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Abstract

Costs related to planting of seedlings and plant material is a major economic obstacle that may impede successful large-scale forest restoration. One way to reduce this cost is to use direct seeding instead of planting. Nut-bearing trees such as oaks have seeds with large nutrient storage that results in competitive seedlings. However, the main problem of direct seeding of oak is the high predation rates on buried acorns by small rodents, which use their well-developed olfactory sense to locate buried seeds.

In this thesis, I therefore, investigated different aspects of rodent predation on acorns and how they use their olfactory sense to locate these seeds. In a review, I synthesised current knowledge about the mechanisms in rodent predation on seeds and acorns. I also snap-trapped rodents to investigate if they use odours from the acorn nut or the shell to find acorns. The headspace of the two seed parts was analysed by using combined gas chromatography and mass spectrometry (GC-MS). Finally, in a laboratory study, I developed a two-choice behavioural assay to quantify lab-bred bank voles' odour preference. I tested if they were attracted to the odour of acorns and their common laboratory diet, and also analysed the odourants emanating from these two foods.

The review showed that a wide variety of protection methods have been tested. Physical protection was among the most successful methods but lacked possibilities for large-scale implementation due to high costs. Few studies have addressed how rodents use odours to find acorns although this knowledge could be used to interfere with rodents' foraging. In my field study, odours from both the nut and the shell were attractive to forest rodents, but the nut was more attractive when cover from avian predation was higher, indicating that rodents may use a trade-off with accuracy for protection when foraging. The seed parts' headspace showed that they shared many compounds. When quantifying bank vole odour preference by the time spent in a choice arm, short time intervals performed best. Furthermore, naïve bank voles were as attracted to acorns as to their common laboratory food, indicating that bank voles have an innate preference for acorn odour. In conclusion, this thesis show that rodents are well-adapted to locate acorns using olfaction. The preference of odourants can be tested in field or laboratory, and since rodents rely heavily on smell, odour manipulations have the potential to provide cost-efficient protection to direct-seeded acorns.

Keywords: Apodemus flavicollis, *Apodemus sylvaticus*, *Myodes glareolus*, seed predation, volatiles, VOC, plant-animal interactions, *Quercus*

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Dofters roll i gnagarpredation på direktsådda ekollon.

Sammanfattning

Kostnaden relaterat till plantering och plantor är ett betydande hinder för att lyckas med storskalig skogsrestaurering. Ett sätt att undvika dessa kostnader är genom att använda sig av direktsådd. Träd med stora nötter såsom ekar har energirika frön vilket resulterar i konkurrenskraftiga plantor. Dock är predationstrycket från gnagare på sådd av till exempel ek mycket högt. Gnagarna använder sig av sina välutvecklade doftsinnen för att hitta de nedgrävda fröerna.

I den här avhandlingen har jag därför undersökt olika aspekter av gnagarpredation på ekollon och hur de använder sig av dofter för att hitta dessa frön. Jag har i en sammanfattning av forskningsläget samlat kunskap om mekanismerna bakom gnagarpredationen på fröer och ekollon. Jag har också fångat gnagare med slagfällor betade med dofter av antingen ekollonets nöt eller skal. Flyktiga ämnen som avges från nöten och skalet har analyserats med kombinerad gaskromatografi och masspektrometri (GC-MS). Slutligen, i en laboratoriestudie, har jag utvecklat en metod för att kvantifiera skogssorkars doftpreferenser. Jag testade om de i en två-valssituation var attraherade av doften av ekollon eller deras laboratorie-anpassade, kommersiella föda samt genomförde analyser av de flyktiga ämnen som avgas av dessa två olika födoämnen.

Sammanfattningen av forskningsläget visade att fysiska metoder för att skydda ekollon var de mest effektiva, men för kostsamma att användas i storskaliga tillämpningar. Få studier hade undersökt hur gnagare använder dofter för att hitta ekollon, trots att denna kunskap skulle kunna användas för att störa gnagarnas födosök. I fällstudien var både dofter från nöten och skalet attraktiva. Men nöten var mer attraktiv när det fanns skydd från rovfåglar, vilket kan bero på att gnagare kan vara mer noggranna över vilka dofter de följer i ett habitat med skydd. Kvantifieringen av skogssorkars doftpreferenser i laboratoriemiljö gjordes bäst med korta mätintervall. Dessutom var oerfarna skogssorkar attraherade av ekollon-doft, vilket indikerar att de har en medfödd attraktion till ekollon. Sammanfattningsvis visar avhandlingen att gnagare är välanpassade till att med doftsinnet lokalisera ekollon. Preferensen för dofter kan undersökas i fält eller i laboratorier, och eftersom gnagare är mycket beroende av sina doftsinnen, så har syntetiska dofter potential att kunna användas som ett kostnadseffektivt sätt att maskera ekollon och på så vis minska predationen på dem.

