

Side Effects of Insecticides on Leaf-Miners and Gall-Inducers Depend on Species Ecological Traits and Competition with Leaf-Chewers

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Abstract: Internal feeding is considered to shield sessile herbivorous insects from exposure to nonsystemic insecticides aerially sprayed against forest defoliators, although this has not been tested. It is, however, established that leaf damage caused by defoliators affects the survivorship and oviposition behavior of sessile herbivores. Thus feeding ecology and competition may mediate nontarget effects of insecticides on these insects. We tested the ecological sensitivity of 3 guilds of sessile herbivores (upper-surface leaf-miners, lower-surface leaf-miners, and gall-inducers) to the lipophilic larvicides diflubenzuron and tebufenozide aerially applied either at operational rates (12 g active ingredient [a.i.]/ha and 69.6 g [a.i.]/ha, respectively) or at maximum legal rates (60 g [a.i.]/ha and 180 g [a.i.]/ha, respectively), in German oak forests. Diflubenzuron affected leaf-miners at different life stages depending on their position on the leaf but had no effect on gall-inducers. Tebufenozide showed a similar, but not significant, pattern in leaf-miners and did not affect gall-inducers. By reducing the incidence of chewing damage on leaves, both insecticides offset the negative effect of competition on leaf-miner and gall-inducers. The net outcome of insecticide treatment was positive for guilds avoiding exposure, but negative for upper-surface leaf-miners. Exposure to insecticides in situ can be mediated by subtle differences in species biology and species interactions, with potential implications for organisms usually considered safe in risk assessment studies. *Environ Toxicol Chem* 2021;40:1171–1187. © 2020 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Insecticide; Sessile herbivores; Population-level effects; Trait-based; Ecological sensitivity; Insect competition; Feeding traits

INTRODUCTION

Forestry's contribution to the global use of pesticides only amounts to <2%, but even this is a matter of great public concern, particularly regarding the practice of aerial applications

(Thompson 2011). Due to the generally negative public perception of aerial spraying, the use of insecticides in forest protection became a last-resort measure and relies mostly on products with an allegedly low environmental footprint. Most insecticides used in forest applications are nonsystemic compounds that are toxic on ingestion, with relatively high target selectivity and short persistence times (Thompson 2011). The main field of application of insecticides in forest management is the control of outbreaking defoliators, such as the gypsy moth *Lymantria dispar* L. (Lepidoptera: Erebidae; Liebhold and McManus 1999). Among the substances frequently used during the last decades are the insect growth regulators diflubenzuron and tebufenozide (Holmes and MacQuarrie 2016; Leroy et al. 2019). Both are lipophilic

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larvicides that must be ingested to be effective. Diflubenzuron nonselectively affects the larval stages of crown-dwelling herbivores (Durkin 2004), whereas the field toxicity of tebufenozide appears to be restricted to caterpillars, that is, the larvae of butterflies and moths (Lepidoptera; Durkin and Klotzbach 2004).

Ecological sensitivity represents the likelihood that a taxon will come into contact with a chemical stressor as well as its ability to recover after exposure. It is mediated by biological traits of the organism, such as the feeding niche of the taxon, and the physicochemical properties of the stressor (Kefford et al. 2012). For sessile herbivores such as leaf-miners and gall-inducers, internal feeding offers enhanced protection against a variety of biotic and abiotic stressors (Price et al. 1987; Connor and Taverner 1997) and may similarly provide a physical barrier against nonsystemic insecticides. In entomology, Lepidoptera are divided into 2 artificial groupings separating large (Macrolepidoptera) and smaller species (Microlepidoptera). In deciduous oak assemblages, the vast majority of Microlepidoptera display either completely (e.g., leaf-miners, acorn-borers, etc.) or partially sheltered feeding habits (e.g., leaf-rollers, web-makers, etc.; Patočka 1980; Marquis et al. 2019). Because Microlepidoptera were found to experience little to no adverse effects from forestry insecticides, it has been hypothesized that their sheltered feeding habit protects them from exposure to insecticide residues (Martinat et al. 1988; Scriber 2004). However, data from agricultural systems partly contradict this hypothesis. Diflubenzuron has shown lethal effects against various leaf-mining pests in orchards and urban trees (e.g., Marshall et al. 1988; Nejmanová et al. 2006), and tebufenozide is included in integrated pest management toolboxes against lepidopteran leaf-miners in vegetable production (Reitz et al. 1999). In contrast, management of gall-inducing pests relies on systemic insecticides that can translocate within the gall instead of lipophilic components that penetrate poorly in the thick and dense galling tissue (Eliason and Potter 2000; Xu et al. 2009). These findings suggest that internally feeding herbivores may be only partly shielded from diflubenzuron and tebufenozide and that the thickness of the surrounding tissue mediates exposure levels.

In addition to the feeding mode of the insect, structural forest characteristics (such as canopy structure) and application conditions (such as dose, frequency, and mode of application) may result in different exposure dynamics in a forest than they do in agricultural and urban settings. Because insecticides are delivered to forests by aircraft, thick and structurally complex canopies may attenuate the deposition of droplets with increasing canopy depth (Richardson and Thistle 2006). Because larger concentrations may accumulate on the upper surface of leaves, species feeding on the lower surface are likely less exposed.

Furthermore, insecticides may also impact herbivores by altering their interactions with other insect species. There is substantial evidence that gall-inducers and leaf-miners respond negatively to competition by defoliating insects. Epidemic densities of the leaf-chewing forest tent caterpillars *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae) were shown to reduce the abundance of several species of

leaf-miners and gall-inducers (Roslin and Roland 2005). At lower, endemic levels of herbivory, leaf-miners preferably selected intact leaves over damaged ones for oviposition (Faeth 1986; Digweed 2006) and had lower survivorship on damaged leaves (Faeth 1986; Cornelissen and Stiling 2008). Because insecticide treatments aim at suppressing leaf-chewers, they may indirectly affect leaf-miner and gall-inducer communities by altering these competition patterns. Thus, subtle differences in species biology, as well as competition and stand structure, likely play important roles in mediating exposure to aerially sprayed insecticides. To account for these mechanisms, the effects of insecticides on internally feeding herbivores must be tested on diverse communities and in realistic field conditions.

Central European deciduous oak (*Quercus robur* L. and *Quercus petraea* Mattuschka) is a particularly suitable system to address these questions. The phytophagous insect community associated with these oak species is characterized by a strong seasonality. Leaf-chewers dominate the spring assemblage such that nearly all chewing damage occurs between early May and late June. Leaf-miners and gall-inducers subsequently dominate the summer assemblage (Southwood et al. 2004; Figure 1), making co-occurrence between internal and external feeding guilds relatively rare and confronting sessile herbivores with a situation in which chewing damage has already occurred. Central European oak stands are susceptible to outbreaks of native leaf-chewing Lepidoptera (Thomas et al. 2002), making them frequently subjected to epidemic levels of leaf damage; hence they are recurrent candidates for aerial spraying operations.

In the present study, we assessed how the leaf-mining and gall-inducing communities of deciduous oak respond to the application of tebufenozide and diflubenzuron, in 2 separate field experiments: one on individual trees sprayed with a helicopter drone, and the other on larger plots sprayed with a manned helicopter. We first assessed the efficacy of the treatment in reducing the population of leaf-chewers and the associated foliar damage in a small-scale experiment whereby individual trees were sprayed with a drone (Figure 1, path A→B). Second, we examined the direct effect of insecticides on different herbivore guilds that we assumed to differ in ecological sensitivity (Figure 1, path A→C). We hypothesized that gall tissue grants full protection from exposure to insecticides, whereas upper-surface leaf-miners experience a stronger negative effect relative to lower-surface species due to the proximity between their feeding site and the leaf surface where insecticide droplets are deposited (Figure 1: D). Third, we addressed the interaction between leaf-chewers and sessile herbivores, anticipating a negative effect of chewing damage on the distribution of sessile herbivores (Figure 1, path B→C). In a second experiment with manned helicopter application, we quantified the indirect impacts of insecticides on sessile herbivores through the alteration of this competitive interaction with chewers (Figure 1, path A→B→C). We then evaluated the relative importance of direct and indirect effects for all 3 focal guilds (gall-inducers, upper-surface leaf-miners, and lower-surface leaf-miners).

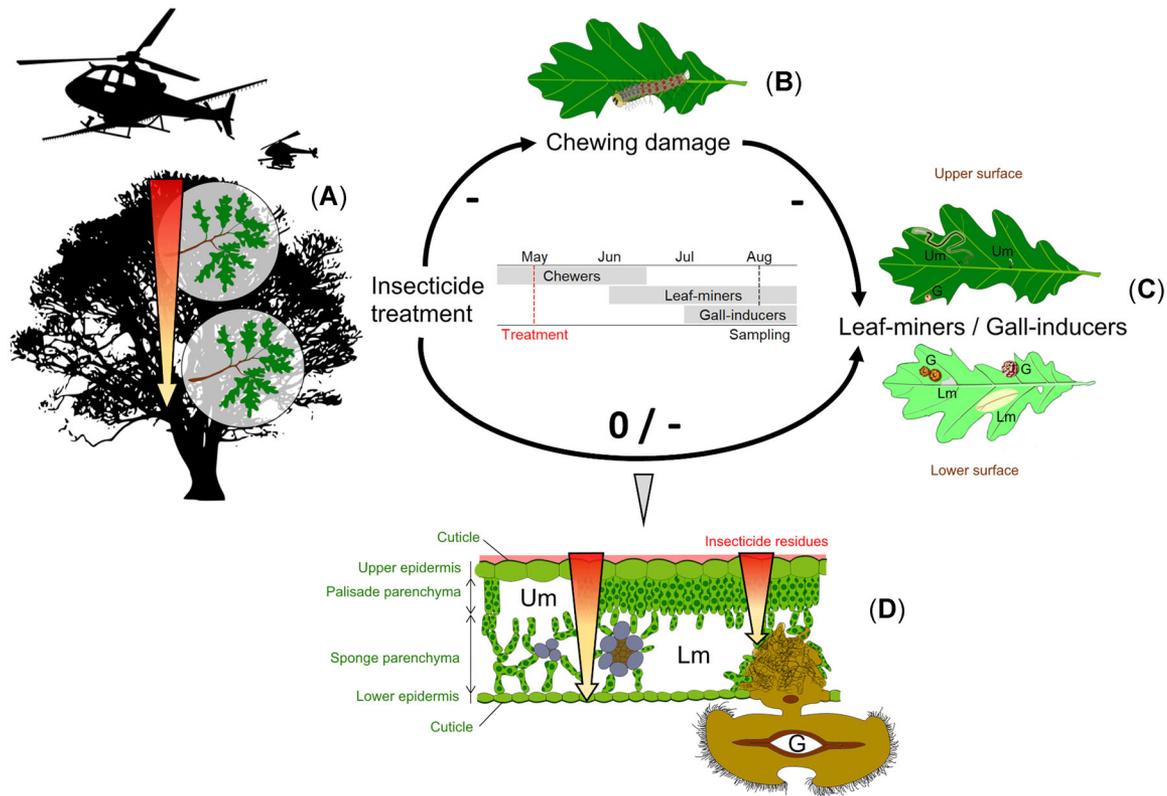


FIGURE 1: Conceptual framework of the present study. Black arrows indicate expected direct effects of the insecticide treatment (A) on chewing damage (path A→B) and leaf-miners and gall-inducers (A→C) as well as the direct effect of chewing damage on leaf-miners and gall-inducers (B→C). The hypothesized direction of the relationship is displayed next to the arrow. The indirect effect of insecticide use on leaf-miners and gall-inducers is indicated by the path A→B→C. (D) A cross-section of an oak leaf and the location of upper-surface leaf-miners (Um), lower-surface leaf-miners (Lm), and gall-inducers (G) in the leaf. Gradient-filled arrows show expected attenuation of insecticide effects at the tree-scale. (A; Filtering of spray droplet along canopy depth) and at the leaf-scale. (D; Penetration of insecticide residues into the leaf mesophyll.) Seasonal peaks of the different herbivore guilds and time stamps of insecticide treatment and leaf sampling are shown in the center of the figure.

