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Do pesticide applications influence feeding and locomotor behaviour of *Pardosa amentata* (Clerck) (Araneae: Lycosidae)?

Влияет ли использование пестицидов на пишевое и локмоторное поведение *Pardosa amentata* (Clerck) (Araneae: Lycosidae)?

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ABSTRACT. Sublethal effects of pesticides can influence the ability of predatory invertebrates to move and feed. *Pardosa amentata* were exposed to several concentrations of cypermethrin including full and field drift concentrations of both the technical grade and a commercial formulation. Locomotor behaviour of individuals was highly variable, while feeding did not differ between treatments. Longevity was significantly reduced after exposure to technical grade applications, with a less pronounced effect following exposure to a commercial field formulation containing the same amount of active ingredient. Behavioural observations identified that paralysis of the fourth pair of legs was associated with the full technical treatment. Of greater importance is the fact that all sublethal effects observed persisted for no more than three days with no long term effects identified.

РЕЗЮМЕ. Сублетальные дозы перстицидов могу влиять на способность хищных беспозвоночных двигаться и питаться. *Pardosa amentata* была подвергнута воздействию нескольких концентраций циперметрина, включая полную и полевую дрифт-концентрации согласно как техническому предписанию, так и коммерческой дозировке. Локомоторное поведение особей сильно варьировало, в тоже время питание не различалось между вариантами обработки. Продолжительность жизни значительно уменьшалась после обработки дозами согласно техническому предписанию, хотя эффект был значительно слабее после воздействия полевой дозы согласно коммерческой дозировке, в которой уровень активного ингредиента был тот же. Наблюдения за поведением выявили, что паралич четвертой пары ног был связан с полной обработкой согласно техническому предписанию. Более важным является факт, что все обследованные сублетальные концентрации персистировали не более чем 3 дня, долговременный эффект не был обнаружен.

KEY WORDS: cypermethrin, sublethal concentrations, ataxia, spray drift.
КЛЮЧЕВЫЕ СЛОВА: циперметрин, сублетальные концентрации, атаксия, дрейф распыления.

Introduction

Arthropod communities in arable land and surrounding marginal habitats can be affected by pesticide applications, either directly as part of crop management or indirectly in the form of spray drift. There is considerable evidence of adverse effects of pesticides on non-target organisms, including predatory invertebrates that may feed on phytophages of potential pest status [Sunderland, 1987]. Spiders are highly susceptible to many pesticides, resulting in depleted populations within agricultural crops [e.g., Everts *et al.,* 1989]. Stark *et al.* [1995] reviewed such effects on spiders in the laboratory and concluded that spiders are highly susceptible to certain pesticides (e.g., synthetic pyrethroids and organophosphates) and show little or no adverse response to some, less synthetic insecticides, fungicides and herbicides. It is important to understand how sublethal effects of pesticides may impact on the role of invertebrate predators within arable crops.

In arthropods, sublethal effects of pesticides include reduced rates of oviposition by mites [Bowie *et al.*, 2001] and an increased rate of dispersal of both mites [Margolies & Kennedy, 1988] and ladybirds [Singh *et al*., 2001] from treated sites. Hyperactivity is a frequently documented effect of pesticide applications to ladybirds [Singh *et al.*, 2001], bees [Barker *et al*., 1980] and woodlice [Bayley & Baatrup, 1996]. Feeding rates, which are of particular importance for potentially beneficial arthropods, can be reduced by sublethal doses of pyrethroids (e.g., in mites [Iftner *et al.*, 1986] and mustard beetles [Hajjar & Ford, 1989]). There is growing evidence that exposure of web building spiders to sublethal levels of pesticides influences web building behaviour [Dinter & Poehling, 1995; Shaw, unpubl. data] with cypermethrin modifying and ceasing web building behaviour in *Araneus diadematus* Clerck, 1757 [Samu & Vollrath, 1992]. These effects can be amplified, because webs are very efficient collectors of pesticides and can therefore lead to increased exposure by direct contact [Samu *et al.*, 1992], especially for those spiders that frequently consume and recycle the web.

