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Subramanyam et al.: Partial grain treatment and spinosad efficacy

Efficacy of partial treatment of wheat with spinosad against *Rhyzopertha dominica* (F.) adults

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Abstract

The efficacy of partial treatment of wheat with spinosad against adults of the lesser grain borer, *Rhyzopertha dominica* (F.), was evaluated by mixing spinosad-treated and untreated wheat kernels in varying proportions. Spinosad was applied to wheat kernels either by dipping in 1 mg (a.i.) ml^{-1} spinosad solution for 1 minute or admixed with dry and liquid spinsoad formulations at 0.1 and the labeled rate of 1 mg (a.i.) kg^{-1} of wheat. In the kernel dipping method, the percentage of kernels treated was increased from 10 to 100 in 10% increments, while keeping the total number of kernels at either 10 or 100. The mortality of introduced adults in independent samples was observed over time at 1 to 209 h post-infestation. In the admixture method, the percentage of spinosad-treated wheat ranged from 10 to 100 in 10% increments by varying amounts of spinosad-treated and untreated wheat to form a total of 50 g. Mortality of introduced *R. dominica* adults was determined after 1, 3, 5, and 7 d. In the kernel dipping method, there was an inverse relationship between lethal times for 50 and 95% mortality of *R. dominica* adults and percentage of kernels treated. In the admixture method, adult mortality increased with an increase in spinosad rate, exposure time, and percentage of kernels treated. The liquid formulation was more effective against *R. dominica* than the dry formulation. At the labeled rate of 1 mg (a.i.) kg^{-1}, treating 20 to 90% of the kernels with liquid or dry formulation of spinosad was as good as treating 100% of the kernels in controlling *R. dominica* adults within 3 to 5 d. In practical situations where uneven distribution of spinosad on kernels is expected, complete control of *R. dominica* adults can be achieved if more than 50% of the kernels receive spinosad treatment.

Keywords: *Rhyzopertha dominica*; Spinosad; Partial grain treatment; Efficacy

1. Introduction

Spinosad is a commercial insecticide based on the fermentation products of a soil microorganism, *Saccharopolyspora spinosa* (Mertz and Yao) (Mertz and Yao, 1990). It was registered by the United States Environmental Protection Agency (US-EPA) in 2005 as a grain protectant at 1 mg (a.i.) kg^{-1} of grain (Hertlein et al., 2011). Spinosad persists for six months up to a year on stored wheat (Fang et al., 2002a; Flinn et al., 2004; Subramanyam et al., 2007), and is extremely effective against the adults of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), including strains resistant to organophosphates (OPs), pyrethroids, or juvenile hormone analogs (Nayak et al., 2005; Daglish and Nayak, 2006; Daglish et al., 2008).

A grain protectant is applied to uninfested grain as it is augured into a bin for long-term protection against insect infestation. During this process, uneven distribution of insecticide on kernels may occur, resulting in some kernels being treated completely while others only partially treated or left completely untreated. For instance, treatment of wheat with spinosad at 1 mg (a.i.) kg^{-1} of grain as it was augured into farm bins resulted in residue deposition of only 0.7 mg (a.i.) kg^{-1} immediately after treatment (Subramanyam et al., 2007). Even in carefully controlled laboratory conditions, treating wheat with spinosad at 0.1, 0.5, 1.0, 3.0, and 6.0 mg (a.i.) kg^{-1}, on an average, resulted in residue deposition of only 75% of the actual rates applied (Fang et al., 2002b). In 4.72 m diameter and 3.35 m high round steel bins holding 30.7 metric tons of 10.8% moisture wheat, Flinn et al. (2004) reported that spinosad deposition soon after treatment at 1 mg (a.i.) kg^{-1} ranged from 0.40 to 0.88 mg (a.i.) kg^{-1}. The actual spinosad residue deposition varied in samples taken near the bin periphery and bin center and in the top, middle, and bottom portions of the grain mass. Despite these variations, the efficacy of spinosad against *R. dominica* was unaffected, because adults of *R. dominica* generally are more susceptible to spinosad than adults of other stored-product insect species (Fang et al., 2002a, b; Flinn et al., 2004; Subramanyam et al., 2007).

The effect of partial treatment of wheat with malathion was evaluated by Minett and Williams (1971, 1976), and with *S*-methoprene by Daglish and Nayak (2010). In these studies, varying proportions (0.1-100%) of wheat kernels were treated with the insecticide at a higher rate and later mixed with the remaining untreated grain mass to get the same overall level of the insecticide for the entire grain mass. However, this method may have resulted in higher insecticide residues on individual kernels. The concept of treating only a portion of wheat kernels proportionately reduces the cost of grain treatment by reducing the amount of insecticide and labor required as well as total residues on kernels.

In the present investigation, a series of laboratory tests was carried out to determine the effect of partial treatment of wheat kernels with spinosad against *R. dominica* adults. Two bioassay methods of treating wheat kernels were used. One involved dipping wheat kernels in an aqueous solution of spinosad, and other involved directly treating kernels with dry and liquid spinosad formulations after which treated kernels were combined with untreated kernels in different proportions. Directly treating kernels with spinosad may still result in some kernels not receiving the insecticide, therefore, the dipping method was used to eliminate this effect. These experiments were conducted to determine time to death of *R. dominica* adults exposed to spinosad. Time to death is of interest because it is important to prevent oviposition before death in this species where the immature stages develop within kernels.