MATERIALS AND METHODS

Drone experiment

Study site. In 2016, we conducted a tree-level field experiment whereby individual trees were treated with diflubenzuron or tebufenozide using an unmanned aerial vehicle (termed hereafter a drone), in an 80-ha oak/hornbeam stand located in the municipality of Willanzheim (Lower Franconia, Bavaria, Germany; 49.6919°N, 10.2409°E). The site was selected based on predicted high gradations of the oak processionary moth *Thaumetopoea processionea* L. (Lepidoptera: Thaumetopoeidae) extrapolated from population survey data collected during winter 2015–2016. Further details on site characteristics, management, and selection are provided in Leroy et al. (2019).

Study design. In early May 2016, we monitored the density of leaf-chewers on individual overstorey oaks by counting frass droppings falling on collection plates exposed below the tree crowns. To control for heterogeneity within the stand, density assessments were carried out in 10 relatively homogenous blocks that differed with regard to topography, overstorey and understorey density, and tree species composition. In each of these blocks, we selected 3 trees with high and 3 trees with low

chewer density. One treatment modality (diflubenzuron, tebufenozide, or unsprayed controls) was randomly attributed to each tree within each density class (Figure 2). Details of the tree selection procedure and the design of the experiment are provided in Leroy et al. (2019).

Treatment application. Insecticides were applied blockwise between 12 and 21 May 2016 with a Sitar Simpel single-rotor helicopter drone (Fa. Sitar and Helicopter-Service Freimut Stephan) equipped with 4 Airmix 110-05 injector nozzles (Sames Kremlin). Diflubenzuron was applied as Dimilin 80WP(R) (Spiess-Urania Chemicals; 800 g active ingredient [a.i.]/kg) at the former operational concentration of 12 g [a.i.]/ha. (NB: As of 2019, the product is no longer licensed for commercial use in Germany.) Tebufenozide was applied as Mimic® (Spiess-Urania Chemicals; 240 g [a.i.]/L) at an operational rate of 69.6 g [a.i.]/ha. The average crown projection area was estimated at 100 m², and this value was used to standardize the amount of product applied to individual trees. To assess the efficacy of the treatment, we monitored caterpillar mortality by collecting caterpillars falling on 3-m² tarpaulin sheets exposed below the crown of each tree during 1 mo after spray (method described in Leroy et al. 2019).

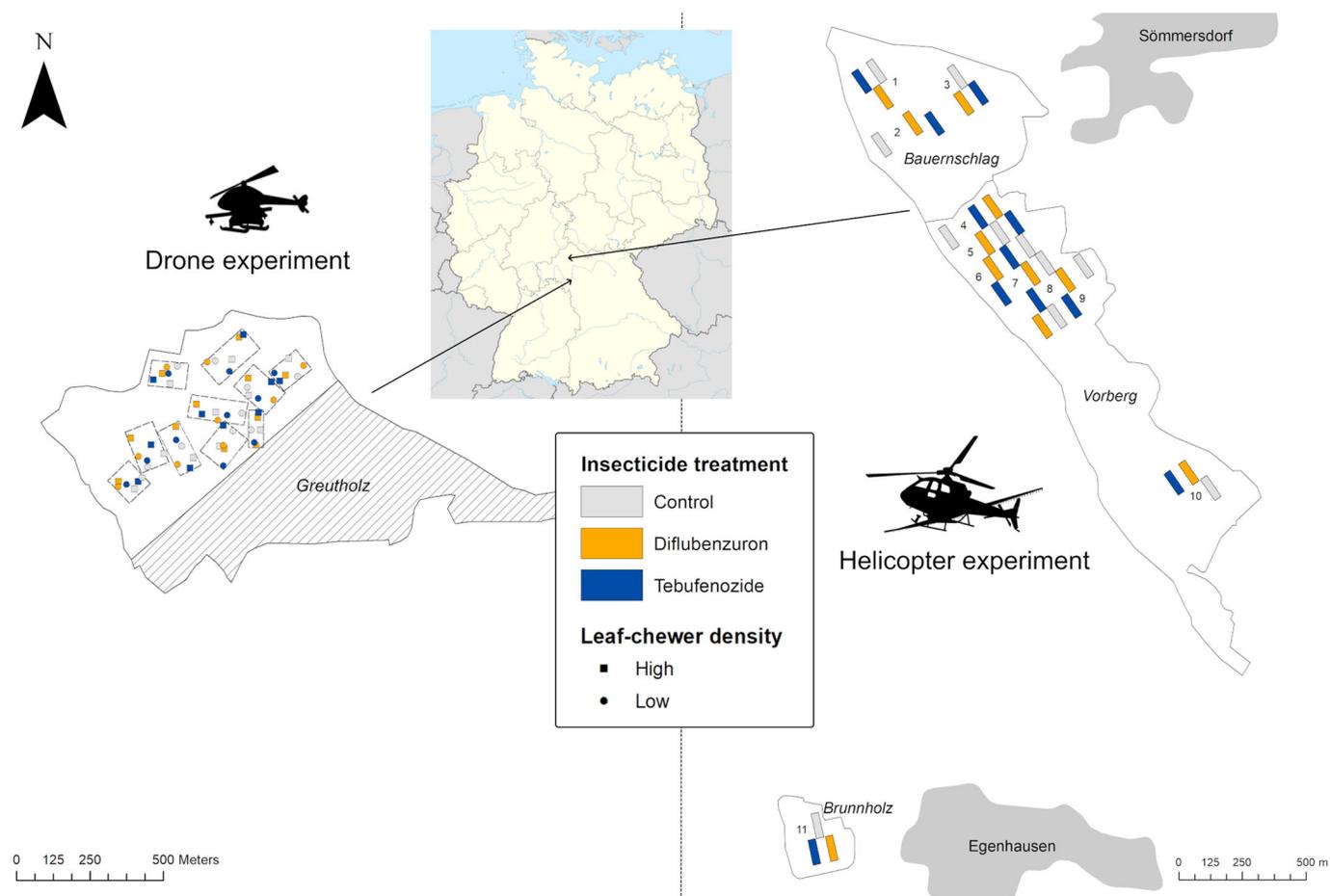


FIGURE 2: Maps of the drone and helicopter experiments. Arrows indicate the location of the study areas in Germany. Drone experiment (left): The hatch pattern indicates forest areas where the understory vegetation was not recently harvested and hence not used in the present study. Colored points designate trees sprayed with tebufenozide (blue) or diflubenzuron (orange), and unsprayed controls (gray). Point shape indicates different chewer density classes (square: high; circle: low) assessed prior to spraying. Blocks are shown as dashed-outlined rectangles. Helicopter experiment (right): Colored rectangles indicate 30- x 100-m treatment plots: tebufenozide (blue), diflubenzuron (orange), or unsprayed control (gray), arranged into blocks numbered from 1 to 11.

Herbivory assessment. We sampled oak foliage in each tree between 22 and 26 August 2016. Two 50-cm-long twigs were cut from the upper and the lower canopy by tree climbers. We haphazardly collected 50 fully developed leaves from the tip to the base of each twig, for a total of 100 leaves/tree. Each leaf was thoroughly inspected for the presence of mines (i.e., scars left by leaf-miner feeding) and galls. Lepidopteran leaf-miners and cynipid and cecidomyiid galls were identified to genus or species whenever possible based on the shape and position of the mines and galls. Chewing damage was scored on each leaf as the percentage of leaf area removed with the help of a set of 67 oak leaf templates of various sizes and shapes with known missing leaf areas. Each leaf was scored in 1 of 13 damage classes (0, 0–1, 2–5, 6–10, 11–20, 21–30, 31–40, 41–50, 51–60, 61–70, 71–80, 81–90, and 91–100%). For analysis, the means of these classes were used to approximate the magnitude of chewing damage, and leaves were further classified into “damaged” and “intact” categories. Leaves were considered intact (i.e., chewing damage was absent) when <1% area had been removed, because such low damage could not be attributed with certainty to chewing insects.

Leaf miner survivorship. Each mine was inspected for the presence of a larva, a pupa, a characteristic exit hole, and predation marks (i.e., mine surface torn apart). The presence of an exit hole or a pupa indicated completed larval development, which was used as a proxy for survivorship. Because some leaf-miners were still developing inside the leaves at the time of sampling, survivorship may have been underestimated. However, because we sampled in the late summer, larval development of the first generation, which should be exposed to higher concentrations of insecticides, was fully completed (Supplemental Data, Table S2-1). Hence, we considered the proportion of exit holes as an appropriate measure of survivorship to test insecticide effects in this system.

Data analysis. The efficacy of diflubenzuron and tebufenozide was assessed for 3 different variables: 1) caterpillar mortality/tree, expressed as the number of individuals fallen from the crown, 2) mean proportion of leaf area removed/branch, and 3) occurrence of chewing damage/leaf. To test the effect of the treatments on the occurrence rate and the magnitude of chewing damage, we fitted linear mixed models

(LMMs) with insecticide treatment (diflubenzuron or tebufenozide, dummy-coded), canopy stratum, and their interaction as fixed effects. To account for spatial heterogeneity and unmeasured sources of variation at the tree level, we allowed random intercepts for tree nested into block. In addition, covariates characterizing the structure of individual trees were considered for inclusion in each model after being checked for multicollinearity and potential confounding effects with fixed predictors. Based on the results, crown volume was included in the models (see the Supplemental Data, S1, for measurement and selection procedures of the covariates). To evaluate the impact of the treatments on the mortality of leaf-chewers, we fitted a log-normal LMM with treatment (diflubenzuron or tebufenozide, dummy-coded) as a fixed effect, block as a random effect (random intercept), and crown volume as a covariate (Supplemental Data, S1). Because the results showed no relationship between chewing damage and treatment in the drone experiment (see *Effect of insecticides on leaf-chewing herbivores* in the Results), follow-up analyses focused exclusively on the separate impacts of insecticides and chewing damage on leaf-miners and gall-inducers.

We assessed the direct effects of insecticides on the abundance and occurrence of sessile herbivores at the branch and leaf levels, respectively. We fitted generalized linear mixed models (GLMMs) with negative binomial (abundance data) and binomial (occurrence data) error distribution for each of the 3 focal guilds: upper-surface leaf-miners, lower-surface leaf-miners, and gall-inducers. We fitted another set of binomial GLMMs to assess the survivorship of the 2 most sampled leaf-miner genera: *Phyllonorycter* and *Stigmella*. In the analyses of leaf-miner occurrence and survivorship, leaves occupied by a sessile herbivore not belonging to the focal guild/genus (i.e., response variable) were excluded, because the presence of another leaf-miner or gall-inducer on the leaf could also influence leaf selection. Fixed predictors included insecticide treatment (diflubenzuron and tebufenozide, dummy-coded), chewing damage, as the percentage of damaged leaves in branch-level models and damage occurrence in leaf-level models, and canopy stratum, as well as the interactions between treatment and canopy stratum, and treatment and chewing damage. The specification of the fixed terms was kept maximal in all models, meaning that no main effect or interaction term was removed regardless of the results from statistical tests. Tree nested into block (branch-level models) and branch nested into tree nested into block (leaf-level models) were added as random effects. When necessary, the random effect structure was simplified to correct convergence problems by removing random terms with near-zero estimated variance (singular fits). Tree diameter at breast height was included in the models as a covariate (Supplemental Data, S1).

