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Comparative lethal and sublethal effects of sulfoxaflor and acetamiprid on *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae): integrating IOBC classification and matrix population modeling

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Following regulatory restrictions on several neonicotinoids, sulfoxaflor, a sulfoximine insecticide proposed as a replacement, has been reported as highly effective against a broad range of sap-feeding pests. However, its impact on key biological control agents, such as the widely used mealybug destroyer *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), remains insufficiently investigated. In this study, we assessed the lethal and sublethal effects of recommended field rates of sulfoxaflor on larvae and adults of *C. montrouzieri* through direct spray treatments and exposure to fresh residues on grapevine plants, comparing its impact with that of acetamiprid. Laboratory bioassays evaluated mortality, development, fecundity, fertility, offspring survival, and demographic parameters following IOBC guidelines and population modeling. Our results show that sulfoxaflor caused significantly lower mortality than acetamiprid, with minimal effects on larvae and no adverse effects on adults. Demographic modeling further indicated that the demographic performance of *C. montrouzieri* populations was not negatively affected by sulfoxaflor, while the impact of acetamiprid on the predator was primarily driven by high short-term mortality, with only marginal contributions from changes in reproductive performance. According to IOBC classification, sulfoxaflor was categorized as slightly harmful (class 2) for larvae and harmless (class 1) for adults, indicating potential compatibility with IPM programs involving augmentative releases of *C. montrouzieri*. Our findings highlight the value of integrating demographic modeling into IOBC assessments to improve ecotoxicological risk evaluation of insecticides on biological control agents.

Keywords: mealybug destroyer, integrated pest management (IPM), neonicotinoids, life table, demographic modeling

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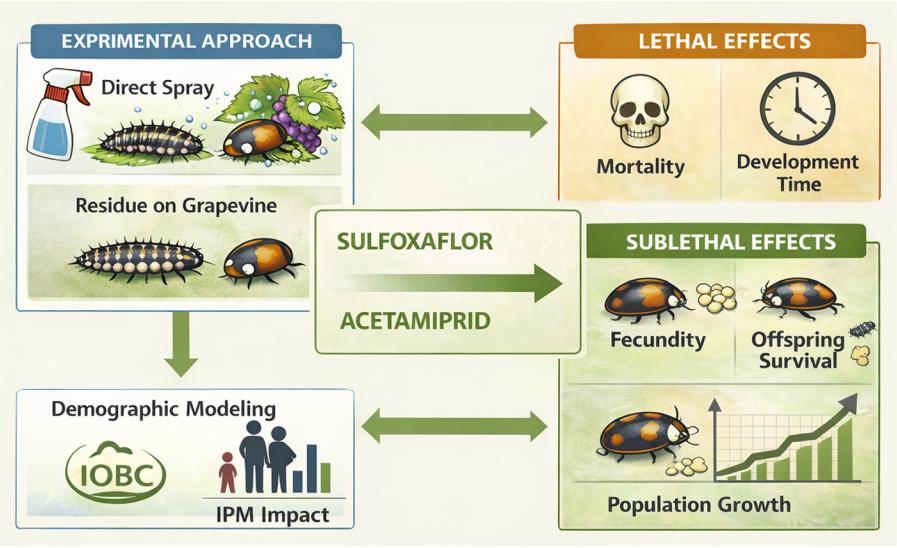
Graphical Abstract



Introduction

Historically, Integrated Pest Management (IPM) programs against mealybugs have relied heavily on insecticide applications. Consequently, a thorough understanding of the selectivity of active substances toward beneficial arthropods is essential to minimize pesticide-induced disruption of the ecological services provided by natural enemies of mealybugs (Mansour et al. 2018). Estimating key population parameters is essential for accurately characterizing how pesticide exposure affects the population dynamics of natural enemies and is therefore crucial for developing effective IPM programs (Stark and Banks 2024).

Coccinellids are among the most important predators in biological control and play a key role in IPM programs (Burgio et al. 2004, Hodek et al. 2012). Among them, the mealybug destroyer *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) is widely released in both classical and augmentative biological control programs targeting mealybugs (Hemiptera: Pseudococcidae) in a variety of cropping systems (Kairo et al. 2013), including vineyards (Burgio et al. 2025) and citrus orchards (Jacas and Urbaneja 2010).

The priority of IPM is the use of biological control (Flint and van den Bosch 1981), therefore, the conservation of natural enemies within agroecosystems, together with the protection of released biological control agents, is considered a major driver of IPM implementation (Gentz et al. 2010, Guedes et al. 2016, van Lenteren 2012).

IOBC classifications are primarily based on corrected mortality and short-term reproductive effects, which may underestimate long-term demographic consequences. Assessing both lethal and sublethal effects of insecticides on biological control agents is therefore crucial for ensuring their compatibility with IPM strategies. Integrating matrix population models allows projection of multigenerational effects and provides a more mechanistic understanding of pesticide impacts on predator population growth. Both the standardized laboratory methodology proposed by the IOBC (Sterk et al. 1999) and a demographic approach based on an age-structured matrix population model (Caswell 2001) were applied.

Neonicotinoids are a class of insecticides that have been widely used to control a variety of agricultural pests. They act selectively on the insect central nervous system as agonists of post-synaptic nicotinic acetylcholine receptors (nAChRs) (Jeschke et al. 2013), thereby disrupting the capability to move, feed, and reproduce (Kundoo et al. 2018). Numerous studies have documented their harmful effects on different non-target organisms, including beneficial arthropods (Lanzoni et al. 2012, Forister et al. 2016, Amjad et al. 2018, Sgolastra et al. 2020, Khurana et al. 2023, Depalo et al. 2025). Over the last decade, increasing environmental and ecological concerns have led to severe restrictions on neonicotinoid use worldwide (Pisa et al. 2015), culminating in the full ban of 3 neonicotinoids for outdoor applications in the European Union (European Commission 2018). As a consequence, acetamiprid remains the only neonicotinoid currently authorized for use in EU outdoor crops.

Sulfoxaflor, the first insecticide belonging to the relatively new chemical class of sulfoximines, has demonstrated high efficacy against a broad range of sap-feeding pests with a pest spectrum largely overlapping that of the neonicotinoids (Babcock et al. 2011, Watson et al. 2021). Sulfoximines interact with nAChRs in a manner distinct from other IRAC Group 4 insecticides, such as the neonicotinoids and may therefore represent a potential replacement for these compounds. Moreover, sulfoxaflor is expected to exhibit a different toxicity profile toward beneficial insects (Depalo et al. 2025), although information on its selectivity remains limited.

The aim of this study was to provide a comprehensive assessment of the lethal and sublethal effects of sulfoxaflor, in comparison with acetamiprid, under the same treatment conditions specified on the commercial product labels for grapevine mealybug control.

Effects were evaluated on larval and adult stages following direct spray applications and exposure to fresh insecticide residues on grapevine leaves. We hypothesized that sulfoxaflor would have lower lethal and sublethal effects on

C. montrouzieri than acetamiprid, and that demographic analysis would reveal population-level effects not captured by the standard IOBC classification.

Materials and Methods

Insects and Experimental Conditions

Second instar larvae of *C. montrouzieri* were purchased from the commercial biofactory Koppert Italia, Bussolengo (VR) Italy. Adults of *C. montrouzieri* were obtained from the commercial biofactory Bioplanet srl, Cesena (FC) Italy. Both larvae and adults were purchased from the vendor once per replicate and used in the experiments on the day of receipt or maintained in a climatic chamber ($T = 10\,°C$) for no longer than 1 day before use. For each experiment, insects were randomly selected from the purchased stock and subsequently randomly assigned to the different treatments, and the containers were equally distributed in the climatic chamber. All trials were conducted under controlled environmental conditions in a climatic chamber (25 ± 1 °C, relative humidity of 60% to 80%, L:D = 16:8 h photoperiod).

Insecticides

Insecticides were applied at the doses indicated on the labels of the commercial products CLOSER (sulfoxaflor 120 g a.i. l^{-1}) and Epik SL (acetamiprid 50 g a.i. l^{-1}) for grapevine mealybug control. The concentrations used in all trials corresponded to the maximum recommended label rates, assuming a spray volume of 1,000 l ha^{-1} water: 48 mg a.i. l^{-1} for sulfoxaflor and 100 mg a.i. l^{-1} for acetamiprid. Tap water was used as the control treatment in all experiments.