2. Materials and methods

2.1. Wheat, insect cultures, and spinosad formulations

Organic, hard red winter wheat (Heartland Mills, Marienthal, KS, USA) was cleaned manually by sieving it over a 2-mm round hole sieve (Seedburo Equipment Co., Chicago, IL, USA) to remove dockage and broken kernels. Cleaned wheat was frozen for one week at -13°C to kill any live insects present. Cultures of *R. dominica* were maintained on organic, hard red winter wheat of ~12% moisture in a growth chamber at 28°C and 65% relative humidity (r.h.) in the Department of Grain Science and Industry, Kansas State University, Manhattan, KS, USA. These insects have been in rearing since 1999. The liquid and dry formulations of spinosad (Bayer CropScience, Research Triangle Park, NC, USA) contained 8.66% (w/v) and 0.5% (w/w) a.i. of spinosad, respectively.

2.2. Wheat kernel treatment by dipping method

In the first experiment, the liquid spinosad formulation was diluted in distilled water to make a 1000 ml solution containing 1 mg (a.i.) ml^{-1} of spinosad. Four 500 g lots of wheat were dipped in the spinosad solution for one minute. Wheat kernels dipped similarly in distilled water served as controls. All dipped kernels were dried on paper towels, and held at 28°C and 65% r.h. for one week to equilibrate the moisture content. There were a total of 11 treatments. The control treatment consisted of gluing 10 water-treated kernels in a grid fashion in 9-cm diameter glass Petri dishes. The spinosad treatments included 1 to 10 treated kernels in 1 kernel increments with 9 to 0 water-treated kernels to give 10 to 100% of spinosad-treated kernels. Ten unsexed, two- to three-week-old adults of *R. dominica* were released in the center of each Petri dish. The dishes were covered with lids and held at room conditions (25°C and 46% r.h.). Each treatment dish was observed over time on 29 occasions, 1 to 105 h at 4 h increments (27 occasions) and at 173 and 209 h after adult introduction to count number of dead and live adults. Each spinosad treatment and observation time combination was replicated four times and separate dishes were used for each treatment combination.

In the second experiment, wheat kernels were treated with 1 mg (a.i.) ml^{-1} of spinosad as described above using 100 spinosad-treated and/or water-treated (control) kernels taken in different proportions in transparent plastic vials (2.5 cm diameter by 5.0 cm high) with a screened lid. The control treatment consisted of 100 water-treated kernels; the remaining 10 treatments included 10 to 100 spinosad-dipped kernels mixed with 90 to 0 water-treated kernels to give 10 to 100% of kernels treated with spinosad. After adding kernels, the vials were closed with lids and turned upside down five times to mix the kernels. In each vial, 10 unsexed two- to three-week-old *R. dominica* adults were released on the top of kernels after which the vials were closed with lids and held at room conditions. These vials were examined at 19 observation times starting at 1 h and ending at 73 h in 4 h increments. Independent vials were examined at each observation time. Each spinosad treatment and observation time combination was replicated three times.

2.3. Residue analysis

In the kernel dipping method, the deposition of spinosad residues on grain may exceed the maximum tolerance level of 1.5 mg kg^{-1} of grain, established by the US-EPA (Hertlein et al., 2011). Therefore, residue analysis of whole wheat kernels dipped in 1 mg (a.i.) ml^{-1} spinosad solution was conducted by Dow AgroSciences, Indianapolis, IN, USA, using methods described by Hastings and Clements (2000). Only one sample was submitted to Dow AgroSciences for residue analysis.

2.4. Wheat treatment with spinosad by admixing

The objective of this experiment was to determine the efficacy of spinosad against *R. dominica* adults when a portion of wheat kernels was treated with spinosad and then mixed with untreated kernels. Wheat kernels were treated with both dry and liquid formulations of spinosad at 0, 0.1 and 1.0 mg (a.i.) kg^{-1} of grain. The total quantity of wheat kernels required for all treatments in a replication (275 g) was treated together either by adding 275 µl of 1 mg (a.i.) ml^{-1} solution (liquid formulation) or 55 mg of dust (dry formulation) to obtain the target rate of 1.0 mg (a.i.) kg^{-1} of grain. Treated and untreated wheat kernels were mixed in different proportions in 150 ml plastic containers by increasing the quantity of treated wheat kernels in 5 g increments, keeping the total quantity of wheat at 50 g i.e. treated: untreated in 0:50, 5:45, 10:40,…45:5, and 50:0 g (equivalent to 0, 10, 20,…90, and 100 percent of kernels treated, respectively). The containers were closed with lids and turned upside down 10 times to mix wheat kernels in each container. Twenty five unsexed (two- to three-week-old) adults of *R. dominica* were introduced into each container and the containers were fitted with screen lids and placed in a growth chamber at 28°C and 65% r.h. Each combination of spinosad formulation, rate, and percentage kernels treated was replicated three times. The adult mortality was examined at 1, 3, 5, and 7 d after adult introduction on the same set of samples.

