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ORIGINAL ARTICLE

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Potential applicability of SPLAT® Verb for management of European spruce bark beetle, *Ips typographus* (L.)

Helge Löcken[1] | Tobias Frühbrodt[2] | Baoguo Du[3] | Christopher J. Fettig[4] | Peter H. W. Biedermann[1] | Jürgen Kreuzwieser[3] | Tim Burzlaff[1] | Horst Delb[2]

[1]Chair of Forest Entomology and Protection, University of Freiburg, Freiburg, Germany

[2]Department of Forest Protection, Forest Research Institute Baden-Württemberg, Freiburg, Germany

[3]Chair of Ecosystem Physiology, University of Freiburg, Freiburg, Germany

[4]Pacific Southwest Research Station, USDA Forest Service, Vallejo, California, USA

Correspondence
Helge Löcken, Chair of Forest Entomology and Protection, University of Freiburg, D-79085 Freiburg, Germany.
Email: helge.loecken@forento.uni-freiburg.de

Abstract

The European spruce bark beetle, *Ips typographus* (L.), is the most important forest pest in Europe due to the profound impacts of periodic outbreaks on ecosystem goods and services. Herein, we evaluated the responses of *I. typographus* to different doses of verbenone (SPLAT® Verb, 10% (–)-verbenone by weight; ISCA Inc., Riverside, CA, USA) in traps baited with its aggregation pheromones. Results are based on 1,492,289 *I. typographus* collected in five experiments over 3 years. SPLAT® Verb inhibited the response of *I. typographus* to baited traps out to 14 m from the point of release (dollop) and for >80 days at a dose of 75 g per dollop. Reductions in trap catch ranged from 34% to 93% depending on the dose of verbenone, age of SPLAT® Verb dollops, distance from dollops and the environment. In forest stands, significant reductions in trap catch were observed at distances up to 14 m from the point of release, with the largest reductions observed at 0 m (93%) and 2 m (64%). In an open area, significant reductions in trap catch were observed at distances up to only 2 m from the point of release, with the largest reduction observed at 0 m (66%). The much lower active inhibitory range of verbenone in the open area appears to be explained by less stable accumulations of verbenone in the surrounding air. There was a significant negative correlation between trap catch and the amount of verbenone measured in air in the vicinity of traps. We also observed inhibition of the sixtoothed spruce bark beetle, *Pityogenes chalcographus* (L.), another important forest pest in Europe, at all doses (20, 40, 75 and 100 g) of SPLAT® Verb that were evaluated. The implications of these and other results to the management of *I. typographus* are discussed.

KEYWORDS
anti-aggregation, forest protection, integrated pest management, pheromones, trap experiment, verbenone

1 | INTRODUCTION

Bark beetles (Curculionidae: Scolytinae) form a species-rich group of weevils, many of which feed on the phloem: a thin, nutrient-rich tissue inside the bark of trees and an important part of the plant vascular system. Colonization of live trees by bark beetles can result in tree death by cutting off the nutrient and assimilate transport between the tree's source and sink organs; as a result, some bark beetle species cause major economic damage in forests worldwide (Biedermann et al., 2019). In Central and Northern Europe, the European spruce bark beetle, *Ips typographus* (L.), is one of the most aggressive bark beetle species. Its primary host is Norway spruce, *Picea abies* (L.) H. Karst (Gregoiré & Evans, 2004). Due to the high proportion of stressed *P. abies* associated with increasingly frequent droughts and other disturbances (e.g., storms), several large outbreaks of *I. typographus* have occurred in Northern and Central Europe in recent years (Hlásny et al., 2021; Netherer et al., 2015; Seidl & Rammer, 2017).

When *I. typographus* disperse from their brood trees, they identify weakened or stressed trees as potential breeding sites guided by visual and olfactory cues (Lehmanski et al., 2023; Netherer et al., 2021). The trees, on the other hand, produce and store chemical defence compounds such as α-pinene, one of the main constituents of resin (Duan et al., 2020; Krokene, 2015). Despite its toxicity and properties as a physical barrier (Franceschi et al., 2005), α-pinene can be oxidized by *I. typographus* to *cis*-verbenol during host colonization. The latter, together with 2-methyl-3-buten-2-ol and ipsdienol, forms an important part of the aggregation pheromone of *I. typographus* (Bakke, 1976; Bakke et al., 1977; Vité et al., 1972). If the tree is unable to prevent the emission of aggregation pheromone through the expulsion of *I. typographus* with resin, a mass attack ensues subsequently killing the tree (Vité & Francke, 1985).

Continuous aggregation would lead to 'over-colonization' (i.e., high levels of intra-specific competition for phloem with deleterious effects on *I. typographus*) of the host tree (Byers, 1984; Raffa, 2001). This is prevented by the release of anti-aggregation pheromones as the infestation proceeds over time (Frühbrodt, Du, et al., 2023; Schlyter et al., 1987). For *I. typographus*, these anti-aggregation pheromones include verbenone (Vn; 4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one) (Bakke, 1981; Byers, 1989; Francke & Vité, 1983) together with a certain ratio of ipsdienol and ipsenol. (Schlyter et al., 1987). Vn is a product of autoxidation as well as metabolization of verbenol by associated microorganisms in the hindgut of *I. typographus* (Byers, 1989) and surrounding host tissues (Cale et al., 2019; Leufvén & Birgersson, 1987).

The discovery of anti-aggregation pheromones led to the idea of using anti-aggregation pheromones to artificially disrupt host colonization (Borden, 1997). To date, most research involving the use of anti-aggregation pheromones has focused on Vn for managing mountain pine beetle (*Dendroctonus ponderosae* Hopkins), southern pine beetle (*D. frontalis* Zimmermann) and western pine beetle (*D. brevicomis* LeConte), in North America and *I. typographus* in Europe (Frühbrodt, Schebeck, et al., 2023). The most successful and widespread application of a bark beetle anti-aggregation pheromone involves the use of 3-methylcyclohex-2-en-1-one (MCH) for the management of Douglas-fir beetle, *D. pseudotsugae* Hopkins, in western North America (Ross, 2021). Although the inhibitory effect of Vn on *I. typographus* has been demonstrated in several studies (Bakke, 1981; Schlyter et al., 1988; Vite & Baader, 1990), its effectiveness for protecting windthrows, downed logs and live trees has been limited (Frühbrodt, Schebeck, et al., 2023; Jakus et al., 2003; Niemeyer et al., 1995). One of the main challenges in using Vn has been its high volatility and UV instability, which made it difficult to obtain Vn dispensers (formulations) with reliable release rates over sufficient periods (months) under field conditions (Fettig et al., 2020; Kostyk et al., 1993; Lobinger, 2006). Another concern is the range of inhibition, also called 'active inhibitory range' (AIR) defined by Zhang and Schlyter (2003) as 'the maximum distance within which a significant inhibitory effect could be observed when compared with response to the pheromone (or attractant) alone'. The AIR of different Vn formulations, which typically comprises only several meters (Frühbrodt, Schebeck, et al., 2023), is a crucial parameter but has only been investigated in a few studies.

