Agricultural and Forest Entomology (1999) **1**, 55–60

The effect of simulated 'wash off' from spot-sprays containing either Malathion or Phloxine B on ground-dwelling arthropods in an orchard.

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Abstract

1 Leaf-litter samples were taken from treated patches of ground in an orchard 7 days after the application of a diluted spot-spray mixture (simulating washed off residue). The mixture contained either 1% Phloxine B or one of two concentrations (1% or 0.1%) of Malathion; the control treatment was water.

2 Half of the replicates of each treatment were left open to immigration and emigration of the leaf litter animals in the assemblage for a week before sampling, the other half were not.

3 Malathion at either concentration altered the assemblage in the treated patches by significantly decreasing abundance and species richness. However, the latter was less affected in treatments open to invasion. Phloxine B had no detectable effect.

4 When patches of leaf litter were completely cleared and replaced with sterilized peat moss (simulating complete insecticide kill without residual effect), the peat moss was rapidly colonized by microarthropods.

Keywords Arthropods, diversity, Malathion, Phloxine B, spot sprays.

Introduction

Phloxine B is a red dye with the potential to be used in the field as a targeted pesticide because it has no effect when topically applied but has a lethal effect in the presence of light when mixed with bait and ingested by tephritid fruit flies and other insects (Clement *et al*., 1980; Bergsten, 1995, 1997; Pimprikar, 1995; Dowell, 1997; Wilson *et al*., 1997; Thomas & Meats, 1999).

Malathion is a contact insecticide that is commonly used in agricultural systems and is effective against a wide range of pests. Its application in bait sprays used as cover sprays for control of fruit flies has been associated with a decrease in arboreal and flying non-target species including beneficial insects (Abdelrahman, 1973; Harris *et al*., 1980; Ehler & Endicott, 1984; Gary & Mussen, 1984; Cohen *et al*., 1988; Daane *et al*., 1990; Hoelmer & Dahlsten, 1993; Messing *et al*., 1995). Pesticide residues, washed from the foliage by rain or leaching from annihilation traps, can also be toxic to the ground-dwelling arthropod community (Whitford & Showers, 1987; Whitford *et al*., 1987; Asquith & Messing, 1992; Stark, 1992; Messing & Seiler, 1993).

In recent years, the use of pesticides has declined as alternatives to cover sprays have been sought (Romoser & Ferro, 1994). In Australia, bait sprays with Malathion are not used as cover sprays against tephritid fruit flies as they are in most parts of the world but are used in spot applications and cover as little as 1% of the infested area (Anon, 1996). This technique reduces the amount of the insecticide required, the cost of application, and also reduces the damage sustained by non-target arthropod species, although these would still be killed if they were attracted to the bait or made random contact (Asquith & Messing, 1992; Messing & Seiler, 1993). It appears from the above, that spot application of bait sprays with Phloxine B would be even less harmful to non-target insects as random contact is not lethal.

The local effects of insecticides on non-target organisms (as would be the case with spot sprays) are known to be difficult to detect (El Titi, 1988; Aebischer, 1990). One important reason shown for this is the tendency for individuals to move into areas after any treatment even if residual toxicity remains (Brown *et al*., 1988; Burn, 1988; Quinn *et al*., 1990; Straalen *et al*., 1992; Ufer *et al*., 1995).

We report here on experiments conducted in an orchard where (a) patches of leaf litter were treated with simulated 'wash off' either with or without insecticide and left open to invasion or not, and (b) the rate of invasion of defaunated patches was estimated in an experiment that simulated complete insecticide kill with no residual effect.

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Methods

Site description

The study was conducted in late autumn in a commercial peach orchard at Glenorie, just beyond the north-west outskirts of Sydney. The trees were 'Flavour Crest' and 'Flame Crest' varieties. They had been planted 1 m apart (for greater yield) along each row but the canopies were up to 3 m diameter in the direction normal to the rows. They were irrigated with a drip-flow system. Leaf-litter covered the ground to a depth of 1–3 cm and it was composed mainly of the dead peach leaves. Grass and weeds surrounded the trees.