Locomotor activity is of great importance to most predacious arthropods, playing a central role in dispersal, reproduction, hunting and predator avoidance. *Pardosa amentata* (Clerck, 1757) is a ubiquitous non-web building, cursorial predator in European agricultural habitats. It is particularly reliant on locomotion for dispersal [Richter, 1971], prey capture [Ford, 1978] and mate location [Vlijm *et al.*, 1963]. Thus any disruption of movement and feeding behaviour of this species could impact on its prey consumption capacity within agricultural crops.

Cypermethrin is a pyrethroid regularly applied to brassica crops, which are of regional importance in the United Kingdom. Pyrethroid insecticides produce various signs of excitatory neurotoxicity [Soderlund *et al.*, 2002] due to their effects on voltage regulated sodium gates [Narahashi *et al.*, 1998]. Symptoms associated with pyrethroid toxicity include intense hyperactivity, ataxia, tetanic contraction or extension of legs and paralysis [Sattelle & Yamomoto, 1988]. This study examines the influence of the pyrethroid cypermethrin, on locomotor and feeding behaviour of *P. amentata,* at concentrations applied to crops and reduced concentrations that simulate drift into field margins.

Material and methods

Females of *P. amentata* (N = 55) were collected from unsprayed tussocky grassland in June 2002. Spiders were sequestered in plastic cups with a moistened inert substrate ('Bathgate' silica sand) and kept at 20°C with a 16 h : 8 h (light : dark cycle). Pedersen *et al.* [2002] showed that sublethal effects were enhanced in starved individuals. We standardized levels of satiation by initially feeding spiders *ad libitum* and then starving them for ten days prior to exposure.

Technical grade cypermethrin (90% cypermethrin) and the commercial formulation 'Toppel 10' (10.8% cypermethrin; United Phosphorus, UK) were used along with a control treatment of distilled water (Table 1). Drift strength treatments were also employed, calculated at 10% of the field strength applications [following Candolfi *et al.*, 2001]. The initial concentration of technical ingredient for each treatment is given in Table 1. The application levels employed were based on the concentration recommended for 'Toppel 10', which for brassica crops is 200 ml/600 l (v/v). Technical grade cypermethrin is a viscous liquid that does not dissolve directly in water, therefore it was dissolved in 5 ml of acetone prior to adding to the water and placing in a sonic bath until full mixing occurred.

Individuals were anaesthetized with carbon dioxide before receiving a droplet (0.5 μl) applied topically, on the dorsum of the opisthosoma. A sample of full field concentration was applied in a single droplet, simulating one large or several small droplets of spray solution hitting the spider. A drift

Table 1.
Treatments used in the study including the amount of active ingredient of cypermethrin applied to individuals prior to observations. Codes refer to the abbreviated terms used throughout this study.
Таблица 1.
Обработки, использованные в исследовании, в том числе количества активного ингредиента циперметрина, примененные к особям до начала наблюдений. Коды обозначают сокращенные термины использованные в данном исследовании.

Treatment	Code	Initial concentration (active ingredient)	Amount of active ingredient applied to each spider
Technical grade	full technical	0.45 g ai/l	0.23 μg ai
Technical grade	technical drift	0.045 g ai/l	0.023 μg ai
'Toppel 10'	full field	0.042 g ai/l	0.021 μg ai
'Toppel 10'	field drift	0.0042 g ai/l	0.0021 μg ai
Distilled water	control	0.00%	0 ml

treatment was also employed, calculated at 10% of the full field treatment as an extreme estimate [G. Weyman, pers. comm.]. It is recognized that drift is, in reality, a reduced volume but this results in a reduced application, so for practical reasons, the treatment in this study was made by reducing the concentration while retaining the same application volume. This direct dosing of the spiders, whilst not necessarily accurately simulating the volume hitting a spider in the field, meant low variability in the dose received by each spider. This also allowed the exact amount of active ingredient applied to each spider, in each treatment, to be calculated (Table 1).

