Synergistic Enhancement of Acetamiprid Toxicity by Herbicide and Fungicide: Triple Mixture Outperforms Binary Combination

Jan Erik Sedlmeier[1*], Emma Gamer[1], Birgit Höglinger[2], Frank Walker[2], Carsten A Brühl[3], Georg Petschenka[1,4*]

[1]Department of Applied Entomology, University of Hohenheim, Stuttgart, Germany
[2]Institute of Phytomedicine, University of Hohenheim, Stuttgart, Germany
[3]Community Ecology & Ecotoxicology, University of Kaiserslautern-Landau, Landau, Germany
[4]KomBioTa – Center for Biodiversity and Integrative Taxonomy, University of Hohenheim and State Museum of Natural History, Stuttgart, Germany

[*]Corresponding authors
Jan Erik Sedlmeier
Department of Applied Entomology, Institute of Phytomedicine, University of Hohenheim, Stuttgart, Germany; jan.sedlmeier@uni-hohenheim.de
Georg Petschenka
Department of Applied Entomology, Institute of Phytomedicine, University of Hohenheim, Stuttgart, Germany; georg.petschenka@uni-hohenheim.de

Author ORCiDs
Jan Erik Sedlmeier: 0000-0002-4694-3894
Emma Gamer: 0009-0003-4241-7840
Frank Walker: 0009-0001-3738-4840
Carsten A. Brühl: 0000-0003-1332-535X
Georg Petschenka: 0000-0002-9639-3042

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Abstract

Pesticides are considered one of the main drivers of insect decline, and complex pesticide mixtures are frequently detected in our landscapes. However, the risk posed by mixtures of active substances is not systematically assessed. In this study using acute contact dose-response testing, we show that binary and triple combinations of relevant surrogates for major pesticide classes can lead to synergistic toxic effects on the non-target insect *Oncopeltus fasciatus*. First, we tested the individual active substances acetamiprid (insecticide), fluopyram (fungicide) and terbuthylazine (herbicide), detecting acute toxic effects only for acetamiprid. However, the combination of acetamiprid and terbuthylazine significantly increased toxicity compared to acetamiprid alone and the combination of acetamiprid and fluopyram with the effective dose 50 being 2.7 and 2.3 times lower respectively. Strikingly, a triple mixture of all active substances exceeded the toxicity of any other treatment. While the exact mechanisms remain unclear, we speculate that increased physiological stress contributes to the observed synergistic effects, as terbuthylazine and fluopyram have been shown to interfere, though to varying extents, with cellular energy metabolism, oxidative balance, detoxification and neurotransmission in several non-target organisms. These results are concerning and highlight the ecotoxicological uncertainty posed by traces of complex pesticide mixtures present in the air, soil, water and plants across landscapes. We therefore propose a systematic assessment of realistic exposure scenarios reflecting both agricultural practices and observed environmental mixtures.

1. Introduction

Pesticide contamination of the environment caused by intensified agriculture is considered to be one of the major drivers of the unprecedented decline in insect biomass, species richness and abundance (Geiger et al. 2010; Sánchez-Bayo and Wyckhuys 2019; Wagner et al. 2021; Dicks et al. 2021). Several recent studies document widespread contamination of landscapes with mixtures of agrochemicals (Silva et al. 2023; Brühl et al. 2024; Mauser et al. 2025). These mixtures, present in different matrices (e.g. soil, vegetation, water), consist of varying combinations of active substances, often representing several major pesticide classes, such as insecticides, herbicides, and fungicides, simultaneously. Such mixtures are found not only in agricultural areas but also in nature conservation zones and even on remote mountain peaks. A recent study found 140 individual mixtures of active substances (containing two or more components) in soil, vegetation and water samples from the Upper Rhine Valley in Germany (Mauser et al. 2025). In these mixtures, individual detected amounts of active substances add up to considerable total concentrations (see e.g. Brühl et al. 2024; Honert et al. 2025; Mauser et al. 2025). Another study detected a total of 109 individual pesticides in the ambient air across Germany. A median of 19 substances was detected per sampling site, with a maximum of 36 substances. The sampling sites included forests and national parks and the substances detected were herbicides, fungicides, insecticides, repellents, growth

regulators, and metabolites of the aforementioned substances (Kruse-Plaß et al. 2021). Based on these measurements, Gandara et al. (2024) exposed *Drosophila melanogaster* larvae to nine pesticides at environmentally relevant concentrations. Although this mixture did not include any insecticides, this treatment resulted in behavioral changes and reduced egg-laying capacity of females. These results suggest that pesticide mixtures at sublethal, environmentally relevant concentrations can impact the fitness of insect populations.

Current risk assessment of plant protection products in the EU focuses on individual active substances and formulated products. Some mixtures are tested as part of the formulation evaluation process. These formulations may contain more than one active substance (e.g. two fungicides with different modes of action for resistance management). In addition, synergistic effects of certain combinations of pesticide classes, such as neonicotinoids or pyrethroids in combination with azole fungicides, on pollinator toxicity are known and therefore considered in application regulations (i.e. tank-mix regulations). However, there is currently no systematic assessment of the ecological risk posed by mixtures of active substances. This is alarming given the 425 active substances currently approved in the EU (European Commission n.d.) and the resulting potential combinations, as well as the numerous mixtures that have been detected in the environment. Similarly, most ecotoxicological research is also focused on the effects of individual substances with a bias towards pollinators and neglecting herbivorous insects. The result is a major knowledge gap regarding combined toxicity of active substances towards non-target insects (Tosi et al. 2022).

In this study, we address this research gap by testing the effects of a combination of three active ingredients as surrogates for the major pesticide classes of (1) insecticides, (2) herbicides and (3) fungicides.

As representatives, we chose the active substances acetamiprid (insecticide, CAS: 135410-20-7), fluopyram (fungicide, CAS: 658066-35-4) and terbuthylazine (herbicide, CAS: 5915-41-3). The substances were chosen based on their agricultural but also ecotoxicological relevance as detailed below.
Acetamiprid is a neurotoxic neonicotinoid insecticide that can act as a contact or systemic insecticide. It binds to the post-synaptic nicotinic acetylcholine receptor, disrupting neural transmission (IRAC Class 4A; IRAC n.d.). We chose acetamiprid because it is the last member of the controversial group of neonicotinoids still registered for field use in the EU and used in arable crops, fruit- and wine growing. Although it is less harmful to pollinators than the previously banned neonicotinoids imidacloprid, thiacloprid, clothianidin and thiamethoxam, recent studies have raised concerns about the environmental safety of acetamiprid regarding non-target insect toxicity (Varga-Szilay and Tóth 2022; Sedlmeier et al. 2025). In addition, acetamiprid was detected in environmental samples in Europe, including protected areas (Brühl et al. 2024; Mauser et al. 2025). It is also known that a combination of neonicotinoids and certain fungicides can result in synergistic toxicity, meaning that the combined effect exceeds the sum of the individual toxic effects. This is particularly true for

fungicides from the class of sterol biosynthesis inhibitors (SBIs; Sgolastra et al. 2017; Schuhmann et al. 2022). However, a recent study by Manzer et al. (2024) demonstrated that even non-SBI fungicides, can exhibit synergistic effects when combined with acetamiprid, leading to enhanced toxicity in non-target insects.

