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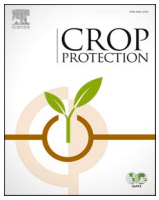

Lethal and sublethal toxicity of sulfoxaflor on the coccinellid *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae)

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A B S T R A C T

The conservation of beneficial insects in agroecosystems, in combination with biological and chemical control, is essential for integrated pest management (IPM) thus, the ban on neonicotinoids has prompted the search for alternative solutions compatible with these principles. Sulfoxaflor, a sulfoximine-based insecticide, has been proposed as a replacement, but its impact on non-target species, such as the two-spotted ladybird *Adalia bipunctata* (L.), remains insufficiently studied. This coccinellid is a versatile predator, primarily feeding on aphids, and commercialized in Europe as biological control agent. This research evaluated the lethal and sublethal effects of sulfoxaflor on *A. bipunctata* compared to imidacloprid. Laboratory bioassays assessed mortality, development, fecundity, fertility, and demographic parameters using a population model. Results showed that sulfoxaflor had significantly lower mortality than imidacloprid, with no adverse effects on larvae and minimal impact on adults. While imidacloprid reduced survival, fecundity, and population growth, sulfoxaflor at higher concentrations slightly increased egg fertility and female fecundity, suggesting potential hormetic effects. Demographic modeling indicated that *A. bipunctata* populations exposed to imidacloprid experienced delayed growth, whereas sulfoxaflor-exposed populations exhibited faster recovery. These findings support sulfoxaflor as a selective and environmentally compatible alternative to neonicotinoids in IPM programs. Further research should explore its long-term ecological effects in field conditions.

1. Introduction

The conservation of beneficial insects is an important aspect of integrated pest management (IPM) as these arthropods play a crucial role in reducing pest populations and preserving the health of the agroecosystem.

Modern IPM programs promote biodiversity, providing habitat and resources for natural enemies, and reducing the use of pesticides. In this sense, the use of selective insecticides is an important tool in IPM programs, as they can effectively control pest populations while reducing the risk of adverse effects on the environment and other non-target species (Gentz et al., 2010; Guedes et al., 2016).

Neonicotinoids are a class of insecticides that are widely used in agriculture to control a variety of pests, they work by targeting the central nervous systems of insects, causing nicotinic acetylcholine receptors (*n*AChRs) blockage and disrupting the ability to move, feed and reproduce (Jeschke et al., 2011, 2015; Kundoo et al., 2018).

This mode of action makes neonicotinoids a subject of concern from an environmental and ecological perspective (Deguine et al., 2021). Studies have shown that neonicotinoids can have harmful effects on a range of non-target species, including bees (Sgolastra et al., 2020), butterflies (Forister et al., 2016), and birds (Li et al., 2020). For example, research has shown that neonicotinoids can reduce the fertility of bees, disrupt their ability to navigate and forage, and impair their learning and memory (Blacquière et al., 2012).

In response to these concerns, several countries, including the European Union, have taken measures to restrict the use of neonicotinoids. In 2018, the European Union introduced a full ban on the use of three neonicotinoid insecticides in outdoor applications (EFSA, 2018), thus acetamiprid, has become the only neonicotinoid that can be applied without restrictions and in open field cultivations in the EU from 2021. In the last years, new molecules, such as sulfoxaflor, have entered the market.

Sulfoxaflor, an insecticide from the sulfoximine chemical class, functions as a competitive modulator of nicotinic acetylcholine receptors in insects, thereby influencing the central nervous system response (Babcock et al., 2010). This molecule was initially used to control aphids, but it is highly effective against a broad range of sap-feeding pests, as plant bugs, white-flies, hoppers, and scales including those resistant to previous insecticides (Watson et al., 2021), thus it has been proposed as a replacement for neonicotinoids (Cutler et al., 2012). In light of the restrictions on neonicotinoids, it is crucial to evaluate the impact of their potential replacements on the most widely used beneficial insects in IPM, particularly those that are commercially available and thus commonly released into agricultural environments (Sahayaraj and Hassan, 2023).

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The two-spotted ladybird *Adalia bipunctata* (L.) is an important natural enemy of many pest species, and is a valuable tool in both conservation and augmentative biocontrol programs. *A. bipunctata*, is a versatile predator, primarily feeding on aphids but also consuming other small insects and even pollen. Since 1999, this species has been commercialized in Europe as biological control agent used in various crops (Depalo et al., 2017).

