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Sehgal et al.: Efficacy of insecticides used for empty bin treatments

Variation in susceptibility of laboratory and field strains of three stored-grain insect species to β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin applied to concrete surfaces

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Abstract

BACKGROUND: The efficacy of commercial formulations of β -cyfluthrin and chlorpyrifosmethyl plus deltamethrin applied to clean, concrete surfaces similar to that of empty bins against field strains of stored-grain insects is unknown. We exposed adults of 16 strains of the red flour beetle, *Tribolium castaneum* (Herbst); 8 strains of the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); and 2 strains of the lesser grain borer, *Rhyzopertha dominica* (F.), collected mainly from farm-stored grain in Kansas, USA, to β -cyfluthrin and chlorpyrifos-methyl plus deltamethrin applied to concrete surfaces and determined knockdown and mortality.

RESULTS: Knockdown and mortality differences among species and strains to the insecticides tested were significant. Mortality of all species was less than that of knockdown, suggesting recovery when placed on food after insecticide exposure. β-cyfluthrin was effective against *R*. *dominica* but ineffective against *T. castaneum* and *O. surinamensis* field strains. Chlorpyrifosmethyl plus deltamethrin was only partially effective against field strains of the three species.

CONCLUSION: Reduced susceptibility in field strains may be due to inherent formulation deficiency and low levels of tolerance or resistance to β -cyfluthrin. No single insecticide provided adequate control of the three species tested.

Keywords: insecticides, empty-bin treatments, stored-grain insects, field strains, efficacy assessment

1 INTRODUCTION

Stored-grain insect management, prior to storing newly harvested grain, begins with removing residual grain debris and application of an approved insecticide to the concrete floor and interior bin surfaces to kill any live insects present. This practice is followed by 78.8% of the 318 Kansas producers storing wheat who responded to a survey in 1987. Among the insecticides currently registered by the United States Environmental Protection Agency (US-EPA) for empty bin treatments, β-cyfluthrin or Tempo® SC Ultra (Bayer CropScience, Research Triangle Park, NC, USA), is new and an alternative to traditionally used cyfluthrin wettable powder (WP) and emulsifiable concentrate (EC) formulations. β-cyfluthrin can be applied to surfaces at low and high application rates of 0.01 and 0.02 g (AI) m⁻², respectively. These rates are 50% less than that of the WP or EC formulations. Chlorpyrifos-methyl at 3 ppm plus deltamethrin at 0.5 ppm (StorcideTM II, Bayer CropScience) was registered in 2004 for direct treatment of barley, oats, rice, sorghum, and wheat intended for storage and for empty bins receiving these grains. This combination product replaced chlorpyrifos-methyl after its tolerances were revoked.

The efficacy of the WP and EC formulations of cyfluthrin at low (0.02 g [AI] m⁻²) and high (0.04 g [AI] m⁻²) labeled rates was evaluated on concrete surfaces against laboratory populations of the red flour beetle, *Tribolium castaneum* (Herbst); confused flour beetle, *Tribolium confusum* Jacquelin du Val; and Indianmeal moth, *Plodia interpunctella* (Hübner).²⁻⁶ The WP formulation was more effective than the EC formulation against adults of *Tribolium* spp. probably due to greater availability of residues on treated surfaces.⁷ Additionally, the WP formulation was more persistent than the EC on both steel⁸ and concrete surfaces.² Exposure of *T. castaneum* adults to the low rate of cyfluthrin WP on concrete surfaces for 0.5 to 4 h in the absence of flour resulted in 100% mortality; similar mortality at the high rate occurred only after

2 h of exposure.³ If *T. castaneum* adults were provided 1 g of flour for 1 wk after a 2 h of exposure to the low rate of cyfluthrin WP, 40% of the adults recovered.⁴ In another study, *T. castaneum* adults exposed for 2 h on concrete surfaces treated with low rate of cyfluthrin WP and then transferred to concrete dishes for 1 wk showed 60% mortality in the absence of food, 49% in the presence of 1 g of wheat kernels, 15% in 1 g of sawdust, and 5% in 1 g of flour.⁹

Differences in insect responses in the presence of flour between the two studies may be attributed to the temperatures used. The former test⁴ was conducted at 22°C and the latter at 28°C.⁹ Pyrethroids such as cyfluthrin generally are known to have a negative temperature coefficient. For example, the toxicity of cyfluthrin to *T. castaneum* adults was found to decrease markedly at 25, 30 and 35°C when compared with 20°C.¹⁰ Limited studies were conducted with the new β-cyfluthrin formulation. β-cyfluthrin was found to be effective against the stored-product psocid species on concrete at a rate of 2.4 mg (AI) m⁻² at 30°C and 70% r.h.¹¹ The efficacy of β-cyfluthrin against stored-product insects other than psocids has not been studied.

Chlorpyrifos-methyl plus deltamethrin was effective against several stored-grain psocids on stored wheat. ¹² It was also effective against the lesser grain borer, *Rhyzopertha dominica* (F.); rice weevil, *Sitophilus oryzae* (L.); *T. castaneum*, and *P. interpunctella* on stored wheat, and against *R. dominica* and *S. oryzae* on short-grain and long-grain rices, ¹³ but studies on its efficacy as a surface treatment are lacking.

All of the studies mentioned above were conducted using laboratory reared stored-grain insects. Field collected insects may differ markedly from laboratory strains in their susceptibility to insecticides applied to empty bins and grains due to natural tolerance or resistance. ¹⁴⁻¹⁸ To date, there are no published studies documenting effectiveness of β -cyfluthrin and chlorpyrifosmethyl plus deltamethrin on concrete surfaces similar to that of empty bins against field strains

of stored-grain insect populations. Such an evaluation is necessary to confirm whether or not an approved insecticide will work in practical field situations at the labeled rates. In the present investigation, we determined susceptibility of adults of *T. castaneum*, *O. surinamensis*, and *R. dominica* field strains from the United States to β -cyfluthrin and chlorpyrifos-methyl plus deltamethrin applied to concrete surfaces in the laboratory.

2 MATERIALS AND METHODS

2.1 Collection of field strains

Cooperating farm sites (Table 1) were visited on one to three occasions between July and November 2011 to collect adults of *T. castaneum*, *O. surinamensis* and *R. dominica* from farm bins in Kansas by inserting five perforated probe traps¹⁹ just below the grain surface to capture live adults of insect species. These traps were removed after 1 to 2 wk. Additionally, 1 to 2 kg sample of mostly wheat, and some corn and sorghum, were collected in 30.5 cm wide and 37.5 cm long plastic Ziploc bags (Assorted Bag Company, Dallas, TX, USA). In the laboratory, 2.38-mm diameter aluminum sieves and pans (Seedburo Equipment Company, Des Plaines, IL, USA) were used to separate live adults of insects from grains. In addition, five strains of *T. castaneum* and one strain of *R. dominica* collected from flour mills in the United States prior to 2011 were also included in this study, along with the laboratory strains of each species, that have been in rearing, without insecticide exposure, since 1999 in the Department of Grain Science and Industry, Kansas State University. These laboratory strains served as the standard reference strains and assumed to be insecticide-susceptible.