Among the agrochemical residues detected in landscapes, the fungicide fluopyram and the herbicide terbuthylazine were often the most frequently detected substances. In addition, fluopyram is often found at comparably high concentrations in vegetation, soil and water (Honert et al. 2025; Mauser et al. 2025).
Fluopyram inhibits the enzyme succinate dehydrogenase (SDH) in the mitochondria of eucaryotic cells. SDH is a membrane-bound protein complex that is part of the citric acid cycle and the respiratory chain (also referred to as complex II). It is therefore directly involved in the electron transport chain of the inner mitochondrial membrane (FRAC Class C2; Sun et al. 2005; FRAC 2025). Besides its function as a fungicide, it is also used as a nematicide, with the same mode of action (Schleker et al. 2022). Terbuthylazine blocks electron transport in photosystem II of plant cells, leading to an accumulation of reactive oxygen that ultimately destroys cell membranes (HRAC Class C1; HRAC 2025).
We used the large milkweed bug *Oncopeltus fasciatus* (Dallas, 1852) (Hemiptera: Heteroptera: Lygaeinae) as a model for a herbivorous non-target insects to test for individual and combined effects of these three active substances in dose-response assays. *O. fasciatus* is closely associated with milkweed (*Asclepias*, Apocynaceae), on whose seeds it feeds to sequester cardiac glycosides (Duffey et al. 1978). Nevertheless, it is also capable of feeding on unrelated, non-toxic plants (Ralph 1976). It can be easily reared in the laboratory on sunflower seeds throughout the year, allowing for a large sample size. In addition, this study does not aim to replicate a specific local non-target scenario, but to investigate the fundamental issue of combined toxicity of several classes of pesticides.

We hypothesized that combinations of an insecticide with either a herbicide, a non-SBI fungicide, or both, exert synergistic acute toxicity on the non-target insect *Oncopeltus fasciatus*, leading to effects stronger than those of the individual compounds alone.

2. Methods

2.1 Bug rearing

O. fasciatus were reared in a climate chamber (APT.line™ KBF-ICH, BINDER GmbH, Tuttlingen, Germany) under long day conditions (16/8h light/dark cycle) at 27 °C and 60%rH. Nymphs were kept in 5.8 l plastic boxes covered with an insect mesh. The bottom of the box was covered with kitchen paper. Tap water was provided in a 15 ml centrifuge tube with a cotton plug in the lid and organic sunflower seeds were provided ad libitum as food. A piece of cotton wool was provided for egg laying.

2.2 Active substances

Analytical grade standards of acetamiprid, fluopyram and terbuthylazine were obtained commercially (acetamiprid: HPC Standards GmbH, Borsdorf, Germany; fluopyram/terbuthylazine: Merck KGaA, Darmstadt, Germany). The compounds were always stored in the dark at 4 °C in their original packaging.

The effect of the three active substances on *O. fasciatus* nymphs was first tested individually (see *Individual substance applications*). In parallel, all possible binary mixtures of the substances and the triple mixture were tested (*Mixed substance applications*).

2.3 Individual substance applications

For each active substance (a.s.), a permitted field concentration of a.s. derived from approved formulated products in the Germany (as of May 2023, see Supplementary Information Table 1) was used as the highest concentration in the dose-response assays. In crop protection, the amount of permitted plant protection product is fixed to a specific rate per hectare, but the water volume used for dilution can vary within a specified range (e.g. 200 to 400 liters per hectare). For fluopyram and terbuthylazine, the lowest volume of water has been used in the calculation to imitate the highest permitted amount of a.s./volume. However, the upper volume limit was used for acetamiprid because acetone was to be used as the common solvent in all assays and acetamiprid has a low reported solubility in acetone (~ 0.16 g/l).

For each a.s., a stock solution equivalent to the permitted field concentration was prepared. Fluopyram and terbuthylazine were dissolved in pure acetone, while acetamiprid was dissolved in a 9:1 acetone:deionized water mixture. The resulting stock concentrations were 83.2 ng/µl (acetamiprid), 990.0 ng/µl (fluopyram) and 3694.0 ng/µl (terbuthylazine). Detailed information on the calculations and volumes used is provided in Supplement Information Tables 2-4. Stock solutions were stored in transparent glass vials sealed with metal screw caps and kept in the dark at 4 °C. A dose-range series of six concentrations was prepared for each a.s., beginning with the stock solution and diluting each subsequent concentration by a factor of three (see table 1). In addition, solvent controls were prepared for each a.s. (fluopyram/terbuthylazine: pure acetone; acetamiprid: 9:1 acetone:deionized water). Dilutions were made by pipetting 250 µl of the respective stock into 1.5 ml ND8 amber glass screw-neck vials (LABSOLUTE—TH GEYER) pre-filled with 500 µl of pure acetone. This step was repeated to generate the full dose-range series, which was prepared once per substance and used for all individual substance assays. Each vial was thoroughly vortexed immediately after every dilution step.

Table 1 Dilution series of acetamiprid, fluopyram and terbuthylazine used in the *O. fasciatus* individual substance dose-response assays. The highest dose corresponds to the permitted field concentrations of terbuthylazine and fluopyram, based on approved formulated products in Germany (as of May 2023). For

acetamiprid, the field-use concentration could not be reached because of its lower solubility in acetone compared to water, resulting in a lower applied concentration.

Field Concentration Equivalent [%]	Active Substance [ng/µl]		
	Acetamiprid	Fluopyram	Terbuthylazine
100	83.2	990.0	3694.0
33.3	27.7	329.7	1230.1
11.1	9.2	109.9	410.0
3.7	3.1	36.6	136.7
1.24	1.0	12.3	45.8
0.41	0.3	4.1	15.1
0.0	0.0	0.0	0.0

For each dose-response assay, L4 nymphs (i.e. 4th instar nymphs) of *O. fasciatus* were isolated from the rearing box and transferred to Petri dishes. Six nymphs were randomly assigned to one of the six treatments (i.e. concentrations) and placed in individual 90 mm Petri dishes. Immediately before topical pesticide application, the nymphs were anesthetized with CO_2. Then, 1 µl of a.s. solution (or pure solvent for the controls) was applied dorsally on the abdomen of each nymph using a Hamilton repeating dispenser with a 50 µl Hamilton syringe (Hamilton Bonaduz AG, Switzerland). All a.s. solutions were thoroughly vortexed immediately before drawing the solution into the syringe. Application started with the control followed by increasing concentrations of the a.s. solutions. The syringe was rinsed six times with pure acetone between concentrations. After the droplet had completely evaporated from the bug's body surface and the nymphs had recovered from anesthesia, each bug was transferred to an individual 55 mm petri dish containing filter paper at the bottom, sunflower seeds and water in a 0.5 ml Eppendorf tube plugged with cotton wool. All Petri dishes within the same a.s. assay were randomized and placed in the same climate chamber under the conditions described above. The number of concentrations, individuals and cohorts tested are detailed in Table 2.

