






Relative impacts of gypsy moth outbreaks and insecticide treatments on forest resources and ecosystems: An experimental approach

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Abstract

1. Gypsy moth outbreaks cause severe defoliation in Holarctic forests, both in North America where it is invasive, and in its native range in Eurasia. Defoliation can hamper timber production and impact ecological communities and processes. Aerial insecticide applications are regularly performed in outbreak areas to mitigate economic losses. These operations can be financially costly and harmful to non-target species and may disrupt species interaction networks. However, replicated studies of the relative impacts of gypsy moth outbreaks and insecticide application on forest growth and animal communities are rare and have yet to be carried out in the species' indigenous range.

2. Here, we review the pathways in which gypsy moth outbreaks and the chemical control of these outbreaks affect forest ecosystems. We then present an experimental design established in South Central Germany in early 2019, aiming to study the ecological and economic consequences of gypsy moth eruptions and insecticide application in oak forests. The study's full factorial design comprises forest stands at high and low defoliation risk, either treated with tebufenozide or left unsprayed, within 12 experimental blocks. Measurements of forest growth and structure, tree mortality, gypsy moth density, and composition of lepidopteran, bird, bat, ground beetle, and canopy arthropod communities will be conducted for several years.

3. One-year intensive monitoring of gypsy moth populations and damage across the selected sites showed substantial differences in population density between plots at high and low defoliation risk and high efficacy of tebufenozide in suppressing gypsy moth populations in treated plots. In the first year of the experiment, gypsy moth density and defoliation in predicted outbreak plots differed strongly, confirming the

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importance of using many replicates and blocking to control spatial heterogeneity. The experiment will be running continuously during the coming years to produce short- and medium-term economic and ecological data to improve our understanding and management of gypsy moth outbreaks.

KEYWORDS

defoliation, experimental design, gypsy moth, insect outbreaks, insecticide, non-target effects, tebufenozide

1 | INTRODUCTION

The gypsy moth *Lymantria dispar* L. (Lepidoptera: Erebidae), a species of tussock moth indigenous to Europe and Asia, is widely acknowledged as one of the most critical defoliators in the Holarctic region. It exhibits cyclical population dynamics with outbreaks that are often periodic and spatially synchronous over large distances (Haynes, Liebhold, & Johnson, 2009) and believed to be driven by one or more of its trophic interactions (Johnson, Liebhold, Bjørnstad, & McManus, 2005). Gypsy moth larvae are voracious folivores that can feed for up to 10 weeks per year on more than 300 species of broadleaf and coniferous trees (Elkinton & Liebhold, 1990). This combination of outbreak dynamics, broad polyphagy and long larval duration granted the species an unmatched ability to rapidly defoliate large forested areas. Multiple large outbreaks have been reported during the last decades throughout its native range, including most of Europe (Alalouni, Schädler, & Brandl, 2013; Lentini et al., 2020; Villemant, 2010; Wulf & Graser, 1996), North Africa (Villemant, 2010), Central Asia (Orozumbekov, Liebhold, Ponomarev, & Tobin, 2009) and Japan (Liebhold, Higashiura, & Unno, 1998). However, the gypsy moth owes its infamous reputation mostly to the disastrous impacts of its eruptions in North American forests. Since its introduction in Massachusetts in 1869, it has colonized most of the Eastern United States and Southeastern Canada and keeps expanding its range (Liebhold, Halverson, & Elmes, 1992; Régnière, Nealis, & Porter, 2009; Tobin et al., 2004). Recent cost estimates including government expenditures and timber losses, appraised the overall financial toll of the gypsy moth invasion in North America to a staggering US\$3.2 billion per year (Bradshaw et al., 2016). In Europe, the gypsy moth problem is generally considered less severe (McManus and Csóka, 2007) although a comprehensive synthesis of its economic impacts is lacking. Estimates of management costs can merely be extrapolated from published reports on the spatial extent of outbreak areas treated with insecticides (Alalouni et al., 2013), while data related to market costs remain scarce and scattered. Likewise, our current knowledge on the ecological impacts of gypsy moth outbreaks and their associated management practices is mostly drawn from studies conducted in the invasive range of the species. Both defoliation and control measures with synthetic and biological insecticides have adverse effects on forest ecosystems (Herms, 2003). Experimental approaches integrating economic and ecological aspects of both outbreaks and control practices would grant a powerful tool to improve the economic and environmental sustainability of gypsy moth

management but are rare. In the present study, we first reviewed the different pathways through which a gypsy moth outbreak and insecticide use affect trees and animal communities in forest ecosystems. We then used the synthesized knowledge to produce biologically sound hypotheses (Figure 1) as a baseline to develop a field experiment that aims to address the relative importance of these processes in the indigenous range of the gypsy moth.

1.1 | Life cycle of the gypsy moth

The gypsy moth has a univoltine life cycle throughout its range. Adult females lay egg masses indiscriminately on bare surfaces in the vicinity of host trees in midsummer, each cluster typically containing 100 to over 1200 eggs (Andresen et al., 2001; Doane & McManus, 1981). The larvae are already fully developed inside the egg 1 month after oviposition and enter diapause in the winter months, eventually hatching during the spring bud-burst. The young caterpillars hang from branch tips with long silken threads, allowing them to spread to other forests via airborne dispersal. The gypsy moth is described as a spring-early summer defoliator. In Central Europe, feeding generally starts late April and continues for up to 10 weeks, until pupation in late June-early July. Pupation takes place in various sheltered places, for example in the ground or between bark ridges, and lasts for approximately 2 weeks. Adults are in wings from July to early August and generally live for 6–10 days. Females produce pheromones to attract males and lay egg masses shortly after mating close to their site of emergence (Doane & McManus, 1981; Wellenstein & Schwenke, 1981).

1.2 | Ecological impacts of gypsy moth outbreaks

Gypsy moth outbreaks primarily affect tree health through defoliation, which can in turn significantly reduce timber production. The primary effects of gypsy moth defoliation on host trees include reduced growth (Muzika & Liebhold, 1999; Naidoo & Lechowicz, 2001), reduced root biomass production (Thomas, Blank, & Hartmann, 2002) and reduced fruit production (Gottschalk, 1990). Defoliation can also induce substantial levels of tree mortality (Campbell & Sloan, 1977; Kegg, 1973; Lobinger, 1999), which vary in intensity depending on the frequency, magnitude and duration of defoliation, the vitality of host trees, as well as the additive action of biotic or abiotic stresses

of the treated area in North America (USDA Forest Service, 2020), tebufenozide is the preferred option some European countries such as Germany, due to its greater reliability in effectively suppressing gypsy moth populations (Lemme, Lobinger, & Müller-Kroehling, 2019). Toxic side-effects of BTK and tebufenozide appear to be restricted to non-target Lepidoptera (Durkin, 2004b and references therein; Durkin & Klotzbach, 2004 and references therein), though environmental risk assessment studies involving tebufenozide in forests are comparatively scarce (but see Butler, Kondo, & Blue, 1997 and Leroy et al., 2019).

