

Food source provisioning and susceptibility of immature and adult *Tribolium castaneum* on concrete partially treated with chlorfenapyr (Phantom[®])

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Abstract A series of experiments were conducted in which adults, pupae, and 4-week-old larvae of *Tribolium castaneum* (Herbst), the red flour beetle, were exposed separately on concrete arenas partially treated (14.4% of the total area) with the insecticide chlorfenapyr (Phantom[®]) at 1.1 g of active ingredient/m². A flour food source (patch) was also provided in the untreated portions of the arenas. In the first trial, adult mortality averaged $60.0 \pm 10.6\%$, but progeny production occurred in the provided food patches. Pupal mortality was only $8.3 \pm 3.1\%$, indicating that when adult emergence occurred, those adults were able to escape exposure, and there was no difference in progeny production from that in untreated controls ($P = 0.27$). In the second trial, few larvae exposed in choice and no-choice arenas were able to emerge as normal adults. In a final trial, residual efficacy declined during a 3-week period, with larvae being more susceptible than adults. Results show mobility of life stage may be a determining factor when assessing susceptibility of *T. castaneum* to contact insecticides.

Keywords *Tribolium castaneum* · Red flour beetle · Contact insecticides · Chlorfenapyr

Introduction

Contact insecticides are often used in the United States (US) as crack and crevice, spot, or surface treatments to control

stored product insects inside flour mills, food processing plants, and warehouses. Two common cosmopolitan stored product pests in the US and many other countries are the red flour beetle, *Tribolium castaneum* (Herbst), and the confused flour beetle, *T. confusum* (Jacqueline DuVal). These species can often be more difficult to control with insecticides compared with other beetle pests that can infest stored products (Arthur 2008, 2009). In addition to that challenge, stored product environments often contain refugial areas where these and other insect species can escape exposure to an insecticide or sites where spilled or accumulated food material allow for continued development of resident insect populations (Campbell et al. 2010a, b; Toews et al. 2010). The presence of food material may also serve as an attractant or oviposition site for female *T. castaneum* (Campbell and Hagstrum 2002; Campbell and Runnion 2003).

The insecticidal pyrrole chlorfenapyr (Phantom[®]) was initially registered in the US to control termites, cockroaches, and nuisance ants. In a previous evaluation for control of adult flour beetles, *T. castaneum* was more tolerant to chlorfenapyr than *T. confusum* (Arthur 2008). In a second test with only *T. castaneum*, the amount of time adults were exposed to treated concrete was a much more important determinant of mortality than the concentration of active ingredient. In addition, the presence of food material after adult exposure caused enhanced survival (Arthur 2009).

When chlorfenapyr, other contact insecticides, or aerosols are applied in commercial facilities, food material that comes into contact with the treated surface may absorb some of the insecticide residues from the treated surface (Arthur 2010). Hence, adults and immatures of *T. castaneum* will encounter these residues as they move through and ingest the food material. This could be considered to be indirect or secondary contact, as opposed to direct contact to a treated surface. There are no published data regarding

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the susceptibility of *T. castaneum* larvae to chlorfenapyr when provided access to food material during or after exposure. Similarly, there are no data regarding the effects of adult exposure on oviposition and resulting development in food material, the effects of refugial areas where adults are not directly exposed to the insecticide, or residual efficacy of chlorfenapyr under these conditions. Therefore, the objectives of this test were to determine: (1) the impact of food material on survival and mortality of the adult and pupal stages of *T. castaneum* exposed to chlorfenapyr, and subsequent progeny production, (2) the exposure of late-stage larvae to food patches treated with chlorfenapyr and subsequent mortality, with and without additional untreated food patches, and (3) residual efficacy of chlorfenapyr when food material is present. Three separate experiments were conducted, as described below.

Materials and methods

Experiment 1

Individual concrete exposure arenas were created in the bottoms of standard plastic 150-mm Petri dishes (143 cm² area in the bottom of the dish), following procedures that have previously been described in detail (Arthur 2009). Briefly, these arenas were constructed using a driveway patching concrete material (Rockite[®]) purchased from a local hardware store. The dry material was mixed with water to create a liquid slurry, which was poured into the Petri dish to a depth of approximately 1.25 cm. Within the dish, a treatment site was constructed consisting of a piece of plastic window trimming material that measured about 9 cm, inserted into the concrete as it was drying (Fig. 1). This was done to provide a barrier and to contain residual food material, in this case 2 g of whole-wheat flour. The actual surface area that was treated with chlorfenapyr was 20.6 cm² or about 14.4% of the total area (Fig. 1).

