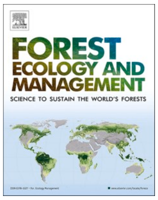
Forest Ecology and Management 548 (2023) 121411

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco



Predator odor can reduce acorn removal by granivorous rodents in mixed oak forest stands

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ARTICLE INFO

Keywords:
Direct seeding
Cost-efficient restoration
Rodents
Repellents
SPLAT® dispenser
Quercus
Predator scent

ABSTRACT

Developing better practices for rodent pest control is of high importance to reduce damage during forest restoration and in crop production. For example, during direct seeding with large and highly attractive seeds such as acorns, most seeds will disappear due to consumption or dispersal if not protected. An unexplored concept in reducing rodent damage is the use of repellents derived from predators. We tested the efficiency of three volatile compounds (2-propylthietane, 2-phenylethylamine and indole) associated with predators as rodent repellent candidates and scored the reduction of acorn (*Quercus petraea*) removal at two field sites in southern Sweden. We further investigated at what distance (5 cm, 10 cm, and 15 cm) from the odor source the odors were efficient in lowering the removal of acorns. Removal was lowest with 2-propylthietane (25–45 % of acorn removal), followed by 2-phenylethylamine (75–95% acorn removal) at 5 cm. Indole failed to decrease acorn removal and did not differ significantly from the control treatments. In the control treatments, almost all acorns (95–100%) were removed from the plots during the 48-hour sessions at both sites, and the acorns were removed faster than in the other treatments. Removal increased with distance from the 2-propylthietane odor dispenser at both field sites. Here, the lowest acorn removal occurred at 5 cm from the odor disperser. Our short time experiment shows for the first time the potential of using 2-propylthietane for short-term protection of acorns from foraging by granivorous rodents in oak woodlands. To help increase the reliability of direct seeding as a method for regenerating and restoring forests, future studies should investigate whether the range and longevity in the field of predator odor formulations can be improved.

1. Introduction

Rodents can cause major economic losses by damaging crops in agricultural lands (John 2014) and in forests (Jacob and Tkadlec 2010). Impacts are likely to accelerate as higher rodent populations are predicted under a warmer climate (Clement et al. 2009; Tersago et al. 2009; Imholt et al. 2015). Sustainable management practices for the control of rodent damage are required to reduce crop damage and to ensure successful forest regeneration (Dey et al. 2008; John 2014; Löf et al. 2019).

In forest ecosystems dominated by oaks, granivorous rodents (e.g., in northern Europe yellow-necked mouse *Apodemus flavicollis* Melchior, the wood mouse *Apodemus sylvaticus* L., and the bank vole *Myodes glareolus* Schreber) are known to play a double role during natural regeneration and in their interactions with oaks (Gómez et al. 2019). The first role is as seed predators (Crawley and Long 1995; Steele et al. 2005) and the second role is as seed dispersers (Pons and Pausas 2007; Gómez et al. 2008; Steele et al. 2011). As scatter hoarders, rodents collect and store acorns in multiple dispersed caches for later consumption (Vander Wall

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https://doi.org/10.1016/j.foreco.2023.121411
Received 14 April 2023; Received in revised form 31 August 2023; Accepted 4 September 2023

1990; Lichti et al. 2017). Many of these acorns can, however, escape predation (Perea et al. 2016), while others may be partly eaten, leaving the embryo intact (Steele et al. 1993; Perea et al. 2011; Yang and Yi 2012). These may germinate and establish if they are hoarded in suitable habitats (Johnson et al. 2019; Gómez et al. 2019). Primary research shows that scatter-hoarding rodents are a key factor in the natural regeneration process of oaks (Jensen and Nielsen 1986), and in years with greater production of acorns higher population sizes of rodents collect and store more acorns that may escape predation (Kellner et al., 2017).

To find acorns or other food on the ground or buried in the soil, granivorous rodents use their sensitive sense of smell (Vander Wall 2003). When comparing artificial regeneration techniques such as direct seeding to planting of nursery-grown seedlings, the former regeneration technique may be a cost-effective alternative where the costs may be only 50% or even lower compared with planting (Bullard et al. 1992). However, foraging behaviors by rodents, to efficiently find acorns and to consume or hoard them, pose major problems during forest restoration using direct seeding of oaks (e.g., Johnson 1981; Birkedal et al. 2009; Birkedal et al. 2010; Leverkus et al. 2013; Villalobos et al. 2020). Moreover, any attempts to mimic natural regeneration of oak by simply sowing high quantities of acorns is often not an economically viable alternative, hence other methods of dealing with granivorous rodents during direct seeding must be developed.