All analyses were conducted in R, Ver 4.0.2. The GLMMs were fitted with the functions “glmer” in the lme4 package (Bates et al. 2015) and “glmmTMB” in the glmmTMB package (Brooks et al. 2017). Nakagawa's pseudo- R^2 s for hierarchical models (Nakagawa et al. 2017) were calculated as a measure of goodness-of-fit with the function “r2” of the performance package (Lüdecke et al. 2020). We tested our hypotheses

(Figure 1) with Wald tests (negative binomial family) with the function “Anova” of the car package (Fox and Weisberg 2011) and likelihood ratio tests (gaussian and binomial family) with the function “mixed” of the afex package (Singmann et al. 2020). Multivariate- t -adjusted pairwise comparisons of estimated marginal means were performed when relevant using the function “contrast” of the emmeans package (Lenth 2020).

Helicopter experiment

The weaker than expected gradations of leaf-chewers in the drone experiment motivated the design of a new experiment to address our questions in a situation of stronger competitive pressure on sessile herbivores. In addition, we designed this experiment to make it suitable for helicopter application, intending to estimate whether the application method could have a strong influence on the ecological sensitivity of the focal guilds.

Study sites. The experiment was conducted in 3 neighboring forest stands in the region of Schweinfurt (Lower Franconia, Bavaria, Germany) including the adjacent stands Bauernschlag (52-ha, oak/beechn) and Vorberg (58-ha oak/hornbeam) located near the village of Sömmersdorf (50.05402°N, 10.08476°E) and the smaller forest patch Brunnholz (6-ha pure oak) near Egenhausen (50.03127°N, 10.08265°E). All stands are dominated by pedunculate (*Q. robur*) and sessile oak (*Q. petraea*). The sites were selected based on the results of population surveys of the gypsy moth, conducted during the previous winter (October 2016–February 2017). Density estimates were inferred by counting egg masses on the 2 lowest meters of the trunks of mature oak trees in successive transects of 10 trees each. We conducted 11 transects in Bauernschlag, 28 in Vorberg, and 23 in Brunnholz. The results predicted low, medium, and high (i.e., outbreak) gypsy moth densities for 2017 in Bauernschlag, Vorberg, and Brunnholz, respectively.

Study design. All forest sites were mapped in January 2017 to identify usable areas for the experiment. Tree species composition, age, and density, as well as the presence of permanent and temporary water bodies (e.g., water tanks, ponds, and ditches), were scored across the forest to delimit homogenous areas. Forty-m-wide spray-free buffer zones were defined along the forest edge and around water bodies to prevent spray spillover to adjacent plots and in compliance with German regulations regarding aerial applications. Within the sprayable area, blocks were designed by randomly assigning individual treatments to 3 neighboring 3000-m² (100- × 30-m) rectangular plots separated by a buffer strip with a minimum width of 40 m. In total, 11 blocks were established across the 3 stands, that is 3 in Bauernschlag, 7 in Vorberg, and 1 in Brunnholz (Figure 2).

Treatment application. Spraying was carried out by a Bell 47 helicopter on 17 May 2017 between 07:00 and 09:00. Diflubenzuron was sprayed as Dimilin 80WP® (Spiess-Urania Chemicals; 800 g[a.i.]/kg) at the former maximum legal rate of

60 g [a.i.]/ha; Tebufenozide was sprayed as Mimic[®] (Spiess-Urania Chemicals; 240 g [a.i.]/L) at a maximum legal rate of 180 g [a.i.]/ha. To measure the efficacy of the insecticides in controlling the pest population, we counted gypsy moth caterpillars resting under bands of barrier tape placed around the trunk of each tree on 12 and 13 June 2017.

Herbivory assessment. We sampled oak foliage in one overstory oak tree located near the center of each plot between 19 and 21 June 2017. Leaves were sampled and processed, and herbivory was scored following the same protocol applied in the drone experiment (see the previous section under *Drone experiment; Herbivory assessment*). However, due to the early sampling timing, which corresponded to the feeding period of the first generation of lepidopteran leaf-miners (Supplemental Data, Table S2-1), no assessment of survivorship was made, because leaves were sampled early in the summer, when most first-generation leaf-miners had not completed their larval development.

Data analysis. The efficacy of diflubenzuron and tebufenozide was tested on gypsy moth density under burlap (i.e., per tree), the mean proportion of leaf area removed/branch, and the occurrence of chewing damage at the leaf level. For the 2 latter variables, the analysis was performed following the procedures described in the previous section (see section *Drone experiment; Data analysis*). The number of gypsy moth caterpillars under burlap bands (one/tree) was fitted as the response variable in a negative binomial GLMM with treatment (diflubenzuron or tebufenozide, dummy-coded) as a fixed effect and block as a random effect. In all 3 models, crown volume and crown density were included as covariates based on results of the covariate selection procedures (Supplemental Data, S1).

To evaluate the relative importance of direct and indirect treatment effects on leaf-miner and gall-inducer incidence, we conducted a separate piecewise structural equation model for each insecticide treatment. Measured variables included incidence of upper-surface mines, lower-surface mines, and galls, as well as the incidence of chewing damage (all binary) at the leaf level. The categorical predictors insecticide treatment and canopy stratum were dummy-coded, with "1" indicating spraying and upper canopy layer, respectively. To account for spatial heterogeneity and among-tree variability, we used GLMMs with branch nested into tree nested into block as a random effect. The random effect structure was then adjusted for every separate path model by removing terms with near-zero variance estimates. Crown volume and crown density were added as covariates following the same selection procedure applied in the analyses of the drone experiment (Supplemental Data, S1). The overall fit of the model was assessed with Shipley *d*-separation tests, summarized using Fisher's *C* statistic. The strength of an indirect treatment effect on leaf-miners and gall-inducers was calculated as the product of the coefficients (at the logit scale) of the paths connecting insecticide and chewing damage and chewing damage and leaf-miner/gall-inducer incidence, provided both were significant. The total effect of treatment was calculated as the sum of the coefficients of significant intermediate paths.

All analyses were conducted in R, Ver 4.0.2. Structural equation models were fitted with the function "psem," Fisher's *C* statistics were calculated with the function "fisherC," and Nakagawa's pseudo- R^2 values were calculated as a measure of goodness-of-fit with the function "rsquared." All the abovementioned functions are part of the piecewiseSEM package (Lefcheck 2016).

RESULTS

Herbivore communities

In the drone experiment, 973 mines and 688 galls belonging to 12 genera (18 identified species) were recorded on 5945 oak leaves. In the helicopter experiment, 473 mines and 688 galls belonging to 12 genera (16 identified species) were recorded on 3304 leaves (Supplemental Data, S3). The most common mines belonged to the genera *Phyllonorycter* (Lepidoptera: Gracillariidae [$n = 648$]) and *Stigmella* (Lepidoptera: Nepticulidae [$n = 509$]). Most galls were of the genus *Neuroterus* (Hymenoptera: Cynipidae [$n = 901$]; Supplemental Data, S3). Leaves were sampled much earlier in the season in the helicopter experiment (late June, that is, first generation of most leaf-miner species; Supplemental Data, Table S2-1) than in the drone experiment (late August, that is, second generation of most leaf-miner species). Despite these differences, the occurrence rate was comparable in both experiments, with 23 and 20% of the scored leaves being occupied by at least one sessile herbivore in the drone and the helicopter experiments, respectively. Levels of chewing damage were also similar, with 77 and 70% of all scored leaves damaged by leaf-chewers in the drone and the helicopter experiments, respectively. Although the same species were found in both experiments, leaf-miners were nearly twice as abundant in the drone experiment (Supplemental Data, Table S3-1). The gall-inducing community of the drone experiment was characterized by the dominance of the agamous generation of *Neuroterus* galls and by the absence of *Cynips* galls. The gall-inducing community in the helicopter experiment was more diverse and included greater numbers of cynipid galls of the sexual generation as well as cecidomyiid galls (Supplemental Data, Table S3-2). Co-occurrence of 2 or more sessile herbivores was observed in 4.2 and 5.0% of the leaves (20.1 and 27.1% of infested leaves) in the drone and the helicopter experiments, respectively.

Drone experiment

Effect of insecticides on leaf-chewing herbivores. In the drone experiment, insecticides effectively killed leaf-chewing Lepidoptera, with 51 and 124% more caterpillars falling from the crown of diflubenzuron- and tebufenozide-treated trees, respectively. Approximately 77% of the leaves presented some chewing damage, with no difference in the magnitude of chewing damage/branch and the incidence of chewing damage/leaf among treatments (Figure 3 and Table 1).

Effect of insecticides and chewing damage on sessile herbivores. At the branch level, application of diflubenzuron resulted in a reduced abundance of upper-surface leaf-miners,

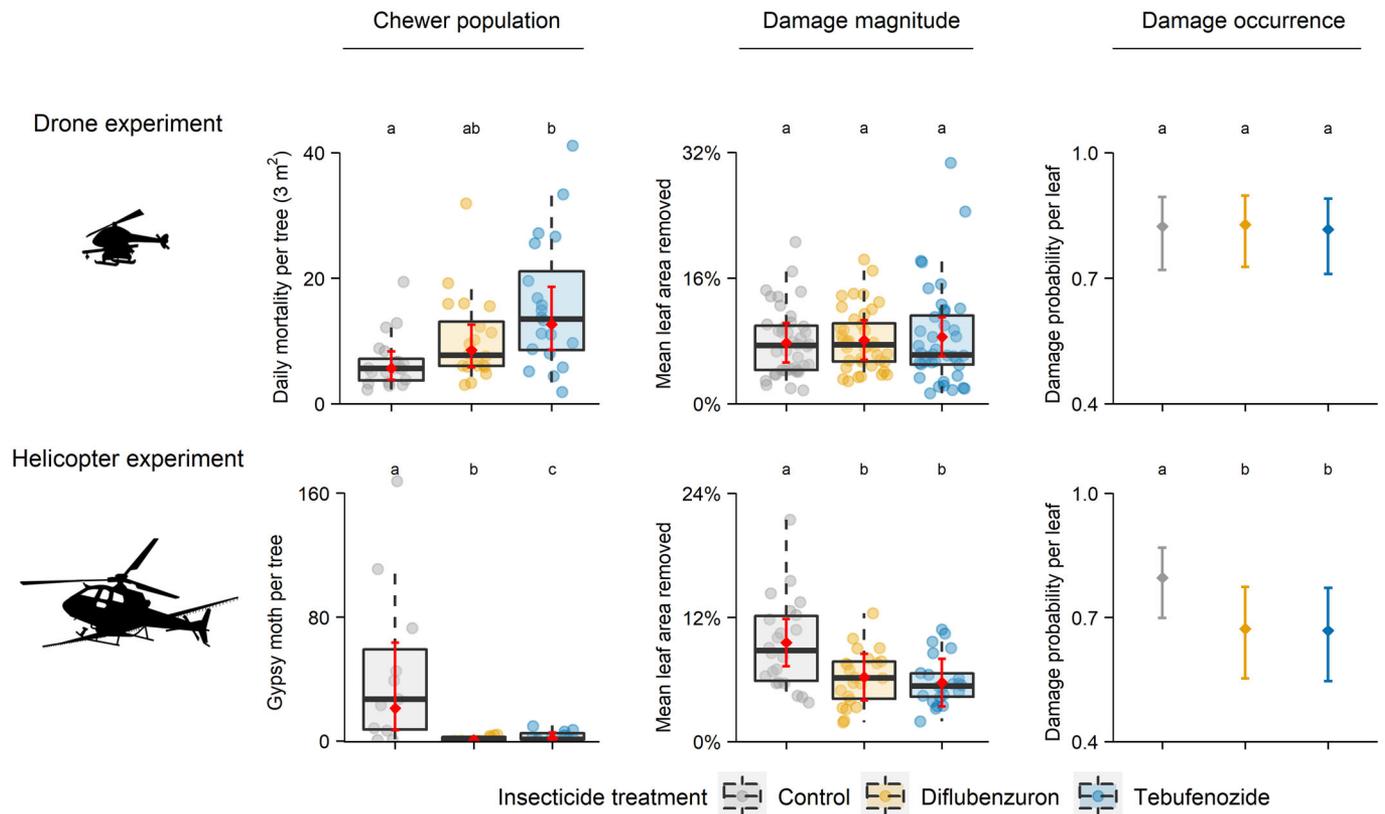


FIGURE 3: Effect of insecticide treatment on lepidopteran leaf-chewer populations and the magnitude and probability of chewing damage in the drone and the helicopter experiment. Insecticides were applied at standard operational rates in the drone experiment (12 g [diflubenzuron]/ha and 69.6 g [tebufenozide]/ha; target species oak processionary moth *Thaumetopoea processionea*) and at the maximal legal rates in the helicopter experiment (60 g [diflubenzuron]/ha and 180 g [tebufenozide]/ha; target species gypsy moth *Lymantria dispar*). Effect on leaf-chewer populations was quantified as the number of fallen caterpillars/tree (i.e., 3 m² sheet) as a proxy for mortality in the drone experiment, and as the density of gypsy moth caterpillars/tree (i.e., under burlap bands) in the helicopter experiment. The magnitude of chewing damage is expressed as the mean percentage of leaf area removed/branch. Chewing damage occurrence is expressed as the probability of chewing damage occurrence/leaf. Colored points (circle shape) and box plots show the actual data for control (gray), diflubenzuron (orange), and tebufenozide (blue). Diamond-shaped points and error bars correspond to estimated marginal means and 95% confidence intervals, respectively. Different lower-case letters denote significant differences among treatments (pairwise comparisons of estimated marginal means with multivariate-t correction of *p* values; $\alpha = 0.05$). Results of the statistical models are given in Table 1.