Imidacloprid was widely recommended in IPM programs against several pests (e.g., mealybugs and aphids) in Europe, since the approval of the EU directive 2009/128/EC on the sustainable use of pesticides, until the recent restrictions. This study aimed to provide a comprehensive picture of the side effects of sulfoxaflor on *A. bipunctata*, in comparison with imidacloprid, comparing the same treatment conditions as indicated on the labels of the commercial products CONFIDOR 200 SL (imidacloprid 200 g/L) and CLOSER ISOCLAST (sulfoxaflor 120 g/L) for pome fruits against aphids.

Acute mortality and sublethal effects were tested on both adults and larvae of *A. bipunctata*. Sublethal effects were assessed by means of life parameters, as these factors directly influence predator population stability in agroecosystems, and disruptions to these parameters can cascade to pest resurgence or secondary outbreaks. The demographic approach was used for assessment of the long-term effects of pesticides, which can be triggered by sub-lethal toxicity (Stark et al., 2007a; Stark et al., 2007b).

Both standardized laboratory bioassays – IOBC methodology (Sterk et al., 1999) and demographic approach by means of an age-structured matrix population model (Caswell, 2001) were performed.

2. Materials and methods

2.1. Insects

Adalia bipunctata individuals were obtained from a laboratory colony established in 2010 at the Department of Agricultural and Food Sciences (University of Bologna). Every year field-collected specimens were introduced into the colony to maintain genetic diversity. Adult ladybird beetles were reared in plexiglas cages (40 x 30 × 50 cm) at 25 ± 1 °C, 60–80 % RH, a photoperiod of 16:8 (L:D) h, and were provided, twice per week, with aphids taken from a colony of green peach aphid, *Myzus persicae* Sulzer (1776) (Hemiptera: Aphididae). Larvae were reared at the same conditions as adults in 100 ml plastic cans and fed *ad libitum* with frozen eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) up to the third instar moult and then with *M. persicae* individuals. Adults and third instar larvae were used to test insecticides side effects.

2.2. Insecticides

The insecticides were applied at the doses indicated on the labels of the commercial products CONFIDOR 200 SL (imidacloprid 200 g/L) and CLOSER ISOCLAST (sulfoxaflor 120 g/L) for pome fruit against aphids.

A. bipunctata was exposed to two concentrations of sulfoxaflor and to imidacloprid. Control specimens were sprayed with tap water only. Rates of active ingredients used in the experiment are the recommended doses for aphid control on pome fruit, as indicated on the European commercial labels. Both doses indicated on sulfoxaflor label were tested, henceforth referred to as “sulfoxaflor 200” and “sulfoxaflor 400”, only one dose of imidacloprid was tested as indicated on label, henceforth referred to as “imidacloprid”; doses and rates are reported in Table 1.

Table 1
Rates of active ingredients tested (considering a volume of 1000 lt of water/Ha).

Commercial name	Active ingredient	Recommended doses	Rates of a.i. tested
Closer Isoclast	Sulfoxaflor	200 ml/Ha – 400 ml/Ha	24 mg AI L^{-1} - 48 mg AI L^{-1}
Confidor 200 SL	Imidacloprid	500 ml/Ha	100 mg AI L^{-1}

2.3. Bioassays on larvae

Adalia bipunctata larvae were directly sprayed, at the IOBC standard application rate (1.5–2 mg insecticide solution/cm^2) following the procedure by Hassan et al. (1994), with the selected compounds into a glass cylinder (diameter 8 cm, height 20 cm) closed on the bottom with a polyester mesh to permit excess liquid evacuation, using a hand-held sprayer (BRAND® atomizer, PE-HD, 400 mL). The rates tested are summarized in Table 1. Control specimens were sprayed with tap water only.

For each treatment, 4 replicates were carried out, each of 10 third instar larvae of *A. bipunctata.* Larvae of each replicate were treated all at once into the cylinder, as stated above, and then transferred singularly into 100 ml plastic cans, sealed with a fine net on the top to allow air circulation, until adult emergence. Larvae were fed *ad libitum* with *M. persicae* provided every 2 or 3 days.

