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INSECTICIDE RESIDUES IN AUSTRALIAN PLAGUE LOCUSTS (*CHORTOICETES TERMINIFERA* WALKER) AFTER ULTRA-LOW VOLUME AERIAL APPLICATION OF THE ORGANOPHOSPHORUS INSECTICIDE FENITROTHION

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Abstract: The need for locust control throughout eastern Australia during spring 2010 provided an opportunity to quantify residues of the organophosphorus insecticide fenitrothion on nymphs of the Australian plague locust, *Chortoicetes terminifera* Walker. Residues were collected across the different physiological states—live, dead, and debilitated (characterized by ease of capture, erratic hopping, and the inability to remain upright)—of locust nymphs observed following exposure to fenitrothion. The time course of residue depletion for 72 h after spraying was quantified, and residue-per-unit dose values in the present study were compared with previous research. Fenitrothion residue-per-unit dose values ranged from 0.2 μg/g to 31.2 μg/g (mean ± standard error [SE] = 6.3 ± 1.3 μg/g) in live *C. terminifera* nymps, from 0.5 μg/g to 25.5 μg/g (7.8 ± 1.3 μg/g) in debilitated nymphs, and from 2.3 μg/g to 39.8 μg/g (16.5 ± 2.8 μg/g) in dead nymphs. Residues of the oxidative derivative of fenitrothion, fenitrooxon, were generally below the limit of quantitation for the analysis (0.02 μg/g), with 2 exceptions—1 live and 1 debilitated sample returned residues at the limit of quantitation. The results of the present study suggest that sampling of acridids for risk assessment should include mimicking predatory behavior and be over a longer time course (preferably 3–24 h postspray) than sampling of vegetation (typically 1–2 h postspray) and that current regulatory frameworks may underestimate the risk of pesticides applied for locust or grasshopper control. *Environ Toxicol Chem* 2013;32:2792–2799. © 2013 SETAC

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INTRODUCTION

Broad-scale locust and grasshopper control programs worldwide rely primarily on the aerial application of chemical insecticides [1]. Many bird species are known to feed on Acrididae, the family of locusts and grasshoppers to which most economically damaging species belong. As such, locust outbreaks provide an important ephemeral food source for birds and are often accompanied by wide and diverse avian assemblages [2,3]. Locusts provide a rich source of protein (62% dry mass) and lipid (17% dry mass) for predators [4,5] and, in regions of inland Australia characterized by low rainfall and variable resource availability, provide valuable nutrients for the many bird species that descend on areas experiencing locust population increases [6]. Several of these species specialize on locust outbreaks and have been observed feeding on this superabundant food source, often in large flocks [7].

Environmental cues that trigger increases in locust populations are also responsible for the physiological mechanisms that stimulate breeding in birds, enabling avian species to manage the natural variation in energy availability and reproduce while conditions are favorable [8]. Consequently, locust outbreaks and avian reproductive events can co-occur [6] and, combined with the ability of avian species to gorge feed, increase the risk to avian populations from pesticide exposure beyond the scope of the standard risk-assessment paradigm. Furthermore, granivorous birds often feed their young with insects, broadening the risk. The field record reflects this with mass intoxications of

Swainson's hawks (*Buteo swainsoni* Bonaparte) witnessed a decade ago when landowners in Argentina began spraying grasshoppers with the organophosphorus insecticide monocrotophos [9]. Cases of bird mortality have also been reported following desert locust (*Schistocerca gregaria* Forskal) control operations, and experimental research on aerially applied fenitrothion and chlorpyrifos under operational conditions has shown this to be lethal to 2% to 7% of individuals from the species assemblages exposed to pesticides in savannah habitat [10].

There is a specific lack of data quantifying residues on locusts and grasshoppers for avian risk assessments. Current recommended default values for residues in insects in the European Union [11] are mainly based on pitfall-trapped insects in a variety of horticultural crops and therefore may not fulfill the requirements for avian risk assessments of locust and grasshopper control given the specific nature of these spray programs (e.g., the use of ultralow-volume formulations in arid and semiarid ecosystems with irregular vegetation structure and high pest densities).

The assessment of risk resulting from pesticide exposure is further complicated by the fact that previous insect collections have not always been designed to mimic the feeding behavior of predatory birds and thus do not accurately reflect their potential dietary intake. Insects are commonly captured by pitfall traps, which may seriously underestimate residue levels by sampling active insects; by sweep netting, which may overestimate the residue level on locusts from contact with contaminated vegetation; or by collecting dead insects that desiccate rapidly, thereby increasing detected residue levels [12].

During spring and summer 2010, population increases of *Chortoicetes terminifera* led landholders, state authorities, and the Australian Plague Locust Commission (APLC) to call for

extensive control operations throughout the states of New South Wales, South Australia, and Victoria. As part of this program, aerial spraying undertaken by the APLC using the organophosphorus compound fenitrothion in New South Wales and Victoria provided the opportunity to quantify residue levels in *C. terminifera* nymphs for this pesticide across different physiological states and to document declining residues over time.

MATERIALS AND METHODS

Study plots

Study plots were chosen from targets identified by APLC staff to be aerially treated. These were Oakdale (70 ha; 34°24.3′S, 142°55.6′E, approximately 75 km east-southeast of Mildura, Victoria) and Koolaman (200 ha; 34°20.1′S, 143°04.5′E, approximately 86 km east-southeast of Mildura, Victoria) in New South Wales, and Karawinna (150 ha; 34°21.2′S, 141°42.3′E, approximately 42 km west-southwest of Mildura, Victoria) and Ouyen (120 ha; 35°10.5′S, 142°16.7′E, approximately 110 km south of Mildura, Victoria) in Victoria. Oakdale and Karawinna were part of larger spray blocks, whereas the other study sites were stand-alone targets.