with 47 and 34% fewer mines found in branches from diflubenzuron-treated trees than from controls and tebufenozide-treated trees, respectively. There was no significant difference in the abundance of upper-surface leaf-miners between control and tebufenozide trees. Lower-surface leaf-miners and gall-inducers were unaffected by either insecticide. The proportion of leaves affected by chewing damage did not influence the abundance of leaf-miners, regardless of which leaf surface they feed on. However, there was a significant negative relationship between chewing damage frequency and the abundance of gall-inducers in diflubenzuron-treated trees, whereas such a pattern was not observed for other treatment groups (Figure 4 and Table 2). At the leaf level, the impact of insecticide treatments on the occurrence of upper-surface leaf-miners varied depending on the occurrence of chewing damage. Upper-surface mines occurred less frequently in leaves from diflubenzuron-treated trees than control leaves, but the difference was only statistically significant on damaged leaves. There were no statistically significant differences between tebufenozide trees and controls.

Upper-surface leaf-miners were 2.4 times more likely to occur on intact than on damaged leaves in control trees. A similar pattern was observed in diflubenzuron-treated trees, with a 3.0 times higher occurrence probability on intact compared with damaged leaves. However, the difference was not significant on tebufenozide-treated trees, with an occurrence probability being 1.6 times higher on intact than on damaged leaves. In contrast, neither treatment had any effect on the incidence of lower-surface leaf-miners and gall-inducers. Lower-surface leaf-miners were 1.9, 2.5, and 1.8 times more likely to occur on intact than on damaged leaves in control, diflubenzuron, and tebufenozide trees, respectively. The same pattern was observed for gall-inducers, with 1.8, 2.1, and 2.0 times higher occurrence probability on intact leaves in control, diflubenzuron, and tebufenozide trees, respectively (Figure 4 and Table 2). Lower-surface mines occurred more frequently on lower canopy leaves, independent of the treatment applied. The incidence of upper-surface mines and galls at the leaf level was not influenced by canopy stratum (Supplemental Data, Figure S4-1 and Table 2).

TABLE 1: Results of (generalized) linear mixed models testing for the effect of insecticide treatments on leaf-chewer populations and leaf-chewing damage on oak trees in the drone and the helicopter experiment^a

| Fixed effects (X ²) ^b | Experiment | | | | Drone experiment | | | | Helicopter experiment | | | |
|--|---|----------------|---------------------------------------|-------------|------------------------------|---------------------------|------------------------------|----------------------------------|------------------------------|---------------------------|------------------------------|----------------------------------|
| | Model elements | Response Level | Caterpillar mortality (n/d/tree) Tree | Sample size | Leaf area removed (%) Branch | Damaged leaves (%) Branch | Damage occurrence (0/1) Leaf | Gypsy moth density (n/tree) Tree | Leaf area removed (%) Branch | Damaged leaves (%) Branch | Damage occurrence (0/1) Leaf | Gypsy moth density (n/tree) Tree |
| | | | | | | | | | | | | |
| | Diflubenzuron (0/1) | 5.17* | 0.09 | 0.21 | 0.01 | 121.04*** | 8.07** | 4.62* | 5.29* | | | |
| | Tebufenozide (0/1) | 17.25*** | 0.40 | 0.04 | 0.03 | 79.76*** | 9.82** | 4.16* | 5.30* | | | |
| | Canopy stratum (Up/Low) | — | 1.87 | 0.23 | 0.69 | — | 1.02 | 0.36 | 0.28 | | | |
| | Diflubenzuron x stratum | — | 0.00 | 0.00 | 0.12 | — | 0.15 | 0.21 | 0.10 | | | |
| | Tebufenozide x stratum | — | 0.64 | 1.00 | 1.27 | — | 0.97 | 0.46 | 0.26 | | | |
| | Crown volume (m ³) ^c | 2.99* | 1.92 | 5.51* | 4.15* | 0.71 | 0.28 | 1.42 | 1.86 | | | |
| | Crown density (%) ^c | — | — | — | — | 13.93*** | 0.28 | 0.27 | 0.10 | | | |
| Random effects (var) ^d | Block | 0.07 | 0.14 | 0.25 | 0.07 | 2.19 | 0.01 | 0.45 | 0.13 | | | |
| | Tree/Block | — | 0.07 | 2.29 | 0.83 | — | 0.04 | 0.75 | 0.08 | | | |
| | Branch/Tree/Block | — | — | — | 0.37 | — | — | — | 0.47 | | | |
| Goodness-of-fit (R ²) ^e | Marginal | 0.24 | 0.04 | 0.08 | 0.02 | 0.58 | 0.24 | 0.12 | 0.03 | | | |
| | Conditional | 0.38 | 0.42 | 0.73 | 0.29 | 0.99 | 0.65 | 0.44 | 0.20 | | | |

^aInsecticides were applied at standard operational rates in the drone experiment (12 g [diflubenzuron]/ha and 69.6 g [tebufenozide]/ha; target species oak processionary moth *Thaumetopoea processionea*) and at the maximal legal rates in the helicopter experiment (60 g [diflubenzuron]/ha and 180 g [tebufenozide]/ha; target species: gypsy moth *Lymantria dispar*). Each column represents one model, characterized by its response variable, hierarchical level of observation, and sample size. Rows indicate the fixed predictors, that is, the independent variables, interaction terms, and covariates included in the models, the random effect structure, and the goodness-of-fit of each model. Numbers indicate results from Chi-square tests (fixed effects), random effect variances (random effects), and Nakagawa's pseudo R²s (goodness-of-fit).

^bLikelihood ratio tests were performed for models with binomial (damage occurrence) and normal (leaf area removed) error distribution. Wald tests were performed for models with negative binomial error distribution (leaf-chewer mortality and density).

^cCovariate selection procedures are detailed in the Supplemental Data, S1.

^dRandom intercepts for tree nested into block, and branch nested into tree nested into block were included as a default random effect structure for branch- and leaf-level models, respectively.

^eMarginal R² indicates the ratio of variance explained by the fixed effects, and conditional R² indicates the ratio of the variance explained by the fixed and the random effects.

***p < 0.001.

**p < 0.01.

*p < 0.05.

+p < 0.1.

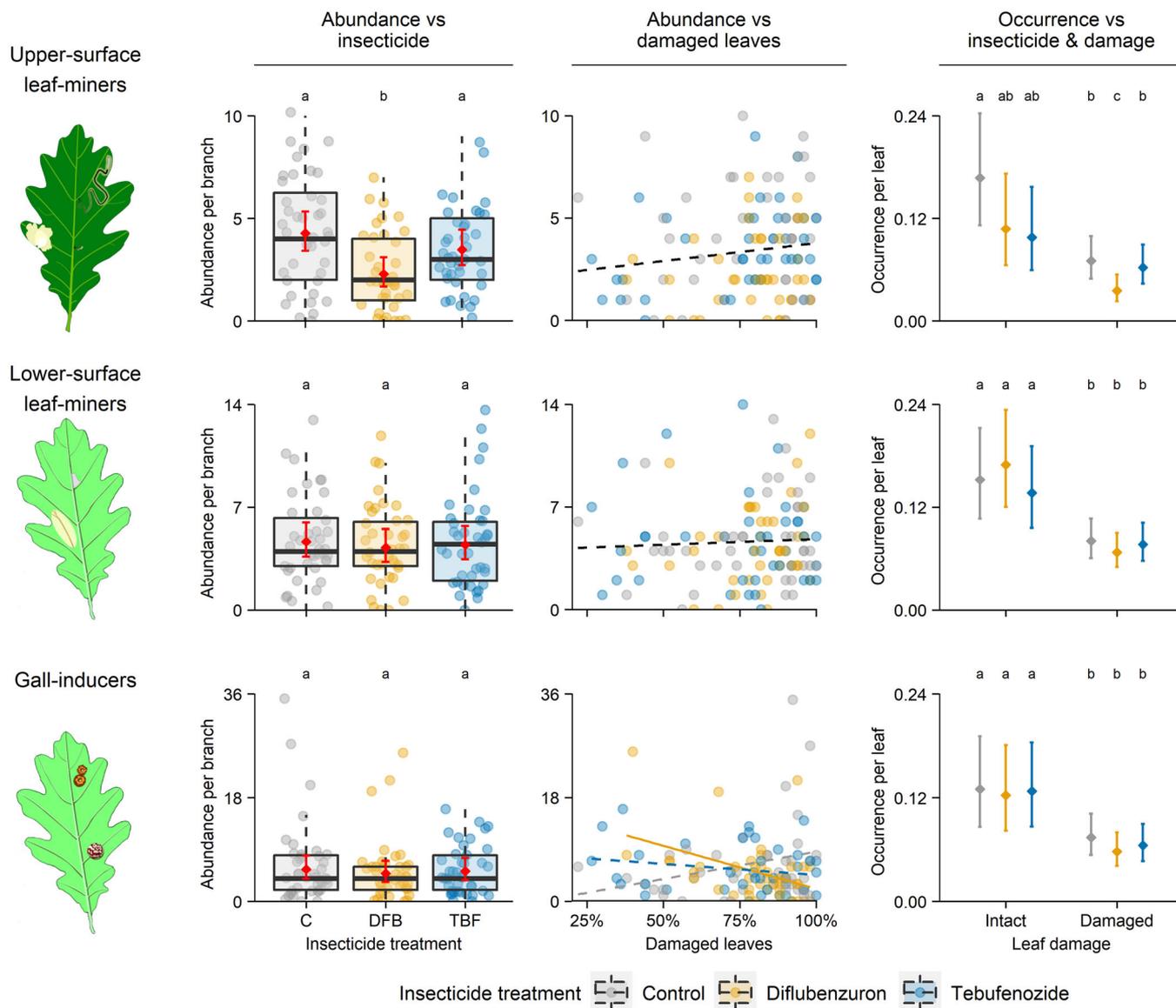


FIGURE 4: Effect of insecticide treatment and chewing damage on upper-surface leaf-miners, lower-surface leaf-miners, and gall-inducers at the branch- and leaf-levels in the drone experiment. Insecticides were applied with a drone between 12 and 21 May 2016 on crowns of individual trees (20/treatment). Treatment groups are control (C; gray; no spraying), diflubenzuron (orange; 12 g [a.i.]/ha), and tebufenozide (blue; 69.6 g [a.i.]/ha). Damage is quantified as the percentage of damaged leaves/branch and as the occurrence of chewing damage on individual leaves (intact leaves: $\leq 1\%$ leaf area removed; damaged leaves: $> 1\%$ leaf area removed). Leaves were collected from one branch in the upper canopy and one branch in the lower canopy of each tree (i.e., 50 leaves/branch). **(Left)** Difference in abundance of sessile herbivores/branch among treatment groups. Colored points (circle shape) and box plots show the actual data. Red points (diamond shape) and error bars correspond to estimated marginal means and 95% confidence intervals, respectively. Different lower-case letters denote significant differences among treatments (pairwise comparisons of estimated marginal means with multivariate- t correction of p values; $\alpha = 0.05$). **(Center)** Relationship between the percentage of damage leaves on the abundance of sessile herbivores/branch. Colored points (circle shape) show the actual data, lines represent the regression curves characterizing the relationship, and dashed lines show nonsignificant relationships. **(Right)** Difference in the probability of presence of sessile herbivores/leaf among treatment groups as a function of chewing damage. Points (diamond shape) and error bars correspond to estimated marginal means and 95% confidence intervals, respectively. Different lower-case letters indicate significant differences among treatments (pairwise comparisons of estimated marginal means with multivariate- t correction of p values; $\alpha = 0.05$). Results of models are given in Table 2.

