ACCUMULATION OF PHORATE BY COTTON PLANTS FROM SOLUTION AND SAND CULTURE

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Phorate, O,O-diethyl S-(ethylthio) methyl phosphorodithioate, is an active plant systemic insecticide when applied as a seed treatment or added to the substrate (7, 10, 11). Its metabolic breakdown within the plant has been thoroughly investigated (2, 3, 9); little information is available, however, concerning the effects of environmental conditions on the absorption of phorate by plants through the root system. The data presented here are concerned mainly with the effects of temperature and humidity on the accumulation of phorate by cotton plants when the insecticide was applied to solution and sand cultures.

Material and methods

Cotton seeds, Deltapine-15 variety, were germinated in flats which contained washed river sand and were transplanted to solutions 3 days after emergence. A modified Hoagland’s nutrient solution was used throughout according to a procedure previously described (6).

Most of the tests were conducted under a controlled environment system, described by BEIRENS and MORTON (1), which has the capability of maintaining four sets of temperature and humidity condi-

1 A co-operative project of the Crops Research Division and Entomology Research Division, USDA, and the Texas Agricultural Experiment Station, College Station, Texas.
2 Crops Research Division, ARS, USDA.
3 Entomology Research Division, ARS, USDA.
tions simultaneously with non-recirculating air. The light intensity was approximately 1800 ft.c., and a photoperiod of 15 hours of light and 9 hours of darkness was maintained.

The radiolabeled phorate* had an initial specific activity of 14.5 mc/gm and had the same infrared spectrum as did chemically pure phorate (3) and a technical preparation of the insecticide. All radioactive measurements were made with a thin-end-window Geiger-Müller tube and a conventional scaling unit. Stainless steel planchetts 1 inch in diameter were used to contain the plant material for radioassay.

Solution Culture.—Cotton plants, 35 days old, grown in nutrient solution were transferred to individual blackened 1-quart glass jars containing 800 ml. of nutrient solution and 4 mg. of P32-labeled phorate (5 p.p.m. phorate). The phorate had a relative specific activity of 180 counts/min/µgm and was added in 1 ml. of acetone. The plants were immediately transferred to four controlled-environment cabinets having the following conditions of temperature and relative humidity: 95° F. and 95% relative humidity, 75° and 95%, 95° and 35%, and 75° and 35%. These conditions will be abbreviated as follows: H1H1, L1H1, H1L1, and L1L1, respectively.

During the experiment the solutions were aerated for 20 minutes three times daily. Aeration was intermittent to reduce the quantity of water vaporized. Two plants from each treatment were harvested 0.5, 1, 2, 4, and 7 days after the addition of the insecticide. The root systems were washed under running tap water and the plants separated into roots, stems, and leaves. The plant parts were dried separately in a forced-draft oven at 80°-85° F. for 24 hours. The dried plant material was weighed and ground in a cutting mill to pass a 60-mesh screen. The plant material was radioassayed in 100-200-ml. aliquots. This amount of plant material resulted in negligible self-absorption of the β-radiation. All radioactivity detected was expressed as micrograms of phorate equivalents. No attempt was made to determine whether P32 was present in phorate or some breakdown product.

During the experiment recorded amounts of water were added to the jars to replace the loss from transpiration and evaporation. Final water-loss measurements were obtained at the time of harvest for each plant.

Sand Culture.—Cotton plants, 35 days old, growing in sand culture in 1-gallon cans, two plants per can, were treated with 8 mg. of P32-labeled phorate per can. The phorate, in 2 ml. of acetone, was added to 250 ml. of water, mixed, and poured around the base of the plants. These plants were placed under the four sets of environmental conditions previously mentioned. The two plants in each can were harvested separately, and each constituted one replicate. Four plants from each set of environmental conditions were harvested 1, 2, 4, and 7 days after treatment, and the leaves, stems, and roots were radioassayed as previously described.

Loss of Phorate from Roots.—Cotton plants, 20 days old, growing in nutrient solution were transferred to 250-ml. glass bottles containing 200 ml. of nutrient solution and 4 mg. of P32-labeled phorate. The plants were exposed to the insecticide for 44 hours under the H1L1 condition, then removed, and the root systems rinsed thoroughly in running tap water. The plants were immediately transferred to bottles containing fresh nutrient solution and 2 mg. of non-radioactive phorate. Four plants each were harvested 0, 3, 5, and 7 days after treatment, and the radioactivity in the dried plant material was determined. The radioactivity also was determined in the nutrient solutions by concentrating the solutions on a hot plate and then evaporating suitable aliquots of the concentrate in 1-inch cupped planchetts. The radioactivity found in the nutrient solutions was not characterized chemically.

