serious injury to the plants. Compared with the controls, the oxygen consumption increased significantly by all concentrations used (3–4 fold).

Fig. 2. \(O_2\) uptake by terminal buds from Dipterex-treated plants. Plants were sprayed every 4th day for 14 days. \(O_2\) uptake was measured in Warburg constant volume respirometer at 35 °C for 4 hours. Data are mean values of duplicate experiments using 20 terminal buds in each measurement.

**Metabolism Studies**

The chloroform extracts proved by paper chromatography in system A to contain only Dipterex \((R_f 0.95)\). The watersoluble hydrolytic product(s) eluted from the anion exchanger at \(p_H 1.0\), proved to contain three components. The radiometric assay of a paper chromatogram developed in system B, showed the presence of two peaks, with \(R_f\) values 0.0 and 0.63 (Fig. 3). The substance with \(R_f 0.63\) was identified as dimethylphosphate, and contributed to 60–70% of the total metabolites output. The activity remaining on the base line is probably due to labelled inorganic P. The formation of inorganic \(^{32}\)P has been confirmed – in a separate experiment – by precipitation from a TCA extract as ammonium magnesium phosphate. After 3 days about 17–24% of the metabolites could be recovered in the ammonium magnesium phosphate precipitate. From this experiment it is obvious that labelled inorganic phosphorus contributed mainly to \(^{32}\)P-activity remaining at the base line in system B.

Fig. 3. Radiopaperchromatogram of the ionic metabolites eluted from Dowex at \(p_H 1.0\) and developed in system B.

**Discussion**

A number of organophosphorus compounds, e.g. Systox and Metasystox, behave as systemic insecticides (cf. 7) which effectively control many pests of the plants. Such compounds must be capable of entering the leaves, stems or roots of the plant and move within its tissues.

From the results obtained, Dipterex is rapidly absorbed through the root system of *Gossypium barbadense* and transported upwards in the xylem to the different parts of the plant. Following the absorption through the root system, the radioactivity present in the leaf increased with time and reached a plateau after 24 hours. This is probably due to transfer of the metabolic products to the phloem and then to different parts of the plant at a rate equivalent to that of entry into the leaf. This may also account for the gradual increase of \(^{32}\)P-activity within the root.

Contrary to systemic organophosphorus insecticides, Dipterex is not resorbed by the leaf when applied topically on the upper surface. From table 1 it is obvious that, the radioactivity entering the leaves is more or less constant, regardless the period following the application of the toxicant. The percentage of applied dose was also too low and never exceeded 6.6 per cent. This activity is presumably not due to proper absorption. It may be due to passage of the insecticide, only through permeability changes, possibly taking place at the area of appli-

7 A. J. Fjelldal, Höfchen-Briefe 8, 1 [1955].
cation. In support of this, it has been observed that the site of application undergoes a visible change in appearance. This explanation would account for the observed fluctuations in the values obtained (cf. Table 1), depending on the area of application.

From the respiration experiments, it is evident that the toxicant enhanced significantly the oxygen consumption. In its action, the insecticide resembles that of certain hormones and in fact, it may act by stimulating some hormone(s) within the plant. Whatever the mode of action is, the increased $O_2$ uptake strongly suggests that oxidative processes are taking place at a higher rate in presence of the toxicant. The latter may have caused a depletion of the energy sources — by inhibiting specific enzyme systems — and for compensation, the oxidation rate of some substrate(s) increased considerably. However, this remains to be clarified, and some parameters — other than respiration — should be investigated.

The metabolism of Dipterex in plants has been previously studied in a qualitative manner by Arthur and Casida. These authors reported the presence of monomethylphosphate and dimethylphosphate as metabolic products of Dipterex in the pea plant tissues.

In the present investigation, a quantitative determination of the degradation products of the insecticide in the cotton plant has been attempted. Under the experimental conditions, about 70% of the toxicant (which was taken up by the plant in 3 days) was changed into a variety of metabolic products; three of which have been identified. These contributed to 80 – 90% of the total metabolites output. Dimethylphosphate accounted for 60 – 70% (cf. Fig. 3), and inorganic phosphate for 17 – 24% of the total metabolites. As monomethylphosphate possesses a low value in system B (0.13), its radioactivity was masked by the activity remaining on the base line, and which is believed to be due to inorganic phosphate. However, the spot on the paper chromatogram corresponding to $R_f$ 0.13 could be easily detected by spraying the chromatogram with Bandurski reagent, which gives a blue colour only with phosphates of organic nature.

A possible mechanism for the detoxification of the insecticide in the cotton plant is illustrated in Fig. 4. The scheme suggests the hydrolysis of the original Dipterex molecule at the phosphonate bond ($C_2\rightarrow P$) through the action of an esterase to give dimethylphosphate. This is in accordance with the postulated mechanism suggested by Arthur et al. for the detoxification of the insecticide. Dimethylphosphate is then subjected to a phosphatase action to from monomethylphosphate, to give in turn inorganic phosphate. However, the possibility that dimethylphosphate be directly metabolised to the inorganic phosphate stage cannot be outruled.

The metabolic formation of inorganic $P$ from the insecticide makes the $P$ atom of Dipterex available for phosphorylation processes, ever taking place in the plant tissues. It is not unlikely that in the course of time, demethylation of the methylated phosphates may continue to transfer most of the phosphorus of the toxicant to available inorganic phosphate. The trichloroest of the molecule should be toxic to the plant tissues, and its fate necessitates further investigation.

As shown in Fig. 4, the production of inorganic phosphate from Dipterex is the result of a chain reaction in which several steps are involved; each proceeding with a special rate constant ($K$). Dimethylphosphate is readily formed from Dipterex, and it is suggested that the production of monomethylphosphate is the rate-determining step in the whole chain, i.e. $K_2 < K_1$ and $K_3$.

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