Table 2 Overview of *O. fasciatus* assays performed for individual active substances assays. The concentrations tested are listed in Table 1. For the acetamiprid individual assay, 12 individuals per concentration were tested in the first cohort, but only 6 in the second due to greater initial availability of test individuals. This reduced sample size was maintained for the fluopyram and terbuthylazine assays. Abbreviations: A = acetamiprid, F = fluopyram, T = terbuthylazine. The

Assay (Individual)	Acetamiprid	Fluopyram	Terbuthylazine	Number of Concentrations Within Dose Range	Individuals Tested per Concentration	Total Number of Individuals Tested	Number of Cohorts
A	dose range	–	–	7	12/6	126	2
F	–	dose range	–	7	6	84	2
T	–	–	dose range	7	6	84	2

concentrations tested are listed in Table 1.

2.4 Mixed substance applications

Dose-response assays with a.s. mixtures were conducted for the following combinations: (1) acetamiprid + fluopyram (AF), (2) acetamiprid + terbuthylazine (AT), (3) acetamiprid + fluopyram + terbuthylazine (AFT, Table 3). Each assay consisted of the six concentration levels of acetamiprid already used in the individual experiments, to which the highest concentration of either fluopyram (990.0 ng/ µl), terbuthylazine (3694.0 ng/ µl) or a combination of the two from the individual experiments was added. The concentrations of fluopyram and terbuthylazine were selected because they correspond to the maximum levels permitted for field application in Germany and showed no effect on *O. fasciatus* in the individual dose-response assays (see Results). A solvent only control (pure acetone) was also included.

To prepare the binary substance combinations (AF and AT), an acetamiprid stock solution was first made at twice the concentration used in the individual substance assay (166.4 ng/µl, i.e. 2 x the permitted field concentration), maintaining a 9:1 ratio of acetone and deionized water. A dose-range series of six concentrations, starting from and including the acetamiprid stock solution, was then prepared as described for the individual substance assays. Consequently, all six concentrations in this dilution series were initially twice as high as those required for testing. In parallel, separate stock solutions of fluopyram and terbuthylazine were prepared in pure acetone at twice their respective maximum permitted field concentrations (i.e. 1980.0 and 7388.0 ng/µl). For each dose of the binary mixtures, 50 µl of one of the six acetamiprid dilutions was pipetted into an empty 1.5 ml ND8 amber glass vial, followed by 50 µl of the fluopyram or terbuthylazine stock solution. Mixing equal volumes (2x 50 µl) yielded the same dose range of acetamiprid as was used in the single substance assay combined with the maximum permitted field concentration of either fluopyram or terbuthylazine.

For the triple combination (AFT), an acetamiprid stock solution was first prepared at three times the concentration used in the individual substance assay (249.6 ng/ul, i.e. 3 x permitted field concentration), maintaining a 9:1 acetone-to-deionized water ratio. A six-point dose-range series, starting from and including this stock solution, was then prepared as described for the individual substance assays. As a result, all concentrations in this series were initially three times higher than the final required levels. In parallel, fluopyram and terbuthylazine stock solutions were prepared in pure acetone at 3 x their maximum field concentrations (2970.0 ng/µl and 11,082.0 ng/µl, respectively). For each dose, 50 µl of each of the six acetamiprid dilutions was pipetted into an empty 1.5 ml ND8 amber glass vial, followed by 50 µl each of the fluopyram and terbuthylazine stock solutions. Mixing equal volumes (3 x 50 µl) yielded the intended acetamiprid dose range, each combined with the maximum permitted field concentration of fluopyram and terbuthylazine.

The double- and triple-concentrated acetamiprid dose-range series, along with the fluopyram and terbuthylazine stock solutions, were used for all assays and stored in the dark at 4°C. Mixtures of A + F, A + T or A + F + T were freshly prepared for each assay.

Topical application of pesticide solutions on L4 *O. fasciatus* nymphs was carried out as described for the single substance assays above. Again, Petri dishes containing L4 nymphs were randomized within the same assay and maintained in the climate chamber under the same conditions. Each concentration in the mixture assays (and a control of pure acetone) was tested on six individuals per acetamiprid concentration and each experiment was replicated three times (n = 126 in total).

In addition to the dose-range series of acetamiprid supplemented with fluopyram or terbuthylazine, a mixture containing only fluopyram and terbuthylazine (each at 100% field application rate) was tested in a supplementary experiment with reduced replication. A volume of 10 µl of each double-concentrated stock solution (200% field concentration) was pipetted into a 1.5 ml ND8 glass vial, resulting in a 100% field concentration FT mixture. Six L4 nymphs were treated with 1 µl of the mixture and six with 1 µl of pure acetone as a control. The application was conducted as described for the individual assays above. Petri dishes containing nymphs were randomized and stored in the climate chamber under the conditions described above.

The number of concentrations, individuals and cohorts tested in all mixed substance assays are detailed in Table 3.

Table 3 Overview of *O. fasciatus* assays performed for mixed active substance assays. A "fixed dose" indicates that the corresponding maximum permitted field concentration was used. Abbreviations: A = acetamiprid, F = fluopyram, T = terbuthylazine.

Assay (mixture)	Acetamiprid	Fluopyram	Terbuthylazine	Number of Concentrations Within Acetamiprid Dose Range	Individuals Tested per Concentration	Total Number of Individuals Tested	Number of Cohorts
AF	dose range	fixed dose	–	7	6	126	3
AT	dose range	–	fixed dose	7	6	126	3
AFT	dose range	fixed dose	fixed dose	7	6	126	3
FT	–	fixed dose	fixed dose	–	6	12	1

2.5 Evaluation of toxic effects

Nymphs were evaluated 96 hours after application. Petri dishes were removed from the climate chamber and placed in the laboratory. Each nymph was classified into one of three categories: dead, affected or unaffected. Classification was aided by gently touching the abdomen with a fine brush. Healthy individuals typically responded with a flight reaction or flinching. Affected individuals reacted slowly, if at all, often only moving a few legs or antennae, or leaning to one side. Nymphs that

appeared dead (typically lying on their back with bent legs and dark discoloration) were further tested with the brush to confirm the absence of any response. This evaluation scheme was applied consistently across all assays. Raw data for all treatments is provided in Supplementary Data.

2.6 Statistical analysis and graphical representation

Using R (version 4.3.0; R Core Team 2023) and R-Studio (version 2023.6.1.524; Posit team 2023) and the 'drc' package (Ritz et al. 2015), a four-parameter log-normal cumulative dose-response model, assuming a binomial distribution, was fitted to the response data at 96 h post application for the data from the individual acetamiprid assay and from the mixed assays AF and AT.

The following data were not included in the model: (1) data from the individual fluopyram and terbuthylazine assays as no effects were observed; (2) data from the fluopyram and terbuthylazine mixed assay (FT) as no effects were observed; (3) data from the acetamiprid, fluopyram and terbuthylazine triple mixed assay (AFT) as the effects were too strong across the dose range (see Results: Mixed substance assays).