2.2 Insect rearing

Laboratory and field strains were reared on standard diets in a growth chamber at 28°C and 65% r.h., respectively. Organic white wheat flour (Heartland Mills, Marienthal, KS, USA) plus 5% (by wt) brewer's yeast diet was used for rearing *T. castaneum*, while clean organic hard red winter wheat (Heartland Mills, Marienthal, KS, USA) and rolled oats plus 5% brewer's yeast diet were used for rearing *R. dominica* and *O. surinamensis*, respectively.

2.3 Concrete-poured Petri dishes

Ready-mix concrete (Rockite, Hartline Products Co., Inc., Cleveland, OH, USA) was mixed with tap water to make a slurry. This slurry was poured into 9 cm diameter, 1.5 cm high, and 62 cm² area plastic Petri dishes (Fisher Scientific, Denver, CO, USA). Concrete (3,810 g) was mixed with 1,905 ml of tap water to make 100 dishes. The slurry was allowed to dry and the inside walls of the Petri dishes were coated with polytetrafluoroethylene (Insecta-a-Slip, Bio Quip Products, Inc., Rancho Dominguez, CA, USA) to prevent insects from crawling on the sides of dishes.

2.4 Treatment of concrete dishes with insecticides

β-cyfluthrin (11.8% purity) and chlorpyrifos-methyl plus deltamethrin (21.6 and 3.7% purity), were supplied by Bayer CropScience and were diluted in distilled water. Concrete surfaces of dishes were treated with β-cyfluthrin at the low labeled rate of 0.01 g (AI) m⁻² and the high labeled rate of 0.02 g (AI) m⁻², and with chlorpyrifos-methyl plus deltamethrin at the labeled rate of 0.12 plus 0.02 g (AI) m⁻² by applying 255 μl spray solution per dish using a Badger 100 artist's airbrush (Model 100, Franklin Park, IL, USA). Dishes sprayed with 255 μl of distilled water served as the control treatment. Treated dishes were allowed to dry under room conditions (25°C and 25% r.h.) for 24 h before exposing insects.

2.5 Time-response tests with laboratory strains

The laboratory strains of *T. castaneum*, *O. surinamensis*, and *R. dominica* were used to establish a time at which 100% or close to 100% knockdown and mortality of adults occurred when exposed to labeled rates of β -cyfluthrin and chlorpyrifos-methyl plus deltamethrin. This time was used to expose field strains of each species to concrete treated with the two insecticides. Ten unsexed (1- to 2-wk-old) adults each of *T. castaneum*, *O. surinamensis*, or *R. dominica* from laboratory cultures were introduced into each dish and the dishes were covered with Petri dish lids. Adults were exposed to treated dishes for 1, 2, 4, 8, 12, 16, 20 and 24 h. Separate dishes were used for each time period. Each exposure time and species combination included an untreated control dish that was sprayed with aliquots of distilled water. Each species, insecticide, rate, and time combination, including the control treatment, was replicated three times. Dishes were arranged on a laboratory table and HOBO® data loggers (Onset Computer Corp., Bourne, MA, USA) indicated the mean \pm SE (n = 4) temperature and relative humidity in the laboratory room during insect exposure were 24.3 \pm 0.04°C (range, 20.0 - 28.5°C) and 23.4 \pm 0.06% r.h. (range, 15.0 - 31.3%), respectively.

At each exposure time, adults of each species that were knocked down and active were counted. After counting, all adults were transferred to 150-ml round plastic containers with 30 g of the respective insect diet. The plastic containers had perforated lids with wire-mesh screens to facilitate air diffusion. Containers were incubated at 28°C and 65% r.h. for 1 wk to determine end-point mortality following insect recovery on rearing diets.

2.6 Exposure of field strains at labeled rates for a fixed time

The time at which knockdown and mortality of adults was 100%, or near 100%, for a given species and insecticide combination was used to expose adults of field strains of stored grain

insect species. Ten adults of each species and field strain, along with the corresponding laboratory strain, were tested following protocols mentioned above. The low labeled rate of βcyfluthrin gave poor control of T. castaneum and O. surinamensis laboratory strains with mortalities of 47 and 83%, respectively, at the maximum exposure time of 24 h. Therefore, adults of these two species were exposed for 24 h to concrete treated only with the high labeled rate of β-cyfluthrin, while R. dominica was exposed to this insecticide for 2 h because of its high susceptibility. All three species were exposed for 8 h to concrete treated with chlorpyrifosmethyl plus deltamethrin. Each insecticide, species, and strain combination was replicated five times. Knockdown and mortality of field strains were determined as explained above. Tests with field strains of the three species and the laboratory strains were performed in the laboratory room where mean \pm SE (n = 4) temperature and relative humidity were 25.4 \pm 0.02°C (range, 23.4 -26.9°C) and 17.2 \pm 0.1% (range, 15.0 - 27.9%), respectively, and also at constant conditions in a growth chamber at 28°C and 65% r.h. At room conditions, low mortality was observed, so the tests were also done at constant conditions to see if there was any temperature effect as these insecticides are applied to empty bins 3 to 4 wk prior to storing new grain after harvest during the summer months of June and July. At room conditions, each strain had its respective control treatment. At constant conditions only the laboratory strain served as the control for all strains because knockdown and mortality of field strains in the control treatments in tests at room conditions were less than 10%.

2.7 Dose-response tests with β -cyfluthrin on laboratory and least susceptible field strains Based on field strain responses to insecticides, three least susceptible strains of T. castaneum and two of O. surinamensis, along with corresponding laboratory strains, were exposed to β -cyfluthrin-treated concrete dishes at one to four times the high labeled rate (0.02 to 0.08 g [AI]

 m^{-2}) to assess knockdown and mortality. Dishes sprayed with distilled water served as the control treatment for all strains. All dose-response tests were performed at 28°C and 65% r.h. There were five replications for each species, strain, and β -cyfluthrin rate combination, and 10 adults were exposed in each replication.