Several techniques for preventing rodent impacts during artificial regeneration using direct seeding have been tested in the last decades. This includes lethal methods such as the use of anticoagulant rodenticides to kill granivores (Jacob and Buckle 2018). However, its application has been discouraged as it also represents a threat to non-target species and therefore has spill-over effects on the environment (Joermann 1998; Gabriel et al. 2018). In forestry and for direct seeding of oaks, several non-lethal acorn protection methods have also been implemented such as coating seeds with deterrent or repellent substances such as mink excrement (Villalobos et al. 2020), seeding during high masting (Dey et al. 2008), using physical protection devices (Reque and Martin 2015; Madsen and Löf 2005; Castro et al. 2015), sowing at different depths (Nilsson et al. 1996) and implementing mechanical site operations like mounding (Birkedal et al 2010). However, these methods are still limited in terms of applicability, efficiency, or economic viability (Löf et al. 2019).

Granivorous rodents rely on their olfactory system for foraging (Vander Wall et al. 2003), identifying plant based toxic or repellent substances (Hansen et al. 2016), and detecting terrestrial predators (Apfelbach et al. 2005; Hegab et al. 2015). Rodents have further evolved several antipredator behaviors triggered by the scent of their predators such as increased avoidance, freezing and vigilance (Kats and Dill 1998; Apfelbach et al. 2005). Such odors are known as kairomones, which are semiochemicals emitted by one species (e.g., predator) whose detection by another species (e.g., prey) induces a response that benefits the latter (e.g., antipredator behavior). Nevertheless, isolating predator odors for use as repellents against rodents have yielded somewhat inconsistent results in lab and field studies (Apfelbach et al. 2005; Parsons et al. 2018).

Studies from Crump (1980), Brinck et al. (1983), and Crump and Moors (1985) identified 15 different chemical compounds in the anal sac secretions of mustelid predators. Some compounds were further classified as sulfurous metabolites derived from meat ingestion, which are characteristic volatile components from feces, urine, and anal gland secretions of several mammal predators (Crump and Moors 1985; Nolte et al. 1994). Some of these (e.g., 2-propylthietane; 2,2-dimethylthietane; indole) were tested as synthetic mixtures for rodent repellency in a series of field studies by Thomas Sullivan and colleagues (Sullivan et al. 1988b; Sullivan et al. 1988a; Sullivan et al. 1988c) or in laboratory experiments as single compounds (Brechbühl et al. 2013; Sarrafchi et al. 2013; Pérez-Gómez et al. 2015; Sievert and Laska 2016). In addition, 2-phenylethylamine was identified as a major volatile of urine from several predators such as bobcats, ferrets, weasels, and triggered defensive responses in both mice and rats (Ferrero et al. 2011).

To our knowledge, there is not a clear conclusion whether complex mixtures of compounds or single compounds had better produce aversive responses in rodents (Apfelbach et al. 2015; Sievert and Laska 2016; Jackson et al. 2018, Villalobos et al. 2022). However, several studies indicate that single compounds trigger strong fear responses in rodents as well as more complex blends of compounds (Saraiva et al. 2016; Jackson et al. 2018; Villalobos et al. 2022).

In a previous laboratory study, our results suggested that 2-propylthietane, 2-phenylethylamine and indole triggered reduced food contact and area avoidance, and that they might be good repellent candidates (Villalobos et al. 2022). However, there is currently no information about the ability of these predator odors to protect acorns against rodents in the field. The purpose of this study was to study these potential repellent odors in the field under natural conditions. We therefore evaluated (1) whether any of three predator odors (2-propylthietane, 2-phenylethylamine or indole) released from a slow-release dispenser would reduce acorn removal by granivorous rodents, (2) if the distance to the odor source influence removal and (3) if removal rates change over time. The ultimate goal of our research is to develop direct seeding of oak towards a more efficient regeneration method, but here we study only the removal rates of acorns.

2. Material and methods

2.1. Site description

The experiment was carried out at two forest sites in Scania, the southernmost part of Sweden. Both Alnarp (55°39′8″N, 13°4′33″E, 11 m. a.s.l.) and Skrylle (55°40′53″N, 13°27′40″E, 101 m.a.s.l.) are mixed forests with stand characteristics that differed somewhat from each other (Table 1). To increase the chances for acorn removal and to be able to test the efficiency of our odor compounds, we put the experiment in closed mixed forests with understories of shrubs and small trees where we expected that granivorous rodent populations and rodent activity could be high. Thus, the sites were chosen for the objectives of our study (to study removal of acorns in relation to potential repellent odors) and not as suitable sites for forest direct seeding or natural regeneration as such. The surrounding vegetation consisted of orchards and public gardens in Alnarp, and production stands of Norway spruce (*Picea abies* L. Karst) and silver birch (*Betula pendula* Roth) in Skrylle.

