Die Bedeutung von sekundären Pflanzeninhaltsstoffen als Abwehrstoffe gegen Nagetiere

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Für meinen Mann

INHALTSVERZEICHNIS

Kapitel 1

Allgemeine Einleitung

Im Jahr 2015 litten laut der Welternährungsorganisation (FAO) über 795 Millionen Menschen weltweit an Unterernährung[1]. Die Ernährung der stetig wachsenden Weltbevölkerung im 21. Jahrhundert erfordert daher eine zunehmende Steigerung der landwirtschaftlichen Produktion. 2014 wurden 2,5 Milliarden Tonnen Getreide weltweit geerntet, jedoch nur 43% des Getreides dienten als Lebensmittel. Der Rest wurde zu Tierfutter, Treibstoffen und Industrierohstoffen verarbeitet. Unser Ernährungssystem ist eine der wichtigsten Ursachen für den Klimawandel, das Artensterben, Umweltverschmutzung, Wasserknappheit und eine Vielzahl an Krankheiten. Heute wird etwa ein Drittel der Landfläche der Erde landwirtschaftlich genutzt[2]. Derzeit werden 11% (1,5 Mrd. ha) der Erdoberfläche für die Getreideproduktion (Ackerland und Dauerkulturland) verwendet. Daten des Umweltbundesamt[3] aus 2014 zeigen, dass in Deutschland knapp über die Hälfte (51,4%) der Gesamtfläche landwirtschaftlich genutzt wird. Die zweitgrößte Flächennutzung bilden die Wälder mit 30,6%; Siedlungs- und Verkehrsflächen stehen mit 13,7% an dritter Stelle. Die Entwicklung der Landwirtschaft hin zu immer größeren Betrieben setzt sich weiter fort und damit auch die Zunahme der Flächennutzung. Diese Flächennutzung durch den Menschen – landwirtschaftlich oder für Siedlungs- und Verkehrsflächen – ist meist mit erheblichen Umweltbelastungen verbunden. Ein Aspekt der Umweltbeeinträchtigung und -zerstörung ist die damit verbundene Habitatfragmentierung und führt damit auch zwangsweise zu Habitatverlusten für oftmals gefährdete oder bedrohte Tier- und Pflanzenarten (Andren, 1994; Haddad et al., 2015). Es wird angenommen, dass die Umweltveränderungen bzw. der Landschaftswandel zu einer Veränderung der Populationsstruktur, Artenrückgang, Populationszusammenbruch oder Massenvermehrungen von Tier- und Pflanzenarten führen können.

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[1] http.//www.fao.org/docrep/005/y4252e/y4252e06.htm

[2] http://data. worldbank.org/indicator/AG.LND.AGRI.ZS

[3] http:// http://www.umweltbundes-amt.de/daten/ flaechen-nutzung/struktur-der-flaechennutzung

Wildtierarten sind integrale Bestandteile der Äcker und landwirtschaftlichen Flächen weltweit. Pflanzenfraßschäden verursacht durch Säugetiere können zu einer verminderten Ernteproduktion führen, gemeinsam mit den Schäden von Insekten und Vögeln oder den Auswirkungen von Krankheiten oder anderen klimatischen Faktoren. Sowohl auf landwirtschaftlichen Flächen mit Kulturpflanzen als auch in landwirtschaftlich genutzten Gebäuden z. B. mit Futtermitteln können Nagetiere erhebliche Schäden verursachen. Um eine möglichst umweltfreundliche Bekämpfung gegen Schadnager zu finden, habe ich den Einfluss des Geruches von sekundären Pflanzeninhaltsstoffen (plant secondary metabolites = PSM (einschließlich ätherischer Öle)) mit zwei unterschiedlichen Nagerarten getestet. Dabei lag der Fokus auf der Identifizierung von abschreckenden pflanzlichen Gerüchen (Repellentien) auf die Nahrungsaufnahme der Nagetiere. Ein weiteres Ziel dieser Arbeit war, festzustellen, ob die effektiv wirkende Repellentien aus den Laborkäfigversuchen auch unter halbnatürlichen Feldbedingungen die Nager von attraktiven Futter abschrecken. Der ganzheitliche Ansatz dieser Arbeit hat es ermöglicht, die bekannten Komponenten der Tier-Pflanze-Interaktion zur Nahrungswahl bei Nagern zu nutzen, um Repellentien für die Anwendung im Schadnagermanagement zu finden und diese auf landwirtschaftlichen Flächen und im Vorratsschutz anzuwenden.

Nager als Schädlinge in der Landwirtschaft

Es gibt bereits aus dem 17. Jahrhundert Belege von Hungersnöten durch Fraßschäden (Aelurius, 1625), die durch die Massenvermehrung von Nagetieren entstanden sind. Heutzutage sind die Ernteverluste eher finanzieller Natur und die Menschen müssen beim Auftreten von Massenvermehrung bei Nagetieren nicht mehr den Hungertod fürchten (Jacob and Tkadlec, 2010). In Forstkulturen und Naturverjüngungen verursachen ausschließlich Wühlmausarten wie Erdmaus (*Microtus agrestis*), Feldmaus (*Microtus arvalis*), Rötelmaus (*Myodes glareolus*) und Schermaus (*Arvicola terrestris*) weitreichende Schäden. Auf Agrar- und Grünlandflächen sind hauptsächlich Scher- und Feldmaus für immense Ernteverluste verantwortlich. Im Jahr 2007 wurden 8,5% der deutschen Weizenernte und 11% anderer Getreideernten während einer Massenvermehrung der Feldmaus in der betroffenen Region vernichtet. Zusammen mit anderen Ackerfrüchten kam es alleine im Jahr 2007 zu einem Verlust von ungefähr 700 Million Euro (Barten, 2009).

Im Vorratsschutz sind es kommensale Nager wie Hausmaus (*Mus musculus*), Hausratte (*Rattus rattus*) und Wanderratte (*Rattus norvegicus*), die Schäden an Futtermitteln, aber auch Gebäudematerialien verursachen. Die Hausratte verunreinigt zudem gelagerte Lebensmittel durch ihren Kot und Urin (Braun and Dieterlen, 2005). Sie verursachen auch in Dämmmaterialien, Isolierungen und weiteren Gebäudeteilen Nageschäden (Pelz, 2006). Die Wanderratte kann durch ihre Nage- und Wühltätigkeiten an Mauern, Wänden, Fundamenten und Kabelleitungen Schäden anrichten. In manchen Gebieten in Deutschland sind mehr als 90% der landwirtschaftlichen Höfe mit Ratten befallen (Endepols et al., 2003) und es konnten Populationen mit mehr als 15.000 Ratten auf Schweinefarmen gezählt werden (Endepols et al., 1989). Die Hausmaus, sowie die Haus- und Wanderratte sind darüber hinaus Träger einer Vielzahl von Viren und Bakterien wie beispielsweise Hantaviren und Leptospiren (Meerburg et al., 2009). Die Krankheitserreger werden über den Kot und Urin oder anhaftend an den Extremitäten weitergegeben (Pelz, 2006).

Bei den Kleinsäugern zählt die Familie der Langschwanz-Mäuse (Muridae) und die Familie der Wühler (Cricetidae) zu den artenreichsten Familien der Säugetiere. In dieser Arbeit wurden Versuche mit je einer Art aus jeder Familie durchgeführt:

Die Feldmaus

Die Feldmaus verbreitete sich mit der Landwirtschaft in Europa und besiedelt nahezu alle geeigneten Lebensräume; bevorzugt lebt sie aber im Gras- und Kulturland. Die Tiere ernähren sich hauptsächlich herbivor und bewegen sich in einem zweistündigen Rhythmus aus ihren unterirdischen Gängen zur Nahrungssuche an die Erdoberfläche (Lehmann, 1976). Man unterscheidet drei Fraßschäden der Feldmaus: abfressen (junger Saaten und Pflanzen), zerstören der Grasnarbe (durch Fraß und Wühlen) und Fraß am Wurzelhals von Obstbäumen. Die Feldmaus ist besonders im Ackerbau eine bekannte Schadnagerart in Europa. In manchen Gebieten kommt es alle 3-4 Jahre zu einem Populationsanstieg und einer sogenannten Massenvermehrung und damit zu massiven Ernteschäden (Jacob et al. 2014). Diese hohen Populationsdichten kann die Feldmaus einerseits durch ihre hohe Reproduktionsfähigkeit erreichen und andererseits durch optimale Umweltbedingungen und Nahrungsverfügbarkeit. Es kann in einem Massenvermehrungsjahr zu einer Populationsdichte von mehr als 1.000 Individuen pro Hektar kommen (Bryja et al., 2005).

Die Hausmaus

Die Hausmaus hat sich durch ihre enorme Anpassungsfähigkeit, in der ganzen Welt verbreitet. Die Tiere leben in Deutschland bevorzugt in unmittelbarer Nähe des Menschen, wo sie sich gerne in der Nähe von Vorratsspeichern für Getreide und Getreideerzeugnisse aufhalten. Hausmäuse sind omnivore Nagetiere und bewegen sich vor allem nachts, können aber auch tagsüber aktiv werden. Die Tiere können sich über das ganze Jahr fortpflanzen. Die verursachten Schäden im Vorratsschutz durch die Hausmaus sind neben Fraßschäden an Futtermitteln des Menschen oder dessen Tiere auch Fraßschäden an Gebäudematerialien (wie Isolierung, Stromkabel, Bewässerungsschläuche). Zudem verunreinigen sie vor allem durch ihren Kot die Lebens- und Futtermittel und führen so zu Gesundheitsrisiken. Zu den häufigsten Krankheiten, die durch Hausmäuse auf diese Weise übertragen werden können, zählt die Salmonellose (Cameron, 1949).

Geruchssinn bei Nagetieren

Das komplexe Geruchssystem von Nagetieren spielt u. a. eine bedeutende Rolle bei der Futtersuche, der Kennzeichnung des Territoriums, der Erfassung von möglichen Gefahren durch Räuber, der Identifikation von abschreckenden oder giftigen Stoffen und dem Fortpflanzungsverhalten (Howard and Marsh, 1970). Ratten benutzen z. B. ihren Geruchssinn um zwischen einer vitaminarmen und einer vitaminreichen Nahrung auszuwählen (Stoddart, 1980). Es gibt zwei Möglichkeiten, durch die Säugetiere mit Gerüchen beeinflusst werden können. Der erste, direkte Weg ist, dass durch den Geruch eine unmittelbare Verhaltensänderung (wie z. B. Vermeiden) ausgelöst wird. Zweitens, wird auf indirektem Weg eine Beeinflussung durch endokrine Aktivität auf das zentrale Nervensystem ausgeübt, wobei Gerüche von (flüchtigen) Pheromonen z.B. die Reproduktivität blockieren.

Kleinsäuger wie Mäuse sind Indikatoren intakter Ökosysteme (siehe Abbildung 1), da sie rasch auf verändernde Umweltbedingungen reagieren. Sie ernähren sich u. a. von Pflanzensamen, Nichtkultur-Pflanzen, der Rinde von jungen Setzlingen und teilweise von Insekten (Engelberger, 2013; Niethammer and Krapp, 1978). Sie können aber auch als landwirtschaftliche Schädlinge in verschiedene Kulturen auftreten (Brown et al., 2007) und sie agieren als wichtige Nahrungsressourcen (Beute) für größere Säugetiere und Vögel (Salamolard et al., 2000).

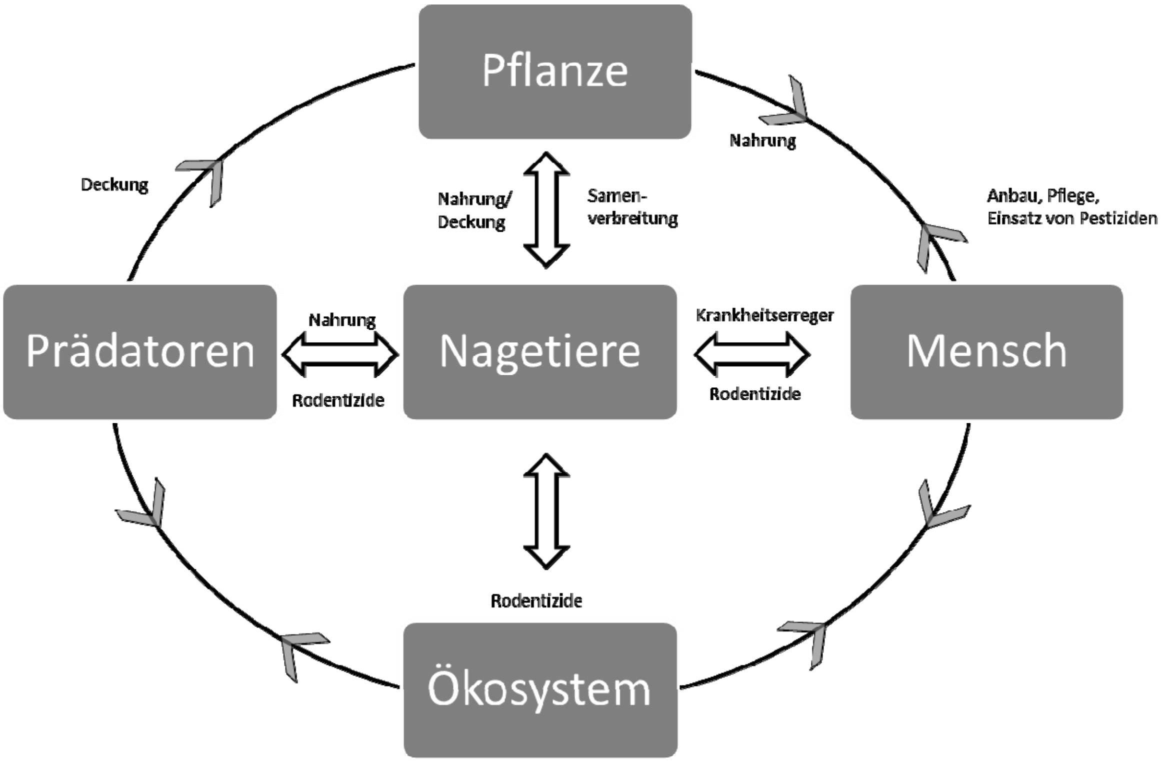

Abbildung 1: Graphische Darstellung der Bedeutung von Mäusen im Ökosystem

Nagerbekämpfung

Es gibt vorbeugende Maßnahmen für die Nagerbekämpfung auf landwirtschaftlichen Flächen, wie z. B. das Ansiedeln von natürlichen Prädatoren (wie z. B. Greifvögel oder Eulen). Heutzutage werden als gezielte Maßnahme vorzugsweise Rodentizide als chemische Nagerbekämpfungsmethode weltweit in der Landwirtschaft eingesetzt. Diese enthalten meistens blutgerinnungshemmenden Wirkstoffe (Antikoagulantien) und werden als Köderpräparate (z. B. Getreideköder) eingesetzt. Wenn Nagetiere diese Köder fressen, führt dass in der Regel nach 3-7 Tagen zum Tod des Tieres durch innerliches Verbluten. Köderpräparate werden in Köderboxen im Vorratsschutz ausgebracht und angewendet, innerhalb und außerhalb von landwirtschaftlich genutzten Gebäuden. Eine weitere Bekämpfungsmethode ist die Begasungsmethode (z. B. durch Aluminiumphosphid) oder der Einsatz von Zinkphosphid. Hierbei handelt es sich um ein Akutgift, das bereits nach einmaliger Aufnahme zum Tod des Tieres führt. Beide Methoden können auf landwirtschaftlichen Flächen zur Bekämpfung von Nagern angewendet werden.

Die Anwendung von chemischen Bekämpfungsmethoden ist allerdings mit Umweltrisiken verbunden. Zum einen können Rückstände von chemischen Bekämpfungsmitteln in Boden und Wasser gelangen (Arias-Estévez et al., 2008). Zum andern besteht die mögliche Vergiftung von Nicht-Zielarten und Nicht-Ziel-Prädatoren durch Antikoagulantien. Köderboxen werden auch z. B. von anderen Kleinsäugern besucht, wie Waldmäuse oder Spitzmäuse, sogenannte Nicht-Zielarten, die das Gift fressen und sterben können (Geduhn et al., 2014). Da Kleinsäuger, wie Nagetiere zu der bevorzugten Beute von Greifvögeln, Katzen und Füchsen gehören, können Antikoagulantien zu einer sekundären Vergiftung bei diesen Nicht-Ziel-Prädatoren führen, wenn sie vergiftete Beutetiere gefressen haben (z. B. Fournier-Chambrillon et al., 2004; Shore et al., 1999). Aber auch die genetische Resistenz einiger Nagetiere gegen einige Antikoagulantien (z. B. Boyle, 1960) stellt seit vielen Jahren eine zunehmende Problematik dar.

Tier- Pflanze-Interaktion

Die Interaktionen zwischen Pflanzen und Herbivoren sind Schlüsselfaktoren in fast allen Ökosystemen, die Wechselwirkungen sind sowohl komplex als auch vielfältig. Mehr als die Hälfte aller Säugetierarten ernähren sich von Pflanzen. Dabei gilt, dass für viele Herbivoren die Qualität des Futters wichtiger ist als die Quantität. Seit der Existenz von Pflanzen und Herbivoren gibt es ein evolutionäres Wettrennen in der Entwicklung der Pflanzen und der Anpassung des Nahrungsverhaltens der Herbivoren. Pflanzen entwickeln sich ständig weiter, um sich vor Fraßfeinden zu schützen. Es haben sich unterschiedliche Abwehrmechanismen bei den Pflanzen entwickelt: die hochregulierte (oder: induzierte) und die permanente (oder: konstitutive) Abwehr.

Bei der induzierten Verteidigung setzten die Abwehrmechanismen der Pflanze verzögert ein und die Pflanze wird zunächst durch den Herbivoren beschädigt, bis sich ein Schutz aufgebaut hat. Der Vorteil dabei ist, dass die Pflanze nur dann ihre Ressourcen beansprucht, wenn sie sich tatsächlich gegen Schädlinge zur Wehr setzen muss (Gatehouse, 2002; Steppuhn and Baldwin, 2007). Die Produktion des Pflanzenhormons Jasmonsäure oder die Freisetzung von flüchtigen Aldehyden, Alkoholen und Estern gehören zu den wichtigen Signalmolekülen bei der induzierten Abwehrreaktion von Pflanzen gegen Fraßfeinde (Mithöfer and Boland, 2012). Pflanzen sind nicht ständig der Beschädigung durch Herbivoren ausgesetzt und müssen daher im Vergleich zur konstitutiven Abwehr relativ wenig Energie und Nährstoffe investieren.

Die konstitutive Abwehr ist bereits vor der Interaktion mit Herbivoren aktiv und wirkt daher vorbeugend. Dazu gehören anatomischen und mechanischen Barrieren, wie z. B. die Blattoberfläche oder Cuticula: sie bilden die erste „Verteidigungslinie“ der Pflanze. Die Cuticula enthält Cellulose, Lignin, Suberin oder Wachs um z. B. Insektenbefall zu verhindern.

Andere mechanische Abwehrmechanismen können Trichome (Haare oder Stacheln), Sekretionskanäle (aus denen Harz oder Latex austritt) und die Blattzähigkeit bzw. Blattfaltung sein. Diese morphologischen Faktoren können sich nach einer Verwundung durch beispielsweise Fraß verändern, z. B. kann die Dichte der Brennhaare auf allen sich neu entwickelnden Blättern zunehmen (Schaller, 2002) und sind somit auch ein Beispiel für eine induzierte Abwehrreaktion. Weitere mechanische Resistenzfaktoren sind verholzte oder verkieselte Zellwände, die als eine physikalische Barriere gegen Blattfraß wirken können. Zu der konstitutiven Verteidigung gehören auch sekundäre Pflanzeninhaltsstoffe, die aus chemischen Komponenten (wie z. B. Alkaloide, Terpenoide, Phenole, etc.) gebildet werden und kontinuierlich von der Pflanze produziert und zusammengesetzt werden.

Sekundäre Pflanzeninhaltsstoffe (PSM)

Pflanzen haben eine Reihe von PSM als Fraßschutz gegen Herbivoren entwickelt. Neben irritierenden Substanzen wie Histamin, Acetylcholin und Ameisensäure gehören beispielsweise Alkaloide, Terpenoide und Phenole zu den PSM. Sie bilden den Hauptteil der chemischen Verteidigung von Pflanzen durch ihre negativen Auswirkungen auf Herbivoren. Im Gegensatz zu den Karnivoren müssen Herbivoren ihrer Nahrung nicht nachjagen oder diese erlegen. Deshalb liegt der Fokus bei Herbivoren auf dem Mechanismus wie die Tiere ihre Nahrungspflanze erkennen, bzw. diese auswählen. Herbivoren treffen zwangsweise durch ihre Ernährungsweise auf PSM und haben gegenüber potentiell toxischen pflanzlichen Metaboliten unterschiedliche Anpassungsmechanismen entwickelt.

PSM können auf verschiedene Weise auf Herbivoren wirken. Es gibt zum einen den Mechanismus vor der Nahrungsaufnahme (prä-ingestiv), d. h. die Abschreckung durch Geruch. Es können aber auch Verhaltensanpassungen sein, wie die Vermeidung oder Regulierung der Nahrungsaufnahme bestimmter PSM. Elche (*Alces alces*) vermeiden z. B. Gerüche von verschiedenen Monoterpenen, die wichtige flüchtige Verbindungen in ätherischen Ölen sind (Edlich and Stolter, 2012). Wiesen-Wühlmäuse (*Microtus pennsylvanicus*) lassen frisch abgenagte Zweige von Nadelbäumen erst mehrere Tage liegen bevor sie die Zweige fressen, um die aufgenommene Konzentration an phenolischen und kondensierten Tanninen zu verringern (Roy and Bergeron, 1989). Es gibt aber auch PSM die nach bzw. während der Nahrungsaufnahme (post-ingestiv) toxisch wirken und so zu einer reduzierten Nahrungsaufnahme führen z. B. durch den Geschmack (Laitinen et al., 2004; Stolter et al., 2013; Stolter et al., 2005). Elche sind in der Lage, Weidenzweige mit einer niedrigen Konzentration bestimmter phenolischer Verbindungen innerhalb einer einzelnen

Pflanze auszuwählen (Stolter, 2008). Eine Untersuchung mit Beuteltieren (*Pseudocheirus peregrinus* und *Trichosurus vulpecula*) zeigt, dass die Tiere ihre Nahrungsaufnahme in Abhängigkeit von der Konzentration des Jensenone, eine Phloroglucinol-Verbindung, regulieren (Stapley et al., 2000). Als post-resorptive (Phase, in der die Nährstoffe gastrointestinal aufgenommen werden) Auswirkungen von PSM können schwere negative Beeinträchtigungen auf den Organismus des Herbivoren auftreten, z. B. wenn Cardenolide die Na/K-ATPase hemmen. Blausäureglykoside (in Farnen, Gymnospermen und mono- und dikotylen Angiospermen) führen zur Lähmung des Atemzentrums. Kumarin, ein natürlich vorkommender PSM, z. B. im Waldmeister (*Galium odoratum*) kann die Blutgerinnung hemmen (Rosenthal and Berenbaum, 1991; Valchev et al., 2008) und zum Tod des Tieres führen. Diese Beispiele zeigen, wie verschieden PSM auf den Stoffwechsel von Herbivoren wirken können.

Eine große Vielfalt von PSM ist toxisch, daher haben sich die meisten Herbivoren koevolutiv durch ihre Physiologie oder ihr Verhalten angepasst. Unter den pflanzenfressenden Säugetieren gibt es Nahrungsspezialisten, die sich nur von einer begrenzten Anzahl von Pflanzenarten oder von nur einer einzigen Wirtspflanze ernähren. Koalas [*Phascolarctos cinereus* (Marsh et al., 2007)] oder Buschratten [*Neotoma stephensi* (Sorensen et al., 2005b)] können höhere Giftkonzentrationen in der Nahrung bewältigen, durch unterschiedliches Fraßverhalten oder ihre anatomischen und physiologischen Möglichkeiten (Marsh et al., 2003). Nahrungsgeneralisten hingegen können sich von verschiedenen Pflanzen ernähren, sind dadurch aber auch einer Vielzahl von PSM ausgesetzt.

Der Stoffwechsel und die Ausscheidung sind physiologische Prozesse im Körper, die (toxische) PSM abpuffern oder eliminieren. Enzyme können beispielsweise Giftstoffe zu Produkten umwandeln und/oder abbauen, die vom Körper schneller ausgeschieden werden (McLean and Duncan, 2006). Die Fähigkeit von Herbivoren, die Absorption von Giftstoffe im Darm zu reduzieren, ist unter den Pharmakologen sehr gut bekannt (Washington et al., 2000). Die Absorption im Darm findet in der Regel durch passive Diffusion statt, entlang eines Konzentrationsgradienten, wobei die Geschwindigkeit von der (Fett-) Löslichkeit des PSM und der Membranpermeabilität abhängt. Einige PSM sind Substrate für aktive Transportermoleküle (z. B. Glykoside). Lipophile PSM werden in den Blutkreislauf aufgenommen und müssen schnell genug abgebaut werden, um eine toxische Anreicherung zu verhindern. Die Spezialisten unter den pflanzenfressenden Säugetieren können durchaus höhere Giftkonzentrationen bewältigen, als die Generalisten, z. B. durch eine reduzierte Absorption von Giften (Dearing et al., 2001; Haley et al., 2007; Sorensen et al., 2005a).