2.8 Data analysis

Adults of each species that were knocked down and those that died after 1 wk recovery on diets out of the total exposed were calculated as a percentage. In the time-response tests with the laboratory strains, there was no knockdown and mortality in the control treatment for T. castaneum and R. dominica during 1 to 24 h exposures. The maximum mean knockdown of O. surinamensis in the control treatment was 3% and mortality was 10%. Therefore, knockdown and mortality data were not corrected for these responses in the control treatment. Linear (y = a +bx) or nonlinear $(y = a + b/x^2)$ models were fit to knockdown and mortality responses over time for each species of laboratory strains by insecticide and rate using Table Curve 2D software (Jandel Scientific, San Rafael, CA, USA). Models were not fit to data when knockdown or mortality was 100% at all exposure times; for example this occurred with R. dominica mortality and with O. surinamensis knockdown at both rates of β-cyfluthrin. Linear or nonlinear models fit to data allowed for statistical comparison of knockdown and mortality responses for each insect species and insecticide, and for comparison of knockdown or mortality responses between insecticides, between the two β -cyfluthrin rates, and between species by insecticide and rate. These pair-wise comparisons involved comparing individual models fit to data²⁰ with a pooled model fit to data of the pairs being compared.²¹ Individual models were considered different from one another if the F-test showed the individual models deviated significantly ($P \le 0.05$) from the pooled model.

The mean knockdown and mortality of all *T. castaneum, O. surinamensis*, and *R. dominica* field strains in the control treatment at room conditions ranged from 0 to 2%, 0 to 7%, and 0 to 4%, respectively. So the knockdown and mortality data of field strains at room conditions were not corrected for these responses in the control treatment. At constant conditions, the mean mortality of *T. castaneum* in the control treatment was 0%, but that of *O. surinamensis* and *R. dominica* was 14% and 21%, respectively. Therefore, the mortality data of *O. surinamensis* and *R. dominica* at constant conditions were corrected for control mortality.²² The knockdown and mortality data of field strains at room and constant environmental conditions at established fixed times were analyzed by species after transformation to angular values²³ for normalizing heteroscedastic treatment variances. For each insecticide and species, knockdown or mortality data at room or constant conditions were analyzed by one-way analysis of variance (ANOVA), and Dunnett's procedure was used to determine if responses of each field strain differed from that of the corresponding laboratory strain.²⁰

In dose-response tests with β -cyfluthrin at constant conditions, knockdown and mortality responses of *T. castaneum* and *O. surinamensis* strains were not corrected for the corresponding control responses as the mean knockdown was 0% and mortality ranged from 0 to 8% for strains of both species. Knockdown or mortality data for each species and strain were subjected to one-way ANOVA, and the least squares means test was used to determine differences ($P \le 0.05$) among the four β -cyfluthrin rates.

3 RESULTS

3.1 Time-mortality responses of laboratory strains

Knockdown of T. castaneum adults during the 1 to 24 h exposures ranged from 20 to 100% at the low β -cyfluthrin rate and 43 to 100% at the high labeled rate (Fig. 1A). Mortality of T. castaneum adults at the low and high β-cyfluthrin rate increased linearly with time but never reached 100% (Fig. 1B). The increase in knockdown and mortality of T. castaneum adults with time when exposed to chlorpyrifos-methyl plus deltamethrin-treated concrete surfaces was nonlinear, and 100% knockdown and mortality were achieved at 4 h and 8 h, respectively. Knockdown of O. surinamensis adults was 100% at all times when exposed to both the low and high labeled rates of β-cyfluthrin (Fig. 1C). Adult mortality increased in a nonlinear fashion and failed to reach 100% at the low rate, and reached 100% only with the high rate at 24 h (Fig. 1D). Complete knockdown of O. surinamensis adults occurred at 8 h (Fig. 1C) and complete mortality at 4 h (Fig. 1D) on chlorpyrifos-methyl plus deltamethrin-treated concrete. Unlike T. castaneum and O. surinamensis, adults of R. dominica were extremely susceptible to β -cyfluthrin, and all adults died at low and high β -cyfluthrin rates irrespective of the exposure time (Fig. 1F). However, with chlorpyrifos-methyl plus deltamethrin, mean knockdown and mortality at all exposure times ranged from 17 to 100% (Fig. 1E) and 7 to 100% (Fig. 1F), respectively. Except for *R. dominica* adults exposed to β-cyfluthrin, adults of *T. castaneum* and *O. surinamensis* exposed to β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin recovered when placed on diets. The overall recovery ranged from 0 to 76%, and the degree of recovery did not follow any consistent trend as it varied with the species, exposure time, and insecticide.

Knockdown and mortality responses for each insect species-insecticide combination were satisfactorily described by linear or nonlinear models ($r^2 = 0.80 - 0.99$) (Fig. 1A-F), and the

model parameters are given in Table 2. Knockdown responses over time for *T. castaneum* exposed to β-cyfluthrin low and high rate were nonlinear, whereas mortality responses at each rate were linear. Therefore, for β-cyfluthrin, at each rate, statistical comparison between knockdown and mortality was not possible. Comparisons between knockdown and mortality at each β-cyfluthrin rate could not be made because of 100% mortality of *R. dominica* or 100% knockdown of *O. surinamensis* at all exposure times. Knockdown and mortality responses of *T. castaneum*, *O. surinamensis*, or *R. dominica* adults exposed to chlorpyrifos-methyl plus deltamethrin were not significantly different from one another (F, range among species = 0.94 - 3.30; df = 2, 12; F, range = 0.072 - 0.417).

β-cyfluthrin at the high rate caused significantly greater knockdown (P < 0.05) of T. castaneum adults than β-cyfluthrin low rate and chlorpyrifos-methyl plus deltamethrin (Table 3). Knockdown responses of R. dominica adults at β-cyfluthrin low and high rate were essentially similar (P > 0.05), but at each rate the knockdown responses were significantly greater (P < 0.05) than that of chlorpyrifos-methyl plus deltamethrin. The high rate of β-cyfluthrin caused significantly greater mortality of O. surinamensis adults when compared with the low rate.

Knockdown responses of R. dominica exposed to β -cyfluthrin at the low or high rate were greater (P < 0.05) than that of T. castaneum (Table 4). Knockdown responses of O. surinamensis exposed to chlorpyrifos-methyl plus deltamethrin were significantly greater (P < 0.05) when compared with that of T. castaneum or R. dominica. Similarly, mortality responses of O. surinamensis were significantly greater (P < 0.05) than that of R. dominica but not T. castaneum.

3.2 Responses of field strains at room conditions

The mean knockdown of all *T. castaneum* field strains exposed to the high rate of β -cyfluthrin-treated concrete ranged from 90 to 98%, and mean mortality ranged 16 to 67% (Fig. 2A) with recovery on diet ranging from 32 to 83%. One-way ANOVA by insecticide showed that knockdown among *T. castaneum* strains exposed to the high rate of β -cyfluthrin was not significant (F= 1.56; df = 16, 73; P = 0.101), but mortality differences among strains were significant (F= 2.14; df = 16, 73; P = 0.015). Although ANOVA showed mortality differences among strains, Dunnett's test²⁰ showed that the mortality of each field strain did not differ significantly (P > 0.05) from that of the laboratory strain.