Forest spraying is usually carried out in mid-spring soon after gypsy moth larvae start feeding on leaves. At this time, insect populations in tree canopies are large and strongly dominated by Lepidoptera larvae (Martinat, Coffman, Dodge, Cooper, & Whitmore, 1988; Southwood, Wint, Kennedy, & Greenwood, 2013). Therefore, concerns have been raised about the impacts of depressed caterpillar abundances on insectivorous predators and parasitoids, mainly on forest birds that strongly rely on caterpillars to fulfil their energy requirements during the breeding season (Cooper, Dodge, Martinat, Donahoe, & Whitmore, 1990). While most studies showed no substantial effect on bird communities, alterations in foraging behaviour, reproductive success and growth were observed in individual species in treated forests (Awkerman et al., 2011; Durkin, 2004a, 2004b and references therein; Holmes, 1998; Lih, Stephen, Smith, Nagy, & Wallis, 1994). Diet shifts and relocation of foraging territories were also documented in rodents (Bellocq, Bendell, & Cadogan, 1992; Seidel & Whitmore, 1995). Indirect effects on predatory arthropods are comparatively poorly known. Klenner (1996) observed reduced activity density of Carabid beetles in stands treated with diflubenzuron but was unable to determine whether this pattern was primarily driven by direct toxicity or reduced prey availability. While host scarcity is expected to negatively affect parasitoid populations, parasitism of sub-lethally intoxicated hosts appears to differ among species and could be influenced by insecticide-specific physiological effects on the gypsy moth hosts (Erb, Bourchier, van Frankenhuyzen, & Smith, 2001; McCravy, Dalusky, & Berisford, 2001; Weseloh et al., 1983).

1.4 | Relative importance of defoliation and insecticides on forest ecosystems: Consequences for an experimental approach

Based on a comprehensive review of the respective effects of defoliation and BTK on non-target Lepidoptera, Scriber (2004) suggested that the decision of non-intervention could be as damaging for Lepidoptera communities as insecticides. This hypothesis has yet to be tested in an integrative experimental approach to address the relative importance of the numerous processes at play (Figure 1). Such an approach should consider the fact that outbreak intensity is spatially variable. Due to differences in abiotic factors and trophic interactions, the development of gypsy moth populations may widely differ among forest stands, potentially leading to a considerable variation in the magnitude of their economic and ecological impacts (e.g. Sample et al., 1996). Because

insecticides must be applied shortly after larvae start feeding in order to be effective, decisions for spraying are mostly based on counts of egg masses on tree trunks conducted during the previous winter. These surveys cannot systematically predict severe defoliation, as most of the damage is typically caused by late instar larvae, up to 10 weeks after the initiation of feeding (Doane & McManus, 1981). Therefore, an experimental approach tailored to address the relative impacts of gypsy moth outbreaks and insecticide treatment should fulfil the following conditions: (1) selection of forest stands with broadly differing egg mass densities, to allow contrasting outbreak from non-outbreak conditions; (2) selection of sufficiently large experimental areas within stands, to be able to study processes over a biologically realistic scale; (3) exclusion of sites with a recent spraying history so that results are not confounded by potential carry-over effects of past treatments; (4) sufficient replication to account for unforeseeable variation in gypsy moth population dynamics among sites; (5) use of a randomized block design with closely located and structurally comparable stands within experimental blocks, to control for spatial heterogeneity among sites; (6) selection of blocks with different stand and climatic conditions, to allow the generalization of the results.

Here we describe a large-scale field experiment in Germany that fulfils these conditions. We established a full factorial block design including 12 blocks each composed of two plots with predicted outbreak densities of gypsy moth, and two plots with low predicted densities. An insecticide treatment with tebufenozide was randomly attributed to one plot in each density class. The experiment has three main objectives: (1) fostering our knowledge of gypsy moth population dynamics and impacts in its native range; (2) providing comprehensive information on the direct and indirect impacts of tebufenozide on forest ecosystems; (3) evaluating the relative impact of gypsy moth outbreaks and tebufenozide-based treatments to help to improve the sustainable management of outbreaks. In the present article, we first introduce the site selection process, the experimental design and the variables that will be measured in the experiment. We then discuss the viability of our design using gypsy moth population and defoliation data from the first experimental year.

2 | MATERIALS AND METHODS

2.1 | Experimental area and gypsy moth population surveys

The experiment was set up in the region of Franconia, in north-western Bavaria, Germany, within an approximately 2400 km² area delimited by the cities of Würzburg in the West, Schweinfurt in the North, Bamberg in the East and Bad Windsheim in the South (Figure 2). The landscape is dominated by a matrix of agricultural land (arable land, vineyards and grasslands) surrounding forest patches of variable size (Figure 2). Forests are dominated by deciduous oaks (*Quercus robur* L. and *Quercus petraea* Mattuschka) and have been subjected to cyclical and spatially synchronous gypsy moth outbreaks since the early 1990s (Lemme et al., 2019).