Direct Sprays of Larvae and Adults

Larvae and adults were directly sprayed with either insecticide solutions or water using a hand-held sprayer (BRAND atomizer, PE-HD, 400 ml), at the IOBC standard application rate (1.5 to 2 mg insecticide solution cm^{-2}) following the procedure by Hassan et al. (1994). Individuals were treated all at once, delivering a spray deposit of approximately 1.5 mg/cm^2 (Van De Veire et al. 2002), inside a cylindrical container (diameter 8 cm, height 20 cm) closed on the bottom with a polyethylene mesh to allow excess liquid to drain and left to dry for 15 min.

Bioassays with Larvae

For each treatment, 20-s instar larvae of *C. montrouzieri* were treated inside the cylinder and then transferred to 150 ml polystyrene urine plastic containers (diameter 60 mm, height 75 mm; Kartell) (5 larvae per container). Larvae were fed *ad libitum* with individuals of *Planococcus ficus* or *Planococcus citri* reared on potato sprouts (*Solanum tuberosum* L.) in the laboratories of the Department of Agricultural and Food Sciences of the University of Bologna and frozen eggs of *Ephestia kuehniella* Zeller (Pyralidae) were purchased from Bioplanet srl, Cesena (FC) Italy. Food was provided every 2 to 3 days. Four replicates were carried out per treatment. Larval mortality was first assessed 3 days after insecticide application. Immature mortality, percentage of pupation, and number of adults emerged were recorded. Cumulative mortality was calculated as the percentage of individuals who failed to reach adulthood. Percentage of pupation was the percentage of formed pupae compared with the total number of treated larvae. Adult emergence was calculated as the percentage of successful emerged adults relative to the number of formed pupae. The weight and the sex ratio (♀/(♂+♀)) of the emerged adults were also recorded.

Bioassays with Adults

For each treatment, 10 adults of *C. montrouzieri* were treated inside the spraying cylinder, and then transferred together into a 100 ml plastic container. Six replicates were carried out for each treatment. Adults were fed frozen *E. kuehniella* eggs. Adult mortality was assessed 3 days after insecticide application.

Exposure on Insecticide Residues

A potted grapevine plant (cv. Famoso BS1 on SO VCR 6 rootstock, approximately 50 cm high) was sprayed outdoor for each insecticide studied, using a hand sprayer (BRAND atomizer, PE-HD, 400 ml) until runoff and allowed to dry completely before the trials. In the same way, a control plant was sprayed with tap water only. For each plant, 1 shoot was enclosed in a plexiglas cylinder (diameter 8 cm; height 20 cm for larvae, 33 cm for adults) covered with mesh at the base to promote air circulation and prevent fungal growth inside the cylinder. The residual effects of insecticides were evaluated by placing 20-s instar larvae or 20 adults of *C. montrouzieri* inside each plexiglas cylinder. Each cylinder represented 1 replicate, and 4 replicates were performed per treatment. Insects were fed *ad libitum* with frozen eggs of *E. kuehniella*, which were glued to a strip of tissue paper (15 × 2 cm) using a mixture honey–water (50% v/v) and hung within the cylinder on the plant. After a 3-day exposure period, the cylinders were dismantled and insect mortality was recorded.

In each replicate, surviving larvae were transferred to 150 ml plastic containers (5 larvae per container) and maintained until adult emergence, following the same feeding and rearing conditions described for the direct spray bioassays. Immature mortality, pupation rate, adult emergence, adult weight, and sex ratio were recorded as previously described. The weight and the sex ratio (♀/(♂+♀)) of the emerged adults were also recorded.

In each replicate, surviving adults were maintained together into a Petri dish (diameter 16.5 cm) and fed *ad libitum* with *Planococcus ficus* or *P. citri*. Adult survival was monitored daily and dead adults were collected and conserved for subsequent scrutiny. Fecundity was assessed by counting the total number of eggs laid by all females daily, over a 21-day period. Eggs were collected daily, and the number of hatched larvae was recorded every day to evaluate fertility. All adults were sexed *a posteriori* to calculate the mean number of eggs laid per female per day.

Demographic Analysis

A complete demographic analysis was conducted on adults exposed to fresh insecticide residues. In each of the 4 replicates per treatment, an age-specific survivorship (l_x) was estimated from data on immature development time obtained from Kairo et al. (2013), from larval survival obtained from the control treatment in the larvae direct exposure bioassay, and from daily adult mortality and egg eclosion rate records from adult direct exposure bioassay. Age-specific fecundity (m_x) was derived

from the number of eggs laid by females considering a sex ratio ♀/♂ = 1. Age-specific survivorship (l_x) and fecundity (m_x) schedules were used to construct ($z \times z$) age-classified population projection matrices (standard Leslie matrix) with a projection interval of 1 day (Caswell 2001).

$$\mathbf{A} = \begin{pmatrix} 0 & F_2 & F_3 & \dots & F_z \\ P_1 & 0 & 0 & \dots & 0 \\ 0 & P_2 & 0 & \dots & 0 \\ \vdots & \dots & \dots & \dots & \vdots \\ 0 & 0 & 0 & P_{z-1} & G_z \end{pmatrix} \quad (1)$$

The only nonzero entries of the matrix, survival probability (P_i) appearing on the subdiagonal and fecundity (F_i) appearing in the first row, were calculated by means of the birth-flow formulas (Caswell 2001) as follow:

$$P_i \approx \frac{l(i) + l(i+1)}{l(i-1) + l(i)} \quad (2)$$

$$F_i = \frac{l(0) + l(1)}{2}\left(\frac{m_i + P_i m_{i+1}}{2}\right) \quad (3)$$

where $l(i)$ is the survivorship from birth to age i and m_i is the mean number of female offspring per female in age class i. Since adult performance were observed for no more than 21 days and *C. montrouzieri* can live longer and keep on reproducing (Kairo et al. 2013) the term G_z was added to the diagonal of the matrix (Tables S1–S3). In each replicate, the most realistic value of G_z was estimate from the characteristic equation of **A** (Caswell 2001).

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, r_m, T, μ_1, DT) were also calculated from the age-classified matrix as follow (Caswell 2001):

$$R_0 = \sum F_i \prod_{j=1}^{i-1} P_j \quad (4)$$

$$r_m = \log \lambda_1 \quad (5)$$

$$T = \frac{\log R_0}{\log \lambda_1} \quad (6)$$

$$\mu_1 = \frac{\sum_i i(\prod_{j=1}^{i-1} P_j) F_i}{\sum_i (\prod_{j=1}^{i-1} P_j) F_i} \quad (7)$$

$$\mathrm{DT} = \frac{\ln 2}{r_m} \quad (8)$$

The time needed to settle to asymptotic dynamics can be denoted by a transient index such as the damping ratio (ρ) calculated, following Caswell (2001), as the ratio of the dominant eigenvalue (λ_1) to the magnitude of the first subdominant eigenvalue ($|\lambda_2|$).

$$\rho = \frac{\lambda_1}{|\lambda_2|} \quad (9)$$

The life expectancy (e_x), ie the mean lifespan that an individual aged x is expected to live if the conditions to which it is exposed were maintained, and the expected life time reproduction, ie the expected number of reproductive events during a female's lifetime, have been calculated treating the transition matrix as an absorbing Markov chain with death as absorbing state (Caswell 2001).

We determined how much the differences in population growth rates (λ) between control and insecticide exposed groups were determined by the differences in each vital rate by means of a life table response experiment (LTRE) analysis (Caswell 2001, Hamda et al. 2012). With this technique, the effects of insecticide treatment on λ, measured respect to the control, was decomposed into contributions from each age-specific fecundity and survival probability terms as follow:

$$\lambda^{(t)} \approx \lambda^{(c)} + \sum_{ij}\left(a_{ij}^{(t)} - a_{ij}^{(c)}\right)\frac{\partial \lambda}{\partial a_{ij}}\bigg|_{\frac{1}{2}\left(A^{(t)} + A^{(c)}\right)} \quad (10)$$

In eq. (10), $\lambda^{(t)}$ and $\lambda^{(c)}$ denote the values of λ for insecticide treatment and control groups, respectively, and each term in the summation represents the contribution of the difference in the matrix element a_{ij} (age-specific fecundity, F_i or survival probability, P_i) to the global effect of insecticide treatment on population growth rate. That contribution is the product of the difference between insecticide treatment and control vital rates and the sensitivity of that vital rate calculated from a matrix halfway between the insecticide treatment matrix $\mathbf{A}^{(t)}$ and the control matrix $\mathbf{A}^{(c)}$ being compared (Logofet and Lesnaya 1997).