Nyckelord: Apodemus flavicollis, Apodemus sylvaticus, Myodes glareolus, flyktiga ämnen, fröpredation, Quercus

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Dedication

To Maja, Kata & Elise

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Löf, M., Castro, J., Engman, M., Leverkus, A. B., Madsen, P., Reque, J. A., Villalobos, A. & Gardiner, E. S. (2019). Tamm Review : Direct seeding to restore oak (*Quercus* spp.) forests and woodlands. Forest Ecology and Management, 448(April), pp 474–489.

II Engman, M., Schlyter, F., G., Olsson, Villalobos A., Birgersson, G., Witzell, J. & Löf, M. (2020). Do foraging rodents use odor cues from non-edible food parts to locate edible food? (submitted)

III Engman, M., Schlyter, F., Koteja, P., Birgersson, G., Olsson, G., Witzell, J. & Löf, M. (2020). Towards streamlined bank vole odor preference evaluation using Y-mazes. *Mammal Research*, 65:1-9.

Papers I & III are reproduced with the permission of the publishers.

The contribution of Mattias Engman to the papers included in this thesis was as follows:

I Participated in the development of the research question and wrote a significant part of the manuscript.

II Developed research questions and design together with co-authors. Performed field and lab work. Compiled and analysed the data. Wrote the manuscript with input from the co-authors.

III Developed research questions and design together with co-authors. Performed field and lab work. Compiled and analysed the data. Wrote the manuscript with input from the co-authors.

1 Introduction

1.1 Background

Globally, forest land is declining with a stunning 3.2 million ha per year, and between 1990 and 2015, forest land cover declined from 31.6% to 30.6% (Ekins *et al.* 2019). To counter this decline, the United Nations has gathered forest restoration initiatives under the Bonn Challenge, which aims to restore 150 million hectares by 2020 and 350 million hectares by 2030. Great restoration efforts are connected with substantial costs (Enserink 1999; Birch *et al.* 2010). One of the significant expenses in forest operations related to restoration is related to planting and plant material. Hence new cost-effective methods to establish forest stands has the potential to reduce costs considerably and to promote forest restoration compared to alternative land uses (Alexander *et al.* 2011).

One possibility to reduce forest regeneration costs is by using direct seeding instead of plants (Bullard *et al.* 1992). Large seeded species, such as many species belonging to the order *Fagales*, are especially suitable for direct seeding operations since the high nutrient reserves present in storage tissue result in competitive seedlings (Palma & Laurance 2015; Löf *et al.* 2019).

As a result of climate change, temperate oaks (*Quercus* spp.), are expected to expand their ranges and have the potential to replace other species adapted to colder climates such as Norway spruce (*Picea abies* L Karst.) (Dyderski *et al.* 2018). As many oak species are drought-tolerant, they are better adapted than many species used in forestry to the exceptional droughts taking place during the last decades (Millar & Stephenson 2015). Also, broad-leaved trees compared to conifers reflect more light and transpire more water and can, therefore, reduce climate warming (Naudts *et al.* 2016).

A primary reason to not use direct seeding is high rodent removal and predation of acorns (den Ouden *et al.* 2005; Birkedal *et al.* 2009, 2010; Leverkus *et al.* 2013). Therefore, cost-efficient methods of acorn protection have the potential to facilitate the transition to climate-adapted forestry.

1.2 The oaks

Around 400 species are belonging to the oak genus *Quercus* (Johnson *et al.* 2019). Oaks are distributed worldwide, and many species are endemic. In USA and Mexico, oaks comprise 20% and 30% of the forest biomass, respectively (Johnson *et al.* 2019). Oaks were dominant species in European forests before intensive human management (Lindbladh & Bradshaw 1998). The two most common oak species in northern-central Europe are pedunculate oak (*Quercus robur* L.) and sessile oak (*Quercus petrea* (Matt.) Liebl.). During the early stages, these species exhibit similar growth rate (Collet *et al.* 2017).

1.2.1 Ecosystem services and biodiversity

Oaks are well known for their timber quality. It has mainly been used in house construction and shipbuilding (Johnson *et al.* 2019). In Scandinavia, Vikings, for example, used large oak (>1 m diameter) trees to build ships (Crumlin-Pedersen 1986). A small vessel could be made out of three trees, while later and larger warships during 16th -19th centuries were each built from thousands of oaks.

Modern uses of oak wood include furniture, flooring and veneer (Johnson *et al.* 2019). It is also used in the production of foodstuff, for example, wood casks release chemical compounds which give taste to wine and whiskey (Cerdán *et al.* 2002). The use of acorns as fodder is responsible for the characteristic taste of Iberian ham (Ruiz *et al.* 1998). In addition., broadleaves such as oaks are also appreciated for their recreational value (Norman *et al.* 2010)

Oaks are also well known as reservoirs for biodiversity which can be explained by the previous wide distribution of oaks (Brändle & Brandl 2001) and the long life span of oaks that extend over several hundred years, providing a temporally stabile habitat for less mobile organisms (Ranius et al., 2009). In Sweden for example, nearly 40 % of all red-listed saproxylic invertebrates are associated with oaks (Jonsell *et al.* 1998). Oaks are also key species for conservation of red listed bryophytes, lichens and fungi (Berg *et al.* 2002).