The mean knockdown of five of the seven *O. surinamensis* field strains exposed to β -cyfluthrin was 100%, whereas it was 71% for AB1 and 76% for AB2 strain (Fig. 2B). The mean mortality for the five strains that showed 100% knockdown ranged from 86 to 100%, whereas it was 36 and 49% for AB1 and AB2 strains, respectively, indicating recovery when placed on diet. The seven strains of *O. surinamensis* exposed to β -cyfluthrin showed significant differences in knockdown (F= 17.71; df = 7, 32; P < 0.0001) and mortality (F= 13.98; df = 7, 31; P < 0.0001). Knockdown and mortality responses of AB1 and AB2 field strains differed significantly from that of the laboratory strain (P < 0.05; Dunnett's test).

β-cyfluthrin was extremely effective against *R. dominica* field strains with more than 98% knockdown and 100% mortality (Fig. 2C). Knockdown responses among strains exposed to the high rate of β-cyfluthrin were not significantly different (F= 1.00; df = 2, 14; P = 0.397).

The knockdown of 11 out of 16 *T. castaneum* field strains exposed to chlorpyrifos-methyl plus deltamethrin was greater than 90%, and only eight strains had mortality greater than 90% (Fig. 2D). Mortality was less than 50% in AB1 and KC field strains, and the overall recovery on

diet ranged from 0 to 50%. One-way ANOVA showed significant differences among field strains in knockdown (F= 4.60; df =16, 73; P < 0.0001) and mortality (F= 4.36; df = 16, 73; P < 0.0001). Knockdown response of AB1strain and mortality of AB1 and KC strains differed from that of the laboratory strain (P < 0.05; Dunnett's test).

The mean knockdown of seven *O. surinamensis* field strains exposed to chlorpyrifosmethyl plus deltamethrin ranged from 68 to 96%, and the mortality ranged from 38 to 98% with a recovery of 0 to 44% on diet (Fig. 2E). Field strains of *O. surinamensis* exposed to chlorpyrifos-methyl plus deltamethrin showed differences in knockdown (F= 2.67; df = 7, 32; P = 0.027) and mortality (F= 5.33; df = 7, 31; P = 0.0004). Knockdown of AB1 strain and mortality of AB1 and CF strains were significantly different from that of the laboratory strain (P < 0.05; Dunnett's test).

The two field strains of R. dominica showed reduced susceptibility to chlorpyrifos-methyl plus deltamethrin (Fig. 2F), because knockdown ranged from 84 to 90% and mortality from 7 to 22%. The recovery of the two field strains on diet ranged from 74 to 92%. One-way ANOVA showed knockdown (F= 17.31; df = 2, 12; P = 0.0003) and mortality (F= 21.31; df = 2, 12; P = 0.0001) responses of laboratory and two field strains (CF and RL) to be significantly different. Mortality responses of both the field strains differed significantly from that of the laboratory strain (P < 0.05; Dunnett's test).

3.3 Responses of field strains at constant conditions

β-cyfluthrin was less effective against *T. castaneum* field strains at constant conditions when compared with room conditions since knockdown was less than 90% in five of the 16 field strains and mortality was less than 51% in all strains including the laboratory strain (Fig.3A). In contrast, at room conditions knockdown of all strains was more than 90% and mortality of only

11 strains was less than 50%. There were significant differences among *T. castaneum* strains exposed to β -cyfluthrin in knockdown (F= 2.14; df = 16, 68; P = 0.016) and mortality (F= 2.26; df = 16, 68; P = 0.011).

The knockdown of six out of eight *O. surinamensis* field strains (one extra strain than those tested at room conditions) exposed to β -cyfluthrin was greater than 94% and for AB1 and AB2 strains it was 53 and 58%, respectively (Fig. 3B). Mortality among the eight field strains ranged from 5 to 82%, and the recovery on diet ranged from 18 to 90%. Knockdown (F = 27.38; df = 8, 36; P < 0.0001) and mortality (F = 15.80; df = 8, 35; P < 0.0001) responses among *O. surinamensis* strains exposed to β -cyfluthrin were highly significant. Knockdown of AB1 and AB2 strains and mortality of AB1, AB2 and MN strains were significantly different from that of the laboratory strain (P < 0.05; Dunnett's test).

 β -cyfluthrin was extremely effective against the two *R. dominica* field strains and the laboratory strain with 98 to 100% knockdown and 100% mortality (Fig. 3C); similar responses were observed under room conditions.

Chlorpyrifos-methyl plus deltamethrin was more effective against T. castaneum field strains at constant conditions than at room conditions with 94 to 100% knockdown. The mortality ranged from 90 to 100% among the strains (Fig.3D). There were significant differences (P < 0.05) among field strains of T. castaneum exposed to chlorpyrifos-methyl plus deltamethrin in knockdown (F = 1.82; df = 16, 68; P = 0.047) and mortality (F = 3.93; df = 16, 68; P < 0.0001). The knockdown and mortality of TP strain differed significantly from that of laboratory strain (P < 0.05; Dunnett's test).

The knockdown and mortality of the eight *O. surinamensis* field strains exposed to chlorpyrifos-methyl plus deltamethrin ranged from 77 to 92% and 67 to 98%, respectively (Fig.

3E). Knockdown (F = 2.41; df = 8, 36; P = 0.034) and mortality (F = 3.29; df = 8, 36; P = 0.007) responses were highly significant among the strains. Dunnett's test showed that none of the strains was different in both knockdown and mortality from that of the laboratory strain. Chlorpyrifos-methyl plus deltamethrin produced higher knockdown (98 to 99%) and mortality (38 to 40%) in the two R. dominica field strains at constant than room conditions (Fig. 3F). The three strains of R. dominica exposed to chlorpyrifos-methyl plus deltamethrin differed significantly in mortality (F = 17.10; df = 2, 12; P = 0.0003), but not in knockdown (F = 0.51; df = 2, 12; P = 0.614). Only mortality responses of both field strains were significantly different from that of the laboratory strain (P < 0.05; Dunnett's test).

3.4 Dose-response tests with β -cyfluthrin at constant conditions

Exposing the three least susceptible strains of *T. castaneum* (CF, PD1, and TP) to up to four times the high rate of β -cyfluthrin resulted in 96 to 100% knockdown and 54 to 90% mortality (Table 5). The knockdown and mortality of the corresponding laboratory strain at all rates of β -cyfluthrin ranged from 96 to 100% and 72 to 90%, respectively. Except for the mortality of TP strain which was different among the four β -cyfluthrin rates (F= 5.55; df = 3, 16; P = 0.008), the knockdown (F, range among strains = 0.76 - 2.67; df = 3, 16; P = 0.083 - 0.532) and mortality (F, range among strains = 0.91 - 2.11; df = 3, 16; P = 0.139 - 0.459) responses of all strains were similar among β -cyfluthrin rates. In the TP strain, there were no significant differences (P > 0.05) in mortality at 0.04 to 0.08 g (AI) m⁻² rates, but only mortality at rates of 0.04 and 0.08 g (AI) m⁻² was significantly greater (P < 0.05) than mortality at 0.02 g (AI) m⁻².