All sites were located within grazing paddocks on farming properties practicing a 3-yr rotation farming cycle (cereal–fallow–sheep). Vegetation within the study sites was made up of a combination of native and introduced grasses with patches of higher grasses (20–30 cm) surrounded by low, sparse vegetation (<5 cm) and bare ground. Also present within the short vegetation were several unidentified medic species (*Medicago* spp.), with the highest densities of locust nymphs being found within these patches. Koolaman and Karawinna had the lowest vegetation density and height and, in part, were almost bare due to previous treatment with herbicides in preparation for the upcoming cropping season.

Sampling sites

In each spray plot, several locust bands (marching groups of second to fifth instar nymphs) were located and flagged before spray (sample sites). Our sampling design called for 3 hopper bands (sample sites) to be sampled per spray plot, well separated from each other and under different flight passes of the spray aircraft. Locust samples for residue analysis were taken at 1 h, 3 h, 6 h, and 24 h postspray on both Oakdale and Koolaman plots, and additional samples were taken at 46 h and 70 h postspray on the Oakdale plot. We found that hopper bands were rather small and sometimes even fractioned before spray and that movements were rather limited. Being aware of their movements away from the initial sampling site, the surrounding area was searched to localize the new front at each sample period. This was, in most cases, less than 10 m from the initial site. Not all samples proved possible to obtain. For example, there were no dead nymphs to sample before 6 h at Koolaman and 24 h at Oakdale, and dead nymphs were no longer visible in sufficient numbers to be sampled at 46 h postspray at Oakdale. Ouyen and Karawinna were sampled only once, 3 h and 6 h postspray, respectively.

Pesticide application

Plots were sprayed cross-wind with ultralow-volume fenitrothion (1.23 kg active ingredient [a.i.]/L; Sumitomo Chemical) by fixed wing aircraft (Cessna 188 for Ouyen and Brave for Oakdale, Karawinna, and Koolaman) using a targeted flying height of 10 m and a track spacing of 100 m. Spray aircraft were equipped with 2 Micronair® AU5000 rotary atomizers (Micron Sprayers) using a flow rate of 7 L/min, an application rate of 0.210 L/ha, and blade angles set at 50° giving 5000 rpm to 5500 rpm at 200 kph. This resulted in a mean dose rate of 260 g a.i./ha [13]. Planes were equipped with a Satloc® differential global positioning system (Hemisphere GPS) for spray guidance using a constant rate flow control. Flight and spray data were recorded by the Satloc system, and these dose rates were used in calculations in the present study.

Sample collection

At each site, pooled locust samples were collected in 200-mL Schott glass vials precleaned with acetone. Caps were lined with aluminum foil. A subsample of the vials was tared before use. Locusts pertaining to 3 different physiological states (live, debilitated, and dead) were collected separately at the postspray intervals outlined in the *Sampling sites* section, above. The first sample was collected by sweep net (hereafter defined as live) and transferred as a single mass by inverting the net into a jar while minimizing contact between the net and the jar itself. The net was changed after each sample. A second sample of live individuals (hereafter defined as debilitated) was collected 1 individual at a time from the ground or from vegetation with fine tweezers. Attempts were made to preferentially catch any individual that appeared to be affected, moving erratically, or falling on its back when landing. The intent was to obtain a sample that closely approximated choices made by a foraging bird. A third sample consisted of freshly dead individuals (evident from state of dehydration, hereafter defined as dead) also picked with tweezers directly from the ground. Tweezers were cleaned with acetone between samples. Samples typically consisted of 25 individuals to more than 100 individuals, with a few exceptions. Samples were placed immediately in a portable Engel freezer (Sawafuji Electric) and transferred to a regular chest freezer each evening (see Table 2).

At both Oakdale and Koolaman, 3 locust nymph samples (13–20 g each) were collected by sweep netting immediately prior to spraying, spiked with 17 μL to 17.4 μL (Oakdale) and 16.6 μL to 22 μL (Koolaman) of the spray formulation, and treated in the same way as the other samples. Three control samples per site were also collected.

In the laboratory, a subsample of dead and debilitated locusts of the same instar from each of the sample plots was counted and weighed individually to correct for eventual water loss of the former. These weights also served to verify whether to correct for weight loss from sample storage until analysis.

During the collection of nymphs for residue analysis, burrowing nocturnal raspy crickets (Gryllacrididae, *Pareremus* sp.) were seen dead or debilitated on the surface at several of our sites, notably on the Oakdale and Karawinna spray plots. Several insectivorous bird species were seen to switch to this species as a food source, so we opportunistically sampled 6 crickets for fenitrothion and fenitrooxon analysis.

Residue analysis

Locust samples were held in a −17 °C freezer until time of analysis. Whole samples were weighed in their original containers (container plus liner and locusts). Whole samples and liner foil rinses were used for analysis. The emptied, dried containers were weighed after rinsing with 10% acetone in hexane solvent added to the respective samples. The difference between the container plus liner and locusts and the dried empty container plus liner was recorded as the whole sample weight. The samples were macerated in 10% acetone in hexane solvent with dried sodium sulfate, using an Ultraturrax blender at

approximately 13 500 rpm. Fenthion was added to the final solution as an internal standard.