1.2.2 Oak regeneration decline

The failure to regenerate oaks have been acknowledged scientifically for at least a century (Watt 1919). However, the decline of oaks has a much longer history. In Scandinavia during the last centuries, oaks have decreased by around 50% (Lindbladh & Foster 2010). Several anthropogenic and biotic factors may have contributed to this. The shade-tolerant and competitive beech began a rapid expansion around 200 years BC, replacing mixtures of oak and lime (Lindbladh & Foster 2010). At the end of the 18th century, the Swedish navy estimated the standing crop of oak trees to 230 000 (Eliasson 2002). Due to extensive exploitation of oak timber for shipbuilding, it had dramatically decreased by 82% to 41 000 trees 30 years later (Eliasson 2002). Expanding agriculture landscape to feed a growing population came at the expense of broadleaved trees growing on fertile soil, such as oaks (Lindbladh & Foster 2010). The suppression of forest fires by human intervention for the last two centuries also negatively affected fire-tolerant oaks (Adámek *et al.* 2016) and benefited competitors such as beech (Fagus sylvatica L.) and spruce (Niklasson *et al.* 2002; Petersson 2019; Spînu *et al.* 2020). During the last decades, rising ungulate populations also restrict the recruitment of oak trees by extensive browsing (Petersson *et al.* 2019).

1.3 The rodents

1.3.1 Rodent species and their ecology

Main acorn predators in southern Sweden are yellow-necked mice (*Apodemus flavicollis* Melchior, 1834), forest mice (*A. sylvaticus* Linnaeus, 1758) and bank voles (*Myodes glareolus* Schreber, 1780) (Jensen 1985; Birkedal *et al.* 2009, 2010).

Bank voles are distributed across Europe (Mitchell-Jones *et al.* 1999) and mainly found in forest habitats (Bergstedt 1966; Hansson 1978). Home ranges of males are around 4 000 m^2 (Korn 1986), larger than the females' which are around 1 000 m^2 (Korn 1986; Koskela *et al.* 1997). Males home ranges are often overlapping with several female territories (Bujalska & Saitoh 2000). The reproduction period typically begins in late March and (Eccard & Ylönen 2001) ceases in September (Bergstedt 1965), although winter breeding can occur when food availability is good (Smyth 1966; Jensen 1982; Eriksson 1984).

Bank voles have an omnivorous diet in between the typical herb diet of voles (*arvicoline*) and the granivorous mice (*murine*) (Butet & Delettre 2011). They consume a wide range of different foods including herbs, mosses, lichens, invertebrates, seeds, fruits and fungi (Watts 1968; Hansson 1971; Zemanek 1972; Gebczynska 1976). Diet composition varies with food availability depending on the season. During the spring and summer, green parts and invertebrates are essential food sources, and during the autumn and the winter, green parts together with seeds are the primary food sources (Hansson 1971).

Both yellow-necked mice and wood mice are in comparison to the bank voles, very mobile species (Bergstedt 1966; Wolton 1985; Mazurkiewicz & Rajska-Jurgiel 1998). A male wood mice home range covers about 5-10 female territories and roughly cover 11 000 m^2. Females' home ranges are approximately 4 000 m^2. The mice species can be found both in forest areas and on arable land (Bergstedt 1966; Hansson 1978). Similar to bank voles, winter reproduction can take place during years with abundant food (Jensen 1982).

Yellow-necked mice and wood mice are considered seed specialists (granivorous) but also eat significant amounts of invertebrates during the summer (Watt 1919; Hansson 1978; Butet & Delettre 2011). Their diet overlap considerably (Abt *et al.* 1998). In common with bank voles, the diet during the winter consists mostly of seeds, while during the summer invertebrates are increasingly important. Mice also consume a smaller range of plants, fungi and fruits (Watts 1968; Hansson 1971).

Both the mice species and the bank voles are bottom-up regulated, where high acorn and beechnut production have a prominent effect on winter survival resulting in rodents surviving food shortage during the winter (Crawley 1970; Jensen 1982; Johnsen *et al.* 2017)

1.3.2 Olfaction

Olfaction is the primary sensory modality that rodents use to collect cues which give them information about their environment (Slotnick 2001). Mice and voles use odour cues to choose mates, establish territories, assess health status of conspecifics, avoid predators, and find food (Howard *et al.* 1968; Yamazaki *et al.* 1979; Rozenfeld & Denoël 1994; Beauchamp & Yamazaki 2003; Kruczek & Gołas 2003; Kwak *et al.* 2010).