Finally, an application of matrix models, the Delay in Population Growth Index (Wennergren and Stark 2000, Stark et al. 2004, 2007a, 2007b), a measure of population recovery, was calculated to compare the time required to a control population and a population exposed to insecticide to reach a predetermined number of individuals. In this study, the population delay was determined by choosing a population size of 100,000 individuals (Stark et al. 2004). In each treatment, population growth was simulated by multiplying the matrix **A** by an initial population vector $\mathbf{n}(t) = [n_1\, n_2\, n_3 \cdots n_z]^{\mathrm{T}}$, containing information on the age distribution of the population. The population projection was started with a vector consisting of 100 individuals distributed according to the stable age distribution and ended when at least 100,000 individuals had been reached. In each treatment, the stable age distribution ($\mathbf{w}_1$) was calculated as the corresponding right eigenvector of the dominant eigenvalue of the matrix **A** (Caswell 2001). All demographic calculations were performed with the software PopTools version 2.7.5 (Hood 2006).

Data Analysis

Mortality at 3 days, cumulative mortality, pupation, and adult emergence of 2nd instar larvae exposed either by direct spray or to fresh insecticide residues on grapevine shoots, were analyzed using generalized linear models (GLMs) with binomial error distributions and logit link function. Treatment was included as a fixed factor. For mortality and pupation, the numbers of dead and pupated individuals, respectively, were modeled relative to the initial number of larvae tested, whereas adult emergence was modeled as the number of emerged adults relative to the number of pupae. To account for overdispersion, the scale parameter was estimated using the Pearson chi-square

statistic. Accordingly, the overall effect of treatment was evaluated using Wald *F* tests. When a significant treatment effect was detected, estimated marginal means were compared pairwise with Bonferroni-adjusted tests. Statistical significance was set at $\alpha = 0.05$.

Mortality at 3 days of *C. montrouzieri* adults exposed either by direct spray or to fresh insecticide residues on grapevine shoots was calculated for each replicate as the percentage of dead adults relative to the total number of adults exposed. Because mortality was 0 in all control replicates and in all replicates exposed to sulfoxaflor residues, resulting in a highly discrete distribution with extensive ties at 0, differences among treatments were analyzed using a Kruskal–Wallis test followed by pairwise rank comparisons with Bonferroni adjustment for multiple comparisons, setting statistical significance set at $P = 0.05$.

Total fecundity was analyzed using a generalized linear model (GLM) with a negative-binomial error distribution and log link function, with treatment as a fixed factor. Treatment effects were assessed by likelihood-ratio tests. Fertility was analyzed using a GLM with a binomial error distribution and logit link function, with treatment as a fixed factor. To account for overdispersion, the scale parameter was estimated from the Pearson chi-square statistic, and treatment effects were assessed using *F* tests.

Oviposition rate and demographic parameters were analyzed using 1-way ANOVA with Tukey's HSD post-hoc test ($P < 0.05$). Before analyses, data were checked for normality (Shapiro Wilk's W test) and homogeneity of variances (Levene's test). Kaplan-Meier method was used to estimate survival in function of time and treatments. The log-linear rank test followed by the Holm-Sidak pairwise multiple comparison procedures was performed to detect significant differences ($P < 0.05$) among survival curves. Individuals alive at the end of the observation period were accounted as censored. Differences and contributions arising from LTREs were tested against 0 by means of 1-sample *t*-test. Statistical analyses were performed using IBM SPSS Statistics (Version 30), STATISTICA software version 10 (StatSoft Inc.) and SigmaStat 3.0 (Systat Software Inc.).

The reduction coefficient (*E*%) for each insecticide was calculated using the different parameters considered according to the following formulas for larval and adult exposure, respectively:

$$E\% = 100 - \left(\begin{pmatrix} 100 - \%_{\text{Abbott cumulative mortality}} \\ \times \text{RV}_{\text{Percent age of pupation}} \times \text{RV}_{\text{Percentage of adult emergence}} \end{pmatrix} \right),$$

$$E\% = 100 - \left(\begin{pmatrix} 100 - \%_{\text{Adult Abbott mortality}} \\ \times \text{RV}_{\text{Total fecundity}} \times \text{RV}_{\text{Fecundity rate}} \times \text{RV}_{\text{Fertility}} \end{pmatrix} \right),$$

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 (Abbott 1925) when significant differences were identified ($P < 0.05$), otherwise, RVs of 1 were assigned. Mortality percentage after the 3-day exposure period and the *E*% value obtained, were used to rank insecticides according to the IOBC classification criteria for pesticide risk assessment in the laboratory (Direct sprays): 1 = harmless (<30%), 2 = slightly harmful (30%–79%), 3 = moderately harmful (80%–99%), 4 = harmful (>99%), and to the IOBC classification for extended laboratory trials (Exposure on insecticide residue): 1 = harmless (<25%), 2 = slightly harmful (25%–50%), 3 moderately harmful (51%–75%), 4 = harmful (>75%) (Sterk et al. 1999).

Results

Direct Sprays of Larvae and Adults

Bioassays with Larvae

Both sulfoxaflor and acetamiprid significantly affected the mortality of second instar larvae of *C. montrouzieri* after 3 days after direct insecticide exposure as well as cumulative mortality, compared with the control (Table 1). Survival analysis of *C. montrouzieri* revealed significant differences among treatments (Log-rank test, $\chi^2 = 59.244$, df = 2, $P < 0.001$) (Fig. 1A). Larvae exposed to sulfoxaflor and acetamiprid survived for a significantly shorter time than control larvae, with mean survival times (±SE) of 14.2 ± 1.5 days (Holm-Sidak test, $P < 0.025$), 9.1 ± 1.3 (Holm-Sidak test, $P = 0.017$), respectively, compared with 28.5 ± 1.3 days for the control (Holm-Sidak test, $P = 0.05$).

Both insecticides caused a significant reduction in the percentage of pupation compared with the control (Table 1). In contrast, no significant differences were observed among treatments in the percentage of adult emergence from pupae formed

Table 1. Mean (±SE; 95% CI) mortality after 3 days, cumulative mortality, percentage of pupation, and adult emergence of *C. montrouzieri* directly sprayed at the second larval instar

	n	Control	Sulfoxaflor	Acetamiprid	Statistics
Mortality after 3 days (%)	80	2.5 ± 1.4a (0–12)	51.3 ± 7.2b [50.0] (39–64)	75.0 ± 8.0c [74.4] (62–84)	$F_{(2,9)} = 39.52$ $P < 0.001$
IOBC class			2	2	
Cumulative mortality (%)	80	30.0 ± 9.6 (17–47)	62.5 ± 6.0b [46.4] (46–77)	80.0 ± 6.1b [71.4] (64–90)	$F_{(2,9)} = 9.95$ $P = 0.005$
Pupation (%)	80	76.3 ± 5.9 (63–86)	37.5 ± 6.0b [0.49] (25–52)	21.3 ± 6.6b [0.28] (12–35)	$F_{(2,9)} = 16.81$ $P < 0.001$
Adult emergence (%)	[a]	90.4 ± 6.0 (81–97)	100 ± 0.0 [1.00] (100–100)	95.8 ± 4.2 [1.00] (66–99)	$F_{(2,9)} = 1.86$ $P = 0.210$
***E*% (IOBC class)**			73.65 (2)	92.04 (3)	

Within each row, values bearing different letters are significantly different for $P < 0.05$ (GLMs, Bonferroni-adjusted tests). Abbott corrected mortality values and reduction factors appear in squared brackets. Insecticides ranked according to 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%).
[a]Control $n = 61$, sulfoxaflor $n = 30$, acetamiprid $n = 17$.