Herbivoren haben u. a. ein vorverdauliches Anpassungsverhalten entwickelt, basierend auf bereits erfahrenem Unwohlsein nach einer Nahrungsaufnahme. Dadurch können sie eine Vergiftung durch PSM vermeiden. Bei Elchen, die in einem Wildpark gehalten wurden, konnte beobachtet werden, dass die Tiere langsamer fraßen, wenn sie dem Geruch einer nicht bevorzugten Nahrungspflanze ausgesetzt waren (Edlich, 2009). Für verschiedene Herbivoren konnte nachgewiesen werden, dass der Geruch einer Pflanze genutzt wird um PSM in der Nahrung zu erkennen, diese zu selektieren und ggf. zu vermeiden, da sie mögliche negative Feedbacks im Körper auslösen (z. B. Burritt and Provenza, 1991; Provenza et al., 2000; Provenza et al., 1992; Villalba and Provenza, 2007). Herbivoren vermeiden negative physiologische Konsequenzen durch PSM durch eine verringerte Nahrungsaufnahme (Hansen et al., 2015; Hansen et al., 2016a; Marsh et al., 2006; Wiggins et al., 2003). Es gibt Untersuchungen, in denen eine selektive Nahrungswahl beobachtet wurde, die Tiere wählten Pflanzen mit niedrigen Giftkonzentration (Frye et al., 2013; Moore and Foley, 2005; Stolter et al., 2013; Stolter et al., 2005). Dieses Verhalten der Meidung von Herbivoren bzw. die Abschreckung durch den Geruch von PSM habe ich mir in meiner Arbeit zu Nutzen gemacht um Repellentien zu finden, die Nagetiere von Kulturpflanzen oder im Vorratsschutz abschrecken.

Einfluss von PSM als Repellentien auf Nagetiere

Der Geruchssinn hat bei Säugern eine fundamentale und charakteristische Rolle in der Verhaltensökologie und in der Nahrungsökologie. Es dient den Nagetieren der Identifikation von abschreckenden oder giftigen Stoffen, aber auch zur Kennzeichnung und Erkennung eines Territoriums, der Erfassung von möglichen Gefahren durch Räuber und dem Fortpflanzungsverhalten (Howard and Marsh, 1970). Es gibt zahlreiche Studien in denen nachgewiesen wird, dass Nagetiere ihren Geruchssinn für die Nahrungswahl gezielt einsetzen (siehe auch Abschnitt **Geruchssinn bei Nagetieren**)

Die Anwendung von flüchtigen pflanzlichen Verbindungen (wie PSM) als Repellentien kann eine gute Alternative zu den chemischen Rodentiziden sein, da sie u. a. durch den Geruch von den Tieren wahrgenommen werden und nicht durch die Nahrung in den Blutkreislauf des Tieres gelangen. Hinsichtlich der Zunahme negativer Auswirkungen durch Rodentizide auf die Umwelt könnten PSM eine umweltfreundlichere Alternative zu den chemischen Bekämpfungsmitteln bieten. Die Anwendung von flüchtigen PSM Gerüchen im

Nagermanagement könnte zu einer Verringerung der negativen Auswirkungen von Rodentiziden auf Nicht-Zielarten und damit auch Nicht-Ziel-Prädatoren führen. Dies würde insbesondere dann gelten, wenn es möglich wäre artspezifische Repellentien zu finden, die gezielt bei der zu bekämpfenden Schadnagerart wirken und andere Arten unberührt bzw. Prädatoren unbeeinflusst lässt.

Ziele dieser Untersuchung

Die erste Fragestellung dieser Arbeit war den Einfluss des Geruches von unterschiedlichen pflanzlichen Verbindungen (wie PSM) bei zwei unterschiedlichen Nagerarten zu untersuchen, um wirksame Repellentien zu finden, die ggf. das Potential zur kommerziellen Anwendung im Nagermanagement haben. Pflanzliche Gerüche beeinflussen Herbivoren bei der Nahrungssuche, -aufnahme, der allgemeinen Aktivität und der Fortpflanzung; dies gilt vor allem bei weiblichen Tieren (Apfelbach et al 2005; Tran und Hinds 2012). Deshalb habe ich zunächst den Einfluss von pflanzlichen Gerüchen auf das Nahrungsverhalten von weiblichen Nagern untersucht. Der Fokus lag bei zwei weit verbreiteten Nagerarten: Feldmäuse (Cricetidae) und Hausmäuse (Muridae) (Kapitel 2). Allerdings spielen Gerüche von pflanzlichen Verbindungen auch bei männlichen Tieren eine wichtige Rolle. Besonders juvenile Männchen, die sich auf der Suche nach neuen Territorien und Ressourcen begeben und dabei von Refugien in landwirtschaftliche Felder einwandern, sind von Bedeutung (delBarco-Trillo et al. 2011).

Als zweite Fragestellung untersuchte ich, ob es einen Einfluss von pflanzlichen Gerüchen auf das Nahrungsverhalten derselben Nagerarten, aber bei männlichen Tieren, gibt. Ich erwartete unterschiedliche Reaktionen auf die pflanzlichen Gerüche zwischen den Geschlechtern, aufgrund der unterschiedlichen Bedürfnisse zwischen Männchen und Weibchen (Kapitel 3).

In der dritte Fragestellunggalt es, artspezifische Unterschiede im Nahrungsverhalten auf pflanzliche Gerüche zu untersuchen. Die untersuchten Nagetierarten leben in verschiedenen Lebensräumen und ernähren sich entsprechend unterschiedlich. Daher erwarte ich, dass die Reaktion auf pflanzliche Gerüche sich zwischen den Arten unterscheiden könnte.

In der vierten Fragestellung untersuchte ich ob effektive Repellentien aus den Laborkäfigversuchen auch unter halb-natürlichen Bedingungen gleiche Effekte auf die beiden Nagetierarten zeigen. Dafür analysierte ich das Besuchsverhalten auf pflanzliche Gerüche beider Nagerarten in unterirdischen Boxen mit attraktivem Futter in Außengehegen. Da die Repellentien aus den Laborversuchen effektiv auf das Nahrungsverhalten bei beiden Nagerarten und in beiden Geschlechtern wirkten, erwartete ich einen ähnlichen Effekt im

Besuchsverhalten (Kapitel 4). Zur der letzten und fünften Fragestellung wird die bestehende Literatur zu PSM als Repellentien gegen Nagetiere evaluiert und ist in einer eine systematische Literaturübersicht (‚systematic Review‘) zusammengefasst. Die Studien, inklusive meiner eigenen, werden hinsichtlich der Anwendung in einem umweltfreundlichen Nagermanagement diskutiert. Es steht deshalb als letztes Kapitel nach der Gesamtdiskussion (Kapitel 6).

Kapitel 2: “The smell to repel: The effect of odors on the feeding behavior of female rodents“

Da pflanzliche Gerüche (wie PSM) einen Einfluss auf die Nahrungsaufnahme bei Kleinsäugern haben, untersuchte ich verschiedene flüchtige pflanzliche Verbindungen als Repellentien bei weiblichen Feld- und Hausmäusen. Weiterhin testete ich die Auswirkung von erhöhten Konzentrationen von wirksamen pflanzlichen Verbindungen und Kombinationen von Einzelsubstanzen, um die Effektivität zu steigern. Zusätzlich untersuchte ich, ob es aufgrund der unterschiedlichen Lebensweise der beiden Nagetierarten auch eine artspezifische Reaktion auf die pflanzlichen Gerüche zeigt.

Kapitel 3: “Effect of plant secondary metabolites on feeding behaviour of microtine and arvicoline rodent species“

In der Studie untersuchte ich den Einfluss von verschiedene flüchtige pflanzliche Verbindungen auf die Nahrungsaufnahme bei männlichen Feld- und Hausmäusen. Auch hier habe ich durch erhöhte Konzentrationen von wirksamen pflanzlichen Verbindungen und Kombinationen von Einzelsubstanzen getestet, ob die Effektivität der Auswirkung gesteigert werden kann. Weiterhin galt es, art- und geschlechtsspezifische Reaktionen auf die pflanzlichen Gerüche zu vergleichen, basierend auf den unterschiedlichen Bedürfnissen von Geschlecht und Art.

Kapitel 4: “Like or dislike - Response of rodents to the odor of plant secondary metabolites“

Ausgehend von der Hypothese, dass wirksame pflanzliche Repellentien in Laborkäfigversuchen effektiv abschreckend auf die Nahrungsaufnahme bei Kleinsäugern wirken, untersuchte ich in dieser Studie, ob vier flüchtige PSM Verbindungen unter halbnatürlichen Bedingungen bei Feld- und Hausmäusen genauso effektiv wirken. Weiterhin galt es zu untersuchen, ob sich die Nager auch bei attraktivem Futter durch den Geruch von pflanzlichen Verbindungen abschrecken lassen. Zusätzlich untersuchte ich, ob sich unter halbnatürlichen Bedingungen art- und geschlechtsspezifische Reaktionen auf die pflanzlichen Gerüche beobachten lassen.

Kapitel 6: “Plant secondary metabolites as rodent repellents: A systematic review”

In der systematischen Literaturübersicht habe ich Literaturdatenbanken mit bestimmten Suchbegriffen zu PSM als Repellentien gegen Nagetiere durchsucht. Der Fokus lag auf der Auswertung der relevanten Studien nach einer bestimmte PSM Gruppe, die am effektivsten abschreckend auf Nagetiere wirkt. Weiterhin wurden die Studien danach evaluiert, ob es pflanzliche Gerüchen gibt, die artübergreifend oder eher artspezifisch wirken. Als Essenz galt es herauszustellen ob es eine optimale experimentelle Methode für die Untersuchung von PSM Gerüchen auf Nagetiere gibt und ob sich aus der Literatur gemeinsame Eigenschaften der PSM Gerüche ableiten lassen, die bei der Abwehr von Nagetiere effizient sein können und wo in Zukunft weiter geforscht werden sollte.

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

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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.

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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

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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.

body mass. The gypsum granules were smaller than wheat and were treated with one of the metabolites or a combination solved in the appropriate solvent (Table 1). Concerning the solubility of the metabolites we used one of two solvents: ethanol and chloroform (Diagonal GmbH & Co. KG, Germany).

2.3. *Experiments with common voles*

For our experiments we used a mixture of adults and sub-adult female animals (Halle and Lehmann, 1987) with a weight range from 15 to 28 g. We compared treatment groups (PSM + solvent) to the corresponding control group (only solvent used). From 2012 to 2014 we tested the effectiveness of the odor of 11 PSM, three of those were tested in two different concentrations and additionally we tested the effect of two combinations, which resulted in 16 test-sets (Table 1). For each test-set we measured the food consumption of eight females each day for four days. The food intake of the control groups was measured for each solvent separately ($n = 8$ individuals for four days for either ethanol or chloroform). This experimental design resulted in testing 144 individuals ($n = 8$ per treatment plus two control groups). The animals were weighed on the first and last day of the experiment to relate food consumption to body weight. At day 0 (0930–1030 h) we added 10 g wheat mixed with 10 g gypsum granules in the feeding rack. On the following days (1–4), and always at the same time, wheat that was not consumed was separated from the granules and weighed and we refilled the feeding racks with a new wheat-granules mixture. If the wheat-granules mixture was contaminated by urine or feces, we separated them with water and dried the wheat in a drying oven for 12 h before weighing. The position of the feeding rack in the cage was changed daily to avoid habituation.

2.4. *Experiments with house mice*

We used also a mixture of adults and sub-adults female animals (DeLong, 1967) with a weight range from 12 to 18 g. The experiments followed the same protocol as that used for the common vole experiments, except that we used 5 g wheat mixed with 5 g gypsum granules. We also used two control groups (ethanol and chloroform) but could run experiments with a lower number of animals. Therefore, we selected a subset of the metabolites/combinations which had already shown an effect in the experiments with the common voles. Ten test-sets were offered to eight female house mice ($n = 80$ individuals including 16 animals for the two control groups): anthraquinone 5%, bergamot oil 5%, black pepper oil (BPO) 2%, bucco oil 3.1%, fennel oil 4%, MNK 25% and the combinations MNK + BPO and MNK + MA + BPO.

2.5. *Statistical analyses*

We examined the effect of treatments on wheat intake in gram per gram body mass [g^*g^{-1}] using a general linear model (GLM) for repeated measurements to test the effectiveness among the different metabolites/combinations. For both species, two GLM analyses were conducted: a) for compounds dissolved in ethanol and b) for compounds dissolved in chloroform and their corresponding control groups. Before using this statistical approach, the data were tested for violation of sphericity using the Mauchly's-Test. If sphericity condition was not met, Greenhouse-Geisser corrections were used (Field, 2013). We included food intake per body mass as a dependent variable. “Treatment” and “Treatment * days” were used as a within subject effect, to test for the influence of different treatments and differences among different experimental days. The difference in food intake among treatments was determined by using within-subject contrasts (P-values were Bonferroni-corrected). All statistical analyses described above were performed using PASW 18 (SPSS 2010, IBM Cooperation).

3. Results

3.1. *Experiments with common voles*

Firstly, we examined the differences between the food intakes for treatments with the solvent ethanol. There was a statistically significant effect of treatments on food uptake ($P < 0.001$), but no day effect (Table 2). Voles reduced food consumption in treatments with BPO 2%, bergamot oil 5%, fennel oil 4%, MNK 25%; MNK + BPO, MNK + MA + BPO, neem oil 20% compared to the control group (solvent ethanol) and this pattern was, with the exception of BPO 2%, consistent over all four experimental days (Fig. 1). Treatment effects were strongest for neem oil (76% reduction in food intake) and weakest for bergamot oil (23%). In contrast to all the other treatments, where common voles showed a lower food intake, they were significantly attracted by bucco oil (Fig. 1). Treatments with higher concentrations of bergamot oil (25%), BPO (12%) and fennel oil (10%) did not further decrease food consumption. The two experiments with combinations (MNK + BPO and MNK + MA + BPO) demonstrated a deterrent effect, as the food intake was significant different to the control group.

Second, the differences between the food intakes for the treatments dissolved in chloroform and the corresponding treatment groups were examined. There was a statistically significant effect of treatment on food intake ($P < 0.001$), but no day effect (Table 3). Voles reduced food consumption in treatments with anthraquinone 5% (47% reduction in food uptake) and abietic acid 5% (33% reduction in food uptake) relative to the control group (solvent chloroform). There were significantly differences between the two treatments (*GLM*: $df = 1$; $F = 18.495$; $P < 0.001$). However, the deterrent effect of abietic acid changed over time (Fig. 2).

3.2. *Experiments with house mice*

Initially the differences in food intake for treatments with the solvent ethanol were examined. There was a statistically significant effect of treatment on food uptake ($P < 0.001$), but no day effect (Table 4). Mice reduced food consumption in treatments with bergamot oil 5%, bucco oil 3.1%, fennel oil 4%, MNK 25%, MNK + BPO and MNK + MA + BPO relative to the control group with the solvent ethanol (Fig. 3). Treatment effects were strongest for bergamot oil (77% reduction in food uptake) and weakest for MNK + MA + BPO (35% reduction). Anthraquinone showed a stronger effect than abietic acid, therefore we tested it with house mice. There was no significant difference between control group chloroform (0.291 ± 0.01) and anthraquinone 5% [0.271 ± 0.01 (*GLM*: $df = 1$; $F = 0.971$; $P > 0.05$)], and no day effect.

Table 4: Results of the feeding experiments with female house mice. Treatment (PSM + solvent) and control (chloroform) granules mixed with wheat. Statistical results are based on a GLM comparing intake of wheat per body mass (P-values are Bonferroni-corrected). Additionally, means and standard error of wheat intake [g] and animal body mass [g] are stated.

4. Discussion

For female common voles we identified nine deterrent essential oils, single compounds or combinations that reduced food intake. In the treatments with BPO 2%, bergamot oil 5%, fennel oil 4%, MNK 25%; MNK + BPO, MNK + MA + BPO and neem oil 20% this pattern was consistent over all four experimental days. Neem oil was the most effective, followed by MNK and its combinations with MA and

Table 2
Results of the feeding experiments with female common voles. Treatment (PSM + solvent) and control (ethanol) granules mixed with wheat. Statistical results are based on a GLM comparing intake of wheat per body mass (P-values are Bonferroni-corrected). Additionally, means and standard error of wheat intake [g] and animal body mass [g] are stated.

GLM repeated measurements					
within-subject-effects					
	df	F	P		
Treatment	5.24	16.475	0.000		
Treatment *day	15.72	0.627	0.856		
within-subject contrasts		F	P	Mean intake [g]	Mean body mass [g]
EtOH				1.97 ± 0.24	18.73 ± 0.49
BPO 2% vs. EtOH		8.67	0.006	1.52 ± 0.31	20.41 ± 0.34
BPO 12% vs. EtOH		2.30	0.140	1.76 ± 0.31	19.83 ± 0.38
Bergamot oil 5% vs. EtOH		6.72	0.015	1.54 ± 0.25	18.42 ± 0.39
Bergamot 25% vs. EtOH		1.82	0.188	1.34 ± 0.3	15.83 ± 0.13
Bucco oil vs. EtOH		16.12	0.000	2.67 ± 0.31	15.95 ± 0.58
Fennel oil 4% vs. EtOH		23.41	0.000	0.64 ± 0.19	24.83 ± 1.47
Fennel 10% vs. EtOH		0.74	0.390	1.88 ± 0.27	24.36 ± 1.11
Grass tree oil vs. EtOH		0.39	0.539	2.25 ± 0.26	15.23 ± 0.27
Limonene vs. EtOH		0.32	0.577	1.72 ± 0.28	13.92 ± 0.24
MNK vs. EtOH		57.37	0.000	0.53 ± 0.21	21.44 ± 0.77
MNK + BPO vs. EtOH		90.25	0.000	0.44 ± 0.11	22.02 ± 0.9
MNK + MA + BPO vs. EtOH		45.05	0.000	0.58 ± 0.12	22.68 ± 0.77
Neem oil vs. EtOH		58.74	0.000	0.75 ± 0.16	28.71 ± 1.42
Tannic acid vs. EtOH		0.37	0.546	1.59 ± 0.27	16.13 ± 0.39

BPO = Black Pepper Oil; EtOH = Ethanol; MNK = Methyl Nonyl Ketone; MA = Methyl Anthranilate.

BPO. MNK could be the main repellent factor, because BPO 2% alone was not avoided and results from other studies suggest repellent properties of MNK in vertebrates (Fischer et al., 2013b; Witmer et al., 2000). Further, the addition of BPO and MA to MNK did not increase its repellency compared to MNK alone. In experiments with higher concentrations, we expected stronger effects, but surprisingly an increase in concentration did not further increase effectiveness. Only with fennel oil was here a significant difference between the different concentrations. Rodents might react to concentration thresholds and exceeding that threshold is ineffective.

Results with house mice showed a different pattern for the eight essential oils, single compounds or combinations: bergamot oil was the most effective, followed by MNK and MNK + BPO. Surprisingly,

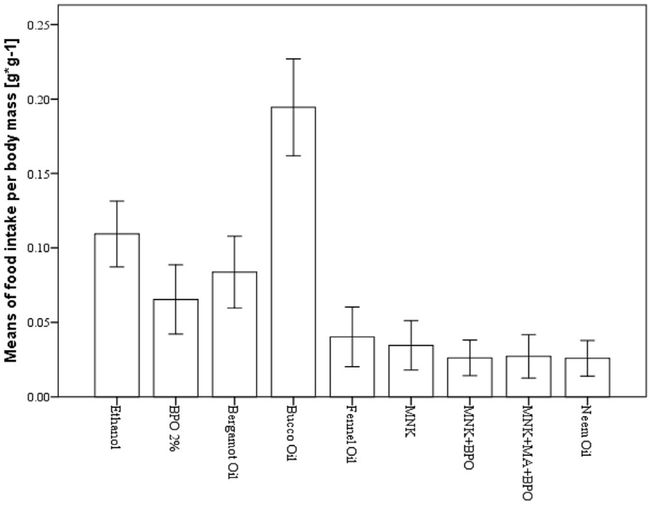

Fig. 1. Means of food intake per body mass and 95% confidence intervals of female common voles (n = 8 animals per treatment) for four days for treatments that reduced or increased (Bucco Oil) significantly food intake compared to the appropriate control group Ethanol. BPO = Black Pepper Oil 2%; MNK = Methyl Nonyl Ketone; MA = Methyl Anthranilate.

Table 3
Results of the feeding experiments with female common voles. Treatment (PSM + solvent) and control (chloroform) granules mixed with wheat. Statistical results are based on a GLM comparing intake of wheat per body mass (P-values are Bonferroni-corrected). Additionally, means and standard error of wheat intake [g] and animal body mass [g] are stated.

GLM (repeated measurements)					
within-subject-effects					
	df	F	P		
Treatment	4	9.124	0.000		
Treatment *day	12	0.785	0.639		
within-subject contrasts		F	P	Mean intake [g]	Mean body mass [g]
Chloroform (CF)				2.41 ± 0.19	16.86 ± 0.31
Abetic acid vs. CF		19.43	0.000	2.22 ± 0.27	25.11 ± 0.44
Anthraquinone vs. CF		23.41	0.000	1.22 ± 0.19	16.52 ± 0.55

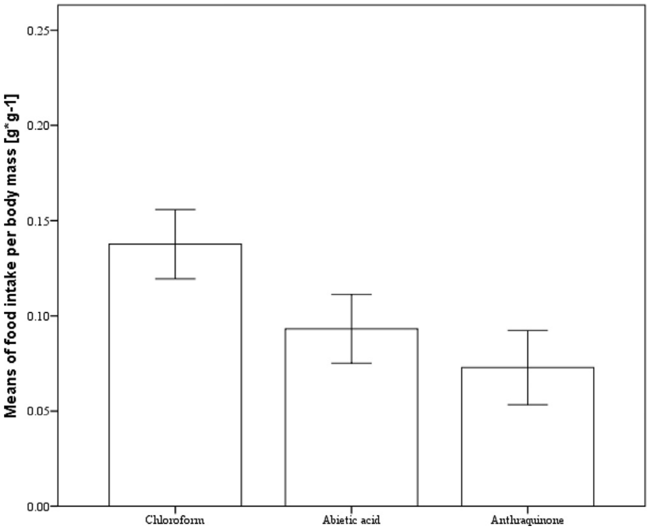

Fig. 2. Means of food intake per body mass and 95% confidence intervals of female common voles (n = 8 animals per treatment) for four days for treatments that reduced significantly food intake compared to the appropriate control group chloroform.

Table 4
Results of the feeding experiments with female house mice. Treatment (PSM + solvent) and control (chloroform) granules mixed with wheat. Statistical results are based on a GLM comparing intake of wheat per body mass (P-values are Bonferroni-corrected). Additionally, means and standard error of wheat intake [g] and animal body mass [g] are stated.

GLM (repeated measurements)					
within-subject-effects					
	df	F	P		
Treatment	3.958	9.22	0.000		
Treatment*day	11.87	0.61	0.828		
		F	P	Mean intake [g]	Mean body mass [g]
Ethanol (EtOH)				2.77 ± 0.33	13.08 ± 0.11
Bergamot Oil 5% vs. EtOH	32.30	0.000	0.69 ± 0.16	14.94 ± 0.63	
BPO 2% vs. EtOH	3.48	0.073	1.97 ± 0.27	14.82 ± 0.61	
Bucco Oil vs. EtOH	25.57	0.000	1.24 ± 0.25	18.3 ± 0.59	
Fennel Oil 4% vs. EtOH	14.26	0.001	0.21 ± 0.07	17.54 ± 0.35	
MNK vs. EtOH	45.84	0.000	1.07 ± 0.24	14.46 ± 0.63	
MNK + BPO vs. EtOH	28.35	0.000	0.84 ± 0.26	12.79 ± 0.22	
MNK + MA + BPO vs. EtOH	4.89	0.035	1.78 ± 0.29	15.18 ± 0.53	

BPO = Black Pepper Oil; EtOH = Ethanol; MNK = Methyl Nonyl Ketone; MA = Methyl Anthranilate.

