Pestic. Sci. 1976, 7, 549–552

Phorate Residues in Aphid Colonies on Broad Bean Plants in Relation to the Site of Application

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(Manuscript received 18 December 1975)

Aphis fabae colonies were established on both leaflets of the second leaf of young broad bean plants. Doses of phorate sublethal to the aphids were applied to the roots or to the third leaf. The aphids, their honeydew and the foliage on which they were feeding were analysed and the quantities of toxic and non-toxic radiolabel determined at intervals. Toxic metabolites were found in the aphids and the leaves but whereas the aphid colonies in all experiments contained approximately similar amounts of the toxic metabolites, the residues in the leaves on which they were feeding differed considerably. The leaves contained more than 100 times as much of these metabolites following the root treatment than after the foliar treatment. These results are discussed in relation to the aphids' feeding site and the probable transport routes of the toxicants.

1. Introduction

Systemically active insecticides move in plants mainly in the apoplast and therefore acropetally.[1] However, some downward movement may also occur and toxic compounds have been found in aphids on lower leaves following phorate application to more distal foliage.[2,3] Although the dose can be increased sufficiently to induce aphid mortality, for practical purposes of insect control this basipetal movement is relatively slight.

Translocation from one leaf to another, especially in a basipetal direction, must occur in the phloem into which there must be some movement if aphids, which are predominantly phloem feeders, are to take up lethal quantities of insecticide. The transfer of an apoplastically moving compound from xylem to phloem may occur as a result of "leakage",[1] though the extent of this phenomenon is largely unknown and probably varies from compound to compound.

Earlier work had shown that the levels of toxic material in foliage supporting aphids was very much greater when the insecticide was applied to the roots,[2] and thus entered the leaf with the transpiration stream in the xylem, than when it was applied to non-infested foliage[3] and was transported in the phloem for part, if not all, of the distance to the infestation. Direct comparisons were not possible since different aphid species were used in each treatment and no attempt was made to standardise the doses in relation to their effect on the aphids.

The experiments described here involved treating plants with what were intended to be sublethal doses of phorate as similar as possible in their effects on the aphids.

2. Experimental

2.1. Plants and insects

The culturing of the broad bean plants, *Vicia faba*, L. cv. "The Sutton", and their handling and nutrient nourishment during experiments have been described previously.[4] Colonies of the aphid, *Aphis fabae* Scop., were established, one on each leaflet, of the bifoliate second leaves of these

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plants by caging alatae from a stock culture[5] on the abaxial surface.[6] After 1 or 2 days the adults were removed leaving the nymphs to develop within the cage and after a further 3 days the cages were exchanged for others with filter paper linings[6] and the insecticide was applied.

Two colony-establishing periods were tried; in the first, 20 alatae were caged for 1 day and in the second, 15 alatae were confined for 2 days. Both methods resulted in a colony of about 100 individuals though the latter was preferred because the times of ecdyses were spread more evenly among the individuals and feeding occurred at a more uniform rate.

2.2. Insecticide treatments

Two experiments were carried out each of which consisted of one foliar treatment and one root treatment with ^{14}C-methylene-labelled phorate ($(EtO)_2PS{\cdot}S{\cdot}^{14}CH_2{\cdot}SEt$),[4] (specific activity 42.5 mCi/g). The foliar treatment in the first experiment was of phorate (6 μg) applied to the adaxial surface of the third leaf (3 μg/leaflet) with 4 days allowed for translocation. In the root treatment phorate (22 μg) was added to the nutrient (100 ml) bathing the roots and 18 h was allowed for translocation. In the second experiment, both treatments (2×3.75 μg to the third leaf, 10 μg to the roots) were allowed to translocate for 2 days. All treatments were replicated on at least three plants. The doses and translocation times used were selected following pilot experiments and were a compromise between the doses that could be applied without seriously affecting the aphids and the amount of radioactivity required to ensure adequate levels for detection.

At the end of these transolcation intervals, the aphids present in each cage were counted and analyses[3,4] for toxic and non-toxic ^{14}C-compounds carried out on each colony, their honeydew and host leaflets. The fractions were not further characterised since in some extracts the levels of toxic ^{14}C were too low for further subdivision by thin-layer chromatography (t.l.c.). An indication of the relative proportions of the oxidation products that may have been present may be gleaned from the results of similar experiments[7] in which phorate (20 μg) was applied to the root nutrient. After 4 days the proportions found using an established t.l.c. technique[8] were: phorate sulphoxide 52%, phorate 18%, phorate sulphone 16%, phorate thioate sulphoxide 10% and sulphone 3% with only a trace of phorate thioate. The water-soluble non-toxic fraction was not further characterised either, and for convenience the results have been expressed in mole equivalents of phorate.

3. Results

The results of analyses are given in Tables 1 and 2. The first foliar treatment (6 μg for 4 days) was applied to four plants and so the values in the tables for this treatment are means of eight leaflets or colonies. In the remaining experiments three plants were treated and thus the values are means derived from six replicates.

Table 1. Total toxic metabolites found in aphids, honeydew and host leaflets following ^{14}C-phorate treatments

Treatment and translocation period	Mean residue ±s.e. (pmol phorate equivalent)			
	In leaflets	In aphids (mean no./colony)	In honeydew	Mean residues/aphid
6 μg to leaf 3 for 4 days	0.83 ± 0.11	2.66 ± 0.29 (66)[a]	2.05 ± 0.14	0.040
7.5 μg to leaf 3 for 2 days	0.41 ± 0.08	3.06 ± 0.55 (132)	0.59 ± 0.08	0.023
22 μg to roots for 18 h	1041 ± 88.0	3.08 ± 0.26 (66)	1.25 ± 0.11	0.046
10 μg to roots for 2 days	290.7 ± 82.6	7.44 ± 0.78 (92)[b]	0.80 ± 0.05	0.081

[a] A few nymphs were also present in the colonies at the end of this experiment; they were analysed with the adults but not included in the census.