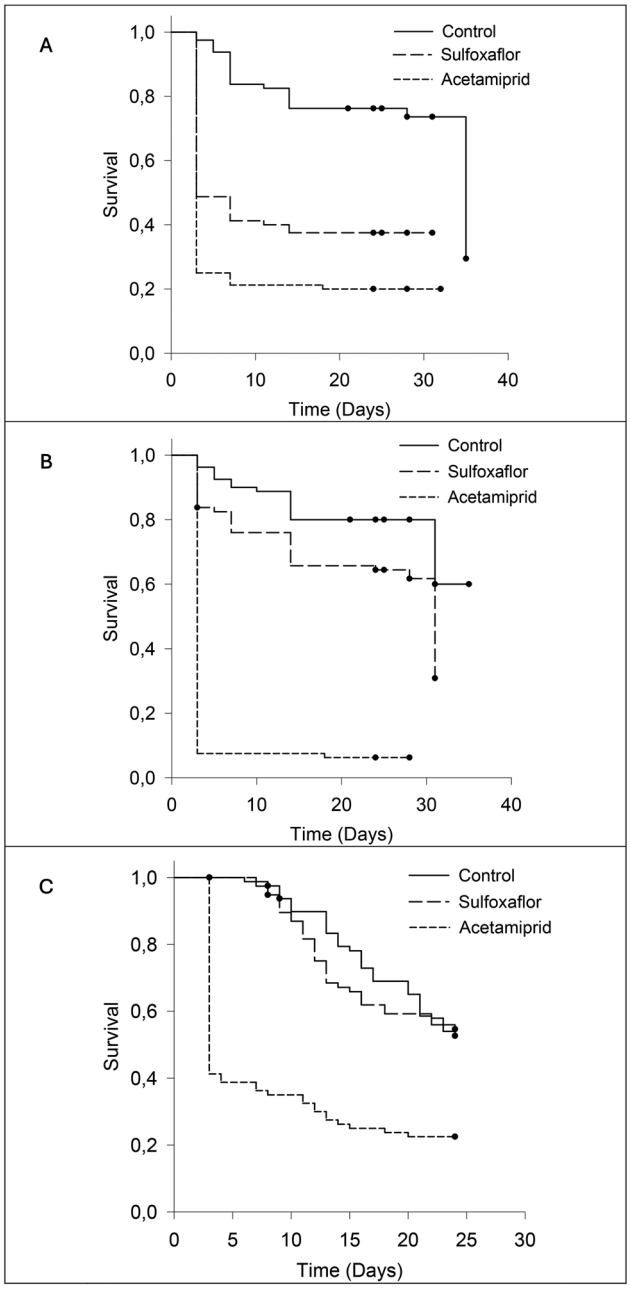


Fig. 1. Survival curves of *C. montrouzieri* (A) directly sprayed at the 2nd larval instar, (B) exposed to fresh residues on grapevine shoot as 2nd instar larvae, and (C) exposed to fresh residues on grapevine shoot as adult. Circles represent censored data. Different letters indicate significant differences ($P < 0.05$, Log rank test followed by Holm-Sidak multiple comparison method). Abbreviations: ACP = acetamiprid, SFX = sulfoxaflor.

by treated larvae (Table 1). Similarly, adult weight did not show any significant differences among treatments (males, $F_{2,8}$ = 0.600, $P = 0.572$; female, $F_{2,8}$ = 0.321, $P = 0.734$; both sexes, $F_{2,9}$ = 0.473, $P = 0.638$) (Fig. S1A).

Based on the coefficient of reduction (E%), sulfoxaflor was classified as slightly harmful (IOBC class 2) to second instar larvae of *C. montrouzieri* in direct spray trials. Acetamiprid, which generally produced more severe effects, was classified as moderately harmful (IOBC class 3), despite not differing significantly from sulfoxaflor when individual parameters were considered separately (Table 1).

Bioassays with Adults

Compared with the control, acetamiprid significantly increased adult mortality 3 days after direct exposure (Table 2). In contrast, no significant increase in the mortality was detected in adults treated with sulfoxaflor. According to IOBC classification criteria, acetamiprid was ranked as slightly harmful (class 2) to *C. montrouzieri* adults, whereas sulfoxaflor was classified as harmless (class 1) (Table 2). Although acetamiprid was ranked as slightly harmful to *C. montrouzieri* adults (class 2), its Abbott-corrected mortality value was close to the upper limit of the class 2 range (30% to 79%).

Exposure to Insecticide Residues

Bioassays with Larvae

Both sulfoxaflor and acetamiprid significantly increased larval mortality 3 days after of exposure to insecticide residues on plants compared with the control (Table 3). However, acetamiprid caused significantly higher short-term mortality than sulfoxaflor and was therefore classified as harmful (class 4), whereas sulfoxaflor was classified as harmless (class 1) based on 3-day mortality (Table 3). In contrast, no significant increase in cumulative mortality was observed in larvae exposed to sulfoxaflor compared with the control was detected, whereas acetamiprid caused a marked increase in cumulative mortality (Table 3).

Survival analysis of *C. montrouzieri* revealed significant differences among treatments (Log-rank test, $\chi^2 = 143.44$, df = 2, $P < 0.001$) (Fig. 1B). Larvae exposed to acetamiprid survived for a significantly shorter time than control larvae, with a mean survival time of 4.8 ± 0.7 days (mean ± SE) (Holm-Sidak test, $P = 0.017$). In contrast, no significant differences were detected between sulfoxaflor-exposed larvae and the control (Holm-Sidak test, $P = 0.05$) with a mean survival time of 22.7 ± 1.3 and 29.0 ± 1.4 days (mean ± SE), respectively.

The percentage of pupation did not differ significantly between sulfoxaflor and control treatments (Table 3), indicating the absence of detectable sublethal effects on this parameter. Conversely, acetamiprid caused a substantial reduction in pupation rate (Table 3). No significant differences were observed among treatments in adult emergence from pupae formed by exposed larvae (Table 3), or in the weight of emerged adults (males, $F_{1,6} = 0.028$, $P = 0.872$; female, $F_{1,6}$ = 0.633, $P = 0.456$; both sexes, $F_{1,6}$ = 0.560, $P = 0.483$) (Fig. S1B).

Based on the coefficient of reduction (E%), sulfoxaflor was classified as slightly harmful (IOBC class 2) to second instar larvae of *C. montrouzieri* exposed to fresh insecticide residues on grapevine shoot, whereas acetamiprid was classified as harmful (IOBC class 4) (Table 3).

Bioassays with Adults

In the adult bioassay, only acetamiprid caused mortality significantly higher than the control during the 3-day exposure period (Table 4). In contrast, sulfoxaflor did not cause any adult mortality following exposure to fresh insecticide residues on grapevine shoots. Neither acetamiprid nor sulfoxaflor significantly affected adult fecundity, oviposition rate and fertility compared with the control (Table 4).

Survival analysis revealed significant differences among treatments (Log-rank test, $\chi^2 = 53.02$, df = 2, $P < 0.001$) (Fig. 1C). Adults exposed to acetamiprid survived for a significantly shorter time than control individuals, with a survival time of

Table 2. Mean (±SE; 95% CI) mortality of *C. montrouzieri* adults 3 days after direct sprays

	n	Control	Sulfoxaflor	Acetamiprid	Statistics
Mortality after 3 days (%)	60	0.0 ± 0.0a	13.3 ± 8.8a	75.3 ± 10.3 b	$H_{(2,N=18)}$ = 14.23
		(0–0)	(0–19)	(46–98)	P < 0.001
IOBC class			1	2	

Different letters indicate significant differences as detected by Kruskal–Wallis test followed by pairwise rank comparisons with Bonferroni adjustment for multiple comparisons for P < 0.05. Insecticides ranked according to 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%).