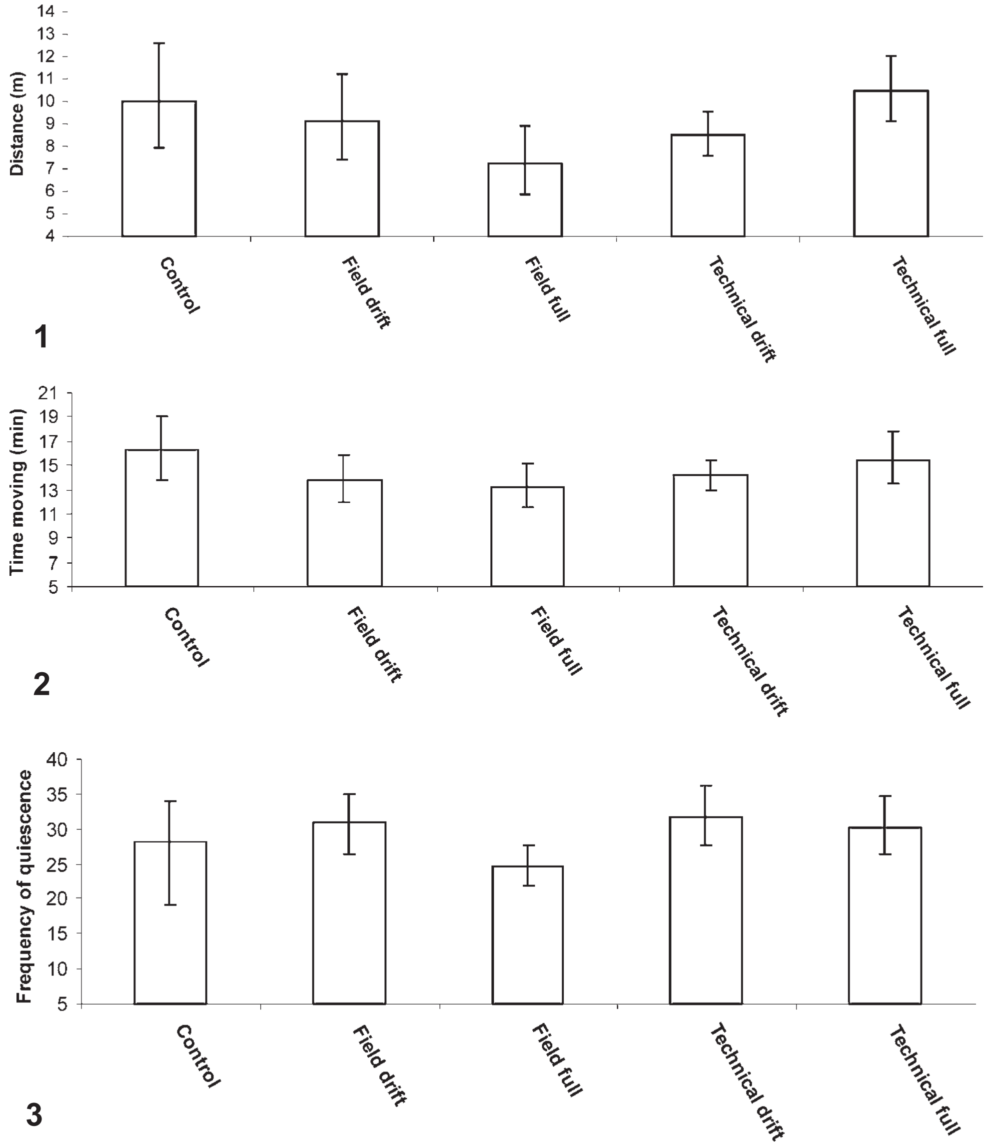
Spiders were videoed in a clear, plastic, circular arena (255 mm diameter) containing 'Bathgate' silica sand (20 mm depth) saturated with water. Each individual was placed in a new arena to exclude the influence of pheromones and faeces from previous spiders. Each spider was filmed for 40 minutes (which included a ten minute acclimation period) immediately following exposure to cypermethrin. Then they were returned to their individual plastic cups and fed immediately with a 'micro' cricket. Feeding continued at a rate of one individual 'micro' cricket daily for 30 days, and then every other day for 50 days. Longevity was monitored over this period.