All the trials were conducted under controlled conditions in a climatic chamber (25 ± 1 °C, relative humidity of 60–80 %, L:D = 16:8 photoperiod).

Mortality of larvae was checked 3 days after insecticide exposure, and the cumulative mortality was recorded by difference from the number of larvae that formed pupae and emerged as adults. The weight and the sex ratio, expressed as the proportion of females on the total of the emerged adults (♀/(♂+♀), were also recorded.

2.4. Bioassays on adults

Acute toxicity and sublethal effects of the insecticides were evaluated by directly exposing 4 replicates each of 10 newly emerged (<24 h aged) *A. bipunctata* females per treatment in a cylinder, as previously described, and then transferred singularly into 100 ml plastic cans, sealed with a fine net on the top to allow air circulation. Females were fed *ad libitum* with *M. persicae* provided every 2 or 3 days. All the trials were conducted under controlled conditions in a climatic chamber (25 ± 1 °C, relative humidity of 60–80 %, L:D = 16:8 photoperiod). The rates tested are summarized in Table 1. Control specimens were sprayed with tap water only.

Female mortality was checked 24 h and 3 days after insecticide exposure.

Females survived at this experiment were used to obtain life parameters. All the females survived were isolated together with an untreated male taken from the colony, in a 100 ml plastic can, sealed with a fine net on the top to allow air circulation, equipped with a piece of bubble wrap as oviposition substrate and daily fed *ad libitum* with *M. persicae* individuals.

The survival of each female was monitored daily, and fecundity was recorded by counting the eggs laid per female each day. The trial ended after 23 days, i.e. shortly before the expiration of the average oviposition period of *A. bipunctata* (24.7 g) reported by Lanzoni et al. (2004). All the eggs laid by each female were collected and the number of hatched larvae was checked every day to evaluate fertility. To reduce cannibalism, newly hatched larvae were provided with frozen *E. kuehniella* eggs.

A sample of 15 newly emerged larvae (first instar) was randomly collected from the offspring produced in each treatment. Three replicates per treatment were carried out. Larvae were individually reared on *M. persicae* until adult emergence. Development time, and sex ratio (expressed as the proportion of females on the total of the emerged adults (♀/(♂+♀)) of the offspring generated by the survived females were determined.

2.5. Demographic analysis

In each treatment age-specific survivorship (l_x) was estimated from

data on immature development and survival and daily schedules of adult mortality whereas age-specific fecundity (m_x) was derived from the number of eggs laid by each female. Age-specific survivorship (l_x) and fecundity (m_x) schedules were used to construct an age-classified population projection matrix (standard Leslie matrix) with a projection interval of one day (Caswell, 2001) (see Supplemental material). The asymptotic population growth rate (λ) was calculated as the dominant eigenvalue of the matrix. The discrete equivalent of the other stable population parameters (R_0, net reproductive rate; r_m, intrinsic rate of increase; T and μ1, two measures of mean generation time) were calculated from the age-classified matrix.

Finally, an application of matrix models, the Delay in Population Growth Index, a measure of population recovery, was calculated to compare the time required to a control population and a population exposed to an insecticide to reach a predetermined number of individuals (Stark et al., 2007a; Stark et al., 2007b; Stark et al., 2004; Wennergren and Stark, 2000). In this study the population delay was determined by choosing a population size of 100,000 individuals (Stark et al., 2004). All demographic calculations were performed with the software PopTools version 2.7.5 (Hood, 2006)

2.6. Statistical analyses

Statistical analyses were performed using STATISTICA software version 10 (StatSoft Inc.).

Data on larvae and adults were analyzed using one-way ANOVA with Tukey's HSD post-hoc test ($P < 0.05$). Percent data were arcsine transformed before analyses. The sex ratio of adults emerged from the bioassay on larvae was analyzed using a 2 × 2 chi-square test (treatment vs control).

Mortality and life history data from the sublethal bioassay were used to calculate a Reduction Coefficient, E%, for each insecticide, using the following formula:

$$E\% = 100 - ((100 - \%_{\text{Adult Abbott mortality}}) \times RV_{\text{Fecundity}} \times RV_{\text{Fertility}} \times RV_{\text{Adult longevity}} \times RV_{\text{Immature survival}} \times RV_{\text{Development time}}))$$

where the reduction values (RV) represent the percentage reduction of the different parameters considered, relative to the control. RV values were calculated according to the Abbott formula when significant differences were identified ($P < 0.05$), otherwise, RVs of 1 were assigned.