2.2. Experimental design

A randomized block design with four blocks and five treatments in each block was used for each of the two selected sites. In addition, at each site we repeated the experiment in two experimental sessions over 48 h measurement periods each (see section 2.5: measurements). The size of each block was ca. 45×45 m (0.2 ha), and except for two blocks in Alnarp, which were 200 m apart, the distance between blocks was ca. 50 m. Our repellent treatments were set up within circular metal mesh cages with a distance of at least 10 m between them. Each mesh cage was 60 cm in diameter and 30 cm high, and the mesh size was 2.5 cm (Fig. 1). The design of the cages allowed small granivorous rodents (e.g., bank voles and wood mice) to enter the cages but excluded larger mammals

Table 1

Characteristics of the two study sites. Min-max temperatures and cumulative rainfall during three consecutive days for the two replications of the experiment in 2019. Basal area (mean ± SE, n = 4) and species composition for canopy trees and understory seedlings (the three most frequent species listed with respect to their abundance). Climatic data obtained from the Swedish Meteorological and Hydrological Institute weather stations at 9.1 and 8.4 km from Alnarp and Skrylle, respectively.

Site	Sampling date	Temp. (°C)	Precipitation (mm)	Basal area (m^2ha^{-1} ± SE)	Canopy	Understory
Alnarp	29–31 July	17.2–26	1.9	19.3 ± 2.4	*Quercus robur/petraea, Acer platanoides/pseudoplatanus, Betula pendula*	*A. platanoides/pseudoplatanus, Sorbus aucuparia, Corylus avellana*
	3–5 August	13.5–24.4	0.3			
Skrylle	6–8 August	14.2–24.8	1.9	20.4 ± 1.7	*Q. robur, Fagus sylvatica, Picea abies*	*B. pendula, S. aucuparia, F. sylvatica*
	14–16 August	12.6–20.8	2.9			

and birds. There was also a roof of the same material and mesh size on top of the cage. All cages were set up at similar micro-sites under the tree canopy, i.e., at micro-sites free from ground vegetation and near to understory bushes and trees (Table 1).

At the center of each cage a plastic pole holding a cardboard delta house was placed at ca. 5 cm height (Fig. 1). Inside the delta house 1 g of SPLAT® (Specialized Pheromone Lure Application Technology, ISCA Technologies' CA, USA) dollop was positioned. Each dollop of SPLAT® functions as a chemical dispenser, releasing slowly the mixed in active ingredient (see Section 2.4: Chemical compounds and SPLAT® preparation). The five treatments were: 2-propylthietane + SPLAT® (2-PT-S), 2-phenylethylamine + SPLAT® (2-PEA-S), Indole + SPLAT® (I-S), control without active volatile compound + SPLAT® (C-S) and control without SPLAT® dollop and delta cage (C).

2.3. *Acorns and food*

Inside the mesh cages, food was available at three distances from the odor source (Fig. 1). These distances were: 5, 10 and 15 cm arranged in four different directions from the center point. At each position an acorn (3.1 g ± 0.7 g) and three pieces of rodent food (ca. 2 g in total) (Versele-Laga, Deinze, Belgium) were placed on the soil surface next to each other. In total 1008 acorns and 3024 pieces of rodent food were used in the experiment. During direct seeding in practice, acorns are normally buried a few centimeters below soil surface, but here they were put on ground to increase chances for acorn removal.

The sessile oak acorns (*Quercus petraea* (Matt.) Liebl) were collected in 2018 in Norway (DK/18/259123/NO/B1801) and stored at Levinsen Treeseed Ltd., Lynge, Denmark until the experiment started. Prior to the experimental start in July 2019, acorns were submerged in water for 12 h and all floating acorns were discarded (Gribko and Jones 1995). The viability of the remaining acorns was determined by a cut-test and was 75% (subsample 100 acorns).

2.4. *Chemical compounds and SPLAT® preparation*

We used volatile synthetic compounds as predator cues that were previously described as semiochemicals with repellent effects against rodents (Apfelbach et al. 2005, Villalobos et al. 2022). The chemical compounds and their concentrations were: 5% of 2-propylthietane (CAS registry number: 70678-49-8; ≥ 95% purity, Chemspace, Riga, Latvia), 5% of indole (CAS registry number: 120-72-9; ≥ 99% purity, Darmstadt, Germany) both found in anal gland secretion of mustelids, and 5% of 2-phenylethylamine (CAS registry number: 64-04-0; ≥ 99% purity, Sigma-Aldrich, Darmstadt, Germany) as a general carnivore scent. The percentage of concentration of neat compounds was determined according to our previous experiments in the laboratory (Villalobos et al. 2022). As dispensers we used SPLAT®, a biodegradable base matrix formulation of inert materials designed to hold and release semiochemicals, while shielding the active compounds from chemical, biological, and environmental degradation (Mafra-Neto et al. 2014). The amounts of material used for the preparation of the SPLAT® mixture for the various treatments are listed in Table 2. To determine that volatile compounds were still active at a relative constant release rate from the SPLAT®, SPME (Solid-Phase MicroExtraction) was performed at the start and Dynamic Headspace Analyses were performed at the end of the first experimental session and from both field sites (4 samples per treatment and per site) (see Supplementary material: *Volatile collection and chemical analysis methods,* Table S1).