Fenitrothion and fenitrooxon were analyzed using the Hewlett Packard HP6890 gas chromatography system coupled to a flame photometric detector, using H_2 and N_2 as the carrier and makeup gases, respectively. Appropriate dilutions of sample aliquots were made, and fenitrothion and fenitrooxon concentrations were determined by high-performance liquid chromatography (HPLC) with mass spectrometric detection using an AB/Sciex AP14000Q mass spectrometer (AB/Sciex Concord), equipped with an electrospray (TurboV) interface coupled to a Shimadzu Prominence HPLC system (Shimadzu). Separation was achieved using a 5-μ, 150×4.6 mm Alltima C_{18} column (Alltech) run at 40 °C and a flow rate of 0.8 mL/min with a linear gradient starting at 40% B for 2 min, ramped to 100% B in 4 min, held for 4 min, and then to 40% B in 0.2 min, and equilibrated for 4 min (A = 10% methanol/HPLC-grade water, B = 90% methanol/HPLC-grade water; both 5 mM in ammonium acetate). The mass spectrometer was operated in the positive ion, multiple reaction-monitoring mode using nitrogen as the collision gas.

The inlet was pulse-pressured at 200 kPa for 0.8 min and purge-flowed at 95.7 mL/min for 0.75 min. The column used was Agilent J&W DB-1701, 30 m, 320-μm diameter, with 0.25-μm film thickness using a nominal initial pressure of 33.6 kPa and average velocity of 38 cm/s. The oven was run at an initial temperature of 50 °C for 1.5 min, at 35 °C/min to 150 °C, and then at 20 °C/min to 260 °C for a run time of 10 min. The detector was run at 250 °C with H_2 flow at 150 °C/min, air (oxidizer) flow at 110 mL/min, and a constant column plus makeup combined flow of 60 mL/min.

Statistical analysis

Because of slight variations in application rates between spray plots, all residue data were normalized to a 1 kg a.i./ha application following the residue-per-unit dose principle [14] and then log-transformed for normality. A paired *t* test was applied to test for water loss in dead individuals, those samples being paired with samples of debilitated nymphs taken at the same time and sample location. Paired *t* tests of log-transformed residue-per-unit dose values were used to compare live, debilitated, and dead locust samples matched for time of collection and sampling sites. Because different sampling locations within sites showed different time courses of residue accumulation, we applied linear regressions to relative log residue-per-unit dose values over time between 3 h and 70 h postspray in debilitated locusts after setting log residue-per-unit dose to 0 at $t = 3$ h and between 1 h and 70 h postspray for live nymphs after setting log residue-per-unit dose to 0 at $t = 1$ h.

RESULTS

Meteorology and spraying parameters

Temperature and wind were generally favorable for spraying (Table 1), and no rain fell during treatments or shortly thereafter.

Spraying parameters used (Table 1) during the present trial were in line with the standard operating parameters employed by the APLC during its standard locust control operations [13]. Effective dose rates ranged from 0.261 kg a.i./ha to 0.307 kg a.i./ha, and the spray direction was generally perpendicular to the wind direction. Observed flying heights were variable because of the presence of mallee (*Eucalyptus* sp.) windbreaks; isolated stands of trees throughout the target area; forested areas bordering plots (e.g., Oakdale on all sides, Koolaman and Ouyen on 3 sides of the spray plot); sand dunes within a plot (Ouyen, Karawinna); or the presence of hazards such as trees, power lines, and property boundaries common in such a highly dissected agricultural area. Consequently, spray aircraft were forced to operate above the optimal 10 m flying height on several occasions while applying fenitrothion on the trial sites used in the present study.

Residue data

Residues in spiked samples ranged from 83.5% to 117.3% of expected values, and thus no adjustments for effects of transport, storage, or extraction efficiency were made to the residue values. Residues in control samples were below the limit of quantitation (>0.1 μg/g) for the fenitrothion analysis.

Freshly dead locusts were found only at 6 h and 24 h postspray. The individual body mass of a subsample of dead locusts was not different from that of debilitated individuals at 6 h postspray (paired *t* test; $t = 0.719$, $df = 4$, $p > 0.05$), but at 24 h an average 24.7% reduction in body mass of dead locusts due to desiccation was apparent ($t = -3.788$, $df = 5$, $p = 0.012$). Therefore, residues in dead locusts at 24 h were corrected for water loss when looking at the time course of residues (Figure 1). Debilitated locusts were found at sampling times ranging from 3 h to 70 h postspray, and live locusts were present at all sampling times.

Fenitrooxon residues in locust nymphs were generally below the limit of quantitation (<0.02 μg/g), with 2 exceptions—1 live and 1 debilitated sample returning residues at the limit of quantitation. Four dead samples contained 0.04 μg/g, 0.05 μg/g, 0.05 μg/g, and 0.1 μg/g fenitrooxon (actual levels not normalized for application rate). Opportunistic samples in other insect species are reported below.

Effect of plot and time

Residue levels were variable and did not always show a monotonic decrease over time, although some of this variation may have been caused by irregular sample sizes and there was no clear correlation in the time taken to reach maximum residue levels between sampling sites (Table 2). Looking at the maximum residues per sampling site, there was a broad overlap of residue levels between the 2 main spray plots, Oakdale and Koolaman (Figure 1) and, in some cases, wide variation across sampling sites within a spray plot (Table 2).