In 2004, ISCA Inc. launched SPLAT® (Specialized Pheromone and Lure Application Technique), an inert carrier matrix of waxes and oils that protect incorporated chemicals from UV radiation and regulates their volatilization over time (Mafra-Neto et al., 2014). Fettig et al. (2015) developed a novel formulation of Vn using SPLAT® (SPLAT® Verb, 10% (−)-verbenone by weight; ISCA Inc., Riverside, CA, USA) for protecting lodgepole pine, *Pinus contorta* var. *latifolia* Dougl. Ex. Loud., from mortality attributed to *D. ponderosae* in western North America. Rather than a single release device, such as the Vn pouch (Seybold et al., 2018), SPLAT® Verb is a flowable emulsion that allows the user to adjust the size of each release point (dollop) according to desired distributions in the field. SPLAT® Verb releases Vn over a sustained period (~8–24 weeks, depending on dollop size and abiotic conditions) at rates suitable to provide significant reductions in levels of *P. contorta* mortality at relatively low doses (Fettig et al., 2020). SPLAT® Verb was registered for use in the United States in 2013 and was first used commercially in 2014. Since then, SPLAT® Verb has been shown to reduce mass attacks by *D. ponderosae* in several host species (Fettig, 2016; Progar et al., 2021), as well as impart inhibition in other tree-killing bark and ambrosia beetles (e.g., Agnello et al., 2021; Gaylord et al., 2020, 2023; Hughes et al., 2017; Martini et al., 2020; Roy et al., 2023).

Herein we examine whether SPLAT® Verb holds promise as a tool for the integrated management of *I. typographus* in Europe. To develop a reliable strategy for the application of SPLAT® Verb to protect *P. abies* from mortality attributed to *I. typographus*, we require answers to three key questions: (1) What is the AIR for a

specific dose? (2) For how long does the inhibitory effect last after initial application? and (3) What is the optimal dose? The answers to these questions will help provide the basic framework to ensure that applied solutions are not only effective but also efficient. We address these questions in a series of field-trapping experiments. The response of the sixtoothed spruce bark beetle, *Pityogenes chalcographus* (L.), another important pest of *P. abies* in Europe (Gregoiré & Evans, 2004), to the aggregation pheromones of *I. typographus* and Vn is also investigated.

2 | MATERIALS AND METHODS

In 3 years, we conducted five field-trapping experiments at three different sites in Germany (Table 1). Two experiments addressed the optimal dose of SPLAT® Verb; one experiment addressed the duration of inhibition; and two experiments addressed the AIR.

2.1 | Study site

Study sites were chosen according to a high proportion of *P. abies*, even stand and the age structure of *P. abies* (≥60 years), and level terrain to avoid thermal effects associated with steep slopes. The 2020 dose experiment was conducted in a closed old-growth *P. abies* forest near Bernau, Germany (Table 1). The 2021 AIR experiment took place in a closed forest dominated by 60–90 year old *P. abies* in Faulenfürst near Schluchsee, Germany. Experiments on the duration of inhibitory effect 2021, application rate 2022 and AIR 2022 were conducted in a ~30-ha clearcut resulting from an ongoing *I. typographus* outbreak near Buggenried/Grafenhausen, Germany. This outbreak started in 2018 and provided ample *I. typographus* abundance ('pressure') for a robust examination of treatments.

2.2 | Experimental design

The basic efficacy of SPLAT® Verb, including duration of inhibitory effect, dose and AIR, was evaluated using the same basic experimental design. Theysohn slot traps (FLÜGEL GmbH, Osterode am Harz, Germany) were placed ≥50 m apart and baited with a commercial lure (Pheroprax®, BASF, Ludwigshafen, Germany; active ingredients: *S*-ipsdienol, S-*cis*-verbenol and 2-methyl-3-buten-2-ol). A dollop of SPLAT® Verb was deployed at 1 m or varying distance and at ~1.6 m in height on a 20-cm wooden crossbar with a 20 × 40-cm roof made of film-faced plywood to protect the dollop from precipitation and solar radiation (Figure 1). Depending on the research question, different doses of SPLAT® Verb, ages of SPLAT® Verb dollops (aged in the lab prior to deployment in the field), and distances from dollops (AIR) were evaluated. To help standardize the effect of wind, dollops were positioned at an azimuth of 240° (upwind, according to the dominant wind direction) to the trap. Temperature

TABLE 1 Overview of experiments and study sites.

Experiment	Sample size (traps × obs.)	Coordinates	Stand characteristic	Local *I. typographus* population (according to the level of number of trap catches)
Dose 2020	1008 (16 × 64)	47.776562 N, 8.078511 E; 890 m a.s.l. (Bernau)	87% *P. abies* (~90 years old); 7% *Fagus sylvatica*, 6% *Abies alba*	Intermediate
AIR 2021	652 (64 × 8)	47.813730 N, 8.200908 E; 1070 m a.s.l. (Faulenfürst)	84% *P. abies* (~90 years old); 6% *A. alba*, <5% *Pinus sylvestris*, <5% *F. sylvatica*	Low
Duration of inhibitory effect 2021	833 (36 × 25)	47.738069 N, 8.268661 E; 830 m a.s.l. (Buggenried)	Calamity after bark beetle outbreak starting in 2018; previous and surrounding forest: 80% *P. abies* (~70 years old); 12% *A. alba*, <5% *F. sylvatica*, <5% *P. sylvestris*	*High*
AIR 2022	432 (16 × 21)			
Dose 2022	580 (24 × 26)			