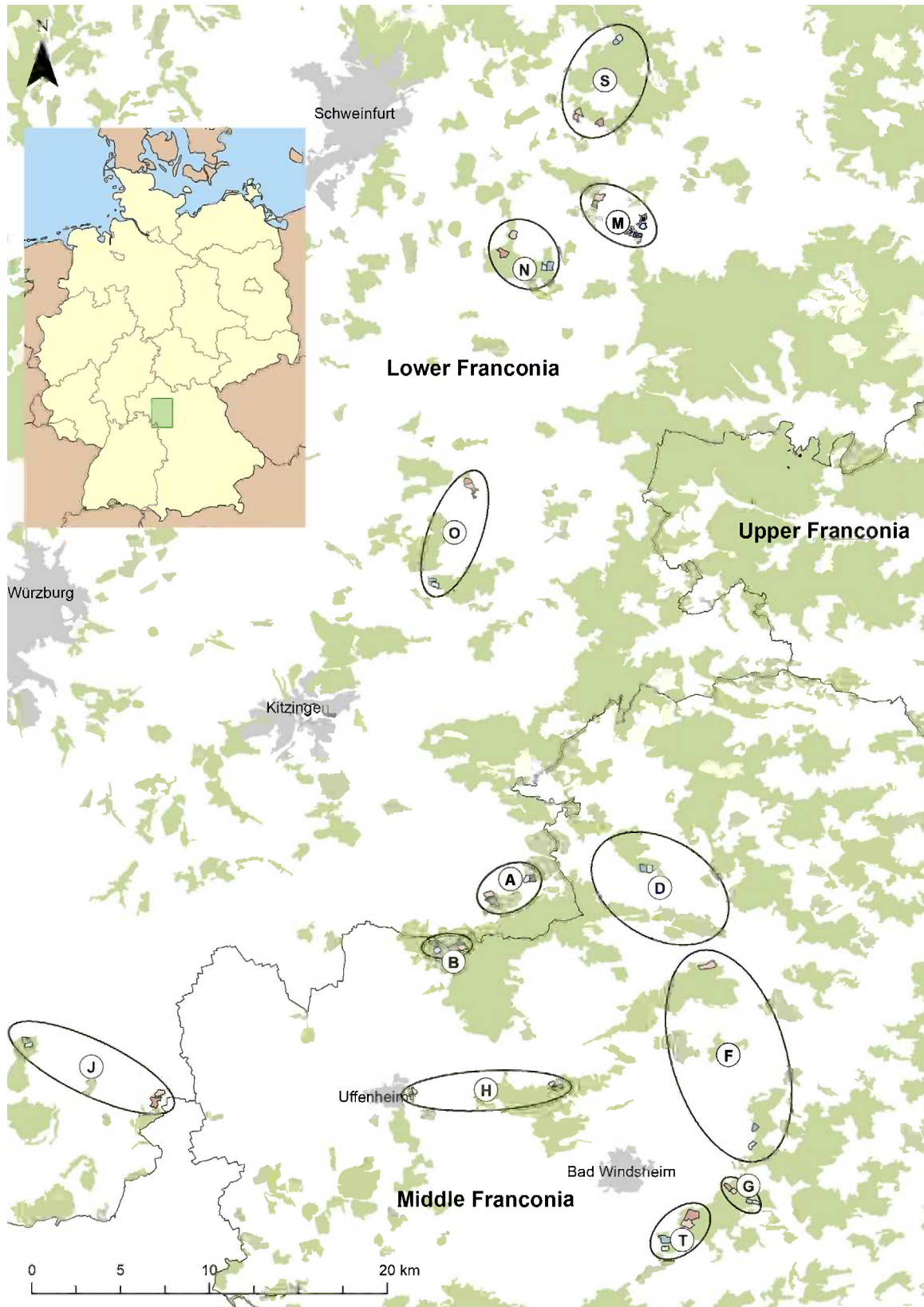


FIGURE 2 Map of the study design. Ellipses with capital letter labels represent the experimental blocks. Plots at high defoliation risk are displayed in red, plots at low defoliation risk in blue. Lighter colours indicate aerial treatment with tebufenozide; darker colours indicate unsprayed controls. Note: Three plots (one in block F, two in block D) are not displayed on the map as local landowners only agreed to take part in the experiment on the condition that the exact location of their stands is not published

Local district foresters carried out surveys of gypsy moth egg masses in four administrative regions – Upper Franconia, Middle Franconia, Lower Franconia and Swabia – during fall 2018, following a standardized protocol. The number of gypsy moth egg masses were counted on the lowest 2 m of tree trunks along a transect comprising, in most cases, 10 trees of the dominant social class. The abundance of egg masses on the underside of lower canopy branches of each tree, stand vitality, stand age and history of previous outbreaks were also reported. These data were used to calculate a ‘defoliation risk index’ (DRI) to identify areas at high or low risk of defoliation in the summer 2019 (Supplementary Information, file S1). In total, 26823 single trees were surveyed along 2802 transects.

2.2 | Experimental design

2.2.1 | Plot selection

We searched for oak-dominated areas at both high (DRI>1) and low (DRI<0.5) risk of defoliation for 2019, excluding young stands (i.e. average overstory tree age <70 years old) as well as sites with a recent spray history, based on the centrally stored application records of Bavaria. Candidate plots were individually checked by on-site visits for stand-structural homogeneity, and heterogeneous areas were excluded. Candidate blocks were to include a minimum of four comparable plots (two high DRI and two low DRI) of at least five hectares each, such that insecticide treatment could be attributed to one random plot per defoliation risk class in a full factorial fashion, that is each block consists of the four following plots: high defoliation risk, unsprayed (further referred to as ‘high-control’); high defoliation risk, sprayed (‘high treatment’); low defoliation risk, unsprayed (‘low-control’); and low defoliation risk, sprayed (‘low-treatment’) (Figures 1 and 2). Overall, 142 areas based on 778 transects (7534 trees) were inspected, of which 22 candidate blocks comprising 108 plots were retained.

2.2.2 | Plot validation

Once a block was considered suitable for the experiment, approval of landowners was sought to include the nested plots into the experimental design. As the aim was to include in each block one sprayed and one unsprayed plot from high risk and low risk stands, attribution of the insecticide treatment was initially done by drawing a random number. However, the process was challenged by several constraints. First, insecticide treatment is generally only allowed in stands at high risk of defoliation. The denomination of stands to be sprayed (‘treatment setting’) is done by the Bavarian State Institute of Forestry (LWF) and is the legal basis for insecticide application in Bavaria. Thus, for all low-treatment plots, and all high-treatment plots falling outside of the treatment setting, permission had to be applied for to the authorities. Second, landowners can decide whether to follow the

recommendation of the LWF or not. Owners’ objections to treatment allocation led to the exclusion of 18 candidate plots and two treatment shifts between plots (Figure S2-1 in the Supporting Information). Third, concurrent with the negotiations, all plots selected for spraying have to be checked for compliance with state guidelines for nature protection. These guidelines prohibit spraying of a stand and apply to a shortlist of species with local conservation value and which populations may be negatively impacted by insecticides either directly (e.g. *Euplagia quadripunctaria*, Lepidoptera: Erebidae) or indirectly (e.g. *Bubo bubo*, Strigidae). We obtained permissions to spray stands falling under this rule for eight plots in which the threat posed by spraying was considered minimal (i.e. indirect threat only and unsprayed habitat available in close vicinity to the plot; Figure S2-1 in the Supporting Information; Supplementary Information, file S3). A final 48-plot study design covering a total area of 647 ha, with 311 ha to be sprayed with tebufenozide, was established as the outcome of the validation process (Figure 2). Around the centre of each plot, we established a 4.5-ha subplot where all investigations will be conducted. A detailed description of all selected plots, that is location, size, tree species composition, soil type, management type, spray date and spray history, is provided in Table S4-1 in the Supporting Information.

2.2.3 | Insecticide application

Tebufenozide was applied in spray plots as Mimic® (Spiess-Urania Chemicals, Hamburg, Germany; 240 g L⁻¹ active ingredient [a.i.]) at the maximal legal rate of 750 mL diluted in 50 L of water per ha (i.e. 180 g a.i. ha⁻¹), between 3 and 23 May 2019. The length of the spraying window was significantly extended due to legal procedures and unfavourable weather conditions. Treatment was applied by a Bell 208 helicopter equipped with a Simplex spraying system (Simplex Aerospace, Portland, Oregon, USA) with nozzle size 5 according to German regulations (German Federal Office of Consumer Protection and Food Safety, 2019) on an area ranging from 6.7 to 27.8 ha, for a total area of 314 ha (Table S4-1 and Figure S5-1 in the Supporting Information). Application proceeded in dry weather and low wind conditions (i.e. wind speed below 2.5 m s⁻¹) and block-wise when applicable.

2.3 | Data collection

During the treatment year (2019), we intensively sampled the study plots in order to measure the response of different components of the ecosystem (trees, non-target fauna) to insecticide and gypsy moth outbreaks. We hereby describe the data collection procedures carried out during the first year of the experiment. These surveys shall be repeated in the post-treatment years to assess the effects of continuing outbreaks and post-treatment recovery. Photographs of all survey methods, an example map of a plot and a list of the associated variables can be found in the Supplementary Information, file S6.