On the basis of the E% value obtained, each insecticide was classified using the IOBC classification criteria for pesticide risk assessment in the laboratory: 1 = harmless (<30 %), 2 = slightly harmful (30 %–79 %), 3 = moderately harmful (80 %–99 %), 4 = harmful (>99 %).

3. Results

3.1. Bioassays on larvae

No significant higher mortality on larvae exposed to sulfoxaflor 200 either sulfoxaflor 400 at both intervals, was detected (Fig. 1A). On the contrary, in comparison with control, imidacloprid significantly increased larval mortality 3 days after insecticide exposure and at adult emergence. (Fig. 1B).

Regarding adult emergence and adult weight, the results did not show significant differences (Table 2). Instead, the sex-ratio of the adults obtained from larvae exposed to imidacloprid was significantly shifted toward females (Table 2).

3.2. Bioassays on adults

In the adult bioassay, imidacloprid and sulfoxaflor 200, but not sulfoxaflor 400, showed a significant higher acute mortality respect to the control, 24 h after direct exposure (Fig. 2A). However, 3 days after exposure, only imidacloprid had an acute mortality significantly higher than the control (Fig. 2B).

Sulfoxaflor 400 and imidacloprid significantly reduced female survival at the end of the 23-day observational

period (Table 3), indicating a prolonged negative effect on this parameter. Unexpectedly, sulfoxaflor 400 significantly increased egg fertility. None of the adult biological parameters were affected by the direct exposure to sulfoxaflor 200 (Table 3).

Parental exposure to both insecticides did not show any significant impact on development time, percentage of preimaginal (larvae to adult) survival, weight and sex ratio of the offspring (Table 3).

Based on the coefficient of reduction (E%), imidacloprid was classified as slightly harmful (IOBC class 2), whereas sulfoxaflor at both concentrations as harmless (IOBC class 1) (Table 3). However, in the case of sulfoxaflor 400, this conclusion arises from a trade-off among negatively affected female survival and positively influenced egg fertility (Table 3).

Table 2
Adult weight, adult emergence and sex ratio of *Adalia bipunctata* directly exposed to insecticides as third instar larvae (mean ± SE).

Treatment	Total weight (mg)	Male weight (mg)	Female weight (mg)	Adult emergence (%)	Sex ratio (% of female)[a]
Control	9.3 ± 0.3	8.9 ± 0.3	9.8 ± 0.5	84.2 ± 3.5	47.9
Sulfoxaflor 200	9.1 ± 0.3	8.7 ± 0.4	9.3 ± 0.4	79.2 ± 4.4	60.0ns
Sulfoxaflor 400	8.9 ± 0.3	8.0 ± 0.4	9.5 ± 0.5	80.5 ± 9.0	57.5ns
Imidacloprid	8.7 ± 0.5	8.3 ± 0.2	8.8 ± 0.6	68.7 ± 6.3	82.4*
Statistics	F3, 143 = 0.698 P = 0.555	F3, 56 = 0.915 P = 0.440	F3, 80 = 0.961 P = 0.415	F3, 12 = 1.206 P = 0.349	–

* = $P < 0.05$, sulfoxaflor 200 vs control and sulfoxaflor 400 vs control ns = $P > 0.05$ (Chi-square test).
According to IOBC classification, imidacloprid was ranked slightly harmful to *A. bipunctata* larvae (class 2) instead sulfoxaflor resulted harmless (class 1).
[a] imidacloprid vs control.