2.5. *Measurements*

Experiments were carried out in 2019 between July 29th and August 5th in Alnarp, and between August 6th and August 16th in Skrylle. Each trial was replicated in two sessions per site (Table 1). We visually determined for each treatment whether acorns and rodent food had been

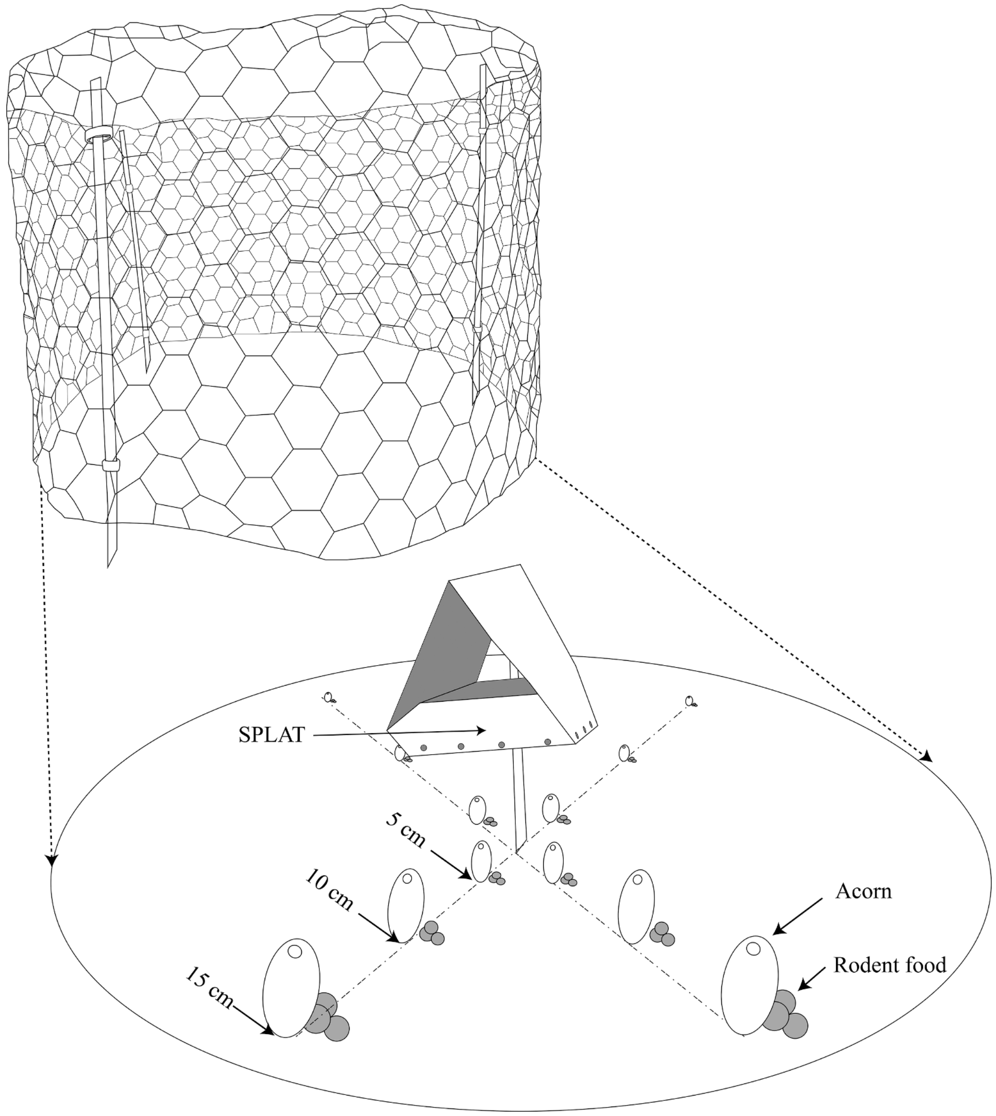

Fig. 1. Design of a treatment plot covered with a wire-mesh cage, and showing SPLAT®, acorns and rodent food arrangement including distances (cm) to the center of the plot. There was also a roof of the same material and mesh size on top of the cage (not shown in the figure). The treated dollop of SPLAT®, the odor mix dispenser, was placed at the center of the treatment five cm aboveground in laminated cardboard house design, derived from moth pheromone traps, to protect from sun exposure and rains.

removed at the start (4 pm), and after 4, 8, 12, 16, 24, 28, 32, 36, 40 and 48 h. For the second experimental session at each site, we randomly changed the treatments within the blocks, and new acorns and rodent food were placed in the treatment plots. Thereafter, data collection was carried out as described above.