Rationale for the insecticide treatment

Bait sprays in Australia are most commonly used against fruit flies and normally consist of 100-mL squirts of an aqueous solution of 1% Malathion and 2% protein hydrolysate applied to the lower foliage of trees at a rate of 100 ha^{-1} (Anon, 1996). The protocol used here assumes that the full amount of insecticide (1 g) is washed off by rain on to an area of soil within the drip-line of 0.25 m^2 ($\approx$ 0.5 m in diameter). One-gram amounts of Malathion or Phloxine B were administered as 1 litre of solution with 0.1% insecticide and 0.2% protein hydrolysate (equivalent to wash off caused by 4 mm of rain). We also used a treatment equivalent to 10 times the normal concentration of Malathion (i.e. 1 litre per patch with 1% Malathion or 10 g per 0.25 m^{-2}). Treatments were applied directly to the ground, the solutions being sprinkled evenly over the area using a watering can.

Effect of insecticides with and without migration of animals permitted into and out of treated leaf litter

Only a small area was allocated for the study. The results of a pilot study indicated that five replicates would constitute a representative sample of the assemblage of animals in the leaf litter. A randomised block design incorporated four treatments: a control (water), Phloxine B, and both concentrated and dilute Malathion. The Phloxine B was a 0.1% solution, dilute Malathion 0.1% and concentrated Malathion 1%. Each treatment was applied to 10 patches (see below) so that, in each case, five samples could be taken immediately and kept closed to immigration and emigration of leaf litter animals and five samples could be taken 1 week later with the treatment areas open to migration.

Each treatment patch was marked out as a square (0.25 m^2) directly beneath a tree, within the drip line of the canopy. Samples consisted of leaf-litter taken from a circular template, 0.5 m in diameter. The template was placed within the treated patch; the area was cleared of loose leaf-litter, scraped with a metal scoop to expose the soil and cleared of grass roots and weeds: all of the foregoing constituted the sample.

Immediately after treatment, one half of each paired sample was collected. These represented the treatments closed to migration; each was placed in a 10-L bucket covered with fine nylon mesh. They were transported to the laboratory and kept under a relatively constant temperature and humidity regime (about 20°C, 45% r.h.) for one week before being sorted. The second half of each paired sample was left open in the field for 7 days before collection and transport to the laboratory for immediate sorting. Treatment of patches for 'open' and 'closed' samples was staggered by a day, because only 20 could be processed per day (by the available Berlese funnels).

On return to the laboratory, the leaf-litter was initially sorted for large motile animals, using a 10-mm grill. Any specimens gained were preserved in ethyl alcohol. Large sticks and other matter were discarded after this sort, and the remaining litter and soil was placed in Berlese funnels (Michael, 1984). The funnels were left for 24 h with a 100-W clear bulb switched on and suspended about 15 cm from the surface of the litter. The specimens extracted fell into 30% ethanol; this was later topped up with a 100% solution.

Invasion of defaunated patches

The second study was conducted in a separate section of the site described above. The investigation ran for 3 weeks. Treatment consisted of replacing patches of leaf-litter with sterilized peat moss. We decided to use the peat moss as a substitute for sterilized leaf litter as we did not have the facilities to sterilize large quantities of the leaf litter with heat and we did not wish to use chemical sterilization because of the risk of having confounding effects of residues from such treatment. A leaf-litter sample was taken immediately before the disturbance. Four samples of peat moss were taken: one on the day after the disturbance and another in each of the subsequent weeks. A randomised block design was used to incorporate four replicates of each sample.

On the first day of the study, the 'before' sample was collected. A circular template (diameter 30 cm) was set down randomly within the drip line. The area was completely cleared of leaf-litter, which was then placed into resealable plastic bags for transport to the laboratory. Peat moss treatments that were to be sampled subsequently were also set up on the first day of the study (16 patches in all, to enable four replicates on each of four occasions). Leaf-litter was cleared from randomly selected areas within the drip line, using the template as above and replaced with peat moss. The peat moss was evenly distributed and as thick as the surrounding leaf-litter (1–2 cm deep). The cleared leaf litter was discarded on the other side of the orchard.