Hydrolysis of Phorate.—The following experiment was performed to establish the duration of the life of phorate in nutrient solution and in distilled water. For a stock solution, 1 gm. each of phorate and of an emulsifier (Triton X-100) were thoroughly mixed and made up to 100 ml. with distilled water. One milliliter of stock solution was added to each of twenty-four bottles containing 49 ml. of distilled water and of twenty-four bottles containing 49 ml. of complete nutrient solution. Each bottle then contained 10 mg. of phorate. Twelve bottles of each solution were held at 95° F. and twelve at 85° F. Samples from each solution at both temperatures were taken at various times after the addition of phorate. Since phorate and its toxic metabolites have a chloroform-water partition ratio of 99 to 1 or greater (2), an equal volume of chloroform was added to each sample and agitated on a mechanical shaker for 1 hour to insure thorough extraction of the insecticide. Three milliliters of the chloroform-phase layer was introduced into a test tube and evaporated; 2 ml. of perchloric acid was added and the contents boiled at 220° C. for 1 hour. Total phosphorus was then determined by a method similar to that of Rockstein and Herron (12) and the phorate equivalents calculated.

Results

Solution Culture.—The transpiration rates of plants held at high temperatures, regardless of

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*Synthesized by Volk Radiochemical Co., 5412 North Clark Street, Chicago 40, Illinois.
humidity, decreased gradually throughout the 7 days of the experiment (fig. 1). The plants held under low temperatures, however, transpired at nearly uniform rates throughout the 7-day experiment. As expected, the plants held under the H1Lh condition showed the greatest transpiration, followed in decreasing order by those under the H1Hh, L1Lh, and L1Hh conditions.

The roots accumulated phorate very rapidly from the solution under all experimental conditions (fig. 2). The amount in the roots of the plants held under the H1Lh condition dropped rapidly following the 0.5-day harvest. Plants under the other conditions accumulated additional quantities of phorate in the roots through the 2-day harvest. Thenceforth, the roots were rapidly depleted of the insecticide, and the rate of depletion was correlated with transpiration.

The accumulation of phorate in the leaves of plants held under all conditions increased rapidly for 4 days; but a reduction was found at 7 days. The fastest rate of accumulation of the insecticide occurred in the leaves of plants subjected to H1Lh, followed in order by H1Hh, L1Lh, and L1Hh, respectively.

The quantity of phorate in the stem remained low under all conditions, indicating that the stem functions as an avenue of transport, not as a storage organ.

Sand culture.—Accumulation of phorate by the leaves of cotton followed a linear pattern with time (fig. 3) and was favored by the environmental conditions which favored transpiration. That is, the leaves of plants in the H1Lh environment accumulated the greatest quantity of insecticide, and successively less amounts were found under H1Hh, L1Lh, and L1Hh, respectively. The root-accumulation data are not presented because they were highly erratic, probably because the entire root system was not recovered from the sand. The data for accumulation of phorate in the stem are not presented, since a consistently small amount of less than 10% of the quantity in the

![Graph](image_url)

**Fig. 1.**—Influence of temperature and relative humidity on transpiration of cotton plants grown in nutrient solution containing 5 p.p.m. phorate.

![Graph](image_url)

**Fig. 2.**—Influence of temperature and relative humidity on accumulation of labeled phorate equivalents by cotton plants grown in nutrient solutions containing 5 p.p.m. P32-labeled phorate.
leaves was found, regardless of environmental condition.

Loss of Phorate from Roots.—The distribution of phorate in the leaves, stems, and roots followed a pattern similar to that reported in the section on phorate accumulation in solution culture. The greatest accumulation in the leaves (table 1) occurred 3 days after treatment, and successive reductions were found at 5 and 7 days. The amount of phorate in the stems and roots dropped rapidly at 5 and 7 days; this phorate was recovered, for the most part, in the nutrient solution rather than in the leaves.

Hydrolysis of Phorate.—Absorption of phorate from solution culture by cotton plants showed that the rate of uptake of the insecticide dropped sharply after a 1- to 2-day interval. This might be partially explained by hydrolysis of the insecticide in the solution. Figure 4 shows that the breakdown of the insecticide in the substrate varies with both the solution medium and the temperature. After 2 days at 95°F, 32% and 25%, of the phorate, and after 6 days 100% and 73%, had hydrolyzed in nutrient solution and water, respectively. The data show that the hydrolysis of phorate was more rapid in nutrient solution than in water.