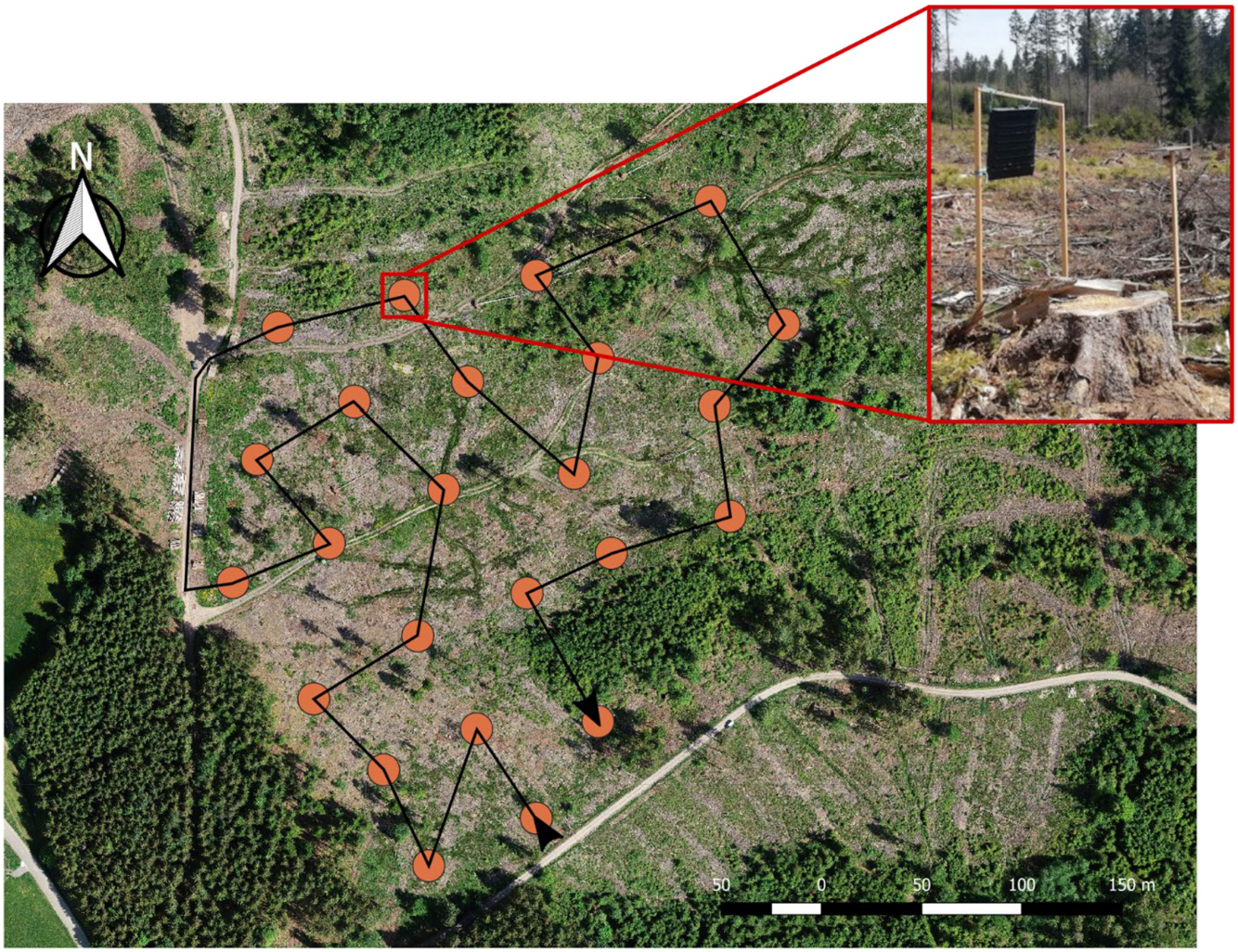


FIGURE 1 Aerial image of the study site near Buggenried/Grafenhausen, Germany (Dose 2022, duration of inhibitory effect and AIR 2022 experiments). The inset shows a trap with the adjacent release (dollop) of SPLAT® Verb (wooden post with a crossbar to the right of the stump). The black line indicates the route along which treatments were rotated between traps (orange dots; positions and route shown for the Dose 2022 experiment).

and solar radiation were recorded through the use of temperature loggers (UA-002-64 HOBO®, Onset, Bourne, USA). Depending on weather conditions, traps were emptied daily or at least twice a week. To better account for location effects, treatments were systematically rotated after each observation in all experiments from 2021 onwards.

2.2.1 | Dose 2020

Three doses of SPLAT® Verb and a positive control (0 = control, 20, 40 and 75 g) were set up next to 16 baited traps resulting in four replicates per time per treatment. Treatments were distributed randomly amongst traps and remained at their fixed position throughout the experiment. Sampling was conducted on 64 days between 13.04.2020 and 03.08.2020.

2.2.2 | Dose 2022

In 2022, 24 baited traps were set up and combined with four doses of SPLAT® Verb and positive control (0 = control, 20, 40, 75 and 100 g). Each treatment was replicated four times. Treatments were rotated as described in 2.2. Sampling was conducted on 26 days between 14.04.2022 and 14.07.2022.

2.2.3 | AIR 2021

For the AIR 2021, we implemented a rotational system similar to the above but modified according to the specific setup required by the research question. We placed 32 trap pairs across four transects (two with an east/west orientation and two with a north/south orientation) to a distance of 24 m. Ground anchors (small

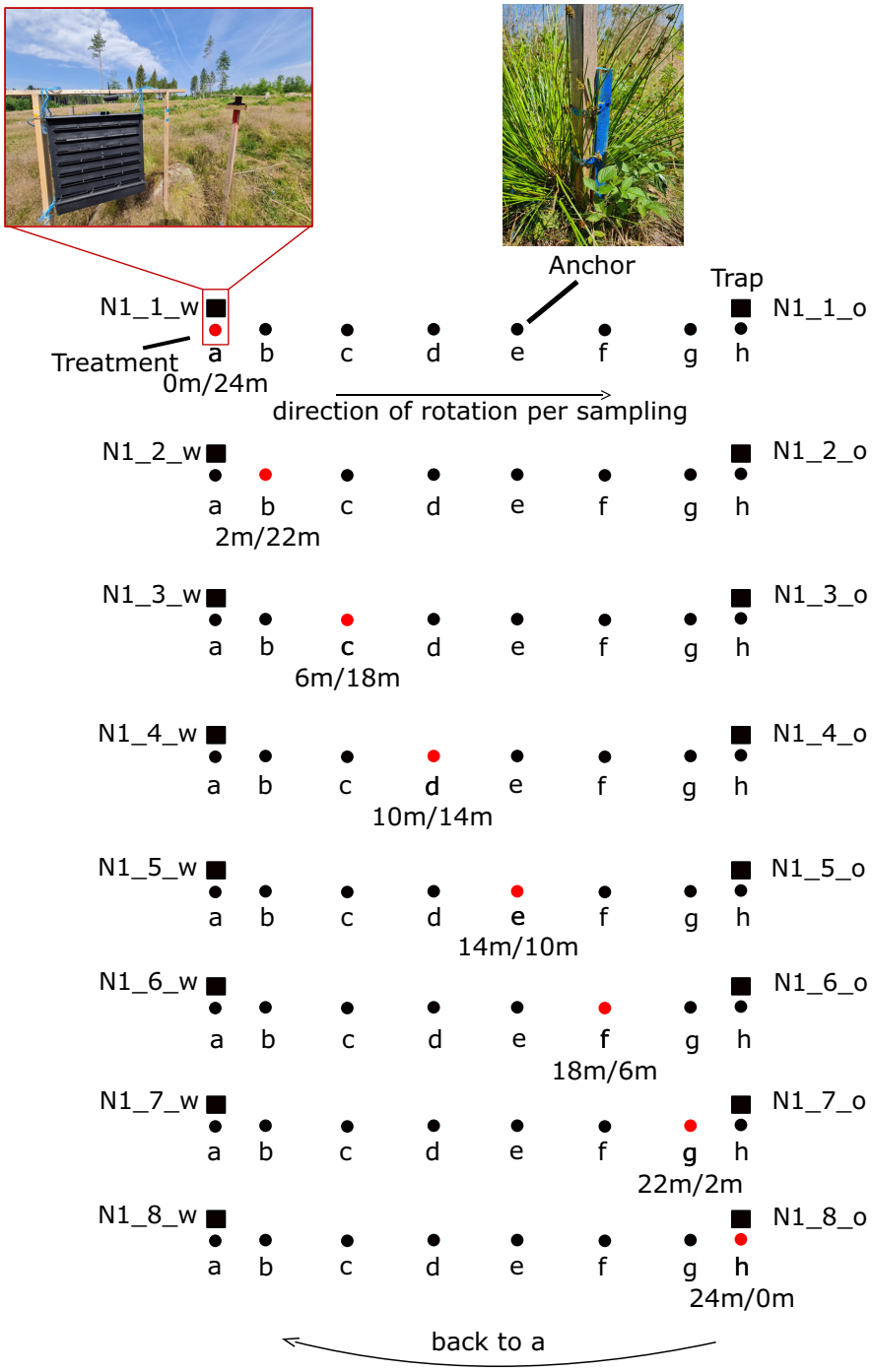


FIGURE 2 Experimental design of the AIR experiment. SPLAT® Verb treatments (red dots) rotated between trap pairs (black squares) by moving one step further (from left to right in the figure) along a line of ground anchors (black dots) after every sample collection. Consequently, each SPLAT® Verb-to-trap distance occurred once during each sample period.

wooden bars with a metal loop) were placed at intervals of 0, 2, 6, 10, 14, 18, 22 and 24 m between pairs of baited traps. The SPLAT® Verb dollop could be placed in these anchors when moved according to the rotation scheme (Figure 2). Sampling was conducted on 8 days between 10.06.2021 and 16.06.2021.

