Research Article

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Toxicities and effects of insecticidal toxic baits to control *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae)

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Abstract

BACKGROUND: *Drosophila suzukii* is a primary insect pest that causes direct damage to fruits with a thin epidermis such as strawberries, cherries and blueberries. In strawberry fields, the co-occurrence of *D. suzukii* and *Zaprionus indianus* has increased production losses. This study evaluated the toxicities and effects of insecticidal baits to control adults and larvae of both *D. suzukii* and *Z. indianus*.

RESULTS: Organophosphate (dimethoate and malathion), spinosyn (spinosad and spinetoram), pyrethroid (lambda-cyhalothrin) and diamide (cyantraniliprole) insecticides exhibited high toxicity to both adults and larvae of *D. suzukii* and *Z. indianus* (mortality >80%) in topical and dip bioassays. However, when the insecticides were mixed with a feeding attractant, a positive effect was observed only for adults of *D. suzukii*. Insecticides containing neonicotinoids (acetamiprid and thiamethoxam) and pyrolle (chlorfenapyr) caused intermediate mortality to adults of *D. suzukii* (40–60%) and low mortality for *Z. indianus* (mortality <23%); however, these compounds reduced the larval infestation of the two species by 55–86%. Botanical (azadirachtin) and sulphur insecticides exhibited low toxicity (mortality <40%) on adults and larvae of both species.

CONCLUSION: Dimethoate, malathion, spinosad, spinetoram, lambda-cyhalothrin and cyantraniliprole are highly toxic to both larvae and adults of *D. suzukii* and *Z. indianus*. The use of toxic baits for adults of *D. suzukii* could be an alternative in management of this species.

Keywords: spotted-wing drosophila; fig fly; chemical control; strawberry; toxic bait; pest control

1 INTRODUCTION

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae), which originates from north-east Asia,[1,2] has recently been detected in Brazil, where it is now considered to be one of the major insect pests of strawberries.[3,4] The detection of *D. suzukii* in South America is consistent with a world outbreak of this species in recent years and follows its invasions of both North America and Europe.[2,5,6] This pest causes particularly high production losses because its larvae feed directly on fruit tissue, making the fruit unmarketable. Hence, as has been observed in the northern hemisphere, it causes economic damage to the host country.[5,6] In the 2014–2015 crop, economic losses in infested Brazilian strawberry fields increased even further after the co-occurrence of *D. suzukii* and *Zaprionus indianus* Gupta (Diptera: Drosophilidae) was observed.[4]

Z. indianus, which originates from Africa, is currently distributed throughout most of the world,[7] and has recently been detected in association with *D. suzukii* infestations.[4,8–12] In Brazil's 2014–2015 strawberry crop, high populations of *Z. indianus* were found in the fields.[4] It has also been found in *D. suzukii* monitoring traps containing apple vinegar[8,10] and in hydrolysed protein Cera Traps™ (Bioibérica, Barcelona, Spain).[12] *Z. indianus* is considered to be an opportunistic insect that is able to attack decaying or mechanically damaged fruit.[9] However, in laboratory bioassays, when tested both with and without alternative choices, *Z. indianus* is able to infest undamaged ripe strawberry fruit. Moreover, a significant increase in *Z. indianus* infestations occurs when the fruit has been previously damaged by *D. suzukii* females.[4]

To avoid economic losses and rapid dispersion of both species, the use of insecticides is still one of the main alternatives available to growers. For *D. suzukii*, organophosphates, pyrethroids and spinosyns have been effective controls when applied by foliar spraying.[13–15] A possible alternative to foliar spraying is the use of toxic baits or low-volume, reduced-risk sprays in conjunction with feeding attractants.[16] For strawberry plants, these techniques are potentially useful mainly during the preharvesting period of the fruit to reduce the incidence of *D. suzukii*[6,17] and *Z. indianus*.[4] The potential use of toxic baits to manage *D. suzukii* became more evident after a commercial formulation of a feeding attractant,

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Suzukii Trap™ (Bioibérica), became available. This formulation is a feeding attractant composed of organic acids and attractant peptides specifically designed to attract *D. suzukii*. The objective of this study was to evaluate the toxicity of various insecticides on adults and larvae of *D. suzukii* and *Z. indianus*, as well as to evaluate the performance of these insecticides on adults in the laboratory when mixed with Suzukii Trap feeding attractant to form toxic baits.

2 MATERIALS AND METHODS

The populations of *D. suzukii* and *Z. indianus* used in the bioassays were obtained from a laboratory population (maintained at a temperature of 25 ± 2 °C, RH 70 ± 5% and a photophase of 12 h) fed on artificial diet as proposed by Emiljanowicz *et al.*[18] and Nava *et al.*[19] The assessed insecticides at various concentrations (see Table 1) were diluted in plastic containers (1 L) either with distilled water or by being mixed with Suzukii Trap feeding attractant. These diluted mixtures were used for topical, dipping and ingestion bioassays respectively. All bioassays were performed in a laboratory under controlled conditions (25 ± 1 °C; RH 65 ± 10% and a photophase of 12 h).