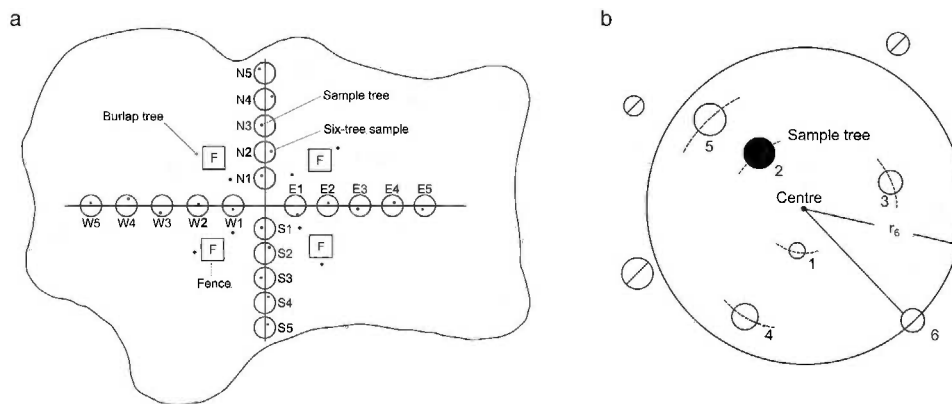


FIGURE 3 Sampling design for defoliation and gypsy moth monitoring. (a) Establishment of 20 six-tree samples along transects in the main cardinal points, starting from the centre of the infested area. Each six-tree sample provides one sample tree for detailed analysis. Eight mature oak trees were selected in the four intercardinal directions close to the plot centre for monitoring of gypsy moth caterpillars under burlap bands. Four fences for the regeneration surveys were set up in the four intercardinal directions within 40 m of the centre of the established coordinate system. In addition to the six-tree-sample surveys, T-Lidar is used for structural surveys along the transects. (b) Six-tree sample to record the sample trees and their environmental conditions. Around the centre tree, the next six trees were selected. The central sample tree is monitored in detail (e.g. crown condition, permanent girth band), the other five trees are used to record stand characteristics, upscaling and estimation of mortality rates. Egg mass surveys are carried out on each centre tree and the five closest oaks

2.3.1 | Tree growth monitoring

We marked 20 oaks in each plot, that is $44 \times 20 = 880$ trees (11 blocks; block T could not be included due to time constraints). In addition to the central tree, its six nearest neighbours with a diameter at breast height higher than 7 cm were included to account for competitive influences (Prodan, 1968). Starting from the centre of each plot, 20 of these 'six-tree samples' were taken along transects in the four cardinal points (Figure 3(a)). Sample circles were positioned at 25, 50, 75, 100 and 125 m from the centre of the infested area (origin of the coordinate system in Figure 3(b)). These sample circles served to record the stand characteristics (e.g. basal area, standing stock). Within each sample circle, the tree with medium diameter was selected as the sample tree for the detailed sampling. Species, diameter, position and crown transparency of the neighbour trees were scored.

In order to investigate long-term effects on the growth pattern of the oaks due to defoliation, the central oaks were additionally equipped with a permanent girth tape. During the 2019 growing season, the tapes were read and checked five times (April, June, July, September and November). The assessment was repeated in 2020 and should be extended for up to three additional years. In the future, the reading and checking will be carried out annually in autumn after completion of annual ring formation and in spring immediately before the start of the growth, in order to eliminate artefacts caused by winter swelling and shrinkage, and defects caused by manipulation or overstretching of the tension springs.

In January 2020, four fences (5×5 m) were installed within 30 m of the centre of each plot to address the effects of defoliation on the development of natural regeneration (Figure 3). An area outside the fence is used as a control to analyse the influence of browsing. Within the fences and on the adjacent control areas, all individuals up to a maximum height of 2 m, separated by tree species, were counted in

height intervals of 20 cm. Additionally, the browsing was addressed on each individual. In order to estimate the biomass of the regeneration, undamaged representative individuals were taken over the entire height spectrum found on the regeneration plots, outside the regeneration areas and separated by tree species. These individuals were dried to constant weight and weighed separately according to root and shoot. The regeneration recordings will be repeated in 2021 and should be extended for another 3 years if possible.

2.3.2 | Periodic changes in vegetation

In winter 2019 (late March-early April; day-of-year (doy): 80–100), before the start of the vegetation season, terrestrial laser scanning (TLS) was performed with a RIEGL VZ-400i laser-scanning system (RIEGL Laser Measurement Systems, Horn, Austria) to measure the three-dimensional structure of the forest canopy before leaf flush. Scanning was conducted at each of the 20 centre trees of the 'tree-cross' sampling design (Figure 3) that were marked with a reflector such that they could later be identified in the point cloud. Scanning was repeated at the expected peak feeding time of the gypsy moth (early July; doy: 180–200) and after reflushing of the defoliated trees (late August-early September; doy: 240–250), such that periodic changes in leaf area could be visualized by comparison of the TLS point clouds of each survey with the baseline. For each time point, we extracted a circular area with a radius of 20 m around the second centre tree starting from the plot centre in each cardinal direction (trees N2, S2, E2, W2; Figure 3). Points lower than 10 m above ground were filtered out to prevent bias generated by understory vegetation and herb layers in the assessment of defoliation and reforescence. The point cloud was then divided into 125 cm^3 voxels, and voxels comprising of three or fewer points were dropped. As a measure of the intensity of changes in leaf

area, we calculated the ratio of the number of voxels at peak feeding (t2) or after refoliation (t3) to the baseline (t1), further referred to as the foliation ratio:

$$\text{Foliation ratio}_i = \frac{\text{Number of voxels}_{t_i}}{\text{Number of voxels}_{t_1}} - 1.$$

With i representing the focal time point: 2 = defoliation peak, 3 = refoliation.

TLS surveys were repeated in 2020 and should be extended for at least one extra year, if possible.

2.3.3 | Non-target fauna

To assess the effect of gypsy moth outbreak and the use of insecticide on other animal species, several measures will be taken: (a) sampling of canopy arthropods by pyrethrum knockdown, (b) sampling of ground-dwelling Carabid beetles with pitfall traps, (c) sampling of adult Lepidoptera with light traps, (d) sampling of the bird community using songbird recorders and nest boxes, and (e) sampling of bat communities using bat call recorders.