Effect of insecticides and chewing damage on the dominant leaf-miner genera.

On damaged leaves, the occurrence of the dominant upper-surface leaf-miner *Stigmella* sp. in diflubenzuron-treated trees was half that in control trees. On intact leaves, the difference was smaller (odds ratio of 1.3) and not significant. No significant difference in the numbers of the

dominant lower-surface leaf-miner *Phyllonorycter* sp. was observed among treatments (Figure 5 and Table 3). Mines of both genera were found more frequently on intact leaves, apart from *Stigmella* in tebufenozide-treated trees (Table 1). Survivorship of *Phyllonorycter* larvae was significantly reduced in diflubenzuron-sprayed trees, but only for individual feeding on

TABLE 2: Results of generalized linear mixed models testing for the effect of insecticide treatments and chewing damage on the abundance at the branch-level and the occurrence at the leaf-level of upper-surface leaf-miners, lower-surface leaf-miners, and gall-inducers on oak trees in the drone experiment^a

| | Model elements | Guild | Upper-surface miners | | Lower-surface miners | | Gall-inducers | |
|--|---|-------------------------------|------------------------------------|---|------------------------------------|---|------------------------------------|---|
| | | Response Level Sample size | Abundance (n) Branch n = 120 | Occurrence (0/1) Leaf n = 5101 | Abundance (n) Branch n = 120 | Occurrence (0/1) Leaf n = 5227 | Abundance (n) Branch n = 120 | Occurrence (0/1) Leaf n = 5176 |
| Fixed effects (X^2) ^b | Diflubenzuron (0/1) | | 16.67*** | 11.68** | 0.33 | 0.31 | 8.66** | 1.02 |
| | Tebufenozide (0/1) | | 2.10 | 2.53 | 0.08 | 0.20 | 2.55 | 0.28 |
| | Chewing damage ^c | | 3.30 ⁺ | 45.12*** | 1.10 | 48.00*** | 2.65 | 36.70*** |
| | Canopy stratum (Up/Low) | | 1.61 | 1.51 | 7.90** | 5.43* | 0.00 | 1.72 |
| | Diflubenzuron × damage | | 0.03 | 0.49 | 0.16 | 1.45 | 9.26** | 0.48 |
| | Diflubenzuron × stratum | | 0.32 | 0.54 | 0.03 | 0.18 | 0.63 | 0.33 |
| | Tebufenozide × damage | | 0.24 | 2.80 ⁺ | 0.15 | 0.06 | 3.01 ⁺ | 0.17 |
| | Tebufenozide × stratum | | 0.15 | 0.14 | 0.20 | 0.34 | 0.01 | 0.07 |
| | Diameter at breast height (cm) ^d | | 1.39 | 3.95* | 2.60 | 0.55 | 0.63 | 0.23 |
| Random effects (var) ^e | Block | | 0.00 | 0.02 | 0.01 | 0.01 | 0.01 | — |
| | Tree/Block | | 0.00 | 0.04 | 0.03 | 0.09 | 0.12 | 0.16 |
| | Branch/Tree/Block | | — | 0.16 | — | 0.11 | — | 0.16 |
| Goodness-of-fit (R^2) ^f | Marginal | | 0.21 | 0.07 | 0.11 | 0.04 | 0.13 | 0.03 |
| | Conditional | | 0.21 | 0.12 | 0.20 | 0.10 | 0.30 | 0.12 |

^aInsecticides were applied by drone at standard operational rates (12 g [diflubenzuron]/ha and 69.6 g [tebufenozide]/ha; target species oak processionary moth *Thaumetopoea processionea*). Each column represents one model, characterized by its response variable, hierarchical level of observation, and sample size. Rows indicate the fixed predictors, that is, the independent variables, interaction terms, and covariates included in the models, the random effect structure, and the goodness-of-fit for each model. Numbers indicate results from Chi-square tests (fixed effects), random effect variances (random effects), and Nakagawa's pseudo- R^2 s (goodness-of-fit).

^bLikelihood ratio tests were performed for models with binomial and normal error distribution (occurrence). Wald tests were performed for models with negative binomial error distribution (abundance).

^cChewing damage defined as the percentage of damaged leaves/branch (branch-level models) and as the occurrence of chewing damage (leaf-level models).

^dCovariate selection procedures are detailed in the Supplemental Data, S1.

^eRandom intercepts for tree nested into block, and branch nested into tree nested into block were included as a default random effect structure for branch- and leaf-level models, respectively. Nested terms with near-zero variance estimates were removed when appropriate.

^fMarginal R^2 indicates the ratio of variance explained by the fixed effects, and conditional R^2 indicates the ratio of the variance explained by the fixed and the random effects.

*** $p < 0.001$. Test values not followed by a symbol are not statistically significant.

** $p < 0.01$.

* $p < 0.05$.

⁺ $p < 0.1$.

intact leaves, with only 56% of the survivorship rate observed on intact control leaves. In contrast, the survivorship of *Stigmella* leaf-miners was not affected by any treatment (Figure 5 and Table 3). Both *Stigmella* and *Phyllonorycter* mines were more abundant on lower canopy leaves, independent of the treatment applied, but the effect was only marginally significant for *Stigmella* (Supplemental Data, Figure S4-1 and Table 3). Conversely, *Stigmella*, but not *Phyllonorycter*, achieved better survivorship on lower canopy leaves (Table 3).

Helicopter experiment

Effect of insecticides on leaf-chewing herbivores. In the helicopter experiment, both diflubenzuron and tebufenozide successfully controlled the gypsy moth population at the tree-level, with a reduction in caterpillar abundance of 98 and 90% relative to controls, respectively. Chewing damage was observed on 68% of the leaves. Mean leaf area removed was the highest in control trees ($9.41 \pm 0.36\%$). Chewing damage was observed on 77% of the control leaves and 65% of the diflubenzuron- and tebufenozide-treated trees. However, the percentage of leaf area removed was significantly and 53 and

68% greater in control trees than in diflubenzuron- and tebufenozide-treated trees (Figure 3 and Table 1).

Direct and indirect effects of insecticides on leaf-miners and gall-inducers.

The structural equation models showed adequate fits to the data for diflubenzuron (Fisher's $C = 4.707$, $p = 0.582$) and tebufenozide ($C = 6.532$, $p = 0.366$). Diflubenzuron directly impacted the occurrence of upper-surface mines, with a 43% decrease in the odds of finding a leaf-miner on a leaf from diflubenzuron trees relative to controls, but not that of lower-surface mines and galls. In diflubenzuron-treated trees, the presence of chewing damage reduced the odds of finding an upper-surface leaf-miner (35%), a lower-surface leaf-miner (34%), and a gall-inducer (48%) compared with control trees. The impact of diflubenzuron on chewing damage led to an indirect increase in the odds of occurrence of upper-surface leaf-miners, lower-surface leaf-miners, and galls of 32, 31, and 53%, respectively (Figure 6A). The relationship between upper-surface leaf-miners and diflubenzuron was the only one involving both a direct and an indirect component, with a net reduction in the odds of finding an upper-surface mine of 24% in diflubenzuron trees relative to controls. Treatment with

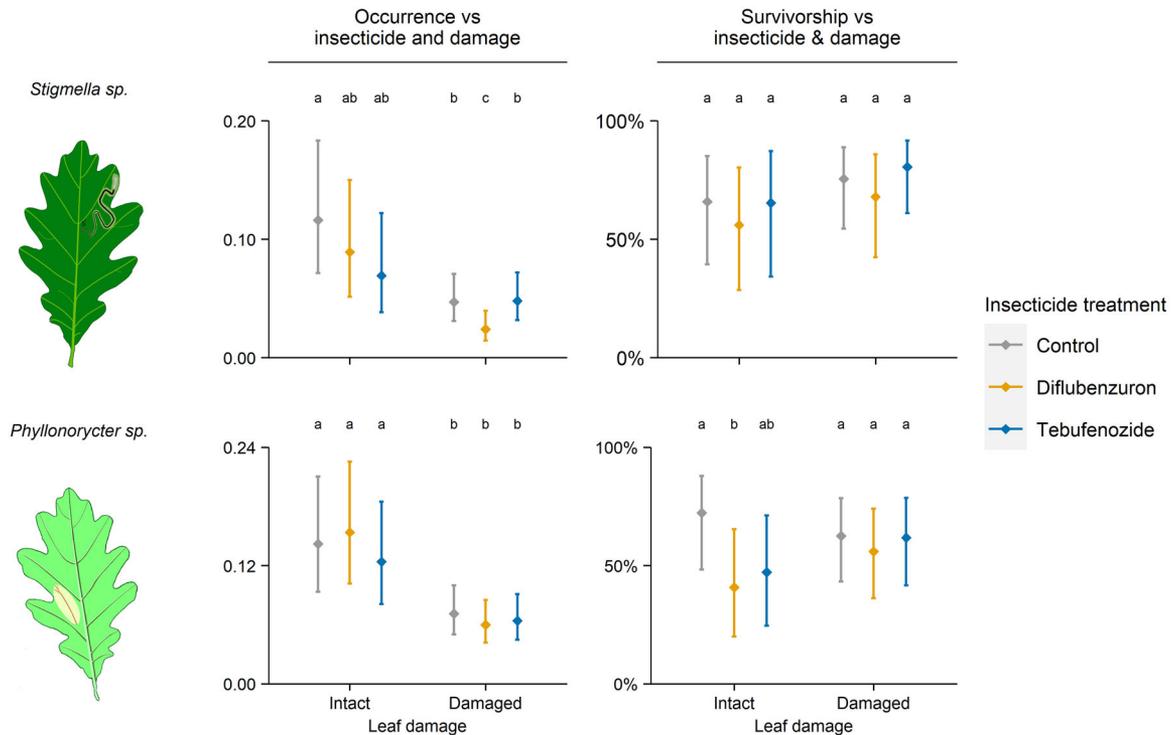


FIGURE 5: Effect of insecticide treatment and chewing damage on the occurrence and survivorship of *Stigmella* and *Phyllonorycter* leaf-miners in the drone experiment. Insecticides were applied with a drone between 12 and 21 May 2016 on individual tree crowns (20 trees/treatment). Leaf-miner occurrence is expressed as the probability of presence on a leaf. Survivorship is expressed as the percentage of mines presenting a characteristic exit hole (*Stigmella* sp.), or a pupa or an edge opening (*Phyllonorycter* sp.). Treatment groups are control (C; gray; no spraying), diflubenzuron (orange; 12 g [a.i.]/ha), and tebufenozide (blue; 69.6 g [a.i.]/ha); leaf damage categories are defined as intact ($\leq 1\%$ leaf area removed by chewers) and damaged ($> 1\%$ leaf area removed by chewers). Two branches (one from the upper and one from the lower canopy) were sampled in each tree, and 50 leaves were haphazardly collected on each branch. Points (diamond shape) and error bars correspond to estimated marginal means and 95% confidence intervals, respectively. Different lower-case letters indicate significant differences among treatments (pairwise comparisons of estimated marginal means with p value correction with the multivariate- t method; $\alpha = 0.05$). Results of models are given in Table 3.

tebufenozide had no statistically significant direct impact on any of the 3 guilds. In tebufenozide-treated trees, the presence of chewing damage resulted in a significant reduction in the odds of occurrence of upper-surface leaf-miners (42%), lower-surface leaf-miners (38%), and gall-inducers (48%). The reduction in chewing damage caused by tebufenozide indirectly contributed to increasing the odds of occurrence of upper-surface leaf-miners by 40%, lower-surface leaf-miners by 34%, and gall-inducers by 49% (Figure 6B).

