Herbal Repellents against Agricultural Rodent Pest Species

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ABSTRACT: Field rodents such as common voles cause significant pre-harvest damage during population outbreaks in European agriculture, and commensal rodents such as house mice are of concern worldwide. Usually, rodenticides are applied to minimize damage by these species. Rodenticides are not species-specific and may cause environmental problems. Plant secondary metabolites (PSM) could be used as a tool for sustainable rodent control, potentially minimizing damage and environmental risk. We screened volatile PSMs in feeding trials and in enclosure trials to identify if the odor of herbal substances repelled the target species. In feeding trials, the odor of two PSMs considerably reduced food intake in both rodent species. The use of underground chambers in enclosures indicated three repellent odors were effective for house mice, based on visitation rates of these rodents. Common voles visited the chambers equally independent of treatment and hence showed no avoiding behaviour. Further PSMs, combinations, and varying concentrations will be screened to support development of products. Effective repellents could be used to treat commodities to be protected from rodents and to develop an "odor barrier" against common voles to reduce migration from refuge areas to crops. Our preliminary findings suggest species-specific effects of some PSMs (impact on common voles but not on house mice, and vice versa); this may offer an option to repel unwanted species. Our results contribute to the development of non-lethal management tools for rodent pest species that are potentially more target-specific than traps or rodenticides.

KEY WORDS: common vole, house mice, *Microtus arvalis*, *Mus musculus*, odor, plant secondary metabolites, repellents, rodent control

INTRODUCTION

Common voles (*Microtus arvalis*) are found throughout most of Europe and are considered one of the most damaging vertebrate pest species in agriculture (Jacob et al. 2014). Damage includes direct mortality of plants through consumption of leaves and roots or sublethal damage to bark. Crops most affected are cereals, rape, and grasslands (Zejda and Nesvadbová 2000). In addition, their burrowing activity can cause problems including damage to infrastructure. Agricultural damage is of particular concern in Europe during common vole outbreaks causing extreme losses, e.g., 50-73% beech damage/loss (Niemeyer and Haase 2003), or monetary losses to apple trees of ~25 million €/year (Heise and Stubbe 1987).

The house mouse (*Mus musculus*) is considered a pest species in many countries due to damage to various farm and urban infrastructure, e.g., drip irrigation tubing and cables (Stansly and Pitts 1990). They are also implicated as carriers of zoonotic diseases (Meerburg et al. 2009).

Common voles and house mice have high reproductive potential because of frequent production of large litters during the breeding period. Therefore, the rodent population can increase extremely fast, quickly causing damage to agricultural fields and infrastructure. Trapping has proven to be an effective tool in mitigating house mouse damage, although it requires increased of labor as compared to toxic bait application, which is frequently used (Timm 1994). For preventing common vole damage, trapping is an inefficient tool because it is too labor-intensive in an agricultural setting. Rodenticides are also commonly used for rodent management because they are easy to handle, and some compounds are lethal after one exposure. But these chemical substances can pose risk to the environment through negative effects on non-target species (Shore et al. 1999, Hosea 2000).

Odor and palatability of plants are major drivers for foraging in mammalian herbivores. Plant secondary metabolites (PSM) play a key role in this interaction and can act as feeding deterrents through regulating the food intake of herbivores (Dearing et al. 2005), influencing foraging behaviour (Roy and Bergeron 1989) or reproduction (Tran and Hinds 2012) in rodents. Consequently, PSM odors may be suitable rodent repellents, potentially useful in creating a first odor barrier to prevent immigration of rodents from refuge habitats to agricultural fields or storage facilities.

Our first study investigated the effect of six PSM odors or combinations on common voles and house mice under laboratory conditions. The second study was designed to test the effect of four repellents, found to be effective in laboratory cage trials, in semi-natural conditions with both rodent species. The tested rodent pest species occur in different habitats; therefore, we assumed the response to PSM odors might be different between the species. For management purposes, it could be helpful to find repellents that are species-specific and do not affect non-target species. We chose in our study PSM volatiles because of their characteristic smell or chemical properties. Some compounds had already been demonstrated to have repellent effects against rodents (Nolte et al. 1994, Fischer et al. 2013, Clapperton et al. 2015).

MATERIALS AND METHODS
Subjects and Compounds

For the laboratory cage trials, we used metabolites of herbal origin, such as essential oils or individual metabolites of plant material: 15% anthraquinone (natural product formed by fungi and seed plants); 2% black pepper oil (BPO) (obtained from *Piper nigrum*); 4% fennel oil (*Foeniculum vulgare*); and 25% methyl nonyl ketone (MNK) (obtained from *Ruta graveolens*). Additionally, we used two combinations of PSMs: MNK+BPO, and MNK+BPO+ methyl anthranilate (MA, a component of various natural essential oils).