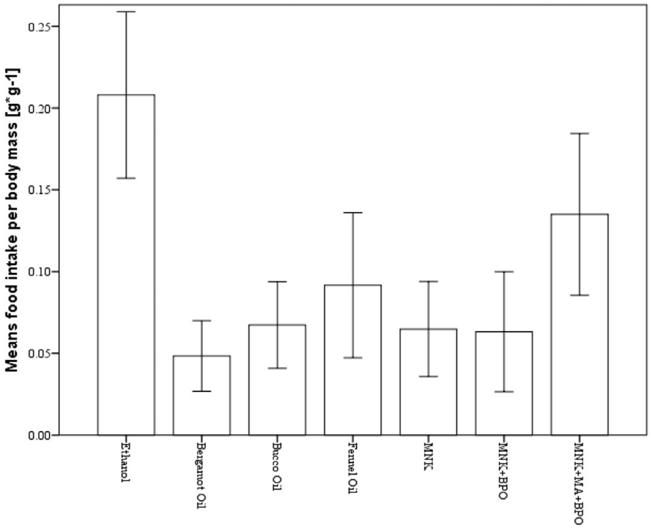

Fig. 3. Means of food intake per body mass and 95% confidence intervals of female house mice (n = 8 animals per treatment) for four days for treatments that reduced significantly food intake compared to the appropriate control group ethanol. BPO = Black Pepper Oil 2%; MNK = Methyl Nonyl Ketone; MA = Methyl Anthranilate.

mice were not affected by anthraquinone and BPO, but were deterred by bucco oil, which increased food intake in common voles. In conclusion, we could detect five deterrent essential oils, single compounds or combinations (bergamot oil, fennel oil, MNK, MNK + BPO and MNK + MA + BPO) whose odors were effective in reducing food intake in both female cricetid rodents (common vole) and female murid rodents (house mouse). There was a similar response to some repellents in both rodent species but also differences (e.g. BPO and bucco oil).

Because of the different habitat preferences of the two rodent species we expected a somewhat weaker response of common voles to the odor of the tested substances compared to house mice. However, the results of the experiments did not confirm this hypothesis. Voles mostly avoid odors that would not naturally occur in their habitat, e. g. bergamot oil, black pepper oil, fennel oil, methyl nonyl ketone and neem oil. Together with bucco oil these odors are all from plants cultivated for their essential oils and originating in dry temperate zones. Our expectation that the volatile terpenoid limonene [in bergamot (Sawamura et al., 2006); in BPO (De Feo et al., 2002); in fennel oil (Karlsen et al., 1968)] could be responsible for the deterrent effect was disproven. Results with limonene when tested singly and bucco oil [also containing limonene (Moolla et al., 2007)] suggested that it acted as an attractant in voles. MNK (also known as 2-undecanone) occurs in rue and although bucco oil was extracted from a plant with the same family (Rutaceae), there were different effects on the feeding behavior in voles.

Mice often live in close proximity with humans and may not encounter some of these plant derived compounds commonly. House mice reduced feeding on all treatments except black pepper oil (Table 4). Consequently, we would suggest a species-specific response for both rodent species, but further experiments are necessary. For logistical reasons we used no-choice experiments for our feeding experiments with both rodent species. Multiple choice experiments could not be conducted with these two rodent species, because the animals always cached wheat in their nest making it impossible to separate remaining treated and untreated food.

For rodents the olfactory sense is important in many respects. Olfaction in rodent control can be a helpful tool to manage rodent pest populations. However, the olfactory sensitivity in rodents (Apfelbach et al., 2005) could be different between sexes as it is in humans (Doty et al., 1984) or herbivorous mammals like deer (Rice and Church, 1974).

Females matter most for reproduction in rodent populations because males can be easily replaced by other males in polygamous mating systems. Therefore, removing females from field sites to be protected should have more severe consequences on rodent population dynamics and consequently damage reduction than removing males. In addition, the fitness of female rodents depends more heavily on foraging than in males, because females have an increased need for food/energy during pregnancy and lactation resulting in higher crop damage (Jacob et al., 2004). Tran and Hinds (2012) showed that various plant extracts can impair reproduction in female rodents. They identified 13 plant extracts which reduced the number and type of ovarian follicles and caused disruption to the oestrous cycle. If females can detect anti-reproductive compounds in plants there should be an aversion. Furthermore, the rodent offspring could learn through the foraging behavior of the mother to avoid certain food (which contains high concentration of PSM) associated with the odor and palatability of the plant (Burritt and Provenza, 1989; Provenza et al., 1992). Although we focused solely on females, further investigations on male rodents are needed.

Several studies discuss the negative impact of anticoagulant use on non-target species, especially in birds and mammals (Eason et al., 2002; Langford et al., 2013). Nonetheless, not all plant compounds are harmless for the environment despite their natural origin. Coumarin, for example, is a natural metabolite found in many plants e.g. *Gallium odoratum* (L.) Scop.. We focused on the odor of PSMs and the avoidance of these, mainly through smell and not through intake. In many rodent species, sense of smell is the most important source of information about the social and nonsocial world (Johnston, 2003).

The application of volatile plant metabolites for crop protection can be very diverse. On the one hand, it should be a timesaving, practical and economic method and on the other hand it should be effective. Attractive odors (e. g. bucco oil) could also be useful in the management of rodent pest species, e.g. by luring rodents away from sites to be protected or could be used to enhance bait acceptance for trapping and baiting. In some circumstances repellent odor (e. g. methyl nonyl ketone) and repellent taste (e. g. bitter tasting compounds like tannins and phenols) could be combined to minimize possible habituation of rodents to the repellent odor, because post-ingestive effects are induced. Otherwise animals could adapt to repellents by recognizing that the odor is not connected to a negative or positive reward. Apfelbach et al. (2005) reviewed the effects of predator odors as repellents for mammalian prey and observed significant habituation. We could observe that in some treatments (e. g. BPO 2% or abetic acid) the deterrent effect seemed to decline in the four days of the trial based on increasing food consumption. However, we could demonstrate that female voles reduced food consumption in treatments with BPO 2%, bergamot oil 5%, fennel oil 4%, MNK 25%; MNK + BPO, MNK + MA + BPO, neem oil 20% and consistently over all four experimental days. Another potential application of species-specific attractants and repellents is the improvement of non-target safety. Attractants specific to common voles could be combined with repellents specific to non-target species in rodenticidal bait to improve efficacy in the target species and limit bait uptake in non-targets.

For the protection of grain storages, for example, dispensers could be used for the application of the deterrent odor to repel the pest species continuously. It may be beneficial to apply the repellents at certain times (e. g. when crops reach an attractive stage or shortly before the commencement of the reproduction season). For protecting cables (e. g. electric) incorporating repellents in the cable insulation may be an option to avoid gnawing damage. However, economical (cost of treatment), practical (dissipation of compounds) and environmental (non-target effects) issues need to be considered before application in agriculture areas.

In conclusion, our study shows that the odor of PSMs can act as repellents in female murid and female arvicoline rodents. We would assume that only metabolites with a high and constant efficacy in the lab cage trials will probably show an effect in field trials. For common voles that would be: neem oil, MNK and their combinations thereof and for house mice: bergamot oil, MNK and bucco oil. From preliminary tests we know that the solvent alone have a negative effect on food intake. Consequently, the deterrent effect of the tested PSMs could be higher in the field trials. These metabolites could be used instead of or supplement the use of commonly applied rodenticides if appropriate products can be developed. Further studies are needed before using the investigated metabolites in rodent management in the field. Their utilization could reduce risk associated with the application of rodenticides e. g. through avoiding poisoning non-target species. Furthermore, our study reveals that attractive odor of plant metabolites might be useful to lure rodents away from crop fields. However, the environmental impact and agricultural consequences of such approaches has to be investigated to ensure environmental safety. Subsequent experiments are required to test promising PSMs under semi-natural and field conditions and optimally with further target rodents.

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ORIGINAL PAPER

Effect of plant secondary metabolites on feeding behavior of microtine and arvicoline rodent species

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Abstract Rodents are among the most damaging pests in agriculture worldwide. High-density populations of rodent pests can kill plants or diminish their growth, and reduce both fruit size and the number of seeds. Therefore, rodent pests can lead to considerable crop loss and require management actions to minimize damage. Optimal management of pest rodents is usually based on the combination of methods to reduce damage. Although rodenticides are usually the most commonly deployed approach, they have undesirable side effects. Consequently, more environmentally benign approaches such as repellents or attractants to lure rodents away from fields are gaining traction. The dispersal of a large number of juvenile males at certain times of year has prompted us to focus on male rodents for our experiments. We used laboratory feeding experiments to measure how the odor of plant secondary metabolites (PSMs) affects two pest rodents: common voles (*Microtus arvalis*, Pallas) and house mice (*Mus musculus*, L.). We tested eighteen different PSMs or combinations thereof with voles. Four PSMs reduced feeding and seven PSMs increased feeding of male common voles. Five of six tested odors were effective as repellents against house mice. However, we assume a species-specific response to volatile PSMs repellents. This study demonstrated that four repellents reduced feeding in both rodent species. Our results contribute to the development of non-lethal management tools for rodent pest species that are potentially more suitable than traps and rodenticides. This approach could be applicable to a variety of crops if effective at field conditions.

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

Key Message

- Plant secondary metabolites (PSMs) odors can act as repellents or attractants in male murid and male arvicoline rodents.
- Methyl nonyl ketone (MNK) was the most effective odor against voles and MNK + MA (methyl anthranilate) against mice.
- Bisabolol seems to cause a species-specific response, attractive to voles and deterrent to mice.
- We recommend that PSM odors should be considered in studies at natural conditions to assess their suitability to manage rodent pest species.

Introduction

Mammalian herbivores are responsible for significant damage to many managed environments worldwide. This includes arable land, forests, grasslands, and orchards. In agricultural areas, pest species can cause crop loss and require expensive management to minimize damage. Rodents are among the most important mammalian pests. Rodent pests contribute to large pre-harvest losses causing

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a global annual loss of about 77 million tons (John 2014). Usually, less than 10 % of rodent species cause substantial negative impacts in cropping (Singleton et al. 2007) and these include deer mice (Borrecco 1976) or pocket gophers (Wiscomb and Messmer 2010) in North America; eastern harvest mice (Sorensen et al. 2007) in South America; grassland melomys (Dyer et al. 2011) and house mice (Brown et al. 2003) in Australia; and common voles in Europe (Jacob and Tkadlec 2010). Rodent population outbreaks, which can occur cyclically (Singleton et al. 2010) can be particularly damaging. During outbreaks, population size irrupts dramatically and common voles in Europe can reach densities of $\geq$1000 individuals per hectare (Bryja et al. 2005; Jacob et al. 2014). Several rodent pest species are also disease vectors. For example, house mice carry diseases (e.g., salmonellosis, leptospirosis) and therefore pose a health risk for humans, especially in urban areas (Cameron 1949). The financial loss due to rodent-borne zoonoses is not known but may be similar to rodent-derived losses in agriculture (Bonnefoy et al. 2008).

Present management methods for rodent pest species focus mostly on the use of rodenticides, fumigants, trapping, and mechanical barriers. However, some of these approaches are impractical, expensive because of time and labor required and the results can be poor. The availability of efficient rodenticides is narrowing and most current approaches are focused on short-term reductions of ongoing damage without much attention to longer term effects.

Popular anticoagulant rodenticides can have negative effects on non-target species and can also pollute the environment (Geduhn et al. 2014; Hosea 2000; Shore et al. 1999). They are usually classified as ‘first generation’ compounds, which act as chronic toxicants, requiring multiple exposures over a short period of time to be effective and have a relatively short half-life (Hadler and Buckle 1992). More effective, ‘second generation’ compounds have a significantly higher-toxicity, and can be lethal after a single dose (Hadler and Buckle 1992). All anticoagulant rodenticides may cause primary (consumption of bait by target and non-target species) and secondary poisoning (non-target species consume poisoned prey). Sometimes farmers prefer acute poisons; these types of rodenticides have a lower risk of secondary poisoning of non-target wildlife and effects are readily visible (Singleton et al. 2007). Resistance to both first and more recently to some of the second generation compounds is a phenomenon which has been known since the 1960s (Buckle et al. 1994) and is an increasing problem for the use of anticoagulants rodenticides in crop and health protection.

There are alternative methods to control rodent pests which may be less harmful to the environment. These include (i) environmental manipulation, e.g. vegetation management or habitat manipulation (Jacob et al. 2008; Witmer et al. 2009); (ii) biocontrol (Howarth 1991; Pavey et al. 2008); (iii) decreasing palatability and increasing resistance of plants to rodent feeding, e.g. constitutive production of secondary plant metabolites (Marquis 1992); (iv) socio-physiological control, e.g. using predator odors as repellents or to inhibit reproduction in small mammals (Apfelbach et al. 2005); (v) specific non-hazardous chemical agents, e.g. using predator odors as natural repellents [in many forms, e.g. fur, skin-derived odors, fecal and urine odors, and odors from scent glands (Sullivan et al. 1988)] or plant-based repellents (Curtis et al. 2002); and (vi) immuno-contraception (Jacob et al. 2008).

Odor, flavor, and palatability provide the basis for foraging selections in mammalian herbivores. PSMs play a key role in this interaction and can act as feeding deterrents through regulating the food intake of herbivores (Dearing et al. 2005). Many studies investigated anti-feedants or repellents for protecting target plant species and most of them used treated or manipulated food (e.g. Glendinning et al. 1990) or water (Nolte et al. 1993; Tordoff et al. 2008) to deter herbivores. Curtis et al. (2002) investigated the effects of extracts of ten plant species on the feeding behavior of prairie voles (*Microtus ochrogaster*, Wagner) to develop a vole repellent. However, the behavioral response of rodents to deterrent (plant) odors other than direct application to the food source has rarely been investigated.

A large body of literature confirms that small mammalian species which are exposed to predator odors show alterations in foraging, feeding, general activity, and reproduction, especially in females (Apfelbach et al. 2005; Tran and Hinds 2012). However, males will also be affected by repellents and they also play an important role for rodent infestations. Particularly juvenile males disperse in search of new territories and resources (delBarco-Trillo et al. 2011) and they may be the first to immigrate from refuges in agriculture fields. Therefore, we firstly evaluated how the odor of PSMs affected the feeding behavior of males in two widespread rodent pest species of two families: common voles (*Microtus arvalis*, Cricetidae) and house mice (*Mus musculus*; Muridae).

Secondly, we asked if there were species-specific differences in the responses to PSMs in common voles and house mice. These rodent species live in different habitats; therefore, we expected that the response to PSM odors might be different between the species. For management purposes, it could be helpful to find repellents that act deterrent to multiple rodent pest species.

In our study, volatile PSMs were chosen because of their characteristic smell, chemical properties, or they had already been demonstrated to have deterrent effects against rodents or other taxa (Edlich and Stolter 2012; Fischer et al. 2013b).

Methods and materials

Subjects and substances

For our experiments, we used metabolites of herbal origin such as essential oils or individual metabolites of plant material: anthraquinone (natural product formed by fungi and seed plants), bisabolol (present in the essential oil of *Matricaria chamomilla*, L.), black pepper oil (BPO; obtain from *Piper nigrum*, L.), capsaicin (alkaloid in *Capsicum* species), carvacrol (present in essential oils, e.g. in *Origanum vulgare*, L.), eugenol (extracted from essential oils from, e.g. *Syzygium aromaticum,* L.), fennel oil (*Foeniculum vulgare*, (L.) Mill.), jatropha oil (seed oil from *Jatropha*, L. plants), juniper oil (*Juniperus communis*, L.), methyl anthranilate (MA; component of various natural essential oils), methyl nonyl ketone (MNK; obtain from *Ruta graveolens*, L.), neem oil (obtain from *Azadirachta indica*, A. Juss.), nettle oil (*Urtica dioica*, L.), and Szechuan pepper oil (*Zanthoxylum piperitum*, DC). Additionally, we used four combinations of PSMs in subsequent experiments to investigate if the effects of different metabolites were synergistic (Table 1). Metabolites and solvents were obtained from Diagonal GmbH & Co. KG, Germany except for bergamot oil, fennel oil, and nettle oil which were obtained from Ronald Reike Spezialversand, Germany and jatropha oil from Vereinigte Werkstätten für Pflanzenöltechnologie, Dr. Gruber/Gruber GbR, Germany. We conducted all feeding experiments in laboratory cages with wild caught male individuals of common voles and house mice or their F1 offspring. We trapped common voles in Gierstaedt, Germany and house mice on pig farms around Muenster, Germany.

Table 1 Compounds, concentrations, and solvents used in feeding experiments with male common voles and house mice

Compounds	Concentration in solvent (%)	Solvent
Anthraquinone	15	Chloroform
Bisabolol	5	Ethanol
Black Pepper Oil	2	Ethanol
Capsaicin	1	Ethanol
Carvacrol	5	Ethanol
Eugenol	5	Ethanol
Fennel Oil	4	Ethanol
Jatropha Oil	5	Chloroform
Juniper Oil	4.4	Ethanol
Methyl Anthranilate	25	Ethanol
Methyl Nonyl Ketone	25	Ethanol
Neem Oil	20	Ethanol
Nettle Oil	2.8	Ethanol
Szechuan Pepper Oil	5	Ethanol
Combinations		
MNK + MA	12.5 + 12.5	Ethanol
MNK + MA + BPO	12.5 + 12.5 + 6.25	Ethanol
MNK + BPO	14 + 14	Ethanol
MNK + Bergamot Oil	18.75 + 18.75	Ethanol

BPO Black Pepper Oil; *MNK* Methyl Nonyl Ketone; *MA* Methyl Anthranilate

Experimental design for feeding experiments

Experiments were conducted to identify responses of rodents to different PSMs odors by measuring the rodents' consumption of wheat mixed with PSM-treated or untreated gypsum granules (Rhône-Poulenc). Animals were held in standard laboratory cages (36 × 21 × 15 cm) with litter and hay in a room at 18-21 °C on a 12 h:12 h light:dark cycle. Commercial food pellets (Altromin 1324; Altromin Spezialfutter GmbH & Co.KG, Lage, Germany) and water ad libitum were supplied at all times. We moved the animals during the experiments in clean cages with cellulose paper, a clay pot, and a cardboard tube for shelter.

Common vole experiments

For each experiment, we fed eight female animals with a weight range from 15 to 28 g. The PSMs were dissolved in one of two solvents: ethanol or chloroform (Table 1). We compared the treatment group (PSM + solvent) with the corresponding control group (solvent only). From 2012 to 2014, we tested the effectiveness of 18 PSMs odors; four of those were combinations of PSMs, which resulted in overall 20 test-sets (including control groups) using 160 individuals ($n = 8$ per experiment). We fed a mix of wheat (10 g) and treated gypsum granules (10 g) in feeding racks (12 × 4.2 × 3.5 cm) for 24 h for 4 days. Uneaten wheat was collected and separated and in case of being wet, dried in a drying oven for 12 h before weighing. Racks were refilled on the following days (1–4) at the same time with a new wheat-granules mixture. Additionally, all animals were weighed on the first and last day of the experiment to relate food consumption to body weight.

House mouse experiments

For the experiments, we used a mixture of adults and subadults female animals with a weight range from 12 to 18 g. The experiments followed the same protocol as that used for the common vole experiments, except that we used 5 g wheat mixed with 5 g gypsum granules. In these experiments, we used also two control groups (ethanol and chloroform). For logistical reasons, we could run experiments with a lower total number of animals. Therefore, we selected a subset of the metabolites/combinations which had already shown an effect in the experiments with the common voles: 15 % anthraquinone, 5 % bisabolol, 4 % fennel oil, 25 % MA, 25 % MNK, and the combinations MNK + BPO and MNK + MA, resulting in overall 9 test-sets (including control groups) using 72 individuals (n = 8 per experiment). In these trials, we aimed to investigate if house mice respond to these odors similar to common voles or if there is a species-specific response.

Statistical analyses

The effect of treatments on wheat intake in gram per gram body mass [g/g] was examined by using a general linear model (GLM) for repeated measurements to test the effectiveness among the different metabolites/combinations. For both species, two GLM analyses were conducted: a) for metabolites dissolved in ethanol and b) for metabolites dissolved in chloroform and their corresponding control groups. Before using this statistical approach, the data were tested for violation of sphericity using the Mauchly's Test. If the sphericity condition was not met, Greenhouse-Geisser corrections were used (Field 2013). We included food intake per body mass as a dependent variable. "Treatment" and "Treatment * days" were used as a within-subject effect, to test for the influence of different treatments and differences among experimental days. All statistical analyses described above were performed using PASW 18 (SPSS 2010, IBM Cooperation).

Results

Common vole experiments

There was a statistically significant effect ($P < 0.001$) of treatments on food intake (Table 2; Fig. 1), but no day effect. Voles reduced food consumption during treatments with 4 % fennel oil, 25 % MA, 25 % MNK, and MNK + MA compared to the control group. Treatment effects were strongest with MNK (70 % reduction in food uptake) and weakest with MNK + MA [9 % (Fig. 1)]. Seven metabolites did not affect the feeding of voles. The treatments with 5 % bisabolol, 2 % black pepper oil (BPO), 1 % capsaicin, 5 % eugenol , 4.4 % juniper oil, 20 % neem oil, and 2.8 % nettle oil resulted in an increased food uptake (Table 2; Fig. 1). Intake was highest in the treatment with bisabolol (41 % increase in food uptake). The experiments with the combinations (MNK + BPO, MNK + MA, MNK + MA + BPO, and MNK + bergamot oil) demonstrated that only the combination MNK + MA was deterrent, as the food intake was significantly lower than in the control group (Table 2; Fig. 1). Treatments with 15 % anthraquinone and 5 % jatropha oil compared to the control group (chloroform) show no significant effect in intake ($P > 0.05$) or the days.

House mouse experiments

There was a statistically significant effect ($P < 0.001$) of treatments on food intake (Table 3; Fig. 2) but no day effect. Mice reduced food consumption in treatments with 5 % bisabolol, 4 % fennel oil, 25 % MA , 25 % MNK , and MNK + MA compared to the control group. Deterrent effects were strongest in MNK + MA treatment (80 % reduction in food uptake) and weakest on in MNK treatment [61 % (Fig. 2)]. The combination of MNK + BPO did not affect the food intake of mice. In contrast to all the other substances, for which house mice showed a lower food intake, the treatment with 15 % anthraquinone, resulted in a greater food uptake (Fig. 3). Mice consumed significantly more food treated with 15 % anthraquinone (0.16 ± 0.01) compared with the control group treated with chloroform [(0.03 ± 0.01) ($P < 0.001$)].

Discussion

Our study focused on the effect of the odor of single plant metabolites or essential oils on the feeding behavior of male rodents. From 18 tested metabolites/combinations, we identified four deterrent and seven attractive metabolites/combinations of PSMs, which affected the feeding behavior of common voles. MNK was the most effective deterrent, followed by MA, fennel oil, and MNK + MA. We expected to see additive effects with the four combinations of metabolites tested because each individual metabolite that contributed to the combinations was deterrent [bergamot oil in a separate study with female common voles (Hansen et al. 2015)]. However, only one combination (MNK + MA) had a deterrent effect on food intake.

Many plants contain PSMs in different concentrations which may vary intraspecifically ontogenetically or temporally (Gershenzon et al. 2012; Moore et al. 2014). Therefore, it is sometimes the whole odor composition of a plant, sometimes just a single metabolite, or the complete plant composition, which affects the foraging behavior of herbivores either as a deterrent or an attractant. That could be a possible explanation for these results. Alternatively, animals may only react to the metabolite until a particular concentration threshold is reached without increasing their aversive response at even higher concentration preventing stronger effects of the combination of odors.

The response of house mice to the six tested PSMs/combinations was different to that of common voles. MNK + MA was the most effective, followed by bisabolol and fennel oil which all reduced food intake. Anthraquinone seems to increase the food intake. House mice were deterred by bisabolol, which was an attractant for common voles. Overall, from 18 tested plant metabolites or combinations

Table 2 Results of feeding experiments with male common voles

GLM (repeated measurements)				
Within-subject effects	df	F	*P*	
Treatment	6.69	14.67	***	
Treatment * day	20.07	0.49	n.s.	
Within-subject contrasts		F	*P*	Differences
Bisabolol versus EtOH		60.91	***	0.099
Black Pepper Oil versus EtOH		9.65	**	0.035
Capsaicin versus EtOH		5.79	*	0.026
Carvacrol versus EtOH		1.87	n.s	0.016
Eugenol versus EtOH		13.60	***	0.045
Fennel Oil versus EtOH		6.37	**	−0.028
Juniper Oil versus EtOH		34.71	***	0.073
Methyl Anthranilate versus EtOH		7.79	**	−0.034
Methyl Nonyl Ketone versus EtOH		23.62	***	−0.049
MNK + Bergamot Oil versus EtOH		0.53	n.s	−0.012
MNK + BPO versus EtOH		0.12	n.s	−0.006
MNK + MA versus EtOH		5.77	*	−0.021
MNK + MA + BPO versus EtOH		0.55	n.s	−0.010
Neem Oil versus EtOH		10.73	**	0.026
Nettle Oil versus EtOH		7.62	**	0.052
Szechuan Pepper Oil versus EtOH		2.77	n.s	−0.020

In treatments granules (PSM + solvent) and in control groups granules (solvent = ethanol only) mixed with wheat were offered

Statistical results are based on a GLM comparing intake of wheat per body mass (P-values are Bonferroni-corrected)

Additionally, differences in gram (food intake per body mass) of means between treatment group and the control group are stated.

