Crop Protection 78 (2015) 270–276

Contents lists available at ScienceDirect

Crop Protection

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

The smell to repel: The effect of odors on the feeding behavior of female rodents

Sabine C. Hansen [a, b, *], Caroline Stolter [b], Jens Jacob [a]

[a] Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Forests, Vertebrate Research, Germany

[b] University Hamburg, Biocenter Grindel and Zoological Museum, Department of Animal Ecology and Conservation, Germany

ARTICLE INFO

Article history:
Received 25 June 2015
Received in revised form
8 September 2015
Accepted 16 September 2015
Available online 29 September 2015

Keywords:
Rodents
Odor
Agriculture damage
Feeding experiments
Plant secondary metabolites
Repellents

ABSTRACT

Rodents can cause extensive damage in agricultural systems. This results in considerable harvest loss as well as damage to agricultural infrastructure. To prevent this, the use of rodenticides has increased worldwide. Rodenticides not only affect rodent pest species but also harm non-target species such as predators and other small mammals. In this paper we show how the odor of plant secondary metabolites (PSMs) can affect the feeding behavior of two rodent species: the common vole (*Microtus arvalis*, Pallas) and house mouse (*Mus musculus*, L.). Common voles are a major vertebrate pest species in agriculture whereas house mice are commensal pests. Both species are well-known to cause severe damage to diverse agricultural enterprises in Europe. We conducted laboratory feeding experiments initially with females because their fitness depends more on their foraging behavior than it does in males. We tested a range of volatile PSMs on voles initially and those compounds that proved effective were later tested on the house mice. Out of 13 PSMs or combinations of PSMs, nine reduced the amount of food eaten and one (bucco oil) increased feeding by voles. In house mice we identified six deterrent PSMs which reduced the food intake including bucco oil and there were two compounds that had no effect on feeding. Those metabolites that were repellent should be tested in field trials for their efficacy and may be suitable alternatives to rodenticides.

1. Introduction

Herbivorous mammals cause significant damage in agriculture and forestry; e.g., squirrels (Rubino et al., 2012), gophers (Engeman and Witmer, 2000), common voles (Briner et al., 2005) and house mice (Stenseth et al., 2003). In Europe, common voles and house mice can be significant agricultural pest species. House mice live in close proximity to humans and cause damage to stored food and infrastructure (e.g. cables), whereas voles primarily live in grasslands and in diverse crops (Jacob et al., 2014), such as alfalfa and grain crops. Because of severe damage these rodents inflict on human foodstuffs, many attempts have been made to establish integrated pest management plans for both species.

Nowadays, the usage of rodenticides is a common tool in rodent pest management worldwide. Acutely toxic compounds such as zinc phosphide and anticoagulants (bromadiolone, diphacinone, brodifacoum) can cause primary poisoning if consumed by non-target species (Geduhn et al., 2014). Anticoagulant rodenticides can also cause secondary poisoning in non-target predators that consume poisoned prey (e. g. Fournier-Chambrillon et al., 2004; Shore et al., 1999). Negative effects on the environment such as these, as well as genetic resistance of some rodent species to some anticoagulant compounds (e. g. Boyle, 1960) dictate a search for alternatives such as antifeedants and repellents. The use of plant secondary metabolites (PSMs) for rodent management could also reduce the negative effect of rodenticides on non-target species. Essential oils or their individual components might be feasible. Furthermore, potential negative impacts on the environment might be minimized by the application of volatile PSMs via dispensers thus avoiding direct contact of compounds with soils and water bodies.

Plants have developed a range of defense mechanisms against herbivores. PSMs, including alkaloids, cyanogenic glycosides, cardenolides, terpenoids and phenols, are part of the chemical

* Corresponding author. Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Forests, Vertebrate Research, Germany.
E-mail address: sabine.hansen@jki.bund.de (S.C. Hansen).

http://dx.doi.org/10.1016/j.cropro.2015.09.019
0261-2194/

defense arsenal of plants, and are supposed to have negative effects on some herbivores. Effects include pre-ingestive effects such as deterrence by odor (Edlich and Stolter, 2012) to post-ingestive consequences such as toxicity, both of which will reduce feeding (Laitinen et al., 2004; Stolter et al., 2013, 2005). The precise mechanisms by which different secondary metabolites work in mammals is known for only a few compounds. Post-absorptive effects can lead to severe negative consequences for the herbivore, e. g. when cardenolides inhibit Na/K-ATPase. Cyanogenic glycosides affect by inhibition of cytochrome oxidase leading to paralysis of the respiratory center and coumarins may inhibit blood coagulation (Rosenthal and Berenbaum, 1992; Valchev et al., 2008).

However, herbivores have evolved mechanisms to cope with exposure to potentially toxic PSMs. These include behavioral adaptations such as avoiding or regulating the intake of some PSMs. For example, moose avoid odors given off by different terpenoids, which are the main volatile compounds in essential oils (Edlich and Stolter, 2012) and they also select twigs with a low concentration of specific phenolic compounds within an individual plant (Stolter, 2008), while captive marsupials (*Pseudocheirus peregrinus*, Boddaert and *Trichosurus vulpecula*, Kerr) regulate food intake depending on the concentration of formylated phloroglucinol compounds (Stapley et al., 2000). Meadow voles (*Microtus pennsylvanicus*, Ord) do not consume freshly cut branches of conifers; they wait for several days before feeding purportedly to reduce the concentration of phenolics and condensed tannins (Roy and Bergeron, 1990). This demonstrates that herbivores can detect PSMs through odor or through taste. For several animal species it has been demonstrated that odor, is used to detect, select or avoid food that could elicit negative feedback (e. g. Burritt and Provenza, 1991; Provenza et al., 1992; Villalba and Provenza, 2000, 2007).