DISCUSSION

Our study confirmed that feeding traits and competition play an important role in determining the ecological sensitivity of internally feeding herbivores to aerial application of insecticides in forests. Consistent with our expectations (Figure 1), gall-inducers were fully shielded from diflubenzuron and tebufenozide and hence were unaffected. In contrast, leaf-miners were negatively affected by diflubenzuron regardless of whether they fed on the upper or lower leaf parenchyma, but not by tebufenozide. Feeding traits were critical in determining whether toxic effects were experienced before mine initiation or during caterpillar development: upper surface mines were reduced in numbers, indicating ovicidal

effects, whereas lower-surface leaf-miners had lower survivorship, indicating larvicidal effects. Our data further corroborated the hypothesis that a nonrandom distribution of leaf-miners and gall-inducers among leaves is driven by active avoidance of leaves previously damaged by leaf-chewers, with these interactions acting as an additional mediator of insecticide effects.

Direct effects of insecticides on leaf-chewers

Insecticide application did not influence the incidence of chewing damage in the drone experiment, despite increasing the mortality of leaf-chewers, implying that a sizeable part of the damage was done before spraying (Figure 3). Family data of externally feeding caterpillars revealed that the Lepidoptera assemblage was dominated by spring species, which were probably responsible for most of the damage, instead of the anticipated *T. processionea* (Leroy et al. 2019). Because spraying was timed for control of *T. processionea*, a species that continues feeding until early summer (Supplemental Data, Table S2-1), it occurred too late to prevent leaf damage by spring species. In contrast, the proportion of damaged leaves and the magnitude of the damage were significantly reduced

TABLE 3: Results of generalized linear mixed models testing for the effect of insecticide treatments and chewing damage on the occurrence and survivorship of *Stigmella* and *Phyllonorycter* leaf-miners on oak leaves in the drone experiment^a

| | Model elements | Response Level Sample size | <i>Stigmella</i> sp. | | <i>Phyllonorycter</i> sp. | |
|--|---|-------------------------------|--------------------------------------|---|--------------------------------------|---|
| | | | Occurrence (0/1) Leaf n = 5161 | Survivorship (0/1) Leaf-miner n = 484 | Occurrence (0/1) Leaf n = 4978 | Survivorship (0/1) Leaf-miner n = 266 |
| Fixed effects (X^2) ^b | Diflubenzuron (0/1) | | 7.81** | 0.56 | 0.27 | 5.80* |
| | Tebufenozide (0/1) | | 0.72 | 0.00 | 0.40 | 3.69 ⁺ |
| | Chewing damage (0/1) | | 36.21*** | 2.68 | 48.58*** | 3.64 ⁺ |
| | Canopy stratum (Up/Low) | | 3.65 ⁺ | 5.39* | 11.26** | 1.40 |
| | Diflubenzuron × damage | | 1.34 | 0.00 | 0.94 | 3.05 ⁺ |
| | Diflubenzuron × stratum | | 0.16 | 0.00 | 0.01 | 0.17 |
| | Tebufenozide × damage | | 3.02 ⁺ | 0.19 | 0.03 | 2.86 ⁺ |
| | Tebufenozide × stratum | | 0.12 | 0.05 | 0.22 | 0.60 |
| | Diameter at breast height (cm) ^c | | 10.73** | 1.04 | 0.12 | 7.02** |
| Random effects (var) ^d | Block | | 0.05 | 0.64 | 0.02 | 0.26 |
| | Tree/Block | | 0.04 | — | 0.18 | — |
| | Branch/Tree/Block | | 0.08 | — | 0.04 | 0.97 |
| Goodness-of-fit (R^2) ^e | Marginal | | 0.05 | 0.06 | 0.08 | 0.08 |
| | Conditional | | 0.11 | 0.31 | 0.13 | 0.23 |

^aInsecticides were applied by drone at standard operational rates (12 g [diflubenzuron]/ha and 69.6 g [tebufenozide]/ha; target species oak processionary moth *Thaumetopoea processionea*). Each column represents one model, characterized by its response variable, hierarchical level of observation, and sample size. Rows indicate the fixed predictors, that is, the independent variables, interaction terms, and covariates included in the models, the random effect structure, and the goodness-of-fit of each model. Numbers indicate results from Chi-square tests (fixed effects), random effect variances (random effects), and Nakagawa's pseudo- R^2 s (goodness-of-fit).

^bLikelihood ratio tests were performed for models with binomial error distribution.

^cCovariate selection procedures are detailed in the Supplemental Data, S1.

^dRandom intercepts for tree nested into block, and branch nested into tree nested into block were included as a default random effect structure for branch- and leaf-level models, respectively. Nested terms with near-zero variance estimates were removed when appropriate.

^eMarginal R^2 indicates the ratio of variance explained by the fixed effects, and conditional R^2 indicates the ratio of the variance explained by the fixed and the random effects.

*** $p < 0.001$. Test values not followed by a symbol are not statistically significant.

** $p < 0.01$.

* $p < 0.05$.

⁺ $p < 0.1$.

by treatment in the helicopter experiment (Figure 3). Even though the dominant species in the helicopter study, the gypsy moth *L. dispar*, has a similar larval phenology to *T. processionea*, the former does not display the same gregarious habit and patchy distribution pattern as the latter. We assume that our sampling design (2 branches/tree) was inadequate to capture the patchy damage caused by *T. processionea* after treatment application, such that spring species caused most of the damage observed in the drone experiment. These discrepancies between both experiments constrained us to restrict the assessment of indirect effects of insecticide on the helicopter experiment.

Direct effects of insecticides on sessile herbivores

Our results showed that although internal feeding protects gall-inducers against diflubenzuron and tebufenozide, it does not fully prevent the exposure of leaf-miners to diflubenzuron. Leaf-miner biology appears to play an essential role in determining the route of exposure, because upper-surface leaf-miners were reduced in numbers without experiencing decreased survivorship, whereas lower-surface species displayed the opposite pattern.

Gall-inducers were not affected by either diflubenzuron or tebufenozide, confirming the hypothesis that gall-forming strongly mitigates exposure to lipophilic insecticides (Figures 4 and 6). Gall wasps (Hymenoptera: Cynipidae) and gall midges (Diptera: Cecidomyiidae) generate outgrowth of thick layers of plant tissues, often surrounded by a tough wall, as an evolutionary adaptation to adverse environmental stressors (Price et al. 1987). Likewise, the toughness and thickness of galls appear to grant the larvae a physical barrier against exposure to insecticide residues. Lipophilic insecticides with contact toxicity can effectively reduce gall formation when sprayed against adult wasps prior to egg-laying (Eliason and Potter 2000). However, both diflubenzuron and tebufenozide are mostly toxic to larval stages and eggs through ingestion or contact, but are harmless to adult insects (Durkin 2004; Durkin and Klotzbach 2004). In Central European deciduous oaks, the spring generation of cynipid gall wasps (Supplemental Data, Table S2-2) would be theoretically vulnerable at the egg stage, but we found no evidence of such an effect in our experiments.

In contrast, the significant reduction of upper-surface leaf-miners in diflubenzuron-treated trees (Figures 4 and 6A) can be linked to the egg stage through increased egg mortality or through alterations of oviposition behavior (i.e., active avoidance of sprayed leaves by gravid females). Ovicidal effects of diflubenzuron have been described for a variety of arthropod

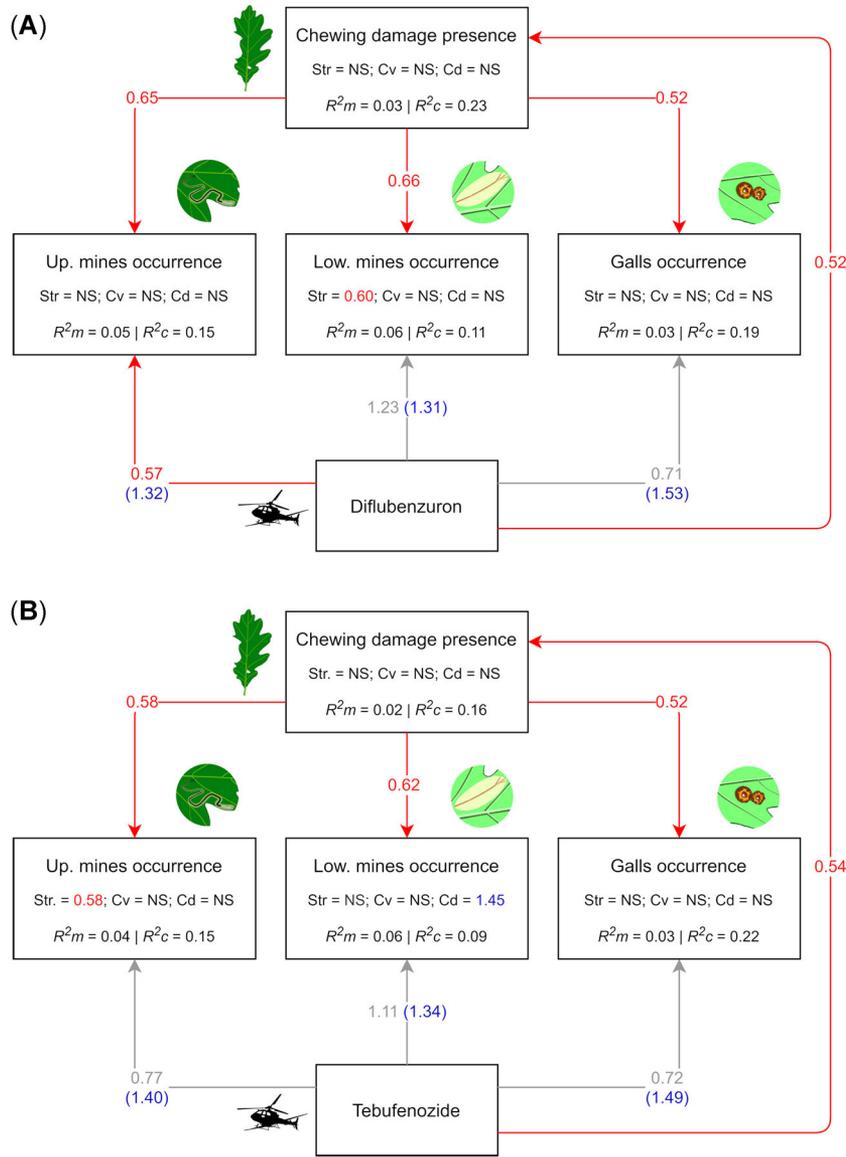


FIGURE 6: Results of the structural equation models of the effects of diflubenzuron (A) and tebufenozide (B) on the incidence of upper-surface leaf-miners, lower-surface leaf-miners, and gall-inducers at the leaf-level in the helicopter experiment. Diflubenzuron (60 g [a.i.]/ha) and tebufenozide (180 g [a.i.]/ha) were applied by helicopter on 15 May 2017 on 3000-m² plots. Oak leaves were collected in late June 2017 on 2 branches (upper and lower canopy) from one mature oak tree at the center of each plot (50 leaves/branch). Effect sizes, expressed as odds ratio, are displayed next to the corresponding path, with indirect effects indicated in parentheses. Statistically significant paths ($\alpha=0.05$) are colored in blue (odds ratio > 1; positive relationship) and red (odds ratio < 1; negative relationship). Statistical tests were performed on the link (logit) scale, and path coefficients were back-transformed to the response scale. Marginal (R^2m) and conditional (R^2c) pseudo- R^2 values are reported in the boxes of the response variables. For clarity, paths of canopy stratum (Str), crown volume (Cv), and crown density (Cd) are not shown (NS), and their odd ratios are given on the boxes of the response variables instead.