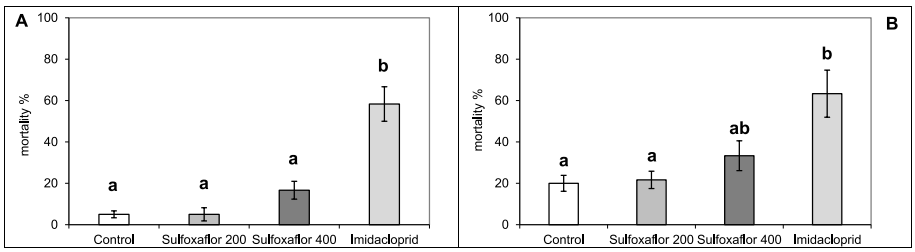

Fig. 1. A. Mortality (mean ± SE) of third instar larvae of *Adalia bipunctata*, 3 days after insecticide exposure. Bars bearing different letters are significantly different for $P < 0.05$ (ANOVA, Tukey HSD post-hoc test); B. Cumulative mortality (mean ± SE) of third instar larvae of *Adalia bipunctata*. Bars bearing different letters are significantly different for $P < 0.05$ (ANOVA, Tukey HSD post-hoc test).

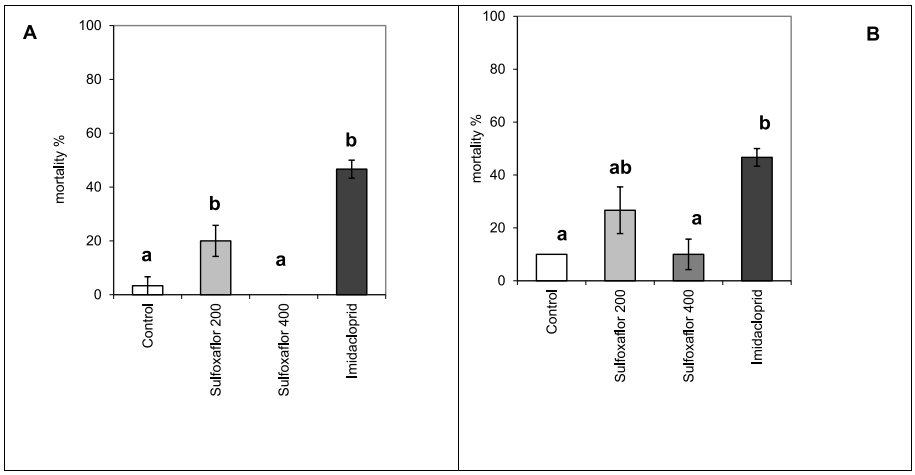

Fig. 2. A. Mortality (mean ± SE) 24 h after insecticide exposure of adult of *Adalia bipunctata*. Bars bearing different letters are significantly different for $P < 0.05$ (ANOVA, Tukey HSD post-hoc test); B. Mortality (mean ± SE) 3 days after insecticide exposure of adult of *Adalia bipunctata*. Bars bearing different letters are significantly different for $P < 0.05$ (ANOVA, Tukey HSD post-hoc test).

Table 3
Effects on biological traits of females of *Adalia bipunctata* and their offspring when directly exposed to insecticides at adult stage.

Parameter	Control	Sulfoxaflor 200	Sulfoxaflor 400	Imidacloprid	Statistics
Fecundity (total eggs/female ± SE)[a]	365.7 ± 40.5	381.6 ± 40.2	395.9 ± 40.7	303.3 ± 54.3	F 3, 8 = 0.4177 P = 0.7452
Oviposition rate (eggs/female/day ± SE)[a]	16.2 ± 1.7	17.2 ± 1.6	20.4 ± 2.0	15.3 ± 2.5	F 3, 8 = 0.5230 P = 0.6484
Female survival (%)[a]	80 ± 5.8a	60.0 ± 10.0 ab	33.3 ± 17.6b	20 ± 5.8b	F 3, 8 = 4.1235 P = 0.0484
Longevity (days ± SE)[a]	20.8 ± 1.1	20.1 ± 1.4	17.3 ± 1.2	16.6 ± 1.7	F 3, 8 = 1.4461 P = 0.3001
Fertility (% hatched eggs/female ± SE)[a]	31.5 ± 5.0a	28.8 ± 5.0a	54.8 ± 3.7b	31.4 ± 5.7a	F 3, 8 = 12.300 P = 0.0023
Ovipositing females % (O/T)	93.1 (27/29)	91.7 (22/24)	96.3 (26/27)	75.0 (12/16)	–
Development time (days ± SE)[a]	18.8 ± 0.21	18.8 ± 0.23	18.5 ± 0.2	18.9 ± 0.21	F 3, 8 = 1.4400 P = 0,3014
Survival larvae to adult (% ± SE)[a]	66.7 ± 3.8	71.1 ± 5.9	71.1 ± 2.2	55.5 ± 5.88	F 3, 8 = 2.371 P = 0.1464
Sex ratio (% of female ± SE)[a]	49.6 ± 10.8	59.8 ± 3.6	56.4 ± 5.6	36.2 ± 6.9	F 3, 8 = 2.0987 P = 0,1788
Reduction coefficient E%	–	25.0	27.5	75,0	–
IOBC Class	–	1	1	2	–