The spray rate used in the study was proportional to the maximum label rate for perimeter treatment using a 0.50% concentration, which gave a final spray volume of 0.40 ml for the area of 20.6 cm² that was treated with chlorfenapyr. Applications were made by holding a piece of cardboard vertically above the top portion of the area to be treated, so that it was touching the concrete, and using a Badger 100 artists' airbrush (Badger Corporation, Franklin Park, IL, USA) to dispense the 0.40 ml of suspension onto the area to be treated. The purpose of the piece of cardboard was to minimize spray drift over to the untreated part of the arena. Six arenas were constructed and treated as described. Three additional arenas were constructed to serve as untreated controls, and in these arenas, the targeted area of 20.6 cm² was sprayed with 0.40 ml of distilled water.

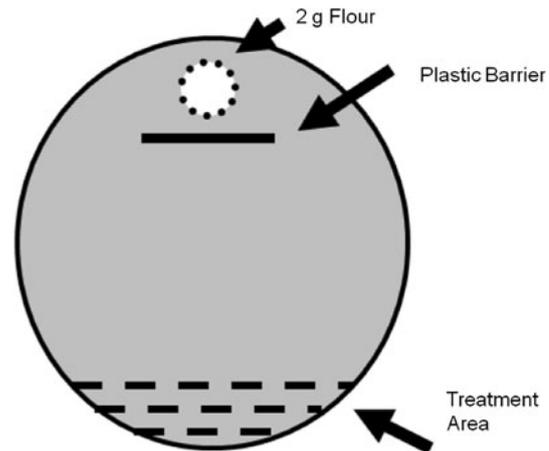


Fig. 1 Experimental treatment arenas used to test the presence of food material on efficacy of chlorfenapyr on adults and pupae of *T. castaneum* (drawing not to scale)

All arenas were allowed to dry for several hours, and a 2-g flour food patch was placed in the approximate position as shown. Ten mixed-sex adult *T. castaneum* (unknown number of males and females) were placed in each arena and held for 1 week inside an incubator (Percival Scientific, Perry, IA, USA) set at 27°C and 60% relative humidity. After 1 week, the beetles in the dish were classified as surviving (actively moving about the arena), knocked down (on their backs but capable of motor movement with legs and antennae) or dead (unable to respond when touched with a probe). After the assessment, dishes were returned to the incubator and held for 6 weeks to record emergence of progeny produced by the exposure of the parental adults.

A test was also conducted by exposing *T. castaneum* pupae, using the same experimental procedures as described above, to 6 treated arenas and 3 untreated arenas. Pupae were sexed according to procedures described by Hinton (1945). Five male pupae and five female pupae were placed in the flour food patch inside the arenas but were not restricted to the food patch. The arenas were held until adult emergence was complete (2–3 weeks). Assessments of survival, knockdown, and mortality were made as described above, the adults removed, and the arenas held for 7 weeks inside the incubators to record progeny produced by the adults that emerged from the exposed pupae. There were four separate replications for the test with the adults and for the tests with the pupae. Data for dead adults and dead pupae in the treated arenas versus dead adults or dead pupae in the untreated controls were analyzed using the *t*-test Procedure of the Statistical Analysis System (SAS 2007). The *t*-test Procedure was also used to determine significance in progeny production in treated arenas versus untreated controls. Correlations

between the number of dead adults and progeny production were carried out using the Correlation Procedure of SAS.

Experiment 2

Test arenas were constructed as described above for exposure of 4-week-old *T. castaneum* larvae, in which three types of arenas were constructed (Fig. 2). The first arena type contained a 1-g flour food patch in the upper portion of the arena, behind the barrier, and a second 1-g patch of food on the treated area (Fig. 2a). A “negative control” was included (Fig. 2b) in which a 2-g food patch was placed on the treated portion of the arena and a “positive control,” in which there was no treatment with the 2-g food patch placed behind the plastic barrier (Fig. 2c).

The actual exposures were made by placing ten 4-week-old larvae in the center of the dish and by giving them free choice and access to the food patches. There were three replicates for the arenas containing the food patch in the untreated portion of the arena (Fig. 2b, c), and six replicates where the larvae were given the choice of the untreated food patch and the food patch in the treated portion of the arena (Fig. 2a). The larvae were allowed to wander throughout the arenas with no restrictions on movement. The arenas were held for approximately 3 weeks inside the incubator at the same conditions as described for Experiment 1. Assessments were made as described above for the adults, and the level of mortality in the larvae and pupal stages was also recorded.