The lower asymptote of the dose-response model was set to '0' because in all included assays, none of the control animals showed any effects or mortality. The upper asymptote was set to '1'. The *plot()* function was used to generate dose-response curves. Model fit was assessed by visual inspection of the regression curve and comparison of the Akaike Information Criterion (AIC) values. LD_{50} values (and respective 95% confidence intervals) were derived from the model using the *ED()* function. The *compParm()* function was used to assess differences in the dose required to affect 50% of the tested population (ED_{50} value) The function compares individual curves (i.e., ED_{50} values) within the model and performs approximate t-tests with $\alpha = 0.05$. Note that all derived ED_{50} values, including those from the mixed assays, refer to the respective acetamiprid concentration in ng/µl. The full R-code is provided in Supplementary Information – Supplementary Code.

Figure 1, including the dose response curves and mean data points from the AFT treatment, was created in R and later edited in Inkscape (version 1.4; Inkscape Project 2020). Figure 2 was created with JMP® Student Edition (version 18.2.2; JMP Statistical Discovery LLC 2025) and later edited with Inkscape.

3 Results

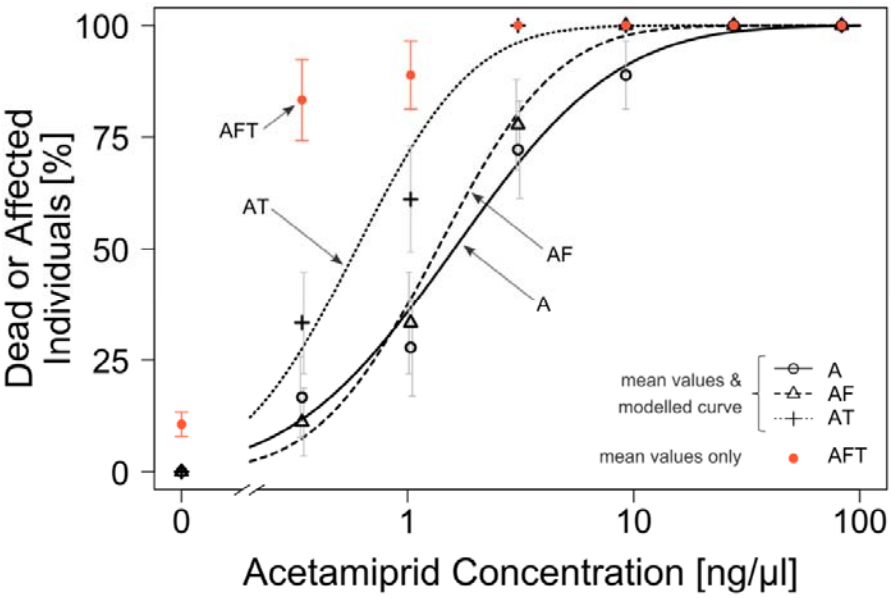
3.1 Individual substance assays

Oncopeltus fasciatus nymphs showed high sensitivity to the insecticide acetamiprid 96 hours after application. At all concentrations tested, dead or affected nymphs were observed. However, at no concentration 100% mortality was observed in the two cohorts tested. The derived ED_{50} for acetamiprid is 1.61 ng/µl (combined '*dead or affected*' data) based on a multiple treatment cumulative dose-response model with a log-normal distribution (Fig. 1).

No effects were observed across concentrations in the single-substance dose-response assays with fluopyram and terbuthylazine 96 hours after application, and no dose-response curves were fitted. However, there were isolated cases of mortality in both experiments. Two nymphs died in the fluopyram assay, one at 33.3% maximum field concentration equivalent (1st cohort) and one at 11.1% (2nd cohort). In the terbuthylazine assay, four nymphs died, two in the control (one in each cohort), one at 3.7% field concentration equivalent (1st cohort) and one at 1.24% (2nd cohort). However, in both assays, there were no nymphs categorized as affected in addition to those that died, supporting that these were random mortality events and these values were not considered further.

3.2 Mixed substance assays

The effect of the AF mixed treatment (insecticide + fungicide) on the nymphs compared to the individual acetamiprid application was not significantly different based on the cumulative dose-response model followed by an ED_{50} comparison (t=0.58, p=0.563; Fig. 1). However, sensitivity towards the AT mixed application (insecticide + herbicide) compared to both acetamiprid alone and the AF treatment was significantly higher based on the ED_{50} comparison (A-AT: t=2.73, p=0.06; AF-AT: t=2.79, p=0.005). The ED_{50} of AT (0.59 ng/µl) was approximately 2.7-fold lower than that of acetamiprid alone (1.61 ng/µl), and 2.3-fold lower than that of AF (1.36 ng/µl). The strongest effects were observed in the AFT treatment (insecticide + fungicide + herbicide). However, the data could not be included in the dose-response model because the observed effects were too pronounced across the entire dose range. At the lowest tested dose (0.34 ng acetamiprid /µl), the mean response was 83.3%, exceeding the inflection point of a typical dose-response curve. As a result, no curve could be fitted. Nonetheless, this suggests that the ED_{50} for the AFT treatment would be well below 0.34 ng/µl.



Derived ED_{50} Values

Treatment	ED_{50} [ng/µl]	95% CI (lower - upper)
A	1.61[a]	0.92 - 2.30
AF	1.36[a]	0.87 - 1.84
AT	0.59[b]	0.36 - 0.82
AFT	<< 0.34	—

Pairwise ED_{50} Comparisons

Treatment Contrast	Estimate	Standard Error	t-value	p-value
A-AF	0.25	0.43	0.58	0.563
A-AT	1.01	0.37	2.73	**0.006**
AF-AT	0.76	0.27	2.79	**0.005**

Fig. 1 *Oncopeltus fasciatus* dose-response curves for individual and mixture assays and comparison of derived ED_{50} values. A**:** acetamiprid individual assay, AF: acetamiprid + fluopyram, AT: acetamiprid + terbuthylazine, AFT: acetamiprid + fluopyram + terbuthylazine. Dose-response relationships were modelled simultaneously using a four-parameter log-normal function. Black symbols represent treatment-specific mean responses per concentration, with grey error bars indicating standard errors (SE). SE indicators were offset in cases of overlap to facilitate interpretation. Red data points (and respective SE indicators) represent mean responses to the AFT treatment but were not included in the dose-response model due to convergence failure. Derived ED_{50} values and respective 95% confidence intervals (CI) for each treatment included in the model are listed in the top right table. For AFT, the ED_{50} is reported as << 0.34 because 83.3% mean response was observed already at the lowest tested concentration (0.34 ng/µl), indicating that the actual ED_{50} must lie well below this value. LD_{50} values derived from the model were compared using the *compParm* function of the *drc* package in R. LD_{50} values of treatments not sharing a common letter are significantly different. A statistical report on the pairwise comparisons is given in the bottom right table with bolt p-values indicating significant differences at α = 0.05. All numbers presented in the adjacent tables are rounded. For all assays, N = 18 biological replicates (nymphs) per concentration

When differentiating between dead and merely affected individuals, mortality in the AFT and AT assay was proportionately higher at lower acetamiprid doses than in the other treatments (Fig. 2). Strikingly, in the AFT treatment, even at the lowest concentration, 67% of nymphs died within 96 hours, compared to 17% in the AT, 0% in the AF, and 4% in the assay with acetamiprid only.
No effects on *O. fasciatus* nymphs were observed in the combined FT treatment (fungicide + herbicide, each at 100% field concentration, see Supplementary Data) 96 hours after application. All nymphs were unaffected in both the treatment and the control group.