Data followed by different letters in the same row are significantly different at $P < 0.05$.
[a] ANOVA, Tukey HSD post-hoc test.

Stable population parameters calculated by means of Leslie matrix showed a similar scenario in comparison with that evinced by the analysis of individual life history parameters. Direct exposure of adult females to imidacloprid caused a significant reduction of the intrinsic rate of increase (r_m) and finite rate of population increase (λ) (Table 4).

The population parameters of *A. bipunctata* were not affected by the direct exposure to sulfoxaflor 200, but significantly higher net reproductive rate (R_0), intrinsic rate of increase and finite rate of population increase along with a shorter mean generation time (either T or μ1) were observed for *A. bipunctata* females directly exposed to sulfoxaflor 400

Table 4
Stable population parameters (mean ± SE) obtained from laboratory cohorts of *Adalia bipunctata* directly exposed to insecticides at adult stage.

Treatment[a]	Net reproductive rate (R_0) (♀♀ offspring)	Mean generation time		Doubling time (DT) (days)	Intrinsic rate of increase (r_m) (days^{-1})	Finite rate of increase (λ) (days^{-1})
		(T) (days)	(μ1) (days)			
Control	44.0 ± 7.0a	30.5 ± 0.4a	32.7 ± 0.3a	5.7 ± 0.3 ab	0.1232 ± 0.0065a	1.1312 ± 0.0074a
Sulfoxaflor 200	42.7 ± 6.4a	30.8 ± 0.6a	32.9 ± 0.3a	5.8 ± 0.3 ab	0.1214 ± 0.0070 ab	1.1291 ± 0.0079 ab
Sulfoxaflor 400	92.1 ± 14.5b	26.2 ± 0.4b	28.1 ± 0.8b	4.0 ± 0.1b	0.1717 ± 0.0044c	1.1874 ± 0.0053c
Imidacloprid	12.6 ± 4.3a	30.4 ± 0.7a	31.6 ± 0.6a	9.6 ± 2.2a	0.0793 ± 0.0154b	1.0828 ± 0.0166b
Statistics	$F_{3, 8} = 13.594$ P = 0.0017	$F_{3, 8} = 17.13$ P = 0.0008	$F_{3, 8} = 18.87$ P = 0.0006	$F_{3, 8} = 8.536$ P = 0.0071	$F_{3, 8} = 16.384$ P = 0.0009	$F_{3, 8} = 17.42$ P = 0.0007

Data followed by different letters in the same column are significantly different (ANOVA, Tukey HSD test, $P < 0.05$).

(Table 4).

The effect of exposure to imidacloprid on survival is chiefly tied to acute mortality and less to sublethal effects on individuals surviving insecticide exposure. Conversely, the sublethal effect seems to be the cause of the reduced life expectancy at adult stage for the individuals exposed to sulfoxaflor 400.

A faster population growth displayed by the sulfoxaflor 400 exposed *A. bipunctata* females can be highlighted by the delay in population growth index to reach 100,000 specimens (Fig. 3). The value of delay in population growth index showed that being exposed to imidacloprid delayed population growth of *A. bipunctata* of 21 days respect to the control (Fig. 3). This delay corresponds to approximately 70 % of the value of generation time (T) calculated for *A. bipunctata* (Table 4). No such a delay can be highlighted for exposure to sulfoxaflor 200. Instead, even an anticipation of 12 days can be seen for exposure to sulfoxaflor 400 (Fig. 3) since a significantly higher population growth index (λ) (Table 4).