2.1 Insecticide toxicity in a topical application bioassay on adults of *D. suzukii* and *Z. indianus*

Five-day-old adults of *D. suzukii* or *Z. indianus* were separated into groups of ten insects (five females and five males) in glass test tubes (2.5 diameter × 8.0 cm length). The tubes were sealed at the top with PVC plastic wrap. At spraying, adults were sedated with CO_2 and placed on a petri dish to be sprayed using a Potter Precision Laboratory Spray Tower (Burkard Scientific, Uxbridge, UK). A quantity of 1 mL of insecticide solution was applied per insect group at each concentration (see Table 1) at a working pressure of 0.70 kg cm^{-2}, resulting in an average residue deposition of 3.0 mg cm^{-2}. Afterwards, the treated insects were placed in cages made of transparent plastic cups (300 mL) flipped upside down on a petri dish (8 cm diameter) with a venting hole (4 cm diameter) at the top covered with *voile* fabric to prevent escape. The treated *D. suzukii* and *Z. indianus* adults were fed with artificial diet and received distilled water on a cotton roll in 10 mL glass bottles throughout the assessment period. The experimental design was completely randomised, with 12 treatments and ten repetitions per treatment. Mortality in each treatment was assessed at 2 h intervals for the first 24 h after exposure to treatments (HAET) and every 24 h thereafter from 24 to 96 HAET. Insects that showed no reaction at the touch of a fine-tipped brush were considered to be dead. Corrected mortality was calculated using the equation of Henderson and Tilton.[20]

2.2 Toxicity of toxic baits on adults of *D. suzukii* and *Z. indianus*

Five-day-old adults of *D. suzukii* and *Z. indianus* were deprived of food for a period of 12 h before the bioassays but provided with water during this period. Next, ten adults (five females and five males) were placed in cages made of transparent plastic cups (300 mL), as described earlier. In each cage the insects were offered two drops (20 µL) of toxic bait formulated from the tested insecticides (commercial product) + Suzukii Trap placed on a small piece of acrylic film (1 cm^2) for 2 h at each concentration (see Table 1). Then, the toxic baits were replaced with artificial diet and distilled water, as described above. The experimental design was completely randomised, with 12 treatments and ten repetitions per treatment for *D. suzukii* and five repetitions per treatment for *Z. indianus*. Adult mortality under each treatment was assessed at 2 h intervals during the first 24 HAET and every 24 h thereafter from 24 to 96 HAET. Insects that showed no reaction at the touch of a fine-tipped brush were considered to be dead. Corrected mortality was calculated using the equation of Henderson and Tilton.[20]

2.3 Insecticide toxicity in a dipping bioassay on larvae of *D. suzukii* and *Z. indianus*

In the laboratory, four ripe strawberry 'Albion' fruits without prior application of insecticides were placed in plastic containers (500 mL) containing ten adults (five females and five males) of *D. suzukii* or *Z. indianus* for a period of 24 h. The containers were sealed on top with Parafilm™ (Bemis Company, Inc., Neenah, WI). Afterwards, the adults were removed and the strawberry fruits were kept for 3 days (the period required for egg hatching and early larval development) in their respective containers in an air-conditioned room. Subsequently, the fruits were immersed in various insecticide solutions (treatments) at each concentration (see Table 1) for 5 s and kept on sheets of filter paper for 3 h to eliminate excess moisture. Then, the fruits were placed in plastic cups (500 mL) on a vermiculite layer (1 cm), sealed on top with Parafilm and stored in an air-conditioned room. The assessment for the presence of dead and living larvae in the fruit was carried out 48 h after immersion (HAI) by macerating the fruit in glass containers with a 10% saline solution. The experimental design was completely randomised, with 12 treatments and ten repetitions per treatment; each repetition used four ripe strawberry fruits.

2.4 Statistical analyses

All data were submitted to studentised residual analysis to confirm the assumption of normality using the Shapiro–Wilk test with the PROC UNIVARIATE procedure in SAS 9.1.[21] The resulting percentage data were submitted to arcsine square root transformation prior to analysis using the SAS function ARSIN (SQRT(x));[21] however, untransformed data and standard errors of the means are presented in the tables and figures. After arcsine square root transformation, the data met the assumption of normality required for ANOVA tests. Then, all data were subjected to analyses using the PROC GLM procedure in SAS 9.1.[21] Treatment differences were determined using least-square means statements (LSMEANS) at a $P = 0.05$ level of significance in SAS 9.1.[21]

