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# Movement of Phorate and Metabolites from Treated Leaflets to Aphid Colonies on Broad Bean Plants

Luis A. Foerster[a] and D. John Galley

*Department of Zoology and Applied Entomology, Imperial College, London SW7 2AZ*[b]

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Aphid colonies were established on one second-node leaflet of young broad bean plants and sublethal treatments of $^{14}C$-labelled phorate were applied to the adjacent leaflets. After 3 days, the amounts of toxic and non-toxic $^{14}C$-labelled compounds in the aphids, their honeydew and the untreated foliage were determined. There were no significant differences between the amounts in leaves at the same level on the plant when infested and aphid-free plants were compared, but the aphids and their honeydew contained two and 23 times as much of the toxic and non-toxic $^{14}C$-compounds, respectively, found in the host leaflets. Following translocation to aphids on leaves above or below treated leaflets, the aphid colonies again contained more labelled compounds than the host-plant leaves. The movement of non-toxic compounds into the roots was reduced when the aphid colonies were situated on foliage between the site of treatment and the roots. More of the toxic and the non-toxic fractions were translocated downwards from the third to the second leaf than in the reverse direction.

## 1. Introduction

The apoplastic translocation characteristics of systemic insecticides and fungicides currently in general use preclude their redistribution within plants since, being restricted essentially to the apoplast they become concentrated at the sites of water loss, the leaf margins.[1–3] While this may be ideal for the control of foliage-consuming insects, mainly lepidopterous larvae, there are many more pests whose destructive feeding habits are associated with the physiological sinks of plants namely the roots, the developing shoots and, in mature plants, the fruits. The Aphididae is one of many families of the Insecta that exploit plants in this way, some species having developed their gregarious feeding habits to the extent that they may induce sink conditions in otherwise normal productive foliage.[4]

Established aphid colonies, feeding predominantly in the phloem, are known to remove considerable quantities of assimilate from plants but appear not to affect greatly the apoplastic transport of an exogenous pesticide.[5] These authors found small amounts of toxic compounds and relatively greater quantities of the water-soluble (non-toxic) metabolites in the aphid colonies and their honeydew; a pattern which occurred when the insecticide was applied either to the roots or to the foliage. However, distribution patterns in these plants were only investigated following root uptake and hence apoplastic movement. The aim of the work reported here was to investigate more closely the fate of foliar-applied phorate in relation to established aphid sinks situated above, below and beside the site of application. Foliar treatments were employed since translocation from the application site must occur in the phloem at least initially and in these tissues its movement is more likely to be affected by the feeding insects. Small doses, sub-lethal to the insects, were used in order that feeding habits might be disrupted as little as possible.

[a] Present address: Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Brazil.
[b] Work carried out at Imperial College Field Station, Silwood Park, Ascot, Berks.

## 2. Experimental

### 2.1. Biological materials

#### *2.1.1. Plants*

Broad bean plants, *Vicia faba* L. cv. The Sutton, were reared in a glasshouse and used in experiments when the third leaf had fully expanded. At this stage the plants were transferred to nutrient solutions and kept in a constant environment (20°C, 16 h photoperoid).[3] Similar plants, but pot grown in potting compost, were used for maintaining the aphid culture.

#### *2.1.2. Insects*

The aphid, *Acyrthosiphon pisum* (Harris), was cultured on bean plants in 500 × 500 × 500 mm Perspex cages in the same constant environment used for the experiments.

In each experiment colonies were established where required on nutrient-fed plants[3] by confining 20 reproducing adults in small cages[6] to the abaxial leaf surfaces. After 24 h the adults were removed and the nymphs allowed to continue development within the cages. Later, when the insecticide was applied to the plants, the cages were exchanged for others to which a plain filter paper lining had been added.[6]

### 2.2. The insecticide, extraction procedures and radioassay

[$^{14}$C]-Methylene-labelled phorate[3] (specific activity 42.5 mCi/g) was spotted on the appropriate leaf surfaces in an acetone solution using a microcapillary syringe. At the end of each experiment the plants, aphids and honeydew samples were stored at −20°C until required. The soluble radiolabel was extracted by homogenising the leaves with aqueous acetone, filtering and washing the filtrate with chloroform. In this procedure, phorate and its toxic metabolites partition into the chloroform fraction and the non-toxic metabolites remain in the aqueous phase.[3]

The aphids were extracted similarly but with smaller quantities of charcoal and reduced volumes of chloroform. Honeydew-bearing filter papers were shaken for 5 min with a 1 + 1 v/v chloroform + water mixture (10 ml) and rinsed with 5 ml aliquots each of chloroform and water. The phases were separated, concentrated and transferred to vials with liquid scintillant for radioassay.[3]

### 2.3. Experiments

#### *2.3.1. Movement from an adjacent leaflet*

Colonies of *A. pisum* were established on one leaflet of the bifoliate second leaf of each of 3 plants and 4 days later $^{14}$C-phorate (3 μg) in acetone (15 μl) was applied to the adjacent aphid-free leaflet. Three plants without aphids were similarly treated. Three days later the experiment was terminated and the first and third leaves, the infested leaflet, the aphids and their honeydew were analysed.