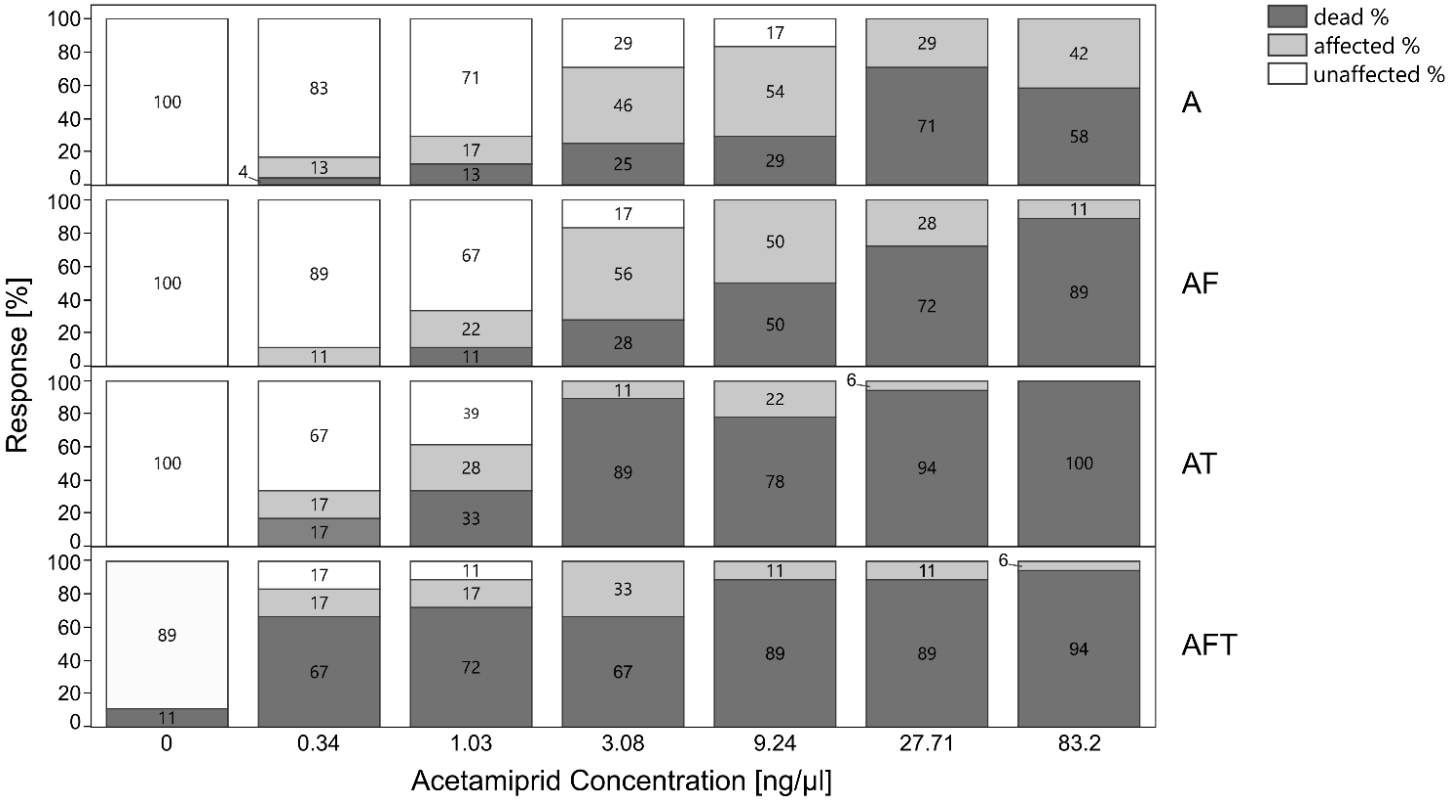


Fig. 2 Response of *O. fasciatus* nymphs to individual and mixed substance treatments containing acetamiprid. Proportions of response types are indicated by different colors for each acetamiprid concentration tested. Numbers inside or left of the bars indicate percentage response (rounded). For all assays, N = 18 biological replicates (nymphs) per concentration

4 Discussion

To test the hypothesis that a combination of an insecticide with either a herbicide, a non-SBI fungicide, or both exerts synergistic acute toxicity on the non-target insect *O. fasciatus*, we investigated mixtures of acetamiprid, terbuthylazine, and fluopyram, all of which are widely used in agriculture. First, we assessed each substance individually in dose-response assays, followed by binary mixtures and finally a combined mixture of all three.

4.1 Synergistic Effects

Previous studies on the combined effects of major pesticide classes have primarily focused on interactions between insecticides and fungicides, with most research being conducted on bees (Tosi et al. 2022). A well-documented example is the synergistic interaction between neonicotinoids such as acetamiprid and SBI fungicides. SBIs have been shown to interfere with insect detoxification pathways (e.g., P450 enzymes), thereby enhancing the toxicity of insecticides (Sgolastra et al. 2017; Schuhmann et al. 2022). In Germany, this has led to additional regulations regarding tank mixtures of certain pesticides in order to protect bees (BVL 2018). However, recent evidence suggests that synergistic interactions may also occur with non-SBI fungicides (Manzer et al. 2024), although the underlying mechanisms remain less well understood. Our results support and extend this perspective.
While our individual substance assays revealed acute toxicity only for the insecticide acetamiprid and not for the fungicide fluopyram (non-SBI fungicide) or the herbicide terbuthylazine, certain mixtures of these compounds induced synergistic effects on the non-target insect *O. fasciatus*. This was observed with the binary mixture of acetamiprid and terbuthylazine, as well as with the mixture of all three compounds. Formal synergy modelling was not possible due to the absence of individual activity of the added compounds and the lack of a complete dose-dose matrix. However, given the significant reduction in ED_{50} (A: 1.61 ng/µl, AT: 0.59 ng/µl) and the drastic effects observed in the triple combination (AFT), although neither fluopyram nor terbuthylazine had an effect on their own, we interpret the enhanced acute toxicity as resulting from a synergistic effect.

These findings support existing indications that synergistic interactions may not be limited to specific pesticide groups such as SBI fungicides. Rather, they could emerge more broadly, including from combinations involving herbicides, a pesticide class less commonly associated with synergism. In addition, complex mixtures (i.e., those containing three or more compounds) appear to be especially problematic due to their unpredictable interaction potential. In the following sections, we focus on

terbuthylazine and fluopyram individually in the context of their combinations with acetamiprid. We then discuss their potential joint contribution to the pronounced toxicity observed in the triple mixture.