3 RESULTS

3.1 Insecticide toxicity in the topical application bioassay

The *D. suzukii* adults were highly susceptible (approximately 100% mortality) to the dimethoate, malathion, lambda-cyhalothrin and spinetoram insecticides during the first 24 HAET (Table 2). These four insecticides differed statistically ($F_{11,107} = 2.11$; $P < 0.0001$) from the other insecticides evaluated. For *Z. indianus*, only dimethoate, malathion and lambda-cyhalothrin were highly toxic to the adult flies (100% mortality) (Table 3), and these differed significantly ($F_{11,96} = 3.23$; $P < 0.0008$) from spinetoram. Over the 96 HAET period, only the spinosad insecticide showed a significant increase in mortality; this increase occurred for both adults of *D. suzukii* (Table 2) and adults of *Z. indianus* (Table 3). At 96 HAET, a greater toxicity was observed for dimethoate, malathion, spinetoram, spinosad and lambda-cyhalothrin insecticides for both *D. suzukii* (Table 2) ($F_{11,107} = 45.26$; $P < 0.0001$) and *Z. indianus*

Table 1. Insecticides evaluated for the management of *D. suzukii* and *Z. indianus* in topical, toxic bait ingestion or dipping bioassays

Active ingredient	Trade name	Chemical classes	Concentration[a]	
			Active ingredient	Commercial product
Acetamiprid	Mospilan™	Neonicotinoid	8	40
Azadirachtin	Azamax™	Tetranotriterpenoid	1.2	100
Chlorfenapyr	Pirate™	Pyrroles	24	100
Cyantraniliprole	Benevia™	Diamide	10	100
Dimethoate	Dimexion™ 400EC	Organophosphates	40	100
Sulphur	Kumulus	Inorganic	240	300
Lambda-cyhalothrin	Karate Zeon™ 50 CS	Pyrethroid	2.5	50
Malathion	Malation™ 1000EC	Organophosphates	200	200
Spinetoram	Delegate™ 250WG	Spinosyns	5	20
Spinosad	Tracer™	Spinosyns	9.6	20
Thiamethoxam	Actara™ 250WG	Neonicotinoid	2.5	10

[a] Concentration in g or mL active ingredient 100 L^{-1} water.

(Table 3) ($F_{11,107} = 94.69$; $P < 0.0001$) (mortality >85%). The other insecticides (including acetamiprid, thiamethoxam, chlorfenapyr and azadirachtin-based insecticide) and sulphur-based fungicide caused medium to low toxicity (mortality between 26 and 50%) for *D. suzukii* adults (Table 2) ($F_{11,107} = 45.26$; $P < 0.0001$) and low toxicity (mortality between 5 and 23%) for *Z. indianus* adults (Table 3) ($F_{11,107} = 94.69$; $P < 0.0001$).

3.2 Insecticide toxicity in the toxic bait ingestion bioassay

In the ingestion bioassay of toxic baits (composed of a mixture of insecticides with Suzukii Trap feeding attractant), adults of *D. suzukii* showed high susceptibility (mortality >85%) to the dimethoate, lambda-cyhalothrin and spinosad insecticides over 24 HAET. This result differed statistically ($F_{11,107} = 30.41$; $P < 0.0001$) from the other tested insecticides (Table 4). After 96 HAET, dimethoate, lambda-cyhalothrin, spinetoram and spinosad insecticides mixed with feeding attractant provided the greatest toxicity on adults of *D. suzukii* (mortality >80%), which differed statistically ($F_{11,107} = 25.67$; $P < 0.0001$) from the other tested insecticides (Table 4). For *Z. indianus*, the ingestion bioassay exhibited low toxicity on adults for all treatments evaluated over time. The largest toxic effect ($F_{11,49} = 5.10$; $P < 0.0001$) occurred with spinosad (mortality 55.5%) at 96 HAET (Table 5).

3.3 Insecticide toxicity in the dipping bioassay

In the dipping bioassay, using strawberry fruit previously infested with larvae of *D. suzukii* or *Z. indianus*, dimethoate, malathion, cyantraniliprole, chlorfenapyr, lambda-cyhalothrin, spinetoram and spinosad insecticides presented greater toxicity on *D. suzukii* larvae (mortality between 85 and 100%) ($F_{11,108} = 34.83$; $P < 0.0001$) (Fig. 1) and *Z. indianus* (mortality between 75 and 100%) ($F_{11,108} = 17.92$; $P < 0.0001$) (Fig. 2) than the other tested insecticides. Lower toxic effects on larvae were observed for the azadirachtin-based insecticide (mortality <40%) and sulphur-based fungicide (mortality <20%) for both species studied (Figs 1 and 2 respectively).