#### *2.3.2. Translocation from sources above or below aphid colonies*

Two experiments were done following procedures similar to section 2.3.1. In the first experiment aphid colonies were established on both leaflets of the second leaf and phorate (3 μg) applied to both leaflets of the third leaf. In the second experiment the aphids were caged on both of the third pair of leaflets and insecticide was applied to the second pair of leaflets. Both the foliage and the aphids were analysed after 3 days and, following the downwards translocation experiment, the roots also.

## 3. Results

### 3.1. Movement from an adjacent leaflet

The results (Table 1) demonstrate that small quantities of the toxic metabolites were translocated to the untreated foliage and that there was significantly more ($P < 0.02$) in the leaflet adjacent to that treated than in other leaflets. The colony had had little effect on these quantities for the amounts

present in the infested leaflet were not significantly different ($P > 0.9$) from those in the equivalent leaflet of aphid-free plants. However, at the end of the experiment the aphids with their honeydew contained more than double the amount present in the leaflet on which they were feeding. Thus the total amounts entering this leaflet were greater when the colony was present than when it was not.

More non-toxic than toxic radiolabel was found and again the amounts present in the leaves of both aphid-free and infested plants were similar. The aphids and their honeydew, however, contained some 23 times the quantities residing in their host leaflet at the end of the experiment again emphasising that considerably more had entered this leaflet when aphids were present.

**Table 1.** Quantities of $^{14}$C-compounds in the foliage and an aphid colony 3 days after a $^{14}$C-phorate application to a leaflet adjacent to the colony

| | Phorate derivatives, pmol/leaflet or aphid colony ± s.e. | | | |
|---|---|---|---|---|
| | Toxic fraction[a] | | Non-toxic fraction[b] | |
| Location | Infested plants | Aphid-free plants | Infested plants | Aphid-free plants |
| Third leaf | 0.51 ± 0.12[c] | 0.60 ± 0.18 | 3.65 ± 0.51 | 3.83 ± 0.75 |
| Second leaflet | 1.51 ± 0.27[c, d] | 1.03 ± 0.27[d] | 4.91 ± 1.04 | 5.41 ± 1.49 |
| Aphids | 2.61 ± 0.51 | — | 46.86 ± 6.71 | — |
| Honeydew | 0.71 ± 0.18 | — | 65.49 ± 9.10 | — |
| First leaf | 0.58 ± 0.18 | 0.47 ± 0.15 | 1.30 ± 0.35 | 1.13 ± 0.37 |

[a] Fraction partitioning into chloroform from aqueous acetone; phorate, its sulphoxide, sulphone and the respective phosphorothioates.

[b] Fraction remaining in aqueous acetone when partitioned with chloroform; non-toxic hydrolysis products of phorate and derivatives.

[c] $t$-test for similarity, $P < 0.02$.

[d] $t$-test for similarity, $P > 0.9$.

### 3.2. Translocation from sources above or below aphid colonies

Similar results were obtained when the insecticide was applied to a leaf above or below the aphid colonies. The aphids did not greatly modify the levels of toxic and non-toxic metabolites present in the leaves, when infested and aphid-free plants were compared (Tables 2 and 3) but in both experiments the aphid colonies contained more radiolabel both as non-toxic and toxic compounds than was found in the leaves supporting the colonies.

In the basipetal translocation experiment (Table 2) the aphids significantly reduced the amounts of the non-toxic fraction moving to the roots though the toxic components were unaffected.

**Table 2.** Quantities of $^{14}$C-compounds in aphid colonies, second leaves and roots 3 days after $^{14}$C-phorate application to the third leaves

| | Phorate derivatives, pmol/leaf, root system or colony pair ± s.e. | | | |
|---|---|---|---|---|
| | Toxic fraction[a] | | Non-toxic fraction[b] | |
| Location | Infested plants | Aphid-free plants | Infested plants | Aphid-free plants |
| Second leaf | 2.74 ± 0.48 | 1.73 ± 0.46 | 15.88 ± 3.91 | 17.85 ± 5.66 |
| Aphids | 6.52 ± 1.34 | — | 188.48 ± 44.18 | — |
| Honeydew | 3.60 ± 0.76 | — | 240.56 ± 39.66 | — |
| Roots | 1.79 ± 0.29 | 1.82 ± 0.52 | 34.05 ± 8.04[c] | 89.62 ± 18.83[c] |

[a], [b] As Table 1.

[c] $t$-test for similarity $P < 0.02$.