Table 2
Amount of ingredients used (g) for the preparation of the five treatments (repellents and two controls) in the experiment. SPLAT® (Specialized Pheromone Lure Application Technology) was used as a chemical dispenser. For a description of the different treatments see text. For release rate measurement from start and midterm of experiments, see Table S1.

Treatment	Compound (concentration %)	Active compound	Oil *	SPLAT® matrix	Total
I-S	Indole (5%)	5	15	80	100
2-PEA-S	2-phenylethilamine (5%)	5	15	80	100
2-PT-S	2-propylthietane (5%)	3**	9	48	60
C-S	–	0	15	85	100
C	–	0	0	0	0

* Rapeseed oil.

** Only 3 g of 2-PT due to stock availability.

2.6. Statistical analysis

During the experiment most of the rodent food was damaged or consumed by isopods (woodlice) and gastropods (slugs) and therefore its consumption was excluded from the analysis. We used mixed effects cox survival models with the R package “coxme” (Therneau 2019) to analyze the effects of treatment and distance on acorn removal. The

Table 3
Fixed factors and their interactions on acorn removal at the two study sites. Interactions were derived *a posteriori* from a mixed-effects Cox survival model using an analysis of deviance (Wald X^2 Type II). For description of treatments (synthetic predator odors) and distance to odor source see text.

Site, fixed factors, and interactions	χ^2	Df	$p\ (> \lvert \chi^2 \rvert)$
Alnarp			
Treatment	163.95	4	<0.01
Distance	6.30	2	0.04
Treatment × distance	21.75	8	<0.01
Skrylle			
Treatment	94.28	4	<0.01
Distance	3.95	2	0.12
Treatment × distance	16.38	8	0.02