2.5. Statistical analyses

The treatment mortality data over time from whole kernel dipping method were corrected for respective control mortality data (Abbott, 1925). The corrected mortality data were subjected to probit regression analysis to estimate the lethal times required for 50% (LT_{50}) and 95% (LT_{95}) adult mortality and associated statistics (SAS Institute, 2008). Linear or nonlinear models were fit to the relationship between percentage of kernels treated and LT_{50} or LT_{95} values using Table Curve 2D software (Jandel Scientific, San Rafael, CA, USA).

Mortality data by day from experiments with spinosad by admixing method were corrected for respective control mortality. The corrected mortality data were transformed to angular values for normalizing variances (Zar, 1984). The data were subjected to a one-way analysis of variance (ANOVA) and means were separated using Bonferroni *t*-tests at $\alpha = 0.05$ (SAS Institute, 2008).

3. Results

3.1. Wheat treatment by dipping whole kernels in spinosad solution

Irrespective of the total number of kernels used, LT_{50} and LT_{95} values decreased as the percentage of kernels treated increased from 10 to 100. However, the magnitude of decrease was higher at LT_{95} than at LT_{50} when total number of kernels was 10 (Table 1), while it was more or less similar at both LT_{50} and LT_{95} when total number of kernels was 100 (Table 2). The Pearson goodness-of-fit Chi-square (χ^2) test showed that the probit model fit to data was significant ($P <$ 0.05) for 18 out of the 20 probit regressions shown in Tables 1 and 2, indicating poor fit of model to data. Fitting logit and complementary log-log models to data (Robertson and Priesler, 1992) also yielded similar results, suggesting that the responses of adults were heterogeneous. In cases where the *P*-value for the test is low, variances and covariances are adjusted by a heterogeneity factor (Chi-square value divided by the degrees of freedom (df)), and a critical value from the *t* distribution is used to compute the confidence limits (SAS Institute, 2008).

The relationship between percentage of kernels treated and LT_{50} or LT_{95} values was satisfactorily described by linear ($y = a + bx$) or nonlinear ($y = a + b/x$) models ($r^2 = 0.51$- 0.94)

(Fig. 1A-D), and the model parameters are given in Table 3. When number of kernels treated was 10, the LT_{50} and LT_{95} values decreased in a non-linear fashion with increase in percentage of kernels treated. However, when number of kernels treated was 100, the LT_{50} values decreased linearly and the LT_{95} values decreased in a non-linear fashion with increase in percentage of kernels treated.

3.2. Residue analysis

Dipping the whole wheat kernels in 1 mg (a.i.) ml^{-1} spinosad solution resulted in a deposit of 11.9 mg (a.i.) kg^{-1} of wheat. This is nearly eight times higher than the tolerance limit of 1.5 mg (a.i.) kg^{-1} on wheat.

3.3. Wheat treatment with spinosad by admixing

The adult mortality in control treatments (0% treated kernels) in tests with both liquid and dry spinosad formulations ranged from 0 to 2.7%. Irrespective of the spinosad formulation and rate used, mortality increased with increase in percentage of kernels treated and exposure time (Tables 4 and 5 and Fig 2A-D). There were significant differences in mortality due to percentage of kernels treated with liquid formulation of spinosad at 0.1 mg (a.i) kg^{-1} at 1 ($F_{9, 20} = 8.38$; $P < 0.0001$), 3 ($F_{9, 20} = 46.84$; $P < 0.0001$), 5 ($F_{9, 20} = 35.71$; $P < 0.0001$), and 7 ($F_{9, 20} = 36.30$; $P < 0.0001$) days after exposure. At 1 d after exposure, the maximum mortality was only 15%. At subsequent observations days, mortality increased several fold. At 3, 5, and 7 d after exposure, the cumulative mortality was ≥50% in treatments with 60, 40, and 30% of kernels treated, respectively (Table 4). Overall, treating ≥30% of kernels resulted in ≥50% mortality after 7 d and higher the percentage of kernels treated, lower the time required for 50% mortality. Treating ≥90% of kernels resulted in 100% adult mortality after 5 d of exposure (Fig. 2A).

At 1 mg (a.i.) kg^{-1}, the adult mortality in all the treatments was higher compared to a similar exposure at 0.1 mg (a.i.) kg^{-1} of grain (Table 4). Significant differences in mortality were observed due to percentage of kernels treated at 1 ($F_{9, 20}$ = 29.60; P < 0.0001), 3 ($F_{9, 20}$ = 16.44; P <0.0001), and 5 ($F_{9, 20}$ = 4.00; P = 0.0047) days after exposure. At 1 day after exposure, >50% adult mortality was obtained by treating 70% of kernels. At 3, 5, and 7 d after exposure even treating 10% of kernels resulted in ≥70, ≥97 and 100% mortality (Table 4). Overall, treating 20 to 60% of kernels resulted in >50 and 100% mortality after 3 and 5 d of exposure, respectively. Treating ≥70% of kernels resulted in >50% mortality after 1 d and 100% mortality after 3 d (Fig. 2B).

Except for the slightly lower mortality with dry spinosad compared to liquid spinosad, the general trend and degree of adult mortality with dry spinosad at 0.1 mg (a.i) kg^{-1} ($F_{9, 20}$ = 4.48-48.48; P < 0.0001- 0.0025) and at 1 mg (a.i) kg^{-1} ($F_{9, 20}$ = 5.89- 24.61; P < 0.0001- 0.0005) was similar to that obtained with liquid spinosad (Table 5). Additionally, the time required for 50 and 100% mortality was similar to that of liquid spinosad at the respective rates (Fig. 2C and D) except that a 2 d longer exposure was required for 100% mortality when 90% of kernels were treated at lower rate and ≥70% of kernels were treated at the higher rate (Fig. 2C and D).