4.2 Terbuthylazine

Herbicides have been shown to exert direct detrimental effects on non-target organisms, including invertebrates (Wan et al. 2025). Moreover, by eliminating food plants, herbicides also impose indirect negative impacts on invertebrate populations (Bohan et al. 2005). However, the mode of action of terbuthylazine does not initially suggest any potential effect on insects, since the target is exclusively found in plants. It blocks electron transport in the photosystem II complex by binding to the QB-binding site of the D1 protein, thereby interrupting the electron transport chain. However, although comprehensive data are limited, there are individual reports of adverse effects of terbuthylazine on both invertebrates and vertebrates. Terbuthylazine can affect the antioxidant defense systems of fish and induce oxidative stress (Semren et al. 2018). A terbuthylazine-containing herbicide formulation caused acute toxic effects on several soil invertebrates, including enchytraeids and mites, despite the pure active ingredient showing no toxicity under the same conditions (Salminen et al. 1996). Notably, no insects were included in this study.
Aside from our study, we are not aware of any published evidence indicating that terbuthylazine can increase the toxicity of insecticides in insects. However, synergistic toxicity has been reported for another s-triazine herbicide, atrazine, in the midge *Chironomus tentans*, when mixed with organophosphate insecticides (Pape□Lindstrom and Lydy 1997). The authors speculated that this effect may be linked to an enhanced metabolic activation of the organophosphates, as most organophosphorus insecticides would require metabolic activation to effectively inhibit the acetylcholinesterase. Since s-triazine herbicides like terbuthylazine were shown to induce the cytochrome P450 machinery (Kao et al. 1995), the authors suggested that such herbicides might accelerate the metabolization of organophosphates and thus results in increased toxicity.

However, acetamiprid does not require metabolic activation to effectively inhibit the nicotinic acetylcholine receptor. Given the known role of cytochrome P450 enzymes in the metabolism of acetamiprid (Iwasa et al. 2004), and the evidence that triazine herbicides like terbuthylazine can interact with detoxification systems in various organisms, it is plausible that co-application could overburden shared detoxification pathways, even if direct metabolic competition remains to be demonstrated in insects. In summary, the synergistic toxicity of the AT mixture in *O. fasciatus* may be explained by a combination of stressors induced by the co-applied herbicide.

4.3 Fluopyram

The fungicide fluopyram targets the mitochondrial SDH, a key enzyme of the respiratory chain and ATP production that is not specific to fungi (Sun et al. 2005).

Unlike other SDH-inhibitor (SDHI) fungicides, fluopyram is also used to affect plant-parasitic nematodes, acting via the same mode of action as in fungi (Schleker et al. 2022). Strikingly, the SDH of *Apis mellifera* was also shown to be sensitive to fluopyram (Bénit et al. 2019) suggesting that it could directly impair cellular metabolism and energy production in insects.

In addition, a recent study conducted on the nematode *Caenorhabditis elegans* showed that fluopyram not only inhibits SDH, but also triggers oxidative stress, intestinal damage, and cellular apoptosis (Liu et al. 2022). The authors observed negative impacts on mobility, feeding, reproduction, and longevity in *C. elegans*. Adverse effects are also reported for other non-target organisms. Fluopyram was proven to be harmful to soil microbial communities (Zhang et al. 2014). In fish, fluopyram exposure can induce altered behavior and metabolic disruption despite a low mitochondrial complex II inhibition in vitro. However, reduced acetylcholinesterase activity was detected in the muscle cells (Bouly et al. 2024). In addition, Zhou et al. (2025) suggest a potential chronic toxicity to fish.

To our knowledge, no synergistic enhancement of insecticide toxicity by fluopyram has been reported to date. However, a recent study by Manzer et al. (2024) demonstrated a synergistic increase in acetamiprid toxicity in honeybees when larvae were orally exposed to a mixture of two other fungicides inhibiting mitochondrial electron transport: the SDHI boscalid (targeting mitochondrial complex II) and dimoxystrobin (targeting mitochondrial complex III). This combination led to increased adult mortality compared to acetamiprid alone, whereas exposure to the fungicide mixture alone had no effect. Interestingly, this synergy was only observed in a low-dose mixture, but not at higher concentrations.

In our study, fluopyram alone did not cause any measurable acute toxic effects in *O. fasciatus* nymphs, and the combination with acetamiprid did not significantly increase toxicity compared to the acetamiprid-only treatment. It remains possible that the experimental design did not capture delayed or developmental-stage-specific effects. Notably, in the study by Manzer et al. (2024), increased mortality was observed only 20-30 days after emergence, following larval-stage exposure.

Although no significant synergistic toxicity was detected in our binary AF treatment, potential interactions between fluopyram and acetamiprid cannot be ruled out. While acetamiprid selectively activates nicotinic acetylcholine receptors, fluopyram has been shown to inhibit the AChE in vertebrates, which may also apply to invertebrates. Such inhibition could lead to endogenous acetylcholine accumulation and overstimulation of cholinergic pathways. In combination with acetamiprid, this may result in dual interference within the cholinergic system, plausibly increasing toxicity. Additionally, the potential inhibition of mitochondrial respiration in invertebrates by fluopyram could reduce the availability of cellular energy, which could compromise detoxification mechanisms. As energy availability is essential for cellular detoxification processes, such as biotransformation and the active transport

of xenobiotics, mitochondrial disruption could indirectly increase susceptibility to insecticides.

While these scenarios remain speculative, it is notable that the proportion of unaffected (i.e., neither dead nor affected) nymphs declined more steeply with increasing acetamiprid concentrations in the AF mixture than in the acetamiprid-only treatment (Fig. 2). Although our dose-response analysis focused on dead and affected individuals, this trend was observed visually but not statistically analyzed, suggesting a potential interaction not captured by ED_{50} values alone. However, significantly increased toxicity in mixtures including fluopyram was only observed in the triple mixture AFT.

4.4 Complex mixtures

The significantly higher toxicity observed in the triple mixture AFT is likely due to the combined physiological stress imposed by all three co-applied pesticides. Previous studies have shown that terbuthylazine and fluopyram can interfere with critical biological processes in several non-target organisms, including cellular energy metabolism, oxidative balance, detoxification capacity and neurotransmission (see above). While many of these pesticide-induced effects have not been confirmed directly in insects, their presence in a broad range of organisms suggests that they may also be observed in insects. Taken together, the potential co-occurrence of neurotoxicity, metabolic disturbances and oxidative stress could overwhelm cellular defense mechanisms. Therefore, the pronounced increase in toxicity observed in the AFT treatment, compared to all other treatments, may be explained by the collapse of critical physiological systems, including ATP-dependent processes such as detoxification and cholinergic regulation.