Videos of sequestered spiders were digitized using a Hoveel digitizer (PMS Instruments, UK). This enabled the path of each individual to be examined, including: the distance covered; number of turns over 90°; time and duration of quiescence; and mean velocity. Measurements were made from 30 minutes of each video recording, excluding the acclimation period. Data were normalized using log transformation and measurements were compared between treatments using one-way analysis of variance ('Statview' version 4.1, Abacus Concepts). In addition, the number of days before feeding and the number of prey eaten throughout the monitoring period were also analyzed.

Changes in behaviour of individuals were noted during filming and the subsequent feeding period. Chi squared analysis was used to examine whether treatments were associated with the occurrence of some of the behavioural characteristics (i.e., leg cleaning and paralysis of the fourth pair of legs). Leg cleaning behaviour is used here to describe the action of the hind pair of legs being passed through the chelicerae and cleaned repeatedly. Paralysis of the fourth pair of legs potentially describes the occurrence of two physiological effects: the tetanic extension of the legs and true paralysis. Chi squared analysis was also employed to examine any association between treatment type and the frequency of feeding immediately after the exposure/monitoring period.

Results

There were no significant differences in the distance covered, duration and frequency of quiescence or the number of turns, between any of the treatments (Figs 1–4; Table 2). This is probably due to the high level of individual variation. However, some trends were apparent. The mean distance travelled was lowest in the full field treatment, increased in both drift level treatments, and was highest in the control and full technical treatments (Fig. 1). Variation within treatments was high, with the distance travelled in the control treatment ranging from 1.37–24.23 m. The amount of time spent moving (Fig. 2) followed a similar pattern, except that control animals moved for longer than those exposed to any of the other treatments. For frequency and duration of quiescence this pattern was different (Figs 3, 4). The full field



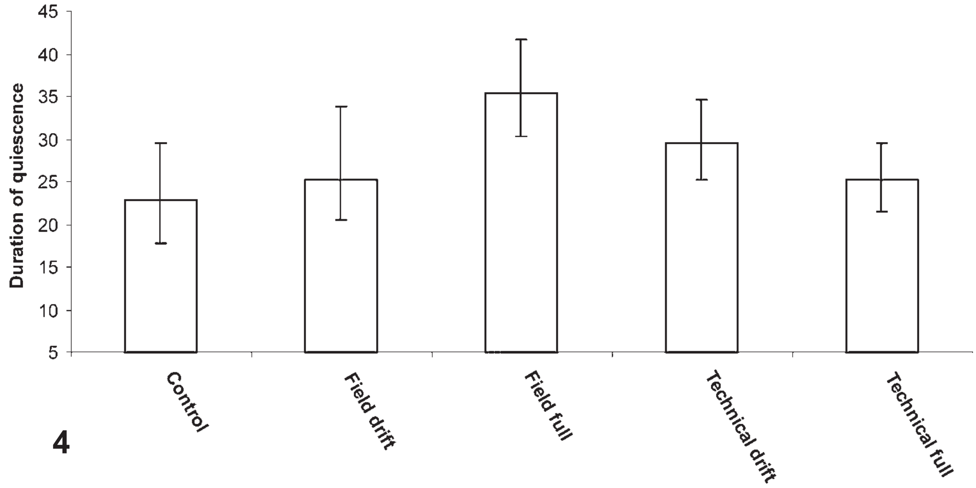
Figs 1–3. Digitizer measurements of a suite of behaviours of sequestered *P. amentata* individuals (N = 55) following exposure to cypermethrin. Back-transformed means (±SE) are as follows: 1 — for mean distance covered; 2 — for mean time spent moving; 3 — for mean frequency of quiescence.