There was complete knockdown and mortality of the laboratory strain of O. surinamensis at all β -cyfluthrin rates (Table 5). The knockdown of field strains AB1 and AB2 among β -cyfluthrin rates was 71 to 100% while the mortality was 36 to 76%. Knockdown responses of

the strains differed significantly among β -cyfluthrin rates (F, range among strains = 3.87 - 4.63; df = 3, 16; P = 0.016 - 0.03), but mortality responses were not different among rates (F, range among strains = 1.22 - 2.62; df = 3, 16; P = 0.087 - 0.336). In strain AB1, knockdown at rates of 0.04 to 0.08 g (AI) m⁻² was similar (P > 0.05) and knockdown at rates of 0.04 and 0.08 g (AI) m⁻² was significantly greater (P < 0.05) than at 0.02 g (AI) m⁻². In strain AB2, knockdown at rates of 0.04, 0.06 and 0.08 g (AI) m⁻² was similar and significantly greater (P < 0.05) than at 0.02 g (AI) m⁻².

4 DISCUSSION

Adults of field strains of *T. castaneum* and *O. surinamensis* were generally less susceptible to chlorpyrifos-methyl plus deltamethrin and β-cyfluthrin. Field strains of *R. dominica* showed reduced susceptibility to chlorpyrifos-methyl plus deltamethrin but not to β-cyfluthrin. Variation in susceptibility of different insect species and strains to insecticides could be due to the bioassay technique used, natural tolerance, and/or resistance. The use of two different bioassay methods, filter paper and treated grain assays, against same strains of *O. surinamensis* revealed that the resistance ratios for pyrethroids from both assays were not correlated. The probit regression slopes were 1.2 - 3 times higher for grain assays indicating greater susceptibility than filter paper assays. Field strains of the granary weevil, *Sitophilus granarius* (L.), from different locations in the former Yugoslavia were 0.5 to 30 times less susceptible to the organophosphates dichlorvos, malathion, chlorpyrifos-methyl, and pirimiphos-methyl, and to the pyrethroids deltamethrin and cypermethrin than the laboratory strain based on discriminating-dose tests with treated filter papers. The susceptible is the papers of the granary strain based on discriminating-dose tests with treated filter papers.

Adults of four field strains of *O. surinamensis* collected from stored barley on Minnesota farms showed 8 to 40% mortality when exposed to chlorpyrifos-methyl at a discriminating dose of 0.09 mg/7 cm diameter filter paper disc (38.5 cm²) compared with a laboratory strain which showed 100% mortality even before the insecticide was registered for use on this commodity, indicating natural tolerance.¹⁷ The wild strains of *O. surinamensis* in Australia showed low resistance levels (<10-fold) to chlorpyrifos-methyl in treated filter-paper assays.²⁵ Strains of *R. dominica* from Brazil were found to be 2 to 874 times more resistant to deltamethrin than a susceptible laboratory strain.²⁶ Resistance to chlorpyrifos-methyl was detected in *R. dominica* strains collected from Brazil and Kansas, USA, with resistance ratios at the median lethal concentration (LC₅₀) ranging from 5.6 to 167.9.²⁷ A low level of resistance (1.2 to 1.8-fold) was observed to deltamethrin in 11 field strains of the maize weevil, *Sitophilus zeamais* Motschulsky, collected from nine states in Mexico.²⁸

In Australia, resistance to cyfluthrin has been reported in *O. surinamensis*²⁴ and *T. castaneum* ²⁹ using filter paper and grain assays. Resistance to cyfluthrin was also reported in other insect species such as the housefly, *Musca domestica* L.;³⁰ German cockroach, *Blattella germanica* (L.);^{31,32} beet armyworm, *Spodoptera exigua* (Hübner),³³ and lesser meal worm, *Alphitobius diaperinus* (Panzer).³⁴In the present study, *R. dominica* showed reduced susceptibility to chlorpyrifos-methyl plus deltamethrin. Some previous studies have reported the field strains of *R. dominica* to be resistant to chlorpyrifos-methyl^{27,35,36} and to deltamethrin²⁶ but not to the binary mixture. Chlorpyrifos-methyl at 3.0 mg(AI) kg⁻¹ of grain was effective against *T. castaneum* and *O. surinamensis* on stored wheat but not against *R. dominica*.³⁷

The results on insect susceptibility to the tested insecticides on concrete surfaces are at variance from that observed with the same insecticides on stored grain. For example, on stored

rice and wheat, chlorpyrifos-methyl plus deltamethrin was effective against the psocids *Lepinotus reticulatus* Enderlein, *Liposcelis entomophila* (Enderlein), *Liposcelis bostrychophila* Badonnel, and *Liposcelis paeta* Pearman.¹² It was also effective at the labeled rate against adults of *R. dominica*, *S. oryzae*, and *T. castaneum* on wheat and *R. dominica* and *S. oryzae* adults on short-grain and long-grain rices.¹³ The reduced susceptibility of adults of *T. castaneum* and *O. surinamensis* strains to chlorpyrifos-methyl plus deltamethrin and β-cyfluthrin on concrete surfaces as opposed to grain could be due to absorption or loss of the sprayed solution into the porous concrete. The concrete surface is also alkaline (pH ~10.5) and may have hydrolyzed the insecticide.³⁸ The persistence of cyfluthrin can be increased by sealing concrete with various commercial sealants.² An additional factor reducing the efficacy of insecticides on concrete may be uneven spray deposition during application, leading to areas with little or no insecticide deposit. Insects seeking such areas may not receive a lethal dose of the insecticide.