In the enclosure trials, we tested with both species two single PSMs (25% MNK and 15% anthraquinone) and the two combinations (MNK+ BPO and MNK+BPO +MA), solved in the appropriate solvent and mixed with soil. The four treatments were chosen based on the results of the laboratory cage trials (Hansen et al. 2015, Hansen et al. 2016).

Experimental Design – Laboratory Cage Trials

Common voles and house mice, live-trapped in northwestern and central Germany, and their offspring were maintained in individual standard rodent cages with litter and hay at 18-21C° on a 12 h:12 h light:dark cycle. The rodents were provided with commercial food pellets (Altromin 1324; Altromin Spezialfutter GmbH & Co.KG, Lage, Germany) and water *ad libitum* at all times. For the experiments, the animals were transferred to fresh cages equipped with cellulose paper, a clay pot, and a cardboard tube for shelter. We conducted feeding trials with both sexes of each species. We fed eight animals in each trial with a mix of wheat and PSM-treated gypsum granules in feeding racks (12×4.2×3.5 cm) for 24 h for four days. Each day at 1000 hr, uneaten wheat was collected and separated and dried in a drying oven for 12 h before weighing. Then, racks were refilled with a new wheat-granules mixture. The PSMs were dissolved in ethanol, except for anthraquinone (dissolved in chloroform). We compared the treatment group (PSM + solvent) with the corresponding control group (solvent only). We tested the effectiveness of six PSM odors, using a total of 128 voles, including two control groups that received a wheat-granules mixture that was treated with either ethanol or chloroform. For logistical reasons, house mice experiments were run with a lower total number of animals, resulting in overall 14 test-sets (including control groups) using 112 individuals (n = 8 per experiment). BPO and MNK+MA+BPO were tested only with females.

A general linear model (GLM) for repeated measurements was used to determine if the odor of PSMs reduced food intake in treatment groups compared to the control group. We included food intake per body mass as a dependent variable. "Treatment" was used as a within-subject effect, to test the influence of PSMs.

Experimental Design – Enclosure Trials

Enclosure trials were conducted with common voles and house mice in four semi-natural enclosures at the premises of Julius-Kühn Institute in Muenster, Germany.

Each enclosure was of about 35 m^2 base area, sown with a grass mix to mimic perennial grassland. In each enclosure, four plastic boxes (32×22×16 cm) with a light-proof lid were buried in each corner one m away from the walls. The boxes had two openings connecting to the surface with a corrugated pipe (40 cm; Ø 25mm). One opening was for entrance and the other one for ventilation (locked with a sieve). At day zero (1100-1200 hr), we put a plastic container (30×20.5×6 cm) in every box with one feeding tray (10×10×3.5 cm) placed in the middle. We weighed 10 g rolled oats in every feeding tray and filled around untreated (control) or treated (with PSMs) soil. In each enclosure, we used two control and two treatment boxes. On the following days (1-4), feeding trays were refilled and leftovers of the rolled oats were weighed. The position of the containers in the boxes was rotated clockwise every day. All four treatments were tested in all four enclosures for four weeks. Our first trials were run with eight adult common voles (six females, two males) in each enclosure, and then with six adult house mice (three females, three males). There was no difference in body weight of animals among enclosures. For the trials with house mice, we equipped each enclosure with two nest boxes with straw inside. We examined the effect of treatments on food intake using a general linear model (GLM) for repeated measurements. The difference in food intake among treatments was determined using within-subject contrasts (P-values were Bonferroni-corrected).

RESULTS
Laboratory Cage Trials

For common voles, food intake differed significantly between treatment and control (P < 0.001) for all tested PSM odors (Table 1). Voles reduced food consumption during all treatments compared to the control group, except for 15% anthraquinone in males. Treatment effects were strongest with MNK+BPO (77% reduction in food uptake) in females and with 25% MNK (67% reduction) in males. For house mice, we could demonstrate a statistically significant effect (P < 0.001) in treatments with 4% fennel oil, 25% MNK, and the two combinations MNK+BPO and MNK+MA+BPO (Table 2). Mice showed a different behaviour to the presented PSM odors. The animals were repelled strongest by 4% fennel oil in both sexes (90% reduction), but were not affected by 15% anthraquinone, and males did not react to 2% BPO.

Enclosure Trials

None of the four potential repellents proved to be effective in reducing food intake in common voles. The rolled oats provided in the boxes to assess food intake were nearly completely consumed in each trial in all underground boxes. Therefore, food intake data were not further analyzed in common voles. House mice reduced food consumption significantly in all four treatments (P < 0.05). Treatment effects were strongest for MNK (68% reduction) and weakest for MNK+BPO (50% reduction) (Table 3).

Table 1. Results of laboratory cage feeding experiments with common voles. Mean intake ± SE of wheat mixed with gypsum granules between control (solvent) and treatments (PSM + solvent). Statistical results are based on a general linear model comparing food intake (P-values are Bonferroni-corrected).