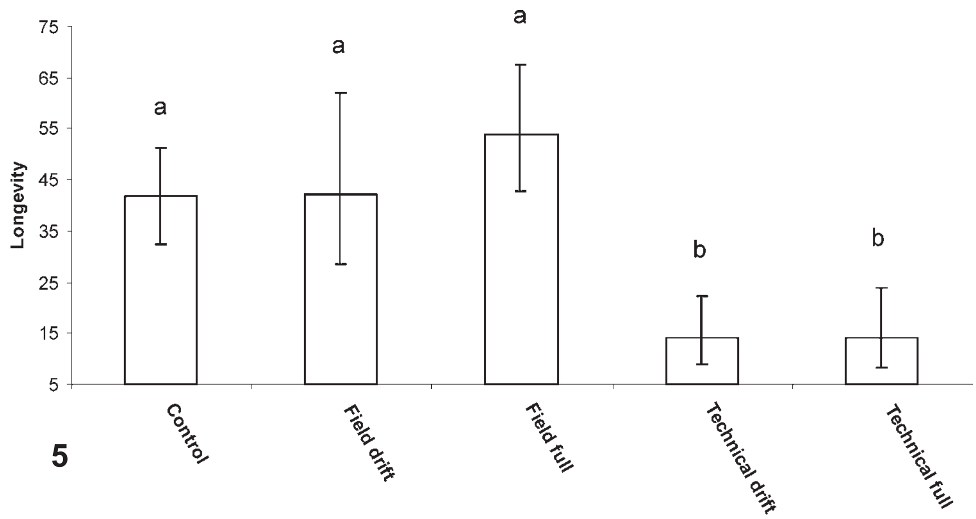
Рис. 1–3. Цифровые измерения схемы последовательностей поведения у особей *P. amentata* (N = 55), которые следовали после обработки циперметрина. Геометрические средние (±SE) показаны: 1 — для средней преодоленной дистанции; 2 — для среднего времени, проведенном в движении; 3 — для средней частоты покоя.

treatment produced the longest mean period of quiescence and the lowest frequency of locomotion, suggesting lower levels of activity interspersed with long periods of quiescence.

Longevity was significantly reduced (Table 1) in both the full technical and technical drift treatments (Fig. 5); mortality in the first four days was 45% and 36% respectively. Those that survived for more than four days generally survived for longer than two weeks post exposure. In all other treatments the first individual to die was approximately ten days after treatment.

The number of individuals feeding immediately following exposure was similar through-





Figs 4–5. Digitizer measurements of a suite of behaviours of sequestered *P. amentata* individuals (N = 55) following exposure to cypermethrin. Back-transformed means (±SE) are as follows: 4 — for mean duration of quiescence; 5 — for mean longevity (N = 50). For longevity, letters denote differences between sample means at P < 0.05.

Рис. 4–5. Цифровые измерения схемы последовательностей поведения у особей *P. amentata* (N = 55), которые следовали после обработки циперметрина. Геометрические средние (±SE) показаны: 4 — для средней продолжительности покоя; 5 — для средней продолжительности жизни (*n* = 50). Для продолжительности жизни буквы обозначают различия между средними образцов при P < 0.05.

out treatments ($\chi^2 = 7.33$, df = 4, P = 0.12) with only 15 spiders taking prey. Those that did not feed immediately took their first prey item an average of two to three days post treatment (Table 2). Frequency of feeding throughout the 80 day monitoring period was also unaffected by treatment (Table 2), with spiders in all treatments consuming an average of 15 'micro' crickets in total.

Leg cleaning behaviour was not significantly associated with treatment type ($\chi^2 = 6.26$, df = 4, P = 0.18). The number of individuals in each treatment displaying this behaviour varied from 3/11 control individuals, to 8/11 full field individuals. In all treatments except the control, leg cleaning was occasionally followed by a state of ataxia that was more pronounced in the full technical and full field treatments. Most indi-

Table 2.
Results of ANOVA tests carried out on log transformed data examining the effect of exposure of *P. amentata* (N = 55) to various formulations containing cypermethrin.

Таблица 2.
Результаты теста ANOVA, проведенные с логарифмами трансформированных данных, которые исследуют эффект экспозиции *P. amentata* (N = 55) к воздействию различных формул, содержащих циперметрин.