4. Discussion

The present study evaluated the efficacy of spinosad against *R. dominica* adults when a portion of wheat kernels were treated either by dipping in spinosad solution or kernels were treated with dry and liquid spinosad formulations at the labeled rate and one-tenth the labeled rate and then admixed treated and untreated kernels in different proportions. In the kernel dipping method where there was uniform coating of spinosad of kernels, there was an inverse relationship between the percentage of kernels treated and lethal times for mortality of *R. dominica* adults, despite the fact that the responses of *R. dominica* adults over time at each percentage of treated kernels were heterogeneous. Heterogeneous responses of adults could be due to age-related or sex-related differences in susceptibility as unsexed adults of two- to three-weeks of age were used in the experiments. Additionally, extent and duration of contact with spinosad-treated kernels or ingestion of spinosad-treated kernels may have contributed to this unexplained heterogeneity, which was also influenced by the kernel density and percentage of kernels treated with spinosad. For example, the lethal times for 50% and 95% mortality of *R. dominica* adults among percentage of kernels treated generally were longer when10 kernels were used as opposed to 100 kernels. This can be attributed to the fact that there were 10 times more kernels with 100 than with 10 kernels. As a result *R. dominica* adults may be coming in frequent contact with treated kernels or feeding more on spinosad-treated kernels when 100 kernels were used. The fact that the lethal times for 50% or 95% mortality of *R. dominica* adults did not change substantially with an increase in percentage of kernels treated from 10 to 100 when 100 kernels were used further supports the above explanation. Carefully designed behavioral experiments may shed light on the unexplained heterogeneity observed in our study.

Kernel treatment by dipping in 1 mg (a.i.) ml^{-1} solution resulted in a spinosad residue deposit nearly eight times higher than the tolerance level of 1.5 mg (a.i) kg^{-1} of grain. In real world situations, uniform coating of kernels with insecticide is rare and uneven distribution of insecticide on kernels and uneven distribution of treated kernels in a grain lot are very common (Flinn et al., 2004; Subramanyam et al., 2007). When grain is treated with an insecticide as it is being augured into the storage bins, not all kernels are treated uniformly and some kernels are not treated at all, and this may result in percentage of treated and untreated kernels to range anywhere between 10 and 100. In a laboratory study, treating only 1% of wheat kernels with a high rate of malathion (1000 mg (a.i.) kg^{-1}) and mixing them with untreated grain mass to give a final rate of 10 mg (a.i.) kg^{-1} of grain resulted in effective control of the rice weevil, *Sitophilus oryzae* (L.), confused flour beetle, *Tribolium confusum* Jacqueline du Val, and *R. dominica* adults and suppression of progeny production for 70 to 100 days. These results were comparable to treating all grains at 10 mg (a.i.) kg^{-1} (Minett and Williams, 1971). Therefore, the next set of experiments were focused on treating the kernels with spinosad dry and liquid formulations at the labeled rate and one-tenth the labeled rate and mixing the treated and untreated kernels in different proportions to simulate variation that could occur under actual practical field applications. The results showed that the liquid formulation of spinosad exhibited a slightly higher activity against *R. dominica* adults than the dry formulation at a given rate. This observation was confirmed in our earlier evaluations on wheat with spinosad (Getchell and Subramanyam, 2008; Subramanyam et al., 2012). There was a positive relationship between percentage of kernels treated or exposure time and adult mortality. At the labeled rate treating only 10% of kernels resulted in near complete to complete mortality of *R. dominica* adults in 5 to 7 d after exposure. However, treating a higher percentage (>10%) of kernels reduced the exposure time for near complete to complete adult mortality to 3 to 5 d.

The near complete to complete adult mortality with only 10% of kernels treated may be attributed to the greater susceptibility of *R. dominica* adults to spinosad and likely transfer of spinosad residues from treated to untreated kernels while mixing. A similar hypothesis for transfer of residues from treated to untreated wheat kernels was proposed by Daglish and Nayak (2010) where treating 2% of wheat kernels with *S*-methoprene to achieve an overall rate of 0.6 mg (a.i.) kg^{-1} of grain. Exposing *R. dominica* adults to this rate for 14 d caused a significant reduction in progeny production. Moreover, a brief exposure to spinosad residues is sufficient to cause mortality of *R. dominica* adults which is typically manifested as delayed toxicity, even after removal of adults from treated grain (Getchell and Subramanyam, 2008; Athanassiou et al., 2010; Boina et al., 2012). Athanassiou et al. (2009) reported that 83% of *R. dominica* adults were dead in 14 d when they were released on surface of wheat kernels in a plastic vial in which only top 1/8th layer was treated with spinosad. This was due to brief exposure of adults to spinosad residues during their downward movement through the thin treated layer (2-3 wheat kernel-deep layer). In our study, *R. dominica* adults were added on the surface of grain in the plastic containers and by virtue of their vertical downward movement (Surtees, 1964; Vardeman et al., 2007a, b), adults were able to pick up sufficient amount of spinosad required for death even in treatments where only 10% of kernels were treated.