BPO Black Pepper Oil; *EtOH* Ethanol; *MNK* Methyl Nonyl Ketone; *MA* Methyl Anthranilate

Significance Levels: * $P < 0.05$; ** $P < 0.01$; *** $P \leq 0.001$

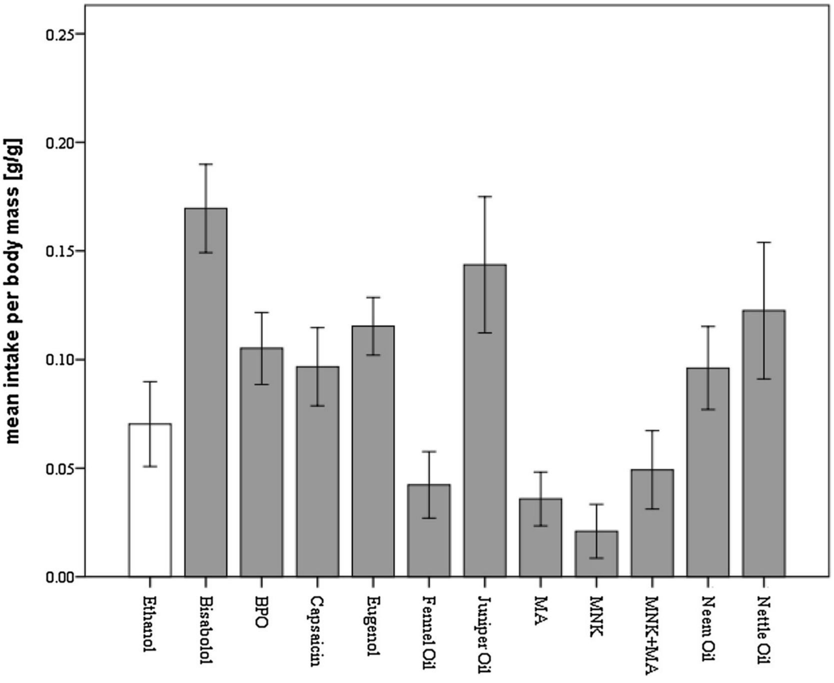

Fig. 1 Means ± SE of food intake per body mass of female common voles (n = 8 animals per treatment) for 4 days for treatments that reduced or increased significantly food intake compared to the appropriate control group (Ethanol). *BPO* Black Pepper Oil 2 %; *MNK* Methyl Nonyl Ketone; *MA* Methyl Anthranilate

Table 3 Results of feeding experiments with male house mice

GLM (repeated measurements)				
Within-subject effects	df	F	*P*	
Treatment EtOH	2.57	30.24	***	
Treatment * day	7.70	1.33	n.s.	
Within-subject contrasts		F	*P*	Differences
Bisabolol vs. EtOH		73.75	***	−0.151
Fennel Oil vs. EtOH		206.44	***	−0.182
Methyl Anthranilate vs. EtOH		117.27	***	−0.173
Methyl Nonyl Ketone vs. EtOH		38.63	***	−0.117
MNK + BPO vs. EtOH		3.48	n.s.	−0.047
MNK + MA vs. EtOH		100.05	***	−0.153

In treatments granules (PSM + solvent) and in control groups granules (solvent = ethanol only) mixed with wheat were offered

Statistical results are based on a GLM comparing intake of wheat per body mass (P-values are Bonferroni-corrected). Additionally, differences in gram (food intake per body mass) of means between treatment group and the control group are stated

BPO Black Pepper Oil; *EtOH* Ethanol; *MNK* Methyl Nonyl Ketone; *MA* Methyl Anthranilate

Significance Levels: * $P < 0.05$; ** $P < 0.01$; *** $P \leq 0.001$

thereof, we were able to identify four deterrents (4 % fennel oil, MA, MNK, MNK + MA) which seem to be effective against both species studied. There was a similar response to the repellents in both rodent families but some odors seemed to be species specific, e.g., bisabolol acts attractive to voles and deterrent to mice.

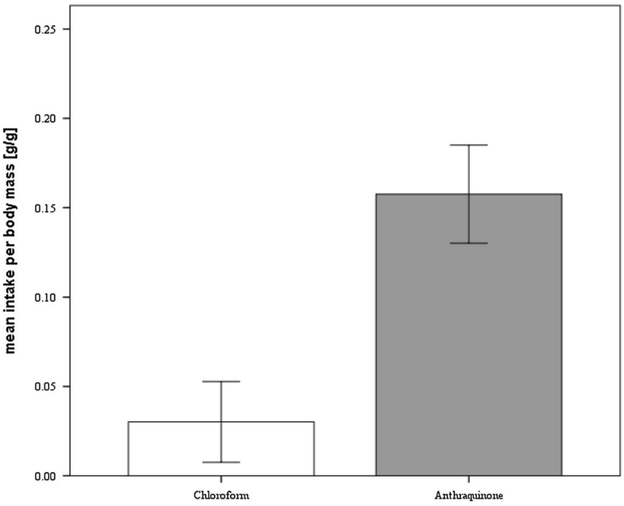

Fig. 3 Means ± SE of food intake per body mass of male house mice for 4 days for anthraquinone that increased significantly food intake compared to the appropriate control group (chloroform)

In addition, there may be a sex-specific difference in the response to odor. Shumake et al. (2000) found that the odor of carbon disulfide affected female rats more than males. In a separate study, we conducted feeding experiments with female rodents of both species (Hansen et al. 2015). Referring to these results, we could also detect that the response to plant odors by male rodents was different compared to females. Based on the data presented in this paper, we can conclude that only the odor of fennel oil and MNK is deterrent in both sexes and in both rodent species.

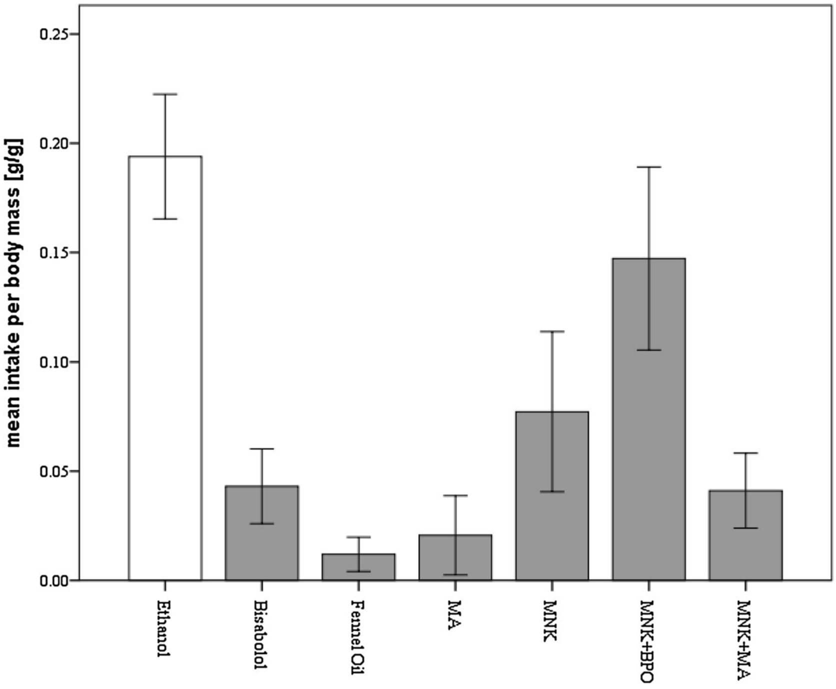

Fig. 2 Means ± SE of food intake per body mass of male house mice for 4 days for treatments that reduced significantly food uptake compared to the appropriate control group (Ethanol). *BPO* Black Pepper Oil; *MNK* Methyl Nonyl Ketone; *MA* Methyl Anthranilate

Our results showed that common voles mostly avoided some herbal odors that are not naturally occurring in their habitat, e.g., bergamot oil, fennel oil, MNK, and MA. Surprisingly, they were attracted by some known deterrent odors, e.g., bisabolol to hares (Reichardt et al. 1990), BPO to voles (Fischer et al. 2013a), or capsaicin to gophers (Sterner et al. 1999).

House mice as commensal species may not encounter some of these plant odors commonly. They avoided all tested PSM odors, except for anthraquinone which increased the food intake and MNK + BPO which seemed to have no effect. Therefore, we would assume a species-specific response for both rodent species but further experiments are necessary. All of our trials were no-choice experiments because animals always cached wheat in their nest. Only in no-choice trials it was possible to separate spilled or cached treated and untreated food. However, other experimental designs (e.g., multiple choice tests using video cameras or using other applications apart from gypsum granules, food dyes) could be used in further work to validate our results in a food choice scenario.

For a non-lethal management tool for rodent pest species, the application of volatile PSMs can be very diverse. It has to be firstly, a very effective and secondly a timesaving, practical and economic method. Repellent odors could be used, for example, in dispensers for protection of grain storages to repel the target pest species continuously or temporarily and possibly with alternating compounds to minimize habituation. All tested substances are slightly soluble in ethanol or chloroform and are therefore well suited for use by deterring through odor. PSM odors could be used as pest controls agent in limited spaces such as storages, greenhouses, or buildings, because of their high volatility, although the effects of these PSMs on humans and environment are yet unknown and need to be tested before application. Other application forms apart from gypsum granules, which are more easily to applicate separately to the food source, e.g. dispensers for vaporization need to be tested in future studies. Our results suggest that PSM odors can be an appropriate carrier for slow release into the air, but there are also needs for a suitable liquid or solid carrier such as lacquer or paraffin for incorporating repellents in cable insulation (e.g., electric) to prevent gnawing damage (Ahn et al. 1995). Fischer et al. (2013b) demonstrated the potential field application of an in-soil repellent by using foam as a carrier directly for MNK with common voles. Thereby, practical (spread of compounds, habituation of rodents, residues of PSMs in soil or water), economical (cost of treatment), and environmental (non-target effects) issues always need to be considered before field application.

Attractive odors (e.g., bisabolol for voles) could be used for luring rodents away from sites to be protected or to enhance species-specific bait acceptance for trapping and for rodenticides. The latter may limit rodenticide feeding and related unwanted knock-on effects on non-target predatory species. The combinations of repellent odor (e.g., methyl nonyl ketone) and repellent taste (e.g., bitter tasting compounds like tannins and phenols) could minimize possible habituation of rodents, because animals associate post-ingestive effects with the repellent odor (Kimball and Taylor 2010). Animals could adapt to repellent odors by recognizing that the odor is not connected to a negative or positive reward. Willoughby (2011) reviewed the effects of different odors as repellents for wood mice (*Apodemus sylvaticus*, L.) and tree squirrels (*Sciurus carolinensis*, Gmelin) and observed habituation. In some of our treatments (e.g., in voles: MNK + MA; in mice: MNK), food consumption seemed to increase in the 4 days of the trial. Additionally, as found in our work and that of others (e.g., Bäumler et al. 1990), the effect of some repellents can decline over time, due to conditioning and environmental degradation. However, we could demonstrate that male voles reduced food consumption in treatments with 4 % fennel oil and 25 % MNK and male mice in treatments with 5 % bisabolol, 4 % fennel oil, 25 % MA , and MNK + MA consistently over all four experimental days.

We focused in our study on male animals, because rodents live in highly social organizations in which males are territorial and juvenile males have to search for a new territory as they mature. In agricultural systems, rodents are reliant on refuge areas where they can live and reproduce undisturbed when crops are absent or unattractive. Juvenile male rodents emigrate from these areas into agricultural fields. In an optimal ecologically based rodent management approach, refuges have to be considered, e.g., through application of deterrent odors or traps with attractants along the crop–refuge interface to intercept migration. Rodenticidal baits typically contain grain components highly preferred by most pest rodent species, bolstered with sweeteners in order to better compete with existing food sources (Kaukeinen and Buckle 1992). When applying attractant odors, the bait palatability may be further enhanced.

For an integrated rodent management, PSMs could be very useful because these metabolites may help avoid risks for non-target species, e.g., through secondary poisoning as well as other unwanted effects on the environment (e.g., contamination of soil and waterbody). In addition, non-lethal deterrents maintain rodent populations as important food sources for predators, which may be desirable in some circumstances.

In conclusion, we could demonstrate in our study that the odor of PSMs can act as repellent or attractant in male murid and male arvicoline rodents. We recommend that herbal substances, e.g., PSMs are investigated as alternative options to manage rodent pest species. They could reduce the environmental risk associated with the application of rodenticides. Before utilization of these metabolites in rodent management, further studies are needed to investigate the environmental impact to ensure environmental safety. Semi-field and field experiments are required to test promising metabolites under semi-natural and natural conditions and with further target rodent species.

Author contribution statement

SH and JJ conceived and designed research. SH conducted experiments and contributed new reagents. SH and CS analyzed data. SH, CS, and JJ wrote the manuscript. All authors read and approved the manuscript.

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Compliance with Ethical Standard

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution and with the permission of the animal ethics committee of LANUV North Rhine-Westphalia (file number 84-02.04.2012.A151).

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Ergänzung zum Manuskript:

“Effect of plant secondary metabolites on feeding behavior of microtine and arvicoline rodent species”

Erratum Liste:

Seite	Originaltext	Korrektur
33	“For each experiment, we fed eight female animals with…”	“For each experiment, we fed eight male animals with…”
33	“For the experiments, we used a mixture of adults and subadults female animals with a…“	“For the experiments, we used a mixture of adults and subadults male animals with a…“
35	“Fig. 1 Means ± SE of food intake per body mass of female common voles…“	“Fig. 1 Means ± SE of food intake per body mass of male common voles…“

Kapitel 4

Like or dislike - Response of rodents to the odor of plant secondary metabolites

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Abstract

Rodents, including common voles (*Microtus arvalis* Pallas, 1778) and house mice (*Mus musculus* Linnaeus, 1758) cause immense pre- and post-harvest losses. Therefore, developing methods that mitigate these losses while maintaining their role in ecosystems is a priority. Several plant secondary metabolites (PSMs) which significantly reduce food intake of both species under laboratory conditions have been identified. However before these can be used in rodent pest management, they must be tested under more natural conditions where other food sources are available. In this study, the odors of four PSMs were evaluated for their repellent effects in experiments conducted in semi-natural enclosures. Soil treated with PSMs or untreated soil (experimental control) was placed in an underground box containing food (rolled oats). We quantified the number of visits to each box and could demonstrate that all four PSMs reduced the number of visits to treatment boxes in both rodent species. For common voles the combination methyl nonyl ketone + black pepper oil was the most repellent PSM. House mice made also fewer visits to all PSM boxes; boxes with the anthraquinone were visited at least. Furthermore, house mice consumed less food from boxes containing soil treated with all four PSMs. Our results suggest that PSMs are repellent in murid and microtine rodents under semi-field conditions. Additionally, the future use of PSM odors for repelling both pest species, especially house mice seems promising. Further investigations with other PSMs, different concentrations as well as alternative application methods are needed to repel common voles from attractive crops.

Key Words – enclosure trials, odor, plant secondary metabolites, repellents, rodents

Introduction

Damage by field rodents is of considerable economic and social importance. In Europe, the principal offenders are common voles (*Microtus arvalis* Pallas, 1778) (Jacob *et al.* 2014), water voles (*Arvicola* spec. L., 1758) (Pelz & Gemmeke 1988), bank voles (*Myodes glareolus* Schreber, 1780) (Hansson & Zejda 1977) and field voles (*Microtus agrestis* L., 1761 (Niemeyer *et al.* 1997)]. All these species feed on agricultural crops, damage forest trees, shelter-belt plantings, and orchard trees (Jacob & Tkadlec 2010). These rodents are responsible for damage through direct consumption and destruction of plants but also through disturbance of the ground's surface and the resulting unwanted changes in plant species composition from subsequent invasion by weeds (Wiscomb & Messmer, 2010). The major European pest rodent, the common vole, can reach population densities of more than 2,000 individuals per ha^{-1} (Bryja *et al.* 2005) during outbreaks. These animals are highly social and can form large colonies inhabiting underground burrows (Frank 1957). House mice (*Mus musculus*, L.) exploit the highly modified agricultural environments of wheat growing areas worldwide. There they occasionally reach high densities and cause significant crop damage and other losses (Singleton *et al.* 2005). As a pest species inhabiting human infrastructure they have direct and immediate effect on the quality of the health and life (e. g. as vectors of diseases such as salmonellosis, leptospirosis) (Cameron 1949).

Previous approaches to the mitigation of damage by rodents include the application of rodenticides or other lethal methods of control (Singleton *et al.* 2007, Jacob *et al.* 2014). Rodenticides are poisons leading to death which may be painful thus raising ethical issues. In addition, short term reduction of populations through isolated poisoning campaigns may be negated by immigration of new animals from source populations (Sullivan 1979). Finally, rodenticides may affect non-target species (e. g. Langford *et al.* 2013, Geduhn *et al.* 2014). Alternative, ecologically-based approaches to the management of rodents are desirable. The use of attractants and repellents may offer poison-free techniques. For example, Pelz (1989) has shown that wood mice (*Apodemus sylvaticus* Linnaeus, 1758) can be lured away from sugar beet seeds by providing an attractive alternative food adjacent to the newly planted crop and concentrated volatiles may drive rodents away from fields or other structures (e. g. Curtis *et al.* 2002, Fischer *et al.* 2013a, Hansen *et al.* 2015).

Plants can be defended against herbivores by mechanical, chemical or biotic mechanisms. In particular, plant secondary metabolites (PSM) can act as feeding deterrents (Glendinning 1992), digestibility reducers (Schmidt 2010), or toxins (Samuni-Blank *et al.* 2013) to rodents.

After ingestion of PSM animals not only need to detoxify but also have to deal with energetic constraints such as reduced food intake, mass loss, increased energy demands, and increased metabolic demands (Sorensen *et al.* 2005). Therefore, mammalian herbivores that eat foods containing certain PSM have behavioral and physiological means to limit negative effects (McArthur *et al.* 2014). Olfaction plays an important role in the foraging behavior of rodents (Howard & Marsh 1970) and their detection of plant metabolites. Amongst burrowing mammals, like voles, odor is an important way of signaling and communicating (Dobly & Rozenfeld 2000).

PSM repellents, if effective, could be used to manipulate the feeding patterns of rodents to protect crops and infrastructure from damage. A great advantage of repellents is that they are either innately aversive in terms of their odor (or taste) or they may trigger conditioned aversion, i.e. the animal associates the odor of the compound or another cue with its post-ingestive consequences and will avoid that odor in subsequent encounters (Chambers & Bernstein 1995).

In several studies, predator odor was used to deter rodents from feeding (Sullivan *et al.* 1988, Shumake *et al.* 2000, Powell & Banks 2004). Johnson *et al.* (1982) evaluated several methods including repellents for mitigating rodent damage in newly planted zero-tillage corn. Previous studies with plant-derived repellents in a laboratory setting (Hansen *et al.* 2015, 2016a) identified several promising compounds against common voles and house mice. However, these have to be tested in less controlled conditions. Few studies have been conducted in outdoor enclosures or in the field to examine the effect of plant-derived repellents on the feeding behavior of rodents (Bäumler *et al.* 1990, Fischer *et al.* 2013b, Witmer *et al.* 1998, Willoughby 2011). The translation of laboratory bioassays into effective operational systems through testing in semi-field or field conditions is missing or has been problematic (Hansen *et al.* 2016b). We investigated whether odors of PSM, which had proven repelling properties in controlled bioassays, are effective in a semi-natural environment in two rodent species. Although the importance of olfaction is broadly accepted (Howard & Marsh 1970, Burda *et al.* 1999, Heth & Todrank 2007), the use of deterrent odors in combination with attractive food has not been widely studied. We used plant-derived compounds with deterrent effects against rodents based on previous cage studies (Hansen *et al.* 2015, 2016a). We hypothesized that the four tested plant-derived odors reduce the number of visits to the underground boxes as well as the food intake in both rodent species under semi-natural conditions. Furthermore, we assumed a species-specific response to the plant-derived odors, based on preliminary trials (Hansen *et al.* 2015, 2016a). The two rodent species have different habitat preferences

(Niethammer & Krapp 1982). Therefore, common voles could be more familiar with plant odors than mostly commensal house mice.

Methods and Materials

Subjects and chemicals

We captured wild common voles with live traps (Ugglan©) in grassland at two locations in Germany (Münster and Gierstädt). House mice were captured with live traps on pig farms around Münster (North Rhine-Westphalia, Germany). Additionally, we used the F1-generation offspring of the wild rodents. Eight common voles (6 females, 2 males) were used in each enclosure; trials were run from 01 till 26 July 2013. The weight range of male common voles was 15-30 g and of female common voles 15-28 g. For the trials with house mice 6 animals (3 females, 3 males) were used; trials were run from 23 September till 18 October 2013. The weight range of male house mice was 13-20 g and female house mice were 14-20 g. There was no difference in mean body weight between enclosures ($P > 0.05$). For house mouse experiments we equipped each enclosure with two nest boxes with straw inside. All animals in the enclosures were marked with a RFID/PIT transponder tag (Lux-IDent, Lanškroun, Czech Republic) subcutaneously between the shoulder blades before they were held in the enclosures for 10 d to habituate. We tested with both species two single plant-derived compounds: methyl nonyl ketone (MNK) and anthraquinone (anthra) and two combinations: methyl nonyl ketone + black pepper oil (MNKBPO) and methyl nonyl ketone + methyl anthranilate+ black pepper oil (MMB) dissolved in the appropriate solvent and mixed with soil (Table 1). We used concentrations based on laboratory studies (Hansen *et al.* 2015, 2016a).

Experimental design for enclosure trials

The experiments were conducted in four rectangular semi-natural enclosures at the premises of Julius Kühn Institute in Muenster, Germany. Enclosures were of about 35 m^2 and sown with a local grass mix to mimic perennial grassland. In each enclosure four plastic boxes (32cm x 22cm x 16cm) with a lightproof lid were buried level with the enclosure surface in each corner 1 m away from the walls. The boxes had two openings where a corrugated pipe (40cm; Ø 25mm) connected the box with the surface. One opening was an entrance/exit equipped with a circular transponder antenna (Francis Scientific Instruments, UK) and the other was used for ventilation and closed with a sieve. The antenna was connected to a data logger (Multi Channel ID Logger, Francis Scientific Instruments, UK) that recorded the presence of an animal individually based on the PIT tag at 10 s intervals (see Figure 1).

Experimental procedure

At day 0 (1100-1200 hr) we placed a plastic container (30 x 20.5 x 6 cm) with one feeding tray (10 x 10 x 3.5 cm) in the centre in every box. We added 10 g rolled oats to each feeding tray and placed ~320 g untreated soil (control) or ~320 g treated soil (with compound + solvent) around the tray. Due to the high volatile character of the solvent we assumed that no solvent was left during the feeding experiments. We used plain soil without solvent as an experimental control because we were interested in the effect of PSM odors compared to no treatment. In each enclosure we used two control and two treatment boxes. The logger station was immediately activated and data were retrieved after 24 h. On the following days (1-4) all remaining oats were weighed and feeding trays were refilled with fresh oats. The repellent applied to the soil was not renewed daily. The position of the controls and treatments was rotated clockwise every day. Each treatment was tested in each of the four enclosures for four days. From the logger data we calculated number of visits (number of times each box was entered). Because of a technical failure there were no data for one enclosure in the third week in common vole trials.

Statistical analyses

Raw numbers of visits (or intake) were converted to a proportional variable of visits to control boxes in relation to the total number of visits (dependent variable). As fixed factors we included sex and substance (four PSMs) into the model. Two random factors were used in the model, one where the animal ID was nested in the respective enclosure to account for the experimental design as well as each experimental day to incorporate the repeated measure approach. The effects of treatments were statistically analyzed using a generalized linear mixed model (GLMM) with binomial error distribution and a logit link function. The best model was selected by using the Akaike information criterion (AIC). To compare within subject contrasts of all PSMs we performed pairwise 'Tukey' comparisons of all PSMs using the glht function from the *multcomp* package. Least square means were obtained from the lsmeans-package back transformed from logit scale and used to visualize PSM-treatment effects. All statistical analyses were conducted with R-project 3.2.2 (R Core Team, 2015).

Results

Experiments with Common voles

There was no statistically significant effect of sex on the proportion of number of visits. The factor sex was consequently excluded from the final model (Table 2). In all PSM treatments individuals showed a higher probability to prefer control boxes compared to the treatment boxes (all PSMs >0.5 in Figure 2a). All random factors explained some variance in the final model. This was greatest when the individual variability (as ID nested in enclosure) was accounted for. The within subject-contrasts based on GLMM results showed that MNKBPO had a significantly larger repelling effect compared to all other PSMs (Table 2, Figure 2a), with an overall ranking of MNKBPO>MNK=anthra>MMB. The rolled oats provided in the boxes were always completely consumed after 24 h so we were unable to use the intake data in further analysis.

Experiments with House mice

All PSMs repelled house mice as shown by the overall higher probability to enter the control boxes compared to treatment boxes (Figure 2b). Mice were mostly affected by soil treated with anthraquinone and MMB treatment effects were less strong for MNKBPO and weakest for MNK (Figure 2b). Therefore, the ranking of visits for the different PSMs in house mice was anthra>MMB>MNKBPO>MNK (Table 2). Males visited control boxes more often than females (Table 2). Similar to the common vole, incorporating individual variability in the random factor explained most variance. The analysis of food intake could only be performed on a population (=enclosure) level, as no observations on individual food intake were made. In general, mice preferred boxes with untreated soil regardless of the different PSMs (Figure 2c), without statistically significant differences between the PSMs (Table 2).