Test	Comparison of treatments		
	F	df	P
Distance (m)	0.61	4,50	0.66
Time moving (min)	0.46	4,50	0.76
Speed (m/min)	0.68	4,50	0.61
Frequency of quiescence	0.46	4,50	0.77
Duration of quiescence (s)	0.71	4,50	0.59
Number of turns	0.67	4,50	0.61
Days before feeding	0.23	4,50	0.9
Number of prey taken	0.25	[1]4,42	0.91
Longevity (days)	2.88	[1]4,45	0.032*

[1] sample size reduced by individuals that were omitted either due to death immediately following exposure or because they escaped.

* = denotes differences between sample means at P < 0.05.

[1] размер образца был уменьшен за счет изъятия особей, которые либо погибли сразу после экспозиции, либо сбежали.

* = показывает отличия между средними образцов при P < 0.05.

Figs 6–7. Examples of the paths taken by *P. amentata* in circular arenas. 6 — specimens exposed to full field treatment of 'Toppel 10' formulation of cypermethrin; this highlights movement of an individual exhibiting ataxia, resulting in reduced thigmotaxis. 7 — the specimen exposed to the control treatment; this individual showed no signs of ataxia and displayed a high level of thigmotaxis.

Рис. 6–7. Образцы перемещений *P. amentata* в круглых аренах. 6 — экземпляры после воздействия полной формулой циперметрина 'Toppel 10'; здесь важно отметить движения особи подверженной атаксии, как результат сниженного тигмотаксиса. 7 — особь, на которую воздействовали контрольной обработкой; эта особь не имеет признаков атаксии и показывает высокий уровень тигмотаксиса.

viduals showed a strong thigmotactic behaviour, keeping very close to the edge of the arena throughout the majority of the experiment, except when exhibiting severe signs of ataxia (Figs 6, 7). Paralysis of the fourth pair of legs was significantly associated with the full technical treatment only ($\chi^2 = 18.64$, df = 4, P = 0.0009). Paralysis occurred in nine full technical individuals, six technical drift individuals, five full field individuals, two field drift individuals, but did not occur in control individuals.

Discussion

Within all treatments a high level of inter-individual variation was found, which is consistent with Baatrup & Bayley [1993a]. A number of factors may be responsible for this, including variations in individual size. Pedersen *et al.* [1999] found that mortality was significantly higher in males of *Pardosa prativaga* (L. Koch, 1870) due to their small size when compared to females. In contrast, Nielsen *et al.* [1999] reported no size effects on individuals caught from field populations. In the current study, size was controlled by selecting only mature females for experimentation. Additionally, individuals caught in the field that were noticeably smaller were not used.

The high level of individual variation may also have been due to starvation. Field collected individuals have an unknown feeding history and determining levels of satiation would have been very difficult. Spiders are known to be relatively resistant to starvation [Wise, 1993], therefore, individuals were fed until satiated and then starved prior to experimentation. However, there may still be variation in the degree of satiation between experimental animals. Pedersen *et al.* [1999] found that starved *P. amentata,* or individuals fed on low quality prey, were more susceptible to the effects of dimethoate. Field starvation in *P. amentata* may result from periods of low prey densities within agricultural crops. Although locomotion in *Pardosa* has been shown to be only mildly affected by levels of satiation or starvation [Walker *et al.*, 1999], Wheater [1991] found that locomotory behaviour in starved invertebrates was very different to that in fully fed individuals.

Toft & Jensen [1998] suggested that high individual variation can be expected in response to dose dependent sublethal effects. In the current study, there were suggestions that full field treatment (analogous to crop application rates) had a mildly suppressing effect on the distance travelled and the time spent moving. This was associated with a low mean number of relatively long periods of quiescence, resulting in slightly reduced distances travelled. Velocity and distance travelled are comparable to those observed by Baatrup & Bayley [1993a] who found that females travelled approximately 300 m in a 24 h period. The frequency of quiescence is typical for many spiders as their locomotion is related to the hydraulic leg extensors that use considerable energy [Foelix, 1996], requiring spiders to move in short bursts interspersed with numerous periods of quiescence. Each treatment, except full field, resulted in around 30 rests during the filming process, equating to approximately one rest per minute (similar to that previously recorded by Baatrup & Bayley [1993a]). Overall, treatments with lower than full technical concentration of cypermethrin, showed similar but less acute effects. Full field and technical drift treatments contained very similar concentrations of cypermethrin, yet technical drift produced more pronounced changes in the behaviour and mortality of individuals compared to full field treatments. This suggests that full field formulation 'Toppel 10' is less toxic to spiders than the technical ingredient alone. There is a paucity of information regarding the effects of the field formulation pesticides, which needs to be addressed in future studies.