Experiment 3

In this test, residual efficacy of chlorfenapyr was assessed at 1-, 2-, and 3 weeks post-treatment. Exposures of adults

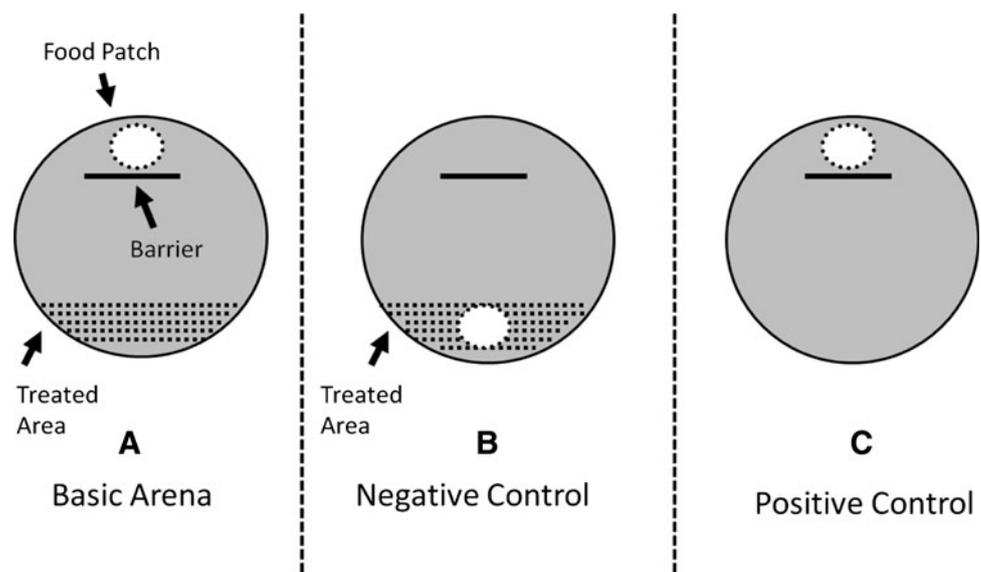
were made as described for Experiment 1 and in Fig. 1, using separate arenas at each of the post-treatment assessments. Arenas were constructed and treated as previously described. Ten adults were placed on respective arenas for 1 week and then removed, and the arenas were held for progeny production as previously described. Dead adults and progeny production at each of the weekly testing intervals were analyzed for differences between treatments and controls using the *t*-test Procedure of SAS. Exposures of 4-week-old larvae were also made as described for Experiment 2, except that for this trial, there were two replicates of the positive and negative controls and four replicates of the choice arenas in which the 1-g food patches were placed on the treated and untreated areas as described. The general linear models (GLM) Procedure of SAS was used to determine significant differences and interactions between treatments (treatment, positive control, and negative control) and time (week) for dead larvae, dead pupae, dead adults (those adults that emerged from the exposed larvae and died), and live adults (those that emerged from the exposed larvae and lived). When significance differences occurred, survival means for treatments were separated using the Waller–Duncan *k*-ratio *t*-test option under the GLM Procedure.

Results

Experiment 1

When adult *T. castaneum* were exposed to the treated experimental arenas, mortality was $60.0 \pm 10.6\%$, in contrast to mortality of $3.3 \pm 3.3\%$ in the untreated controls. Progeny production occurred in all of the food

Fig. 2 Three types of exposure arenas used for testing of 4-week-old larvae of *T. castaneum*. The dashed lines in the first two figures (a and b) delineate the treated portion of the arena, and the dashed line in the positive control (c) delineates the area that would have been treated, but was not treated so as to serve as the true untreated control (drawing not to scale)



patches in the 9 arenas, though it was greater in controls (126.0 ± 14.8 larvae) than in the treatments (48 ± 17.1 , $P < 0.05$). However, the number of dead adults in the treatments was not correlated with progeny production ($P = 0.12$). Mortality of pupae exposed in the treated arenas was $8.3 \pm 3.1\%$ compared with 0 in controls, but the average of progeny in the treatments, 48.2 ± 8.1 , was not different than the average progeny production in the controls (74.0 ± 27.5 , $P = 0.27$). However, this lack of significance was due in part to the variation in progeny production in the untreated controls, as shown by the high standard error of the mean.