Discussion

The current study focused on the assessment of the repellent effects of PSMs under semi-field conditions using wild caught common voles and house mice. The PSMs tested were previously determined under laboratory settings as having potential for these two rodent species (Hansen *et al.* 2015, Hansen *et al.* 2016a). We have demonstrated that, behavior in cage trials could be nearly translated to the semi-natural environmental conditions of these experiments. Previous laboratory cage trials with both rodent species regarding the four compounds tested were very promising, especially for females. Hansen *et al.* 2015 results for female common voles shows a ranking of the four tested PSM odors MMB < MNKBPO < MNK < anthra. We could demonstrate in this study that common voles and house mice responded differently to the tested plant-derived odors, but were effectively repelled from treatment boxes. On average, voles had a higher probability to visit control boxes compared to PSM treated boxes. Common voles were more likely to visit control boxes than treatment boxes (Figure 2). Fischer *et al.* (2013) showed the repelling effect of MNK under field conditions. Although our study could confirm this, we suggest the effect could be enhance by incorporating black pepper oil. Rolled oats were always completely consumed by the voles,

which suggests that the tested PSM odors did not prevent the uptake of this highly attractive food.
House mice were deterred from visiting boxes treated with all PSMs (anthra, MNK, MNKBPO and MMB). MMB and anthraquinone were the most repellent compounds (Figure 2). Attractive food provided in the underground boxes, which may have prompted the animals to ignore the potentially repellent odors could be an explanation for the different rankings. House mice results indicate a sex-specific activity, but further investigations are necessary to underpin this assumption. Females of both species react to all four PSM odors in cage trials (Hansen *et al.* 2015); male common voles are repelled by MNK and male house mice by anthra and MNK (Hansen *et al.* 2016a). There are also other studies which confirmed that both sexes can respond differently to the same odor (Shumake & Hakim 2000). The effect of repellents can decline over time, due to conditioning, environmental degradation (Bäumler *et al.* 1990) or alternatively, dissipation of compounds over time. However, there are also studies which demonstrate reduced food consumption (Hansen *et al.* 2016a) or reduced burrowing activity in common voles for at least two to six days (Fischer *et al.* 2013b).

We examined the food intake as another method to investigate the efficacy of the potential repellents, but analysis was only possible for house mice. House mice not only visited treated boxes less, they also showed reduced food intake in all treatments in comparison to the control boxes, with anthraquinone having the largest effect. However, food intake was only measured at a population level in this trial. Food consumption however can strongly vary between individuals and sexes (Clapperton 2006), but is difficult to obtain in enclosure trial. Given our model could not account for such variability; this might have precluded detection of any underlying differences between the PSMs. Consequently, we suggest the combination of these two methods or using, in addition, video cameras (with individually marked animals) for the investigation of the effects of potentially deterrent odors on rodents.
It is possible that animals had learned over the four days that there was no post-ingestive effect of the compounds. Conditioned food aversions have been shown to have powerful influences on the diets of herbivores (Provenza ***et al.*** 1998, Lawler ***et al.*** 1999). If animals in field trials received no negative feedback (e. g. nausea) associated with a particular PSM odor**,** then an aversion is unlikely to develop and animals would start to eat more readily. Furthermore, our results show that there is a species-specific response to the PSM odors. Interspecific variation in olfactory sensitivity could be an explanation for the observed differences in foraging behavior (Bramley & Waas 2001). PSM (or indeed other xenobiotics) can affect the foraging behaviour through different ways, ranging from pre-ingestive stimuli

(e.g. taste and odor) to post-ingestive mechanisms to post-absorptive effects (Foley ***et al.*** 1999). The initial recognition and detection of the compounds and their concentration through odor or taste need to occur rapidly to determine if food can be eaten or not. Olfactory sensitivity in rodents (Apfelbach ***et al.*** 2005) could be different between sexes as it is in humans (Doty ***et al.*** 1984). In our enclosure experiments the number of animals (males and females) was restricted to account for possible competition and the social structure. A larger number of males could have yielded clearer patterns in sex-specific responses to treatements. Wang & Liu (2001) determined that predator odor of the steppe polecat (*Mustela eversmanni* Lesson, 1827) affected breeding and significantly lowered the frequency of copulation of female root voles (*Microtus oeconomus* Pallas, 1776). Further studies are needed to investigate the influence of plant-derived odors on rodent reproduction in enclosure or field trials – potentially these odors could have a major influence on populations of rodents and could be used to mitigate damage (Jacob ***et al.*** 2008).

In our experiment, the individual response explained most of the random variance, highlighting that individuals can react differently to certain stimuli. In more natural field settings, the ability of rodents to find buried food using their smell appears to vary depending on a wide range of environmental factors (e. g. humidity, predator pressure) or social factors (e. g. receptiveness) (Kotler 1984, Ostfeld 1985, Heth ***et al.*** 2002, Wolff 2007). Our previous studies in laboratory cages demonstrated reduced intake of an attractive food (wheat mixed with treated gypsum granules) (Hansen ***et al.*** 2015, 2016a). Hence, the final outcome may depend on the odor of the available food and how attractive it is and also perhaps what kind of post-ingestive experiences the animals associate with this odor.

PSM and other xenobiotics have the capacity to affect herbivorous mammals both as individuals and populations. In house mice, odors of all four tested PSMs (anthra, MNK, MNKBPO and MMB) could be utilized as a non-lethal management tool that has the potential to reduce house mouse damage to crops and infrastructure and for application in public health management. In addition, it seems appropriate to determine the compound's potential for the management of other rodent pest species e.g., pocket gophers (Baldwin ***et al.*** 2015), Günther's voles and Tristam jirds (Moran 1981), because these rodents can cause considerable damage to plants through root consumption or damage to various forms of infrastructure (e.g. subsurface-drip irrigation tubing, buried cables).

The use of volatile plant-derived odors as a tool in ecologically-based rodent management to minimize rodent damage needs to be based on firm knowledge of effects in target species at realistic environmental conditions. Fischer *et al.* (2013b) repelled rodents with MNK as an in-soil foam application under field conditions. Future field experiments with these potential repellents are required to elucidate details for field application including the minimal effective dose for relevant compounds and the duration of efficacy. Our results support the hypothesis that PSM odors can deter some rodents from consuming otherwise attractive foods.

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Table 1: Compounds, weight of soil, weight of compounds + weight of solvent used for two boxes in enclosure experiments with common voles and house mice. We used concentrations based on preliminary laboratory studies (Hansen *et al.* 2015, 2016a).

Compounds	**Soil [g]**	**Substance [g]**	**Solvent [g]**	
Anthraquinone (anthra)	640	3.2	76.8	Chloroform
Methyl nonyl ketone (MNK)	640	20	60	Ethanol
Methyl nonyl ketone +Methyl anthranilate + Black pepper oil (MMB)	640	4.5+4.5+4.5	66.5	Ethanol
Methyl nonyl ketone + Black pepper oil (MNKBPO)	640	4.5+4.5	71	Ethanol

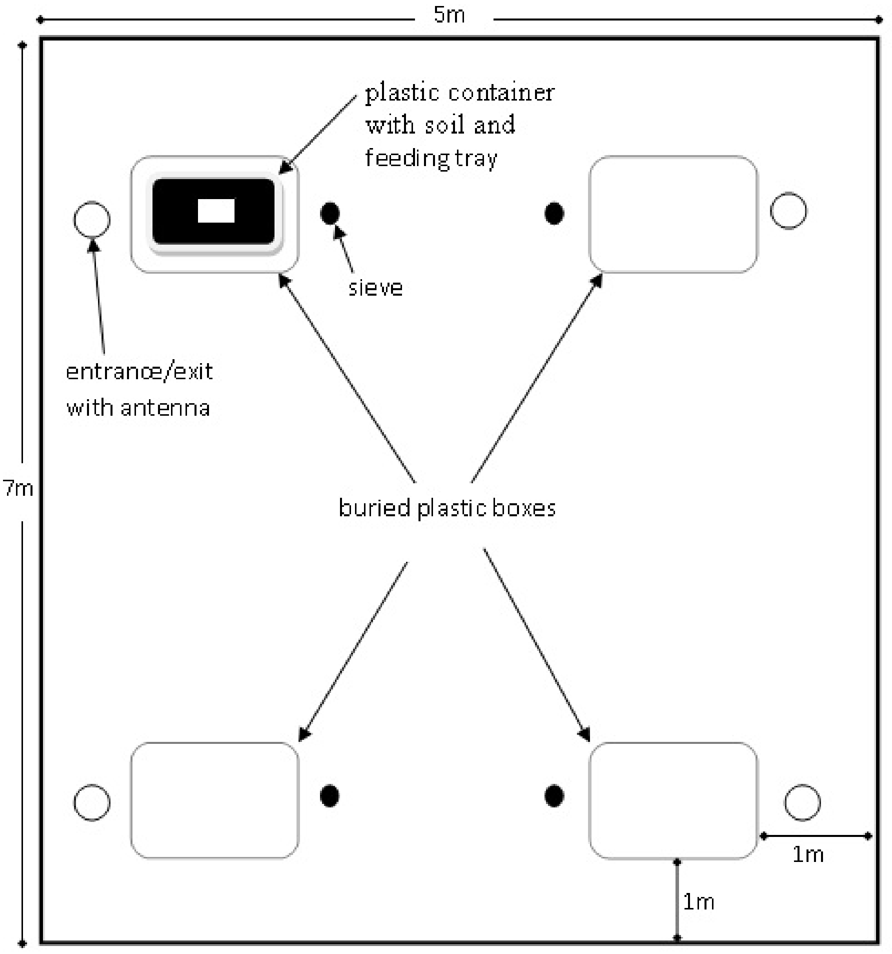

Figure 1: Experimental setup in the enclosure experiments with two different rodent species to test for effects of plant-derived odors on numbers of visits of rodents to control boxes (untreated soil) and treatment boxes (soil + compound + solvent).

Table 2: Model results indicating correlations between numbers of visits, sex and substance (here results of post hoc comparisons among PSMs are presented). Parameter coefficients from generalized linear mixed models with binomial error distribution used to examine the influence of PSM odors on number of visits of common vole and house mice as well as food intake of house mice are stated.

	Common vole visits				House mouse visits				House mouse intake			
Source of variation	Coef. ß	SE (ß)	z	p	Coef. ß	SE (ß)	z	p	Coef. ß	SE (ß)	z	p
Intercept	0.226	0.247	0.913	0.361	**0.955**	**0.269**	**3.546**	**<0.001**	**0.304**	**0.314**	**0.969**	**0.333**
Sex [M]	/	/	/	/	**0.358**	**0.085**	**4.206**	**<0.001**	/	/	/	/
MMB vs. Anthra	**-0.154**	**0.029**	**-5.305**	**<0.001**	**-0.162**	**0.056**	**-2.916**	**0.018**	0.466	0.244	1.909	0.221
MNK vs. Anthra	-0.034	0.030	-1.143	0.662	**-1.051**	**0.051**	**-20.575**	**<0.001**	0.167	0.247	0.676	0.905
MNKBPO vs. Anthra	**0.091**	**0.030**	**3.037**	**0.013**	**-0.591**	**0.046**	**-12.885**	**<0.001**	0.469	0.227	2.069	0.160
MNK vs. MMB	**0.120**	**0.030**	**4.001**	**<0.001**	**-0.889**	**0.041**	**-21.938**	**<0.001**	-0.299	0.183	-1.632	0.357
MNKBPO vs. MMB	**0.245**	**0.032**	**7.755**	**<0.001**	**-0.429**	**0.040**	**-10.804**	**<0.001**	0.003	0.200	0.016	1.000
MNKBPO vs. MNK	**0.125**	**0.032**	**3.871**	**<0.001**	**0.460**	**0.032**	**14.181**	**<0.001**	0.302	0.193	1.567	0.393
Random	σ^2	StDev			σ^2	StDev			σ^2	StDev		
Enclosure	0.091	0.302			0.056	0.237			0.047	0.217		
ID*Enclosure	0.253	0.503			1.347	1.161			/	/		
Day	0.121	0.348			0.03	0.173			0.217	0.466		

Anthra= Anthraquinone; MNK = Methyl nonyl ketone; MNKBPO = Methyl nonyl ketone + Black pepper oil; MMB = Methyl nonyl ketone +Methyl anthranilate+ Black pepper oil

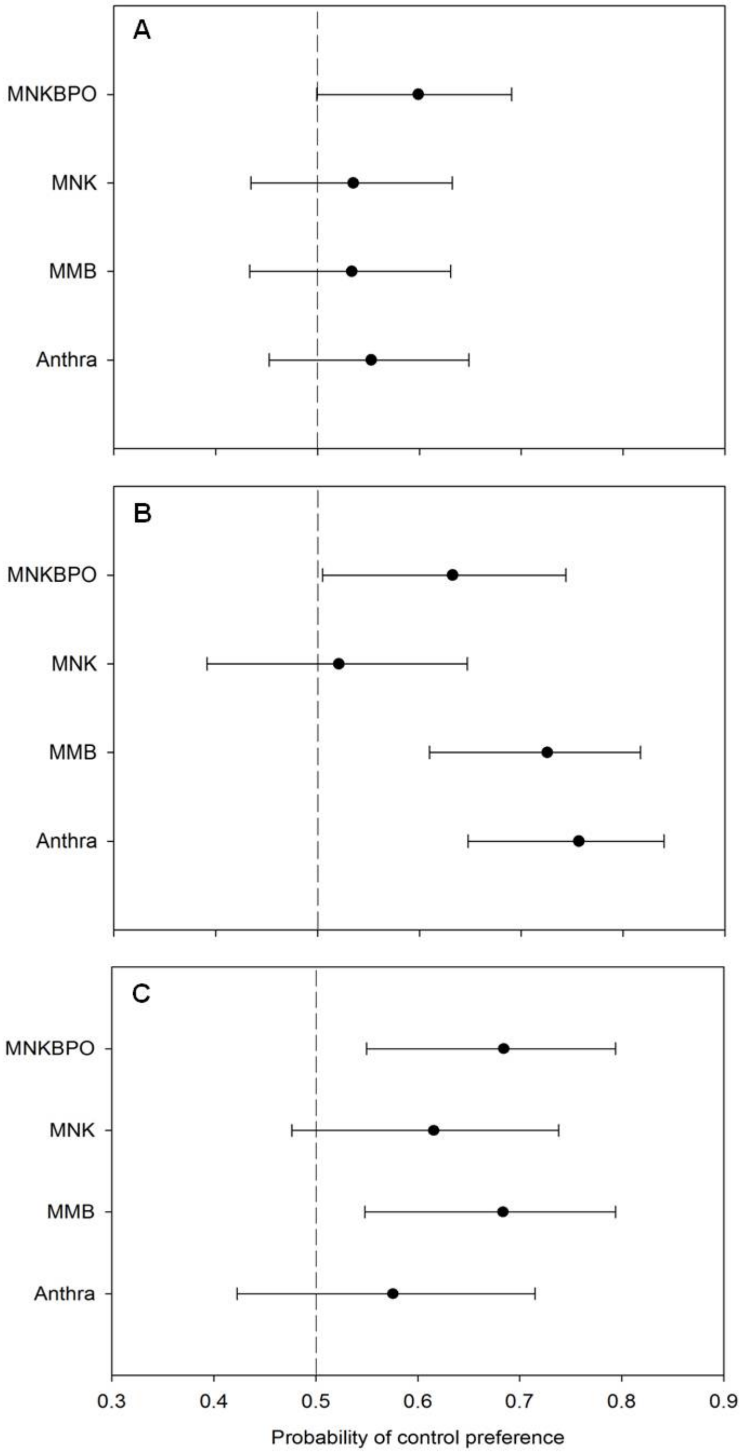

Figure 2: Effects of four PSMs on the numbers of visits of rodents to two control and two treatment boxes (proportional to total visits).
A: Probability of common voles visiting control boxes (n=32) in 4 enclosures.
B: Probability of house mice visiting control boxes (n=24) in 4 enclosures.
C: Probability of food intake by mice in control boxes (n=24) in 4 enclosures.
0 = high probability of visits (intake) to treatment boxes; 1 = high probability of visits (intake) to control boxes; line at probability 0.5 indicates no preference for treatment or control boxes
Anthra= Anthraquinone; MNK = Methyl nonyl ketone; MNKBPO = Methyl nonyl ketone + Black pepper oil; MMB = Methyl nonyl ketone + Methyl anthranilate + Black pepper oil.

Kapitel 5

Gesamtdiskussion - experimentelle Arbeit

In einem Ökosystem interagieren Organismen wie Pflanzen immer mit anderen Organismen, die sich in ihrer Umgebung befinden, wie beispielsweise mit Fraßfeinden, Parasiten oder Wirten. Als Konsequenz unterliegen sie dem natürlichen Selektionsdruck durch diese anderen Organismen ausgesetzt und damit der Evolution. Wenn die Entwicklung einer bestimmten Art zu der Entwicklung eines entsprechenden Gegenstückes führt, und umgekehrt, spricht man von einem koevolutiven Prozess oder auch vom „evolutivem Wettrüsten“ (Dawkins and Krebs, 1979; Thompson, 1994). Kleinsäuger, wie z. B. Nagetiere können eine Schlüsselrolle in einem terrestrischen Ökosystem (besonders in der Kulturlandschaft) einnehmen, als diejenigen die fressen (Konsumenten) und gefressen werden. Die Interaktion der Nagetiere mit ihrer Umwelt beeinflusst nicht nur ihren eigenen (Nahrungs-)Erfolg, sondern auch die Fitness der Pflanzen, die sie fressen. Es beeinflusst aber auch die Prädatoren, die ihrerseits Nagetiere fressen, mit potentiellen weitreichenden ökologischen und evolutionären Auswirkungen.

Zu den Grundbedürfnissen eines jeden Tieres gehört die Nahrungsaufnahme (für z. B. Proteine, Wasser, Mineralien, Vitamine oder essentielle Fettsäuren), die die Fitness des Individuums wesentlich beeinflusst. Die Nahrungssuche hängt von mehreren äußeren Umweltfaktoren ab: Kosten der Nahrungssuche, einschließlich Energie- und Zeitaufwand der Suche, Bearbeitung und Nahrungsaufnahme, erhöhte thermoregulatorische Kosten, Prädationsrisiko, reduzierte Reproduktion oder territoriale Lebenserhaltungsaktivitäten oder die potentielle Aufnahme von toxischen oder hemmenden Pflanzenverbindungen. Herbivoren entscheiden je nach Nahrungsverfügbarkeit über die Aufenthaltsdauer an einem Ort, welche Pflanzen sie dort fressen und wann sie wieder weiterziehen. Das Ganze beruht auf einer fundamentalen Nutzen-Kosten-Analyse der Nahrungssuche. Herbivoren müssen mit Pflanzengemeinschaften leben, die saisonalen und menschlichen Änderungen unterliegen und stellen daher für sie keine stabile Umwelt dar. Es gibt Pflanzen und Pflanzenteile (wie Blätter), deren Verfügbarkeit nur zu bestimmte Jahreszeiten gegeben ist. Die Nahrungssuche aus der Perspektive des Herbivoren ist bestimmt durch Nährstoffe (primäre Pflanzenmetaboliten) und deren Energie und durch PSM, wie beispielsweise Phenole oder Tannine. PSM können das Nahrungsverhalten von Herbivoren auf unterschiedliche Art beeinflussen, angefangen von prä-ingestiven Reizen (z. B. dem Geruch), über post-ingestiven

Mechanismen (z. B. dem Geschmack), bis hin zu post-resorptiven (gastrointestinal) Effekten (Foley et al., 1999). Der Mechanismus der Registrierung durch Geruch oder Geschmack erfolgt meist sehr schnell, um festzustellen, ob die Nahrung gefressen werden kann oder nicht. Die Nahrungswahl des einzelnen Herbivoren hängt dabei individuell von seinen Erfahrungen ab. Es kann unterschieden werden zwischen a) neu und mit unbekanntem Nährstoffgehalt; b) vertraut-gefährlich; c) vertraut-gefahrlos und d) vertraut-nützlich (Rozin, 1976). Die Generalisten unter den Herbivoren müssen kontinuierlich ein breites Spektrum von verfügbarer Nahrung sammeln und dabei gleichzeitig essentielle Nährstoffe, Fasergehalt, PSM und Verfügbarkeit beachten.

Die Nahrungswahl der nicht kommensalen Nagetiere findet bevorzugt in geschützten Mikrohabitaten (oft unter hoher Vegetationsdichte) statt, in denen das Risiko von möglichen Prädatoren niedriger ist. Diese natürlichen Habitate sind durch Habitatverlust oder Habitatfragmentierung allerdings begrenzt, dafür bieten landwirtschaftliche Flächen Nahrung und Schutz und werden deshalb von manchen Nagetieren gerne besiedelt. Schadnager, wie die Feldmaus (in Europa) oder die Hausmaus (in Australien) können auf diesen Flächen erheblichen Schäden und damit immense Ernteverluste verursachen. Kulturlandschaft und Ackerfläche bilden dann das Primär- oder Sekundärhabitat der Feldmaus, auf welches sie vor allem in Jahren der Massenvermehrung, die periodisch alle 2-5 Jahre stattfindet (Cornulier et al., 2013), ausweicht (Niethammer and Krapp, 1982; Stein, 1958) und für enorme Schäden und Ernteausfälle verantwortlich ist. Die kommensalen Hausmäuse verursachen vor allem Schäden in Vorratsspeichern, an Gebäudematerialien und sind als Überträger von Krankheiten (z. B. Leptospirose, Salmonellose) eine Gefahr für die menschliche Gesundheit. Als Mittel zur Bekämpfung von diesen Schadnagetieren werden meist Rodentizide eingesetzt.

Die gerinnungshemmenden Rodentizide, sogenannte Antikoagulantia, wurden in den 1940er Jahren entdeckt und ihre Vorteile der Wirksamkeit und Sicherheit führten schnell zu deren Verwendung, bevorzugt in der Bekämpfung von Nagetieren in Ländern mit gemäßigtem Klima (Hadler and Buckle, 1992). Es handelt sich um Wirkstoffe aus der Gruppe der Kumarine. Diese Vitamin-K-Antagonisten hemmen die Blutgerinnung und führen damit zum Tod des Tieres. Es wird in Wirkstoffe der ersten Generation (FGAR= first generation anticoagulant rodenticides) und Wirkstoffe der zweiten Generation (SGAR= second generation anticoagulant rodenticides) unterschieden. Warfarin und Coumatetralyl gehören zu den Rodentiziden der ersten Generation. Difenacoum, Brodifacoum, Flocoumafen und Bromadiolon sind Wirkstoffe der zweiten Generation, die giftiger und biologisch schwerer abbaubar sind als die der ersten Generation. Antikoagulante Rodentizide werden zunehmend sehr kritisch bewertet. Zum einen hat sich bei manchen Nagetierarten eine Resistenz gegenüber einigen gerinnungshemmenden Wirkstoffen entwickelt (Boyle, 1960). Aber auch die umweltgefährdenden Eigenschaften wie Persistenz oder Bioakkumulation, sowie die Schädigung von Nicht-Zielarten durch antikoagulante Rodentizide konnte nachgewiesen werden (Geduhn et al., 2014; Hosea, 2000; Shore et al., 1999). Es gibt bereits Untersuchungen zur Nagerbekämpfung mit weniger umweltgefährdenden Alternativen. Dazu gehören:

a.) Habitatmanagement, z. B. Vegetationsmanagement oder Habitatmanipulation (Jacob, 2008; Witmer et al., 2009);
b.) die Verringerung der Attraktivität von potentiellen Futterpflanzen und zunehmende Resistenz von Pflanzen, z. B. die konstitutive Produktion von sekundären Pflanzenstoffen (Marquis, 1992);
c.) die sozial-physiologische Kontrolle, z. B. mit Gerüchen von Prädatoren als Repellentien (Apfelbach et al., 2005); d.) Empfängnisverhütung (Jacob et al., 2008);
e.) Einsatz von nicht umweltgefährdenden chemischen Mitteln, z. B. Gerüche von Prädatoren als natürliche Repellentien [z. B. Fell, Haut, Fäkalien, Urin oder aus den Stinkdrüsen (Sullivan et al., 1988)] oder
f.) Repellentien auf pflanzlicher Basis, wie PSM (Curtis et al., 2002).