Canopy arthropods. Crown-dwelling arthropods are sampled by pyrethrum knockdown with SwingFog SN50 fogging machines (Swingtec GmbH, Isny, Germany). In each plot, one mature oak tree from the dominant social class was selected as the centre of the fogging area. Fogging areas were also selected at least 30 m away from trees used for gypsy moth monitoring and assessment of tree response, to prevent pyrethrum knockdown from impacting the natural development of focal gypsy moth populations. Four tarpaulin sheets (3 × 5 m) are laid on the forest floor below the crowns of the focal tree and its neighbours, within a distance of 20 m, for a total sampled area of 60 m². The tree canopy above the sheets is fogged for 3–12 min depending on the wind conditions, until the fog cloud coats the targeted tree crowns. Arthropods are collected from the sheets after a 30-min exposure period and stored at –18°C. In 2019, the operation was repeated at three different time points: pre-spray baseline (25 April–8 May 2019; 38 plots), acute insecticide toxicity (i.e. 1–3 weeks post-spray, 23 May–7 June 2019; 48 plots) and peak feeding by the gypsy moth (1–4 July 2019; 48 plots) in different but comparable fogging areas. Gypsy moth caterpillars were counted and separated from the rest of the catch. Barcoding will be performed on all individual caterpillars, including gypsy moth, to identify each specimen and its associated parasitoids to species. The by-catch will be sorted to order for further analyses. The second and third sampling rounds, that is the acute toxicity phase and the peak defoliation sampling were repeated in 2020 in the same fogging areas that were swapped between both time points. As the post-treatment recovery of oak-dwelling Lepidoptera is expected to take more than 2 years, canopy arthropod surveys should be extended for at least one additional year.

Carabid beetles. Pitfall traps (i.e. 200 mL plastic yoghurt pots) are used to sample ground-dwelling arthropod fauna, in all 48 plots. In spring 2019, three traps were placed in individual holes in a line between the plot centre and the S1 tree under an alveolar polycar-

bonate plate mounted on 3 wooden sticks to protect them from rain-water. The traps are filled with 150 mL vinegar solution (5%) mixed a few drops of dishwashing liquid, acting both as a killing and conservation agent. In 2019, the traps were exposed for about 3 weeks and sampled continuously from spring to early summer (24 April–31 May 2019; 31 May–25 June 2019; 25 June–16 July) and one more time in late-summer (29 August–24 September 2019). As the main focal group, Carabid beetles were separated from the by-catch, counted and identified to species. The assessment was repeated in 2020 to measure potential recovery from insecticide impacts, with one additional trap per plot. For this second survey, the traps were moved inside the recently installed regeneration fences (one trap per fence; section 2.3.2. 'Periodic changes in vegetation') to reduce trap losses to wildlife damage. Conditional on patterns observed in 2020, the survey may be continued in 2021 to investigate longer-term carry-over effects.

Adult Lepidoptera. Adult moths are sampled by automatic light trapping in 44 plots. One light trap per plot is mounted at the height of approximately 1.6 m in proximity to the plot centre. Each trap consists of one fluorescent tube (12 V, 15 W; up to 40 m attraction range; Truxa & Fiedler, 2013) powered by a lead storage battery (12 V, 12 Ah) and equipped with a light sensor to enable automated activation and switch-off controlled by daylight intensity. Insects attracted by the lighted fluorescent tube fall through a plastic funnel into a bucket containing a chloroform-soaked wick. During each survey campaign, traps are set up for a single night in each plot, under conditions favouring flying activity of insects, that is temperature above 9°C, low precipitation, wind speed below 27 km h⁻¹ and fullness of the moon below 85%. We use 32 individual light traps so that up to eight blocks can be sampled in one night. Insects are collected in the following morning and stored at –20°C. Light traps were operated in five occasions throughout the spring and summer to cover most of the species assemblage. In 2019, light trapping was performed at the end of April, May, June, July and August 2019. Light trapping was repeated in 2020, excluding the May session, and will be repeated in 2021, with a survey frequency conditional of the results obtained in the previous years. Male and female gypsy moth are separately identified and counted in the laboratory, while other macrolepidopteran species are identified by an expert lepidopterologist. Full species composition of the samples is achieved by morphological identification of the Coleoptera and metabarcoding of the remaining by-catch.

Bats. We use autonomous bat call recorders of the type 'Batcorder 2.0' and 'Batcorder 3.0' (ecoObs, Nuremberg, Germany) to quantify activity and species diversity of bats in 44 plots. Batcorders are set up in the same nights as the light traps, that is we use up to 32 batcorders simultaneously. One batcorder is tied to a tree located near the plot centre at the height of 1.5 m with a distance of at least 15 m from the illuminated light trap in order to avoid interference. Batcorder microphones are set up to point towards an open space in the forest stand away from the light trap. Bat calls are automatically recorded from dusk until dawn for one night. Batcorders are set with a quality less than or equal to 20 and a maximum critical frequency of 16 kHz. From all recordings, bat calls within a threshold of –27 dB are

automatically analysed and assigned to a species or species group using bcAdmin4, batIdent1.5 and bcAnalyze3 (ecoObs, Nuremberg, Germany). Bat activity indices of each species or group are generated in 1-min intervals using bcAdmin4. Bat recording was repeated in 2020 and is planned for the second post-treatment year (2021), simultaneously with light trapping sessions.

Birds. We intend to examine the reproductive success of cavity-nesting birds in nest boxes and monitor songbird communities using sound recorders. Bird monitoring was conducted during the treatment year (2019), repeated identically in 2020 and planned for one additional year (2021) to address recovery of birds following disturbance.

Cavity B1 nest boxes (entrance hole diameter: 32 mm; Schwegler, Schorndorf, Germany) are used to study the breeding success of cavity-nesting species such as tits (*Parus major*, *Cyanistes caeruleus*), flycatchers (*Ficedula hypoleuca*, *Ficedula albicollis*) and nuthatches (*Sitta europaea*). In March and April 2019, eight nest boxes were deployed in 44 study sites for a total of 352 nest boxes, with an inter-box distance ranging from 30 to 90 m. In each location, half of the nest boxes were hung freely onto a tree branch while the other half was placed in direct contact to an oak trunk to measure the effect of nest invasion by gypsy moth caterpillars that commonly occurs early in the summer during outbreaks. Nest boxes were checked four times between late April and mid of July 2019, covering the first and second broods of nesting birds. Breeding success was measured by the number of fledged nestlings and the number of successful broods, which we defined as broods with at least one fledged nestling.

Communities of vocalizing bird species were detected continuously from late April to September 2019 via autonomous recording units (Bioacoustic Audio Recorder, Frontier Labs, Salisbury, Australia). At 44 locations, one recorder per site was permanently mounted at the height of 2.5 m near the centre of the plot. On four occasions between late April and mid-June, 10 min of the recorded sounds were identified to species for each study site and occasion by an experienced ornithologist.

2.3.4 | Gypsy moth population monitoring

We monitor gypsy moth populations by intensively sampling different life stages, including different larval instars. In 2019, all four life-stages (egg, larva, pupa and imago) were sampled throughout the spring and summer. In April, shortly before egg hatch, egg masses laid on oak trunks up to 2 m high were counted on 48 trees comprising the 'six-tree samples' centred around the trees 1 and 2 in each cardinal direction starting from the plot centre (Figure 3). To sample late-instar larvae, we installed 50-cm-wide burlap bands (polypropylene wood fleece, DuPont™ Plantex® Gold) on eight trees per site, that is two trees in each intercardinal direction starting from the plot centre, with an inter-tree distance ranging from 20 to 40 m. Live larvae, dead larvae and pupae sheltered below the bands were counted on two 300 cm² windows orientated north and south on each banded tree in two occasions (11–20 June and 02–18 July 2019) (Figure 3). This type of survey was conducted in 10 of the 12 blocks (i.e. 40 plots) in

2019. Early-instar larvae and imagines were sampled by pyrethrum knockdown and light-trapping together with the associated non-target species (see section 2.3.3. 'Non-target fauna'). Gypsy moth monitoring was conducted at the same intensity in 2020. As no outbreak population remained in any plot after 2020, lighter surveys (e.g. on a smaller number of trees per plot) will be performed during the next years as routine monitoring of population density.