Longevity was significantly reduced in the full and drift technical grade treatments. Both treatments resulted in relatively high levels of mortality in the first four days following exposure (for all other treatments mean longevity was over 30 days). The full technical treatment had a concentration of technical ingredient equivalent to ten times the level applied to a crop, representing a scenario unlikely to occur in natural situations. At this level, five individuals died within the first four days, which may have been a direct result of the exposure to cypermethrin.

The full technical treatment produced the most negative effects in *P. amentata*. Mortality, leg extensions, ataxia and paralysis of the fourth pair of legs sometimes attributed to poisoning by a pyrethroid insecticide [Sattelle & Yamomoto, 1998] were observed most frequently in the full technical treatment. Individuals that were not lethally influenced recovered within two to three days of exposure (as also found by Baatrup & Bayley [1993b]).

Paralysis occurred only in the fourth pair of legs and in some cases there were still some minor movements in these legs, but on the whole they dragged behind the spider. *Pardosa milvina* (Hentz, 1844) locomotion and prey capture have been found to be unaffected by the loss of legs [Brueseke *et al.*, 2001] and therefore paralysis that effects only the fourth pair of legs may not necessarily inhibit hunting success in the laboratory. Whether this is directly applicable to individuals in the field is not known. However, it is unclear whether dragging paralyzed legs adversely influences prey capture behaviour. Spiders exposed to the full technical treatment travelled a similar distance to all other treatments and the feeding rate was unaffected. Paralysis was only associated with the fourth pair of legs which may be due to direct contact with the pesticide during the cleaning of the abdomen with these legs. During this cleaning behaviour the legs are pulled through the chelicerae, after being pulled over the dorsal part of the opisthosoma in what appears to be an attempt to remove the droplet, possibly leading to the ingestion of the pesticide. Ingestion of pesticides in the field also occurs through the consumption of contaminated prey. Observations suggested that those individuals which cleaned their legs were affected to a greater extent than those not displaying this behaviour. Further analysis of the acclimation period and the duration and frequency of cleaning behaviour is currently underway. The level of variation in individuals and the trends that have been presented need further investigation, possibly with the inclusion of chemical analysis of the spiders to determine levels of pesticide uptake.

Field drift treatment resulted in a lower frequency of the occurrence of sublethal effects. Six individuals were observed for leg cleaning behaviour, with only two of those displaying signs of leg extension or paralysis. There were no significant effects of the treatment on locomotion or feeding. Drift levels of pesticides affect uncultivated areas of agricultural landscapes, such as field margins, that are known to be overwintering sites for many beneficial predators [Holland *et al.*, 1999] and represent refugia in an ephemeral ecosystem [Kampichler *et al.*, 2000]. Spiders inhabiting these areas will be affected to a lesser extent than field populations, providing potential immigrants to re-populate crop fields depending on the adjacent vegetation structure [Shaw *et al*., 2004]. The results obtained from the field drift treatment represent levels of cypermethrin that may be higher than those experienced in the field, due to the influence of wind speed and direction as well as by vegetation density and margin width [Longley *et al.,* 1997].

In conclusion, cypermethrin had minimal effects on *P. amentata* at field application levels, with even less impact when applied at drift levels. Frequency of feeding and amount of movement remained unaffected, even in those individuals experiencing ataxia and paralysis in the fourth pair of legs. Only adult females were included in this study and further work is needed on juveniles and males, specifically in terms of the effects on mating behaviour as this will impact on the success of future populations. However, the current study suggests that this particular insecticide may be selectively used as part of an integrated pest management program that utilizes *P. amentata*.

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