Odours consist of one or several volatile compounds, commonly called odorants or semiochemicals, often present in a more or less specific blend. The rodent olfactory system consists of the main olfactory epithelium (MOE) and the vomeronasal organ (VNO) (Figure 1). The MOE sense volatile compounds like the human nose while the VNO detect less or non-volatile compounds that are important in sexual communication and predator avoidance. The olfactory receptor (OR) is the most common receptor type in the MOE and can sense a wide variety of volatile compounds. Mice have 1063 functional OR genes, more than both dogs (822) and humans (388), suggesting that mice can detect a broader variety of odour ligands (Yoshikawa & Touhara 2015). Each OR may respond to multiple odorants, and each odorant often activates several different ORs, depending on the dose (Malnic *et al.* 1999; Kajiya *et al.* 2001). Furthermore, the concentration of the odorant will affect how it is perceived, as ORs differ in their affinity to an odorant (Kajiya *et al.* 2001). These two properties of the ORs allows rodents to detect a vast amount of compounds and their blends (Bushdid *et al.* 2016).

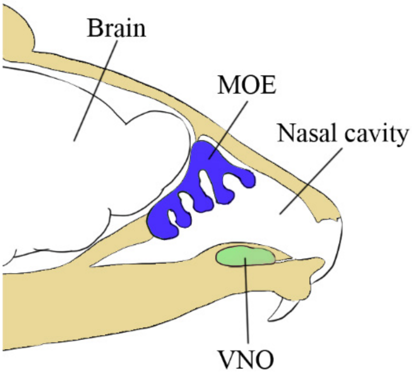

Figure 1. Schematic drawing of the mouse nasal cavity, main olfactory epithelium (MOE) and vomeronasal organ (VNO).

Animals follow odour trails up-winds in order to locate a fragmented plume of odour packets (Conover 2007; Vergassola *et al.* 2007; Khan *et al.* 2012). When rodents approach close to food, they follow the odour plume gradient (Gire *et al.* 2016).

However, little is known of what compounds rodents use to locate food from a distance. Research on “flavours”, which essentially are odour blends, suggest that food compounds are derived from nutritive compounds from primary metabolism such as proteins, fats, carbohydrates, and vitamins (Goff & Klee, 2006). In contrast, herbivores are known to locate food using plant secondary chemicals (or plants secondary metabolites), such as volatile plant defensive compounds (Bedoya-Pérez et al., 2014). Many seeds emit a minimal amount of odours, and rodents searching for them might not have many options than to target the few compounds present (Paulsen et al., 2013). Rats (*Rattus rattus* Linnaeus, 1758 & *R. norvegicus* Berkenhout, 1769) are attracted to compounds that are present in many different preferred food items, indicating that there are general compounds which are food cues for this oligophagous animal (Jackson *et al.* 2016).

1.4 The oak-rodent interaction

It is well established that animals disperse oaks, so-called zoochory (Bossema 1979; Jensen & Nielsen 1986). A mutualistic relationship where the trees gain dispersal and burial of their seeds and the animals gain a storable and nutritive food source. Corvids are famous for their ability to collect several thousands of acorns and store them in spatially distributed caches (Clayton *et al.* 1992). The benefit of rodents to oaks is more ambiguous since rodents are also very effective consumers of acorns. Therefore, oak-rodent interactions can be aligned on a mutualism–antagonism continuum (Zwolak & Crone 2012; Gómez *et al.* 2019). Studies show that the rodent consumption of acorns can be so high that as little as 1 % of all acorns may germinate (Gómez *et al.* 2008; Pérez-Ramos *et al.* 2013). Furthermore, the main benefit of acorn burial to oaks may rather be decreased predation by other rodents rather than a favourable germination site (Bogdziewicz *et al.* 2019).

The long evolutionary history of oak-rodent interactions has resulted in a co-evolutionary arms race with numerous adaptions in both plant and animals (Vander Wall 2010; Lichti *et al.* 2017). For example, tannins, a group of polyphenolic antifeedants, are in acorns are concentrated to the apical part where the embryo is located, leading to many rodents only partially consuming acorns leaving the embryo intact (Steele *et al.* 1993). The partially consumed acorn has a reduced energy reserve but can, on the other hand, germinate quicker (Perea *et al.* 2011b). Many rodents remove the embryo before caching acorns, making the seed unviable (Steele *et al.* 2006; Zhang *et al.* 2014). In response, *Q. mongolica* has acorns where the embryo is placed deeper into the acorn (Zhang *et al.* 2014).

The synchronized reproduction effort followed by several years of low reproduction by plants is called masting. Masting in animal-dispersed plants can lead to an increased seedling establishment (Vander Wall 2002; Jansen *et al.* 2004). An increased establishment can be explained by the predation satiation hypothesis which proposes that rodents, birds, and invertebrates cannot respond in numbers to the high acorn production, and therefore many acorns will not be consumed (Crawley & Long 1995). In mast years, *Q. robur* seed fall amounts to 400 000 acorns/ha (Shaw 1968), which is much higher than forestry recommendation of a sowing density of 15 000 acorns/ha (Birkedal & Madsen 2007).