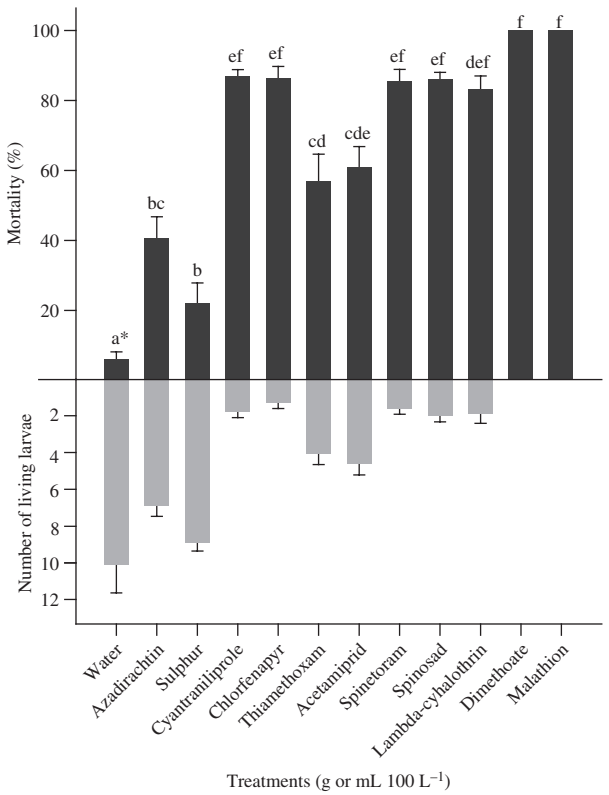

Figure 1. Larval mortality and mean number of living larvae of *D. suzukii* in the dipping bioassay with strawberry 'Albion' in the laboratory. * Bars (± SE) with the same letter are not significantly different (LSMEANS with Tukey's adjustment; $P > 0.05$).

4 DISCUSSION

The tested insecticides based on organophosphates, pyrethroids and spinosyns showed high toxicity on both *D. suzukii* and *Z. indianus* adults and larvae in laboratory bioassays. Similar results have been reported for these three chemical groups in both laboratory and field bioassays aimed at management of *D. suzukii*,[13,22–24] but little is known about *Z. indianus* control. According to the authors of those studies, products that showed high toxicity in laboratory bioassays were also effective in the field. The tested insecticides based on cyantraniliprole, acetamiprid and thiamethoxam showed intermediate toxicity on *D. suzukii* and *Z. indianus* adults compared with those based on organophosphates, pyrethroids and spinosyns. Similar results have been observed on *D. suzukii*

Table 2. Average numbers of live *D. suzukii* adults ($N \pm$ SE) and mortality percentages (M) at 0, 24, 48, 72 and 96 h HAET in the topical application bioassay in the laboratory

	Concentration[a]		0 HAET	24 HAET		48 HAET		72 HAET		96 HAET	
Active ingredient	AI[b]	CP[c]	$N \pm$ SE[d]	$N \pm$ SE[d]	M[e]	$N \pm$ SE[d]	M[e]	$N \pm$ SE[d]	M[e]	$N \pm$ SE[d]	M[e]
Acetamiprid	8	40	8.6 ± 0.5 ABa	5.5 ± 0.6 ABCb	34.5	5.3 ± 0.6 ABb	33.8	4.2 ± 0.5 Bb	39.3	3.8 ± 0.4 BCb	44.3
Azadirachtin	1.2	100	8.8 ± 0.3 ABa	5.6 ± 0.8 ABCb	33.1	5.6 ± 0.8 ABb	33.1	5.6 ± 0.8 ABb	33.1	5.6 ± 0.8 ABb	33.1
Chlorfenapyr	24	100	8.9 ± 0.4 ABa	4.3 ± 0.7 DCb	49.0	4.3 ± 0.0 BCb	49.0	4.2 ± 0.7 BCb	50.0	4.2 ± 0.3 BCb	50.1
Cyantraniliprole	10	100	9.2 ± 0.6 Aa	2.7 ± 0.5 Db	70.0	2.5 ± 0.5 CDb	70.8	1.9 ± 0.5 Cb	74.3	1.2 ± 0.3 DEb	83.5
Dimethoate	40	100	9.3 ± 0.4 Aa	0.0 ± 0.0 Eb	100	0.0 ± 0.0 Eb	100	0.0 ± 0.0 Db	100	0.0 ± 0.0 Fb	100
Lambda-cyhalothrin	2.5	50	9.1 ± 0.4 Aa	0.0 ± 0.0 Eb	100	0.0 ± 0.0 Eb	100	0.0 ± 0.0 Db	100	0.0 ± 0.0 Fb	100
Malathion	200	200	8.9 ± 0.4 ABa	0.0 ± 0.0 Eb	100	0.0 ± 0.0 Eb	100	0.0 ± 0.0 Db	100	0.0 ± 0.0 Fb	100
Spinetoram	5	20	8.5 ± 0.3 ABa	0.2 ± 0.1 Eb	97.5	0.1 ± 0.1 Eb	98.7	0.1 ± 0.1 Db	98.7	0.1 ± 0.1 Fb	98.7
Spinosad	9.6	20	9.4 ± 0.4 A	2.7 ± 0.5 Da	70.6	1.0 ± 0.3 DEb	88.5	0.3 ± 0.1 Dc	95.8	0.3 ± 0.1 EFc	95.8
Sulphur	240	300	8.9 ± 0.4 ABa	7.5 ± 0.5 ABab	13.7	5.8 ± 0.7 ABb	30.0	5.2 ± 0.7 ABb	27.3	5.2 ± 0.7 ABb	26.3
Thiamethoxam	2.5	10	6.9 ± 0.5 Ba	5.1 ± 1.0 BCDab	24.3	4.5 ± 1.1 BCab	29.5	3.8 ± 1.0 BCb	31.5	3.2 ± 0.8 CDb	41.5
Control (water)	–	–	8.3 ± 0.5 ABa	7.9 ± 0.5 Aa	–	7.9 ± 0.5 Aa	–	7.9 ± 0.5Aa	–	7.9 ± 0.5Aa	–

