

Dosage rate, temperature, and food source provisioning affect susceptibility of *Tribolium castaneum* and *Tribolium confusum* to chlorfenapyr

Frank H. Arthur

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Abstract A series of experiments were conducted to evaluate residual efficacy of the insecticidal pyrrole chlorfenapyr (Phantom[®]) on treated concrete for control of *Tribolium castaneum* (Herbst), assess development of progeny from exposed parental adults, and to determine if starvation before exposure with or without a flour food source increased susceptibility of adult *T. castaneum* and adult *Tribolium confusum* Jacqueline du Val to chlorfenapyr. No adults survived exposure on concrete treated with chlorfenapyr at the maximum label rate of 1.1 g active ingredient (AI)/m², and no progeny were produced in bioassays conducted at 0–8 weeks posttreatment. In the second test, application rates were reduced, and bioassays were conducted at 27 and 32 °C. Adult survival and progeny production decreased as the application rate increased from 28 to 225 mg AI/m², and survival and progeny production were generally lower at 32 °C than at 27 °C, but at higher rates survival was <1 %, and no progeny were produced. In the final test, adult *T. castaneum* and adult *T. confusum* were starved for 1–7 days and then exposed either with or without flour on concrete treated with 3.9 and 27.5 mg AI chlorfenapyr/m². Mortality generally increased with starvation time, the presence of a food source led to decreased mortality at both application rates, and *T. confusum* was the more susceptible of the two species. Results show that chlorfenapyr could effectively control both species, but precise dosage levels need to be

determined. Also, the presence of a food source greatly compromises adult control.

Keywords Chlorfenapyr · Insecticides · Control · Sanitation · Starvation · *Tribolium castaneum* · *Tribolium confusum*

Introduction

The insecticidal pyrrole chlorfenapyr is currently labeled in the United States (USA) for control of nuisance ants, termites, cockroaches, and also has provisions for use in general pest control for a variety of species, including some stored product insects. It has been evaluated as a grain protectant (Kavallieratos et al. 2011) and as a residual surface treatment for flour beetles (Arthur 2008a, 2009; Arthur and Fontenot 2012a). When adults of both *Tribolium castaneum* (Herbst), the red flour beetle, and *Tribolium confusum* Jacqueline du Val, the confused flour beetle, were exposed from 1 to 8 h on concrete treated with 11 mg active ingredient (AI)/cm² of chlorfenapyr, survival of both species decreased as exposure interval increased; however, survival was reduced when those adults were provided with a flour food source after they were exposed (Arthur 2009). The application rate in the above study was based on label directions for formulating a 0.05 % AI solution, and applying that solution in equivalent ratio to the maximum volume rate specified on the label, which is 194 ml of formulated solution/m². *Tribolium confusum* was the more susceptible of the two species.

The application rate used in the above studies was consistent with the insecticide label, but more detailed testing would help us define more precisely the actual rates needed for residual control. In addition, there is also evidence that

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F. H. Arthur (✉)
USDA, Agricultural Research Service, Center for Grain and Animal Health Research, 1515 College Avenue,
Manhattan, KS 66502, USA
e-mail: frank.arthur@ars.usda.gov

immature stages of *T. castaneum* and *T. confusum* are more susceptible to insecticides compared with the adults (Arthur and Campbell 2008). Studies involving evaluation of insect growth regulators (IGRs) on a treated surface usually involve the addition of a food source to the treatment arena, and assessing the resulting adult emergence (Arthur et al. 2009; Sutton et al. 2011; Wijayaratne et al. 2012). The larvae may come into direct contact with the residues on the treated surface, and the food source may absorb some of those residues, thus resulting in reduced adult emergence.

Arthur and Fontenot (2012b) evaluated the IGR methoprene and the chitinase inhibitor novaluron by exposing adult *T. castaneum* for 1 week, along with wheat flour, on treated concrete. The adults were removed after 1 week, and production of F1 progeny adults was determined after an 8-week holding period. This methodology would also be useful for evaluating a contact insecticide, as it would assess adult mortality and also progeny production on a food source that was in contact with a treated surface. Evaluations done in this manner may also mimic how flour beetles would be exposed on food patch deposited on a treated surface inside a flour mill or food warehouse. There are no recent studies published in the scientific literature, which have examined this aspect of exposure on a treated surface.