Animals stealing other individuals caches' is referred to as pilfering, and rodents are the main pilferers using their olfactory sense to locate hidden caches (Vander Wall & Jenkins 2003; Dittel *et al.* 2017). Rodents prefer to cache acorns in open habitats where predation risk is high, in order to reduce the likelihood of pilferers finding the caches (Muñoz & Bonal 2011; Steele *et al.* 2013, 2015). Site preparation techniques in direct seeding can be viewed as a cache protection strategy where removal of vegetation and elevation of soil increases the risk of predation (Birkedal *et al.* 2010; Zwolak *et al.* 2016).

Several rodent species remove the seed shell before caching seeds (Jansen *et al.* 2010; Jenkins & Devenport 2014; Yang *et al.* 2018). These deshelled seeds experience less pilfering than whole seeds (Jansen *et al.* 2010; Jenkins & Devenport 2014; Yang *et al.* 2018), which may be explained by lower emissions of several volatile compounds in deshelled seeds (Yang *et al.* 2018). Removal of the shell in acorns also have implications for germination in some oak species, as deshelled acorns can germinate quicker and have a higher germination rate (Finch-Savage & Clay 1994; Yang *et al.* 2018).

Although smell is the primary sense that rodents use to locate acorns, its' role in foraging is very little studied (Vander Wall 2010). How odours migrate from the seed to the ground is dependent on the soil (Vander Wall 2003). Small particle soils like clay have a higher potential of binding volatile compounds, and could potentially reduce the odours that reach the ground surface. Pinyon mice (*Peromyscus truei*) and brush mice (*P. boylii*) prefer to cache seeds in small particle soil (< 1 mm diameter) rather than in large particle soil (>2 mm diameter) (Pearson & Theimer 2004). Subsequently, pilfering is lower in the small particle soil (Briggs & Vander Wall 2004; Pearson & Theimer 2004).

Rodents prefer to cache seeds that smell less (Yi *et al.* 2016), and also invest more energy in memorizing low odour caches indicating that low-odour seeds have a higher value (Li *et al.* 2018). Reducing the odours released by acorns could to some extent be accomplished by removing the shell (see above). However, since rodents may at close range use odour gradients to locate food (Carthey *et al.* 2011; Gire *et al.* 2016), another possibility to reduce acorn predation would be to disrupt odour gradients by distributing artificial acorn odour evenly across direct seeding sites.

2 Objectives

The main objective of this thesis was to examine how small forest rodents use odours to locate buried acorns and find uses of such knowledge in forest restoration using direct seeding.

In the first part of this thesis (Paper I), we review the current state of knowledge about direct seeding of temperate oaks and identify directions for future research.

In the second part of this thesis (Paper II) we experimentally investigate which part of the seed small forest rodents use to locate acorns and also tried to identify the volatile compounds from the two seed parts shell and nut.

In the last part of this thesis (Paper III) we develop a method to identify which odours are attractive to the bank vole and how this method can be streamlined in order to assess the broad array of compounds identified from the acorn headspace.

The following research questions are addressed in the papers that are included in the thesis:

I What is known about direct seeding of temperate oaks, and which are the knowledge gaps?
II Which part of the seed is used by rodents to locate acorns, how does it depend on habitat, rodent species and sex, and which are the volatile compounds released by the shell and nut, respectively?
III How can the evaluation of attractive food odours be streamlined in order to screen a large number of volatile compounds?

3 Methods

One review study and two research studies were conducted in order to address the main objectives of the thesis. Their methods are briefly outlined below. Further details can be found in the individual articles in the thesis (Paper I-III).

3.1 Review study

To summarise the current state of knowledge regarding direct seeding of oaks and identify potential areas where new research should be directed, we conducted a qualitative review study. The target group was researches but also practitioners who wish to apply direct seeding of oak. Therefore, we aimed to describe the biology and ecology of acorns, acorn predation by rodents, acorn germination and early seedling growth. We also provided general guidelines of acorn collection, storage, and direct seeding techniques. The databases Web of Science, Scopus and Google Scholar were used to find references. In addition, we searched for articles through reference lists. References were also chosen subjectively depending on their quality and the relevance for the review.

3.2 Field study

The experimental field study was conducted in two oak forests in southernmost Sweden (Paper II). We examined if small forest rodents use odour cues from the nut (nuts) or the shell (pericarp) from acorns and how this depends on habitat, rodent species, and sex. Both forests were the result of afforestation and had been established on former agricultural land. They were parts of small fragmented forests in a landscape dominated by arable lands. The two forest sites were of different age, where the young forest was established 24 years ago the old forest was 80 years old. They, therefore, varied in canopy cover and shrub layer.