Experiment 2

All of the larvae exposed in the three positive control arenas (Fig. 2) emerged as normal adults. In the arenas where the flour food was placed on the treated spot (negative control), there were no live healthy adults. Mortality in the larvae and pupal stages was 80.0 ± 5.7 and $13.3 \pm 3.3\%$, respectively. Two out of the total of 30 exposed larvae reached the adult stage but died upon emergence. When the larvae were given the choice of the treated and the untreated food patch, they exhibited no avoidance behavior of the spot treated with chlorfenapyr. Average mortality of larvae exposed in these six choice arenas was $93.3 \pm 3.3\%$, pupal mortality was 1.0 ± 0.5 , and $3.3 \pm 0.5\%$ of the exposed larvae reached the adult stage but died upon emergence. A small portion of the exposed larvae, $2.2 \pm 0.6\%$, were able to emerge as normal, healthy adults, indicating little escape of exposure to chlorfenapyr.

Experiment 3

The results of this experiment show a slight decline in efficacy during the 1–3-week-post-treatment interval

Table 1 Percentage (mean \pm SEM) of dead adult *T. castaneum* after 1 week of exposure to arenas partially treated with chlorfenapyr (Phantom) and progeny production resulting from the exposure of the 10 parental adults

Week		Dead (%)	Progeny
1	Treatment	66.7 ± 12.5	34.0 ± 16.9
	Control	$5.0 \pm 5.0^*$	$81.5 \pm 0.5^*$
2	Treatment	41.1 ± 10.8	59.7 ± 6.2
	Control	$9.5 \pm 0.5^*$	$106.5 \pm 30.5^*$
3	Treatment	25.0 ± 5.0	84.0 ± 22.9
	Control	$5.0 \pm 5.0^*$	128.5 ± 12.5

Tests were carried out at 1, 2, and 3 weeks post-treatment. Means between treatments and controls denoted by an asterisk are significantly different ($P < 0.05$, *t*-test in SAS)

(Table 1), as determined by the percentage of the exposed parental adults that died and the resulting progeny production. Mortality was significantly lower ($P < 0.05$) in untreated controls at all three residual testing intervals, but at week 3, there was no difference in progeny production in the treated arenas versus untreated controls.

The exposure studies with larvae produced different results than the test described in Experiment 2 in that few larvae died in that stage or in the pupal stage; however, even though the exposed larvae emerged as adults, most of them died (Table 2). The model for treatment, week, and the interaction was not significant for percentage of dead larvae ($F = 1.5$, $df = 8, 22$, $P = 0.22$) or dead pupae ($F = 2.2$, $df = 8, 15$, $P = 0.10$). The model was significant ($P < 0.01$) for dead adults ($F = 11.9$, $df = 8, 15$) and live adults ($F = 22.8$, $df = 8, 15$). In both types of exposures to the treated arenas, adult survival was significantly lower than in untreated controls. At weeks 1 and 2, survival at the adult stage was greater when the larvae were placed on the choice arenas compared to the negative control, but there was no difference between the negative controls and the choice arenas at week 3.

Discussion

There are a number of recent studies with insecticides and treated surfaces that have documented increased survival of adult *T. castaneum* when provided with a food source during or after exposure (Toews et al. 2009). However, the results of this series of studies show that the presence of the refugial food source compromised the effectiveness of chlorfenapyr, as adult *T. castaneum* females that encountered the residues on the treated portion of the arenas were able to locate the untreated food source and oviposit before mortality occurred. When the contact insecticide was applied as a banding treatment around a refugial area with a food source, inside a shed artificially infested with *T. castaneum*, dead adults were present on the floors but the overall populations in the food source remained constant (Toews et al. 2005). Hence, in field sites where contact insecticides are applied, insecticide effectiveness must be evaluated cautiously, especially when basic decisions hinge on the presence of dead adults or adults collected in pitfall or pheromone traps (Toews et al. 2009).