It is important to note that our study only assessed acute toxicity. Potential sublethal effects, such as behavioral or physiological alterations, impaired development or communication, and reproductive deficits, were not investigated but are commonly reported, including for pesticide mixtures (Müller 2018; Gandara et al. 2024). The potential for complex mixtures to cause disproportionately stronger sublethal effects than binary combinations has not yet been systematically addressed and demands further investigation.

4.5 Realistic Scenarios and Risk Assessment

Although this study was not designed to reflect a specific local exposure scenario, but rather to address the general question of combined toxicity across pesticide classes, the combination of the tested substances is not merely hypothetical. There are realistic conditions under which such mixtures can be encountered by non-target organisms.

Firstly, it is noteworthy that ecotoxicological studies of environmental samples, such as soil, vegetation, water and crop plants, conducted in recent years have detected

all three of the substances tested here across all sample types (Honert et al. 2025; Mauser et al. 2025).

Secondly, environmental measurements clearly show that the tested combinations that showed synergistic effects (AT, AFT) are indeed present in the landscape. Soil samples from agricultural areas in Rhineland-Palatinate (Germany) analyzed by Honert et al. (2025) contained the a.s. combination AT in topsoil. The combination AFT was detected only once. However, FT was often found in combination with one or more other neonicotinoids, such as thiamethoxam, clothianidin, or imidacloprid.

These local examples of potential exposure to hazardous a.s. combinations clearly indicate a landscape-level risk. Brühl et al. (2021) further demonstrate this risk by finding that insects in nature conservation zones in Germany were contaminated, on average, with 16.7 pesticides. While this finding is alarming, it is not unexpected, given that contamination of areas surrounding fields – which in some cases might be conservation zones – is generally anticipated (Rautmann et al. 2001). Given the results of our study, these findings are alarming and demand further attention from research, legislation and risk assessment.

While EU regulations acknowledge the importance of assessing cumulative and synergistic effects in the context of simultaneous or sequential applications, current risk assessment procedures still predominantly focus on individual active substances (EFSA PPR 2015). As a result, the ecological risks arising from the potential for multiple substances to co-occur in the environment forming complex mixtures remain insufficiently addressed. Such mixtures may arise in-field due to common plant protection practices and the long environmental persistence of some substances (e.g., fluopyram, DT90 = 833 days in soil in field at 20 °C; Lewis et al. 2016), or off-field via various input pathways (e.g., spray drift; Honert et al. 2025; Mauser et al. 2025).

Investigating combined pesticide effects is inherently challenging: the number of possible compound combinations is vast, and the range of potential concentration scenarios is practically infinite. Our results show that complex mixtures are a major source of uncertainty in ecotoxicological assessment due to their unpredictable interaction potential. Although environmental pesticide concentrations are often comparatively low, our study demonstrates at the mechanistic level that interactions among active substances may occur more broadly than previously assumed, making interactions at lower environmental doses plausible. In addition, a recent study demonstrated that environmentally relevant combinations of pesticides at realistic concentrations do exert adverse effects on non-target insects (Gandara et al. 2024). Moreover, a.s. interactions may manifest differently across species, further increasing the complexity of this field. We therefore propose testing realistic exposure scenarios based on (1) plant protection practices, including crop type, application method, and degradation behavior, and (2) environmental monitoring of actual a.s. combinations. These tests should consider both likely individual

concentrations and potentially affected non-target organisms, depending on the environmental matrix (soil, plant, water, air).

We believe a reformed ecotoxicological assessment is urgently needed, given that evidence, such as this study, increasingly suggests environmental pesticide mixtures may be influencing the ongoing biodiversity crisis.

References

Bénit P, Kahn A, Chretien D, et al (2019) Evolutionarily conserved susceptibility of the mitochondrial respiratory chain to SDHI pesticides and its consequence on the impact of SDHIs on human cultured cells. PLOS ONE 14:e0224132. https://doi.org/10.1371/journal.pone.0224132

Bohan DA, Boffey CWH, Brooks DR, et al (2005) Effects on weed and invertebrate abundance and diversity of herbicide management in genetically modified herbicide-tolerant winter-sown oilseed rape. Proceedings of the Royal Society B: Biological Sciences 272:463–474. https://doi.org/10.1098/rspb.2004.3049

Bouly L, Jacquin L, Chapeau F, et al (2024) Fluopyram SDHI pesticide alters fish physiology and behaviour despite low *in vitro* effects on mitochondria. Ecotoxicology and Environmental Safety 288:117400. https://doi.org/10.1016/j.ecoenv.2024.117400

Brühl CA, Bakanov N, Köthe S, et al (2021) Direct pesticide exposure of insects in nature conservation areas in Germany. Sci Rep 11:24144. https://doi.org/10.1038/s41598-021-03366-w

Brühl CA, Engelhard N, Bakanov N, et al (2024) Widespread contamination of soils and vegetation with current use pesticide residues along altitudinal gradients in a European Alpine valley. Commun Earth Environ 5:1–9. https://doi.org/10.1038/s43247-024-01220-1

BVL (2018) Neue Vorgaben zum Bienenschutz bei Tankmischungen von Insektiziden mit Fungiziden. In: Bundesamt für Verbrucherschutz und Lebenmittelsicherheit. https://www.bvl.bund.de/SharedDocs/Fachmeldungen/04_pflanzenschutzmittel/2018/2018_11_12_Fa_Bienenschutz_Tankmischung_Insekt_Fung.html. Accessed 25 Aug 2025

Dicks LV, Breeze TD, Ngo HT, et al (2021) A global-scale expert assessment of drivers and risks associated with pollinator decline. Nat Ecol Evol 5:1453–1461. https://doi.org/10.1038/s41559-021-01534-9

Duffey SS, Blum MS, Isman MB, Scudder GGE (1978) Cardiac glycosides: A physical system for their sequestration by the milkweed bug. Journal of Insect Physiology 24:639–645. https://doi.org/10.1016/0022-1910(78)90127-0

EFSA PPR P on PPP and their R (PPR) (2015) Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods. EFSA Journal 13:3996. https://doi.org/10.2903/j.efsa.2015.3996

European Commission (n.d.) EU Pesticides Database (v3.3) - Active substances. https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances. Accessed 1 Apr 2025

FRAC (2025) SDHI fungicides. In: Fungicide Resistance Action Committee. https://www.frac.info/frac-teams/working-groups/sdhi-fungicides/. Accessed 22 July 2025

Gandara L, Jacoby R, Laurent F, et al (2024) Pervasive sublethal effects of agrochemicals on insects at environmentally relevant concentrations. Science 386:446–453. https://doi.org/10.1126/science.ado0251

Geiger F, Bengtsson J, Berendse F, et al (2010) Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. Basic and Applied Ecology 11:97–105. https://doi.org/10.1016/j.baae.2009.12.001

Honert C, Mauser K, Jäger U, Brühl CA (2025) Exposure of insects to current use pesticide residues in soil and vegetation along spatial and temporal distribution in agricultural sites. Sci Rep 15:1817. https://doi.org/10.1038/s41598-024-84811-4