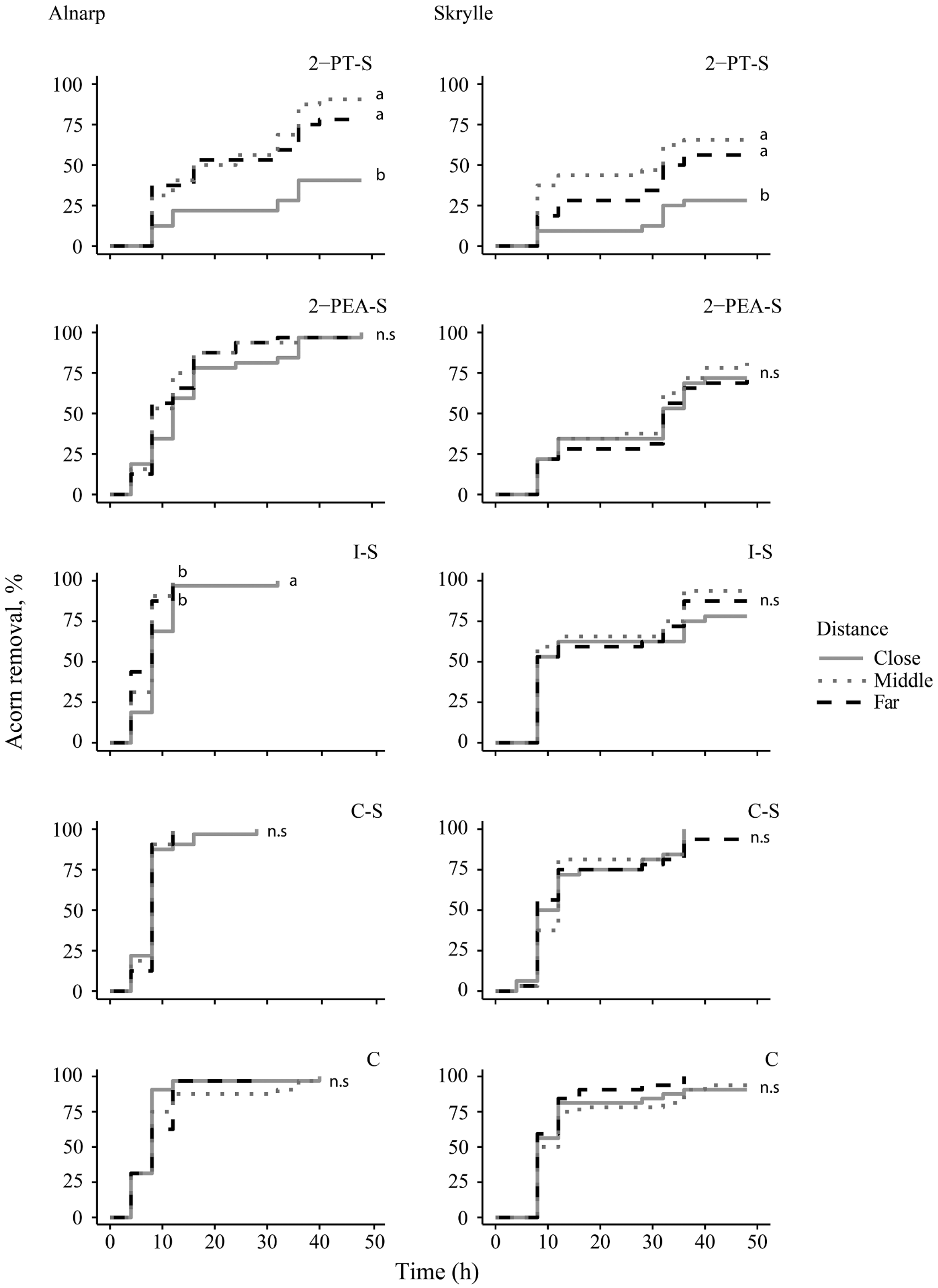

Fig. 2. Acorn removal at the three distances (*close*: 5 cm*, middle*: 10 cm, and *far*: 15 cm) from the odor source in all the treatments for Alnarp (left) and Skrylle (right). Data from the two sessions are pooled in the figure. All curves have censored data. Different letters represent significant differences ($p < 0.05$) based on estimated marginal means analysis.

response ("survival") variable represents acorn removal status (1 = removed, 0 = non-removed) over time. Since not all seeds were removed by the end of the trials remaining seeds were right-censored. We included treatment (5 levels) and distance (3 levels) as fixed factors and their interactions. The random factors were included as nested session/block. Already with the first model, we observed that site (2 levels) as a fixed factor had a significant effect ($\chi^2 = 11.44$, Df = 1, $p < 0.01$) on the outcome. Therefore, we decided to model each site separately. To evaluate the assumptions for proportional hazards (Schoenfeld residuals diagnostics), and the influence of outliers (deviance residuals), we performed a separate cox survival model with all the fixed effects but without the random factors (Velho et al. 2012) with the R package "survival" (Therneau and Lumley 2014).

To determine the main and interactive effects of the fixed factors, an analysis of deviance (Wald X^2 Type II) was performed *ad post*. Pairwise comparisons between factors were done using estimated marginal means with the “emmeans” package (Lenth 2018). For graphical representation, we used Kaplan-Meier curves (R package “survminer” (Kassambara et al. 2017)) on the proportion of acorns removed per treatment and distance against time at the two sites separately. For analytical statistics the alpha level was set at $\alpha = 0.05$ for all tests. All statistical analyses were performed using R version 3.5.0 (R Core Team 2018).

3. Results

Our results showed a significant interaction of treatment × distance at both sites (Table 3), which implies that the effect of treatment was dependent on the distance from the odor source (see below). Indeed, both 2-PT-S and 2-PEA-S reduced seed removal at both sites (Fig. 2, Table S2), with <45–25% and 95–75% removal, respectively at the closest distance to the odor source. For 2-PT-S, the rate and total seed removal increased with distance. Such an effect was not observed with 2-PEA-S. The treatment I-S failed to decrease acorn removal rates at all distances and did not differ significantly from the controls. At the site Skrylle it took longer time for rodents to remove acorns close to the odor source (Fig. 2, Table S2). After 38 h, however, all acorns were removed for this treatment. Except for the 2-PT-S and 2-PEA-S treatments in Skrylle, most acorns were removed irrespective of distance to the odor source by the end of both sessions and at both sites. In addition, the rate of removal was different between treatments, and the rate was highest in the control treatments, followed by I-S, 2-PEA-S, and 2-PT-S. For all distances to the odor source, there was no difference between the two control treatments on removal rates of acorns (Fig. 2).