When peat moss samples were collected, the original template was used as a guide. It was gathered up (in the same way as the first leaf-litter samples had been) for immediate transport to the laboratory where it was sorted as described above. To ensure that it was not contaminated by arthropods before deployment in the field, the peat moss had been heated to a temperature of $\approx$ 50°C for 24 h before it was used in the field. Two additional sets of peat moss were kept in the laboratory and sampled at one and 7 days, respectively, after the heat treatment. A single psocopteran was found in this material. Although psocopterans had been recorded in the field during pilot studies, this taxon was excluded from further analysis. At some time during the 24-h period in which the 'before' replicates were being processed in the funnels, one of the bulbs burnt out; hence the affected replicate was excluded from further analysis. Several days before the last sample was taken, the surrounding grass in the orchard was cut (by the farmer), using a ride-on lawn mower, which severely disturbed

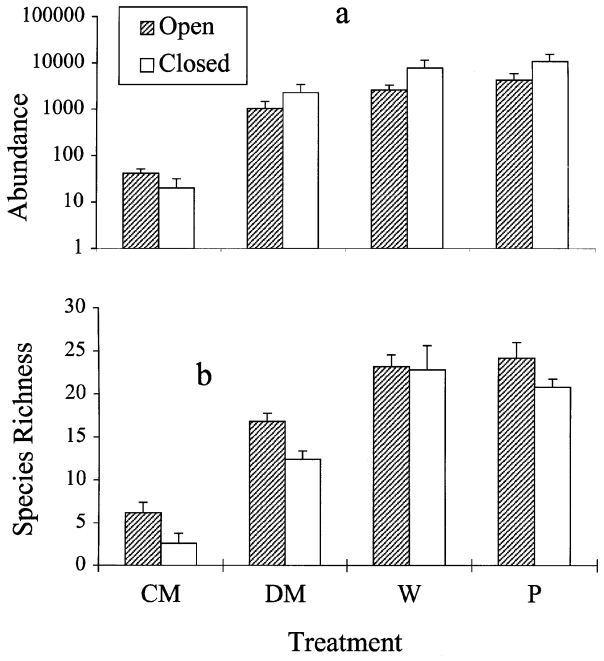

Figure 1 Effect of insecticide component added to bait spray mixture applied to leaf litter at concentrations equivalent to wash off. Differences in (a) abundance and (b) species richness of the entire assemblage sampled. Counts were done 7 days after treatment: in one series the leaf litter had remained open to migration during this period and in the other it was closed to migration. Values are means ± SEM, $n = 5$. Treatments: CM = 1% Malathion, DM = 0.1% Malathion, P = 1% Phloxine B and W = water.

all remaining treatment areas and surrounding areas of leaf litter.

Identification and exclusion

All specimens of animals in the samples were identified to order, sorted into morphospecies and counted. Later, these identifications were checked, and an attempt was made to refine taxonomic clarity. Individuals in classes Insecta and Collembola were identified to family or subfamily (Andersen, 1991; CSIRO, 1991). All spiders were treated as one morphospecies, as were larvae of Coleoptera, Diptera and Lepidoptera. The Acarina were identified courtesy of G. Hunt (of the Australian Museum) and D. Walter (The University of Queensland). Specimens were preserved in 70% ethyl alcohol.

Adult dipterans, thysanopterans and winged hymenopterans were excluded because it was thought that they did not represent the ground-dwelling assemblage. To improve the consistency of the sorting effort, all species less than 1 mm in length were also excluded. In both studies exclusions amounted to less than 0.1% of total abundance.

Analyses

The abundance and species richness of the sampled assemblages in both studies were examined using analyses of variance and differences among means were examined with Student–Newman–Keuls tests (Winer *et al.*, 1991). Similar analyses examined the effects of the treatments in the three most dominant taxa, as well as those seen in the remaining assemblage once these groups had been excluded. Data were transformed appropriately if shown, by Cochran's test, to have heterogeneous variances.

Multivariate methods of analysis (Clarke, 1993) were also used to examine differences between treatments and sample times. Non-metric multidimensional scaling (MDS) produced a two-dimensional ordination to represent the relative differences between samples graphically. The stress value calculated during the MDS represents the degree to which the relative distances in the two-dimensional ordination diverge from the actual relative distances between the points, which can be in three or more dimensions. A stress value of less than 0.20 is conventionally thought to be desirable.