species (Grosscurt 1978), including tree-dwelling leaf-miners such as *Cameraria ohridella* Deschka & Dimic (Lepidoptera: Gracillariidae; Nejmanová et al. 2006). The ovicidal activity of tebufenozide appears to be weaker, because it varies strongly depending on the targeted species and the method of application (Smagghe et al. 2012). Oviposition deterrent effects are less studied but were also reported for the leaf-miner *Liriomyza trifolii* (Diptera: Agromyzidae) when exposed to diflubenzuron (Smith 1986). Consistent with our hypothesis, the absence of a reduction in the abundance of lower-surface leaf-miners (Figures 4 and 6) suggests that the aerial mode of application

favors the exposure of species that oviposit on the upper surface of the leaf. Interestingly, we obtained similar results in both experiments for all insecticides and all 3 guilds of sessile herbivores. Helicopters have been praised for their better performance in crop-dusting operations relative to fixed-winged aircraft, because downward-moving air currents generated by the rapid movement of the rotor blades allow coverage of both sides of leaves with spray droplets (McGowen 2005). In contrast, our findings suggest that droplet distribution was likely strongly biased toward the upper surface of the leaves and did not point to major differences between

helicopter- and drone-based spraying. With the increasing popularity of drones for pesticide application, direct comparisons of spray deposition patterns generated by both types of aircraft would be desirable to identify potential differences in exposure dynamics between application methods. Our results show that exposure at and after oviposition is a critical aspect of ecological sensitivity and mediates diverging responses of species with different oviposition habits. Ovicidal effects should be considered when one is drafting hypotheses on nontarget impacts of ingestion insecticides like diflubenzuron, because most species assumed to be not ecologically sensitive, such as leaf-miners and sucking herbivores, might well be vulnerable at the egg stage. Although the absence of significant differences from controls suggests a low egg toxicity of tebufenozide, the pattern observed is very similar to that of diflubenzuron-treated trees, such that we cannot dismiss the potential field toxicity of tebufenozide against the egg stage of leaf-miners (Figure 4).

Reduced survivorship of the lower-surface *Phyllonorycter* in diflubenzuron-treated trees (Figure 5) indicates that the active ingredient was absorbed into the mesophyll in sufficient amounts to induce larvicidal effects. Residues of diflubenzuron undergo substantial losses within few weeks of application, followed by a stabilization of remaining residues until leaf fall (Wimmer et al. 1993), such that they are still present in appreciable concentrations in summer when leaf-miners start feeding. A fraction of the residues is gradually absorbed into the mesophyll and hence becomes available to leaf-miners (Bull and Ivie 1978). The absence of a similar response of the upper-surface *Stigmella* (Figure 5) suggests an asymmetric exposure between upper- and lower-surface species, likely resulting from uneven distribution of residues across the mesophyll. As an upper-surface species, *Stigmella* leaf-miners feed exclusively in the tightly packed layer of cells composing the palisade parenchyma, whereas the lower-surface *Phyllonorycter* feed on the more loosely arranged cells composing the spongy parenchyma (Figure 1). Lipophilic pesticides bind with cuticular waxes on the leaf surface and penetrate leaf tissue mostly by moving along cell walls in the intercellular space, where they accumulate (Currier and Dybing 1959; Sundaram 1995a). Because of the larger size of the intercellular space in the spongy parenchyma, oils spread between cells into large globules, whereas they concentrate in thin films in the palisade parenchyma (Rohrbaugh 1934), suggesting that lipophilic insecticides may accumulate in higher concentrations in the spongy parenchyma. As for ovicidal effects, the absence of statistically significant evidence of larvicidal effects of tebufenozide in the present study should be interpreted with caution, because we still observed a comparable, albeit weaker, pattern in tebufenozide-treated trees (Figures 4 and 5). Diflubenzuron and tebufenozide have similar lipophilicity (log octanol/water partition coefficient [K_{ow}] values of 4.25 and 3.83, respectively; Marsella et al. 2000; Radović et al. 2016) and foliar degradation dynamics (Wimmer et al. 1993; Sundaram 1995b), and are therefore expected to follow similar exposure routes. Differences in field toxicity between both compounds may instead pertain to differences in within-plant metabolism (Bull and

Ivie 1978; Smirle et al. 2004) and physiological sensitivity of the focal species at the applied concentrations.

Contrary to our initial hypothesis, the vertical distribution of leaf-miners and gall-inducers in the tree crown did not influence ecological sensitivity, because the interaction between canopy stratum and treatment was not significant in any model (Tables 2 and 3). Studies addressing the degradation of diflubenzuron and tebufenozide in forests reported that most residues lost in the first postapplication weeks were washed off by rainfall (Wimmer et al. 1993; Sundaram 1995b). As a result, the initial filtering of insecticide deposition along the canopy depth may be gradually canceled by a slower degradation of residues in the lower canopy due to high interception of rainfall in the upper crown layers. Due to their late phenology, sessile herbivores may hence be only exposed to a fraction of the applied insecticide, regardless of their position in the crown. Alternatively, it is plausible that the doses used to control leaf-chewers far exceed the threshold above which a vertical stratification of insecticide effects would become visible.

Our results with oak leaf-miners suggest that the toxicokinetics and environmental dynamics of insecticides are critical mediators of the exposure of species dwelling outside the target ecological niche and the acute phase of exposure. In the absence of residue analysis, we speculated on the probable mechanisms underlying the response of leaf-miners by drawing on existing knowledge on the fate and the persistence of diflubenzuron and tebufenozide in forests. However, explicit testing of these mechanisms would be necessary to reach conclusions that could be generalized to a broader range of organisms and substances. With the ever-growing interest of ecologists in the field of ecotoxicology, future field studies that use integrative approaches combining toxicology, chemistry, and ecology should be encouraged.

Indirect effect of insecticides through alteration of competition patterns

Consistent with previous studies (Faeth 1986; Digweed 2006), we observed that leaf-miners and gall-inducers occurred more frequently on intact leaves than on damaged ones (Figures 4 and 6). Interestingly, insecticides indirectly nullified the negative effect of chewing damage, such that the net outcome was only negative for species experiencing direct toxicity, that is, upper-surface leaf-miners in diflubenzuron-sprayed trees (Figure 6A). Beyond the effect of leaf-chewers on leaf-mining activity, several studies have reported higher survivorship of leaf-miners feeding on intact leaves, which was attributed to induced plant defenses or intensified parasitism through increased production of physical and chemical cues on damaged leaves (Faeth 1986; Cornelissen and Stiling 2008). However, we did not detect a clear effect of chewing damage on the survivorship of leaf-miners in the present study (Figure 5). Faeth (1986) noted that differences in the relationship between survivorship and leaf damage were mediated by increased rates of parasitism, whereas deaths from other causes were significantly less in damaged leaves than in intact

ones. We propose that the absence of a correlation between survivorship and chewing damage in the drone experiment could be related to a low activity of parasitoids. However, it is unclear why *Phyllonorycter* survivorship was reduced by diflubenzuron only on intact leaves (Figure 5). Insecticides may conceivably influence herbivore fitness by altering leaf chemistry, but these interactions have been studied for only a few compounds and plant species, showing widely contrasting results depending on the study system (Silfver et al. 2013; Zhou et al. 2019).

Ecological significance of insecticide and competition effects on sessile herbivores

Despite relatively large effect sizes, our data were characterized by large variations, such that the percentage of the variance of leaf-miner distribution explained by insecticides and chewing damage was relatively modest. This was particularly striking for the leaf-level analysis, with marginal R^2 values <0.10 . At the branch level, the fixed predictors explained a larger proportion of the variance but it still did not exceed 21% (Table 2). The sessile nature of leaf-miners and gall-inducers and the high structural complexity of tree crowns likely causes a broad variation in exposure among individuals, due to uneven deposition and degradation of insecticide residues among leaves. In our experiments, insecticide effects may have been further diluted by the relative scarcity of leaf-miners and gall-inducers, with infestation rates not exceeding 23% in both experiments. Whereas chewing damage significantly influenced the incidence of sessile herbivores at the leaf level, it had no impact on their abundance at the branch level apart from the weak significant interaction effect of treatment and chewing damage on gall-inducers, which seems to be mostly driven by influential outliers (Figure 4). In line with previous studies (Faeth 1986; Digweed 2006), these findings confirm that chewing damage only interacts with the leaf selection process and does not have a major impact over larger spatial scales at endemic levels of damage such as those recorded in the present study. Interestingly, after repeating the drone experiment in 2017, when leaf-chewer densities were exceptionally low, we observed that although insecticide effects were relatively consistent with those recorded in 2016, chewing damage no longer had any influence on the occurrence of sessile herbivores (Supplemental Data, S5). This result suggests that leaf-miners stop discriminating between intact and damaged leaves when the majority of leaves are intact. However, competition is expected to exert much stronger pressure on sessile herbivores when leaf-chewers reach epidemic densities. Faeth (1986) proposed that changes in parasitism rates induced by chewing damage should be inversely density dependent, because parasitoids become swamped with search cues in the presence of large numbers of damaged leaves. During defoliator outbreaks, competition between sessile herbivores and leaf-chewers should hence shift from being primarily driven by active avoidance (i.e., decreased leaf-miner occurrence) and top-down control (i.e., reduced survivorship) to bottom-up control via exploitation (i.e., reduced available foliage due to

substantial defoliation) or interference competition (i.e., death-induced leaf abscission or direct destruction; Roslin and Roland 2005). In such conditions, fully shielded gall-inducers should likely experience the greatest benefit from the suppression of chewer outbreaks. The net effect of insecticide application may also benefit leaf-miner populations, provided their density is low enough to prevent strong direct insecticide effects at the population scale. In our view, such a density-dependent response makes unlikely the possibility of insecticide-driven secondary infestation by leaf-mining pests such as *Tischeria ekebladella* (Lepidoptera: Tischeriidae; Molnár et al. 2012).

Differential response to insecticides between upper- and lower-surface guilds could conceivably lead to intensifications or shifts in ecological dominance between competing leaf-miners through selective exposure (Cordeiro et al. 2014). However, such an outcome seems rather unlikely to profoundly affect the structure of the oak leaf-miner community, because intraguild competition was shown to play only a minor role in shaping leaf-mining communities at realistic field densities in this system (Tack et al. 2009).