4. Discussion

Our field experiment demonstrated that the compound 2-propylthietane (2-PT), found in mustelid anal glands (Crump and Moors 1985) can significantly reduce acorn removal by rodents. This result is in line with our previous laboratory findings where 2-PT increased area avoidance and reduced contacts to rodent food in bank voles (Villalobos et al. 2022). To our knowledge, this is the first time that 2-PT has been tested as a single compound to control the removal of acorns in forests. In laboratory settings, 2-PT-treated rodent food pellets were less consumed by Long Evans rats (*Rattus norvegicus domestica* Berkenhout) (Heale and Vanderwolf 1994), and 2-PT-sprayed seedlings of *Pinus radiata* D. Don were less damaged from common brushtail possums (*Trichosurus vulpecula* Kerr) (Woolhouse and Morgan 1995). Furthermore, in laboratory trials, mice avoided areas treated with 2-PT (Sarrafchi et al. 2013; Sievert and Laska 2016), and heightened levels of the stress hormone corticosterone were measured in laboratory rats at the exposure of this compound (Perrot-Sinal et al. 1999). Only a few experiments have tested the effect of 2-PT as a repellent against rodents in the field. For example, 2-PT combined with 3-propyl-1,2-dithiolane reduced bark and vascular tissue feeding on apple trees by meadow voles (*Microtus pennsylvanicus* Ord) (Sullivan et al. 1988a), and reduced stem damages to Scots pine (*Pinus sylvestris* L.) by the red-backed vole (*Myodes gapperi* Vigors) (Sullivan et al. 1991).

We found only a moderate reduction of acorn removal for the compound 2-phenylethylamine (2-PEA). This is in line with previous results obtained in laboratory trials by Wernecke (2016), where only a moderate avoidance was observed in rats. One explanation can be that since 2-PEA is a compound found in predator’s urine, it only triggers strong avoidance responses in rodents when presented in a complete blend of urine odor and not as a single compound (Wernecke 2016). It could also be possible that the concentration of 5 % 2-PEA used into the SPLAT® matrix in our experiment was not appropriate. Wernecke (2016) only observed avoidance in rats at lower concentrations of 2-PEA. Contrary to our results, Ferrero et al. (2011) found a strong area avoidance in mice and rats triggered by 2-PEA.

Indole was the least effective treatment in this study. There are no previous studies on rodents using indole as a single compound. However, in accordance with our results are the findings from Arnould et al. (1998) who found that domestic sheep (*Ovis aries* L.) did not avoid areas in the presence of indole alone, but showed avoidance when indole was part of a synthetic mixture of 14 compounds. In line with this, indole has shown positive effects for suppressing feeding behavior in meadow voles and montane voles (*Microtus montanus* Peale) when applied in a 16:1:4 mixture with 2-propylthietane and 3-propyl-l,2-dithiolane (Sullivan et al. 1988a). Moreover, Swihart and Mattina (1995) found no avoidance of either woodchucks (*Marmota monax* L.) or meadow voles in areas treated with a mixture of nitrogenous compounds, including indole. These results may be explained by the fact that indole is a more general major nitrogenous compound found across many different organisms such as mustelids (Brinck et al. 1983; Crump and Moors 1985), canids (Arnould et al. 1998), bacteria, invertebrates (Tomberlin et al. 2017) and different plants (Bischoff et al. 2015) and alone does not signify the presence of a predator to rodents.

Under natural conditions the probability of detecting odor plumes rapidly decrease with distance from the odor source (Gire et al. 2016). This is both due to shifting winds, which are particularly high in forests (Elkinton et al., 1987), and due to a diminishing concentration over distance due to diffusion. Diffusion likely dilutes the concentration of, for example 2-PT already at a short distance to a level where it is either not repellent or the repellency is not strong enough to hinder seed removal. This could explain the significant interaction between repellent and distance in our results. For example, in our study, the lowest rate of acorn removal for the compound 2-PT was at a close distance (5 cm). Indeed, a previous laboratory experiment with bank voles shows repellents effects of 2-PT at 10 cm from the odor source (Villalobos et al 2022). In contrast, a semi-field study by Sundell et al. (2004) used fecal material from mustelids at two different distances (1 m and 3 m) and did not observe a lower seed removal at the closer distance. However, compared to distances in our experiment (5–15 cm) 1 m seems long. Therefore, our results suggest that dollops of SPLAT® with 2-PT need to be placed in closed proximity (<5cm). It is likely that the price of SPLAT® would be negligible and easily applicable as a paste using a caulking gun (Fettig et al 2020) but the selling price per gram of the active compound 2-PT at the time of this experiment (2019) was around 500 euros. At this price point, the cost to protect 100 acorns will be around 2500 euros if one single dollop is to be placed near each acorn. Therefore, cost-effective synthesis of 2-PT for lower prices is still necessary before this compound can be recommended for direct applications. Alternatively, the compound 2-PT could be put directly on the acorns by attaching the SPLAT dispenser on the acorns, thus reducing the amount of active compound needed. However, germination tests should first be performed to observe if the SPLAT, or the 2-PT itself may have negative effects on germination or seedling performance.