Our results show that with uneven distribution of insecticide resulting from treating only a few (10%) kernels, complete mortality of *R. dominica* adults could still be achieved with a longer exposure time (7 d). However, adults surviving until day 7 in grains with uneven distribution of insecticide could still mate and lay eggs as well as continue further infestation and grain damage (Athanassiou et al., 2010). The results of the present study indicated that treating all wheat kernels in a grain mass at a rate 10 times lower than labeled (0.1 mg kg^{-1}) or treating <50% of kernels at labeled rate results in survival of adults for a longer period of time than treating all kernels at labeled rate. This may increase the chances of further infestations and kernel damage. Daglish and Nayak (2010) also reported that treating a portion of wheat kernels with *S*-methoprene at a lower rate of 0.03 mg (a.i.) kg^{-1} than the recommended of 0.6 mg (a.i.) kg^{-1}, resulted in survival of adults and subsequent progeny production.

Finally, it can be concluded that treating 20 to 90% of kernels at the labeled rate of 1 mg (a.i.) kg^{-1} is as good as treating 100% of kernels in controlling *R. dominica* adults to reduce cost of grain treatment. However, this compromises the intended quick mortality of adults and may lead to some progeny production and kernel damage from adults surviving until 7 d after treatment. In practical situations where uneven distribution of spinosad on kernels is expected, complete control of *R*. *dominica* adults can be achieved if more than 50% of the kernels receive spinosad treatment, which is more likely to prevent progeny production, subsequent infestation and kernel damage from surviving adults.

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Table 1

Probit regression estimates for *R. dominica* adults exposed to wheat with varying percentage of kernels treated by dipping in liquid spinosad formulation at 1 mg (a.i.) ml^{-1} (total number of kernels = 10).

Percentage of kernels treated	Intercept ± SE	Slope ± SE	Lethal time (LT) in hours		χ^2 (df)[a]	*P*-value
			LT_{50} (95% CL)	LT_{95} (95% CL)		
10	-6.50 ± 0.28	3.57 ± 0.16	66.11 (63.30-69.06)	190.94 (173.30-214.02)	38.08 (28)	0.097
20	-7.00 ± 0.38	4.14 ± 0.22	49.26 (46.63-51.86)	123.06 (112.59-136.95)	61.36 (28)	0.0003
30	-5.60 ± 0.34	3.35 ± 0.19	47.11 (43.90-50.30)	145.98 (129.70-168.83)	72.78 (28)	<0.0001
40	-6.47 ± 0.65	4.09 ± 0.38	38.19 (33.79-42.35)	96.39 (83.58-117.04)	208.35 (28)	<0.0001
50	-7.61 ± 0.46	4.97 ± 0.28	33.85 (31.72-35.89)	72.51 (67.27-79.26)	71.41 (28)	<0.0001
60	-6.21 ± 0.24	3.99 ± 0.14	35.92 (34.46-37.35)	92.71 (87.66-98.68)	19.42 (28)	0.885
70	-7.64 ± 0.37	4.99 ± 0.23	34.06 (32.40-35.66)	72.76 (68.37-78.17)	45.64 (28)	0.019
80	-7.49 ± 0.37	5.03 ± 0.23	30.82 (29.26-32.33)	65.47 (61.59-70.21)	44.27 (28)	0.026
90	-6.82 ± 0.35	4.70 ± 0.22	28.37 (26.80-29.89)	63.56 (59.57-68.47)	44.43 (28)	0.025

100	-8.38 ± 0.44	5.78 ± 0.29	28.21 (26.80-29.57)	54.33 (51.19-58.21)	45.44 (28)	0.020

[a]Chi-square value for goodness-of-fit of probit model to data.

Table 2

Probit regression estimates for *R. dominica* adults exposed to wheat with varying percentage of kernels treated by dipping in liquid spinosad formulation at 1 mg (a.i.) ml^{-1} (total number of kernels = 100).

Percentage of kernels treated	Intercept ± SE	Slope ± SE	Lethal time (LT) in hours		χ^2 (df)[a]	*P*-value
			LT_{50} (95% CL)	LT_{95} (95% CL)		
10	-9.93 ± 0.85	6.46 ± 0.53	34.50 (32.17-36.76)	62.03 (56.55-70.14)	67.04 (17)	<0.0001
20	-11.22 ± 0.70	7.39 ± 0.45	33.00 (31.54-34.42)	55.11 (51.86-59.35)	32.51 (17)	0.013
30	-10.60 ± 0.97	6.87 ± 0.61	34.97 (32.60-37.23)	60.70 (55.43-68.65)	71.47 (17)	<0.0001
40	-11.97 ± 0.76	7.84 ± 0.48	33.64 (32.18-35.05)	54.53 (51.40-58.63)	33.77 (17)	0.009
50	-12.34 ± 1.15	8.09 ± 0.73	33.60 (31.47-35.62)	53.67 (49.53-59.81)	71.04 (17)	<0.0001
60	-10.29 ± 0.84	6.85 ± 0.54	31.79 (29.79-33.71)	55.26 (50.87-61.53)	57.14 (17)	<0.0001
70	-10.67 ± 0.89	7.10 ± 0.57	31.94 (29.93-33.87)	54.47 (50.19-60.62)	59.67 (17)	<0.0001
80	-12.05 ± 0.99	8.12 ± 0.65	30.54 (28.86-32.17)	48.70 (45.28-53.56)	51.84 (17)	<0.0001
90	-9.66 ± 0.68	6.55 ± 0.44	29.87 (28.17-31.51)	53.27 (49.43-58.48)	42.36 (17)	0.0006

100	-9.20 ± 0.66	6.21 ± 0.42	30.29 (28.49-32.01)	55.73 (51.55-61.47)	43.39 (17)	0.0004

[a]Chi-square value for goodness-of-fit of probit model to data.