[a] Concentration in g or mL AI 100 L^{-1} water.
[b] AI = active ingredient.
[c] CP = commercial product.
[d] Mean number of living flies ± SE. Means followed by the same upper-case letter in the columns and by the same lower-case letter in the rows do not differ significantly (LSMEANS with Tukey's adjustment; $P > 0.05$).
[e] Mortality corrected by Henderson and Tilton's formula.

Table 3. Average numbers of live *Z. indianus* adults ($N \pm$ SE) and mortality percentages (M) at 0, 24, 48, 72 and 96 HAET in the topical application bioassay in the laboratory

	Concentration[a]		0 HAET	24 HAET		48 HAET		72 HAET		96 HAET	
Active ingredient	AI[b]	CP[c]	$N \pm$ SE[d]	$N \pm$ SE[d]	M[e]	$N \pm$ SE[d]	M[e]	$N \pm$ SE[d]	M[e]	$N \pm$ SE[d]	M[e]
Acetamiprid	8	40	9.2 ± 0.3 ABa	9.0 ± 0.3 Aa	2.2	9.0 ± 0.3 Aa	0.9	8.8 ± 0.3 Aa	3.2	8.7 ± 0.4 Aa	4.2
Azadirachtin	1.2	100	8.6 ± 0.6 ABa	7.9 ± 0.8 ABa	8.1	7.8 ± 0.8 Aa	8.1	7.8 ± 0.8 Aa	8.1	7.8 ± 0.8 Aa	8.1
Chlorfenapyr	24	100	9.4 ± 0.3 ABa	7.5 ± 0.5 ABab	20.2	7.3 ± 0.5 Ab	21.4	7.1 ± 0.4 Ab	23.5	7.1 ± 0.4 Ab	23.5
Cyantraniliprole	10	100	9.7 ± 0.3 ABa	4.0 ± 0.5 Cb	58.7	3.5 ± 0.5 Bb	63.4	3.3 ± 0.5 Bb	65.6	3.2 ± 0.5 Bb	67.0
Dimethoate	40	100	10.0 ± 0.0 Aa	0.0 ± 0.0 Db	100	0.0 ± 0.0 Db	100	0.0 ± 0.0 Db	100	0.0 ± 0.0 Db	100
Lambda-cyhalothrin	2.5	50	10.0 ± 0.0 Aa	0.0 ± 0.0 Db	100	0.0 ± 0.0 Db	100	0.0 ± 0.0 Db	100	0.0 ± 0.0 Db	100
Malathion	200	200	10.0 ± 0.0 Aa	0.0 ± 0.0 Db	100	0.0 ± 0.0 Db	100	0.0 ± 0.0 Db	100	0.0 ± 0.0 Db	100
Spinetoram	5	20	9.8 ± 0.1 ABa	5.5 ± 0.7 BCb	43.8	1.9 ± 0.3 BCc	80.4	1.4 ± 0.3 Cc	85.5	1.4 ± 0.3 Cc	85.5
Spinosad	9.6	20	9.7 ± 0.2 ABa	4.0 ± 0.8 Cb	58.7	2.0 ± 0.6 Cbc	79.1	1.4 ± 0.5 Cc	85.4	1.4 ± 0.5 Cc	85.3
Sulphur	240	300	9.6 ± 0.2 ABa	9.4 ± 0.3 Aa	2.0	9.4 ± 0.3 Aa	0.8	9.3 ± 0.3 Aa	1.9	9.3 ± 0.3 Aa	1.9
Thiamethoxam	2.5	10	8.4 ± 0.4 ABa	8.3 ± 0.4 Aa	1.2	8.1 ± 0.4 Aa	2.3	7.9 ± 0.5 Aa	4.79	7.9 ± 0.5 Aa	5.0
Control (water)	–	–	8.2 ± 0.5 Ba	8.2 ± 0.5 Aa	–	8.1 ± 0.6 Aa	–	8.1 ± 0.6 Aa	–	8.1 ± 0.6 Aa	–