Table 3. Mean (±SE; 95% CI) mortality after 3 days, cumulative mortality, percentage of pupation and adult emergence of *C. montrouzieri* exposed to fresh insecticide residues on grapevine shoot as second instar larvae

	n	Control	Sulfoxaflor	Acetamiprid	Statistics
Mortality after 3 days (%)	80	2.6 ± 1.5a	16.6 ± 3.8a [14.4]	92.5 ± 6.0b [92.3]	$F_{(2,9)}$ = 49.02
		(0–15)	(8–31)	(80–97)	P < 0.001
IOBC class			1	4	
Cumulative mortality (%)	80	20.3 ± 6.5a	42.4 ± 5.3a [27.7]	93.8 ± 4.7b [92.2]	$F_{(2,9)}$ = 26.00
		(10–36)	(28–58)	(81–98)	P < 0.001
Pupation (%)	80	80.9 ± 5.7a	65.3 ± 3.6a [1.00]	15.0 ± 10.0b [0.19]	$F_{(2,9)}$ = 26.26
		(66–91)	(49–79)	(2–21)	P < 0.001
Adult emergence (%)	[a]	98.2 ± 1.8	87.9 ± 5.8 [1.00]	90.0 ± 10.0 [1.00]	$F_{(2,9)}$ = 2.66
		(88–100)	(75–95)	(33–98)	P = 0.139
***E*% (IOBC class)**			27.75 (2)	98.55 (4)	

In each row values bearing different letters are significantly different for P < 0.05 (GLMs, Bonferroni-adjusted tests). Mortality values corrected by Abbott's formula and reduction factors appear in squared brackets. Insecticides ranked according to the IOBC classification for extended laboratory trials: 1 = harmless (<25%), 2 = slightly harmful (25%–50%), 3 moderately harmful (51%–75%), 4 = harmful (>75%).
[a]Control n = 64, sulfoxaflor n = 51, acetamiprid n = 6.

Table 4. Mean (±SE; 95% CI) mortality after 3 days and effects on biological traits of females of *C. montrouzieri* exposed to fresh residues of insecticides as adults on vine shoot

	n	Control	Sulfoxaflor	Acetamiprid	Statistics
Mortality after 3 days (%)	80	0.0 ± 0.0a	0.0 ± 0.0a	58.4 ± 12.3b [58.4]	$H_{(2,N=12)}$ = 10.46
		(0–0)	(0–0)	(19–97)	P = 0.005
IOBC class			1	3	
Fecundity (total eggs)	80	388.5 ± 127.2	396.5 ± 91.7 [1.00]	134.5 ± 50.0 [1.00]	$\chi^2_{(2)}$ = 2. 63
		(142–1014)	(145–1034)	(49–351)	P = 0.268
Oviposition rate (eggs/ female/day)	80	2.59 ± 0.31	2.49 ± 0.93 [1.00]	3.35 ± 1.18 [1.00]	$F_{(2,9)}$ = 0.28
		(1.59–3.59)	(0–5.45)	(0–7.09)	P = 0.7601
Fertility (% hatched eggs)	80	70.4 ± 2.73	68.6 ± 5.8 [1.00]	57.4 ± 6.5 [1.00]	$F_{(2,9)}$ = 0.70
		(64–80)	(62–78)	(48–76)	P = 0.52
***E*% (IOBC class)**			0.0 (1)	58.4 (3)	

In each row, values bearing different letters are significantly different for P < 0.05 (Kruskal–Wallis test followed by pairwise rank comparisons with Bonferroni adjustment for multiple comparisons). Abbott mortality values and reduction factors appear in squared brackets. Insecticides ranked according to the IOBC classification for extended laboratory trials: 1 = harmless (<25%), 2 = slightly harmful (25%–50%), 3 = moderately harmful (51%–75%), and 4 = harmful (>75%).

9.3 ± 1.0 days (mean ± SE) (Holm-Sidak test, P = 0.017). In contrast, no significant differences were detected between sulfoxaflor-exposed adults and control adults (Holm-Sidak test, P = 0.05) with mean survival times of 19.0 ± 0.7 and 20.1 ± 0.6 days (mean ± SE), respectively. Based on the coefficient of reduction (*E*%), acetamiprid was classified as moderately harmful (IOBC class 3), whereas sulfoxaflor was scored as harmless (IOBC class 1) (Table 4).

No detrimental effects compared to the control was detected for the stable population parameters calculated using Leslie matrix models for either insecticide treatments (Table 5). Specifically, no significant differences among treatments were observed for any of the demographic parameters analyzed. During immature stages, life expectancy (e_x) of *C. montrouzieri* exposed to acetamiprid was lower than that of individuals in the control and sulfoxaflor treatments (Fig. 2A). These differences decreased for individuals older than 36 days, corresponding approximately to the adult stage. The expected number of reproductive events during a female's lifetime was lower than the control until approximately 35 days of age but became higher from 37 days onward in adults exposed to acetamiprid residues (Fig. 2B). Using LTREs decomposition, the effect of insecticide exposure on λ were decomposed into contributions arising from the effect on each age-specific vital rate (ie survival, P_i and fecundity, F_i) (Figs 3 and 4). LTREs showed that sulfoxaflor exposed *C. montrouzieri* females

Table 5. Stable population parameters (mean ± SE; 95% CI) relative to cohorts of *C. montrouzieri* exposed to fresh residues of insecticides on grapevine shoot at adult stage

Treatment	*n*	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}	Damping ratio, ρ
			T, days	μ_1, days				
Control	80	11.4 ± 3.7 (0–23.2)	44.6 ± 1.0 (41.4–47.7)	45.3 ± 1.2 (41.5–49.1)	6.1 ± 0.6 (4.1–8.1)	0.0515 ± 0.0063 (0.0315–0.0715)	1.0529 ± 0.0066 (1.0318–1.0741)	1.0069 ± 0.0020 (1.0006–1. 0133)
Sulfoxaflor	77	8.7 ± 2.2 (1.8–15.5)	44.4 ± 1.7 (38.9–49.9)	45.0 ± 1.9 (38.9–51.1)	8.1 ± 2.2 (0.9–15.2)	0.0440 ± 0.0079 (0.0188–0.0692)	1.0451 ± 0.0082 (1.0189–1.0712)	1.0057 ± 0.0015 (1.0009–1.0105)
Acetamiprid	80	4.9 ± 1.2 (1.2–8.6)	46.2 ± 0.9 (43.5–49.0)	46.5 ± 0.9 (43.5–49.5)	9.8 ± 1.4 (5.5–14.2)	0.0325 ± 0.0047 (0.0176–0.0473)	1.0330 ± 0.0048 (1.0176–1.0484)	1.0034 ± 0.0008 (1.0008–1.0060)
ANOVA		$F_{2,9}$ = 1.62 P = 0.2515	$F_{2,9}$ = 0.65 P = 0.5473	$F_{2,9}$ = 0.33 P = 0.7305	$F_{2,9}$ = 1.47 P = 0.2802	$F_{2,9}$ = 2.23 P = 0.1637	$F_{2,9}$ = 2.23 P = 0.1633	$F_{2,9}$ = 1.00 P = 0.2959

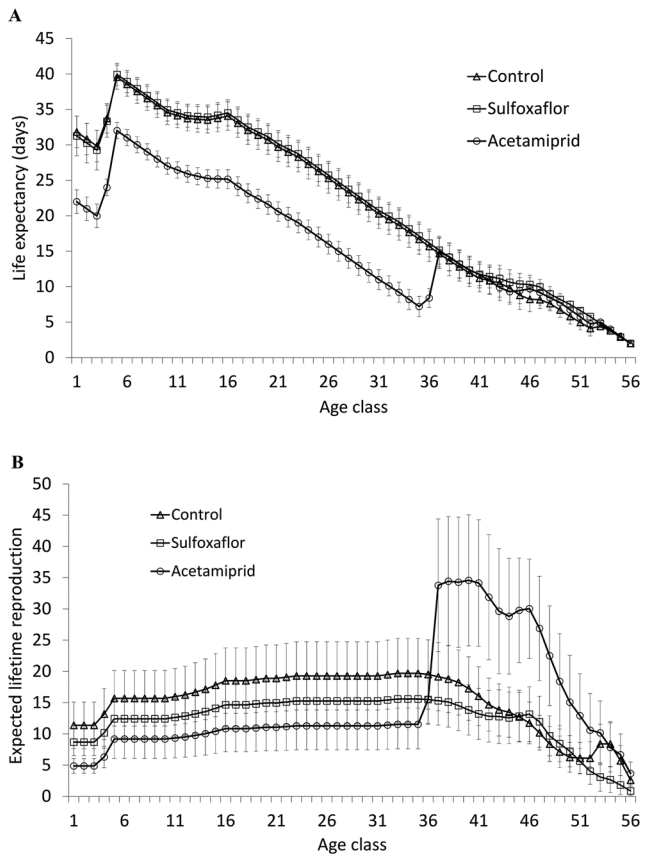


Fig. 2. Life expectancy (mean ± SE) (A) and number of lifetime reproductive episodes (mean ± SE) (B) of *C. montrouzieri* populations exposed to fresh residues of insecticides on vine shoot at adult stage.

suffered no significant survival or fecundity reductions or increases during the entire adult life (from 35 days onward) or fertility variations (considered as egg survival at age 3 and 4 days) with respect to the control (Fig. 3). Conversely, acetamiprid exposed females suffered a significant fecundity reduction between 35 and 37 days of age and a fecundity advantage later in adult life, along with a sharp survival disadvantage earlier in adult life corresponding to the 3-day insecticide exposure period (Fig. 4). The trade-off between initial fecundity reduction and later increase and survival reduction barely balanced, anyhow keeping λ not significantly different in comparison to the control.