Table 1. Spray and meteorological parameters on the day of treatment for each study plot

| Property/location	Treatment date	Treatment start time	Plot size (ha)	Dose rate (kg a.i./ha)	Spray height (m)	Temperature (min–max; °C)	Wind direction	Wind speed (m/s)
Oakdale	26 October 2010	12:07	70	0.284	15–20	7–29	NW	3.5
Koolaman	28 October 2010	12:33	200	0.307	10–15	9.9–25.8	NNE	1–2
Karawinna	2 November 2010	11:36	150	0.261	15–20	7.1–20	WSW	3.1–10
Ouyen	9 November 2010	10:00	120	0.291	15–25	10.5–31.2	E–N	2.5–5.6

a.i. = active ingredient.
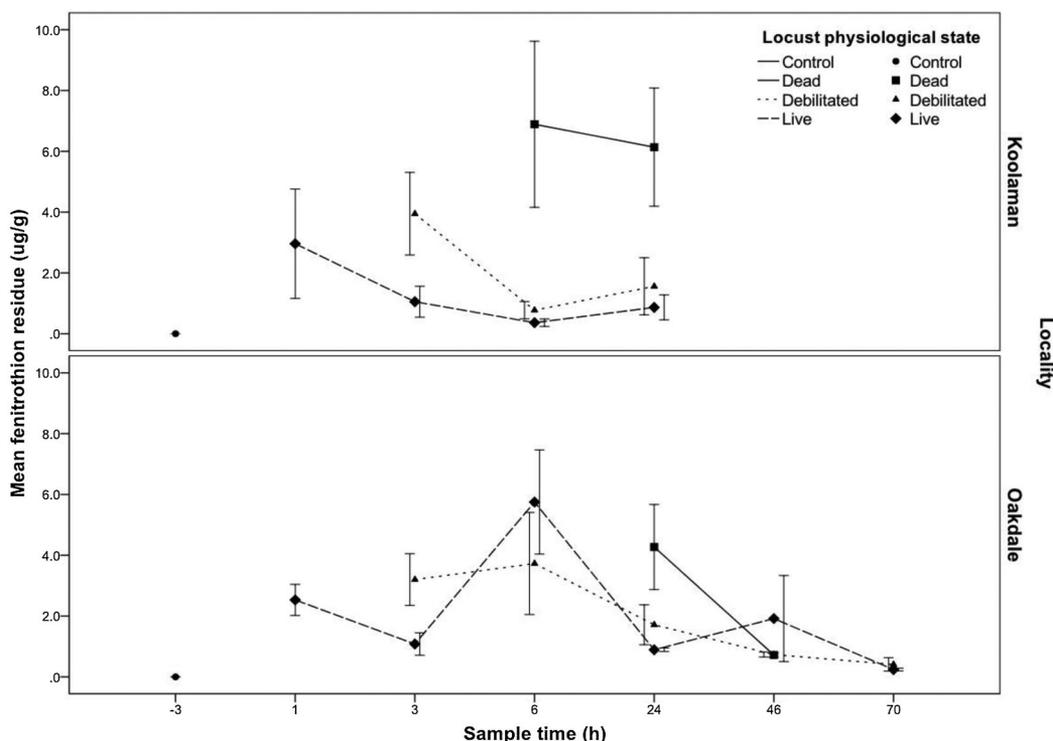
Figure 1. Mean residue levels (adjusted for an application rate of 1 kg active ingredient/ha) on the 2 main spray plots: Koolaman (B4, B5, and B7 sites) and Oakdale (L1, L3, and L7 sites) given with 1 standard error. Standard error bars are offset for visual clarity. Residues in dead hoppers are corrected for moisture loss.

Differences between live, debilitated, and dead locust nymphs

For this comparison, data from Karawinna and Ouyen spray plots were added to the 2 other, more complete data sets. These data cannot be used to infer maximum residue levels from these plots because only 1 collection was made. Residue levels were highest in dead nymphs, followed by debilitated and then live (Table 2). Paired *t* tests showed significant differences in log residue-per-unit dose values between live and debilitated

locusts ($t = -2.5767$, $df = 28$, $p = 0.02$), live and dead locusts ($t = -4.3071$, $df = 10$, $p = 0.01$), and debilitated and dead locusts ($t = -3.2641$, $df = 10$, $p = 0.01$).

A comparison of the log residue transformed concentrations in dead or debilitated nymphs with those collected live shows an increase of 0.24 log concentration units for debilitated nymphs (range = -0.81–1.8; standard deviation [SD] = 0.50) and 0.80 log concentration units for dead nymphs (range = 0.07–2.0;

Table 2. Fenitrothion residues (µg/g; adjusted for an application rate of 1 kg active ingredient/ha) on the 2 main spray plots: Koolaman (B4, B5, and B7 sites) and Oakdale (L1, L3, and L7 sites)[a]

Physiological state	Site	Time postspray					
		1 h	3 h	6 h	24 h	46 h	70 h
Live	B4	21.11[b]	6.28	1.66	3.68		
Live	B5	5.89[b]	3.45	1.50	4.59		
Live	B7	1.89[b]	0.55	0.39	0.20		
Live	L1	5.82	2.50	8.64[b]	3.21	1.02	1.16
Live	L3	8.88	29.33	31.98[b]	2.75	16.71	0.60
Live	L6	12.06[b]	5.11	11.14	3.46	2.54	0.78
Debilitated	B4		21.67[b]	3.19	1.50		
Debilitated	B5		7.87[b]	3.68	2.57		
Debilitated	B7		8.98[c]	0.68[c]	11.16[b]		
Debilitated	L1		5.29	6.06[b]	5.01	2.08	0.85
Debilitated	L3		14.46	24.89[b]	10.47	2.57	3.00
Debilitated	L6		14.10[b]	8.46	2.64	3.10	0.49
Dead	B4			39.82[b]	26.12		
Dead	B5			16.82[b]	7.32		
Dead	B7			10.61	26.45[b]		
Dead	L1				5.89[b]		
Dead	L3				15.76[b]		
Dead	L6				10.01[b]		

[a]Residues in dead hoppers are not corrected for moisture loss.
[b]Value is maximum residue at each sample site for live, debilitated, or dead hoppers.
[c]Small sample weight representing fewer than 10 individuals. May be less representative.

SD = 0.61) over live samples. Returning these calculated values to a linear scale, the increased concentration in debilitated and dead (corrected for desiccation) nymphs represents an average increase of 62% and 224%, respectively, over the concentration measured on live locusts collected by sweep netting.