We arranged snap traps baited with either the acorn nut or the acorn shell in small quadrats modified from Myllymäki *et al.* (1971) in order to study which part of the seed was used by rodents to locate acorns (Figure 2). Baits were prepared by separating the nut and shell and then inserting 3g of either seed part into unused tea paper bags. Nuts were cut into smaller pieces in order to compensate for the larger surface to area of the shell. Two pairs of snap trap baited with nut and shell were placed in the corner of a 50 x 50 cm quadrat. The trap quadrats were separated with 20 m and aligned along transects. Transects were separated by at least 150 m. Traps were placed for three consecutive nights and checked every morning. In total, we placed five transects and 84 trap quadrats. This resulted in 608 traps and 1824 trap nights.

To collect volatiles from the nut and shell, we used dynamic headspace extraction and solid-phase microextraction (SPME). In dynamic headspace extraction, volatiles were trapped on aeration columns consisting of a Teflon® tube filled with the adsorbent Porapak® (Figure 3). Volatiles were collected during four hours and then extracted from the aeration column using 500 µL pentane.

In SPME, a fused silica fibre is coated with an extraction phase layer, that can either be a liquid polymer or a solid sorbent, to which the volatiles adsorb. It is a sensitive method but difficult to quantify as compounds compete for space and will adsorb depending on the affinity of the extraction phase, the so-called matrix-effect (Rocha *et al.* 2001). We sampled both intact seed parts at room temperature and seed parts which had been ground in a ball mill and heated to 35 °C during sampling. Volatiles were collected onto the fibre for 45 min and then thermally desorbed in GC-MS injection port.

We analysed the volatile profile of the seed parts using a combined gas chromotograph and mass spectrometer (GC-MS) (Figure 3). The GC-MS was equipped with a DB-Wax coated fused silica capillary column (60m * 0,25mm, df = 0,25µm) and used helium at a constant flow of $35 \mathrm{cm}^3 \mathrm{s}^{-1}$ as the mobile phase. Compounds were identified using mass spectral libraries and Kovats indices. We quantified the compounds in the dynamic headspace samples using an internal standard.

Figure 2. Some methods used in the design of the behavioural experiments in the field study and the two-choice laboratory study. From top left corner: a trap with bait concealed by a paper tea bag, placement of traps within a trap group (marked with red); a y-maze with a bank vole; one of the lab-bred bank voles.

3.3 Behavioural study

We conducted a two-choice laboratory study on odour preference in bank voles (Paper III). Test animals were the control line of bank voles belonging to an experimental colony at the Jagiellonian University (Kraków, Poland) (Sadowska *et al.* 2008). The behaviour of individuals was video recorded in Y-mazes, of which two arms had odour inlets connected to them while the third arm had an odour outlet and material from the focal animals' home cage (Figure 2). Odours were tested pairwise in the following combinations: acorns to active charcoal-filtered air (Acorn – Blank), acorns to acorn aeration extracts solved in pentane (Acorn – Extract), acorns to standard lab rodent chow (Acorn – Rodent chow), the solvent pentane to active charcoal filtered air (Solvent – Blank) and acorn aeration extract solved in pentane to solvent (Extract – Solvent).

Odour preference was scored using two different behaviours, approaching and investigation. Approaching was defined as the time spent in each of the odour arms. The behaviour was extracted from the recordings using video analysis software and measured for the cumulative time spent in the odour arms during intervals of 15 s, 30 s, and 60 s. Investigation was defined as the frequency of nose pokes into the odour inlets during 180 s after the first nose poke was performed. To assess the difference in efficacy of the approaching time intervals and investigation, we also extracted the time taken to end a trial using either interval and for investigation.

Volatile compounds from acorns and the standard laboratory food (rodent chow) were collected on aeration columns consisting of Teflon® tubing filled with the adsorbent Porapak®Q. The volatiles was then extracted using pentane to create the aeration extracts.

We analyzed the aeration extracts using GC-MS, as described above, in order to identify potential volatile compounds and their quantity. Peaks in the chromatograms were identified using mass spectral libraries and Kovats indices. An external reference mix was used to quantify compounds.

Figure 3. Some of the methods used to analyse volatiles in acorn headspace. From top: Acorn headspace collection, combined gas chromatograph and mass spectrometer with autosampler. Aeration extracts in blue capped vials.

4 Main results and discussion

4.1 Small mammals limit the success of direct seeding

We highlighted predation by small mammals as the major obstacle to successfully direct seed oaks (Paper I). Several mammalian taxa feed on acorns, but small rodents were, consistently among studies, the main predators of sown acorns. The rate of predation varied between sites and burial depth but had in common that within four months, most acorns were removed (Figure 4). Some of the cited references dated back to the 19th and early 20th century and described successful direct seeding, which, however, was labour-intense and used large amounts of nuts. Thus they rendered incompatible with modern standards.