Population projection starting from stable age distribution ($\mathbf{w}_1$, see supplemental material) was used to calculate the delay in population growth index (Fig. S2). However, since the population growth index at the asymptote (λ) was not significantly different among the 3 treatments (Table 5), the short-term population projection modeled starting from an initial cohort of 100 young adults (Fig. 5), should result more informative. This scenario is particularly relevant because adults represent the life stage more frequently released in the augmentative biological control programs of *C. montrouzieri* in vineyards (Burgio et al. 2025) and this stage naturally colonizes vineyards. Acetamiprid exposed population showed a decrease until day 9 mainly due to acute mortality, whereas sulfoxaflor exposed adults did not exhibit a similar trend (Fig. 5).

Discussion

This study provides a comprehensive assessment of the lethal and sublethal effects of sulfoxaflor on the mealybug destroyer *C. montrouzieri* by integrating standardized IOBC laboratory protocols with demographic modeling and LTRE analyses. Overall, sulfoxaflor was substantially less harmful than acetamiprid to both immature and adult stages, although larvae were consistently more susceptible than adults. Demographic analyses indicated that sulfoxaflor did not significantly affect the population performance of surviving adults, whereas the effects of acetamiprid were mainly associated with acute mortality occurring immediately after exposure. These findings highlight that insecticides sharing similar mode of action as nicotinic acetylcholine receptor (nAChR) agonists may differ considerably in their selectivity toward biological control agents and reinforce the importance of evaluating pesticide compatibility using multiple biological endpoints rather than acute mortality alone.

The lower toxicity of sulfoxaflor observed in the present study agrees with previous literature reporting relatively high selectivity of this sulfoximine insecticide toward adult coccinellids. Similar responses have been described for *Adalia bipunctata* (Garzón et al. 2015, Depalo et al. 2025), *Hippodamia convergens* (Colares et al. 2016), *Harmonia axyridis* (He et al. 2020), *Coccinella septempunctata* (Atta et al. 2021, Wang et al. 2023) and, more recently, *C. montrouzieri* (Singh et al. 2025). Although differences among studies exist

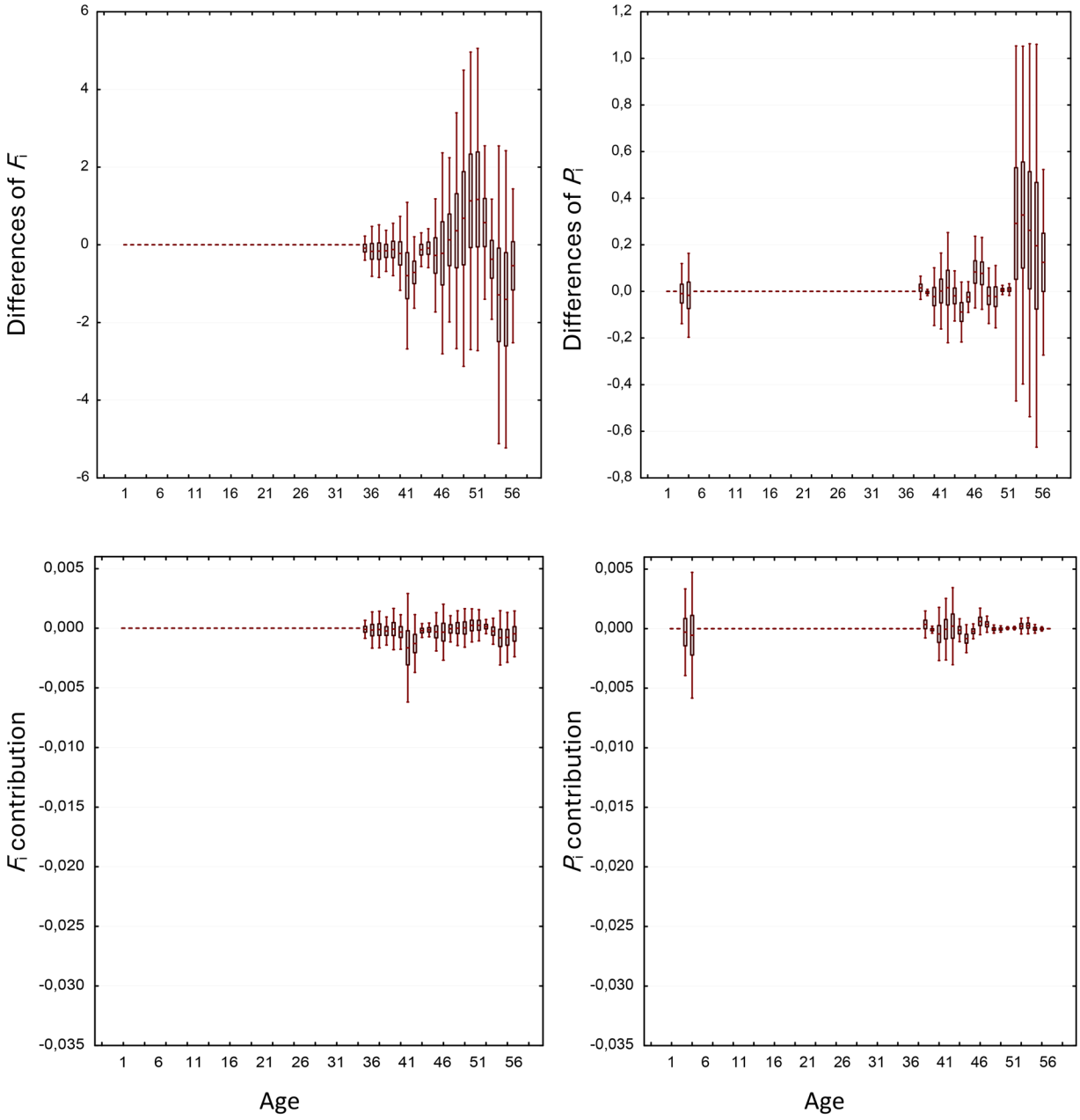


Fig. 3. LTRE decomposition analysis of treatment effects on population growth rate (λ). (Top) Differences in age-specific fecundity (F) and age-specific survival (P_i) between populations of *C. montrouzieri* exposed at the adult stage to fresh residues on grapevine shoot either of sulfoxaflor or water control. (Bottom) Contributions of these differences on population growth rate (λ). Negative values indicate disadvantages or negative contributions to λ of sulfoxaflor relative to the control. Lines represent mean ($n = 4$), boxes represent ± SE and whiskers ±95% confidence interval. In all values $P > 0.05$, 1 sample *t*-test against 0.

because of variations in exposure routes, experimental protocols, developmental stages, and application rates, adult coccinellids generally appear considerably less susceptible to sulfoxaflor than to conventional neonicotinoids. In contrast, the high toxicity of acetamiprid observed in the present work is consistent with previous investigations demonstrating its poor compatibility with *C. montrouzieri* and other coccinellid predators used in augmentative biological control (Cloyd and Dickinson 2006, Halappa et al. 2013, Halikatti et al. 2015, Rahmouni et al. 2015). Together, these studies suggest that sulfoxaflor may pose a lower laboratory hazard to beneficial predators than conventional neonicotinoids. However, this evidence should be regarded as an indication for further assessment rather than as demonstration of compatibility within IPM programs.