Die meisten PSM Gruppen die im Zusammenhang mit der Pflanzenabwehr auf Fraßschäden durch Herbivoren genannt werden sind Cyanide, Alkaloide, Glucosinolate, Terpenoide, Kumarine, Cardenolide, Ätherische Öle, Tannine und Flavonoide. Terpenoide gehören zu der größten und vielfältigsten Gruppe der PSM und kommen in höheren Pflanzen, Moosen, Leberblümchen (*Hepatica nobilis*), Algen und Flechten vor (Charlwood and Banthorpe, 1991). Terpenoide werden auch als Isoprenoide bezeichnet, weil das chemische Baugerüst aus Isopren-Einheiten (2-Methylbutan) besteht. Sie besitzen eine enorme chemische Vielfältigkeit und Komplexität durch ihr Kohlenstoffgerüst (5 Kohlenstoffeinheiten = C_5). Je nach der Anzahl dieser Einheiten wird unterschieden in Mono- (zwei C_5 Einheiten), Sesqui- (drei C_5 Einheiten), Di- (vier C_5 Einheiten), Sester, Tri-, Tetraterpene, sowie Polyterpene (Rosenthal and Berenbaum, 1991). Mono- und Sesquiterpenoide kommen in ätherischen Ölen in einer Vielzahl an Pflanzen vor und sind besonders charakteristisch durch ihren markanten Geruch (Elliott and Loudon, 1987), wie z. B. Limonen in Citrusfrüchten (Bergamotte). Repellente Gerüche aus pflanzlichen Verbindungen könnten in der Schadnagerbekämpfung genutzt werden, da diese das Geruchssystem der Nager und damit ihre Nahrungsökologie beeinflussen.

In meinen Versuchen (Laborkäfig- und Außengehegeversuche) habe ich hauptsächlich ätherische Öle und Substanzen aus der Gruppe der Terpenoide gewählt, vereinzelt aber auch Substanzen aus der Gruppe der Alkaloide, Carbonsäuren und Tannine.

Der Einfluss von ätherische Ölen oder Terpenoide wurden bei unterschiedlichen Herbivoren untersucht: Schneeschuhhasen [*Lepus americanus* (Sinclair et al., 1988)], Feldmäuse [*Microtus arvalis* (Fischer et al., 2013b)] oder Taschenratten [*Geomys bursarius* (Epple et al., 1996)]. Die Wirkung von ätherischen Ölen und Terpenoiden wird oft als giftig für die Tiere beschrieben, weshalb sie auch von den Tieren gemieden werden (Langenheim, 1994; Wittstock and Halkier, 2002). Einige Terpenoide sind in der Literatur auch bekannt als Insektenabwehrmittel (z. B. Neemöl, Thymianöl) oder als Attraktanzien für Insektenschädlinge; diese werden größtenteils auch kommerziell produziert und eingesetzt (Rehman et al., 2014). Obwohl es umfangreiche Literatur zu Repellentien gegen Nagetiere gibt, sind nur wenige kommerzielle Produkte erhältlich, die gegen Schadnager eingesetzt werden können. In der EU sind zur Verwendung im Biozidbereich bisher acht Produkte registriert (European Chemicals Agency, zuletzt am 15. April 2016 abgerufen). Eins davon ist 2-Undecanon oder Methylnonylketon (MNK), dass bereits in Studien mit Feldmäusen getestet wurde und dem eine vergrämende Wirkung nachgewiesen wurde (Fischer et al., 2013b). Auf dem Pflanzenschutzsektor der EU sind 20 Repellentien zugelassen, vier Verbindungen davon sind auf pflanzlicher Basis (Kleeöl, Knoblauchextrakt, Methylnonylketon, Pfeffer) (EU Pesticides database, zuletzt am 15. April 2016 abgerufen).

Einfluss von PSM als Repellentien bei zwei unterschiedlichen Nagerarten

Die Ergebnisse der Laborkäfigversuche zeigten zwei pflanzliche Gerüche, die bei beiden Nagetierarten (Feldmaus und Hausmaus) und in beiden Geschlechtern abschreckend auf die Nahrungsaufnahme wirkten: Fenchelöl und MNK. Weibliche Feldmäuse reduzierten bei neun von 13 getesteten pflanzlichen Gerüchen signifikant die Nahrungsaufnahme (Hansen et al., 2015). Neemöl, ein ätherisches Öl aus den Samen des Niembaumes gewonnen, mit dem Terpenoid Azadirachtin als Hauptwirkstoff, wirkte am effektivsten. Ebenfalls effektiv abschreckend wirkte MNK oder auch 2-Undecanon, aus der Gruppe der Ketone und Hauptbestandteil des Weinrautenöls, das auch in den Kombinationen mit MA (Methylanthranilat; Carbonsäureester) und Pfefferöl (BPO= black pepper oil, ätherisches Öl) eine negative Wirkung zeigte. Weibliche Feldmäuse mieden meist Gerüche von Pflanzen, die nicht natürlich in ihrem Lebensraum vorkommen, z. B. Bergamotte (Hauptkomponenten: Limonen und Linalyacetat, Terpenoide), Pfeffer (Hauptkomponenten: Piperin, ein Alkaloid und Pinene, ein Terpenoid), Fenchel (Hauptkomponenten: Anethole, ein Phenylpropanoid und Fenchone, ein Terpenoid), Weinraute (Hauptkomponente: MNK, ein Keton) und Neemöl. Diese pflanzlichen Verbindungen werden aus Pflanzen gewonnen, die dem Ursprung nach eher in trockenen, gemäßigten Zonen wachsen. In den ätherischen Ölen der Bergamotte (Sawamura et al., 2006), dem Pfeffer (De Feo et al., 2002) und dem Fenchel (Karlsen et al., 1968), ist das Monoterpenoid Limonen die gemeinsame Hauptkomponente und wurde deshalb einzeln getestet, zeigte jedoch keine abschreckende Wirkung in den Versuchen. Männliche Feldmäuse reagierten auf vier von 18 getesteten PSM mit einer abnehmenden und auf sieben PSM mit einer erhöhten Nahrungsaufnahme (Hansen et al., 2016a). MNK war hier das wirksamste Repellent, gefolgt von MA (Carbonsäureester), Fenchelöl (ätherisches Öl) und MNK+MA. Im Gegensatz zu den weiblichen Feldmäusen wirkte Neemöl bei den Männchen attraktiv auf die Nahrungsaufnahme.

Hausmäuse zeigten generell ein anderes Fraßverhalten als die Feldmäuse. In den Laborkäfigversuchen der Weibchen wirkte Bergamottöl (ätherisches Öl) effektiv abschreckend auf das Fraßverhalten, gefolgt von MNK und MNK+Pfefferöl (Hansen et al., 2015). Überraschenderweise zeigte Anthrachinon (ein vom Anthracen abgeleitetes Chinon) und Pfefferöl bei weiblichen Hausmäusen keine abschreckende Wirkung, wogegen Buccoblätteröl (gewonnen aus *Agathosma betulina* mit der Hauptkomponente Isomenthon, ein Terpenoid) eine anziehende Wirkung bei der Nahrungsaufnahme auf die Hausmäuse hatte. Männliche Hausmäuse mieden alle sechs getesteten pflanzlichen Gerüche (Hansen et al., 2016a). Die Kombination MNK+MA wirkte am effektivsten auf die Nahrungsaufnahme, gefolgt von Bisabolol (monocyclischer Sesquiterpen-Alkohol) und Fenchelöl, die alle zur reduzierten Nahrungsaufnahme führten. Hausmäuse mieden fast alle pflanzlichen Gerüche. Bei der Mehrzahl der getesteten pflanzlichen Gerüche konnte eine artspezifische Reaktion bei der Nahrungswahl beobachtet werden. Wie bereits angenommen, könnten die verschiedenen Lebensräume und die Ernährung der zwei Nagetierarten eine mögliche Erklärung für die unterschiedliche Reaktion auf die Gerüche sein. Die herbivoren Feldmäuse bevorzugen Grünlandflächen und sind daher vielleicht besser an pflanzliche Gerüche angepasst als omnivore Hausmäuse, die als kommensale Art meist in Speichern oder Gebäuden leben.

Wenn einzelne pflanzliche Gerüche eine effektiv abschreckende Wirkung auf die Nahrungsaufnahme bei den Tieren in den Käfigversuchen zeigten, nutzte ich teilweise höhere Konzentrationen oder verwendete Kombinationen aus effektiven Verbindungen, um zu untersuchen, ob sich ein synergistischer Effekt einstellt. Wenn die Mischung aus verschiedenen individuellen Terpenen zusammen synergistisch agieren kann („defense mixtures"), könnte es zu einer größeren Giftigkeit oder Abschreckung kommen, als bei einer äquivalenten Menge einer einzelnen Verbindung (Gershenzon and Dudareva, 2007). Diese Hypothese konnte ich allerdings in meinen Versuchen nur mit Fenchelöl 10% bei weiblichen Feldmäusen und mit der Kombination MNK+MA bei männlichen Feldmäusen beobachten

Die Laborkäfigversuche wurden als ‚No-Choice' Versuche durchgeführt, d. h. die Tiere bekamen im Versuch eine Futterraufe mit dem zu testenden Substanz-Gipsgranulat-Weizengemisch. Eine zusätzliche Beobachtung, z. B. mit Videokameras, um die Nahrungsaufnahme der Tiere zu beobachten war aus logistischen Gründen nicht möglich.

Das beobachtete unterschiedliche Fraßverhalten zwischen den Männchen und Weibchen kann an die unterschiedliche Geruchsempfindlichkeit bei Nagetieren (Apfelbach et al., 2005) zwischen den Geschlechtern liegen, wie auch bei Menschen (Doty et al., 1984) oder bei anderen herbivoren Säugetieren, z. B. bei Hirschen [*Odocoileus hemionus* (Rice and Church, 1974)]. Shumake and Hakim (2000) konnten bei ihren Untersuchungen mit Ratten (*Rattus norvegicus*) beobachten, dass der Geruch von Schwefelkohlenstoff Weibchen mehr als Männchen in ihrer Nahrungsaufnahme beeinflusst. Weibliche Feldmäuse reagierten unterschiedlich in der Geruchswahrnehmung auf Pfefferöl und Buccoblätteröl als Hausmäuse. Bei den männlichen Hausmäusen wirkte Bisabolol abschreckend, im Gegensatz zu den Feldmäusen, die mit einer erhöhten Nahrungsaufnahme reagierten.

Damit Repellentien evtl. auch als kommerzielles Produkt in der Anwendung im Schadnagermanagement in Frage kommen, sollte die Effektivität im Feld unter möglichst natürlichen Bedingungen nachgewiesen sein. Ich wählte aus den Laborkäfigversuchen vier effektive pflanzliche Verbindungen (MNK, Anthrachinon, MNK+BPO, MNK+MA+BPO) für die Außengehegeversuche mit beiden Nagerarten aus, um die Effektivität der Repellentien unter halbnatürlichen Bedingungen zu untersuchen. Die Wirkung in Feldversuchen von MNK (als Schaum Applikation) konnte bereits bei Feldmäusen nachgewiesen werden (Fischer et al., 2013b). Bei den Feldmäusen hatten alle getesteten pflanzlichen Gerüche einen geringen Einfluss auf das Besuchsverhalten der unterirdischen Boxen mit behandelter Erde (siehe Kapitel 4). Die Tiere suchten bevorzugt die Kontrollboxen auf. Im Vergleich aller getesteten pflanzlichen Verbindungen zeigte sich die Kombination MNK+BPO am effektivsten. Die Auswertung über die Nahrungsaufnahme (Haferflocken) war nicht möglich, da diese in den unterirdischen Boxen an jedem Versuchstag leer gefressen waren. Möglicherweise wirkte sich das attraktive Futter auch auf die Besuchshäufigkeit aus. Bei den Hausmäusen konnte ein eindeutiges Vermeidungs-Verhalten beobachtet werden, die Tiere zeigten eine höhere Besuchswahrscheinlichkeit der Kontrollboxen in allen vier getesteten Substanzen (siehe Kapitel 4). Die Tiere mieden am meisten die Boxen mit Anthrachinon behandelter Erde. Darüber hinaus fraßen die Hausmäuse weniger Haferflocken aus den Boxen mit der behandelten Erde in allen vier getesteten PSM Gerüchen.

Beide Nagetierarten reagierten dennoch unterschiedlich auf die vier getesteten pflanzlichen Verbindungen in den Außengehegen. Die Feldmäuse ließen sich nur gering von den Besuchen in den Boxen mit der behandelten Erde abschrecken. Bei den Hausmäusen hatte die repellente Wirkung der Gerüche in den Außengehegen die gleiche Effektivität wie in den Laborkäfigen. Demnach konnte auch in den Außengehegeversuchen eine artspezifische Antwort auf pflanzliche Gerüche nachgewiesen werden. Die Hypothese einer geschlechtsspezifischen Antwort auf die Gerüche konnte ich in den Außengehegeversuchen nicht eindeutig bestätigen. Bei den Feldmäusen zeigte sich keinerlei Einfluss des Geschlechtes auf die Besuchswahrscheinlichkeit, was auch auf die geringe Anzahl der männlichen Tiere zurückzuführen ist. Lediglich bei den Versuchen mit den Hausmäusen wurden die Kontrollboxen von den Männchen häufiger besucht als von den Weibchen. Obwohl das Geschlechterverhältnis bei den Hausmäusen 1:1 gewählt wurde, kann jedoch ein Konkurrenzverhalten nicht ausgeschlossen werden und eine Erklärung für die Besuchshäufigkeit der Männchen gegenüber den Weibchen sein.

Weitere Einflüsse von sekundären Pflanzeninhaltsstoffen

Weibliche Nagetiere spielen in der Dynamik einer Population eine wichtigere Rolle als männliche Tiere, denn in einem polygamen Paarungssystem können diese leicht durch andere Männchen werden. Darüber hinaus hängt die Fitness der weiblichen Tiere stärker von der Futtersuche ab, als die der Männchen. Weibliche Nager haben einen erhöhten Bedarf an Nahrung und Energie während der Trächtigkeit und Laktation (Migula, 1969). Tran and Hinds (2012) zeigen in ihrer Studie, dass verschiedene Pflanzenextrakte die Fortpflanzung bei weiblichen Nagern beeinträchtigen. Sie identifizierten 13 Pflanzenextrakte, die die Anzahl und Art der Eifollikel reduzierten und Zyklusstörungen verursachen. Weibliche Tiere können fortpflanzungshemmende Substanzen in Pflanzen erkennen, und meiden diese. Während der vier Wochen Versuchsdauer in den Außengehegen (Feldmäuse im Juli 2013; Hausmäuse im

September 2013) und eine Woche danach, konnte keine Reproduktions-Aktivität, sprich keine Trächtigkeit und keine Nachkommen bei beiden Nagetierarten beobachten werden. Um diese Hypothese zu bestätigen, sind allerdings weitere Außengehege- und/oder Feldversuche erforderlich, um den Einfluss von pflanzlichen Verbindungen auf die Reproduktion von Nagetieren zu untersuchen.

Kleinsäuger, wie Nagetiere gehören in das Beutespektrum von Greifvögeln und Füchsen. Antikoagulante Rodentizide, die als gezielte Bekämpfungsmaßnahme von Schadnagern in der Landwirtschaft eingesetzt werden, hemmen die Blutgerinnung und führen damit zu inneren Blutungen, auch bei sogenannten Nicht-Zielarten. Untersuchungen belegen, dass antikoagulante Rodentizide zu einer sekundären Vergiftung bei Nicht-Ziel-Prädatoren führen, die vergiftete Beutetiere gefressen haben (z. B. Fournier-Chambrillon et al., 2004; Geduhn et al., 2016; Shore et al., 1999). Pflanzliche Gerüche als Repellentien können eine gute Alternative zu antikoagulante Rodentiziden sein, da die Wirkstoffe in der Regel nicht vom Zielorganismus aufgenommen werden, sondern durch den Geruch wahrgenommen werden. Die Anwendung von diesen Repellentien im Nagermanagement könnte somit eine umweltfreundlichere Alternative zu den chemischen Bekämpfungsmitteln sein, auch hinsichtlich der Zunahme negativer Auswirkungen auf die Umwelt und der genetischen Resistenz einiger Nagetiere gegen einige antikoagulante Rodentizide. Um eine Verringerung der negativen Auswirkungen von Rodentiziden auf Nicht-Zielarten durch die Verwendung von Repellentien in Form von pflanzlichen Verbindungen beim Einsatz im Nagermanagement zu belegen, bedarf es weiterer Untersuchungen.

Abschließend zeigt diese Arbeit, dass der Geruch von pflanzlichen Verbindungen als Repellentien bei beiden Nagetierarten wirkt. Es hat sich herausgestellt, dass besonders ätherische Öle oder Terpenoide eine hohe Wirksamkeit zeigen. Vorversuche haben gezeigt, dass die Lösungsmittel allein einen negativen Effekt auf die Nahrungsaufnahme haben. Folglich könnte die abschreckende Wirkung der getesteten pflanzlichen Verbindungen in der Anwendung höher sein. Im nachfolgenden Kapitel wird die bestehende Literatur zu pflanzlichen Verbindungen als Repellentien gegen Nagetiere hinsichtlich einer optimalen Untersuchungsmethode evaluiert. Weiterhin stellte diese systematische Literaturübersicht dar ob es geeignete PSM Gruppen als Repellentien gegen Nagetiere gibt. Abschließend werden die Studien mit möglichen Repellentien (inklusive meiner) hinsichtlich einer möglichen Anwendung in einem umweltfreundlichen Nagermanagement diskutiert.

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Plant Secondary Metabolites as Rodent Repellents: a Systematic Review

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Abstract The vast number of plant secondary metabolites (PSMs) produced by higher plants has generated many efforts to exploit their potential for pest control. We performed a systematic literature search to retrieve relevant publications, and we evaluated these according to PSM groups to derive information about the potential for developing plant-derived rodent repellents. We screened a total of 54 publications where different compounds or plants were tested regarding rodent behavior/metabolism. In the search for widely applicable products, we recommend multi-species systematic screening of PSMs, especially from the essential oil and terpenoid group, as laboratory experiments have uniformly shown the strongest effects across species. Other groups of compounds might be more suitable for the management of species-specific or sex-specific issues, as the effects of some compounds on particular rodent target species or sex might not be present in non-target species or in both sexes. Although plant metabolites have potential as a tool for ecologically-based rodent management, this review demonstrates inconsistent success across laboratory, enclosure, and field studies, which ultimately has lead to a small number of currently registered PSM-based rodent repellents.

Keywords Essential oils · Herbivory · Plant secondary metabolites · Rodentia · Repellents · Review · Vertebrate pest management

Introduction

Satisfying the dietary needs of the world's growing population in the twenty-first century requires an increase in agricultural production. In 2014, about 2.5 billion tons of grains were harvested worldwide, with only 43 % of the crop used as food because grain also is processed for animal feed, fuel, and industrial raw materials. Today, about one third of the Earth's land mass is used for agriculture (http://data.worldbank.org/). Data from the Food and Agriculture Organization of the United Nations demonstrate that 3 % of the globe's land surface (13 billion ha) is used for permanent crops (Roser 2016). To elevate food production in accord with population growth, the utilization of existing agricultural land needs to be optimized, for example, by minimizing the effect of disease and crop pests.

Significant agricultural pests include arthropods and vertebrates, especially rodents. The number of rodent species causing problems that require management is small. Only about 5 % of rodent species worldwide pose a significant risk to humans (Singleton et al. 2007). These species include commensal rodents that are present close to man-made infrastructure, such as Norway rats (*Rattus norvegicus*), black rats (*R. rattus*), and house mice (*Mus musculus*), as well as field rodents including ricefield rats (*R. argentiventer*), multimammate mice (*Mastomys* spp.), and the lesser bandicoot rat (*Bandicota bangalensis*) that can cause chronic damage (Buckle and Smith 2015). In some cases, damage only occurs when massively overabundant populations build up during population outbreaks in temperate regions (Delattre

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et al. 1992; Singleton and Brown 2005; Singleton et al. 2007) and in the tropics (Doungboupha et al. 2003; Leirs et al. 1997).

Adverse effects of rodents are manifold and include pre-harvest crop damage, and post-harvest damage to stored products and infrastructure. Pre-harvest rodent damage is particularly prevalent in Asia and Africa (Singleton et al. 1999). Pre-harvest losses in Asia result in a reduction in rice yield of 5–20 % (Singleton et al. 2003). This equals an annual loss of 77 million tons (John 2014) – enough food to feed 200 million people for a year (Singleton et al. 2003). Population densities of both house mice in Australia and common voles (*Microtus arvalis*) in Europe can exceed 2000 animals per hectare (Bryja et al. 2005; Saunders 1983). In such a scenario, pre-harvest damage in Europe probably tops several hundreds of millions of Euros (€) (Jacob et al. 2014) and 60 million US$ in Australia (Brown et al. 2000). Outbreaks of bamboo rats in Asia have more dramatic effects because food competition of rodents with livestock and people that can result in widespread famine (Singleton et al. 2010). Post-harvest losses occur when commensal rodents consume stored goods or through the contamination of produce. Rodent damage to infrastructure by musk rats (*Ondatra zibethicus*) is estimated at about 599 million € and 3.35 billion € by *Rattus* spp. per year in the EU (Kettunen et al. 2009).

In addition, rodents contribute to health problems for humans, livestock, and companion animals through the transmission of zoonotic disease (Meerburg et al. 2009). Pathogens include viruses: e.g., hantavirus, tick-borne encephalitis virus, hepatitis E virus; bacteria (e.g., *Leptospira*, *Borrelia*, *Rickettsia*) and parasites (e.g., toxoplasmosis, giardia infection, echinococcus infection) (Meerburg et al. 2009). The cost associated with the transmission of rodent-borne diseases is assumed to be similar to the losses due to rodents in plant production (Bordes et al. 2015). The annual cost of rodent related pre-harvest and post-harvest damage, losses of stored goods, and expenditure for disease prevention and treatment is likely to exceed 23 billion US$ (Jacob and Buckle 2016).

Due to the adverse effects of rodents on plant production and public health, rodent management is critical in many cropping systems and in urban situations around the world (Buckle and Smith 2015). Although large-scale measures of rodent management in an agricultural context include agricultural practices that render habitat temporally unsuitable for rodents (e.g., ploughing) and bio-control (e.g., promoting predation), they rely mainly on the application of rodenticides to reduce damage to crops. For the management of commensal rodents in urban and rural areas, anticoagulant rodenticides often are the weapon of choice, although the increased occurrence of resistant populations poses an enormous challenge to future strategies. A major problem caused by the use of poison is the risk to non-target species either by direct ingestion of poisonous bait or indirectly through the consumption of poisoned prey or carrion. There are reports of secondary poisoning caused by anticoagulants worldwide (e.g., Eason et al. 2002; Geduhn et al. 2015) that are of conservation and biodiversity concern.

The problems associated with the use of poison to manage populations and efforts to increase efficacy of rodent control have culminated in a call for ecologically-based rodent management (Singleton et al. 1999) that uses a toolbox of techniques that are ecologically, socially, and economically appropriate.

Potential of Plant Secondary Metabolite as Rodent Control Tools

One of the promising rodent control tools is the application of plant secondary metabolite (PSM) odor mixtures that deter rodents from feeding on crops or destroying infrastructure. Despite numerous candidate compounds that affect individual fitness (Ostfeld and Canham 1995), reproduction (Diawara and Kulkosky 2003), or behavior (Takahashi and Shimada 2008), the development of products for field application is hampered by a complex mosaic of confounding factors, recently laid out by DeGabriel et al. (2014). These factors include profound differences in adaptations of different herbivore species to different PSMs. These differences reach even higher complexity because of differences in sex, age, and reproductive status of the animals, and may vary seasonally with the spatial and temporal availability of food and changes in food chemical composition (DeGabriel et al. 2014).

On small scales, repellents based on PSMs that are less toxic than rodenticides may provide a viable, cost-effective, and ethically acceptable alternative to the use of poison or labor intensive trapping. For example, Epple et al. (1996) successfully repelled pocket gophers (*Geomys bursarius*) from gnawing on cables by using pine needle oil, which also has potential as a commercial repellent for snowshoe hares and voles (Bell et al. 1987). Willoughby et al. (2010) used capsaicin to deter wood mice (*Apodemus sylvaticus*) from consuming valuable tree seeds.

Some compounds are regarded as highly toxic (e.g., cardenolides common in Plantaginaceae, alkaloids in Solanaceae, cyanogenic glucosides in Fabaceae), while others have negative impacts on fecundity (e.g., plant steroids in Ranuculaceae) or on various compounds that affect enzymes (e.g., polypeptides and non-protein amino acids in Fabaceae). If such compounds are ingested, there may be negative effects on predators or on the environment similar to anticoagulant rodenticides. To prevent such negative unintentional effects, less harmful compounds that function before or shortly after ingestion by their odor (e.g., terpenoids or glucosinolates) or taste (e.g., tannins, low molecular phenolics) seem to be more promising substances. Some compounds, especially those with a characteristic repellent odor (essential oils and terpenoids), may be useful for prevention against rodent damage, because these odors disseminate through the environment without further action required. Such sensory repellents are highly volatile and affect the mucous membranes of the eyes, nose, mouth, and throat of animals before feeding (Mason et al. 1996). Taste-based plant-derived repellents generally include a bitter or hot-tasting ingredient that affects the same membranes, but they act after ingesting.

There also are described predator odors that are repellent to rodents (Nolte et al. 1993, 1994a, b), especially when they match the odors of the target species' natural predators (Apfelbach et al. 2005). Mostly, compounds from urine, feces, and anal gland secretions are used, but these products are difficult to obtain, and often commercial products are based on chemicals that contain sulfur (Wagner and Nolte 2001), which seem to trigger avoidance behavior in rodents. There have been some applications of such products for the protection of crops and other resources. Essential oils and terpenes may function similar to "predator odors" as post-ingestive repellents because they cause rodents to become place aversive or food aversive. Phenols can be used only as food aversive, as the animal has to ingest them first and then be repelled by the taste.