Dietary stress after stored product insects have been exposed to various toxicants, including insecticides and fungal pathogens, increases susceptibility to those toxicants (Lord 2010). Many studies have documented decreased insecticidal efficacy when adult stored product insects, including adult *T. castaneum* and *T. confusum*, are provided with a flour food source during or after exposure to an insecticide (Toews et al. 2003; Arthur 2008a, 2009). As a corollary, there have been no recent studies to determine if starvation before exposure affects susceptibility of *T. castaneum* and *T. confusum* to chlorfenapyr or any other residual contact insecticide. The objectives of this study were to determine (1) residual efficacy of chlorfenapyr for control of exposed adults of *T. castaneum*; (2) efficacy of reduced application rates for adult control and prevention of progeny production of this same species; and (3) selected rates to evaluate effects of starvation before exposure on susceptibility of *T. castaneum* and *T. confusum* to chlorfenapyr.

Materials and methods

Experiment 1

Concrete exposure arenas were created using a dry commercial driveway patching material that contains Portland cement (Rockite®) and was purchased from a local

hardware store. This material was mixed with tap water in an approximate ratio of 0.5 g of product/ml water to create a slurry, which was then poured into the bottom portion of plastic Petri dishes measuring 62 cm² in area. Each dish was filled to an approximate height of 0.5 cm, and all prepared dishes were allowed to dry on a laboratory counter for several days.

The commercial formulation of chlorfenapyr used for this experiment was Phantom®, 21.45 % AI, 240 mg/ml, obtained from BASF Corporation (Research Triangle Park, NC, USA). Label directions specify creation of a 0.5 % diluted concentration by mixing 89 ml of product in 3,784 ml of water. This dilution can be applied at a maximum of 35.4 ml per 1,858 cm² as an outside perimeter spray. Hence, the equivalent of this rate for the 62 cm² area of the concrete arena was 1.2 ml, which was 1.1 g AI/m². For our study, the 0.5 % dilution was formulated in 50-ml volumetric flasks, in proportion to the label directions for mixing the larger volume of 3,784 ml.

A replicate consisted of a series of 18 treated arenas. For each of 6 replicates of these 18 arenas (108 total), an insecticide solution was created in the 50-ml flask as described above, and a Badger 100 Artist's airbrush (Franklin Park, IL, USA) was used to spray each arena with 1.2 ml of the solution. For each replicate, there were six post-exposure treatment intervals, 0, 2, 4, 6, 8, and 10 weeks. For the 0-month bioassay, three arenas were selected from each replicate (three sets of 6: 18 total arenas). Approximately, 500 mg of flour was added to each arena by placing the flour in a small pile in the center of the arena. Ten adults of 1–2-week-old mixed-sex adult *T. castaneum* were added to each arena. One set of six arenas was placed in an incubator set at 22 °C, another placed in an incubator set at 27 °C, and the third placed in an incubator set at 32 °C (18 total). For each set placed in each incubator, an untreated control arena was set up in the same manner. The remaining treated arenas were split evenly among the three incubators to be used for residual bioassays.

The adults placed on the arenas used for the 0-week bioassays were allowed to remain on the arenas for a week. At that time, the arenas were removed from the incubator, adult mortality assessed and recorded, all insects live and dead removed from the arenas, and the arenas returned to the incubator for another 6 weeks, at which time, all larvae, pupae, and adults in controls and treatments were tabulated. This process was repeated at each of the remaining residual bioassay intervals.

The test was analyzed using the general linear models (GLM) procedure of the statistical analysis system (SAS, version 9.1, Cary, NC, USA) to determine treatment means. Means were separated at $P < 0.05$ using the Waller-Duncan k -ratio t test under the GLM procedure.

Experiment 2

In this test, chlorfenapyr was applied at lower concentrations than those used in [Experiment 1](#), using new sets of concrete arenas created as described above. The concentrations were 0, (untreated control) 10, 20, 40, 60, 80, and 100 % of the label rate listed for the outside perimeter treatment. Hence, the rates in succession were 0, 0.11, 0.22, 0.44, 0.66, 0.88, and 1.1 g AI/m², respectively. There were two sets of six separate subreplications for an initial trial (84 total arenas), with each diluted solution for each rate, applied at the equivalent label volume rate of 1.2 ml solution per arena. Treatments were done as described above, with 500 mg of flour and 10 adult *T. castaneum* being added as described above. One set of the six arenas for each concentration was held at 27 °C (42 arenas), and the other held at 32 °C (42 arenas). These arenas were removed from the incubators after 1 week, adult mortality assessed, and the arenas returned to the incubator.