When 4-week-old larvae of *T. castaneum* were exposed to concrete treated with chlorfenapyr, there was little adult survival, even with the presence of the food source. Some larvae that came into contact with the chlorfenapyr were able to emerge as adults, but they were not viable and died very quickly after emergence. Most published studies with stored product insects exposed to treated surfaces involve adult insects, and there are few direct comparisons between

Table 2 Percentage of dead larvae, dead pupae, dead adults (those that died after emergence) and live adults (those living after emergence (mean \pm SE) resulting from the exposure of 10 4-week-

old larvae of the red flour beetle on concrete arenas partially treated with chlorfenapyr (Phantom)

Week	Treatment	Dead larvae	Dead pupae	Dead adults	Live adults
1	Positive	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^c	100.0 \pm 0.0 ^a
	Negative	7.1 \pm 7.1 ^a	0.0 \pm 0.0 ^a	92.8 \pm 7.1 ^a	0.0 \pm 0.0 ^c
	Choice	5.0 \pm 2.9 ^a	0.0 \pm 0.0 ^a	58.6 \pm 0.0 ^b	36.4 \pm 7.6 ^b
2	Positive	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^c	100.0 \pm 0.0 ^a
	Negative	17.5 \pm 17.5 ^a	0.0 \pm 0.0 ^a	63.7 \pm 23.2 ^a	18.7 \pm 18.7 ^c
	Choice	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	21.1 \pm 9.4 ^b	53.4 \pm 5.8 ^b
3	Positive	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^c	100.0 \pm 0.0 ^a
	Negative	0.0 \pm 0.0 ^a	5.5 \pm 5.5 ^a	72.2 \pm 16.7 ^a	22.2 \pm 11.1 ^b
	Choice	0.0 \pm 0.0 ^a	2.7 \pm 2.7 ^a	65.0 \pm 4.8 ^a	32.2 \pm 3.4 ^b

Tests were carried out at 1, 2, and 3 weeks post-treatment. Arenas were as described for Experiment 2 with untreated controls (positive control, positive), arenas with a flour food patch placed on the treated portion of the arena (negative control, negative), and food patches on the treated portion of the arena and upper portion of the arena (choice, see diagrams in Fig. 2)

^a Means within columns for each week for each category that are followed by different lower-case letters are significantly different ($P < 0.05$, Waller–Duncan k -ratio t -test in SAS)

immatures and adults of a particular species. In a simulated field trial with pyrethrin applied as an aerosol, larval and pupal *T. castaneum* and *T. confusum* were far more susceptible than the adults (Arthur and Campbell 2008). This same type of concrete exposure arena was utilized in an aerosol study where residual efficacy of 1% active ingredient [AI] pyrethrin or 3% pyrethrin + the same amount of the insect growth regulator (IGR) methoprene (Diacon II[®]) was compared (Arthur 2010). The pyrethrin appeared to rapidly dissipate because there was little control of adult *T. castaneum* after the exposed arenas were removed from the field site. Residues were active for up to 4 weeks, as assessed by adult emergence from eggs, larvae, and pupae placed with food on the exposed surfaces, but in all cases, adult emergence was reduced at the higher rate of pyrethrin. This would seem to show some persistence of pyrethrin at levels that would not affect adults, or an additive effect of the pyrethrin and the IGR, or both.

In previous studies with adult *T. castaneum* (Arthur 2008, 2009) and in studies with Argentine ants *Linepithema humile* (Mayr) (Wiltz et al. 2009), chlorfenapyr exhibited delayed rather than immediate toxicity. Chlorfenapyr affects oxidative phosphorylation and inhibits ATP synthesis (McLeod et al. 2002); hence, it may be slower acting compared with conventional neurotoxins. In the current study, exposed parental adults eventually died but the delay provided enough time for oviposition in the provided food source. Slow-acting compounds would therefore seem to be poor choices for surface treatments; however, in our tests, we were also assessing residual efficacy during a two-week time period, larval susceptibility to the toxicant, and avoidance behavior of larvae to

the insecticide. As stated by Wiltz et al. (2009), rapidity of kill is not the only desirable quality of a surface toxicant, but in their study, chlorfenapyr was more slower acting than either bifenthrin or fipronil, and chlorfenapyr did not give the same level of control as the other two insecticides.

Consideration of delayed toxicity and resulting progeny production might be useful in evaluations of contact toxicants used as surface treatments, because insects may experience limited exposures on treated surfaces in commercial flour mills and food storage sites. However, if *T. castaneum* oviposit on food sources deposited on a treated surface, the residues could be absorbed by the food source; hence, population development would occur on the contaminated food source. This could lead to reductions or delays in adult emergence, and this method of evaluation also warrants further consideration.

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