2.4 | Statistical procedures

Statistical analyses will be performed in R 4.0.2 and upcoming versions (R Core Team, 2020).

2.4.1 | Missing data

In cases of non-random missing data (e.g. an entire block could not be sampled) in independent variables, missing data will be dropped, and the analysis only performed on complete data. Covariates with non-random missing data could be dropped depending on their importance in the analysis. For data missing at random in essential variables, individual data points will be dropped when the proportion of missing values is below 5%. For randomly missing data ranging from 5 to 30% of the total, multiple imputations ($n = 500$) will be performed with the R package mice to impute NAs with realistic values computed based on information from relevant variables in the dataset (Buuren & Groothuis-Oudshoorn, 2011). Statistical models will then be applied to each of the imputed datasets and the results pooled by Rubin's rules (Rubin, 1987).

2.4.2 | Statistical models

In order to analyse the effects of gypsy moth outbreaks and tebufenozide application on the various focal variables, we will use the following core linear mixed model based on the characteristics of the experimental design:

$$\text{Response} \sim \text{Defoliationrisk} \times \text{Treatment} + (1|\text{Block}).$$

For variables with non-normal error distribution, generalized linear mixed models will be used with the family distribution that best fits the response variable. Zero-inflation models will be used to investigate the post-spray response of groups for which strong insecticide effects are expected, that is gypsy moth and non-target Lepidoptera. Adjustments to the core model, such as inclusions of relevant covariates or nested random effects (e.g. 1|block/plot) will be made on a model-to-model basis, considering the existence of specific hypotheses justifying the inclusion of additional variables and the hierarchical level at which the effects are investigated (e.g. plot or individual tree). Because our experiment has a full factorial design, (generalized) linear models may be performed instead of mixed effect models in the absence of

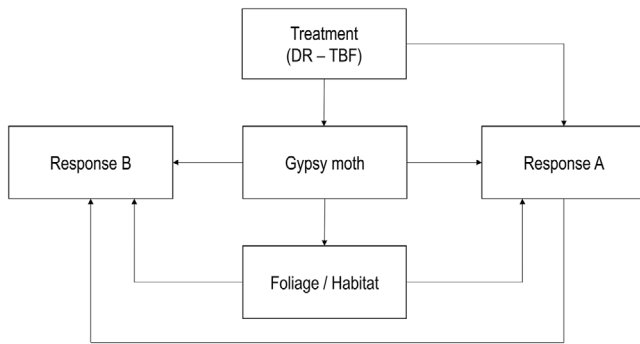


FIGURE 4 Structural equation model to test the relative impact of direct and indirect effects of gypsy moth outbreaks and tebufenozide on the response variables. The ‘treatment’ predictor depicts the combination of defoliation risk (DR; high/low) and tebufenozide application (TBF; control/sprayed) characterizing the experimental plots. Two different outcomes (A and B) may be integrated into the model to study cascading effects (e.g. trophic interactions). Each arrow represents a (generalized) linear or generalized additive (mixed) model with a random effect structure relevant to the scale at which the analysis is conducted, when applicable. Additional covariates are not shown but may be included when relevant

nested structure (i.e. plot-level analysis) and strong block effect. Lastly, generalized additive (mixed) models may be used to fit nonlinear relationships between the dependent and independent variables.

To separate direct and indirect effects of tebufenozide on the various non-target groups under study, we will perform structural equation models with gypsy moth density or foliation rate as mediators of the relationship between treatment and the independent variable(s). The choice of the appropriate mediator(s) will be motivated by the pathways through which indirect effects on the independent variable are expected to occur, namely whether they are related to change in resources (foliage or caterpillars) or habitat degradation (Figure 4).

2.4.3 | Covariates

A covariate will be included in a model only if it complies with the following criteria: (1) expected biological relevance, that is there is a sound hypothesis motivating its consideration in the analysis; (2) independence, that is the effect of the focal variable is not confounded with that of treatment and other covariates. The presence of confounding effects will be tested for each covariate in regression models with treatment as a predictor. Failure to accept the null hypothesis (i.e. no significant correlation between the covariate and the treatment) will lead to the exclusion of the focal covariate. To test for multicollinearity of covariates, we will perform a principal component analysis (PCA) on all potential covariates using the subset of plots considered for the analysis. In cases when two or more covariates are highly correlated, we will favour the variable which best describes the expected relationship with the independent variable. The covariates potentially included in further analyses are listed in the overview of all measured variables in Table S6-1 in the Supporting Information.

2.4.4 | Post-fitting procedures

Model diagnostics. (Generalized) linear (mixed) models and generalized additive (mixed) models must fulfil the assumptions of normality and independence of the residuals and homogeneity of the variance among groups. Each model will be graphically checked for compliance with these assumptions using base R plotting functions (linear models), and model diagnostic functions built-in the R package DHARMA (linear mixed models; Hartig, 2020) and mgcv (additive models; Wood, 2017)

Influence measures. To assess the influence of outliers on the results of the regression models, we will compute Cook’s distance (D) for each observation and examine the observations with values of D that are substantially outstanding from the rest. These observations will be dropped from the data, and the model refitted. Outliers will be considered influential if they substantially bias the estimates, in which case they will be dropped from the analysis.

Inference. To test our hypotheses, we will use t -, F -, Wald or likelihood ratio tests depending on the family error distribution and the structure of the focal model. For (generalized) linear mixed models, we will use the options currently available, following recommendations from Bolker (2020), namely Kenward–Roger F tests for the normal family, likelihood ratio tests for the Poisson family and Wald tests for the beta and negative binomial families and zero-inflation models. To test for differences among groups, we will perform comparisons of estimated marginal means with multivariate- t adjustment of p -values for multiple comparisons.

2.5 | Evaluation of the experimental design

In order to assess the suitability of the study design for addressing our research questions, we measured gypsy moth population density and the intensity of defoliation in the study sites during the treatment year (2019). We gathered data on gypsy moth population density at various stages of its development: egg masses, early-instar larvae (canopy; pre-spray and post-spray), late-instar larvae (burlap bands; live and dead), pupae (burlap bands) and imagines (see section 2.3 ‘Data collection’ for a description of the sampling methods). The data were analysed following the statistical procedures described in the previous section. Characteristics of the individual models are shown in Table S7-1 in the Supporting Information.