Upon examination of the arenas after 1 week, treatment mortality was 100 % at all application rates, and no progeny were produced. Subsequently, evaluations were done using application rates of 0.3 ml per area of the Petri dish (0, 27.5, 55, 111, 165, 220, and 275 mg AI/m²) and 0.6 ml per area of the Petri dish (55, 110, 222, 330, 440, and 550 mg/m²) instead of the volume of 1.2 ml used in the initial series. Four separate replicates (356 total arenas) were done on different weeks, as described above. After the 1-week assessments, all adults live and dead were removed from the arenas before they were returned to the incubator.

The test was analyzed using the GLM procedure of SAS, with application rate, concentration (including untreated controls), and temperature as main effects. The variables analyzed were adult mortality after 1 week of exposure and progeny production after 7 weeks. Means were separated with the Waller-Duncan *k*-ratio *t* test using the GLM procedure as described for [Experiment 1](#).

Experiment 3

For this test, a total of 288 concrete arenas were prepared as described above. Solutions of chlorfenapyr were prepared at 2.5 and 10 % of the maximum label rate, using the standard volume rate of 1.2 ml per the area of the arena. A replicate consisted of a set of 48 arenas, with 16 arenas untreated, 16 sprayed as described above with 0.3 ml of 2.5 % solution (low rate, 6.9 mg AI/m²), and 16 sprayed with 0.3 ml of 10 % solution (high rate, 27.5 mg AI/m²) (three treatments). For each treatment set, 500 mg of whole-wheat flour was placed in each of the eight arenas and no flour in the other eight arenas. Adult *T. castaneum* and/or *T. confusum* that had been held for 1, 2, 5, or 7 days

(four starvation times) without a flour food source were placed in each of four of the eight arenas with or each of the four arenas without 500 mg of flour. All arenas were held on a laboratory counter, temperature was ~25 °C, and relative humidity (RH) was ~50 %. Knockdown of adults, defined as beetles that were on their backs, was assessed at 15, 30, 60, 90, and 120 min after exposure. After the final knockdown assessment was made, the arenas were transferred to an incubator set at 27 °C–60 % RH. These arenas were removed from the incubator ~24 h after exposure, and mortality (adults unable to move when prodded) was recorded. All observations were made on the same set of arenas. All beetles were discarded and the arenas returned to the incubator.

This entire process was repeated weekly for 6 weeks (six separate replicates), using 48 new arenas each time, with new insecticide solutions, as was described above. This experiment was analyzed with knockdown at the different time periods as a repeated measure using the GLM procedure of SAS, because all observations were done on the same set of arenas. The mortality counts were analyzed separately as independent observations. It was difficult to distinguish beetles that had fallen down in the flour from those that were affected by exposure to chlorfenapyr; therefore, data for percentage of adult beetles knocked down at the different assessment times were analyzed only for those arenas that did not contain flour. Also, because of the difficulty of determining knockdown in the arenas with flour, mortality was used as the evaluation criterion. Mortality was defined as those individuals on their backs, exhibiting no visual movement, and no mobility or reflexive response when touched with a thin probe. The mortality data were analyzed with species, rate, starvation duration, and the presence of flour as main effects, using the GLM procedure of SAS. Analysis of the variables of interest was done on both the raw data and by arc-sine transformation of the raw data. There were no differences with respect to significance of the main effects regarding the method of statistical analysis. Hence, the analysis for raw data is reported for the results. Means for mortality of adults of each species with respect to starvation time at each concentration, and means for mortality exposed with or without flour at each individual starvation time, were separated using the Waller-Duncan *k*-ratio *t* test as part of the GLM analysis.