Table 3

Parameter estimates of the regression models describing the relationship between percentage of kernels treated with spinosad by kernel dipping method and the times for 50% (LT_{50}) and 95% (LT_{95}) mortality of *R. dominica* adults.

Number of kernels treated	LT value	Mean ± SE for parameter[a]		r^2
		a	*b*	
10	LT_{50}	27.06 ± 1.46	414.11 ± 37.12	0.9395
	LT_{95}	55.48 ± 8.56	1443.77 ± 217.38	0.8464
100	LT_{50}	35.37 ± 0.56	-0.05 ± 0.01	0.8186
	LT_{95}	52.53 ± 1.32	96.08 ± 33.59	0.5055

[a]Ten observations were used when fitting regression models to LT_{50} and LT_{95} versus percentage of kernels treated data.

Table 4

Mortality of *R. dominica* adults on wheat with varying percentage of kernels treated with two rates of spinosad liquid formulation at different exposure times.

Rate (mg a.i. kg^{-1})	Percentage of kernels treated	Mean ± SE mortality at exposure time in days[a]			
		1	3	5	7
0.1	10	0 ± 0d	5.3 ± 3.5f	10.7 ± 5.8e	14.7 ± 6.7e
	20	1.3 ± 1.3cd	5.3 ± 1.3f	10.7 ± 3.5e	18.7 ± 1.3de
	30	1.3 ± 1.3cd	12.0 ± 4.0ef	26.7 ± 7.1de	56.0 ± 6.9cd
	40	2. 7 ± 1.3bcd	32.0 ± 2.3de	53.3 ± 13.5cd	70.7 ± 11.4bc
	50	2. 7 ± 1.3bcd	46.7 ± 2.7cd	69.3 ± 7.1bc	89.3 ± 3.5abc
	60	4.0 ± 0.0abcd	65.3 ± 2.7bc	88.0 ± 4.0abc	94.7 ± 3.5ab
	70	6.7 ± 1.3abc	57.3 ± 7.1cd	84.0 ± 4.6abc	97.3 ± 2.7a
	80	9.3 ± 1.3ab	88.0 ± 4.0ab	94.7 ± 1.3ab	98.7 ± 1.3a
	90	10.7 ± 1.3ab	90.7 ± 2.7ab	100 ± 0a	100 ± 0a
	100	14.7 ± 1.3a	93.3 ± 3.5a	100 ± 0a	100 ± 0a
1	10	10.7 ± 1.3e	72.0 ± 2.3d	97.3 ± 1.3b	100 ± 0
	20	14.7 ± 2.7de	77.3 ± 1.3cd	100 ± 0a	100 ± 0
	30	20.0 ± 2.3de	86.7 ± 1.3bcd	100 ± 0a	100 ± 0
	40	25.3 ± 3.5de	89.3 ± 4.8bcd	100 ± 0a	100 ± 0
	50	29.3 ± 2.7de	93.3 ± 3.5abc	100 ± 0a	100 ± 0
	60	36.0 ± 2.3cd	94.7 ± 2.7ab	100 ± 0a	100 ± 0
	70	57.3 ± 9.3bc	100 ± 0a	100 ± 0a	100 ± 0

80	66.7 ± 9.3ab	100 ± 0a	100 ± 0a	100 ± 0
90	73.3 ± 3.5ab	100 ± 0a	100 ± 0a	100 ± 0
100	84.0 ± 2.3a	100 ± 0a	100 ± 0a	100 ± 0

[a]For each spinosad rate and exposure time, means followed by different letters are significantly different ($P < 0.05$; by Bonferroni t-tests).

Table 5

Mortality of *R. dominica* adults on wheat with varying percentage of kernels treated with two rates of spinosad dry formulation at different exposure times.

Rate (mg a.i. kg^{-1})	Percentage of kernels treated	Mean ± SE mortality at exposure time in days[a]			
		1	3	5	7
0.1	10	1.3 ± 1.3b	2.7 ± 1.3e	6.7 ± 1.3e	13.3 ± 3.5e
	20	5.3 ± 1.3ab	6.7 ± 1.3e	14.7 ± 3.5de	24.0 ± 2.3de
	30	4.0 ± 2.3ab	9.3 ± 2.7e	20.0 ± 4.6de	46.7 ± 7.4cd
	40	8.0 ± 2.3ab	14.7 ± 3.5de	42.7 ± 4.8cd	72.0 ± 6.1bc
	50	10.7 ± 1.3ab	34.7 ± 4.8cd	62.7 ± 7.1c	85.3 ± 7.1ab
	60	5.3 ± 1.3ab	45.3 ± 7.4c	74.7 ± 8.1bc	93.3 ± 3.5ab
	70	14.7 ± 3.5a	57.3 ± 4.8bc	93.3 ± 4.8ab	98.7 ± 1.3a
	80	12.0 ± 2.3ab	84.0 ± 2.3ab	94.7 ± 3.5ab	97.3 ± 2.7a
	90	18.7 ± 7.1a	85.3 ± 5.3a	96.0 ± 0.0ab	100 ± 0a
	100	13.3 ± 1.3a	85.3 ± 4.8a	100 ± 0a	100 ± 0a
1	10	2.7 ± 2.7e	62.7 ± 8.1c	93.33 ± 2.7b	100 ± 0
	20	16.0 ± 2.3de	77.3 ± 3.5bc	100 ± 0a	100 ± 0
	30	14.7 ± 2.7de	93.3 ± 3.5ab	100 ± 0a	100 ± 0
	40	22.7 ± 3.5cde	90.7 ± 3.5abc	100 ± 0a	100 ± 0
	50	24.0 ± 2.3cd	89.3 ± 1.3abc	100 ± 0a	100 ± 0
	60	37.3 ± 1.3bcd	92.0 ± 4.6ab	100 ± 0a	100 ± 0
	70	58.7 ±13.9abc	94.7 ± 1.3ab	100 ± 0a	100 ± 0