On grain, both contact and ingestion toxicity are important, whereas on concrete there is only contact toxicity. On grain, insects are typically exposed for 1 wk or more, but on concrete surfaces the maximum exposure time in our study was 24 h. Such short exposures may have been sublethal³⁹ and allowed insect recovery when placed on diets. The exposure of *R. dominica* adults for 24 h or less on wheat treated with an emulsifiable concentrate of cyfluthrin at 1, 2, and 4 mg(AI) kg⁻¹ gave less than 90% mortality.⁴⁰

The poor effectiveness of β -cyfluthrin against T. castaneum field strains at 0.02 to 0.08 g (AI) m⁻² rate in this study is in contrast to excellent control shown by cyfluthrin wettable powder at 0.04 g (AI) m⁻² against a laboratory strain of T. castaneum.³ The wettable powder formulation gave 90% mortality of T. castaneum adults when exposed for 0.5 to 4.0 h on deposits aged for 8 to 24 wk.³ Similar data on O. surinamensis with a wettable powder

formulation are not available for comparisons. β-cyfluthrin, an enriched isomeric form of the two biologically active diastereoisomeric pairs of isomers of cyfluthrin, should perform better or as well as the wettable powder formulation. For example, β-cyfluthrin showed high short-term efficacy with time to 95% mortality (LT₉₅) of 12 to 15 h against stored-product psocids, *L. bostrychophila* and *L. entomophila*, when applied at a low rate of 0.002 g (AI) m⁻² on concrete. Kaufman and Rutz⁴¹ reported the wettable powder formulation of cyfluthrin to be more toxic than the suspension concentrate formulation when applied to painted and unpainted plywood panels against *M. domestica* collected from dairies in the State of New York.

Except for *R. dominica* strains exposed to β-cyfluthrin, in our study percentage mortality of *T. castaneum* and *O. surinamensis* strains was generally lower than knockdown indicating recovery when placed on diets. Recovery of insects on food after a brief insecticide exposure may be due to absorption of insecticide from the insect's integument by the food particles, or an increase in insect's ability to detoxify the insecticide after removal from treated substrates. The time for 90% mortality of beetles (LT₉₀) placed on wheat flour for 1 wk after exposure to 0.02 g(AI) m⁻² of cyfluthrin wettable powder for 0.5 to 2.0 h was 195 min, whereas LT₉₀ for those without flour was 19 min. The presence of wheat flour on methoprene-treated concrete surfaces reduced the efficacy of methoprene against *T. castaneum* larvae (10 to 12 d old after eclosing from eggs). Therefore, sanitation of empty storage surfaces is very important to improve effectiveness of residual insecticides. The fact that there is recovery indicates that the insecticides did not exhibit any delayed effects. Delayed mortality effects have been reported in *R. dominica*^{43,44} but not in *S. oryzae* after short exposures to spinosad-treated wheat.

In the present study, β -cyfluthrin was found to be more effective at the room temperature of 24-25°C than at 28°C against *T. castaneum* and *O. surinamensis*. These strains and those of *R*.

dominica were more susceptible at 28°C than at 25°C when exposed to chlorpyrifos-methyl plus deltamethrin. In tests at 20, 25, 30, and 35°C, cyfluthrin toxicity was negatively correlated with temperature in tests with adults of T. castaneum, ¹⁰ T. confusum, the larger grain borer, Prostephanus truncatus (Horn) and larvae of P. interpunctella and the almond moth, Cadra cautella (Walker)⁴⁵. Cyfluthrin toxicity was unaffected by temperature in tests with R. dominica adults. 45 Organophosphate insecticides such as chlorpyrifos-methyl were more toxic at higher (25°C) than lower (17.5 and 10°C) temperatures against adults of O. surinamensis, ⁴⁶ T. castaneum, and S. granarius. 47 A positive correlation was found between temperature and effectiveness of organophosphate insecticides such as malathion, pirimiphos-methyl, and fenitrothion against adults of *T. confusum*. ⁴⁸ Deltamethrin displayed a negative temperature coefficient (more toxic at 15.6 than at 37.8°C) against the third instar larvae of cabbage looper, Trichoplusia ni (Hubner), and adults of the boll weevil, Anthonomus grandis grandis Boheman, but exhibited either a neutral or a positive temperature coefficient against third instar larvae of the fall armyworm, Spodoptera frugiperda (J. E. Smith), and tobacco budworm, Heliothis virescens (F.). 49,50 An overall negative temperature coefficient was observed for deltamethrin emulsifiable concentrate formulation when tested against 2nd instar nymphs of the grasshopper Melanoplus spp. at 15.6 to 37.8°C, with a slight positive temperature coefficient at 21.1 to 26.7°C.51 However, a neutral or positive temperature coefficient was reported with a flowable formulation of deltamethrin when tested at 15 to 31°C against *Melanoplus* spp. ⁵² Based on these studies we hypothesize that deltamethrin in the combination product may have exhibited either neutral or positive temperature coefficient.

Our results show that β -cyfluthrin is an ideal insecticide to use in clean, empty bin floors prior to storing wheat only to control R. *dominica* adults but not T. *castaneum* and O.

surinamensis strains. The reduced susceptibility of field and laboratory strains of the latter two species may be due to an inherent formulation deficiency or resistance, since four times the labeled rate failed to provide complete control. Chlorpyrifos-methyl plus deltamethrin was only partially effective against strains of all three species. There is documented evidence of resistance in field strains of these three species to one or both active ingredients. This is the first report that characterized susceptibility, or lack thereof, of field strains of three insect species from Kansas and other parts of the United States to two approved insecticides used for empty-bin treatments. According to surveys of wheat stored on-farm and elevators in Kansas, the most common insect species associated with stored wheat are R. dominica, S. oryzae, and T. castaneum. In addition, Oryzaephilus spp. and Cryptolestes spp. are also found in stored grain in Kansas. 53,54 Based on our results, no single insecticide can be recommended to provide adequate control of all species tested. More work is needed on the mechanism of detoxification of these chemicals by the three species to understand why some chemicals are effective against some species and strains and not against others. Evaluation of other recommended empty-bin insecticides with the field strains is also needed to identify a broad-spectrum insecticide that is effective against species commonly found in empty bins.

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Table 1. Sites and year of collection of adult *T. castaneum*, *O. surinamensis*, and *R. dominica* field strains

Species	Strain ID	County, State	Location	Commodity	Collection year
T. castaneum	AB1	Dickinson, KS	Abilene	Wheat	2011
	AB2	Dickinson, KS	Abilene	Wheat	2011
	AZ	Maricopa, AZ ^a	b	Flour mill	2009
	CF	Washington, KS	Clifton	Wheat	2011
	CN	McPherson, KS	Canton	Wheat	2011
	GH	Russell, KS	Gorham	Wheat	2011
	HN	Stafford, KS ^a		Flour mill	2001
	IL	Cook, IL ^c	Bridgeview	Rice facility	2011
	KC	Jackson, MO ^a		Flour mill	2005
	MN1	Ottawa, KS	Minneapolis	Wheat	2011
	MN2	Ottawa, KS	Minneapolis	Wheat	2011
	PD1	Russell, KS	Paradise	Wheat	2011
	PD2	Russell, KS	Paradise	Corn	2011
	PO	KS^a		Flour mill	2001
	SR	Dickinson, KS ^a		Flour mill	2001
	TP	Mitchell, KS	Tipton	Wheat	2011
O. surinamensis	AB1	Dickinson, KS	Abilene	Wheat	2011
	AB2	Dickinson, KS	Abilene	Wheat	2011
	CF	Washington, KS	Clifton	Wheat	2011
	CN	McPherson, KS	Canton	Wheat	2011

	MN1	Ottawa, KS	Minneapolis	Wheat	2011
	PD1	Russell, KS	Paradise	Wheat	2011
	PD2	Russell, KS	Paradise	Corn	2011
	TP	Mitchell, KS	Tipton	Wheat	2011
R. dominica	CF	Washington, KS	Clifton	Wheat	2011
	RL	Riley, KS ^a		Flour mill	2007

^aThese strains were collected prior to 2011 and were provided by Dr. James Campbell, USDA-ARS, Center for Grain and Animal Health Research, Manhattan, KS, USA.