[a] Concentration in g or mL AI 100 L^{-1} water.
[b] AI: active ingredient.
[c] CP: commercial product.
[d] Mean number of living flies ± SE. Means followed by the same upper-case letter in the columns and by the same lower-case letter in the rows do not differ significantly (LSMEANS with Tukey's adjustment; $P > 0.05$).
[e] Mortality corrected by Henderson and Tilton's formula.

adults in both laboratory and field bioassays.[13,22] However, this intermediate effect should be considered, because studies have shown high systemic activity of these products after application in cherry and blueberry fruit on *Rhagoletis indifferens* Curran (Diptera: Tephritidae).[25] These products directly influenced adult behaviour (reduced mobility),[13] and ingestion caused sublethal effects on larvae and adults,[26] which can contribute to population suppression over time.[25]

A major concern with insecticide applications over an entire area of a strawberry field for drosophilid control is the risk of contamination from chemical residues in fruit. This risk is highest during the preharvesting or ripening periods, when the likelihood of infestations by *D. suzukii*[27–29] and *Z. indianus* is greater.[4] Therefore, the use of toxic baits can be a viable alternative for managing *D. suzukii*, as has been demonstrated by Van Steenwyk *et al.*,[16] as well as for *Z. indianus*. The potential field effectiveness of toxic baits is evident because insecticides containing organophosphates, pyrethroids and spinosyns were highly toxic to *D. suzukii* adults in the ingestion bioassay when mixed with a feeding attractant, leveraging the use of these insecticides in formulations as toxic baits. Because the feeding attractant used in this study is specifically formulated to attract *D. suzukii* adults, it did not exhibit attractiveness to

Table 4. Average numbers of live *D. suzukii* adults ($N \pm$ SE) and mortality percentages (M) at 0, 24, 48, 72 and 96 h HAET in the toxic bait ingestion bioassay in the laboratory

	Concentration[a]		0 HAET	24 HAET		48 HAET		72 HAET		96 HAET	
Active ingredient	AI[b]	CP[c]	$N \pm$ SE[d]	$N \pm$ SE[d]	M[e]	$N \pm$ SE[d]	M[e]	$N \pm$ SE[d]	M[e]	N ± SE[d]	M[e]
Acetamiprid	8	40	8.1 ± 0.5 ABa	3.6 ± 0.4 BCb	51.5	3.6 ± 0.4 BCDb	51.5	3.5 ± 0.4 BCDb	51.3	2.7 ± 0.4 BCb	58.9
Azadirachtin	1.2	100	8.2 ± 0.8 ABa	7.2 ± 0.9 Aa	4.3	7.0 ± 0.9 ABa	6.9	6.5 ± 0.8 ABa	10.2	5.5 ± 0.7 ABa	17.3
Chlorfenapyr	24	100	9.2 ± 0.3 Aa	6.8 ± 0.4 ABab	19.4	6.5 ± 0.5 ABCab	23.0	6.2 ± 0.5 ABCb	23.6	4.8 ± 0.7 ABb	35.7
Cyantraniliprole	10	100	8.4 ± 0.5 ABa	3.0 ± 0.5 Cb	61.0	3.0 ± 0.5 Db	61.0	3.0 ± 0.5 Db	59.5	2.7 ± 0.5 BCb	60.4
Dimethoate	40	100	6.4 ± 0.6 Ba	0.0 ± 0.0 Eb	100	0.0 ± 0.0 Fb	100	0.0 ± 0.0 Gb	100	0.0 ± 0.0 Eb	100
Lambda-cyhalothrin	2.5	50	8.8 ± 0.5 ABa	1.0 ± 0.2 DEb	87.6	0.9 ± 0.3 EFb	88.8	0.7 ± 0.3 EFb	90.9	0.5 ± 0.2 DEb	93.6
Malathion	200	200	6.4 ± 0.5 Ba	2.0 ± 0.4 CDb	65.9	1.8 ± 0.5 DEb	69.3	1.7 ± 0.4 DEFb	69.9	1.7 ± 0.4 CDb	67.2
Spinetoram	5	20	9.4 ± 0.2 Aa	2.6 ± 0.6 Cb	69.9	2.1 ± 0.5 Db	75.6	1.8 ± 0.5 DEb	78.3	1.4 ± 0.4 CDb	81.6
Spinosad	9.6	20	7.9 ± 0.9 Aa	0.6 ± 0.3 DEb	91.7	0.6 ± 0.3 EFb	91.7	0.6 ± 0.3 FGb	91.3	0.3 ± 0.2 DEb	95.3
Sulphur	240	300	9.2 ± 0.3 Aa	7.7 ± 0.5 Aa	8.8	7.5 ± 0.5 Aab	11.2	7.2 ± 0.6 Aab	11.3	5.6 ± 0.6 ABb	25.0
Thiamethoxam	2.5	10	8.8 ± 0.4 ABa	3.5 ± 0.4 Cb	56.6	3.4 ± 0.5 CDb	57.9	3.2 ± 0.5 CDb	58.8	2.9 ± 0.6 BCb	59.4
Control (water)	–	–	8.5 ± 0.3 AB	7.8 ± 0.5 Aa	–	7.8 ± 0.4 Aa	–	7.5 ± 0.5 Aa	–	6.9 ± 0.4 Aa	–