Despite the overall low toxicity of sulfoxaflor, our results clearly demonstrate that susceptibility depends strongly on predator developmental stage. Larvae were consistently more affected than adults irrespective of the exposure route, a pattern that has repeatedly been reported for other coccinellid species. Garzón et al. (2015) observed that *A. bipunctata* larvae were considerably more susceptible than adults after residual exposure, while Colares et al. (2016), He et al. (2020), Wang et al. (2023), and Skouras et al. (2026) similarly reported more sensitivity of

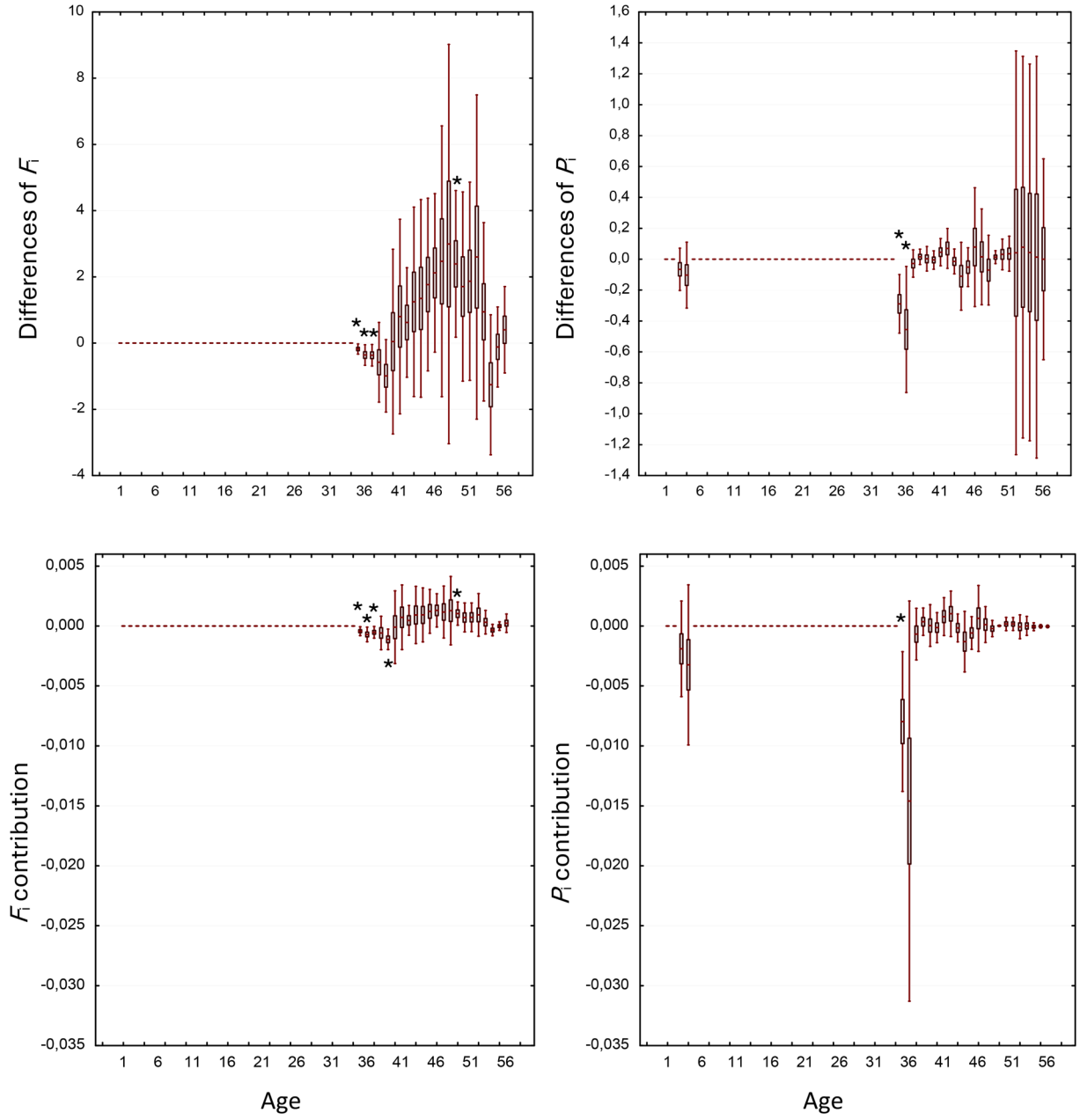


Fig. 4. LTRE decomposition analysis of treatment effects on population growth rate (λ). (Top) Differences in age-specific fecundity (F_i) and age-specific survival (P_i) between populations of *C. montrouzieri* exposed at the adult stage to fresh residues on grapevine shoot either of acetamiprid or water control. (Bottom) Contributions of these differences on population growth rate (λ). Negative values indicate disadvantages or negative contributions to λ of acetamiprid relative to the control. Lines represent mean ($n=4$), boxes represent ± SE and whiskers ±95% confidence interval. * $P<0.05$, 1 sample *t*-test against 0.

immature stages in *H. convergens*, *H. axyridis*, and *C. septempunctata*. Such consistency across several coccinellid species suggests that stage-specific susceptibility represents a general biological pattern rather than a species-specific response.

Several mechanisms may explain this difference. Larvae spend most of their time actively searching for prey on treated plant surfaces, thereby increasing the probability of contact with fresh insecticide residues. Physiological differences can contribute as well. Developmental changes in detoxification capacity have been proposed to increase tolerance during adult stages, while differences in cuticular characteristics and body size may further influence insecticide penetration and distribution (Rocha et al. 2011, Hodek et al. 2012). Compounds considered moderately lipophilic have an octanol-water partition coefficient (log P_{ow}) value between 0.5 and 3.5 (Jeschke et al. 2013). The relatively low log P_{ow} value of sulfoxaflor (0.8) should contribute to limited penetration into *C. montrouzieri* larvae after direct spray exposure, but the waxy covering of *C. montrouzieri* larvae does not provide a continuous protective barrier and therefore cannot completely prevent insecticide uptake following direct spray applications. The greater toxicity observed after direct application than after exposure to residues further suggests that the initial dose received by larvae is a key determinant of subsequent mortality.

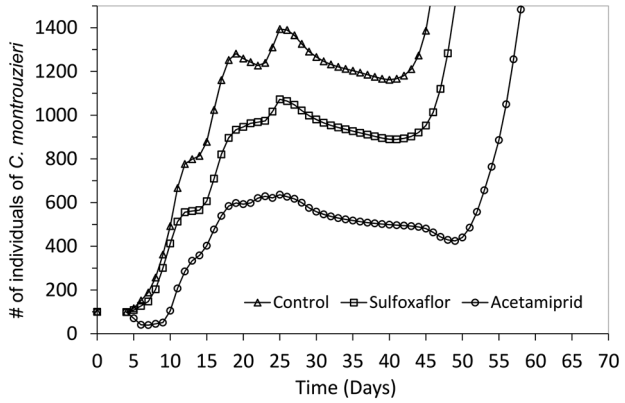


Fig. 5. Projection of total population size of *C. montrouzieri* exposed to fresh residues on grapevine shoot at adult stage starting from an initial population of 100 young adults.

Interestingly, while the greater susceptibility of larvae agrees with previous investigations, the magnitude of sublethal responses differed from those reported for other coccinellids. Recent studies on *C. septempunctata* demonstrated that topical exposure to sulfoxaflor not only reduced larval survival but also significantly decreased adult body mass, fecundity, intrinsic rate of increase, finite rate of increase, and net reproductive rate, ultimately impairing projected population growth (Skouras et al. 2026). Also, demographic analyses performed on *Hippodamia variegata* showed reductions in longevity, reproductive performance and population growth parameters following sublethal exposure to sulfoxaflor (Skouras et al. 2023). By contrast, no significant reductions in fecundity, fertility, or asymptotic demographic parameters were detected in adult *C. montrouzieri* exposed to sulfoxaflor residues in the present study. These contrasting responses indicate that susceptibility to sulfoxaflor cannot be generalized across Coccinellidae and likely depends on species-specific physiological, behavioral, and ecological characteristics. Such differences emphasize the importance of evaluating each biological control agent individually rather than extrapolating pesticide selectivity among related taxa.