	80	64.0 ± 10.6ab	96.0 ± 2.3ab	100 ± 0a	100 ± 0
	90	70.7 ± 3.5ab	98.7 ± 1.3a	100 ± 0a	100 ± 0
	100	81.3 ± 6.7a	98.7 ± 1.3a	100 ± 0a	100 ± 0

[a]For each spinosad rate and exposure time, means followed by different letters are significantly different ($P < 0.05$; by Bonferroni *t*-tests).

Figure Captions

Fig. 1. Relationship between the percentage of kernels treated with spinosad by kernel dipping method and the times for 50% (LT_{50}) and 95% (LT_{95}) mortality of *R. dominica* adults.

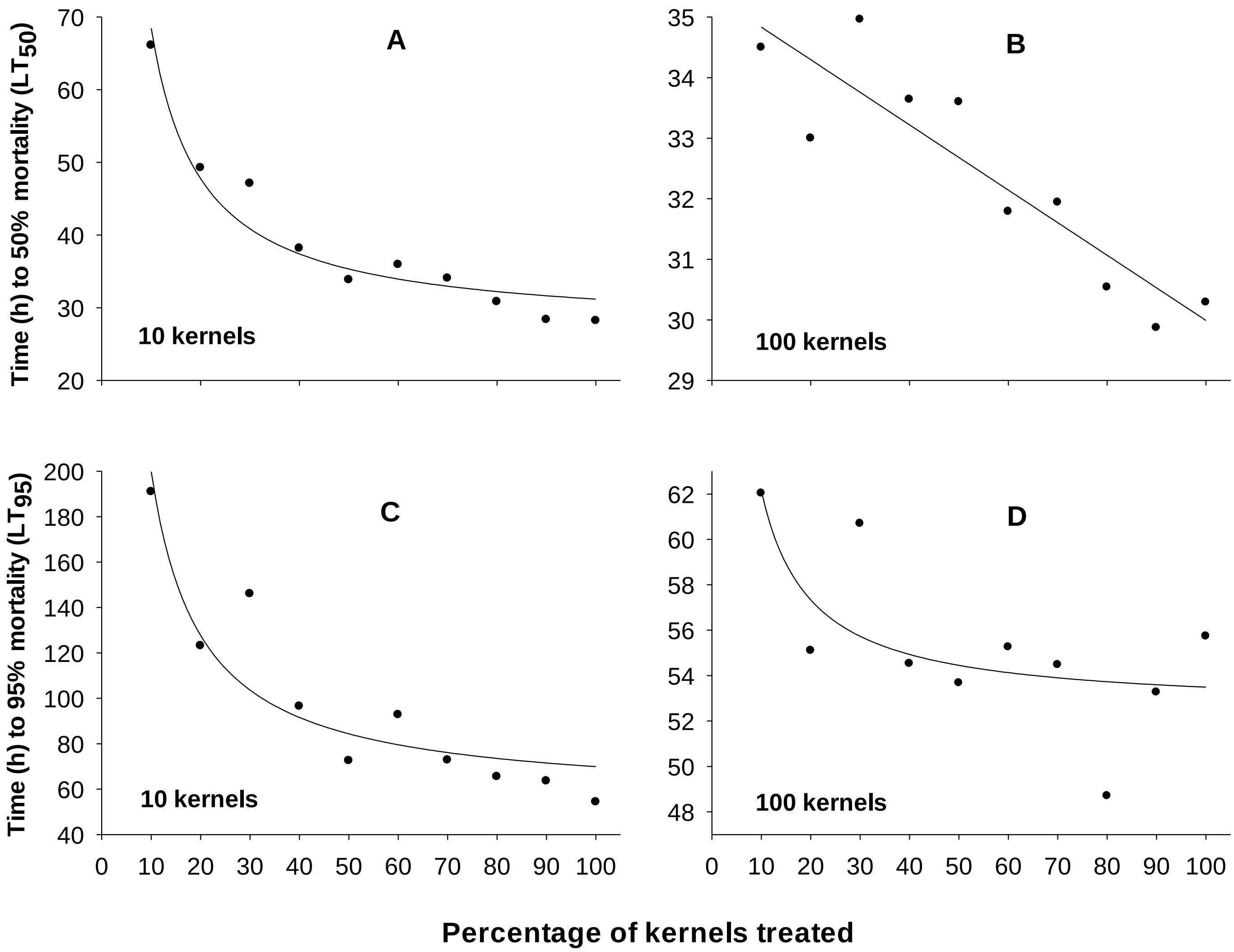
Fig. 2. Relationship between the percentage of kernels treated with two spinosad formulations by admixture method and time for >50% and <100% mortality or 100% mortality of *R. dominica* adults.