Analysis of similarities (ANOSIM) was used to test the null hypothesis that the treatments produced no effect on the community structure of the sampled assemblage. All multivariate techniques were conducted using PRIMER v.4.0 and are described further in Clarke (1993). The similarity matrices between samples used by the MDS and ANOSIM were constructed using the Bray–Curtis similarity coefficient. Data were converted using a log transformation; this retained information concerning relative abundance across samples, while reducing large differences in scale.

With all analyses, null hypotheses were rejected using a 0.05 probability of a type one error.

Results

Effect of insecticides with and without migration of animals permitted into and out of treated leaf litter

The sampled assemblage was dominated by high density, less speciose groups: the class Collembola and the order Acarina made up 99.3% of total abundance but accounted for only 28.3% of species sampled. One species of Collembola (family Neanuridae) represented over 95% of the fauna.

Univariate analyses showed a significant difference in abundance between some treatments (Fig. 1a). The analyses revealed that the assemblage treated with 1% Malathion had significantly fewer individuals than that treated with the 0.1% solution. Both had significantly fewer individuals than the 'control' and 'Phloxine B' samples. There was no significant difference in abundance between each 'open' and 'closed' treatment (Fig. 1a).

Species richness showed a similar pattern (Fig. 1b). However, with the Malathion treatments, there were significantly fewer species in samples that were closed to invasion when compared to those left open in the field for 7 days after treatment. When different taxonomic groupings were examined separately (i.e. as Collembola, Acarina and 'others'), the results were essentially the same as above.

The graph of the MDS ordination (Fig. 2) shows three groups pertaining to 1% Malathion, 0.1% Malathion, and remaining treatments, respectively. The 'Phloxine B' and 'control' points form a tight cluster, with the scatter of points from both treatments overlapping. The plot only gives information about

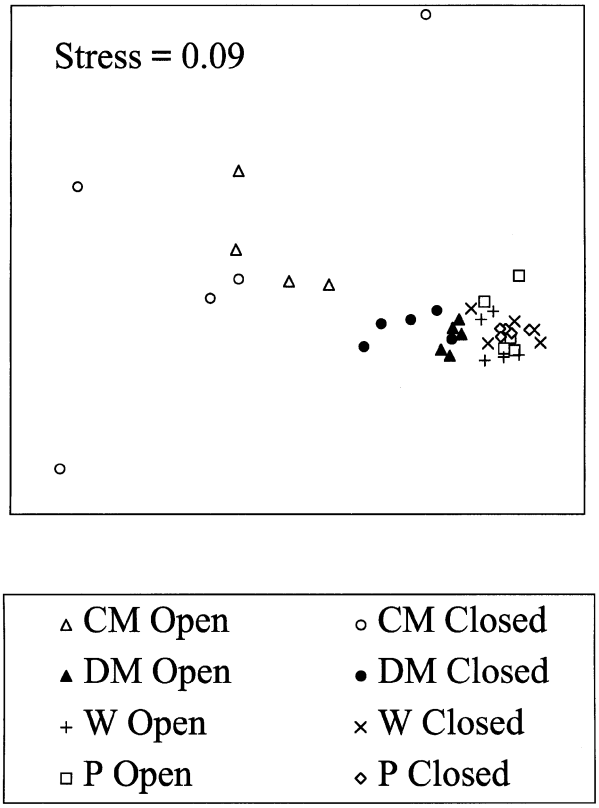

Figure 2 The MDS ordination of the assemblage sampled for Fig. 1, in each of the treatments both open and closed to migration. Treatments: CM = 1% Malathion, DM = 0.1% Malathion, P = 1% Phloxine B and W = water. The plot only gives information about the relative distances between the points; the values on the axes are irrelevant.

the relative distances between the points; the values on the axes are irrelevant. The stress value (representing the degree of distortion of distances due to the two-dimensional representation) was acceptably low (see Methods).