Modes of Action and Adaptation Several modes of action determine the effectiveness of PSMs in repelling herbivores. Pre-ingestive effects cause deterrent impact on animals before the food item is ingested. These are due mainly to the odor of volatile compounds (e.g., terpenoides). Deterrent effects caused by taste (e.g., bitterness of low molecular phenolics, alkaloids, or astringency of tannins) usually are regarded as post-ingestive effects. Some of the latter have negative impact on the digestion of nutrients or on the bacterial community of the gastro-intestinal tract (e.g., tannins, terpenoides, pre-absorptive effects), while others have negative impact due to their toxicity (e.g., cardenolides post-absorptive effects) with far reaching physiological consequences (Palo and Robbins 1991).

Herbivores have evolved adaptations in feeding behavior that avoid malaise or toxication caused by PSMs. Individual responses to PSMs are governed by several mechanisms (reviewed by Dearing et al. 2005) and include conditioned aversion that dictates how the individual will react to the compound at the next encounter (Baker et al. 2007). Animals can learn to associate taste with discomfort, which leads to reduced food intake. This process is known as conditioned food aversion (Garcia et al. 1955). It has been demonstrated in different herbivorous mammals, e.g., house mice (Watkins et al. 1998), goats (Provenza et al. 1990), and sheep (Kyriazakis et al. 1998). Simply avoiding or reducing consumption of plants or parts of plants with particular compounds is the most obvious herbivore behavior (Marsh et al. 2006; Wiggins et al. 2003). Another is choosing plants with low toxin concentrations (Moore and Foley 2005; Stolter et al. 2005, 2013). Still another is decreasing the feeding rate when exposed to the odor of a non-preferred food plant, (pre-ingestive effect, Edlich and Stolter 2012; Hansen et al. 2015, 2016) or to increased concentrations of PSMs (Stapley et al. 2000; Wiggins et al. 2003). Negative physiological consequences of compounds also affect the food intake of an animal via a feedback loop. Enhanced plasma concentration of the toxin (McLean et al. 2007), activation of the emetic system (Provenza et al. 1994), or possibly acidosis (Foley 1992) can lead to negative consequences. Consequently, animals can learn through physiological feedback to manage intake of plants with toxic metabolites.

Metabolism and excretion, the physiological processes used to eliminate ingested compounds from the body, are better adapted in animals with a high PSM intake (e.g., specialists and browsers) than in generalists and grazers, which encounter only a limited amount of PSMs (Iason and Villalba 2006). For example, enzymes metabolize toxins into products for rapid excretion (McLean and Duncan 2006). Thus, herbivore specialists, such as koalas [*Phascolarctos cinereus* (Marsh et al. 2007)] or woodrats [*Neotoma* spec. (Sorensen et al. 2005)] can cope with higher toxin concentrations than generalists either through different feeding behavior or by anatomical and physiological means (Marsh et al. 2003). The ability of herbivores to reduce the absorption of toxins via the gut is well known to pharmacologists (Wagner and Nolte 2001) but such studies are under-represented in the plant-animal scientific literature.

In this review, we systematically scanned the literature for PSM-based principles of plant defenses against rodents. We evaluated this information to highlight common features of compounds that are efficient in repelling rodents, and we discuss where to focus future work to develop effective repellents based on PSMs.

Methodology

Literature Search We performed a systematic literature search to retrieve relevant publications from the ISI Web of Science (WoS) scientific publication database via the Thomson Reuters Web of Knowledge platform (http://www.webofknowledge.com). Within the platform, we included six databases (Web of Science ™ Core Collection, Biological Abstracts ®, CABI: Cab Abstracts®, FSTA®- the food science resource, KCI- Korean Journal Database, and SciELO Citation Index). We considered publications associated with "rodents and repellents and with plant secondary metabolites", from documents published January 1910 to January 2016. No language restrictions were applied. The following topics were used to search titles abstracts and keywords: Topic 1: rodent* or mice or mouse or rat* or vole or squirrel or beaver or gopher or hamster or dormice or "guinea pig"; and Topic 2: repel* or repellent or avoid* or deterrent or antifeedant or defense; and Topic 3: plant secondary metabolite or plant secondary compound or secondary plant metabolite. The search was run on 14th January 2016. The initial database search yielded 977 publications.

Selection of References For identifying the relevant studies, one author (SH) reviewed all titles and abstracts that resulted from the search. From these, 917 publications were rejected on initial screening because title and/or abstract indicated that the publication did not meet the topics. The evaluation of the full text articles was conducted by SH, CS, and CI to further refine results based on the PSM group considered in the publication. After evaluation of the full text, six further publications were excluded because they were not related directly to rodent feeding behavior. In total, 54 relevant studies were included in the systematic review that belonged to eleven PSM groups (Fig. 1). The publications were organized according to PSM group (Fig. 1), compound, or plant of concern, animal species, reference and year of publication, type of study (laboratory, enclosure, field), and major result regarding repellent properties (Supplementary Table S1).

Summary of Chemical Results

The literature search showed that there has been increased scientific interest in PSM application in rodent management during the last 15 years. This may be a result of increasing conservation and ethical concerns regarding the use of lethal methods and associated economic, public, and political pressure to develop suitable alternatives. The main focus of all studies has been the effect of potential plant-derived repellents on rodent feeding behavior.

Effectiveness of PSMs

Plants and Plant Materials

Some studies have aimed to quantify the effect of PSMs on feeding and some to examine the exact mechanisms for these plant-animal interactions by using different methodological approaches. In 19 of 22 studies, fresh parts of plants were offered directly to animals; in four, food intake was analyzed through manipulated diets. There were only two studies that identified specific plants that act as feeding deterrent (Curtis et al. 2002; Dearing et al. 2001) and 12 where plant material had an effect on rodents (Table 1; Supplementary Table S1). Apart from studies that used whole plants or plant material, several groups of compounds have been considered in rodent research. They include essential oils and terpenoides, alkaloids, alkylamides, (di)carboxylic acids, glucosinolates, and phenolics.

Essential Oils and Terpenoides

This diverse group includes compounds from a range of plant species that were studied almost exclusively in laboratory experiments (Table 2; Supplementary Table S1). Epple et al. (1996), for example, used pine needle oil, containing α- and β-pinene, to repel pocket gophers from gnawing on cable insulation. Kelsey et al. (2009) demonstrated the avoidance of PSMs by rodents by the removal of needle resin ducts before consumption. Fischer et al. (2009, 2010, 2011a, b, 2013a) reported that a multitude of terpenes (as well as ketones) prominent in essential oils have a repelling effect in a laboratory two choice test (e.g., geranium oil, black pepper oil), although combinations of both substances did not increase the repelling effect. Similar results were achieved by Hansen et al. (2015) who identified four plant odors of essential oils (black pepper, bergamot, fennel, neem) that repelled common voles. Three of the tested essential oils also repelled house mice (bergamot, bucco, fennel) under laboratory conditions (Table 2). In a field trial, Fischer et al. (2013b) demonstrated that terpenoides can be used in an outdoor application to repel the fossorial common vole from experimental plots. The observed effect lasted for about four to five days.

Alkaloids, Alkylamids

Guimarães et al. (2003) found a repelling effect of the total quinolizidine alkaloids (QA) of *Ormosia arbora* seeds in a field experiment. Feeding trials by Janzen et al. (1990) revealed only a mild effect of polyhydroxypyrrolidine alkaloid (DMDP) found in seeds of *Lonchocarpus* species in the Brazilian rainforest (Table 3; Supplementary Table S1). Alkylamides from extracts of Szechuan pepper (*Zanthoxylum piperitum*) had a strong post-ingestive repelling effect on Norway rats (Epple et al. 2001) (Table 3; Supplementary Table S1).

(Di) Carboxylic Acids

Fanson et al. (2008) demonstrated that several environmental factors influence the foraging behavior of two African rodents. Oxalic acid treated popcorn kernels were avoided by woodland thicket rats (*Grammomys dolichurus*) and Cairo spiny mice (*Acomys cahirinus*) (Supplementary Table S1).

Glucosinolates

This group has been intensively studied in laboratory trials by Samuni-Blank et al. (2012, 2013a, b, 2014). They elucidated different aspects of the multi-layered effects of glucosinolates

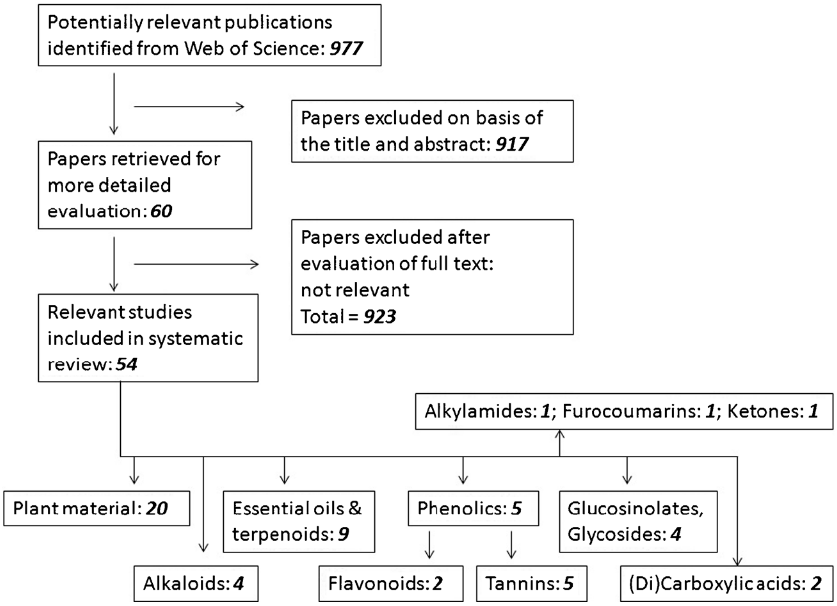

Fig. 1 Flow chart of publication selection process (adapted from Van Cauwenberghe et al. 2010)

(GLS) in the seeds of the *Ochradenus baccatu* tree. Glucosinolates are found in many plant families, and usually are thought to have little repellency. However, as part of a compartmentalized defense system that is activated by damage to plant tissue and subsequent hydrolysis of GLS, several toxic compounds are released that affect herbivores. This "mustard bomb" has been demonstrated to be effective in its interspecific repelling effect, although there are species-specific evolutionary adaptations to this defense mechanism (Samuni-Blank et al. 2013a). While seed predators show an increased physiological tolerance towards the released toxins, others have developed behavioral responses and avoid damaging intact seeds (Samuni-Blank et al. 2013a). In this series of publications, the authors have highlighted the ecological divergence in responses to plant defense mechanisms (Supplementary Table S1).

Phenolics – Low Molecular Phenolics and Flavonoids

Seven specific compounds, two phenolic groups (stilbenes, phenolic resins), and total phenolics have been tested against nine rodent species (Table 4; Supplementary Table S1). For three compounds, a negative impact on feeding behavior was reported (cinnamamide, anthraquinone, and one unknown). The results show deterrent effects on female common voles but not on male voles or house mice (Hansen et al. 2015, 2016). These results underpin the necessity of validating effects for all target species and sexes. Cinnamamide showed a strong repellent effect against wild Norway rats (Crocker et al. 1993) and is known as 'non-lethal mouse repellent' (Gurney et al. 1996) because of its low toxicity. Other studies have confirmed the repellent effect of cinnamic aldehyde against house rats (Babbar et al. 2015). It could not be determined whether the specific mechanism of the cinnamic aldehyde effect is via taste or odor. Despite this fact, these compounds should be useful in rodent management for protecting crops, stored products, and farm structures where other pesticides would be too hazardous. All the other tested compounds (e.g., salicortin, salicin) showed no repellent effects. Additionally, stilbenes and creosote resin are reported to have negative effects on field voles (*Microtus agrestis*) and woodrats (*Neotoma lepida, N. stephensi, N. albigula*), and the concentration of total phenolics seems to be important for the avoidance of these plants by *Microtus oeconomus* (Dai et al. 2014) (Table 4). Bergeron and Jodoin (1991) tested quercetin with *Microtus pennsylvanicus,* and Basey et al. (1988, 1990) tested tremulacin and tremuloidin with *Castor canadensis.* These studies showed no repellent effect of these specific flavonoids. The secondary metabolites of *Lonchocarpus*, however, contain seven types of flavonoids, which were found to be responsible for the strong avoidance of *Lonchocarpus* seeds by spiny pocket mice (Janzen et al. 1990).

Phenolics – Tannins

Of the nine studies dealing with tannins, seven were conducted with the hydrolyzable tannin, tannic acid, using eight rodent species (Table 5; Supplementary Table S1). Four were conducted as laboratory experiments; the others were field studies, and all of them used artificial food. Due to the fact that tannic acid has a negative post-ingestive effect it has been assumed that rodents should decrease their food intake when exposed. However, only one of the experiments (Bozinovic 1997 with *Octodon degus* and *Phyllotis darwini*) demonstrated explicit repellent results. Two studies used plant materials

Table 1 Effect of plant material reported in 20 of 54 studies based on laboratory experiments (L), enclosure experiments (E) or field experiments (F)

Compound(s) / Plant(s)	Animal species	Author	Year	L/ E/ F	Result
Betula pendula	*Microtus agrestis*	Rousi et al.	1993	L	Fertilization increase & shading decreased palatability of seedling bases
Betula pendula	*Microtus agrestis*	Tiainen et al.	2006	E	No effect
several *Betula* species	*Microtus agrestis*	Laitinen et al.	2004	E	Birch species differed in general susceptibility to browsing
Capsicum spp.	Mammals	Levey et al.	2006	F	Deterred by naturally occurring fruits
Carex aquatilis, *Salix pulchra*, *Ledum palustre*	*Lemmus sibiricus*, *Dicrostonyx torquatus*, *Microtus oeconomus*	Jung and Batzli	1981	L	No effect; *L. palustre* toxic
Several plants	*Microtus ochrogaster*	Curtis et al.	2002	L	Repellent effect*
Helianthus annuus, *Arachis hypogaea Juglans regia*	*Rattus norvegicus*	Grant-Hoffman and Barboza	2010	L	Repellent effect to walnuts
Helleborus foetidus	*Apodemus sylvaticus*	Fedriani and Boulay	2006	L	Repellent effect of unripe fruits
Quercus rotundifolia, Quercus faginea, Quercus suber	*Apodemus sylvaticus*	Rosalino et al.	2013	E	Repellent effect of acorn with tannins
Juniperus monosperma	*Neotoma stephensi, N. albigula*	Dearing et al.	2001	L	Repellent effect*
Juniperus monosperma	*Neotoma stephensi, N. albigula*	Boyle and Dearing	2003	L	No effect
Several plants	*Neotoma fuscipes*	Brooke McEachern et al.	2006	L	Repellent effect of novel-chemically-defended plants
Several plants	*Microtus ochrogaster*, *M. pennsylvyticus*	Lindroth and Batzli	1986	E/L	No intake effect, reduced growth rate
Picea abies, *Abies alba*	*Sciurus vulgaris*	Rubino et al.	2012	F	Limonene in fir seeds seemed to act repellent
Several plants	*Microtus agrestis*, *Clethrionomys glareolus*	Vehvilainen and Koricheva	2006	F	Target tree species mixed with a less palatable species, damage is reduced
Several plants	*Microtus arvalis orcadensis*	Hartley et al.	1995	L	Voles can detect variation in chemical composition
Pinus sylvestris	*Clethrionomys glareolus*	Iason et al.	2011	E	No effect
Several *Salix* species	*Myodes glareolus*	Shaw et al.	2013	F	No effect
Several plants	*Geomys attwateri*	Rezutek and Cameron	2011	F	No effect
Several plants	*Microtus agrestis, Clethrionomys glareolus*	Hjältén and Palo	1992	F	Repellent effect of high nitrogen concentrations

*to a experimental control

Table 2 Effect of essential oils and terpenoids reported in 10 of 54 studies based on laboratory experiments (L) or field experiments (F)

Compound(s) / Plant(s)	Animal species	Author	Year	L/ E/ F	Result
α-pinene, β-pinene, myrcine	*Geomys bursarius*	Epple et al.	1996	L	Repellent effect*
α-pinene	*Neotoma stephensi, N. albigula*	Sorensen and Dearing	2003	L	No effect
Black pepper oil (BPO), bergamot oil, buchu oil, fennel oil, grass-tree oil, (*R*)-(+)-Limonene, neem oil	*Microtus arvalis, Mus musculus*	Hansen et al.	2015	L	Repellent effect in voles*: BPO, bergamot oil, fennel oil, neem oil; repellent effect in mice*: bergamot oil, bucco oil, fennel oil
Black pepper oil, Chinese geranium oil, onion oil	*Arvicola amphibius*	Fischer et al.	2009, 2010, 2011a, b; 2013a, b	L	Repellent effect*
Monoterpene, sesquiterpene, diterpene from *Pseudotsuga menziesii*, *Tsuga heterophylla* and *Picea sitchensis*	*Arborimus longicaudus*	Kelsey et al.	2009	F	Resin avoided by removing ducts

*to a experimental control

containing tannins that were tested against *Apodemus speciosus* and *Microtus oeconomus*. They either produced ambiguous results or showed no effects. Tannins, especially "condensed tannins", are complex molecules, and nearly every deciduous plant species has a specific pattern. This complexity hampers testing tannins as possible feeding deterrents because specific compounds are often unknown and not available on the market.

Differences in Experimental Design and Impact on Results

Of the 54 relevant references identified in our systematic literature search, there were 32 cage studies, 6 studies in outdoor enclosures, and 13 in the field. In three studies, rodents were not involved directly in any experiments (Kelsey et al. 2009; Levey et al. 2006; Rubino et al. 2012) but since they were based on feeding observation of animals that avoided certain plants they were considered in the review.

Laboratory Studies Laboratory cage studies allow the screening of compounds/concentrations with relatively small numbers of animals, and they can yield rapid results. A comparison of laboratory studies shows considerable differences in methodology in regard to sample size. Sometimes sample size was unspecified (Diawara and Kulkosky 2003; Schmidt et al. 1998; Tiainen et al. 2006), or minimal ($N = 3$ of one sex, Dai et al. 2014). Sometimes only females were considered because of their importance in population dynamics (Diawara and Kulkosky 2003; Hansen et al. 2015). Most studies, however, included both sexes, although sex-specific effects were not always reported (Bergeron and Jodoin 1991; Boyle and Dearing 2003; Curtis et al. 2002; Dai et al. 2014a; Epple et al. 1996; Fedriani and Boulay 2006). However, such reporting is confounding because males and females may react differently to PSMs (Hansen et al. 2016; Shumake and Hakim 2000;), and combining data for males and females masks sex-specific effects.

Secondary metabolites often are investigated by providing either fresh or thawed plants, or plant parts, or food manipulated with metabolites to experimental animals. Feeding experiments usually are carried out in no-choice, two-choice, or cafeteria feeding experiments. Generally, we prefer multiple choice tests, as animals almost always have a choice of several food sources in nature. In no-choice tests, where the only

Table 3 Effect of alkaloids, alkylamides reported from 5 of 54 studies based on laboratory experiments (L) or field experiments (F)

Compound(s) / Plant(s)	Animal species	Author	Year	L/ E/ F	Result
Caffeine, rauwolfia alkaloids	Laboratory ICR Swiss mouse	Freeland and Saladin	1989	L	No effect
Gramine	*Microtus pennsylvanicus*	Bergeron and Jodoin	1991	L	No effect
Polyhydroxypyrrolidine alkaloid (DMDP)	*Liomys salvini*	Janzen et al.	1990	L	Mild repellent effect*
Quinolizidine alkaloids (QA); sparteine, angustifoline, lupanine, ormosanine, panamine	*Dasyprocta leporina*	Guimarães et al.	2003	F	Repellent effect for seeds with QA
Zanthoxylum piperitum	*Rattus norvegicus*	Epple et al.	2001	L	Strong post-ingestive repellent effect*

*to a experimental control

Table 4 Effects of phenolics (low molecular phenolics and flavonoids) reported in 8 of 54 studies based laboratory experiments (L) or field experiments (F)

Plants/ Compounds	Animal species	Author	Year	L/E/ F	Result
Anthraquinone	*Microtus arvalis, Mus musculus*	Hansen et al.	2015	L	Repellent effect in female common voles*
Cinnamic acid, Cinnamamide, ferulic acid	*Rattus norvegicus*	Crocker et al.	1993	L	Repellent effect of cinnamide
Creosote resin (phenolic resin)	*Neotoma lepida*	Meyer and Karasov	1989	L	Repellent effect*
Creosote resin (phenolic resin)	*Neotoma stephensi, Neotoma albigula*	Torregrossa et al.	2012	L	Repellent effect*
Several plants	*Microtus oeconomus*	Dai et al.	2014	L	Repellent effect total phenolics
Salicin, salicortin	*Castor canadensis*	Basey et al.	1988	F	Site dependent effect
Salicin, salicortin Unknown phenolic	*Castor canadensis*	Basey et al.	1990	F	Repellent effect of unknown compound of plant regrowth
stilbenes	*Microtus agrestis*	Virjamo et al.	2013	(F)	Repellent effect for higher concentrations of stilbene

*to a experimental control

option is to eat or to starve, animals can overcome their initial reluctance and learn to accept foreign odors and mild adverse effects of PSMs (Gurney et al. 1996).

Enclosure Studies Enclosure experiments are conducted under conditions resembling the natural environment more closely than laboratory cage trials and may provide more robust results. However, they are time consuming and require appropriate infrastructure. Most outdoor enclosure experiments have focused on rodent damage to seedlings, assessing the effect of culture conditions (Iason et al. 2011; Laitinen et al. 2004), replanting (Virjamo et al. 2013), or seedling size (Tiainen et al. 2006).

Field Studies Field experiments should yield the ultimate information whether a particular compound or a combination of compounds effectively repels rodents, but they require substantial effort and are subjected to more variability than cage and enclosure trials. Field methods range from observational studies (Basey et al. 1988; Guimarães et al. 2003; Levey et al. 2006; Rubino et al. 2012), manipulated field plots (Hjältén and Palo 1992; Rezsutek and Cameron 2011; Shaw et al. 2013), and feeding experiments (Barthelmess 2001; Basey et al. 1990; Fanson et al. 2008; Samuni-Blank et al. 2012; Schmidt et al. 1998) to the application of product prototypes, e.g., methyl nonyl ketone (Fischer et al. 2013b).

Methodology Recommendations Differences in methodology are due primarily to differences among target species, management systems, and based on the general aim of the study that is related to rodent management. However, some general strategies for screening potential PSM are possible. First, wild caught (DeGabriel et al. 2014) or possibly the F_1 generation males and females of the target rodent pest species should be tested in laboratory cage trials. Second, promising plant-derived repellents need to be tested under semi-natural conditions, and if successful, in the field with the specific target species. We recommend this sequential approach of cage – enclosure – field trials, and only continuing the study with a particular metabolite when results are positive. Lindroth and Batzli (1986), however, used the reverse approach, starting with field work, continuing with plant analyses, enclosure

Table 5 Effect of tannins reported in 9 of 54 studies based on laboratory experiments (L) or field experiments (F)

Compound / Plant	Animal species	Author	Year	L/ F	Result
Tannic acid	*Octogon degus*	Bozinovic et al.	1997	L	No effect
	Grammomys dolichurus; *Acomys cahirinus*	Fanson et al.	2008	F	No effect
	Mus musculus	Freeland and Saladin	1989	L	No effect
	Sciurus niger, *Sciurus carolinensis*	Schmidt et al.	1998	F	Middle deterrent effect
	*Sciurus carolinensi*s	Barthelmess	2001	F	Concentration dependent negative effect
	Microtus pennsylvanicus	Bergeron and Jodoin	1991	L	No effect
	Octogon degus, *Phyllotis darwini*	Bozinovic	1997	L	Repellent effect*
Quercus crispula	*Apodemus speciosus*	Takahashi and Shimada	2008	L	Experience dependent negative effect
Several plants	*Microtus oeconomus*	Dai et al.	2014	L	No effect

* to a experimental control

trials and finally laboratory feeding trials to examine differences in plant-animal relationships.

Caveats - Feeding Behavior and Impact on Efficacy Among mammalian herbivores are specialists that feed on a single host plant or on a limited number of species, and generalists that can feed on various plant species. Therefore, it is perhaps easier to find plant-derived repellents against specialists, as they are restricted to a limited range of food by nature. For a generalist, however, it is more difficult to find plant-derived repellents, as they are exposed to a range of PSMs and therefore may be adapted to possible repellents.

Additionally, sensitivity to odors differs among rodent species (Apfelbach et al. 2005) and other herbivorous mammals, e.g., black-tailed deer [*Odocoileus hemionus* (Rice and Church 1974)], is of coures is mirrored in differences in food choice. Another aspect that must be considered in examining foraging behavior is the different food requirements of females and males. Food quality and quantity have a stronger influence on feeding strategies of females (Ostfeld and Canham 1995), and pregnant or lactating females that have higher energetic demands (Jacob et al. 2006). Thus, effects of food quality on reproductive output and hence fitness are more pronounced than in males. However, in the establishment of new infestations, males play a major role because particularly juvenile males disperse in search of new territories and resources (DelBarco-Trillo et al. 2011).