2.2.4 | AIR 2022

Analogue to AIR 2021, we implemented two transects, one facing north/south and one east/west, with four trap pairs per transect and 10 m between baited traps. Distances of 0, 1, 2, 4, 6, 8, 9 and 10 m were evaluated. Sampling was conducted on 21 days between 22.04.2022 and 21.06.2022.

2.2.5 | Duration of inhibitory effect

To assess the duration of the inhibitory effect of SPLAT® Verb, dollops were pre-aged under controlled conditions in the laboratory for up to 80 days. For this purpose, four replicates of 75 g of SPLAT® Verb were applied at 10-day intervals to a 20-cm × 5-cm crossbar (*see* Supplement

1). On the day the experiment started, the pre-aged dollops were taken to the study area and distributed randomly (2020) or systematically (2021) to the trap sites. An additional treatment included a fresh dollop of SPLAT® Verb applied in the field at the beginning of the experiment ('age = 0'). Four baited traps were kept as positive controls. In 10-day intervals, the oldest dollops (80 days) were removed and disposed of, and new dollops were applied ('age = 0'). This process automatically established the temporal repetition of treatments with a systematic rotation of the treatments in space. Sampling was conducted on 25 days between 26.04.2021 and 01.07.2021.

2.3 | Processing of trap catches

Trap catches were stored at −20°C and later sorted to *I. typographus*, *P. chalcographus* and other bycatches of insects. For trap catches with ≤100 *I. typographus* (or *P. chalcographus*), beetles were counted individually. For trap catches of >100 *I. typographus* (or *P. chalcographus*), the numbers of beetles were estimated with a measuring cylinder using 40 *I. typographus* mL^{-1} and 400 *P. chalcographus* mL^{-1}.

2.4 | Verbenone release from SPLAT® Verb under controlled conditions

To evaluate Vn release characteristics from SPLAT® Verb, release rates were determined at different temperatures under controlled conditions in the laboratory. Twelve glass Petri dishes (5 cm diameter, 1.2 cm inner depth) were filled with ~21 g of SPLAT® Verb each with a levelled surface. Four of the 12 Petri dishes were incubated at 16, 22 and 30°C, respectively. Vn abundances released from SPLAT® Verb were determined with a self-made sampling system. Petri dishes with SPLAT® Verb were placed in a glass cuvette with a top lid that had an inlet and outlet. The closed system was flushed with synthetic air (Linde, Pullach, Germany) at 200 mL min^{-1} for 1 min by an air sampling pump (220-1000 TC, PocketPump, SKC Inc., Pennsylvania, USA) connected to the outlet. A thermodesorption tube (Gerstel, Mülheim a.d.R., Germany) filled with 30 ± 2 mg Tenax (Sigma–Aldrich, Munich, Germany) was placed between the outlet and air sampling pump for 10 s to adsorb Vn. The Petri dishes were immediately brought back and incubated at the previous temperature as described above. The system was opened and aerated with ambient air between each sample. Sampling was conducted 13 times, starting at day 0 and lasting for 97 days. Volatile samples were stored at 4°C until analysed by gas chromatography–mass spectrometry (GC–MS) to determine Vn concentrations.

2.5 | Verbenone concentrations at trap sites, released from SPLAT® Verb under field conditions

To evaluate the concentration of Vn at the trap sites during beetle collection, ambient air was sampled directly above the traps of the AIR experiment 2022 (see Section 2.2.4). For this purpose, an air sampling pump (220-1000 TC, PocketPump) connected to a thermodesorption tube (Gerstel) filled with 30 ± 2 mg Tenax (Sigma–Aldrich) was placed on the crossbar directly above the trap to absorb Vn. The flow rate was adjusted to 200 mL min^{-1} and sampling occurred for 5 h (11:00–16:00), hence, in total 60 L of ambient air passed through each adsorbent. The thermodesorption tubes were transported to the laboratory and stored at 4°C until analysed by GC–MS to determine Vn concentrations. The Theysohn traps were emptied before each air sampling. Beetles caught during (5 h) sampling were collected after sampling and processed as described above (see Section 2.3).

2.6 | Verbenone determination

To quantify the amount of Vn, the thermodesorption tubes from the laboratory and field were analysed on a GC–MS (GC model 7890; MSD, 5975C, Agilent, California, USA), equipped with a thermodesorption/cold injection system (TDU-CIS4, Gerstel, Mülheim an der Ruhr, Germany). Briefly, Vn was released from the Tenax adsorbent by heating the tubes containing Tenax to 220°C in the TDU, volatiles were directly channelled into the CIS4 and cryotrapped at −40°C. The CIS4 was then heated to 240°C, releasing the volatiles onto the separation column (DB-5 ms UI, 60 m × 0.25 mm × 0.25 µm film thickness, Agilent). Helium was used as a carrier gas at a flow of 1 mL min^{-1}. The oven programme started at 100°C, which was kept for 0.5 min, and then increased stepwise to 170 and 300°C at rates of 12 and 18°C min^{-1}, respectively. The MSD ran at 70 eV and an ion source temperature of 230°C and a quadrupole temperature of 150°C. The mass-selective detector was run in SIM mode detecting *m/z* 55, 67, 77, 79, 93, 107, 109, 121, 122, 134, 135, 136, 150 and 152 with GC–MS conditions described previously (Byron et al., 2022; Frühbrodt, Du, et al., 2023). Vn was identified and quantified according to a standard of ((1*S*)-(−)-verbenone, >94%) (Sigma–Aldrich, Munich, Germany) analysed in the same manner with the MassHunter Software (Version B.07.00, Agilent) as described in Frühbrodt, Du, et al. (2023).

2.7 | Statistical analysis

All statistical analysis was done in R statistical Software (v4.2.1; R Core Team, 2022). Reductions per treatment were calculated as mean comparisons between treatments and the control using the Abbotts formula (Abbott, 1925): $\text{Reduction} = (C - T) \times C^{-1}$, where C and T are the expected means of the control and treatment group, respectively. Generalized mixed models (GLMM) were fitted using the glmmTMB package (Brooks et al., 2017). For multiple group comparisons, posthoc tests were performed as a Tukey HSD test using the glht function of the multcomp package (Hothorn et al., 2008). Model diagnostics were performed using the DHARMa

package (Hartig, 2020). Plots were created using the sjPlot package (Lüdecke, 2021).

Model predictors were selected by a top-down approach, starting with the most complex model structure, and, always retaining the treatment, reducing it for the sake of model convergence. Model selection took place by considering conspicuous patterns in the residual plots and the lowest AIC.

2.7.1 | Dose 2020

We fitted a GLMM (tweedie family with a log link) (estimated using ML and nlminb optimizer) to predict trap catches with dose (formula: trapcatches ~ dose + offset(log(dailycatch))). The model included trap and date as random effects. Also, the sample processing person was added as a random factor, since the compaction rate in the measuring cylinder (used to estimate the numbers of beetles) varied by person and it improved the model fit (formula: list(~1|trap, ~1|date, ~1|protocoller)).