Generally, the same pattern was seen among the treatments regardless of whether the points pertained to samples being 'open' or 'closed' to migration. The majority of pairwise comparisons in each ANOSIM showed a significant difference between the assemblages treated with 1% and 0.1% Malathion solutions; both were significantly different from the control and Phloxine B samples.

Invasion of defaunated patches

Again, Collembola and Acarina dominated the assemblage sampled. A new morphospecies (Acarina, Tydeidae) was recorded in the sample taken 7 days after disturbance. This species was the second most abundant in weeks 1, 2 and 3. The family Tydeidae is comprised of opportunistic predators, scavengers and fungivores (D. Walter, pers. comm.).

The abundance in the 'before' sample was greater than in any of those taken after disturbance. No significant differences were seen between post-disturbance samples (Fig. 3a). Species richness showed a similar pattern; the 'before' sample contained

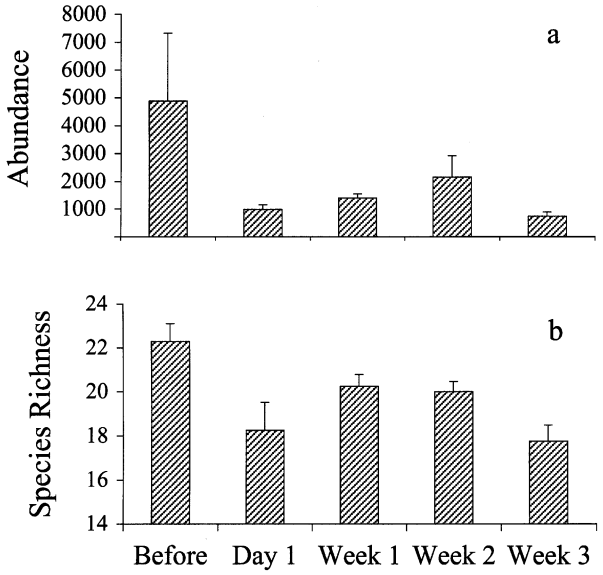

Figure 3 Invasion of defaunated patches. Effect of replacing the leaf litter with sterilized peat moss: the abundance (a) and the species richness (b) of the assemblage pre- and post-disturbance. Values are means ± SEM, $n = 4$. Note there was an additional unintended disturbance before taking the samples for week 3 (see text).

a significantly greater number of species than those collected on day 1 or week 3. However, no difference was seen between the 'before' sample and weeks 1 and 2 (Fig. 3b).

The MDS ordination of the assemblage sampled pre- and post-disturbance shows three groups (Fig. 4). The points representing the 'before' sample are clearly distinct from those of the first day after disturbance, and those of samples of weeks 1–3. The majority of pairwise comparisons in the ANOSIM showed a significant difference between pre- and post-disturbance samples. No difference was seen between the assemblages sampled in weeks 1–3.

Discussion

The insecticidal treatments reported here did not exactly mimic the effects of spot sprays for tephritid fruit flies because they were applied in late autumn rather than in the period from early spring to late summer. We can, however, draw some general conclusions about the effect of spot-spray wash-off on the fauna of leaf litter.

Spot-spray wash-off containing Malathion can have a significant effect on the structure of the local arthropod community in the leaf litter. This is consistent with the findings of previous investigations concerned with the effects of washed-off residues on soil and ground fauna (Whitford & Showers, 1987; Whitford *et al.*, 1987; Asquith & Messing, 1992; Stark, 1992; Messing & Seiler, 1993). Malathion, in a 1% solution, is about 10 times more potent than the wash-off that would actually occur with standard practice (Anon, 1996). Although the impact was most dramatic with the application of 1% Malathion, the 0.1% solution (a strength expected, with our assumptions, to 'wash-off' from standard practice) also had a slight effect.