Common Voles	Female Food Mean Intake [g]	P	Male Food Mean Intake [g]	P
Chloroform (control)	2.41 ± 0.19		2.25 ± 0.24	
Anthraquinone 15%	1.22 ± 0.19	0.000	1.63 ± 0.13	0.124
Ethanol (control)	1.97 ± 0.24		1.87 ± 0.28	
Black Pepper Oil 2%	1.52 ± 0.31	0.006	2.70 ± 0.22	0.004
Fennel Oil 4%	0.64 ± 0.19	0.000	1.08 ± 0.2	0.018
MNK 25%	0.53 ± 0.21	0.001	0.61 ± 0.18	0.000
MNK+BPO	0.44 ± 0.11	0.000	0.92 ± 0.22	0.000
MNK+MA+BPO	0.58 ± 0.12	0.000	0.99 ± 0.19	0.023

BPO = black pepper oil; MNK = methyl nonyl ketone; MA = methyl anthranilate

Table 2. Results of laboratory cage feeding experiments with house mice. Mean intake ± SE of wheat mixed with gypsum granules between control (solvent) and treatments (PSM + solvent). Statistical results are based on a general linear model comparing food intake (P-values are Bonferroni-corrected).

House Mice	Female Food Mean Intake [g]	P	Male Food Mean Intake [g]	P
Chloroform (control)	4.7 ± 0.1		0.61 ± 0.23	
Anthraquinone 15%	3.52 ± 0.22	0.346	2.65 ± 0.21	0.346
Ethanol (control)	2.77 ± 0.33		3.19 ± 0.24	
Black Pepper Oil 2%	1.97 ± 0.27	0.073	not tested	
Fennel Oil 4%	0.21 ± 0.07	0.001	0.24 ± 0.73	0.000
MNK 25%	1.07 ± 0.24	0.000	1.18 ± 0.27	0.000
MNK+BPO	0.84 ± 0.26	0.000	2.54 ± 0.3	0.07
MNK+MA+BPO	1.78 ± 0.29	0.035	not tested	

BPO = black pepper oil; MNK = methyl nonyl ketone; MA = methyl anthranilate

Table 3. Results of enclosure experiments with house mice (n = 24 animals in 4 enclosures). Mean intake ± SE of rolled oats between control (untreated soil) and treatment boxes (soil + PSM + solvent). Statistical results are based on a general linear model (GLM) comparing food intake (P-values are Bonferroni-corrected).

GLM (Repeated Measurements) Within-subject-effects	Mean Food Intake Control Boxes ± SE	Mean Food Intake Treatment Boxes ± SE	P
Anthraquinone	3.71 ± 0.53	2.48 ± 0.41	0.04
MNK	5.91 ± 0.55	4.03 ± 0.44	0.003
MNK+BPO	5.07 ± 0.37	2.53 ± 0.39	0.000
MNK+MA+BPO	4.98 ± 0.46	2.58 ± 0.43	0.000

BPO = black pepper oil; MNK = methyl nonyl ketone; MA = methyl anthranilate

DISCUSSION

Rodent pest species are detrimental in agricultural areas because of the variety and intensity of damage associated with them. Lethal methods, such as traps and rodenticides, have been used for a long time as the "golden standard" method in rodent management. However, with resistance to some compounds (e.g., bromadiolone) and the evidence of residues widespread in non-target species (Geduhn et al. 2014), the search for eco-friendly rodent management methods is increasing. Effective PSM odor repellents would be a great addition to the rodent management toolbox for rodent pest species. They would allow farmers to repel rodent pest species in storage, for protecting produce, or using them as an odor barrier around fields to minimize immigration of pest animals. We could identify three effective PSM repellents against both sexes in both species in our laboratory cage study: 4% fennel oil, 25% MNK, and MNK+BPO. Additionally, there was one repellent combination for common voles: MNK+MA+BPO.

In our enclosure study, we tested four promising PSM repellent odors from laboratory cage trials with both species. None of these had effectively repelled voles from food intake in the underground chambers; hence, we cannot recommend using these PSMs for further (field) trials – at least not in the concentration/combination tested. Mice decreased food intake in all 4 treatments. The use of anthraquinone, MNK, MNK+BPO, and MNK+MA+BPO as potential candidates in repelling mice could result in very promising results. Researchers have shown that rodents respond to semiochemicals of conspecifics' odors in different ways, depending on age, sex, social dominance, or breeding condition (Drickamer 1997). We assumed a different response to volatile PSM repellents for the different species, and we could demonstrate a species-specific and sex-specific response to 15% anthraquinone. In other work with additional PSM odors, we found similar results (Hansen et al. 2015, Hansen et al. 2016). For management purposes, it could be helpful to find repellents that act deterrent to the target rodent pest species only. Consequently, further laboratory cage and enclosure trials will be required to identify additional metabolites for application in rodent management.

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