Table 3. Quantities of $^{14}$C-compounds in aphid colonies and in the third leaves 3 days after $^{14}$C-phorate application to the second leaves

| Location | Phorate derivatives, pmol/leaf or colony pair ± s.e. | | | |
|---|---|---|---|---|
| | Toxic fraction[a] | | Non-toxic fraction[b] | |
| | Infested plants | Aphid-free plants | Infested plants | Aphid-free plants |
| Third leaf | 1.77 ± 0.27 | 2.20 ± 0.55 | 22.90 ± 3.44 | 31.25 ± 9.02 |
| Aphids | 3.70 ± 0.44 | — | 104.54 ± 21.09 | — |
| Honeydew | 1.33 ± 0.33 | — | 130.44 ± 27.69 | — |

[a], [b] As Table 1.

## 4. Discussion

### 4.1. Aphid-free plants

Some loss of the insecticide by evaporation from the leaf surface is to be expected especially when simple solvent solutions are employed, as in these experiments. Thus the amounts available for translocation are likely to be somewhat less than the dose applied.

The results of the treatments to aphid-free plants clearly demonstrated that the amounts of toxic material appearing in the untreated foliage were very small even when compared with the quantities probably available for translocation. Similar differences between treated and untreated leaves were found in previous experiments[5] where, after 6 days, between 50 and 100 times more of the toxic fraction was found in a treated second node leaflet than in an adjacent untreated leaflet.

These observations confirmed the apoplastic tendencies of phorate and its toxic metabolites and emphasise that only a small proportion of the toxic derivatives within a treated leaf is exported to other parts of a bean plant.

The non-toxic metabolites were present in greater abundance than the toxic metabolites but they, too, formed a relatively small proportion of the applied dose. In these experiments the fate of all the translocated radiolabel was not investigated. It would be expected, however, that the natural plant sinks in the young leaves and stem apex also contained a proportion of the non-toxic label, as was found in the roots (Table 2).

### 4.2. Aphid-infested plants

The toxic and non-toxic residues found in the untreated foliage of aphid-infested plants were not significantly different from those in the aphid-free plants but appreciable quantities of labelled material, greater than the amounts in the host foliage, were found in the aphids and their honeydew. Thus the colonies had withdrawn both toxic and non-toxic metabolites from the leaves on which they were caged without greatly influencing the accumulation of these compounds within the host leaf. The non-toxic fraction was more affected by the aphids in this way than the toxic fraction, though, from these experiments alone, it is not possible to ascertain to what extent the appearance of hydrolysed phorate derivatives in the honeydew was due to imbibing these compounds from the plant in that form or to hydrolysis within the insect. There are, however, a number of reasons which suggest that they were most likely imbibed after hydrolysis. Firstly, most of the hydrolysed label probably appeared as such in the leaf since, before it could reach the infested area, the label must have been exported from the leaf to which it was applied, a process which the toxic metabolites do not readily undergo. Support for this lies with the existing evidence for the apoplastic characteristics of this type of systemic compound[1] and earlier experiments[3] suggesting that the major route for loss of this particular label from accumulations in leaves is in hydrolysis products. Secondly, the results following basipetal movement (Table 2) showed that the aphid colonies on the second leaf reduced the normal passage of this label to the roots, the amounts of label found here in infested

plants being significantly less ($P < 0.02$) than in aphid-free plants. There was one apparently conflicting result here; the toxic radiolabel found in the roots was not affected by the aphids in the same way; however, the lesser amounts involved might have been more prone to redistribution in the plant by partitioning back into the apoplast.

Although the toxic metabolites do not readily partition into phloem tissue, some must do so to be exported from treated leaves, but once exported from the treated leaf, they might be expected to return to the xylem relatively quickly. This would appear not to be so, however, for in the infested leaves most of both the toxic and non-toxic fractions was found in the aphids and honeydew, whereas with apoplastic translocation following root application most of the extractable label was in the leaf and not in the aphid colony.[5] Further evidence may be found in a comparison of the amounts present in the leaves above and below the site of application. If significant partitioning back into the xylem were to occur in the stem, then greater quantities would be expected in a leaf above that treated. Little, if any, would reach a lower leaf. In these experiments no such trend was found with the toxic fraction though this possibility cannot be eliminated completely, since the non-toxic label derived at some time from the toxic fraction was found in greater quantity in the higher leaflets.

## 5. Conclusions

The residues of both toxic and non-toxic label found in the leaves of infested plants were not significantly different from those in the equivalent leaves of aphid-free plants. The aphids had, however, altered the pattern of distribution of both fractions because, when present, they withdrew considerable quantities of labelled products from the plants, far greater than the residual amounts in the host leaflets. When interposed between the root and the site of application the colonies also reduced the amounts in the root. Both the toxic and non-toxic fractions appeared to move from the treated areas to the aphid colonies in the phloem with little partitioning into the xylem.

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