Rodents can use visual landmarks to allocate food (Pyare and Longland 2000; Zhang et al. 2016) or they might experience neophobia to new objects (Tanaś and Pisula 2011). Therefore, the presence of the delta house could have influenced the behavior of rodents. However, both control treatments (with and without delta house) showed a significantly higher removal of acorns and therefore it can be discarded as a visual cue artifact in our study. This result also demonstrates that the SPLAT® itself as a chemical dispenser does not have any deterrent or repellent effect to granivorous rodents. In addition, and according to our experimental design, the acorns that were placed furthest away from the chemical dispenser could potentially have a higher probability of being removed since it would be the first acorns (acorn placed in the outer circle) encountered by rodents. However, the results from our controls (where no distance effect was observed) support our hypothesis of repellency for 2-PT and reject satiation effects on rodents as an explanation. Moreover, these results from our controls also reject any influence from the short-term human presence during data collection.

It may be expected that an increase of the concentration of a chemical compound could increase its repellent effect towards a target species. Indeed Jackson et al. (2018) and Apfelbach et al (2005) suggested that behavioral responses in rodents are more dose-dependent, where intermediate doses could give stronger deterrent or repellent effects to rodents (Apfelbach et al 2005). However, odor saturation due to strong concentration may negatively affect behavioral responses on rodents, causing fatigue (Jackson et al 2018). Therefore, our field study has focused on a single concentration derived from our previous laboratory study on bank voles (Villalobos et al 2022), where no fatigue was observed at 5% concentration. However, to determine a proper dose, a dilution series study should be carried out for each species of granivorous rodent in order to identify their olfactory thresholds (Villalobos et al 2022).

We observed a trend of higher acorn removal in Alnarp compared to Skrylle. A mixture of open areas, orchards, and crop fields surrounds the experimental plots in the forested areas of Alnarp. This might indicate a richer habitat with more rodents compared to Skrylle, which is a more ordinary mixed forest managed for timber production. Although we did not estimate rodent populations in our field sites, our study was conducted the year after a heavy mast. This may suggest a sizable population of granivorous rodents. Moreover, we are confident that all removal of acorns was due to rodents as the mesh size in our cages prevented other animals such as magpies, jays, or wild boars to reach the acorns.

Even though our results on 2-PT show a significant reduction of acorn removal, its direct application for practical direct seeding operations needs further studies. For example, further studies should compare the proposed technique at different years where the rodent or acorn abundances may differ in the field (i.e., higher, or lower removal risks). Our experiments were short-term and each of them lasted only 2 days. This was done to verify any immediate repellent effects of the active chemical compounds, but in practice this repellency needs to be verified over much longer time intervals (i.e., 30–150 days, depending on the sowing season). The dispenser SPLAT® can hold an active ingredient from 2 weeks up to 6 months (Mafra-Neto et al 2014). However, this might vary depending on the weather, form of application, its formulation and the chemical compound used. Furthermore, the protection of acorns can be diminished if habituation effects occur, and thus rodents will no longer be repelled. Therefore, laboratory and field trials should address any habituation of 2-PT in future studies.

Our results suggest that 2-PT has potential as an acorn protection technique to be applied during direct seeding. This technique could present advantages compared to other methods available. For example, where a physical protection device needs to be removed after germination of acorns. There is no need to remove the chemical dispenser SPLAT® due to its biodegradable properties (Mafra-Neto et al. 2014). Moreover, expensive mechanical site preparation to create areas free from ground vegetation and other rodent habitats may not be needed (Löf et al. 2019). However, the mode of application and the synthesis of the organic compound needs to be optimized to ensure economic viability and a long-lasting repellent effect.

5. Conclusion

Our study shows that 2-PT as a single compound can reduce the foraging pressure on acorns during field conditions and the effectiveness of this potential repellent was reduced with distance from the odor source. These are promising initial results for the future development of candidate repellents against rodents in field settings. However, our results need to be addressed with caution, since acorn removal progressed over time and our study was only conducted over relatively short 48-h sessions. It is important to protect seeds until germination, which may take much longer than two days (i.e., 30–150 days). If the application of semiochemicals is to be recommended for operational use, further research is required into optimizing release rates of compounds in field settings with the use of chemical dispensers such as, SPLAT® and into different ways to disperse odors in small droplets to protect areas, instead of single acorns. In addition, we need more studies to determine effective distances to the odor source. Any repellent applied in a similar way as in this study needs to be effective at longer distances. Otherwise, the costs for using them will make them impractical compared to other approaches.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We are grateful to Jeanne Badin, Max Jensen and Ali Quasim for their support during field work; Jan-Eric Englund and Adam Flöhr for useful comments on the statistical analysis and experimental design; and Paul Becher and Wiebke Feindt for useful comments on the manuscript. This project was financially supported by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (2014-00955). FS was further supported by EXTEMIT-K project financed by OP RDE at the Czech University of Life Sciences, Prague (CZ.02.1.01/0.0/0.0/15-003/0000433).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2023.121411.

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