Maximum residue values at each sample site

Peak residue-per-unit dose values in live nymphs at Koolaman or Oakdale ranged from 1.9 μg/g to 32 μg/g, whereas the range in debilitated nymphs was 6.1 μg/g to 25 μg/g (Figure 2; $n = 6$ sample sites). Two samples of dead locust nymphs collected at Karawinna 6 h postspray (not necessarily peak values) had residue-per-unit dose values of 31.7 μg/g and 16.7 μg/g, and when combined with the Oakdale and Koolaman values, the full range of residue-per-unit dose values for dead samples from 8 sample sites ranged from 5.9 μg/g to 39.8 μg/g.

Time course of residues in locusts, debilitated individuals

Relative residue-per-unit dose values were calculated by expressing each data point as a change relative to the 1st sample (3 h postspray) taken on the sampling site. Forcing the regression through the origin, the reduction in log residue-per-unit doses over time is highly significant ($F_{1,23} = 35.223, p < 0.00001$) with a slope of −0.0146 log units of concentration per hour, an approximate 10-fold reduction in residues over approximately 74 h.

Time course of residues in locusts, live individuals

Maximum residues were found in nymphs from 4 sampling sites at 1 h postspray and in 2 sites at 6 h. One of the sites at Oakdale, however, peaking at 1 h, had similar values at 6 h; and another at Koolaman that peaked at 1 h had only slightly lower values 24 h postspray. At the latter site, locust bands moved rapidly through the plot at a rate of 200 m to 350 m in the 4 d to 5 d prior to spraying. If they were sprayed in an area with low vegetation cover or even bare ground, they might have moved postspray into areas with a higher vegetation cover, which likely had intercepted higher spray deposits. Regardless of the actual reasons, more variation in the site-specific rate of loss was seen in

live nymphs relative to debilitated ones (Figure 3). Forcing the regression through the origin, the temporal reduction in log residue-per-unit doses was again highly significant, with an almost identical slope of −0.0145 log units of concentration per hour, resulting in a 10-fold reduction of residues in approximately 75 h to 76 h ($F_{1,29} = 30.472, p < 0.00001$).

Residues in insects other than locust nymphs

Postspray, a number of large (1.5–2 g) burrowing nocturnal raspy crickets (Gryllacrididae, *Pareremus* sp.) were collected for analysis from the Oakdale and Karawinna spray plots. Two opportunistic samples taken 24 h postspray had residue-per-unit dose values (uncorrected for desiccation, because only fresh specimens were collected) of 7.85 μg/g and 28.56 μg/g. A sample of dead nymphs collected at the same time and location as the latter had a residue-per-unit dose level less than half of that of the cricket (11.5 μg/g). Another cricket collected 46 h postspray had a residue-per-unit dose value of 2.79 μg/g, and a fourth sample had a residue-per-unit dose of 15.2 μg/g (collection time not noted). Three of the cricket samples had measurable oxon concentrations ranging from 0.03 μg/g to 0.09 μg/g, uncorrected for application rate. Finally, a carabid beetle (0.5 g) found on the Oakdale plot 46 h postspray had a residue-per-unit dose of 2.29 μg/g uncorrected for desiccation.

DISCUSSION

Physiological state of locust nymphs and time course of residues

Residues in dead, debilitated, and live nymphs were all statistically different from one another, with dead samples containing the highest and live samples the lowest residues. Changing the collection method from sweep netting to picking out individuals with tweezers resulted in an average increase of 62% in residue concentration. Dead nymphs contained 224% more residues than paired sweep net samples.

We consider residue levels in debilitated locust nymphs to best represent the risk to avian predators postspray. Debilitated individuals certainly attracted our attention by their erratic
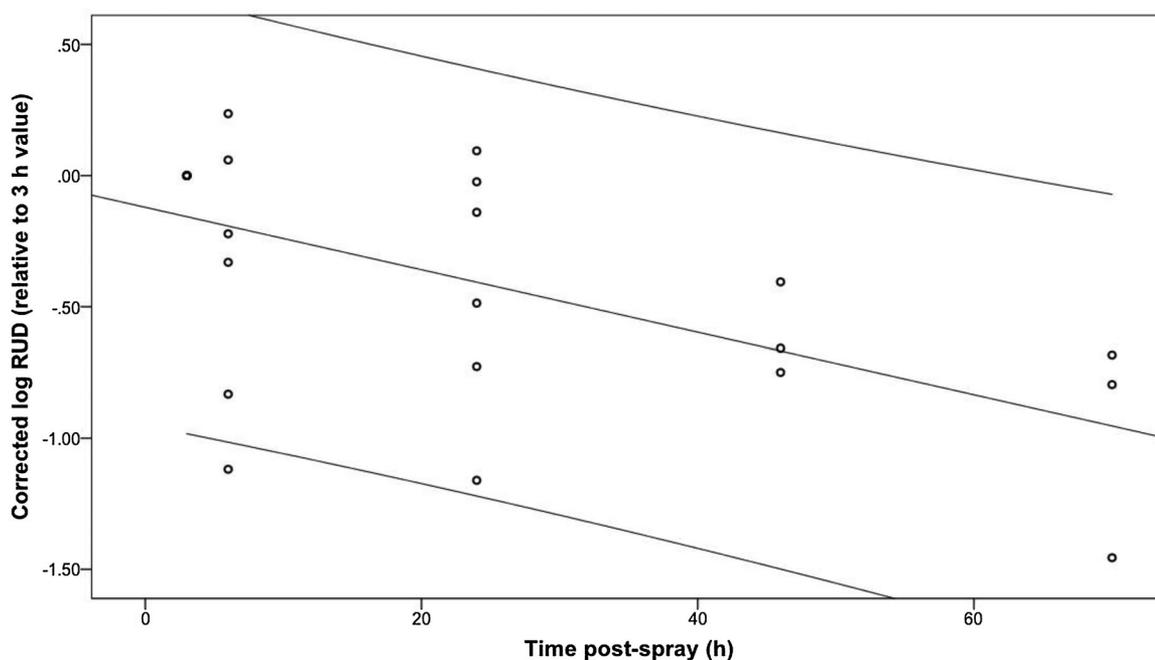