2.7.2 | Dose 2022

GLMM (tweedie family with a log link; estimated using ML and nlminb optimizer) were fitted to explain trap catches with application rate (formula: Beetlecatches ~ applicationrate + poly(pheropraxage, 2) + poly(SPLATVerbage, 2)). The terms 'pheropraxage' and 'SPLATVerbage' refer to the period of time that aggregation dispensers and SPLAT® Verb dollops were exposed in the field before the sampling date. This was necessary since we assume changes in the release rate of the aggregation pheromone over time, as observed with Vn (see Section 3.5). The model included date, trap site, and sample processing person as random effects (formula: list(~1|date, ~1|trap, ~1|protocoller)).

2.7.3 | AIR 2021

We fitted GLMM (tweedie family with a log link) containing the trap catches as a response variable and distance, mean temperature, the orientation of the trap (i.e., its orientation to the dollop) and date as fixed effects (formula: trap catches ~ distance + meantemp. + orientation + date). The model included trap, nested in trap pair, as random effects (formula: list(~1|trap: pair, ~1|pair)).

2.7.4 | AIR 2022

Similar to AIR 2021, we fitted a GLMM (tweedie family with a log link) containing trap catch as the response variable with distance, orientation of the trap (i.e., its orientation to the dollop) and, similar to the dose 2022 analysis, the pre-exposure time (age) of the aggregation dispenser and SPLAT® Verb dollop were included with their quadratic terms as fixed effects. Also, as it increased the model fit, we included the sample processing person as a fixed effect because the model did not converge when included as a random effect as done above (formula: trapcatches ~ distance + poly(pheropraxage, 2) + poly(SPLATVerbage, 2) + meantemp. + orientation + date + protocoller). The model included a trap, nested in the transect, as random effects (formula: list(~1|trap: transect, ~1|transect)).

To correlate beetle catches with the amount of Vn at the trap, we fitted a GLMM (tweedie family with a log link) containing again trap catch as the response variable but this time the measured amounts of Vn as the predictor variable. The model included date and trap as random effects (formula: list(~1|date, ~1|trap)). As we had very few data points at high levels of Vn (five datapoints at >25 ng 60 L^{-1} Vn), we excluded all data points >25 ng 60 L^{-1} air Vn to be conservative and prevent misinterpretation due to a lack of data (compare model with full dataset in Supplement 2).

2.7.5 | Duration of the inhibitory effect

We fitted a GLMM (tweedie family with a log link; estimated using ML and nlminb optimizer) to explain trap catches with an exposure time of SPLAT® Verb. To include the control in the analysis, we grouped exposure times in 10-day blocks (1–10, 11–20, 21–30, 31–40, 41–50, 51–60, 61–70 and 71–80 days, respectively) to have a categorical factor rather than continuous. As the age of the aggregation pheromone dispenser influences attraction, this was considered a polynomial fixed effect. In addition, as it improved the model fit, log-transformed daily catch was included in the model as an offset (formula: trapcatches ~ group + poly(pheropraxage, 2) + offset(log(totalcatch))). The model included date and trap as random effects (formula: list(~1|date, ~1|trap)).

3 | RESULTS

Results are based on captures of 1,492,289 *I. typographus* in 3802 samples over 131 sampling day in 3 years.

3.1 | Dose 2020

Whilst we observed no significant reductions in trap catch with the 20 g (tweedie glmm: $z = -1.77$, $p = 0.077$) and 40 g treatments (tweedie glmm: $z = -1.62$, $p = 0.107$), there was a significant reduction in trap catch (96%) in the 75 g treatment compared to the control (tweedie glmm: $z = -4.682$, $p < 0.001$). Catches in the 75 g treatment were also significantly lower than in the 20 g (Tukey's contrast for multiple comparisons of means: $z = -2.924$, $p = 0.018$; reduction: 71% compared to control) and 40 g treatments (Tukey's contrast for multiple comparisons of means: $z = -3.079$, $p = 0.011$; reduction: 68% compared to control) (Figure 3).

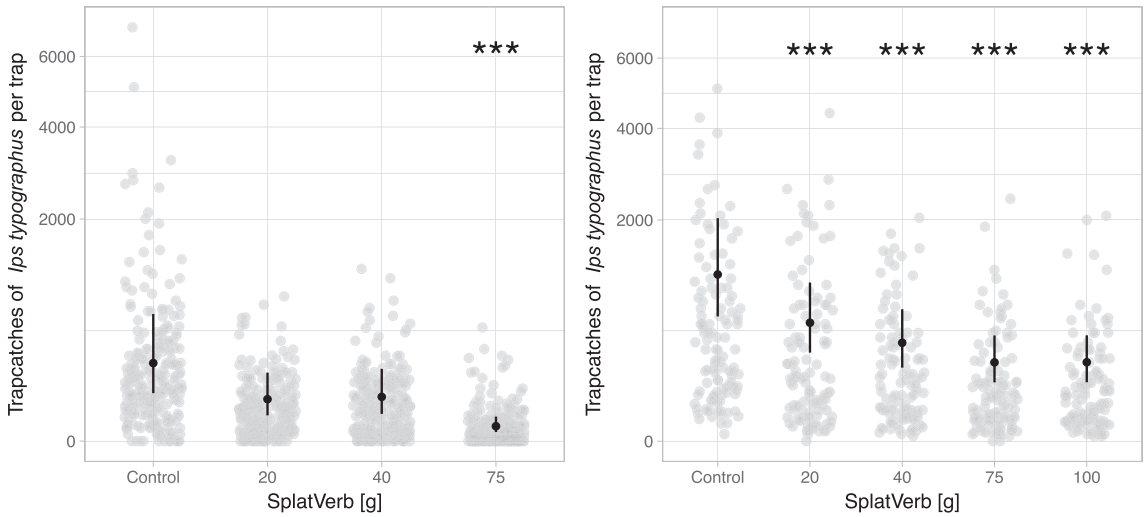


FIGURE 3 Trap catches of *Ips typographus* in the dose experiments 2020 (left) and 2022 (right). Black dot with 95% confidence intervals shows the predicted means given by the models. Grey dots in the background show the observed data with a jitter for improved visibility. Asterisks indicate different levels of significance of a group mean compared to the positive control (***$p < 0.001$).

3.2 | Dose 2022

There was a significant reduction in trap catch observed for all (20, 40, 75 and 100 g) doses of SPLAT® Verb compared to the control, with the highest reduction in trap catch (78%) in the 75 g treatment (tweedie glmm: $z = -16.25$, $p < 0.001$) and the lowest reduction (50%) in the 20 g treatment (tweedie glmm: $z = -7.97$, $p > 0.001$). There was no difference in trap catch between the 75 and 100 g treatments (Tukey's contrast for multiple comparisons of means: $z = 0.035$, $p > 0.1$) (Figure 3).

3.3 | AIR 2021

Inside the forest stand, significant reductions in trap catch were observed at distances up to 14 m from the SPLAT® Verb dollop (tweedie glmm, $z = -2.02$, $p = 0.04$). The largest reductions in trap catch were observed at 0 m ($z = -15.76$, $p < 0.001$; 93%) and 2 m ($z = -6.87$, $p < 0.001$; 64%), whilst at 14 m the reduction was only 24% ($z = -0.27$, $p = 0.035$) (Figure 4).

3.4 | AIR 2022

In the open field, significant reductions in trap catch were observed at distances up to only 2 m from the SPLAT® Verb dollop (tweedie glmm, $z = -3.220$, $p = 0.028$). The largest reduction in trap catch was observed at 0 m (tweedie glmm: $z = -10.262$, $p < 0.001$; 66% compared to the mean catches in the furthest distance of 10 m). There were no significant differences amongst trap catches at 4, 6, 8, 9 or 10 m (post hoc Tukey HSD: all $p > 0.9$) (Figure 4). There was a significant negative correlation between trap catches and the amount of Vn measured at traps (tweedie glmm: $z = -3.565$, $p < 0.001$; Figure 5).