3 | DISCUSSION

Gypsy moth outbreaks and insecticide treatments can strongly impact tree health and forest animal communities and are thus an important subject in forest ecology and management. Our understanding of these effects, however, is still somewhat limited due to a number of methodological shortcomings. Designing a suitable experiment to study these effects is a challenging undertaking that requires a careful selection of forest plots with sharp differences in gypsy moth densities while controlling for spatial heterogeneity and confounding factors such as past

TABLE 1 Summary of statistical tests for all models

Variable	Defoliation risk	Treatment	Defoliation risk: Treatment
Egg masses (pre-treatment)	$\chi^2_{(1, N=40)} = 73.53, p < .001$	$\chi^2_{(1, N=40)} = 0.23, p = .628$	-
Larvae (L1-L3) - Fogging (pre-treatment)	$\chi^2_{(1, N=40)} = 51.57, p < .001$	$\chi^2_{(1, N=40)} = 0.27, p = .602$	-
Larvae (L3-L4) - Fogging	$\chi^2_{(1, N=48)} = 37.27, p < .001$	$\chi^2_{(1, N=48)} = 49.4, p < .001$	$\chi^2_{(1, N=48)} = 3.63, p = .057$
Larvae (L4-L5) - Burlap bands	$\chi^2_{(1, N=40)} = 5.93, p = .015$	$\chi^2_{(1, N=40)} = 16.31, p < .001$	$\chi^2_{(1, N=40)} = 3.88, p = .049$
Dead larvae (L4-L5) - Burlap bands	$\chi^2_{(1, N=40)} = 29.42, p < .001$	$\chi^2_{(1, N=40)} = 28.63, p < .001$	$\chi^2_{(1, N=40)} = 2.16, p = .142$
Pupae (L4-L5) - Burlap bands	$\chi^2_{(1, N=40)} = 7.22, p = .007$	$\chi^2_{(1, N=40)} = 22.20, p < .001$	-
Adults - Light trapping	$\chi^2_{(1, N=44)} = 23.41, p < .001$	$\chi^2_{(1, N=44)} = 4.00, p = .045$	$\chi^2_{(1, N=44)} = 0.64, p = .423$
Foliation ratio	$\chi^2_{(1, N=44)} = 43.13, p < .001$	$\chi^2_{(1, N=44)} = 61.49, p < .001$	$\chi^2_{(1, N=44)} = 16.10, p < .001$

All response variables were fitted to generalized linear mixed models with negative binomial error distribution, except the foliation ratio that was fitted to a beta regression model (Table S7-1 in the Supporting Information). Fixed effects include defoliation risk, accounting for pre-spray gypsy moth density and stand vulnerability to defoliation (Supplementary Information, file S1), and insecticide treatments. The interaction between defoliation risk and insecticide treatment was added when treatment effects were hypothesized to vary with the pre-spray density of the gypsy moth. Significant effects are highlighted in bold. All analyses were conducted on data aggregated at the plot-level when applicable. Wald tests were used to test the significance of fixed effects. Further information on the models are given in the Supplementary Information (Table S7-1)

spraying, within a sufficiently large region to allow the generalization of results. We successfully implemented an experimental approach that overcomes these hurdles to address the relative impacts of gypsy moth outbreaks and the insecticide-based management of these outbreaks. While a factorial design crossing high and low gypsy moth densities with insecticide treatment has already been implemented in a previous study (Sample et al., 1996), our design should considerably increase the robustness of this approach, notably with the inclusion of twice as many replicates and the use of blocking to account for spatial heterogeneity. Additionally, our high-resolution monitoring of gypsy moth population across the included stands offers a unique perspective on the different trajectories followed by different spatially synchronized gypsy moth populations. This data should help us investigating critical factors driving gypsy moth densities and closely follow the ecosystem response as a function of the outbreak magnitude.

Multiple obstacles face insecticide trials in forests: aerial pesticide applications are highly regulated practices that require considerable administrative effort to obtain clearance and involves complex organization and logistics. High heterogeneity among stands and unfavourable issues in negotiations with landowners often prevent the inclusion of stands within an experimental design, such that a considerable number of stands must be surveyed in order to reach reasonable objectives with regard to replication. These problems are usually overcome with pseudo-replication (Schönfeld et al.,), reduced numbers of true replicates (Cadogan & Scharbach, 2003) or small-scale approaches (Leroy et al., 2019). In a research field where the compliance with the principles of replication, randomness and blocking are more often the exception than the rule the inclusion of 12 replicates in a large-scale aerial forestry trial constitutes a great effort.

One year of gypsy moth monitoring showed that our site selection met the conditions required for the success of the approach. Tebufenozide applications worked as intended by successfully suppressing gypsy moth populations, which remained low until adult emergence in all sprayed plots (Figure 5). In line with our objectives, the selected high- and low-defoliation risk classes sharply differed

in terms of gypsy moth densities, with egg masses and early instar larvae in average 12- and 10-fold more abundant in high- than in low-defoliation risk plots, respectively (Table 1 and Figure 5). Outbreak plots experienced the highest defoliation, with foliation rates in high-control 71%, 67% and 64% that of low-control, high-treatment and low-treatment plots, respectively (Figure 5). However, defoliation was characterized by considerable variation among outbreak populations. In eight out of the 11 scanned high control stands, the gypsy moth population rose to very high densities, causing near-complete defoliation of the oaks but also showed clear signs of collapse, with high larval mortality and a drop in pupal abundance in seven plots (Supplementary Information, file S8). On average, nearly two-thirds of the larvae collected under burlap bands in high-control plots were dead, while mortality was comparatively low in plots at low defoliation risk (Figure 5). Such high mortality suggest an infestation of the caterpillar populations by pathogens such as the nuclear polyhedrosis virus, which often plays a crucial role in the collapse of erupting populations (Elkinton & Liebhold, 1990). These findings emphasize the importance of a large sample size to account for the highly variable and poorly predictable course of multiple outbreak populations within the same region.

Unlike the other life stages, the behaviour of the adult populations significantly deviated from our initial expectations based on defoliation risk and insecticide application. The number of adult gypsy moths caught in high-risk plots was twice as high as in low-risk plots, while tebufenozide only reduced adult catches by 25%, such that the difference between sprayed and control plots was not statistically significant within defoliation risk class (Table 1 and Figure 5). This pattern was driven by male moths, while females did not differ among treatments (Figure S9-1 in the Supporting Information). This result suggests spillover between sites, likely facilitated by the pairing of adjacent plots at similar defoliation risk as control and treatment areas within eight out of the 12 blocks (Figure 2). Such short distances between treatment and control plots may be considered as a lack of independence. However, this is consistent with the practice of aerial spraying in Bavaria,