Results

Experiment 1

Adult survival in untreated controls after the 1-week exposure period was 98.3 ± 1.7 , 90.0 ± 6.3 , and 98.3 ± 1.7 %

at 22, 27, and 32 °C, respectively, averaged over all residual bioassays. In contrast, there was no adult survival on any of the arenas treated with chlorfenapyr. Similarly, progeny emergence occurrences (larvae, pupae, and adults combined) in the untreated controls at the three temperatures were 55.6 ± 13.7 , 30.2 ± 8.9 , and 56.8 ± 7.8 %, respectively. No progeny were in the treatment arenas, while in the control arenas, there were visible tracks made by the larvae as development progressed, and the flour was distributed throughout the arena from the activity of the beetles. In the treatment arenas, the flour virtually remained in the position where it was placed on the arena, with the only disturbance of the flour occurring from the movement of the parental adults before they died. The maximum label rate of 1.1 g AI/m² produced complete mortality and complete suppression of progeny production.

Experiment 2

Adult survival was significant at $P < 0.001$ for main effects application rate, concentration, and temperature ($F = 24.2$, $df = 1$, 392; $F = 358.5$, $df = 6$, 392; $F = 61.0$, $df = 1$, 392), and all interactions were significant at $P < 0.05$ except for the interaction of all main effects. Survival on untreated controls for the series of application rates of 28–275 mg AI/m² was 97.2 ± 1.4 % at 27 °C and 95.5 ± 1.7 % at 32 °C, and ranged from 0 to 31 % in the treatments, depending on the rate and temperature (Fig. 1a). Survival in the treatments was generally less at 32 °C compared to 27 °C. Adult progeny in untreated controls at 27 and 32 °C were 62.5 ± 10.8 and 92.4 ± 10.4 , respectively, while few progeny adults were produced in any treatment arena at either temperature (Fig. 1b). Adult survival on untreated controls at 27 and 32 °C for the series of application rates of 55–555 mg AI/m² was 98.3 ± 1.2 and 99.2 ± 1.8 %, respectively, with little survival in the treatments (Fig. 2). Progeny production in the untreated controls at 27 and 32 °C were 47.8 ± 4.6 and 67.2 ± 4.0 , but no progeny adults occurred in any of the treatment arenas.

Experiment 3

The percentage knockdown on the arenas with no flour was not different among species, concentration (including untreated controls), or the four starvation periods ($F = 3.1$, $df = 1$, 23, $P = 0.09$; $F = 0.1$, $df = 2$, 24, $P = 0.89$; $F = 1.0$, $df = 3$, 24, $P = 0.42$, respectively). Percentage knockdown was significant with time ($F = 3.1$, $df = 6$, 887, $P < 0.004$), with time as the independent variable being marginally nonsignificant (regression procedure, SAS, $P = 0.053$). However, knockdown was only 2.5 ± 0.2 % even when all time assessments were combined.

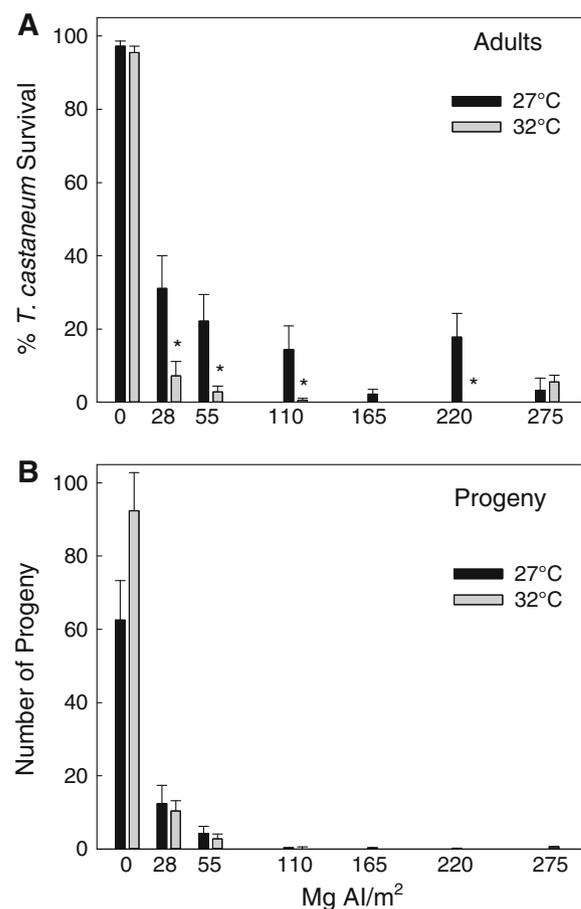


Fig. 1 Percentage survival of mixed-sex adult *Tribolium castaneum* (a, mean \pm SE) exposed for 1 week on untreated concrete and concrete treated with 28–275 mg AI of chlorfenapyr/m². Ten parental adults were exposed in concrete treatment arenas with 500 mg of wheat flour, and tests were conducted at 27 and 32 °C. Number of adult progeny (b, mean \pm SE) in the arenas. Significant differences in survival or progeny production between the two temperatures ($P < 0.05$) are denoted with asterisks