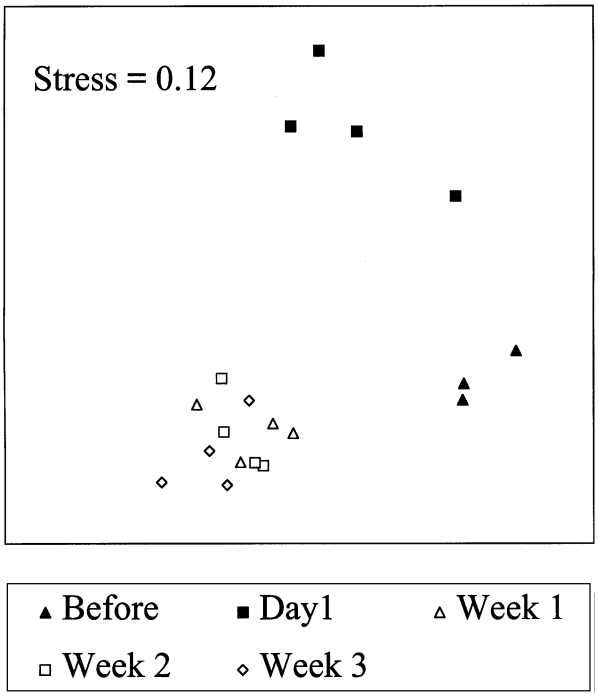

Figure 4 Invasion of defaunated patches. MDS ordination of the assemblage, sampled for Fig. 3, before and after replacement of leaf litter with sterilized peat moss.

However, it must be remembered that spot-sprays are normally only applied at 100 spots per hectare (Anon, 1996) which would affect (at most) only 100 m^2 of ground per hectare, or 1% of ground surface. Also, our assumption that the whole of the insecticide content of a spot-spray gets washed off on to an area of ground of only 0.5 m diameter may mean that our treatments that simulated the effects of spot-sprays of normal dilution were more concentrated than wash off from real spot-sprays.

It has been argued that movement of arthropods from untreated areas can cancel out the effect of an insecticide in a treated patch (Quinn *et al*., 1990). Such a phenomenon can be detected by comparing 'open' and 'closed' treatments, as in this paper, but there must be also a significant insecticidal effect in the treatment that is closed to invasion. The present study shows that there is an effect of Malathion when invasion of treated litter is prevented. Invasion of the Malathion treatments that were left 'open' resulted in this effect appearing less evident but only with respect to species richness (Fig. 1).

Phloxine B produced no detectable change in community structure in terms of abundance and species richness, whether the treatments were left open to invasion or not. When plotted as a two-dimensional ordination, the assemblage treated with Phloxine B appeared almost identical to the control. The dye was applied as 0.1% solution which is a concentration within the range that can produce a lethal effect in tephritid fruit fly (Thomas & Meats, 1999). If applied in a spot spray at this concentration, the strength of any wash off would be probably only a tenth of this (see Materials and methods). Phloxine B is generally regarded as a photo-activated insecticide, thus it may not impact on the leaf litter fauna that is not exposed often (if at all) to bright light. It is possible that a delayed reaction could take place, as the chemical can still be effective at low light intensities but takes longer to work (Thomas & Meats, 1999). No such effect was detected within the period of the present study.

It would appear from our experiments with peat moss that when the effects of a spot-spray deplete a treated patch, migrants could invade it, compensating for its loss. This is to be expected (Brown *et al*., 1988; Burn, 1988; Quinn *et al*., 1990; Ufer *et al*., 1995). However, it is also possible that immigrants would be killed subsequently by the residual insecticide. If this process did occur and was left to continue for several weeks or months, the difference seen between treated and untreated patches could decrease according to the availability of fresh immigrants and the level of residual toxicity in the treated patches. It also follows that a single spot treatment could kill more insects than were present in the treated patch at the time of application. The true environmental impact could be assessed using methods such as those proposed by Straalen *et al*. (1992).

The composition of invading faunal assemblage in our peat moss experiment did not exactly resemble the original one. This may have been due in part to the peat moss itself, which may not have been an appropriate substitute for defaunated leaf litter. Alternatively, different species may invade patches at different rates or similar changes could have also occurred simultaneously in the untreated leaf litter (surrounding the peat moss patches). The latter is unlikely, since the period involved was very short, but we cannot fully discount it because no simultaneous control sample was taken later in the experiment.

Acknowledgements

We thank David Walter of the University of Queensland and Glen Hunt of the Australian Museum for their help in identification.

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Accepted 9 February 1999