3.5 | Duration of the inhibitory effect

In the open field, the largest reduction in trap catch (~70%) occurred within the first 10 days of application (tweedie glmm, $z = -11.05$, $p < 0.001$). Thereafter, reductions in trap catch gradually decreased over time. Nonetheless, even after 80 days, trap catches were significantly lower (34%) in the SPLAT® Verb treatment (by 34%; $z = -4.69$, $p = 0.001$) compared to the control (Figure 6).

3.6 | Pityogenes chalcographus

Analogue to the results on *I. typographus* obtained in the open field, we observed significant reductions in the catch of *P. chalcographus* at all doses of SPLAT Verb® compared to the control, with the highest reduction (59%) in the 75 g treatment (tweedie glmm: $z = -7.892$, $p < 0.001$) and the lowest reduction (36%) in the 20 g treatment (tweedie glmm: $z = -4.127$, $p < 0.001$; Figure 7).

4 | DISCUSSION

We examined how different doses of SPLAT® Verb, ages of SPLAT® Verb dollops and distances from SPLAT® Verb dollops (AIR) affect the response of *I. typographus* to baited traps in multiple experiments over a 3-year period. To the best of our knowledge, this is the first study to evaluate the response of *I. typographus* to SPLAT® Verb in trapping experiments. Our data clearly demonstrate the

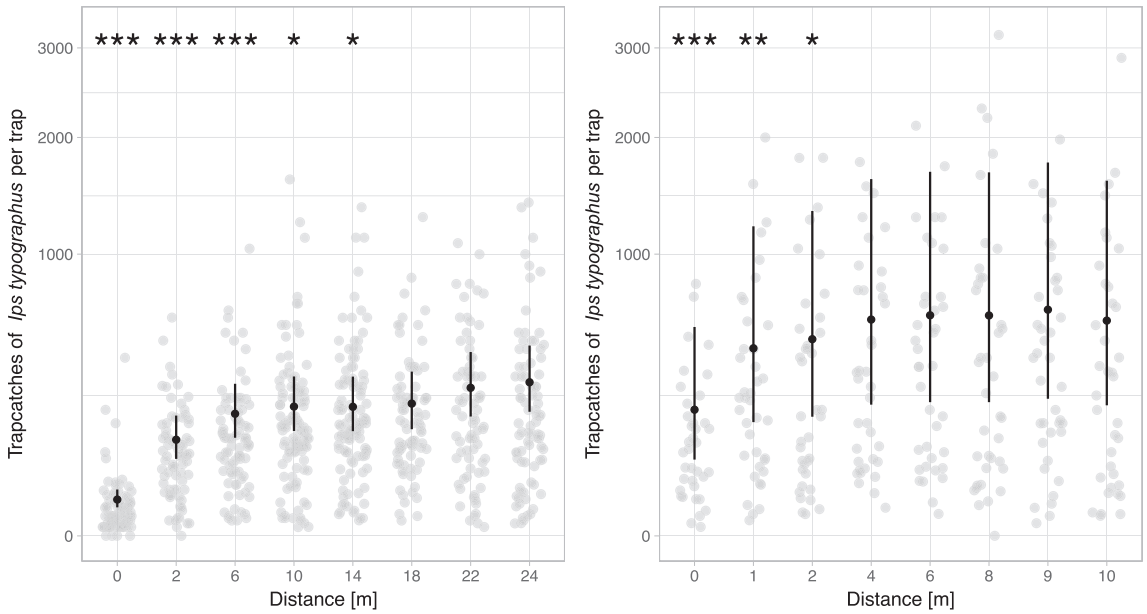
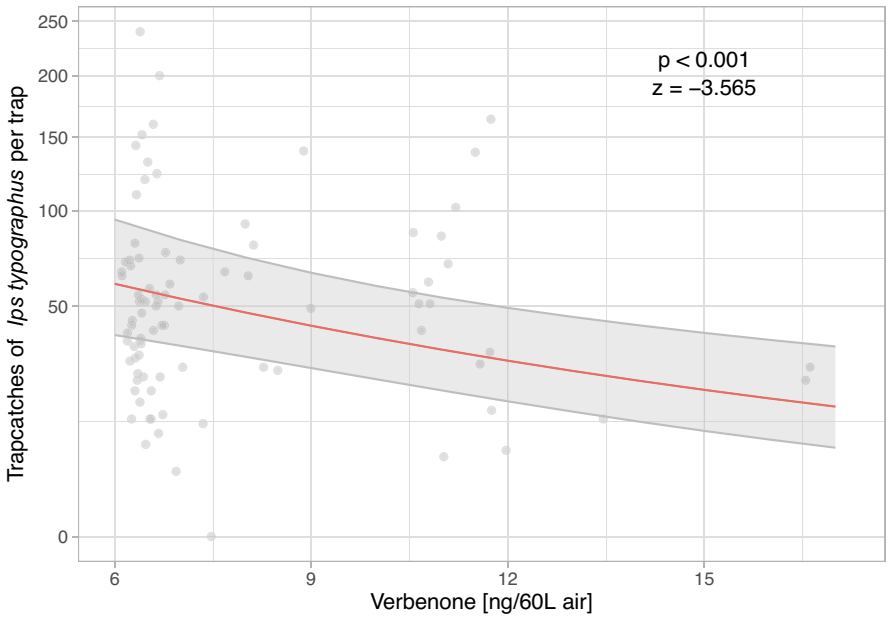


FIGURE 4 Trap catches of *Ips typographus* in the AIR experiments 2021 (left) and 2022 (right). Black dots with 95% confidence intervals show the predicted means given by the models. Grey dots in the background show the observed data with a jitter for improved visibility. Asterisks indicate different levels of significance of a group mean compared to the positive control (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

FIGURE 5 Trap catches of *I. typographus* in relation to the amount of verbenone measured at the trap during the sampling period (5 h). Red line shows the predicted mean with a 95% confidence interval. Grey dots show the observed data.



effectiveness of SPLAT® Verb for reducing *I. typographus* catches in baited traps. Levels of reduction ranged from 34% to 93%, depending on the dose, pre-exposure (age), distance to the dollop and environment (open area vs. closed stand). These results are in line with previous studies evaluating the effects of other formulations of Vn on *I. typographus* (Bakke, 1981; Schlyter et al., 1987, 1988). SPLAT® Verb has the advantage of being more biodegradable than most other formulations of Vn that contain plastics (Mafra-Neto et al., 2014; Seybold et al., 2018).

Our data suggest an optimal dose of 75 g of SPLAT® Verb for reducing the response of *I. typographus* to baited traps. This corresponds to 7.5 g of (–)-Vn, at which a reduction in trap catch of 78%–96% can be expected. Generally, trap catch reductions of >75% are indicative of levels of inhibition sufficient to impart tree protection in other bark beetle-host systems (e.g., Fettig, 2012). No further reductions in *I. typographus* trap catch were observed at a higher dose (100 g; Figure 3). About 1.5 kg 0.41 ha^{-1} is needed to protect stands of *P. contorta* from *D. ponderosae* in western North America (Fettig et al., 2020), although such comparisons are tenuous given our work focused on traps (not trees) and there are large differences between bark beetle species, forest structures and climatic conditions.