Mortality after 24 h of exposure was analyzed as an independent measure for all the main effects and interactions, on arenas with and without flour. Main effects species, rate (including untreated controls, starvation days, and the presence of flour) were all significant at $P < 0.001$ ($F = 34.2$, $df = 1$, 238; $F = 101.3$, $df = 2$, 238; $F = 21.0$, $df = 3$, 238; $F = 31.5$, $df = 1$, 238, respectively). All associated interactions were significant ($P < 0.05$) except flour by days, species by rate by flour, rate by flour by days, and the overall interaction of all four main effects. Control mortality averaged 0.3 ± 0.1 % over all combinations; hence, the controls were eliminated from further analysis of the different factors with one-way ANOVAs.

Mortality of *T. castaneum* starved for the four time periods and those exposed on arenas treated with the low rate of chlorfenapyr was two to threefold less when adults were provided with flour (Fig. 3a). There was a general pattern of

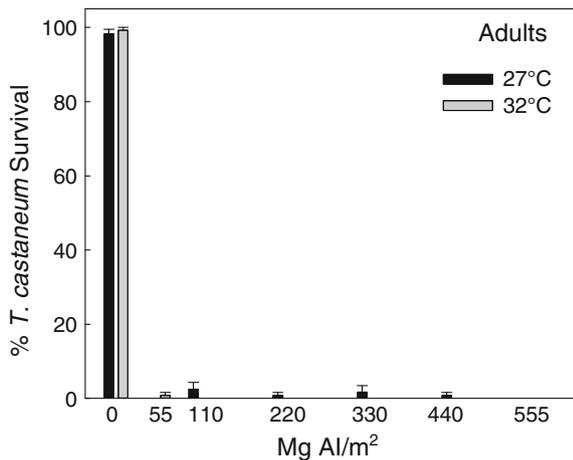


Fig. 2 Percentage survival of mixed-sex adult *Tribolium castaneum* (mean ± SE) exposed for 1 week on untreated concrete and concrete treated with 55–550 mg AI of chlorfenapyr/m². Ten parental adults were exposed in concrete treatment arenas with 500 mg of wheat flour, and tests were conducted at 27 and 32 °C. Significant differences in survival between the two temperatures ($P < 0.05$) are denoted with different lower case letters

increased mortality with increased starvation regardless of the flour treatment, but the only significant difference was for the increased mortality after starvation for 7 days compared to the other intervals (Fig. 3a). Maximum mortality was $66.7 \pm 6.7 \%$ for *T. castaneum* starved for 7 days and not given flour after exposure. In contrast, there was little or no mortality for adult *T. confusum* starved and provided with flour when exposed on the arenas treated with the low rate of chlorfenapyr, starvation duration did not affect mortality when exposed with or without flour, and maximum mortality was only $16.7 \pm 11.1 \%$ for adults starved for 7 days, and then exposed without flour (Fig. 3b).

Mortality of *T. castaneum* exposed on the arenas treated with the high rate of chlorfenapyr was generally greater at the 5- and 7-day starvation periods compared with 1 and 2 days, and was less on flour compared with arenas without flour for two of the starvation durations (Fig. 4a). Mortality of *T. castaneum* when starved for 7 days and not given flour was $96.7 \pm 3.3 \%$. While mortality of *T. confusum* generally increased with starvation duration on arenas both with and without flour, less mortality occurred on flour compared with the no-flour arenas for only one comparison, and overall mortality was two to fivefold less than corresponding mortality of *T. castaneum* (Fig. 4b). There was a general increase in mortality of both species at the high rate compared to the low rate.