Olfaction plays a major role in rodent behavior and thus could be utilized for rodent control. The complex olfactory systems of rodents are used not only for foraging but also for marking territory, detecting predation risk, identifying repellent or toxic substances and for reproductive behavior (Howard and Marsh, 1970). Odors of PSMs might function as antifeedants/repellents before they are ingested, hence avoiding damage to crops and infrastructure. Few studies have examined the role of odor of PSMs as repellents on the feeding behavior of rodents (Curtis et al., 2002; Fischer et al., 2013a, b; Heidecke et al., 2005). In this study we used the odor of single PSMs or essential oils some of which are already known as deterrent against rodents, e. g. methyl nonyl ketone (Fischer et al., 2013b). Other compounds and essential oils were chosen by their characteristic aromatic smell: e.g. bergamot oil, bucco oil, grass-tree oil and (R)-(+)-limonene.

We investigated if pre-ingestive effects of 11 mainly volatile PSM compounds (different concentrations and combinations thereof) have an impact on the food intake of female common voles and house mice. Our experiments focused initially on common voles because of the extensive background data on other species of microtine rodents (Lindroth, 1988) and because they are the most important agricultural vertebrate pest species in Europe (Jacob et al., 2014). Both rodent species prefer different habitats; therefore we assumed that the response to odors might be different. Common voles, which live in an agricultural environment, might be more familiar with plant compounds. In contrast, house mice live more closely with humans may not be as familiar with these “natural odors”. We focused on females because the effects of food quality on the reproductive output and hence fitness are more pronounced that in males. In addition, food quality and quantity have a stronger influence on feeding strategies of females (Ostfeld and Canham, 1995).

2. Methods and materials

2.1. Subjects and chemicals

We used the following compounds in our experiments: abietic acid (primary component of resin acid), anthraquinone (natural product formed by fungi and seed plants), bergamot oil (*Citrus bergamia*, Risso & Poit), black pepper oil (gain from *Piper nigrum*, L.), bucco oil (*Agathosma buchulina*, Lina L.), fennel oil (*Foeniculum vulgare*, (L.) Mill.), grass-tree oil (*Xanthorrhoea preissii*, Endl.), (*R*)-(+)-limonene (gain from citrus fruits), methyl anthranilate (component of various natural essential oils), methyl nonyl ketone (gain from *Ruta graveolens*, L.), neem oil (gain from *Azadirachta indica*, A. Juss.), and tannic acid (gain from *Rhus coriaria*, *L.* leaves). We modified concentrations in three cases and used two combinations in subsequent experiments to investigate if the effects are synergistic (Table 1). Metabolites were obtained from Diagonal GmbH & Co. KG, Germany except of bergamot oil, bucco oil, fennel oil, grass-tree oil that were obtained from Ronald Reike Spezialversand, Germany. We conducted all experiments with wild-caught female individuals of common voles and house mice or their F1 offspring. We captured common voles with live traps (Ugglan©) at two locations in Germany (51° 2′ 28.73″N, +10° 51′ 44.88″E and 51° 58′ 8.80″N, +7° 32′ 41.42″E). The house mice were captured with live traps on pig farms around Muenster (North Rhine-Westphalia, Germany).

2.2. Experimental design for feeding experiments

The animals were housed separately in standard laboratory cages (36 × 21 × 15 cm) with litter and hay before and after the experiments. They were fed with commercial food pellets (Altromin 1324; Altromin Spezialfutter GmbH & Co. KG, Lage, Germany) and water *ad libitum* at all times including during the experiments. Animals were held at a 12 h light/dark cycle at 21 °C keeping experimental conditions consistent at all times.

Rodents were acclimatized to the experimental environment for at least five days before feeding experiments commenced. For the experiments we moved the animals to clean standard cages with cellulose paper, a clay pot and a cardboard tube for shelter. Feeding racks (12 × 4.2 × 3.5 cm) were used to offer a mix of wheat and treated gypsum granules (Rhône-Poulenc, USA). Cage experiments were conducted to identify responses to different odors of PSMs by measuring the rodents' consumption of food mixed with treated or untreated gypsum granules and the data was expressed relative to

Table 1
Compounds, concentrations and solvent used in feeding experiments with female common voles and house mice.

Compounds	Concentration in solvent [%]	Solvent
Abietic acid	5	Chloroform
Anthraquinone	5	Chloroform
Bergamot oil	5/25	Ethanol
Black Pepper Oil (BPO)	2/12	Ethanol
Buchu oil	3.1	Ethanol
Fennel oil	4/10	Ethanol
Grass-tree oil	3.5	Ethanol
(*R*)-(+)-Limonene	5	Ethanol
Methyl Nonyl Ketone (MNK)	25	Ethanol
Neem oil	20	Ethanol
Tannic acid	25	Ethanol
combinations		
MNK + BPO		Ethanol
MNK + MA[a] + BPO		Ethanol

[a] MA = Methyl Anthranilate.