Figure Captions

Fig. 1. Relationship between the percentage of kernels treated with spinosad by kernel dipping method and the times for 50% (LT_{50}) and 95% (LT_{95}) mortality of *R. dominica* adults. The *y*-axis scale is different among the four graphs.

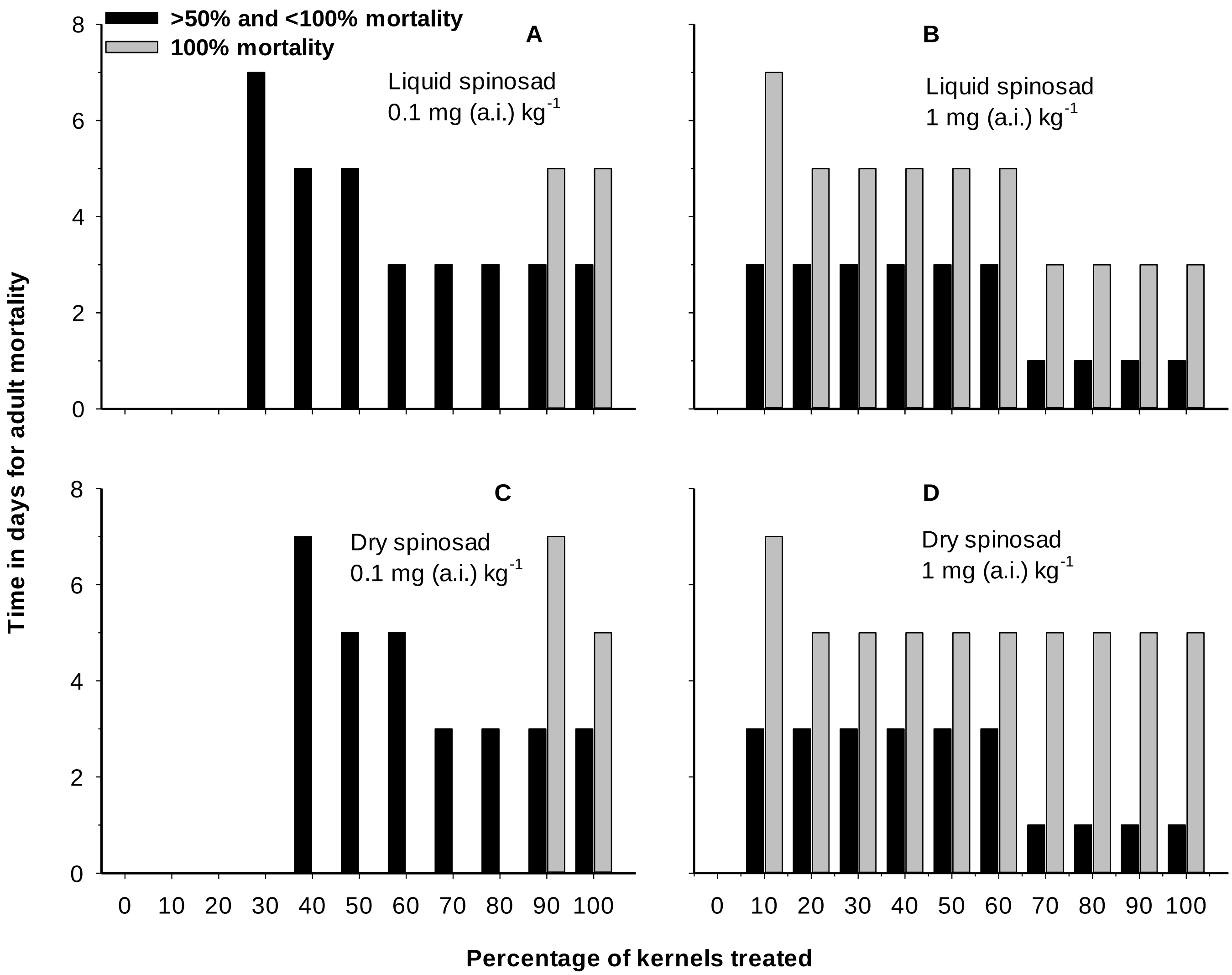
Fig. 2. Relationship between the percentage of kernels treated with two spinosad formulations by admixture method and time for >50% and <100% mortality or 100% mortality of *R. dominica* adults.

Fig. 1



A
B
C
D
10 kernels
100 kernels
Time (h) to 50% mortality (LT_{50})
Time (h) to 95% mortality (LT_{95})
Percentage of kernels treated

Fig. 2


>50% and <100% mortality
100% mortality
A
Liquid spinosad
0.1 mg (a.i.) kg-1
B
Liquid spinosad
1 mg (a.i.) kg-1
C
Dry spinosad
0.1 mg (a.i.) kg-1
D
Dry spinosad
1 mg (a.i.) kg-1
Time in days for adult mortality
Percentage of kernels treated