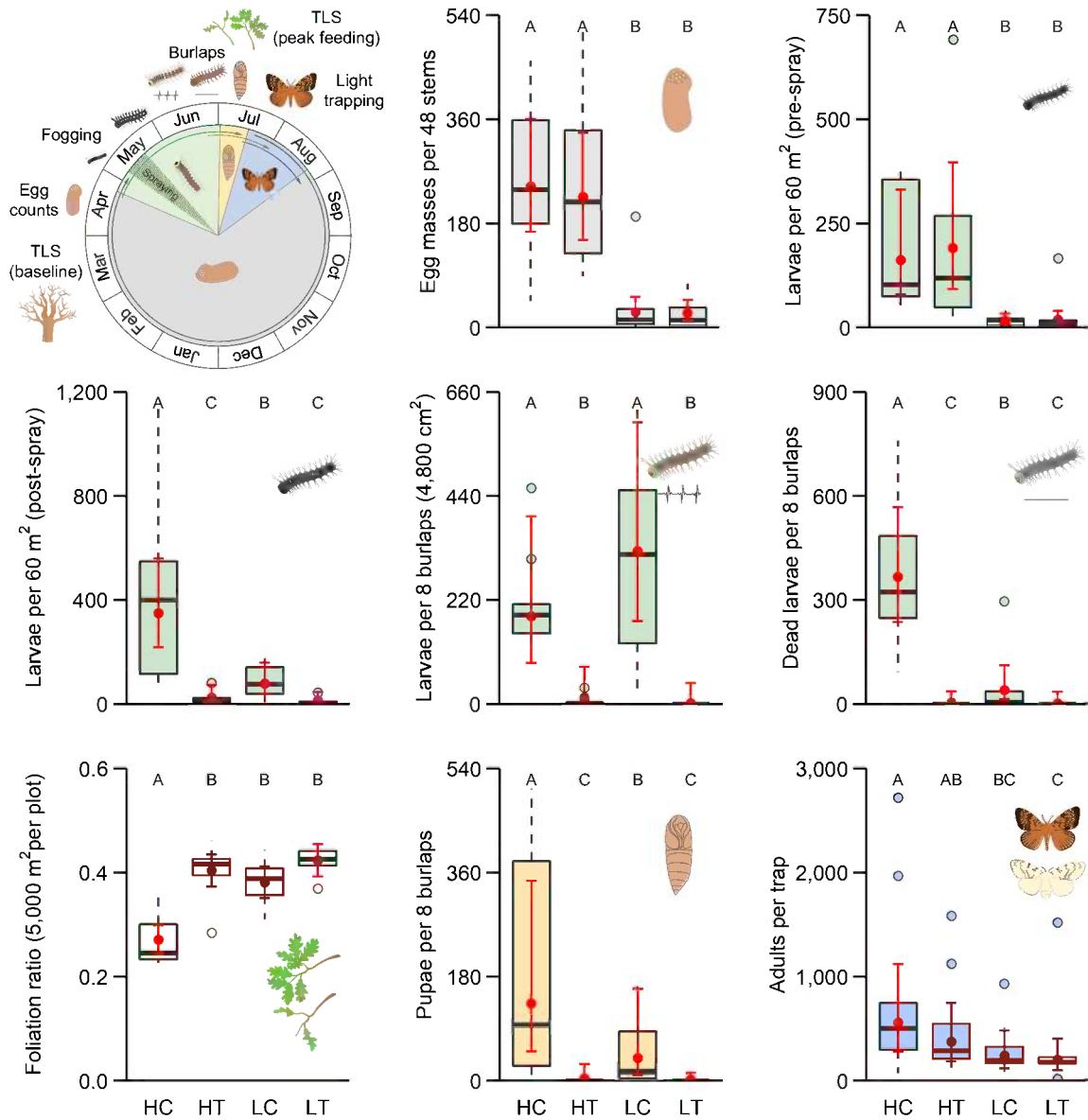


FIGURE 5 Plot-level abundance of different life stages of the gypsy moth and foliation ratio in the treatment year as a function of predicted defoliation risk (H = high; L = low) and insecticide treatment (C = unsprayed control, T = tebufenozide). From left to right, top to bottom: gypsy moth life cycle with time points corresponding to the sampling of response data; egg masses; canopy larvae (pre-spray); canopy larvae (post-spray); burlap larvae (live); burlap larvae (dead); foliation ratio; pupae; imagines (males + females). Boxplots show the raw data aggregated to the plot-level with the type and number of sampling units per plot indicated in the y-axis title of each graph. Points and error bars correspond to estimated marginal means and 95% confidence intervals for each treatment group. Different letters indicate significant differences among groups (multivariate-*t*-adjusted comparisons of estimated marginal means, $\alpha = 0.05$)

where treated areas are usually small (e.g. 7.5 ha median plot size in the operational application conducted in parallel to our project in 2019; Figure S5-1 in the Supporting Information), and entire stands are rarely treated due to environmental safety requirements regarding aerial applications (Supplementary Information, file S3). Hence, we interpret this result as an early sign of recolonization of sprayed areas by adult gypsy moths with potential implications for the management of outbreaks in the region. Adult males are known to fly over long distances to find mates and hence expectedly dominated the light trap catches. However, we surprisingly caught a fair number of females as well (Fig-

ure S9-1 in the Supporting Information). While females of the European subspecies *Lymantria dispar* are described as flightless and static (Doane & McManus, 1981; Zhang et al., 2019), our data suggest some flight activity in female gypsy moths in our sites. Moreover, we caught an unexpectedly high number of adult females in treated plots, while the juvenile population was nearly totally suppressed by tebufenozide, further suggesting stronger female movement than anticipated based on current knowledge. These results challenge our understanding of the movement behaviour of the European gypsy moth and call for further investigations in this direction.

Insecticide applications in forests are controversially discussed in the political sphere, on the one hand because of the potential longer-term effects of insecticides on biodiversity and on the other hand because of the potential income loss of forest owners when outbreaks are left unmanaged. Insect outbreaks in forests can have long-lasting consequences for ecosystems (Carson, Cronin, & Long, 2008), such as a decrease in tree growth following defoliation, or higher susceptibility of defoliated trees to secondary biotic and abiotic stresses. Similarly, defoliation and insecticide application may have long-lasting effects on the structure of animal communities. To better understand the implications of these processes for forest management, it is essential to monitor the impacts of gypsy moth outbreaks and insecticide treatments over several years. Our experiment provides the opportunity to study both the effects of gypsy moth outbreaks and insecticide application on short and medium timescales. Data produced as part of this effort during the coming years should help decision-makers to develop well-informed management strategies considering both the economic and ecological impact of gypsy moth outbreaks.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

Original idea and funding acquisition: Wolfgang Weisser, Jörg Müller, Hans Pretzsch, Hannes Lemme. **Site selection and study design:** Benjamin Leroy, Hannes Lemme, Sebastian Kienlein, Torben Hilmers, Kilian Stimm, Sophia Hochrein, Wolfgang Weisser, Jörg Müller, Hans Pretzsch, Sebastian Seibold. **Data collection:** Philipp Braumiller, Hannes Lemme, Benjamin Leroy, Sebastian Seibold, Jessica Jaworek, Sebastian Kienlein, Sophia Hochrein, Martin Jacobs, Torben Hilmers. **TL**

data processing: Martin Jacobs. **Statistical analysis:** Benjamin Leroy. **Artwork:** Sophia Hochrein, Benjamin Leroy. **Writing direction:** Benjamin Leroy. **Writing contribution:** Hannes Lemme, Wolfgang Weisser, Jörg Müller, Philipp Braumiller, Torben Hilmers, Martin Jacobs, Sophia Hochrein, Sebastian Seibold, Hans Pretzsch, W. Andreas Hahn.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.1vhmgqr> (Leroy et al., 2021).

PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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