Discussion

Several recent studies have documented decreased insecticidal efficacy when stored product insects, including adult

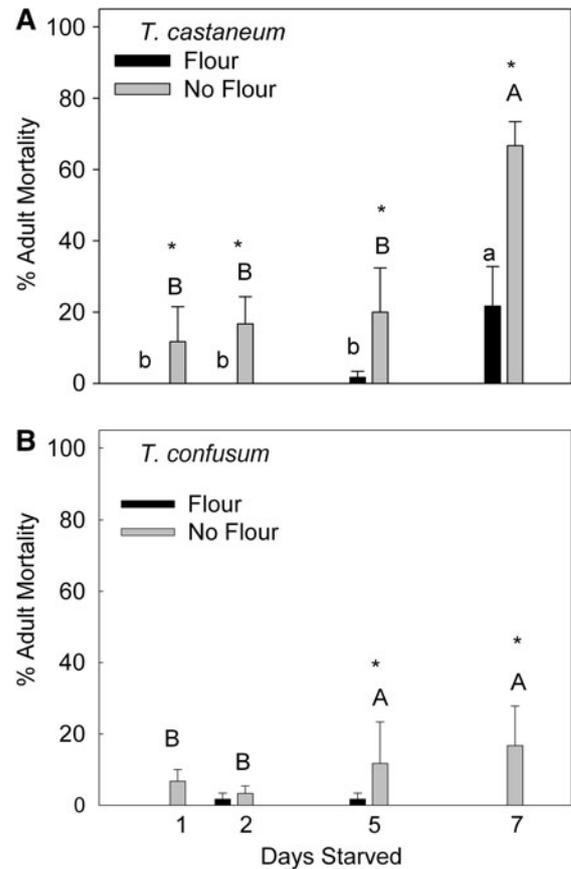


Fig. 3 Mortality (mean ± SE) of adult mixed-sex *Tribolium castaneum* (a) or *Tribolium confusum* (b) starved for 1, 2, 5, or 7 days, and exposed for 24 h either with or without wheat flour on concrete treated with 6.9 mg AI chlorfenapyr/m². A significant difference in mortality between days of starvation with and without flour is denoted with different lower and upper-case letters, respectively; a significant difference in mortality either with or without flour at each starvation day is denoted with an asterisk ($P < 0.05$)

T. castaneum and *T. confusum*, are provided with a flour food source during or after exposure to an insecticide (Toews et al. 2003; Arthur 2008a, b, 2009). This is the first study evaluating effects of starvation before exposure to chlorfenapyr, and although there was a definite increase in mortality of adult beetles starved before exposure, the presence of a food source will lead to increased survival even when *T. castaneum* and *T. confusum* are starved before insecticidal exposure. Flour mills and other stored product environments can often contain refugial areas whereby stored product insects may escape exposure to residual surface treatments, either through limited contact to a treated surface or insufficient exposure to produce adult mortality. Cleaning and sanitation are an important component of pest management programs for flour mills, and can help in reducing beetle populations inside mills, and increasing the efficacy of insecticide treatment, including fumigations (Campbell et al. 2010a, b). The

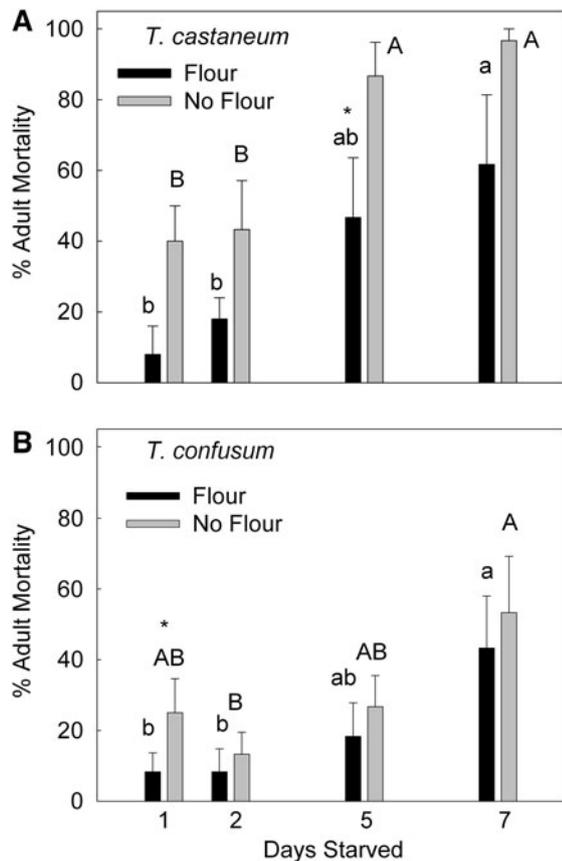


Fig. 4 Mortality (mean \pm SE) of adult mixed-sex *Tribolium castaneum* (a) or *Tribolium confusum* (b) starved for 1, 2, 5, or 7 days, and exposed for 24 h either with or without wheat flour on concrete treated with 27.5 mg AI chlorfenapyr/m². A significant difference in mortality between days of starvation with and without flour is denoted with different lower and upper-case letters, respectively; a significant difference in mortality either with or without flour at each starvation day is denoted with an asterisk ($P < 0.05$)