Figure 2. Scatter plot of time course of residue concentrations, given as corrected log residue-per-unit dose (RUD) values (relative to 3 h value) in debilitated (tweezer-collected) *Chortoicetes terminifera* nymphs, shown with 95% confidence interval. $R^2 = 0.35$ ($y = 0.12 + 0.01 \times x$).
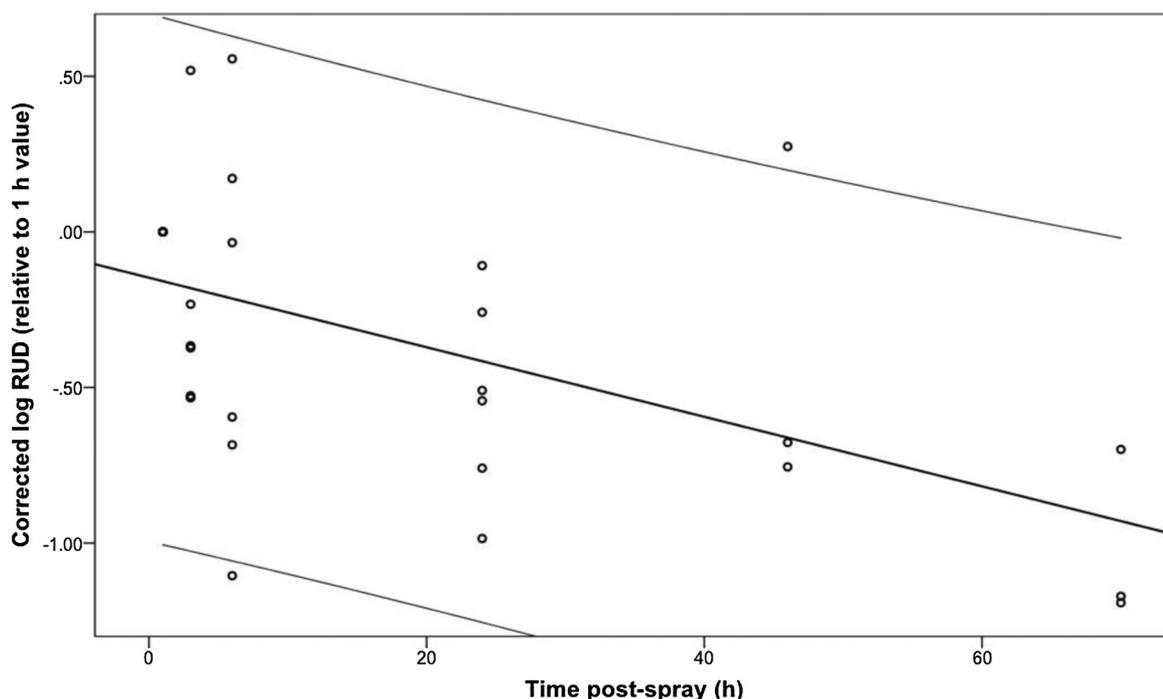
Figure 3. Scatter plot for time course of residue concentrations, given as corrected log residue-per-unit dose (RUD) values (relative to 1 h value) in live (sweep-netted) *Chortoicetes terminifera* nymphs, shown with 95% confidence interval. $R^2 = 0.29$ ($y = 0.15 + 0.01 \times x$).

movements and we believe that this conspicuousness, as well as the relative ease with which they could be captured, increases the possibility that affected nymphs could be picked out selectively by foraging birds.

Residues in dead insects reported in the literature may not represent the best choice for avian risk assessment as insects tend to desiccate rapidly after death (water loss of 25% between 6 h and 24 h postspray in the present study) and may be less attractive as a food source in a desiccated state. This is supported by a study addressing avian food preferences for live, freshly dead, or desiccated insects under laboratory and field conditions. That study concluded that, when given a choice, wild birds did not consume desiccated prey but had a strong preference for live insects, followed by freshly dead individuals [12]. In line with this observation, captive white ibis (*Threskiornis molucca*) have been shown to prefer live locusts over freshly killed or dried locusts [15]. The latter 2 categories were consumed in higher quantities after the live locust supply was exhausted. However, the same author observed during spray trials that straw-necked ibis (*Threskiornis spinicollis*) appeared to favor freshly sprayed paddocks containing "sluggish" locusts over unsprayed areas. Once desiccated, however, sprayed locusts did not appear to be attractive to ibis. Observing birds that feed heavily on desert locusts (*S. gregaria*), Symens [16] noted that those species appearing to favor dead locusts were the small-bodied ones. In line with that observation, a study using clay-colored sparrows (*Spizella pallida*) maintained in indoor cages and fed fresh dead and live grasshoppers (*Melanoplus sanguinipes*) simultaneously [17] concluded that *S. pallida* showed a preference for dead (over live) *M. sanguinipes* and that this resulted in a greater consumption of pesticide-exposed (over unexposed) grasshoppers. Although these studies were conducted under different conditions and degrees of rigor, the apparent disparity highlights the importance of appropriate consideration of the species under consideration, their energetic requirements, and feeding behav-

ior when formulating risk assessments for gorge-feeding avian species.