![Graph](image)

**Fig. 3.—Influence of temperature and relative humidity on accumulation of P32-labeled phorate equivalents in leaves of cotton plants grown in sand drenched with 4 mg. P32-labeled phorate per plant.**

Discussion

Phorate is a compound which is absorbed by plant roots and translocated to the shoot, even though it is a relatively large, slightly water-soluble, non-polar, organic molecule. LINDQUIST et al. (8) obtained data which showed that the quantity of phorate absorbed by young cotton seedlings was directly related to the volume of a section of radicle tissue exposed to the insecticide. TIEZ (13), invoking the lipid-sieve theory, stated that demeton, a systemic insecticide chemically related to phorate, was apparently preferentially absorbed by the roots of Phaseolus vulgaris because of its lipid-soluble properties. This reasoning seems plausible in light of results reported by COLLANDER and BURLUND (4), who showed that, within

**TABLE 1**

<table>
<thead>
<tr>
<th>TABLE 1 DISTRIBUTION OF P32-LABELED PHORATE EQUIVALENTS IN COTTON GROWN IN SOLUTION CULTURE*</th>
<th>DAYS AFTER TRANSPLANTATION</th>
<th>Per gram of dry weight</th>
<th>Per plant</th>
<th>Loss to solution (per plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Stems</td>
<td>Roots</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>312</td>
<td>224</td>
<td>1266</td>
<td>208</td>
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<td>3</td>
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<td>189</td>
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</tr>
<tr>
<td>7</td>
<td>355</td>
<td>76</td>
<td>128</td>
<td>109</td>
</tr>
</tbody>
</table>

* Plants were grown in 200 ml of nutrient solution containing 4 mg. P32-labeled phorate for 44 hours and then transferred to fresh solution containing 2 mg. of non-radioactive phorate.

![Graph](image)

**Fig. 4.—In vitro hydrolysis of phorate**

limits, the accumulation of organic compounds by cells increased as their polarity decreased. In addition to its lipid-soluble properties, phorate has a water solubility of about 50 p.p.m., which would permit it to be readily moved in the conducting elements of the plant.

The very rapid accumulation of phorate in the roots of plants grown in solution culture undoubtedly can be attributed, at least in part, to its lipid-soluble properties, because it fits the pattern of passive accumulation rather than that of an active or metabolic process. Without question, movement to the leaves was directly correlated with transpiration: plants grown in solution or sand culture at a given tempera-
tured; more insecticide in the leaves at a low than at a high relative humidity. The increase in phorate concentration in leaves of plants in solution cultures was at the expense of the phorate in the roots; the total concentration per plant, regardless of treatment, was also essentially the same, and only the distribution between root and shoot varied.

The apparent loss of phorate from the leaves at the 4- to 7-day harvest of plants in solution culture prompted the study which determined that at least a portion of the absorbed P³² was being lost from the root system into the nutrient solution. Tietz (13) reported that demeton (Systox) was recreted by roots of Phaseolus plants, but no explanation for this phenomenon was offered. It was concluded in the present investigation that phorate injured the root system by reacting with the lipids of the plasma membrane to alter its semi-permeability in a manner similar to that described by van Overbeek and Blondeau (14) for oils. Hackshaw (5) reported that anthocyanins were leached from cubes of beet root in direct proportion to the concentration of phorate in the bathing medium. These findings add support to the hypothesis that the alteration of cellular permeability is an important factor in the responses of plant tissues to phorate.

The possibility that some of the radioactivity lost from the root system (table 1) resulted from an exchange reaction between the non-radioactive phorate from the nutrient solution and the radioactive phorate in the roots cannot be excluded. The data presented, however, show, for the most part, a decrease in P³²-labeled phorate equivalents with time within the plant parts assayed. Since the concentration of insecticide within the plant initially was substantially greater than in the nutrient solution, the diffusion gradient would be in favor of the nutrient solution. Again, these results would tend to indicate that the loss of phorate from the root systems was passive and that a leakage of not only the insecticide but also of other cellular constituents could occur.

The hydrolysis of phorate in nutrient solution should be considered in evaluating these data, since all radioactivity was expressed in phorate equivalents. The data in figure 4 show that phorate starts to hydrolyze immediately in nutrient solution and that hydrolysis is nearly complete after 6 days at 95°F. This indicates that some of the radioactivity is perhaps absorbed as P³²O₄. If, however, all the phosphorus in the insecticide were in the form of inorganic phosphorus, it would contribute a maximum of about 6% P³² while that in the nutrient solution would contribute about 94% to the plants. The results presented should be little affected by the accumulation of inorganic P³²O₄ obtained from the original insecticide over the short time interval of these experiments.

Summary

1. Accumulation of P³²-labeled phorate from nutrient solutions in the roots of cotton plants was very rapid initially but decreased sharply with time.
2. The rate of movement of phorate from roots to aerial portions was facilitated by increased transpiration; with subsequent root exposure, however, not only did the plants fail to absorb additional quantities of insecticide, but a leakage from roots to nutrient solution occurred.
3. The accumulation of phorate in leaves of plants grown in treated sand cultures was linear with time and was directly associated with the environmental conditions which favored transpiration.

LITERATURE CITED