CONCLUSIONS

Our results indicate that biological traits (in particular the feeding niche) and competition with leaf-chewers regulate the ecological sensitivity of leaf-miners and gall-inducers to tebufenozide and diflubenzuron. Gall-forming completely negates exposure to lipophilic insecticides, whereas leaf-miner biology mediates the exposure pathways, likely depending on the physicochemical and toxicological properties of the tested substance. Both leaf-miners and gall-inducers displayed non-random distribution patterns among leaves, which can be explained by a selection bias toward leaves not affected by prior feeding by leaf-chewers, leading to positive outcomes of insecticides for guilds that were able to avoid exposure. We demonstrate that insecticide applications can affect insect communities beyond direct toxic effects by altering patterns of interactions between species. Insecticides can have significant impacts on nontarget species and can affect organisms traditionally presumed to be safe, through subtle interactions with their biological traits and interaction networks, even at residual concentrations. These findings encourage the extension of pesticide risk assessment beyond the targeted ecological niche and the testing of alternative impact pathways. Recent developments in ecotoxicology have encouraged the inclusion of ecological factors such as species traits and species interactions in risk assessment models (van den Brink 2008). Our results support this approach and further call for greater integration of toxicological, chemical, and ecological data within the framework of pesticide risk assessment.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at <https://doi.org/10.1002/etc.4969>.

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REFERENCES

- Bates D, Mächler M, Bolker BM, Walker S. 2015. Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48.
- Brooks ME, Kristensen K, Benthem KJ, van, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Mächler M, Bolker BM. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J* 9:378–400.
- Bull DL, Ivie GW. 1978. Fate of diflubenzuron in cotton, soil, and rotational crops. *J Agric Food Chem* 26:515–520.
- Connor EF, Taverner MP. 1997. The evolution and adaptive significance of the leaf-mining habit. *Oikos* 79:6–25.
- Cordeiro EMG, Corrêa AS, Guedes RNC. 2014. Insecticide-mediated shift in ecological dominance between two competing species of grain beetles. *PLoS One* 9:e100990.
- Cornelissen T, Stiling P. 2008. Clumped distribution of oak leaf miners between and within plants. *Basic Appl Ecol* 9:67–77.
- Currier HB, Dybing CD. 1959. Foliar penetration of herbicides—Review and present status. *Weeds-U* 7:195–213.
- Digweed SC. 2006. Oviposition preference and larval performance in the exotic birch-leaf-mining sawfly *Profenusa thomsoni*. *Entomol Exp Appl* 120:41–49.
- Durkin PR. 2004. Control/eradication agents for the gypsy moth—Human health and ecological risk assessment for diflubenzuron (Dimilin). SERA TR 04-43-05-03b. Final Report. US Department of Agriculture, Forest Service, Forest Health Protection, Arlington, VA, USA.
- Durkin PR, Klotzbach J. 2004. Control/eradication agents for the gypsy moth—Human health and ecological risk assessment for tebufenozide (Mimic). SERA TR 04-43-05-06c. Final Report. US Department of Agriculture, Forest Service, Forest Health Protection, Arlington, VA, USA.
- Eliason EA, Potter DA. 2000. Impact of whole-canopy and systemic insecticidal treatments on *Callirhytis cornigera* (Hymenoptera: Cynipidae) and associated parasitoids on pin oak. *J Econ Entomol* 93:165–171.
- Faeth SH. 1986. Indirect interactions between temporally separated herbivores mediated by the host plant. *Ecology* 67:479–494.
- Fox J, Weisberg S. 2011. *An {R} Companion to Applied Regression*, 2nd ed. Sage, Thousand Oaks, CA, USA.
- Grosscurt AC. 1978. Diflubenzuron: Some aspects of its ovicidal and larvicidal mode of action and an evaluation of its practical possibilities. *Pestic Sci* 9:373–386.
- Holmes SB, MacQuarrie CJK. 2016. Chemical control in forest pest management. *Can Entomol* 148:270–295.
- Kefford BJ, Liess M, Warne MStJ, Metzeling L, Schäfer RB. 2012. Risk assessment of episodic exposures to chemicals should consider both the physiological and the ecological sensitivities of species. *Sci Total Environ* 441:213–219.
- Lefcheck JS. 2016. piecewiseSEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods Ecol Evol* 7:573–579.
- Lenth R. 2020. emmeans: Estimated marginal means, aka least-squares means. R package Ver 1.5.2-1. CRAN. [cited 2020 December 7]. Available from: <https://github.com/rvleth/emmeans>
- Leroy BML, Gossner MM, Lauer FPM, Petercord R, Seibold S, Jaworek J, Weisser WW. 2019. Assessing insecticide effects in forests: A tree-level approach using Unmanned Aerial Vehicles. *J Econ Entomol* 112: 2686–2694.
- Liebholt AM, McManus ML. 1999. The evolving use of insecticides in gypsy moth management. *J Forest* 97:20–23.
- Lüdecke D, Makowski D, Waggoner P, Patil I, Ben-Shachar MS. 2020. Performance: Assessment of Regression Models Performance. R package Ver 0.5.1. CRAN. [cited 2020 December 7]. Available from: <https://easystats.github.io/performance/>
- Marquis RJ, Passoa SC, Lill JT, Whitfield JB, Le Corff J, Forkner RE, Passoa VA. 2019. Illustrated guide to the immature Lepidoptera on oaks in Missouri. FHAAS-2018-05. US Department of Agriculture, Forest Service, Forest Health Assessment and Applied Sciences Team, Morgantown, WV, USA.
- Marsella AM, Jaskolka M, Mabury SA. 2000. Aqueous solubilities, photolysis rates and partition coefficients of benzoylphenylurea insecticides. *Pest Manag Sci* 56:789–794.
- Marshall DB, Pree DJ, McGarvey BD. 1988. Effects of benzoylphenylurea insect growth regulators on eggs and larvae of the spotted tentiform leafminer *Phyllonorycter blancardella* (Fabr.) (Lepidoptera: Gracillariidae). *Can Entomol* 120:49–62.
- Martinat PJ, Coffman CC, Dodge KM, Cooper RJ, Whitmore RC. 1988. Effect of diflubenzuron on the canopy arthropod community in a central Appalachian forest. *J Econ Entomol* 81:261–267.
- McGowen SS. 2005. *Helicopters: An Illustrated History of Their Impact. Series: Weapons and Warfare*. ABC-CLIO, Santa Barbara, CA, USA.
- Molnár BP, Tröger A, Tshova TB, Subchev M, van Nieukerken EJ, Koster JC (Sjaak), Szócs G, Tóth M, Francke W. 2012. Identification of the female-produced sex pheromone of *Tischeria ekebladella*, an oak leaf-mining moth. *J Chem Ecol* 38:1298–1305.
- Nakagawa S, Johnson PCD, Schielzeth H. 2017. The coefficient of determination R^2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *J R Soc Interface* 14:20170213.
- Nejmanová J, Cvačka J, Hrdý I, Kuldová J, Mertelík J, Muck AJ, Nešněrová P, Svatoš A. 2006. Residues of diflubenzuron on horse chestnut (*Aesculus hippocastanum*) leaves and their efficacy against the horse chestnut leafminer, *Cameraria ohridella*. *Pest Manag Sci* 62:274–278.
- Patočka J. 1980. *Die Raupen und Puppen der Eichenschmetterlinge Mitteleuropas*. Series: Monographs to Applied Entomology, Supplements to Journal of Applied Entomology. Paul Parey, Hamburg, Germany.
- Price PW, Fernandes GW, Waring GL. 1987. Adaptive nature of insect galls. *Environ Entomol* 16:15–24.
- Radović TT, Grujić SD, Kovačević SR, Laušević MD, Dimkić MA. 2016. Sorption of selected pharmaceuticals and pesticides on different river sediments. *Environ Sci Pollut Res* 23:25232–25244.
- Reitz SR, Kund GS, Carson WG, Phillips PA, Trumble JT. 1999. Economics of reducing insecticide use on celery through low-input pest management strategies. *Agric Ecosyst Environ* 73:185–197.

- Richardson B, Thistle HW. 2006. Measured and predicted aerial spray interception by a young *Pinus radiata* canopy. *T Asabe* 49:15–23.
- Rohrbaugh PW. 1934. Penetration and accumulation of petroleum spray oils in the leaves, twigs, and fruit of citrus trees. *Plant Physiol* 9:699–730.
- Roslin T, Roland J. 2005. Competitive effects of the forest tent caterpillar on the galls and leaf-miners of trembling aspen. *Écoscience* 12:172–182.
- Scriber JM. 2004. Non-target impacts of forest defoliator management options: Decision for no spraying may have worse impacts on non-target Lepidoptera than *Bacillus thuringiensis* insecticides. *J Insect Conserv* 8:241–261.
- Silfver T, Autelo M, Paaso U, Koikkalainen K, Mikola J. 2013. Use of an insecticide in field-scale plant-herbivore studies: No side effects of synthetic pyrethrin on *Betula pendula* growth or chemistry. *Ann Bot Fenn* 50:337–346.
- Singmann H, Bolker BM, Westfal J, Aust F, Ben-Shachar MS. 2020. *afex: Analysis of Factorial Experiments*. R package Ver 0.28-0. CRAN. [cited 2020 December 7]. Available from: <https://github.com/singmann/afex>
- Smagghe G, Gomez LE, Dhadialla TS. 2012. Bisacylhydrazine insecticides for selective pest control. In Dhadialla TS, ed, *Advances in Insect Physiology*, Vol 43—Insect Growth Disruptors. Elsevier Academic, Oxford, UK, pp 163–249.
- Smirle MJ, Lowery DT, Zurowski CL. 2004. Chemical residues and bioactivity of tebufenozide applied to apple foliage. *Pest Manag Sci* 60:1137–1142.
- Smith RF. 1986. Efficacy of selected insecticides against *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae), a leafminer of *Chrysanthemum*. *Can Entomol* 118:761–766.
- Southwood TRE, Wint GRW, Kennedy CEJ, Greenwood SR. 2004. Seasonality, abundance, species richness and specificity of the phytophagous guild of insects on oak (*Quercus*) canopies. *Eur J Entomol* 101:43–50.
- Sundaram KMS. 1995a. Persistence and fate of tebufenozide (RH-5992) insecticide in terrestrial microcosms of a forest environment following spray application of two Mimic[®] formulations. *J Environ Sci Health B* 30:321–358.
- Sundaram KMS. 1995b. Photostability and rainfastness of tebufenozide deposits on fir foliage. In Hall FR, Barry JW, eds, *Biorational Pest Control Agents*. ACS Symposium Series, Vol 595. American Chemical Society, Washington, DC, USA, pp 134–152.
- Tack AJM, Ovaskainen O, Harrison PJ, Roslin T. 2009. Competition as a structuring force in leaf miner communities. *Oikos* 118:809–818.
- Thomas FM, Blank R, Hartmann G. 2002. Abiotic and biotic factors and their interactions as causes of oak decline in Central Europe. *Forest Pathol* 32:277–307.
- Thompson DG. 2011. Ecological impacts of major forest-use pesticides. In Sánchez-Bayo F, van den Brink PJ, Mann RM, eds, *Ecological Impacts of Toxic Chemicals*. Bentham eBooks, Sharjah, EAU, pp 88–110.
- van den Brink PJ. 2008. Ecological risk assessment: From book-keeping to chemical stress ecology. *Environ Sci Technol* 42:8999–9004.
- Wimmer MJ, Smith RR, Wellings DL, Toney SR, Faber DC, Miracle JE, Carnes JT, Rutherford AB. 1993. Persistence of diflubenzuron on Appalachian forest leaves after aerial application of Dimilin. *J Agric Food Chem* 41:2184–2190.
- Xu T, Jacobsen CM, Hara AH, Li J, Li QX. 2009. Efficacy of systemic insecticides on the gall wasp *Quadraslichus erythrinae* in wiliwili trees (*Erythrina* spp.). *Pest Manag Sci* 65:163–169.
- Zhou Q, Cheng X, Wang S, Liu S, Wei C. 2019. Effects of chemical insecticide imidacloprid on the release of C6 green leaf volatiles in tea plants (*Camellia sinensis*). *Sci Rep* 9:625.