Few freshly dead locusts were found after 24 h, despite intensive searches by the authors. We attribute this to high scavenging rates of dead and debilitated hoppers in the period when they contain the highest residues. Scavenging was most likely both by diurnal and, probably more importantly, by nocturnal scavengers/predators, reducing the number of nymphs available for birds. While other studies suggest that predators such as arachnids and ground beetles rapidly remove prey from fields [12], it is likely that small mammals and the significant number of reptile species present in Australia's arid and semiarid regions must also be contributing to scavenging of moribund and dead locusts [1].

Transfer of residues

Through scavenging, risks may be transferred from locusts to alternative prey, thereby extending exposure to the birds that feed on these scavenger species. Similarly, contamination of other invertebrates such as raspy crickets increases the scope of vertebrate exposure. Based on a single point of comparison, the crickets may accumulate higher residue levels than locust nymphs. The presence of measurable oxon levels in 3 of the 4 samples also contrasts with samples where the majority had nondetectable levels of the oxon.

Secondary uptake

The loss of fenitrothion residues in live and debilitated nymphs was approximately 10-fold over the 70 h to 75 h postspray, or an apparent first-order dissipation half-life of approximately 48 h. This should not be equated to degradation of the chemical because of the dynamic sampling situation, the movement of locusts in and out of sample plots, and the gradual disappearance of dead and affected individuals. Locusts acquire insecticide residues both from direct droplet impingement and

from uptake by touching and/or ingesting contaminated vegetation [18]. Residue levels at Koolaman tended to peak later, usually 3 h to 6 h postspray and, in a single sampling site, 24 h postspray. The state of the vegetation, ranging from almost bare to low grasses, probably was the main factor accounting for differences in secondary uptake and hence for insecticide residue peaking at different time intervals after spraying. In the context of risk assessment, such variation is likely to be important for gorge-feeding species.

Avian risk assessments using invertebrate residue data

The geometric mean concentrations of residues (1 peak value per sampling site) normalized to a 1-kg a.i./ha application were 9.6 µg/g, 12.6 µg/g, and 14.8 µg/g in live, debilitated, and dead nymphs, respectively. The residue-per-unit dose values reached maxima of 32 µg/g in live and debilitated nymphs and 40 µg/g in dead nymphs (fresh wt). There are surprisingly few published data with which to compare these values. Average residue-per-unit dose values of 2.3 µg/g and 23.5 µg/g for live and of 15.9 µg/g and 29.5 µg/g for dead grasshoppers in 2 yr of aerial spraying with carbofuran have been reported previously [19]; and similarly, in a study using acephate [20], the average calculated residue-per-unit dose values in live and debilitated ground-sprayed locusts were 17.6 µg/g and 22.8 µg/g, respectively. Our values may therefore be low compared with other values for orthoptera.

Residue values calculated from nomograms [14,21] have long dominated the data contributing to avian risk assessments concerning pesticides. It was initially proposed that values for seeds could be used as a proxy for insects [22]. If we consider residue-per-unit dose values for large insects to be applicable to acridids, these would range from 2.7 µg/g to 4 µg/g for mean (or typical) values to 11 µg/g to 13 µg/g for maximum values (following Hoerger and Kenaga [14] and Kenaga [22]). The Ecological Committee on FIFRA Risk Assessment Methods (ECOFRAM) exercise [23] used data from terrestrial field studies conducted by industry in the late 1980s and early 1990s, mainly based on 2 previous studies [24,25]. Their range of 3.7 µg/g to 5.4 µg/g for large insects falls roughly within the nomogram range, as does the range recently given elsewhere [26] of 7.5 µg/g (mean) to 13.8 µg/g (90th percentile) in ground-dwelling arthropods without interception by vegetation. The latter values were derived to be used in European tier 1 avian risk assessments.

However, all proposed regulatory values underestimate residues found following acridid control. Possible reasons for this might be the use of ultralow-volume formulations and a finer droplet spectrum resulting in better interception by the insects, as well as the typical acridid habitat consisting of scattered and irregular vegetation structure. Furthermore, past insect collections have not always been designed to mimic the feeding behavior of insectivorous birds and therefore accurately reflect their potential dietary intake. It has been argued that sweep netting may remove residues from vegetation and transfer them to the insects [26]. Our data show that locusts captured by sweep nets had *lower* residues than individuals picked by tweezers. If we unwittingly contaminated those samples by sweeping vegetation, then the difference between live and debilitated nymphs would be greater than we reported. It is also reasonable to assume that insects falling into pitfall traps (the other common method of collection) are the most mobile individuals and may therefore reflect the least affected portion of the exposed population, whereas insects affected by insecticide residues are likely to become immobilized. Yet, immobilizing insects under

the spray or placing them in enclosures [25] gave insect residue levels that were lower than those measured in pitfall traps [24].

CONCLUSIONS

The present study shows that locusts accumulate residues by secondary uptake before they become debilitated and that maximum residues are generally reached 3 h to 24 h postspray. Therefore, it is unlikely that artificially immobilized insects reflect realistic maximum residue levels any more than pitfall traps can. Our data suggest that sampling of (debilitated) acridids for risk assessment should include mimicking predation and take place over a longer and different time course (preferably 3–24 h postspray) than sampling of vegetation (typically 1–2 h postspray). This is particularly important in arid or semiarid habitats, where vegetation is naturally patchy and residue uptake variable. The present study, in concert with previous research, shows that applying existing regulatory (European Food Safety Authority or US Environmental Protection Agency) standard values for residues in insects will likely underestimate the risk of pesticides applied for locust or grasshopper control and possibly other insects.