HRAC (2025) Global Herbicide Classification Lookup. In: Herbicide Resistance Action Committee. https://hracglobal.com/tools/classification-lookup. Accessed 22 July 2025

IRAC (2025) Acetamiprid. In: Insecticide Resistance Action Committee. https://irac-online.org/active-ingredient/acetamiprid/. Accessed 22 July 2025

Iwasa T, Motoyama N, Ambrose JT, Roe RM (2004) Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. Crop Protection 23:371–378. https://doi.org/10.1016/j.cropro.2003.08.018

Kao LM, Wilkinson CF, Brattsten LB (1995) In-vivo effects of 2,4-D and atrazine on cytochrome P-450 and insecticide toxicity in southern armyworm (Spodoptera eridania) larvae. Pesticide Science 45:331–334. https://doi.org/10.1002/ps.2780450407

Kruse-Plaß M, Hofmann F, Wosniok W, et al (2021) Pesticides and pesticide-related products in ambient air in Germany. Environ Sci Eur 33:114. https://doi.org/10.1186/s12302-021-00553-4

Lewis KA, Tzilivakis J, Warner DJ, Green A (2016) An international database for pesticide risk assessments and management. Human and Ecological Risk Assessment: An International Journal 22:1050–1064. https://doi.org/10.1080/10807039.2015.1133242

Liu Y, Zhang W, Wang Y, et al (2022) Oxidative stress, intestinal damage, and cell apoptosis: Toxicity induced by fluopyram in Caenorhabditis elegans. Chemosphere 286:131830. https://doi.org/10.1016/j.chemosphere.2021.131830

Manzer S, Thamm M, Hilsmann L, et al (2024) The neonicotinoid acetamiprid reduces larval and adult survival in honeybees (*Apis mellifera*) and interacts with a fungicide mixture. Environmental Pollution 360:124643. https://doi.org/10.1016/j.envpol.2024.124643

Mauser KM, Wolfram J, Spaak JW, et al (2025) Current-use pesticides in vegetation, topsoil and water reveal contaminated landscapes of the Upper Rhine Valley, Germany. Commun Earth Environ 6:166. https://doi.org/10.1038/s43247-025-02118-2

Müller C (2018) Impacts of sublethal insecticide exposure on insects — Facts and knowledge gaps. Basic and Applied Ecology 30:1–10. https://doi.org/10.1016/j.baae.2018.05.001

Pape Lindstrom PA, Lydy MJ (1997) Synergistic toxicity of atrazine and organophosphate insecticides contravenes the response addition mixture model. Environmental Toxicology and Chemistry 16:2415–2420. https://doi.org/10.1002/etc.5620161130

Ralph CP (1976) Natural food requirements of the large milkweed bug,Oncopeltus fasciatus (Hemiptera: Lygaeidae), and their relation to gregariousness and host plant morphology. Oecologia 26:157–175. https://doi.org/10.1007/BF00582894

Rautmann D, Streloke M, Winkler R (2001) New basic drift values in the authorization procedure for plant protection products. Workshop on Risk Assessment and Risk Mitigation Measures (WORMM), vol 13 3–141

Salminen J, Eriksson I, Haimi J (1996) Effects of Terbuthylazine on Soil Fauna and Decomposition Processes. Ecotoxicology and Environmental Safety 34:184–189. https://doi.org/10.1006/eesa.1996.0062

Sánchez-Bayo F, Wyckhuys KAG (2019) Worldwide decline of the entomofauna: A review of its drivers. Biological Conservation 232:8–27. https://doi.org/10.1016/j.biocon.2019.01.020

Schleker ASS, Rist M, Matera C, et al (2022) Mode of action of fluopyram in plant-parasitic nematodes. Sci Rep 12:11954. https://doi.org/10.1038/s41598-022-15782-7

Schuhmann A, Schmid AP, Manzer S, et al (2022) Interaction of Insecticides and Fungicides in Bees. Frontiers in Insect Science 1:

Sedlmeier JE, Grass I, Bendalam P, et al (2025) Neonicotinoid insecticides can pose a severe threat to grassland plant bug communities. Commun Earth Environ 6:1–12. https://doi.org/10.1038/s43247-025-02065-y

Semren TŽ, Žunec S, Pizent A (2018) Oxidative stress in triazine pesticide toxicity: a review of the main biomarker findings. Archives of Industrial Hygiene and Toxicology 69:109–125. https://doi.org/10.2478/aiht-2018-69-3118

Sgolastra F, Medrzycki P, Bortolotti L, et al (2017) Synergistic mortality between a neonicotinoid insecticide and an ergosterol-biosynthesis-inhibiting fungicide in three bee species. Pest Management Science 73:1236–1243. https://doi.org/10.1002/ps.4449

Silva V, Gai L, Harkes P, et al (2023) Pesticide residues with hazard classifications relevant to non-target species including humans are omnipresent in the environment and farmer residences. Environment International 181:108280. https://doi.org/10.1016/j.envint.2023.108280

Sun F, Huo X, Zhai Y, et al (2005) Crystal Structure of Mitochondrial Respiratory Membrane Protein Complex II. Cell 121:1043–1057. https://doi.org/10.1016/j.cell.2005.05.025

Tosi S, Sfeir C, Carnesecchi E, et al (2022) Lethal, sublethal, and combined effects of pesticides on bees: A meta-analysis and new risk assessment tools. Science of The Total Environment 844:156857. https://doi.org/10.1016/j.scitotenv.2022.156857

Varga-Szilay Z, Tóth Z (2022) Is acetamiprid really not that harmful to bumblebees (Apidae: Bombus spp.)? Apidologie 53:2. https://doi.org/10.1007/s13592-022-00909-6

Wagner DL, Grames EM, Forister ML, et al (2021) Insect decline in the Anthropocene: Death by a thousand cuts. Proceedings of the National Academy of Sciences 118:e2023989118. https://doi.org/10.1073/pnas.2023989118

Wan N-F, Fu L, Dainese M, et al (2025) Pesticides have negative effects on non-target organisms. Nat Commun 16:1360. https://doi.org/10.1038/s41467-025-56732-x

Zhang Y, Xu J, Dong F, et al (2014) Response of microbial community to a new fungicide fluopyram in the silty-loam agricultural soil. Ecotoxicology and Environmental Safety 108:273–280. https://doi.org/10.1016/j.ecoenv.2014.07.018

Zhou L, Li Y, He C, et al (2025) Toxicity, bioaccumulation and metabolism of fluopyram in zebrafish (*Danio rerio*). Aquatic Toxicology 285:107420. https://doi.org/10.1016/j.aquatox.2025.107420

Data Availability

All data generated in this study are provided in the supplementary materials.

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Contributions

J.E.S., E.G. and G.P designed the experiments. E.G. and J.E.S. conducted the experiments. B.H. and F.W. developed the active substance mixing strategies in a common solvent based on chemical properties. J.E.S. and E.G. performed the analysis of the experiments. J.E.S. wrote the original draft of the manuscript and created the figures. G.P. supervised the research and edited the manuscript.