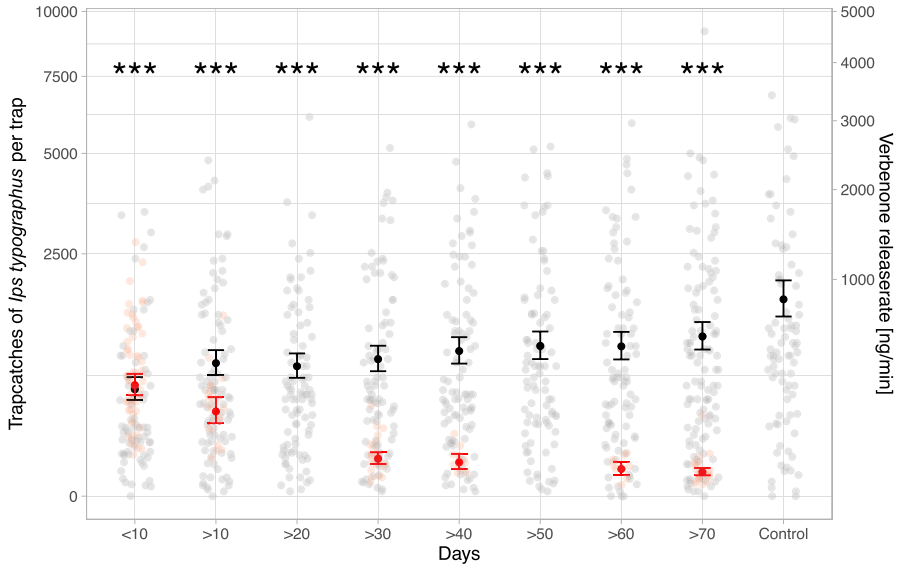


FIGURE 6 Trap catches of *Ips typographus* in the experiment on the duration of inhibitory effect combined with the data on verbenone release rates as measured under laboratory conditions at three temperatures (16, 22 and 30°C). Black dots indicate the estimated mean trap catches with 95%-confidence intervals at exposure times from 0 to 80 days and red dots indicate the estimated mean release rates of verbenone from the SPLAT® matrix at exposure times from 0 to 69 days (missing bins are due to missing measurements in given time period). Dots in the background show the observed data (grey: Trap catches, red: Release rates) with a jitter for improved visibility. Asterisks indicate different levels of significance of a group mean compared to the positive control (***$p < 0.001$) and refer to the catch data only.

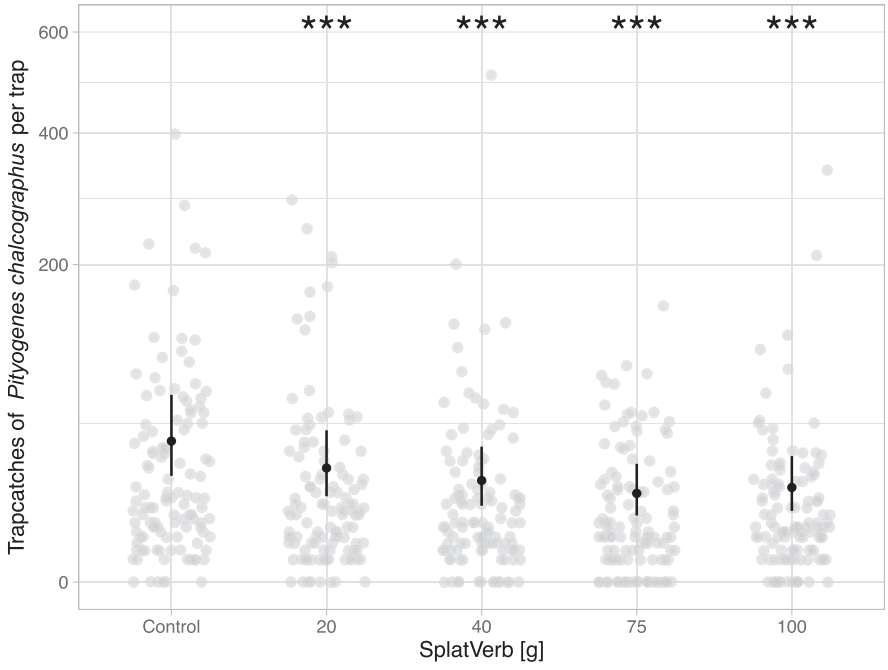


FIGURE 7 Trap catches of *Pityogenes chalcographus* in the dose experiment 2022. Black dots with 95% confidence intervals show the predicted means given by the models. Grey dots in the background show the observed data with a jitter for improved visibility. Asterisks indicate different levels of significance of a group mean compared to the positive control (***$p < 0.001$).

Significant reductions in trap catch occurred up to 14 m from the release of SPLAT® Verb inside the closed forest (AIR 2021) which would correspond to an area of 0.06 ha. However, the magnitude of the effect dropped rapidly within the first 2 m. This decrease was even stronger in an open area (AIR experiment 2022), where no significant reduction in trap catch was obtained at a distance of ≥2 m. A possible explanation is the higher dilution of Vn in the surrounding air as a function of increased volatile drift due to increased air movement in the open field (Cardé, 2021; Murlis et al., 2000). In forests, canopy density affects mean and turbulence flow fields (Russell et al., 2018), with turbulence and wind speeds increasing following reductions in canopy density. Furthermore, trees may act as 'buffers' for volatiles through adhesion and subsequent release over time (Himanen et al., 2010; Wall & Perry, 1983). However, we also observed large differences in the relative abundance of *I. typographus* between sites based on the

total number of *I. typographus* captured in the controls (dose experiments: mean of 247 vs. 1137 $trap^{-1}\,day^{-1}$ inside the stand and in the open, respectively). It is likely that levels of inhibition imparted by SPLAT® Verb decline with increasing abundances of *I. typographus* (Bakke, 1987; Bentz et al., 2005; Progar et al., 2013), thus helping to explain the lower reduction in trap catch observed in the open field. Furthermore, the appearance and thus attractiveness of baited traps may be enhanced in more open areas since bark beetles in general are attracted to vertical, contrast-rich silhouettes (Lehmanski et al., 2023), although data specific to *I. typographus* are lacking. Ultimately, we think the much lower AIR in the open area is primarily explained by less stable accumulations of Vn, which have been implicated as a factor affecting the efficacy of Vn for tree protection in other studies (e.g. Fettig et al., 2009) and are visible in the reduced inhibitory effect at low concentrations of Vn in the ambient air as observed in our volatile data (Figure 5). A potential solution is applying more dollops per unit area, for example, by manually applying higher numbers of smaller dollops or by spraying SPLAT® Verb. Both warrant further evaluation.