REFERENCES

1. Story PG, Walker PW, McRae H, Hamilton JG. 2005. A case study of the Australian Plague Locust Commission and environmental due diligence: Why mere legislative compliance is no longer sufficient for environmentally responsible locust control in Australia. *Integ Environ Assess and Manag* 1:245–251.
2. Story PG, Oliver DL, Deveson T, McCulloch L, Hamilton JG, Baker-Gabb D. 2007. Estimating and reducing the amount of plains-wanderer (*Pedionomus torquatus* Gould) habitat sprayed with pesticides for locust control in the New South Wales Riverina. *Emu* 107:308–314.
3. Szabo JK, Astheimer LB, Story PG, Buttemer WA. 2003. An ephemeral feast: Birds, locusts, and pesticides. *Wingspan* 13:10–15.
4. Bodenheimer FS. 1951. *Insects as Human Food: A Chapter of the Ecology of Man*. W. Junk, The Hague, The Netherlands.
5. Ekop EA, Udoh AI, Akpan PE. 2010. Proximate and anti-nutrient composition of four edible insects in Akwa Ibom State, Nigeria. *World J App Sci Tech* 2:224–231.
6. Szabo JK, Davy PJ, Hooper MJ, Astheimer LB. 2009. Predicting avian distributions to evaluate spatiotemporal overlap with locust control operations in eastern Australia. *Ecol Appl* 19:2026–2037.
7. Piersma T, Hassell C. 2010. Record numbers of grasshopper-eating waders (Oriental pratincole, Oriental plover, little curlew) on coastal west-Kimberley grasslands of NW Australia in mid February 2010. *Wader Study Group Bulletin* 117:103–108.
8. Fildes KJ, Astheimer LB, Story PG, Buttemer WA, Hooper MJ. 2006. Cholinesterase response in native birds exposed to fenitrothion during locust control operations in eastern Australia. *Environ Toxicol Chem* 25:2964–2970.
9. Goldstein MI, Lacher TE Jr, Woodbridge B, Bechard MJ, Canavelli SB, Zaccagnini ME, Hooper MJ, Cobb GP, Tribolet R. 1999. Monocrotophos-induced mass mortality of Swainson's hawks in Argentina 1995–1996. *Ecotoxicology* 8:201–214.
10. Mullie WC, Keith JO. 1993. The effects of aerially applied fenitrothion and chlorpyrifos on birds in the savannah of northern Senegal. *J Appl Ecol* 30:536–550.
11. European Food Safety Authority. 2009. Guidance of European Food Safety Authority—Risk assessment for birds and mammals. Question number EFSA-Q-2009-00223. *European Food Safety Authority Journal* 7:1438–1577.

12. Stafford JM, Brewer LW, Gessaman JA. 2003. Avian food selection with application to pesticide risk assessment: Are dead and desiccated insects a desirable food source. *Environ Toxicol Chem* 22:1335–1339.

13. Australian Plague Locust Commission. 2010. *Australian Plague Locust Commission Operations Manual*. Department of Agriculture, Fisheries and Forestry, Canberra, Australia.

14. Hoerger F, Kenaga EE. 1972. Pesticide residues on plants: Correlation of representative data as a basis for estimation of their magnitude in the environment. In Coulston F, Korte F, eds, *Environmental Quality and Safety*, Vol 1—Global Aspects of Chemistry, Toxicology, and Technology as Applied to the Environment. Academic, New York, NY, USA, pp 9–29.

15. Cowling SJ. 1974. Observations on ibis feeding on the Australian plague locust. *Emu* 74:256–257.

16. Symens P. 1990. Effects of the mass migration of desert locusts *Schistocerca gregaria* on birds in the Taifarea, Saudi Arabia. *Sandgrouse* 12:3–7.

17. Forsyth DJ, Hinks CF, Westcott ND. 1994. Feeding by clay-colored sparrows on grasshoppers and toxicity of carbofuran residues. *Environ Toxicol Chem* 13:781–788.

18. Nguyen NT. 1981. Insecticide acquisition by spray drift hoppers. Australian Plague Locust Commission Research Supplement (1981) Department of Primary Industries and Energy. Canberra, Australia, pp 79–85.

19. Forsyth DJ, Westcott DA. 1994. Carbofuran residues in grasshoppers and vegetation from aerially applied sprayed prairie pastures: Potential effects on wildlife. *Environ Toxicol Chem* 13:299–306.

20. Stromborg KL, McEwne LC, Lamont L. 1984. Organophosphate residues in grasshoppers from sprayed rangelands. *Chem Ecol* 2:39–45.

21. Fletcher JS, Neilsen JE, Pfleger TG. 1994. Literature review and evaluation of the EPA food chain (Kenaga) nomogram, an instrument for estimating pesticide residues on plants. *Environ Toxicol Chem* 13: 1383–1391.

22. Kenaga EE. 1973. Factors to be considered in the evaluation of the toxicity of pesticides to birds in their environment. *Environmental Quality and Safety* 2:166–181.

23. Ecological Committee on FIFRA Risk Assessment Methods. 1999. ECOFRAM terrestrial draft report. US Environmental Protection Agency, Washington, DC.

24. Fischer DL, Bowers LM. 1997. Summary of field measurements of pesticide concentrations in invertebrate prey of birds. *Poster*, Society of Environmental Toxicology and Chemistry 18th Annual Meeting, San Francisco, CA, USA, November 16–20.

25. Brewer LW, Sullivan JP, Akins JM, Kamiri LK, Mihaich EM. 1997. Comparison between measured insect residue values and those predicted according to USEPA standard procedures. *Poster*, Society of Environmental Toxicology and Chemistry 18th Annual Meeting, San Francisco, CA, USA, 16–20.

26. Luttik R. 2001. Residues of plant protection products on food ingested by birds and mammals. In Luttik NR, van Raaij MTM, eds, *Fact Sheets for the (Eco)Toxicological Risk Assessment Strategy of the National Institute of Public Health and the Environment (RIVM)* 601516 007. Amsterdam, The Netherlands, pp 83–94.