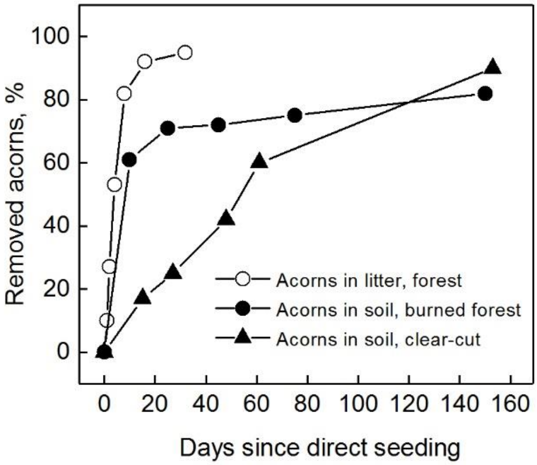

Figure 4. Cumulative removal of acorns by rodents from three reviewed studies denoted by an open circle, filled circle, and filled triangle (from paper I).

How rodents move in their surroundings is mainly dependent on the landscape of fear, a concept which maps spatial variation of movements depending on how rodents perceive fear (Krijger *et al.* 2017). Several studies found that the landscape of fear is of great importance to the rates of predation on sown acorns. This phenomenon is also illustrated by the successful application of direct seeding on arable land, which many rodents avoid due to the absence of slash piles, stones, and the destruction of burrows by ploughing. Another critical aspect of acorn predation is the fluctuations of the rodent population. After a mast year, these populations are at their peak, and therefore direct seedings have a higher risk of failing. Within years, rodent population peak during fall and are at their lowest in early spring as winter mortality usually is high. The landscape of fear, together with knowledge of rodent population fluctuations are knowledge about rodent ecology that we suggest could help practitioners chose suitable sites and time of direct seeding oak.

Although the importance of olfaction to rodents searching for buried seeds was established several decades ago (Howard *et al.* 1968), we still did not find many studies investigating the mechanisms of this behaviour and if it can be disrupted. Martelletti *et al.* (2018) suggested that lower predation on acorns in the vicinity of the legume scotch broom (*Cytisus scoparius* L.) could be caused by the plant masking the acorns' odours. The volatile profile of flowering branches of scotch broom and acorns have a few compounds in common, and possibly could the nitrogen fixating bacteria in the root nodules of the plant also emit volatile compounds common to acorns (Orozco-Mosqueda *et al.* 2013; Cavaleiro *et al.* 2018). Still, knowledge about which volatile compounds rodents sense when they locate acorns is unknown. Such information could be used to develop synthetic camouflage or to increase understanding of how different soils transport these compounds to the surface where rodents can sense them.

Several attempts to protect acorns were identified and included chemical ecologically-based, physical and silvicultural techniques. Plant-based chemical repellents are often used in organic farming but have shown to harm germination, and are therefore not a viable option at the moment. Rodents use olfaction not only to find food but also to avoid predators (Apfelbach *et al.* 2005), therefore predator odours have the potential to protect acorns. One option which showed potential in the laboratory was mink excrement, which in contrast to the plant-based compounds, also could benefit oak growth by the addition of nitrogen-containing organic compounds (Villalobos *et al.* 2019). In the field, predator compounds have not yielded success. The lack of success could partly be explained by that animals eventually will have to feed regardless of predation risk (Lima & Bednekoff 1999). In addition, most predator odour research has not taken into consideration that odour cues from predators quickly dissipate

(Bytheway *et al.* 2013). Long-lasting release devices of synthetic odours could solve this issue. Previous studies have mainly used single compounds to deter rodents which could underestimate the effect since recent research suggests that blends of several predator-indicating compounds are more effective (Apfelbach *et al.* 2015)

Little research has been conducted on ecologically-based tools for the protection of acorns. Birkedal *et al.* (2009) installed raptor perches at sowing sites in order to attract rodent predators which however did not decrease predation on acorns. Several physical protection devices have been developed, and Farlee (2013) suggested to re-use beverage cans. Physical protection is a promising method as it can be very effective in reducing rodent predation (Castro *et al.* 2015). The main obstacle remaining is to use biodegradable material and to reduce costs. Previously used degradable tubes are easily gnawed through by rodents (Löf *et al.* 2004) and several devices are costly to produce or need a high amount of labour to place and remove from the field. As mentioned earlier, rodents avoid habitats with few structures where they can hide. Several silvicultural practices that reduce these habitats were found useful to decrease rodent predation on acorns. Herbicides remove vegetation that can be used for hiding and also reduce competition for the oak seedling. As applying herbicides in forests is prohibited in many countries, mechanical preparation has been used with moderate success (Nilsson *et al.* 1996; Birkedal *et al.* 2010). Many authors recommended removing rodent habitat such as slash piles from reforestation sites (Birkedal *et al.* 2010) although this may facilitate browsing on young seedlings by larger mammals such as ungulates (van Ginkel *et al.* 2013).

Finally, we concluded that oaks are suited for direct seeding as the acorns have high energy and nutrients reserves which result in large and robust seedlings. The use of direct seeding could substantially reduce the costs of regeneration and is suitable for large-scale restoration efforts. However, direct seeding is a more uncertain method of establishing oak stands. One major obstacle is rodent predation, but several promising techniques are available but need further development. Often have these techniques have been studied singularly, but by combining them even lower rates of predation may be obtained.