Fettig et al. (2015) reported the mean release of Vn from 17.5-g dollops of SPLAT® Verb over 30 days was 28.6 $mg\,day^{-1}$. The dollops were placed in a forest environment in California, USA (mean min = 18.7°C, mean max = 33.0°C). As expected, release was greater during the first 10 days (56 mg/day) than the following 20 days (14.7 mg/day). This trend is also observed with other formulations of Vn. For example, Fettig et al. (2009) reported the mean release of Vn from 7-g pouches at 30°C was 132.5 $mg\,day^{-1}$ on day 3 and 65.3 $mg\,day^{-1}$ on day 30. This is also in line with our data. When comparing volatile release over time (as measured at the headspace of the SPLAT® Verb matrix under laboratory conditions) with *I. typographus* catches during the duration of the inhibitory effect experiment, a comprehensive pattern is apparent (Figure 6). During the first 10–20 days, the highest quantities of Vn are emitted, with subsequent decreases to a relatively stable, lower rate. Concurrent with this, the highest reductions in trap catch of *I. typographus* occurred shortly after the deployment of fresh dollops. However, although reliably detectable releases of Vn were limited after ~60 days, inhibition of *I. typographus* occurred for longer periods of time (Figure 6) demonstrating the remarkable sensitivity of *I. typographus* to Vn (Andersson et al., 2009). Significant reductions (34%) in trap catch were observed for dollops that were aged for 80 days (Figure 6). Hence, the total duration of the inhibitory effect was not reached in our trapping experiments, although the level of inhibition observed at day 80 may not be sufficient to impart protection of windthrows, downed logs, or live trees. However, a delay of colonization of 1 month could represent an important gain of time for forest management operations within the context of integrated bark beetle management that is often limited by logistic constraints (Wermelinger & Seifert, 1998).

A potential concern is that Vn applied to protect windthrows from colonization by *I. typographus*, or woodpiles awaiting transport to mills, may act as a 'switching signal' and divert *I. typographus* towards adjacent live trees (Jakuš et al., 2022). Whilst this relationship has not been adequately studied, adjacent trees may (or may not) be of sufficient health to resist colonization from these beetles (Krokene, 2015). However, beetles would be obliged to infest live trees, increasing the risk of beetle mortality compared to more optimal breeding sites with reduced host resistance (e.g., windthrows or woodpiles) likely reducing local beetle abundances rather than allowing for population growth (Komonen et al., 2011).

In the complex realm of bark beetle communication, Vn is not the only inhibitory olfactory cue (Sun et al., 2006). Nonhost volatiles have received a lot of attention as an additive or alternative to anti-aggregation pheromones for inhibiting bark beetle aggregation (Dickens et al., 1992; Zhang & Schlyter, 2004). Early research focused on green leaf volatiles (i.e., six-carbon alcohols, aldehydes and related esters that function in plant defence). Field-trapping experiments in North America, Europe and Asia have demonstrated that the nonhost signal is generic, redundant and additive (Huber et al., 2021). That is, in most studies removing one behaviorally active compound and replacing it with another behaviorally active compound imparts similar levels of inhibition in the target species. An exception is *trans*-conophthorin, which synergized the inhibitory effect of other nonhost volatiles in *I. typographus* (Zhang & Schlyter, 2004). The main nonhost compounds evaluated for inhibition of *I. typographus* have been thujanol (Jirošová et al., 2022), (*Z*)-3-hexenol (Deganutti et al., 2023), *n*-hexanol (Deganutti et al., 2023; Lindmark et al., 2022) and conophthorin (Jakuš et al., 2022; Lindmark et al., 2022). Preliminary results from trapping experiments evaluating SPLAT® Verb combined with nonhost volatiles (1 hexenol, 3-octanol and *trans*-conophthorin) showed no synergistic inhibitory effect over Vn alone (Löcken et al., unpubl. data). Nevertheless, combining behaviorally active nonhost volatiles with SPLAT® Verb to increase inhibition of *I. typographus* and obtain consistent effects across different environmental conditions warrants further study.

The inhibition effects shown here are based on responses to pure and synthetic *I. typographus* aggregation pheromones in traps. In an applied scenario, the volatile organic compound bouquet emanating from host (and nonhost) trees in forests is far more complex (Kalinova et al., 2014; Lehmanski et al., 2023); it involves many different volatile organic compounds in differing quantities and/or ratios, likely changing the quality and strength of the signal. This suggests caution should be exerted when extending results from trapping experiments to applied scenarios (e.g., for tree protection). This is especially important given the limited effectiveness of other formulations of Vn for protecting *P. abies* windthrows, logs and live trees from *I. typographus* (Jakus et al., 2003; Niemeyer et al., 1995). A next crucial step towards evaluating SPLAT® Verb for the management of *I. typographus* involves testing on host material (Frühbrodt et al., 2024). In North America, there is some evidence that SPLAT® Verb is more effective than the Vn pouch for protecting trees from *D. ponderosae*, which has been attributed to a larger AIR provided by SPLAT® Verb and the flexibility of applying dollops of varying doses at high densities (e.g., Fettig et al., 2015; Progar et al., 2021).

Since we are intervening in a complex multitrophic system, the study of the effects of high doses of Vn on non-target species is

warranted (Hulcr et al., 2006). Our analysis of insect bycatch in the AIR 2021 experiment found an increase in captures of *Rhizophagus* spp., known predators of *I. typographus* larvae, in traps closest to SPLAT® Verb dollops (*see* Supplement 4). Shifts in the ratio of bark beetles and their predators (Aukema et al., 2000) may be advantageous from a management perspective if, in addition to reducing *I. typographus* aggregation, higher predatory pressures result in reductions in brood production over time (Wegensteiner et al., 2015; Wermelinger, 2004).

Pityogenes chalcographus was also attracted to the aggregation pheromone used in our experiments and likewise was inhibited by Vn (see Figure 7; Supplement 3). These species share the same habitat and hence compete for resources (Schebeck et al., 2023). Whilst the smaller *P. chalcographus* typically colonizes the upper bole of *P. abies* and the larger *I. typographus* typically colonizes the lower bole, this differentiation is not strict and overlaps occur (Göthlin et al., 2000; Grünwald, 1986, pers. obs.). To our knowledge, the inhibitory effect of Vn on *P. chalcographus* has only been reported twice (Byers, 1993; Byers et al., 1998). From a management perspective, this is encouraging as *P. chalcographus* is also an important pest on *P. abies* in Europe (Gilbert et al., 2005; Gregoiré & Evans, 2004). Contrary to *I. typographus*, *P. chalcographus* also imposes a threat to young trees and has a larger host range (Berthelot et al., 2021). The effect of SPLAT® Verb on the response of *P. chalcographus* to traps baited with its own aggregation pheromone remains to be examined.

Overall, SPLAT® Verb strongly inhibited the response of *I. typographus* to baited traps up to 14 m from the point of release and for >80 days at a dose of 75 g per dollop. Whilst SPLAT® Verb is not a 'silver bullet', with further study SPLAT® Verb may add a valuable, environmentally friendly tool for the integrated management of *I. typographus* in *P. abies* forests of Europe.

AUTHOR CONTRIBUTIONS

Helge Löcken: Conceptualization; methodology; data curation; formal analysis; visualization; writing – original draft. **Tobias Frühbrodt:** Conceptualization; methodology; writing – review and editing. **Baoguo Du:** Methodology; data curation; formal analysis; writing – review and editing. **Christopher J. Fettig:** Methodology; writing – review and editing. **Peter H. W. Biedermann:** Writing – review and editing; supervision. **Jürgen Kreuzwieser:** Conceptualization; methodology; writing – review and editing; project administration. **Tim Burzlaff:** Conceptualization; methodology; funding acquisition; writing – review and editing; project administration; supervision. **Horst Delb:** Conceptualization; methodology; writing – review and editing; project administration; funding acquisition; supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no relevant financial or non-financial interests to disclose that might be perceived as affecting the objectivity of this study.

DATA AVAILABILITY STATEMENT

The data included in this study is available at https://doi.org/10.6084/m9.figshare.25472020.v1 (Löcken, 2024).

ORCID

Helge Löcken https://orcid.org/0009-0002-9862-3283

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