4. Discussion

The results of this study reveal the toxicity profiles of sulfoxaflor and imidacloprid on the beneficial coccinellid *Adalia bipunctata*. Sulfoxaflor demonstrated substantially lower toxicity compared to imidacloprid, both at larval and adult stages, making it an alternative to the neonicotinoids. These findings align with earlier research suggesting that sulfoximine-based insecticides are selective in targeting pest species while minimizing impacts on non-target beneficial insects (Barbosa et al., 2017; Watson et al., 2021).

Considering larvae, the lack of significant larval mortality in sulfoxaflor treatments, as opposed to the harm caused by imidacloprid, underscores the relatively benign nature of sulfoxaflor. This reduced lethality is crucial in IPM programs, as larval survival is directly linked to the sustainability of predator populations (Serrão et al., 2022). The observed harmless classification of sulfoxaflor, according to IOBC criteria found in our study, further supports its suitability in IPM frameworks. A slightly higher mortality was reported for *Hippodamia convergens* Guerin-Meneville (Coleoptera: coccinellidae) fourth-instar larvae after sulfoxaflor topic exposure but using significantly higher concentrations - 275 mg a.i. L^{-1} and 550 mg a.i. L^{-1} - that is more than ten-fold higher than utilized in our study (Colares et al., 2016). Since the general lack of sublethal effects on development or reproduction on the survived larvae, the Authors concluded that sulfoxaflor does not result as toxic as other nicotinic acetylcholine receptor agonists, providing an improved level of selectivity for coccinellids. In addition, a higher dose of sulfoxaflor 180 mg a.i. L^{-1}, directly sprayed on insects, was moderately harmful to second-instar larvae of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) (He et al., 2020). On the contrary, Garzon et al. (2015) found sulfoxaflor highly toxic to fourth-instar larvae of *A. bipunctata* when exposed to fresh insecticide residues on glass surface. The higher concentration (63.6 mg a.i. L^{-1}) respect our study and the high impact exposure method (glass surface) used, can explain the high toxicity found. Similar results were found by Wang et al. (2023a) on *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), with similar exposure method. Indeed, larvae under this experimental conditions, may have experienced a close adhesion to substrate as they actively walk on treated surfaces during foraging activity.

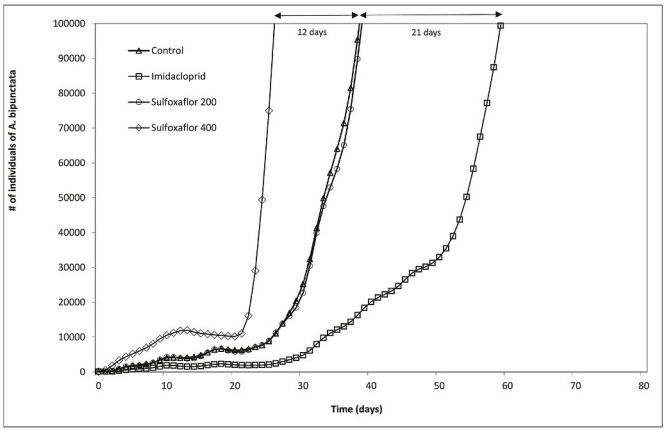

Fig. 3. Matrix model projections and comparison of the delay in population growth to reach 100,000 specimens for insecticide exposed and control populations of *Adalia bipunctata*. Simulation started with 100 specimens at a stable age distribution.

Imidacloprid's consistent reduction in survival, fertility, and fecundity confirms its incompatibility with IPM (Di Vitantonio et al., 2018). Sulfoxaflor's impact on adult survival, particularly at the lower concentration, indicates its reduced acute toxicity in accordance to other studies carried out on *A. bipunctata* (Garzon et al., 2015), *H. convergens* (Colares et al., 2016), and on *H. axyridis* (He et al., 2020).