[b] 0.9% mortality.

Table 2. Non-toxic ^{14}C found in aphids, honeydew and host leaflets following ^{14}C-phorate treatments

Treatment and translocation period	Mean residues ± s.e. (pmol phorate equivalent/ leaflet or colony)		
	In leaflet	In aphids	In honeydew
6 μg to leaf 3 for 4 days	5.4 ± 0.76	39.0 ± 4.5	126.5 ± 14.4
7.5 μg to leaf 3 for 2 days	2.9 ± 0.74	6.16 ± 1.0	119.6 ± 13.6
22 μg to roots for 18 h	100.4 ± 12.6	26.3 ± 5.0	21.5 ± 3.2
10 μg to roots for 2 days	87.4 ± 10.9	12.8 ± 2.4	153.4 ± 18.6

The most important feature of these results is the disparity between the total toxic residues found in the host leaflets following the two types of treatment (Table 1). The leaflets receiving insecticide from the root treatment contained more toxic residues than those of the foliar treatment by a factor of at least 100. In contrast, the amounts found in the aphids feeding on these leaflets differed relatively little. The same pattern was reflected in the levels of non-toxic labelled compounds though here there was more variation and the differences were less marked (Table 2). Overall, the root treatments led to greater quantities of both fractions in the leaflets but, whereas the non-toxic fraction present after the two-day root treatment was about 30 times greater than in the foliar treatments, there was barely a two-fold difference in the total amounts present in the aphid "sinks" suggesting that this non-toxic label was more readily available to the aphids when the foliage had been treated. There was certainly more non-toxic than toxic label in the leaflets after the foliar treatment whereas the reverse was true in the root-treated plants.

Very little of the radiolabel applied to the plants was recovered in the extractions. Most was found following root treatment where in both experiments the infesting colonies and host leaflets combined contained between 1 and 2% of the initial dose. In the foliar treatments only 0.5 and 0.8% of the initial dose appeared in the extracts.

4. Discussion

A degree of similarity in the toxic residues found in each colony might perhaps be expected since the doses applied to the plants were intended to be sublethal to the same extent and, as had been found in the pilot experiments, had any of these doses been increased or had the experiments been allowed to run for a longer time more casulaties would have been recorded. The 2-day root treatment was marginally above the intended level; five moribund aphids were discovered and more of the individuals in these colonies were found walking round the cages, a behaviour pattern which typically precedes more definite symptoms of poisoning. Consistent, too, with these observations was the fact that these aphids contained more toxic residues than the others.

The residues of toxic compounds found in the aphids did not necessarily represent the total amounts imbibed since some was excreted in the honeydew and no account was taken of hydrolysis within the insects. However, these quantities represented the toxicologically important proportion of the imbibed label at the time of sampling and were comparable when expressed on a *per capita* basis (Table 1).

An earlier study[3] suggested that most of the toxic compounds from a foliar treatment applied above or below an infestation arrive in the infested leaves in the phloem. The results reported here reinforce this hypothesis for the amounts in these leaflets were less than in the infesting colonies whereas after xylem translocation from the roots far greater quantities were found in the leaflets. A similar pattern would also be expected in a more simple system with no interconnection between phloem and xylem. A mature leaf normally exports assimilate but with aphids drawing on the phloem, material entering the leaf in this tissue to satisfy the demand of the aphid "sink" would be withdrawn at least in part from the plant; when entering the xylem it would remain in the leaf unaffected by the aphid colony. If a small leakage between xylem and phloem occurs, as is probable,

then these results agree closely with the expected pattern. However the situation which exists in the plant is complex because the toxic fraction as a whole is being continually augmented from its source and is simultaneously being degraded to non-toxic products. Also the proportions of the various toxic metabolites that constitute this fraction are continually changing[7] and, though this is a secondary factor, it would probably affect the toxicity of the residues.

The quantities of water-soluble radiolabel present in the leaves at the end of the experiments also support the view expressed previously[3] that this non-toxic residue is readily translocated in the phloem. After movement from the roots and hence xylem transport there was less non-toxic than toxic label in the leaflets but, after the foliar treatment non-toxic label predominated and appears to have been more readily available to the aphids, for they contained more than their host leaflets in these treatments and less after the root treatments.

Understandably, these results, obtained at one instant in a dynamically complex system, cannot elucidate the progress of the absorption, translocation, activation and hydrolytic mechanisms involved. However, the differing leaf residues and the relative constancy of the potentially toxic residues present in the aphids together with the variety of treatments and times employed emphasise the importance of translocation pathways in the distribution and availability of this compound to the aphids.

5. Conclusions

Phorate, when mobile in the phloem, was potentially more toxic to infesting aphids than when translocated to the insects in the xylem. The total phorate residue in a leaf was not necessarily a true indication of the toxic potential of that residue.

The results confirmed the poor transfer of phorate and its toxic residue components from xylem to phloem.

Acknowledgements

The authors wish to thank American Cyanamid Company for the radiolabelled insecticide and the Agricultural Research Council for their support for the liquid scintillation spectrometer. L.A.F. acknowledges the British Council for financial support, some of these experiments formed part of a thesis submitted by him for a Ph.D. in the University of London.

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