The mechanisms underlying these interspecific differences remain poorly understood but likely involve more than differences in acute neurotoxicity. Sulfoxaflor and neonicotinoids share the same primary target, acting as agonists of insect nicotinic acetylcholine receptors, but their receptor interactions differ substantially (Watson et al. 2011, 2021, Sparks et al. 2013). Consequently, compounds belonging to IRAC Group 4 may exhibit different toxicological profiles despite affecting the same receptor family. Beyond direct effects on survival, increasing evidence indicates that nAChR agonists may alter a wide range of physiological processes influencing predator fitness. Laboratory studies have shown that sulfoxaflor exposure may reduce prey consumption (Dai et al. 2020, Wang et al. 2023, Skouras et al. 2026), delay development (Skouras et al. 2023, 2026), decrease body mass and reproductive performance (Skouras et al. 2026), and impair demographic fitness even when mortality remains relatively limited (He et al. 2020, Skouras et al. 2023). At the molecular level, Wang et al. (2023) demonstrated that sulfoxaflor suppresses vitellogenin expression in *C. septempunctata*, providing evidence that reproductive impairment may result from disruption of endocrine regulation rather than solely from reduced food intake. Similarly, behavioral alterations affecting locomotion, prey searching, and feeding activity have been reported following exposure to systemic insecticides in several coccinellid species (Guedes et al. 2016, Dai et al. 2020). Although none of these physiological mechanisms were directly investigated in the present study, they provide plausible explanations for the demographic responses reported in the literature and highlight that apparently small sublethal effects may translate into consequences over successive generations.

Nevertheless, our results also demonstrate that demographic consequences are not general among coccinellids. Contrary to previous studies in which reductions in reproduction represented an important component of pesticide toxicity, adult *C. montrouzieri* surviving sulfoxaflor exposure maintained reproductive performance comparable to untreated individuals. This finding suggests that the demographic resilience of this predator may be greater than that reported for several aphidophagous coccinellids and further supports the value of combining demographic analyses with conventional toxicity tests when characterizing pesticide effects on population performance under laboratory conditions.

A major strength of the present study is the integration of conventional IOBC protocols with demographic modeling. Current laboratory risk assessment procedures developed by the IOBC primarily rely on corrected mortality and short-term reproductive parameters (Sterk et al. 1999). These standardized assays constitute primary laboratory evaluations and are valuable for comparative hazard assessment, but they do not reproduce the complexity of exposure and population dynamics under field conditions. Recent work has questioned whether current IOBC procedures may underestimate or overestimate ecological effects because they do not explicitly identify which demographic processes are responsible for changes in population growth (Stark and Banks 2024). Matrix population models complement this approach by integrating survival and reproduction across the entire life cycle and by evaluating how individual vital rates contribute to population dynamics.

In the present study, demographic modeling indicated limited effects of sulfoxaflor on adult *C. montrouzieri* under the tested laboratory conditions. Neither the asymptotic population growth rate nor the remaining demographic descriptors differed significantly from the control, indicating that surviving adults maintained their reproductive potential and population performance. More importantly, LTRE decomposition allowed identification of the demographic mechanisms underlying pesticide responses. Although sulfoxaflor produced negligible contributions of both survival and fecundity to changes in population growth, acetamiprid primarily affected λ through a marked reduction in adult survival immediately after exposure, while changes in reproductive performance contributed comparatively little. Consequently, although acetamiprid temporarily reduced life expectancy and expected lifetime reproduction, the surviving females largely maintained their reproductive capacity. This distinction could not have been identified using conventional IOBC classifications alone and demonstrates the additional explanatory value of demographic decomposition analyses within a laboratory assessment framework.

These findings also provide an interesting perspective when compared with recent demographic studies on other coccinellids. Skouras et al. (2026) concluded that mortality alone underestimated the ecological consequences of sulfoxaflor because significant reductions in predation, fecundity, and

life-table parameters contributed substantially to population decline in *C. septempunctata*. Similarly, studies on *H. axyridis* and *H. variegata* reported measurable demographic consequences arising from sublethal exposure despite relatively limited mortality (He et al. 2020, Skouras et al. 2023). By contrast, our LTRE analysis indicates that, in *C. montrouzieri*, demographic responses were determined principally by acute mortality, whereas surviving adults experienced little evidence of persistent reproductive impairment. Rather than contradicting previous investigations, these contrasting outcomes demonstrate that the demographic pathways through which insecticides affect predator populations differ among coccinellid species. Consequently, understanding pesticide selectivity requires not only estimating population growth rates but also identifying the demographic mechanisms responsible for those changes.

The transient population projections further reinforce this interpretation. Stable population parameters describe long-term population dynamics once the stable age distribution has been reached, but recently established predator populations rarely satisfy this assumption under field conditions. Colonization of vineyards by naturally occurring *C. montrouzieri* or augmentative releases generally begin with adult individuals, generating transient population dynamics that differ substantially from asymptotic growth (Koons et al. 2005, Stott et al. 2011). Under these circumstances, even relatively short delays in population development may have important ecological consequences. In our simulations, acetamiprid delayed predator population development by approximately 9 days, whereas sulfoxaflor produced no comparable effect. Considering that several economically important mealybug species may double their populations within only a few days under favorable environmental conditions, a delay of this magnitude could potentially weaken biological control if comparable dynamics occurred under field conditions. Transient demographic responses may therefore provide useful information beyond asymptotic population parameters, but these laboratory-based projections should not be interpreted as direct predictions of predator performance in augmentative biological control programs.

Although the demographic performance of *C. montrouzieri* was largely unaffected by sulfoxaflor under our experimental conditions, the present results should not be interpreted as evidence that the insecticide is harmless under field conditions. The laboratory design standardized exposure and therefore could not reproduce several processes that may alter risk in cropping systems, including degradation of residues over time, repeated applications, behavioral avoidance of treated surfaces, changes in prey availability, and variation in temperature and other environmental factors. In addition, sublethal effects on biological functions beyond the endpoints measured here may influence predator efficacy. Previous studies have demonstrated that sulfoxaflor and other systemic insecticides may reduce prey consumption, alter functional response, impair locomotion and prey-searching behavior, delay development, modify reproductive physiology, and induce transgenerational effects in coccinellids (Dai et al. 2020, Skouras et al. 2023, 2026, Wang et al. 2023). Such alterations may reduce the ecological efficiency of predators without necessarily affecting their immediate survival or asymptotic population growth, thus future investigations should address the physiological mechanisms responsible for the interspecific differences observed among coccinellids.

From an applied perspective, the comparatively low laboratory toxicity of sulfoxaflor to adult *C. montrouzieri* identifies this insecticide as a candidate for further semi-field and field evaluation rather than establishing its compatibility with IPM programs. The lower toxicity observed for adults is potentially relevant because augmentative biological control programs commonly release adults into vineyards and other perennial crops, whereas the greater susceptibility of larvae indicates that developmental stage may strongly influence risk. These laboratory results can therefore help define conditions to examine in higher-tier studies, including application timing and the extent of direct exposure to larval stages. Recommendations on selective use or application timing, however, require validation under realistic exposure scenarios in which residue dynamics, repeated treatments, predator behavior, prey availability and environmental variability can modify both exposure and biological response.

Finally, our results reinforce the value of expanding pesticide risk assessment beyond conventional mortality-based evaluations. Integrating demographic modeling with standardized IOBC protocols provided mechanistic information that improved interpretation of insecticide effects on predator populations under controlled laboratory conditions. At the same time, IOBC laboratory assays represent first-tier testing and cannot by themselves establish compatibility with biological control or IPM programs. Field performance may differ because residues degrade over time, applications may be repeated, predators may avoid treated surfaces, prey availability may change, and temperature, humidity, and other environmental conditions may alter both exposure and toxicity. Semi-field and field studies are therefore needed to determine whether the patterns observed here persist under realistic cropping conditions. Combining laboratory, demographic, semi-field, and field approaches will provide a more realistic basis for evaluating pesticide selectivity toward biological control agents and for informing IPM decisions.

Author Contributions

Laura Depalo (Conceptualization [equal], Data curation [equal], Investigation [equal], Writing—original draft [equal]), Antonio Masetti (Writing—review & editing [equal]), Edison Pasqualini (Supervision [equal]), and Alberto Lanzoni (Data curation [equal], Investigation [equal], Methodology [equal], Writing—original draft [equal])

Supplementary Material

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Conflicts of Interest

The authors declare no competing interests.

Data Availability

The data that support the findings of this study are openly available in Zenodo at https://doi.org/10.5281/zenodo.20136631

Declaration of Generative AI and AI-Assisted Technologies in the Manuscript Preparation Process

During the preparation of this work the authors used ChatGPT in order to improve the accuracy of the English language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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