Interestingly, the higher concentration of sulfoxaflor (48 mg a.i. L^{-1}) increased egg fertility and female fecundity while slightly reducing adult survival. This trade-off suggests a potential population compensation, where short-term reproductive gains may offset survival losses. Since the observed reproductive stimulation and enhancement of some demographic parameters, on various target pests, after exposure to sublethal concentration of sulfoxaflor (Feng et al., 2025; Tang et al., 2015; Wang et al., 2023b; Yu, 2014), it could be possible to infer an hormetic effect of sulfoxaflor on *A. bipunctata* in our study. A possible insecticide-induced hormesis mechanism, evoked as explanation of the observed reproductive stimulation of the higher concentration of sulfoxaflor, could be tied to a low insecticide penetration after the direct spray exposure of the *A. bipunctata* adults. Indeed, penetration of the insecticides in insect integument can result from molecular properties such as polarity (Yu, 2014). The relative low octanol-water partition coefficient of sulfoxaflor (log $P_{ow} = 0.8$) could account for a low insecticide penetration under our exposure method. Lipophilicity refers to the ability of active ingredients to dissolve in fats, oils, and lipids. Insecticides that are lipophilic (log $P_{ow} > 4$) are generally not systemic, whereas those compounds considered moderately lipophilic have a log P_{ow} value between 0.5 and 3.5. (Jeschke et al., 2013). The consequence of this moderate lipophilicity can lead to a low penetration, thus making the higher concentration, used in our study, acting as a sublethal concentration able to foster an insecticide-induced hormesis (Cutler, 2013).

Unexpectedly in this study sulfoxaflor caused significant adult mortality, the same was not found on the larvae even though they are generally considered more sensitive. In our case, a possible explanation for this result may be the larger body surface area of the adults, which could correspond to greater product uptake than the larvae. A similar situation was found by Youn et al. (2003) on *H. axyridis,* topically treated with the neonicotinoid thiamethoxam. However, the literature on sulfoxaflor is currently still limited to provide a conclusive reason for this result.

The impact of pesticides on natural enemies of crop pests in ecotoxicological studies has highlighted the need for more ecologically relevant endpoints to measure their effects on beneficial species (Elhamalawy et al., 2024). Therefore, assessments require further investigation and should consider not only individual mortality and field performance of a given natural enemy, but also its population dynamics. Indeed, population modeling is a proper approach to study the effects of toxicants on biocontrol agents critical to ecosystem services. In this study, demographic analyses reveal that sulfoxaflor at the higher concentration may foster population growth through enhanced reproductive metrics. The intrinsic rate of increase (r_m), finite population growth rate (λ), and net reproductive rate (R_0) were all significantly improved in the higher concentration sulfoxaflor treatment compared to imidacloprid and control. This highlights sulfoxaflor's potential to support the ecological role of *A. bipunctata*. Conversely, imidacloprid induced delays in population growth, confirming the long-term risks posed by this

insecticide to beneficial insect populations, as already proved on *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) (Skouras et al., 2019) as well as on *C. septempunctata* (Xiao et al., 2016). Allowing 21 days for *A. bipunctata* to recover after a spray with imidacloprid, would give, for example, to *M. persicae*, its most common aphid prey on peach, or to *Dysaphis plantaginea* (Passerini) (Homoptera: Aphididae), its most common aphid prey on apple, enough time to complete ≈2 or ≈1.4 generations, respectively, based on the value of generation time of those aphids (Angeli and Simoni, 2006; Hong et al., 2019).

The faster population growth and enhanced reproductive output associated with sulfoxaflor suggest it is a safer alternative to traditional neonicotinoids. However, the slightly reduced survival observed at higher sulfoxaflor doses warrants further investigation to optimize its use and minimize unintended ecological impacts. This balance is critical in agroecosystems, where maintaining predator-prey dynamics is essential for sustainable pest management (Guedes et al., 2016).

Overall, this study demonstrates that sulfoxaflor may be a less harmful alternative to imidacloprid for the control of pests while preserving populations of the beneficial coccinellid *A. bipunctata* a key predator in IPM programs. However, the positive effect of sulfoxaflor 400 on egg fertility and young female fecundity needs further investigation to clarify the underlying mechanisms.

Compared to imidacloprid, sulfoxaflor increased reproductive metrics and population growth while limiting adverse effects. These findings pose sulfoxaflor as a viable alternative to neonicotinoids in environmentally sensitive pest management strategies.

Future research should focus on: field experiment to confirm laboratory findings under natural conditions, long-term ecological assessments to identify cumulative effects on beneficial insect populations and investigating synergistic effects of sulfoxaflor with other IPM practices, such as habitat diversification.

CRediT authorship contribution statement

Laura Depalo: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Edison Pasqualini:** Supervision, Methodology. **Alberto Lanzoni:** Writing – review & editing, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2025.107366.

Data availability

Data will be made available on request.

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