[a] Concentration in g or mL AI 100 L^{-1} water.
[b] AI: active ingredient.
[c] CP: commercial product
[d] Mean number of living flies ± SE. Means followed by the same upper-case letter in the columns and by the same lower-case letter in the rows do not differ significantly (LSMEANS with Tukey's adjustment; $P > 0.05$).
[e] Mortality corrected by Henderson and Tilton's formula.

Table 5. Average numbers of live *Z. indianus* adults ($N \pm$ SE) and mortality percentages (M) at 0, 24, 48, 72 and 96 h HAET in the toxic bait ingestion bioassay in the laboratory

	Concentration[a]		0 HAET	24 HAET		48 HAET		72 HAET		96 HAET	
Active ingredient	AI[b]	CP[c]	$N \pm$ SE[d]	$N \pm$ SE[d]	M[e]	$N \pm$ SE[d]	M[e]	$N \pm$ SE[d]	M[e]	$N \pm$ SE[d]	M[e]
Acetamiprid	8	40	8.6 ± 0.8 ABa	7.8 ± 0.9 ABCa	9.3	7.8 ± 0.9 ABa	21.7	7.8 ± 0.9 ABa	26.8	7.6 ± 0.8 ABa	28.7
Azadirachtin	1.2	100	9.4 ± 0.2 ABa	8.6 ± 0.5 ABa	8.5	8.6 ± 0.5 Aa	21.0	8.2 ± 0.6 Aa	17.7	8.0 ± 0.7 ABa	19.7
Chlorfenapyr	24	100	9.8 ± 0.2 AABa	9.8 ± 0.2 Aa	0.0	9.8 ± 0.2 Aa	13.7	9.6 ± 0.2 Aa	11.8	9.6 ± 0.2 Aa	11.8
Cyantraniliprole	10	100	8.8 ± 0.4 ABa	7.4 ± 0.5 ABCa	15.9	7.4 ± 0.5 ABa	27.4	7.4 ± 0.5 ABa	34.1	7.4 ± 0.5 ABa	34.1
Dimethoate	40	100	8.4 ± 0.4 ABa	6.6 ± 1.1 BCa	21.4	6.6 ± 1.1 ABa	32.2	6.6 ± 1.1 ABa	43.0	6.6 ± 1.1 ABa	43.0
Lambda-cyhalothrin	2.5	50	7.4 ± 0.5 Ba	7.0 ± 0.6 ABCa	5.4	7.0 ± 0.6 ABa	18.3	7.0 ± 0.6 ABa	39.5	7.0 ± 0.6 ABa	39.5
Malathion	200	200	9.2 ± 0.3 ABa	6.8 ± 0.9 ABCb	26.0	6.8 ± 0.9 ABb	36.0	6.8 ± 0.9 ABb	41.0	6.8 ± 0.9 ABb	41.0
Spinetoram	5	20	9.8 ± 0.1 Aa	9.8 ± 0.1 Aa	0.0	9.7 ± 0.1 Aa	1.0	9.5 ± 0.2 Aa	3.0	9.4 ± 0.2 Aa	4.1
Spinosad	9.6	20	9.0 ± 0.3 ABa	5.8 ± 0.7 Cab	35.6	5.0 ± 0.8 Bb	52.0	5.0 ± 0.9 Bb	55.5	5.0 ± 0.9Bb	55.5
Sulphur	240	300	10.2 ± 0.2 Aa	10.0 ± 0.00 Aa	1.9	9.8 ± 0.2 Aa	17.1	9.8 ± 0.2 Aa	11.9	9.8 ± 0.2Aa	11.9
Thiamethoxam	2.5	10	9.0 ± 0.7 ABa	8.0 ± 0.6 ABCa	11.1	7.6 ± 0.6 ABa	27.1	7.4 ± 0.5 ABa	23.9	7.2 ± 0.5ABa	26.0
Control (water)	–	–	9.5 ± 0.3 ABa	9.5 ± 0.4 ABa	–	9.5 ± 0.37 Aa	–	9.5 ± 0.37 Aa	–	9.5 ± 0.4Aa	–

[a] Concentration in g or mL AI 100 L^{-1} water.
[b] AI: active ingredient.
[c] CP: commercial product.
[d] Mean number of living flies ± SE. Means followed by the same upper-case letter in the columns and by the same lower-case letter in the rows do not differ significantly (LSMEANS with Tukey's adjustment; $P > 0.05$).
[e] Mortality corrected by Henderson and Tilton's formula.