Which Plant Secondary Metabolite Groups Have Potential as Rodent Repellents?

The most promising groups, which may act across rodent species, are the essential oils and terpenoids. Effective pre-ingestive repellent properties have been identified (e.g., for pine needle oil, terpenoides) that repel pocket gophers, voles, red tree voles (*Arborimus longicaudus*), and house mice (Table 2). As already stated above, these pre-ingestive effects are due to their volatility resulting in characteristic deterrent odors.

The complex olfactory system of rodents is important and used in foraging, predator avoidance, and social interactions (Howard and Marsh 1970). In mammals, two olfactory pathways are influenced by odor. The direct route triggers immediate feeding behavior through avoidance, (Hansen et al. 2015, 2016), and the indirect route acts through endocrine activity via the central nervous system e.g., as pheromones that inhibit reproduction (Stowers and Liberles 2016). Compounds should be preferred that affect the behavior of rodents directly.

Species-specific compounds and mixtures include for example: Quinolizidine alkaloids (QA) in agoutis (Guimarães et al. 2003) and dihydroxymethyldihydroxypyrrolidine (DMDP) together with several flavonoids in pocket mice (Janzen et al. 1990); two flavonoids, kaempferol-3-O-β-glucoside and quercetin-3-O-β-glucoside, in Sprague-Dawley rats (Halaweish et al. 2003); oxalic acid in African rodents (Fanson et al. 2008) and squirrels (Schmidt et al. 1998); and glucosinolates in African spiny mice (Samuni-Blank et al. 2013a).

Alkylamides from the fruit of *Xanthoxylum* produce a strong tingling sensation in the mouth and work as a feeding repellent through taste. Cinnamamide, a synthetic derivative of cinnamic acid, is known as a post-ingestive repellent for birds and mammals (Gill et al. 1995) as well as slugs (Watkins et al. 1996). Further investigations on the deterrent effects of the "phenolics group" seem promising, especially low molecular specific phenolics (Cheynier et al. 2013; Virjamo et al. 2013), which are easily extracted from plant material. Their mode of action is via taste and hence, the compounds have to be eaten. As post-ingestive compounds they are suitable for rodent management only in situations where some damage is acceptable. Additionally, they also may negatively affect non-target species when consumed and lead to death (post-absorptive effect) similar to rodenticides (Geduhn et al. 2014).

There are other PSM groups that are part of plant defense mechanisms but were not detected in our literature review. For example, non-protein amino acids are known for their repellent effect on non-specialist herbivores often have deleterious effects on many animals (Bennett and Wallsgrove 1994; Levin 1976). Cyanide and cyanogenic glycosides have acute toxic as well as chronic effects and lead to the death of non-adapted animals (Seigler 1991). Coumarins and furanocoumarins occur in many plants and are toxic in low concentrations to rodents (Berenbaum 1991) and all other warm-blooded organisms. This group already is known in rodent management, because their derivates are used for anticoagulant rodenticides that inhibit blood coagulation (Rosenthal and Berenbaum 1991; Valchev et al. 2008).

When comparing the most promising rodent PSM groups found in the literature to other classes of pest species considerable overlap becomes apparent. Worldwide, insect pests are responsible for pre-harvest loss of 8–15 % of wheat, rice, maize, potato, soybean, and cotton (Oerke 2006) and for up to 10–40 % of food grains loss in granaries and storehouses (Upadhyay and Ahmad 2011). In their review of PSM-based insecticides, Adeyemi (2010) highlighted several potent anti-feedants, with various essential oils and terpenoids showing strong repelling characteristics as in rodents. The methodological approaches and problems are similar to those of rodent researchers who are struggling with the implementation of products for commercial use. These often are hindered by interspecific differences in compound efficacy, environmental pollution, negative effects on ton-target organisms, and costs of application. There are more registered products with natural active ingredients against insects available (see Nerio et al. 2010) than against rodents.

Commercial Products

Despite the extensive literature concerning PSMs for repelling rodents, there are just a few commercial products. Only cinnamon, methyl nonyl ketone (MNK), and pepper oil are available commercially. The registration of these compounds is based partially on the knowledge generated by studies mentioned in this review. Curcumol and triptolide are registered as plant source steriliants for rodent management in China (Huang 2014). Only one rodent repellent based on extracts from hot pepper (*Capsicum annuum*) is registered in China (Z. Zhang, personal communication). In Australia, two plant based products are registered as rodent repellents, i.e., 1. a mixture of white pepper (food flavor) and garlic oil (oil-plant extract), and 2. a mixture of corn mint oil, campher white oil, eucalyptus oil (all oil-plant extracts), and methyl salicylate (phenolic) (https://portal.apvma.gov.au; accessed 12 January 2016). Garbage bags are treated with the latter mixture to repel rodents. The latter mixture also is approved by the USA EPA, which also has authorized the use of products based on capsaicin (derivatives) and on balsam fir oil that is marketed as a botanical rodent repellent (npic.orst.edu/NPRO/;accessed 12 January 2016). Further products (exempt from registration) are available in the USA that are based on white pepper, capsaicin (DeTour for Rodents 2016), garlic, cinnamon, clove, white pepper, rosemary, thyme, peppermint (Nature's defense 2010), castor oil, rosemary oil, mint oil, garlic oil (Thorp 2011), and habañero peppers (Etscorn and Torres 1997).

In the EU, eight repellent or attractant compounds are registered for *biocidal use* against several vertebrate species (European Chemicals Agency's database; accessed 12 January 2016). One of them is methyl nonyl ketone – one of the few PSMs that has been shown to repel rodents under field conditions (Fischer et al. 2013b). However, no product has been registered specifically for rodent use based on this compound and hence no plant based repellent is available for rodents for biocidal use. In the EU plant protection sector, 20 repellent actives are authorized. Four are plant based (clover oil, garlic extract, methyl nonyl ketone, pepper) (EU Pesticides database; accessed 12 January 2016). None of the supported uses by the authorization of these compounds, however, covers rodents, but garlic extract and methyl nonyl ketone are registered for European rabbit (*Oryctolagus cuniculus*) management.

Few repellents are available to effectively minimize rodent damage compared to the variety of rodenticidal compounds and products registered worldwide for rodent management to protect crops and health (Jacob and Buckle 2016). Compared to the number of PSM-based rodent repellent products registered in China, Australia, the USA, and the EU, the number of rodenticidal products authorized is about 300 times higher. As stated above, in the EU there is no PSM-based product registered for repelling rodents, but >3000 rodentical products are authorized (this includes multiple registrations of some products in several member states; according to ECHA Database; accessed 1 March 2016). In the EU, efficacy of biocidal rodenticidal products has to be demonstrated by the applicant to the competent authority of the member state where registration is sought, and must accord to EU Biocides Regulation 528/2012.

Applications - Directions for Further Research

There are many plant metabolites that have been tested under laboratory conditions and found to be efficacious in rodents but have not been translated into commercial products. Many factors must be considered in the decision to develop and market a repellent for rodent management. First, the efficacy under natural conditions (e.g., in the field) must be proven for the target species. Second, the toxicological and environmental properties for the range of concentrations must be considered. The natural origin of a plant-derived compound does not ensure that it is environmentally safe. In many cases, risk assessment must be conducted for potential PSM rodent repellents similar to the risk assessment mandatory for pesticides. Third, application must fit the target species requirements. Production, be it via chemical synthesis or by extraction from plants needs to be cost-effective. In contrast to rodenticides, some volatile compounds and mixtures can act via odor without compounds entering the food chain. Potential negative impact on the environment can be minimized by the application of volatile PSMs via dispensers, thus possibly avoiding direct contact with soil and water bodies.

From a practical point of view, the application of volatile compounds seems promising on a small-scale in confined spaces, such as storage facilities because evaporation of volatile substances can have a strong effect on rodents (Epple et al. 1996; Fischer et al. 2013a; Hansen et al. 2015, 2016). One disadvantage of essential oils and terpenoids is their high volatility. Dilution of the gas phase of the compound soon after application can result in a reduced effect. Research is needed to improve stability of formulations to ensure long-lasting emission of volatiles.

The use of plant-derived repellents for large-scale field application seems challenging to implement, and will require different application strategies. One option is the use of drip irrigation to apply repellents similar to the application of pesticides (Ghidiu et al. 2012). As for other management tools, it needs to be demonstrated that benefits in terms of damage prevention outweigh the cost of management.

Summary

Our review indicates that PSMs can repel several rodent species. There is potential for use as a tool for rodent management in several situations. These include reducing damage to crops in fields and to grain in storage facilities. In many cases, there has been no translation of the results of the testing of metabolites for repelling rodents from laboratory cage to enclosure and field studies. Often, the efficacy found in cage trials cannot be repeated in enclosure or field trials. This lack of successful transitions from laboratory to field applications is the likely reason why there are only a small number of PSM-based repellents registered for rodent management worldwide.

Promising groups of rodent repellents are essential oils and terpenoids, because they operate pre-ingestively and can act across species. Cinnamamide (phenolics) and alkylamides also have potential, but they act post-ingestively and some crop damage will occur before aversion sets in. Other compounds and mixtures appear to be species-specific, which provides the opportunity to focus management on a particular target species and to minimize unwanted non-target effects. Potential uses of PSMs include the protection of crops and storage facilities by repelling rodents and the use of attractive odors that lure them away to other habitats (push-and-pull strategy). Attractants are also useful for improving trap success, or to enhance bait acceptance (Shumake and Hakim 2000). A few studies suggest that PSM odors have reproductive effects (Tran and Hinds 2012). Effects of PSMs in rodents can contribute to an ecological friendly rodent pest management system as they can be used as an addition to an integrated rodent management tool box. Currently, there is neither a general approach for the study of PSMs as rodent repellents nor a harmonized protocol for identifying rodent repellent effects among species. Field use of PSMs should be preceded by detailed laboratory work that elucidates the mechanistic relationship between a compound and rodent response, thus enabling an appropriate application. Repelling individuals from a target (e.g., a valuable plant or a certain area) will inevitably turn them to alternative food sources in surrounding areas. This response needs to be incorporated into potential application schemes.

Although there has been significant progress in the identification of the functional mechanisms that cause effects of natural products on rodent behavior research needs to be expanded before being utilized on a broad scale for management purposes. Among the challenges are achieving sustainable efficacy, preventing unwanted environmental effects, and basic economical considerations. However, a rigorous stepwise approach (cage, enclosure, field trials) that concentrates on the most promising PSM groups may increase the likelihood of identifying effective and safe metabolites, and the development of a range of repellent products for crop and hygiene protection from rodents.

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Zusammenfassung

Kleinsäuger, wie Nagetiere, können erhebliche Schäden in landwirtschaftlichen Systemen verursachen. Besonders in Jahren der Massenvermehrung, wenn die Tiere in erhöhter Individuendichte auftreten führen die Schäden zu erheblichen Ernteverlusten, sowie zu Schäden an der landwirtschaftlichen Infrastruktur. Häufig werden Rodentizide angewandt, die aber nicht nur die Schädlingsarten beeinflussen, sondern auch Nicht-Zielarten, wie andere Kleinsäuger oder sie können sogar Prädatoren schädigen. Eine Alternative zu der chemischen Bekämpfungsmethode könnte die Anwendung von sekundären Pflanzeninhaltsstoffen (plant secondary metabolites = PSM) sein. In dieser Arbeit wurde untersucht, wie der Geruch von verschiedenen pflanzlichen Verbindungen (wie PSM), das Fraßverhalten von zwei Nagetierarten beeinflusst: der Feldmaus und der Hausmaus. Feldmäuse gehören in Europa zu den bekannten Schädlingsarten in der Landwirtschaft, während Hausmäuse eher als kommensale Schädlinge im Vorratsschutz auftreten. Diese Arbeit besteht aus insgesamt vier Manuskripten.

Das ersten Manuskript (**Kapitel 2**) beschreibt Fütterungsversuche in Laborkäfigen mit zunächst weiblichen Tieren beider Nagerarten. Die Fitness der weiblichen Tiere hängt stärker von der Nahrungsaufnahme und der -suche ab als bei den Männchen, besonders, wenn sie tragend oder laktierend sind. Bei den weiblichen Feldmäusen konnte ich bei neun von 13 getesteten pflanzlichen Gerüchen (oder Kombinationen) eine stark reduzierte Nahrungsaufnahme beobachten und bei einer Substanz (Buchu Öl) eine erhöhte Aufnahme. In den Versuchen mit den weiblichen Hausmäusen wurden alle sechs getesteten pflanzlichen Gerüche gemieden, einschließlich des Buchu Öls.

Allerdings spielen pflanzlichen Gerüche auch bei männlichen Tieren eine wichtige Rolle, besonders bei juvenile Männchen auf Territoriumssuche. Das zweite Manuskript (**Kapitel 3**) beinhaltet die Fütterungsversuche in Laborkäfigen mit den Männchen beider Nagerarten. Es wurden insgesamt 18 verschiedene flüchtige pflanzliche Verbindungen und Kombinationen mit Feldmäusen getestet. Vier pflanzliche Gerüche hatten einen negativen Einfluss auf die Futteraufnahme und sieben pflanzliche Gerüche wirkten attraktiv. Bei den Hausmäusen konnte bei fünf von sechs getesteten pflanzlichen Gerüchen eine wirksame Abneigung beobachtet werden. Insgesamt zeigten meine Fütterungsversuche unter Laborbedingungen, dass zwei pflanzliche Gerüche (Fenchelöl und MNK) in beiden Nagetierarten und in beiden Geschlechtern zu einer reduzierten Nahrungsaufnahme führten.

Das dritte Manuskript (**Kapitel 4**) bildet die „Brücke“ vom Labor zum Feld. Hier wurden pflanzliche Gerüche unter halbnatürlichen Bedingungen getestet. Aus den Ergebnissen der Laborkäfigversuche wählte ich vier effektive pflanzliche Gerüche (MNK, Anthrachinon, MNK+BPO, MNK+MA+BPO) für Außengehegeversuche mit beiden Nagerarten aus, um die Effektivität der Abschreckung zu untersuchen. Die Tiere hatten in den Gehegen die Wahl unterirdische Boxen mit Haferflocken und behandelter (mit PSM) oder unbehandelter Erde (experimentelle Kontrolle) aufzusuchen. Bei den Feldmäusen hatten alle getesteten pflanzlichen Gerüche einen geringen Einfluss auf das Besuchsverhalten der unterirdischen Boxen mit behandelter Erde. Die Kombination MNK+BPO wirkte am effektivsten. Eine Auswertung der Nahrungsaufnahme war nicht möglich, da die Haferflocken in jedem Versuch leer gefressen wurden. Bei den Hausmäusen konnte ein eindeutiges Vermeidungs-Verhalten beobachtet werden, die Tiere zeigten eine höhere Besuchswahrscheinlichkeit der Kontrollboxen in allen vier getesteten Substanzen. Anthrachinon wirkte als effektivstes Repellent. Darüber hinaus fraßen die Hausmäuse weniger Haferflocken aus den Boxen mit der behandelten Erde in allen vier getesteten PSM Gerüchen. Die Ergebnisse zeigen, dass PSMs bei kommensalen Nagetiere unter Feldbedingungen abschrecken wirken können. Die Ergebnisse aus den drei Manuskripten könnten ein Beitrag zur Entwicklung von nicht-tödlichen Abwehrstoffen (Repellentien) zur Bekämpfung von Schädlingsnagern sein, die möglicherweise besser geeignet sind als Fallen oder Rodentizide.

Das letzte und vierte Manuskript (**Kapitel 6**) ist eine systematische Literaturübersicht in der die bestehende Literatur zu PSM als Repellentien gegen Nagetiere evaluiert wird. Mit einer systematischen Literaturrecherche wurden relevante Publikationen auf ihr Potenzial für den Einsatz in der Schädlingsbekämpfung ausgewertet. Um eine bessere Übersicht zu erlangen, welche PSM bei der Abwehr von Nagern einen negativen Einfluss haben, wurden die Publikationen nach den getesteten PSM Gruppen eingeteilt und ausgewertet. Es wurden Informationen gesammelt welche PSM Gruppe(n) effektiv wirken, um das Potenzial für die Entwicklung von kommerziellen Produkten abzuschätzen. In 54 Publikationen wurden verschiedene PSM, Pflanzen, Pflanzenteile oder manipuliertes Futter auf das Nahrungsverhalten, Wachstum oder den Stoffwechsel von Nagern untersucht. Bei dem Vergleich der Publikationen fiel eine sehr diverse Methodik und Herangehensweise auf. Man kann drei grundsätzliche methodische Herangehensweisen bei der Untersuchung von PSM auf das Fraßverhalten von Nagern erkennen: 1. Fraßversuche mit Einzeltieren in Laborkäfigen, 2. Versuche mit mehreren Tieren in Außengehegen und 3. Beobachtungen und Untersuchungen im Feld. Der Grund für die großen methodischen Unterschiede sind die unterschiedlichen Fragestellungen, mit der die Autoren, die Wirkung von PSM auf das Nahrungsverhalten von Nagern untersucht haben. Ein weiterer Grund für die unterschiedliche Herangehensweise sind die jeweils unterschiedlichen Zielnagerarten und ihre unterschiedlichen Habitate bzw. Nahrungsansprüche. Die Auswertung der Publikationen zeigt, dass PSMs sowohl artspezifisch als auch geschlechtsspezifisch auf Nagetiere wirken können. Für weitere Untersuchungen nach einem artübergreifenden, anwendbaren pflanzlichen Repellent, empfiehlt sich zunächst ein systematisches Screening von PSMs mit der Ziel-Nagerart durchzuführen. Die PSM Gruppe der ätherischen Öle und Terpenoide hat dabei das größte Potential für den Einsatz in einem ökologisch-basierten Schadnagermanagement. Die Literaturübersicht zeigt ein Missverhältnis von den Erfolgen in Käfigversuchen und den nicht fortgeführten Versuchen in Außengehegen und/oder Feldstudien. Das Ergebnis führt letztlich zu einer derzeit sehr kleinen Zahl von registrierten pflanzlich-basierten Abwehrstoffen für Nagetiere. Obwohl es unterschiedliche Herausforderungen bei der Suche nach geeigneten pflanzlichen Repellentien gibt, könnte eine weitere Forschung mit zukunftsträchtigen pflanzlichen Verbindungen, mit der richtigen PSM Gruppe, reproduzierbare Ergebnisse und mögliche kommerzielle Produkte hervorbringen.

Conclusion

Rodents can cause extensive damage in agricultural systems. This results in considerable harvest loss as well as damage to agricultural infrastructure. Commonly rodenticides were applied, but they did not only affect rodent pest species but also harm non-target species such as predators and other small mammals. To prevent this, the search of rodent repellents has increased worldwide. In this thesis, I investigated how the odor of mainly plant secondary metabolites (PSMs) can affect the feeding behavior of two rodent species: the common vole (*Microtus arvalis*) and house mouse (*Mus musculus*). Common voles are a major vertebrate pest species in agriculture, whereas house mice are commensal pests. Both rodent species are well-known to cause severe damage to diverse agricultural enterprises in Europe.

Firstly (**chapter 2**), I conducted laboratory feeding experiments initially with females because their fitness depends more on their foraging behavior than it does in males. A range of volatile PSMs were tested 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 female voles. In female house mice, I identified six deterrent PSMs which reduced the food intake including bucco oil and there were two compounds that had no effect.

Secondly (**chapter 3**), I conducted laboratory feeding experiments with males of both species, eighteen different volatile PSMs and combinations thereof with voles. However, males will also be affected by repellents and they also play an important role for rodent infestations. Four PSM odors reduced feeding intake and seven PSM odors increased feeding of male common voles. Five of six tested herbal odors were effective as repellents against house mice. Overall, my studies under laboratory conditions demonstrated that two PSM odors (fennel oil and MNK) reduced feeding intake in both rodent species and both sexes.

Thirdly (**chapter 4**), I evaluated four promising PSM odors for their repellent effects in experiments conducted in semi-natural enclosures. Soil treated with PSMs or untreated soil (experimental control) was placed in underground boxes containing food (rolled oats). I quantified the number of visits to each box and could demonstrate that all four PSMs reduced the number of visits to treatment boxes in both rodent species. For common voles, the combination MNK+BPO was the most repellent PSM. House mice made fewer visits to all PSM boxes; boxes with the anthraquinone were visited least. Furthermore, house mice consumed less food from boxes containing soil treated with all four PSMs. Our results suggest that PSMs are repellent in murid and microtine rodents under semi-field conditions. Results of these three studies contribute the development of non-lethal management tools for rodent pest species that are potentially more suitable than traps and rodenticides. This approach could be applicable to a variety of crops if effective at field conditions.

The last and fourth manuscript (**chapter 6**) is a systematic literature review to retrieve relevant publications and evaluated these according to PSM groups to derive information about the potential for developing herbal rodent repellents. We screened a total of 54 publications where different compounds or plants were tested regarding rodent behavior/metabolism. In the search for widely applicable products, we recommend multi-species systematic screening of PSMs, especially from the essential oil and terpenoid group, as laboratory experiments have uniformly shown the strongest effects across species. Other groups of compounds might be more suitable for the management of species-specific or sex-specific issues, as the effects of some compounds on particular rodent target species or sex might not be present in non-target species or in both sexes. Although plant metabolites have potential as a tool for ecologically-based rodent management, this review demonstrates inconsistent success across laboratory, enclosure, and field studies, which ultimately has lead to a small number of currently registered PSM-based rodent repellents.

Beitrag der Autorin zu den vorliegenden Manuskripten

Kapitel 2:

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

Sabine C. Hansen, Caroline Stolter, Jens Jacob
Crop Protection (78) 270-276

Die gesamten Laborkäfigversuche wurden von mir konzipiert. Dazu gehörte auch die Feldarbeit zum Fang der Mäuse und die Suche und Beschaffung der Substanzen. Das Manuskript wurde allen Autoren angefertigt. Besonders Caroline Stolter half mir das erste Manuskript auf den richtigen Weg zu bringen, analysierte mit mir die Daten und stand mir in statistischen Fragen unermüdlich zur Seite. Jens Jacob betreute die Arbeit, diskutierte mit mir die Aufbereitung des Manuskriptes und las Korrektur. William Foley half mir bei der englischen Korrektur.

Kapitel 3:

Effect of plant secondary metabolites on feeding behaviour of microtine and arvicoline rodent species

Sabine C. Hansen, Caroline Stolter, Jens Jacob
Journal of Pest Science (89) 955–963

Mein Anteil an dieser Arbeit war die Konzeption und Durchführung der gesamten Laborkäfigversuche. Die Suche nach Substanzen, sowie die Feldarbeit wurden von mir durchgeführt und das Manuskript von mir verfasst. Caroline Stolter half mir bei statistischen Fragen. Jens Jacob betreute die Arbeit, stand mir für Diskussionen zur Seite und las Korrektur. William Foley half mir bei der englischen Korrektur.

Kapitel 4:

Like or dislike - Response of rodents to the odor of plant secondary metabolites

Sabine C. Hansen, Caroline Stolter, Christian Imholt, Jens Jacob
Integrative Zoology (submitted am 10.06.2016)

Die gesamte Arbeit, angefangen von der Konzeption, über die Feldarbeit und die Durchführung der Außengehegeversuche bis hin zum Verfassen des Manuskriptes, wurden von mir durchgeführt. Caroline Stolter war bezüglich des Versuchsdesigns und statistischen Fragen ein sehr kompetenter Ansprechpartner. Christian Imholt half mir die Daten statistisch aufzubereiten und zu analysieren und er stand mir in statistischen Fragen unermüdlich zur Seite. Jens Jacob betreute die Arbeit, stand mir für Diskussionen zur Seite und las Korrektur. William Foley half mir bei der englischen Korrektur.

Kapitel 6:

Plant secondary metabolites as rodent repellents: A systematic review

Sabine C. Hansen, Caroline Stolter, Christian Imholt, Jens Jacob
Journal of Chemical Ecology – Semiochemicals in Pest Management II, (42) 9, 970–983

Mein Anteil an dieser Arbeit war die Literatursuche, das Lesen der Literatur, sowie das Verfassen des Manuskriptes. Von Caroline Stolter kam die Konzeption, zu einem systematischen Review mit Hilfe von Schlüsselwörtern. Caroline Stolter und Christian Imholt halfen mir beim Lesen der umfangreichen Literatur und trugen somit zum Ergebnis- und Diskussionsteil bei. Jens Jacob betreute die Arbeit, half bei der Gliederung des Manuskriptes und las Korrektur. Alle Coautoren standen mir für wertvolle Diskussionen zur Seite und halfen bei der Erstellung des Manuskriptes. Alex Stuart half mir bei der englischen Korrektur.

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„Wer sich Steine zurechtlegen kann, über die er stolpert, hat Erfolg in den Naturwissenschaften.“
(Erwin Chargaff (*1905), östr.-amerik. Biochemiker u. Schriftsteller)

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