^bExact city name is not disclosed at the request of the mill manager.

^cStrain collected by one of the authors (Bhadriraju Subramanyam) during a visit to a riceprocessing facility in 2011.

Table 2. Parameter estimates for regression models fit to knockdown and mortality data of the laboratory strains of three insect species exposed to insecticide deposits on concrete

Species	Insecticide	Response	n	Mean ± Sl	r^2	
				a	b	_
T. castaneum	β-cyfluthrin low rate	Knockdown	8	99.5 ± 1.1	-78.9 ± 3.0	0.99
		Mortality ^b	6	22.3 ± 7.8	3.2 ± 0.8	0.81
	β -cyfluthrin high rate	Knockdown	8	99.6 ± 1.7	-54.7 ± 4.6	0.96
		Mortality ^b	8	21.6 ± 5.1	2.4 ± 0.4	0.87
	Chlorpyrifos-methyl	Knockdown	8	102.8 ± 2.2	-86.4 ± 6.1	0.97
	plus deltamethrin	Mortality	8	99.6 ± 0.9	-84.1 ± 2.4	0.99
O. surinamensis	β-cyfluthrin low rate	Knockdown		c	_	
		Mortality	6	91.0 ± 2.4	-23.9 ± 5.9	0.80
	β-cyfluthrin high rate	Knockdown		_	_	
		Mortality	5	101.5 ± 1.7	-1655.8 ± 211.6	0.95
	Chlorpyrifos-methyl	Knockdown	8	100.0 ± 0.2	-39.6 ± 0.6	0.99
	plus deltamethrin	Mortality	8	98.2 ± 3.7	-56.8 ± 10.1	0.84
R. dominica	β -cyfluthrin low rate	Knockdown	8	100.4 ± 0.4	-9.9 ± 1.0	0.94
		Mortality		_	_	
	β-cyfluthrin high rate	Knockdown	8	100.4 ± 0.4	-9.6 ± 1.0	0.94
		Mortality		_	_	
	Chlorpyrifos-methyl	Knockdown	8	103.1 ± 2.6	-82.8 ± 7.1	0.96
	plus deltamethrin	Mortality	8	89.4 ± 5.8	-87.6 ± 15.9	0.83

^aAll regression ANOVA values were significant indicating that the slope (*b*) is not equal to zero (*F*, range among species, insecticides, and responses = 17.32 - 1232.66; df = 1, 6 except for *T*. *castaneum* and *O. surinamensis* mortality with β-cyfluthrin at low rate where df = 1, 4; and *O. surinamensis* mortality with β-cyfluthrin at high rate where df = 1, 3; $P \le 0.016$).

^bLinear equation y = a + bx was fit to the data; all other responses were fit to the nonlinear equation $y = a + b/x^2$.

^cKnockdown or mortality at all observation times was 100%.

Table 3. Comparison of knockdown or mortality responses of three insect species between insecticides and rates

Species	Response	Insecticides compared	<i>F</i> -value	df	<i>P</i> -value ^a
T. castaneum	Knockdown	β-cyfluthrin high rate vs β-cyfluthrin	12.16	2, 12	0.001
		low rate			
		β-cyfluthrin low rate vs Chlorpyrifos-	1.05	2, 12	0.380
		methyl plus deltamethrin			
		β-cyfluthrin high rate vs	8.81	2, 12	0.004
		Chlorpyrifos-methyl plus			
		deltamethrin			
	Mortality	β -cyfluthrin low rate vs β -cyfluthrin	1.52	2, 10	0.266
		high rate			
O. surinamensis	Mortality	β -cyfluthrin high rate vs β -cyfluthrin	9.18	2, 7	0.011
		low rate			
		β-cyfluthrin low rate vs Chlorpyrifos-	3.59	2, 10	0.067
		methyl plus deltamethrin			
		β-cyfluthrin high rate vs	3.77	2, 9	0.065
		Chlorpyrifos-methyl plus			
		deltamethrin			
R. dominica	Knockdown	β -cyfluthrin low rate vs β -cyfluthrin	0.03	2, 12	0.971
		high rate			
		β-cyfluthrin low rate vs Chlorpyrifos-	59.83	2, 12	< 0.001
		methyl plus deltamethrin			

β-cyfluthrin high rate vs	60.39	2, 12	<0.001
Chlorpyrifos-methyl plus			
deltamethrin			

^aIn cases where the P-values were significant (< 0.05), the insecticide listed first caused significantly greater knockdown and/or mortality than the other in the pair being compared. Some insecticide combinations were not compared because of 100% knockdown or mortality at all observation times.

Table 4. Comparison of knockdown or mortality responses between insect species by insecticides and rate

Insecticide	Response	Species compared	<i>F</i> -value	df	P-value ^a
Cyfluthrin low rate	Knockdown	R. dominica vs T. castaneum	307.95	2, 12	< 0.0001
Cyfluthrin high rate	Knockdown	R. dominica vs T. castaneum	60.44	2, 12	< 0.0001
Chlorpyrifos-methyl	Knockdown	T. castaneum vs R. dominica	0.11	2, 12	0.895
plus deltamethrin		O. surinamensis vs T. castaneum	31.90	2, 12	< 0.001
		O.surinamensis vs R. dominica	19.87	2, 12	0.0001
	Mortality	T. castaneum vs R. dominica	2.15	2, 12	0.159
		T. castaneum vs O. surinamensis	3.87	2, 12	0.050
		O. surinamensis vs R. dominica	3.91	2, 12	0.049

^aIn cases where the P-values were significant (< 0.05), the insect species listed first showed significantly greater knockdown and/or mortality than the other in the pair being compared.