Z. indianus adults, resulting in the low mortality of those insects in all the evaluated toxic bait treatments. Therefore, it is important to evaluate other feeding attractants for *Z. indianus*, such as apple vinegar,[8] which has shown efficiency in field monitoring programmes of this pest.

The availability of several chemical classes with different modes of action and with biological activity on *D. suzukii* and *Z. indianus* can effectively help in rotating insecticides for insect resistance management (IRM).[30,31] Owing to factors such as high multiplication capacity over short durations,[32] high polyphagia[33] and dispersion capacity,[34,35] managing *D. suzukii* requires several chemical applications during a harvest season, which increases the selection pressure on insects and, consequently, may accelerate resistance evolution.

The insecticide azadirachtin and the sulphur-based fungicide showed low toxicity on both adults and larvae of *D. suzukii* and *Z. indianus*. This low toxicity was also observed for the insecticide chlorfenapyr on adults, corroborating Bruck *et al.*[13] and Beers *et al.*[22] However, even though these products have low toxicity for these pests, they might favour pest suppression by causing repellence or by reducing pest oviposition capacity, as has

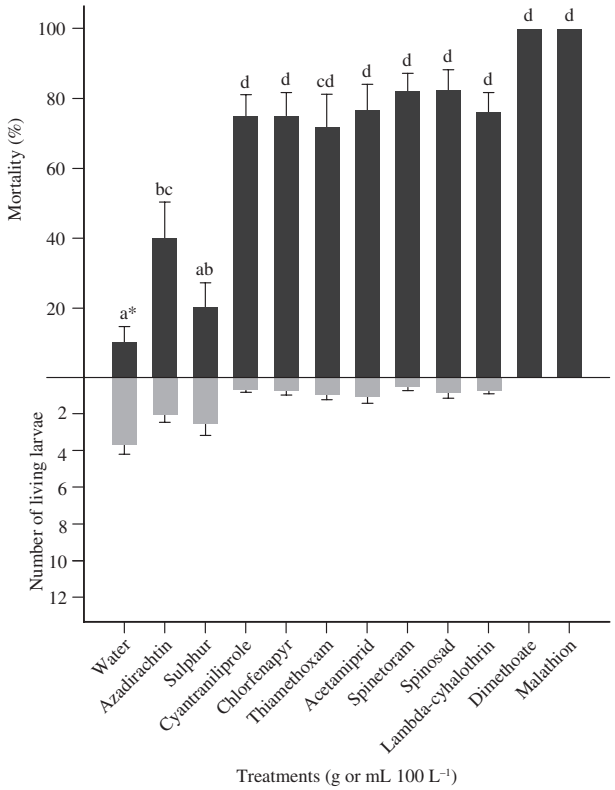

Figure 2. Larval mortality and mean number of living larvae of *Z. indianus* in the dipping bioassay with strawberry 'Albion' in the laboratory. * Bars (± SE) with the same letter are not significantly different (LSMEANS with Tukey's adjustment; $P > 0.05$).

been observed in other species,[36] particularly in organic production systems, where synthetic products usually cannot be sprayed.

In our tests, in addition to the organophosphates, the insecticides containing spinosyns (spinosad and spinetoram) also demonstrated effective control through all three modes of action (contact absorption and ingestion for adults and in-depth activity within the fruit tissue for larvae). Hence, they can provide significant benefits for the management of *D. suzukii* and *Z. indianus*.[37] A great advantage of using spinosyns is their low rate of chemical residues in fruit, as these chemicals degrade rapidly (within 3 days after application), allowing them to be applied during the fruit preharvesting period. In contrast, insecticides based on organophosphates and pyrethroids degrade much more slowly (14–21 days).[38]

This study resulted in the identification of several active ingredients with high toxicity on larvae and adults of *D. suzukii* and *Z. indianus* and showed that toxic baits can be a viable alternative for replacing insecticide sprays over an entire cropped area, especially during fruit preharvesting periods. Our results, along with other cultural control tactics such as reductions in fruit harvesting intervals under infestations of *D. suzukii*[27,29] and *Z. indianus*[6] and the destruction of infested fruit,[4] may contribute to reductions in pest populations and reduce growers' losses.

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