maximum starvation interval used in our current test was 7 days, and hence longer starvation intervals before adult beetles encounter a treated surface without a food source would most likely produce greater mortality. However, when adults encounter a food source, it would correspondingly negate some of the effects of the starvation and resulting exposure to residual insecticides.

The continued presence of refugial areas with a food source also allows resident populations of *T. castaneum* to persist even though residual insecticidal treatments appear to be effective (Toews et al. 2005, 2009). This could particularly be important when an insecticide such as chlorfenapyr is used as a residual spot or crack and crevice treatment. The adults could be exposed for a limited time, and if mortality is not immediate, then female adults may be able to locate the food source and oviposit before death. In addition, the protected population may be able to persist even when adult mortality occurs. Recent studies with

aerosol insecticides, which would be expected to give more complete coverage to a given area compared to a crack and crevice application, also demonstrate population persistence. Toews et al. (2010) and Arthur et al. (2013) conducted studies in which resident populations of *T. castaneum* were established in food patches underneath shelves in experimental sheds. Fewer live adults were trapped in treatment sheds compared with controls, and more dead adults were collected in treatments compared with controls, yet the resident populations continued to develop and were not totally eliminated.

Previous studies with these same laboratory insect strains showed *T. confusum* was less susceptible to chlorfenapyr than *T. castaneum*, which was consistent with results for studies in which adults of the same laboratory strains of the two species were exposed to the pyrethroid cyfluthrin, but the reverse was true when these strains were exposed to diatomaceous earth (DE) and pyrethrin aerosol (Arthur 2008a, b). However, in the current test, *T. castaneum* was the more susceptible species than *T. confusum* to chlorfenapyr. These particular strains of *T. castaneum* and *T. confusum* have been in laboratory culture for about 30 years, and have been used for a number of published studies regarding evaluation of different insecticides. Field strains of stored product insects will vary in their response to contact insecticides (Huang et al. 2004; Kljajic and Peric 2006; Athanassiou et al. 2008a), but the results of the current study show some variation in susceptibility with time could exist in a laboratory strain as well.

Results of the second study indicated that chlorfenapyr was more effective at 32 °C compared to 27 °C. Kavallieratos et al. (2011) evaluated chlorfenapyr as a grain protectant at different application rates on barley, corn, rough rice, and wheat held at 20, 25, and 30 °C. Adult mortality trends were not consistent, but overall mortality was generally greater at 25 and 30 °C than at 20 °C. Differences in toxicity have been described for different insecticide classes, but in other instances, results are conflicting and inconsistent (Athanassiou et al. 2008b). Increases in toxicity with temperature have been attributed to greater insect activity at higher temperatures, thereby causing increased absorption and metabolism of toxicants, but variation among different species regarding optimum temperatures for development may also affect susceptibility to insecticides (Kavallieratos et al. 2010). Therefore, the apparent increase in toxicity of chlorfenapyr with temperature could result from increased movement of *T. castaneum* at the higher temperature, and not due to any properties of chlorfenapyr or pyrolles in general.

The final series of studies with reduced rates of chlorfenapyr indicate further studies for control of stored product insects in flour mills and food warehouses should be done with lower application rates than what is specified on the

product label. One factor requiring additional evaluation is the time required to knock down and incapacitate a stored product insect on a surface treated with different application rates of chlorfenapyr. Little knockdown occurred when adult *T. castaneum* or *T. confusum* were exposed for 3 h on the concrete surface when treated with 6.9 and 27.5 mg of chlorfenapyr/m². However, since this insecticide has a different mode of action compared with more traditional insecticides such as organophosphates and pyrethroids (Hunt 1996), caution must be exercised when making direct comparisons with other residual insecticides or their labeled usage rates.

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