Table 5. Knockdown and mortality of laboratory and select least susceptible field strains of *T. castaneum and O. surinamensis* exposed to concrete surfaces treated at or above the high labeled rate of β -cyfluthrin^{a,b}

Strain	Mean ± SE kno	Mean \pm SE knockdown (%) at β-cyfluthrin rate (g[AI]m ⁻²) of:			Mean \pm SE mortality (%) at β-cyfluthrin rate (g[AI]m ⁻²			(g[AI]m ⁻²) of:	
Strain	0.02	0.04	0.06	0.08	0.02	0.04	0.06	0.08	
T. casta	T. castaneum								
Lab.	96.0 ± 2.4^{c}	100	98.0 ± 2.0	98.0 ± 2.0	72.0 ± 10.7^{d}	74.0 ± 5.1	84.0 ± 10.3	90.0 ± 7.7	
CF	98.0 ± 2.0^{c}	100	100	100	54.5 ± 12.6^{d}	61.0 ± 10.5	55.1 ± 17.0	77.6 ± 12.4	
PD1	98.0 ± 2.0^{c}	100	100	100	66.0 ± 12.5^{d}	90.0 ± 7.7	72.0 ± 7.3	78.0 ± 4.9	
TP	96.0 ± 2.4^{c}	100	100	100	$54.0 \pm 9.3b$	$82.2 \pm 5.0a$	$74.7 \pm 7.1ab$	$90.0 \pm 3.2a$	
O. suri	namensis								
Lab.	100	100	100	100	100	100	100	100	
AB1	$71.3 \pm 3.9b$	$93.9 \pm 2.5a$	85.8 ± 6.8 ab	$92.0 \pm 3.7a$	58.7 ± 4.9^{e}	76.9 ± 9.3	57.1 ± 10.7	70.9 ± 9.9	
AB2	$80.0 \pm 8.4b$	$98.0 \pm 2.0a$	100.0a	$96.0 \pm 2.4a$	$36.0 \pm 10.3^{\rm e}$	71.4 ± 7.2	60.0 ± 10.5	70.2 ± 6.5	

^aEach mean is based on n = 5.

^bFor each strain and response (knockdown or mortality), means among rates followed by different letters are significantly different (*P* < 0.05; by least squares means test).

^cFor each *T. castaneum* strain, knockdown among rates was not significant (*F*, range among strains = 0.76 - 2.67; df = 3, 16; *P*, range = 0.083 - 0.532; one-way ANOVA).

^dFor *T. castaneum* Lab., CF, or PD1 strain, mortality among rates was not significant (F, range among strains = 0.91 - 2.11; df = 3, 16; P, range = 0.139 - 0.459; one-way ANOVA).

eFor *O. surinamensis* AB1 or AB2 strain, mortality among rates was not significant (F, range between strains = 1.22 - 2.62; df = 3, 16; P, range = 0.087 - 0.336; one-way ANOVA).

Figure captions

Figure 1. Mean \pm SE (n=3) observed and predicted adult knockdown and mortality of laboratory strains of three insect species as a function of time when exposed to β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin treated concrete surfaces at 24.3°C and 23.4 % r.h.

Figure 2. Mean \pm SE (n = 5) knockdown and mortality of adults of laboratory and field strains of three insect species exposed to β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin treated concrete surfaces at room conditions (25.4°C and 17.2% r.h.) For each species and response, means for a strain followed by an asterisk (*) is significantly different from the corresponding laboratory strain (P < 0.05; by Dunnett's test).

Figure 3. Mean \pm SE (n = 5) knockdown and mortality of adults of laboratory and field strains of three insect species exposed to β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin treated concrete surfaces at constant conditions (28°C and 65% r.h.). For each species and response, means for a strain followed by an asterisk (*) is significantly different from the corresponding laboratory strain (P < 0.05; by Dunnett's test).

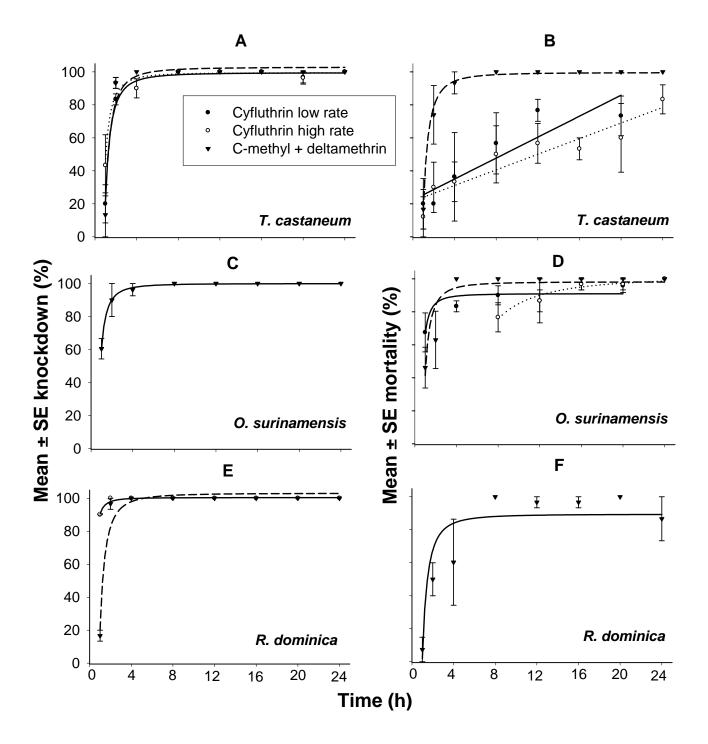


Figure 2

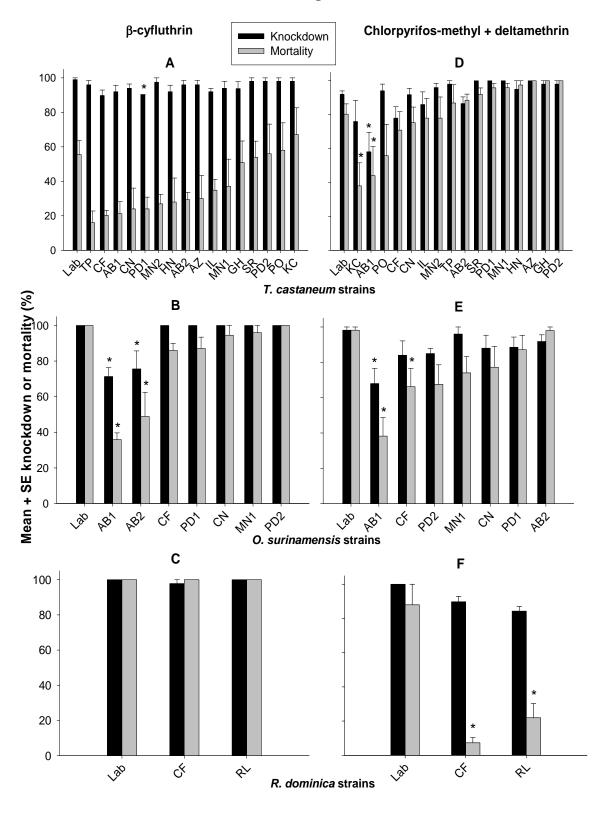


Figure 3