4.2 Odour-guided foraging in relation to habitat, sex and species

Both odour cues from the nut and the shell were used by the rodents (Paper II). However, at the old forest site, nut odour (Pr[1] = 0.048) was three times more likely to attract a rodent compared to shell odour (Pr[1] = 0.015, $p < 0.01$) (Figure 5). One major difference between the young and the old forest was that the latter had a higher canopy cover and shrub abundance compared to the young site. Canopy cover and shrubs are used as cover by rodents in order to avoid predation (Longland & Price 1991). When rodents forage in safe habitats like under shrubs, they are more selective than when they forage in open habitats (Perea *et al.* 2011a). Such behaviour may stem from a speed-accuracy trade-off (Chittka *et al.* 2009), where rodents in high-risk environments prioritize speed over accuracy in order to minimize the exposure to predators. Accuracy, in this case, is correctly finding cues from the edible seed parts – the nut. Therefore, it is likely that rodents have the sensory ability to find food accurately, but instead adopt a more sloppy foraging strategy to increase their foraging speed (Chittka *et al.* 2003; Charalabidis *et al.* 2017).

Males showed more accurate foraging in the old forest. One reason could be that males were less affected than females by differences in perceived risk at the four traps constituting the trap group. Thus they were more prone to follow accurate cues into unsafe microhabitats, which could be reflected in higher activity, exploration and risk-taking. Laboratory tests on other species of rodents show that males sometimes can be more active and explorative, but not more prone to take risks (Auclair *et al.* 2013; Eccard & Herde 2013; Gracceva *et al.* 2014). In a study by Maiti *et al.* (2019), there was no difference between bank vole males and females in neither activity, exploration, nor risk-taking. Similarly, Lodewijckx (1984) found no sex-dependent difference in wood mice activity and exploration. In the field, one apparent sex difference is the higher avian predation pressure on male voles (Beacham 1979; Korpimäki 1985; Halle 1988; Mappes *et al.* 1993; Koivunen *et al.* 1996; Norrdahl & Korpimäki 2000). One suggested explanation is that males use open microhabitats to a greater extent than females (Mappes *et al.* 1993). In a study by Peles & Barrett (1996), survival and body mass of male meadow voles (Microtus pennsylvanicus) were much less affected than females in low-cover manipulations, indicating that males are more willing to use open microhabitats. In the young forest, there was no difference how accurately the sexes foraged. Possibly males also reduced their time and accuracy during foraging in the total absence of shrubs. If that is

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[1] Probability

the case, then also females could be more accurate during foraging in habitats with higher shrub coverage than was present in the old forest.

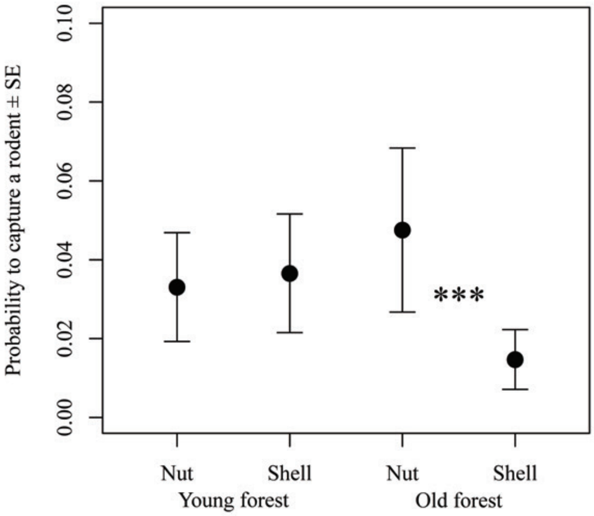

Figure 5. The probability of a snap trap catching a rodent baited by either the nut or the shell divided on the two sites. Error bars represent standard error, filled circles the estimated mean, and *** indicate a difference between factors ($p < 0.001$) (from paper II).

There was no difference between generalists (bank vole) and specialists in odour accuracy (wood mice or yellow-necked mice). How generalists and specialists gather information is less studied in comparison to diet selection, habitat breath and detoxification of plant secondary metabolites (Bernays & Wcislo 1994). Specialists are predicted to have higher sensory acuity to the more narrow range of diet (Linz *et al.* 2013), and also make faster decisions due to that they have to select from a more narrow range of stimuli (Bernays & Funk 1999). Although bank voles are not as strictly granivorous, they are highly dependent on seeds for survival (Jensen 1982, 1985; Zwolak *et al.* 2016). Therefore, a possible explanation of the lack of differences may be that the mutual high dependence on seeds results in equal sensory specialization. Both voles and mice detoxify tannins to similar extent (Shimada *et al.* 2006; Wereszczyńska *et al.* 2007), highlighting that both taxa are adapted to this diet. Although there was no difference between species in the selectivity for nut cues in the shrub abundant old forest, another possibility could be that risk of predation is a more important factor than the ability to interpret foraging cues (Lima & Dill 1990). For example, grey squirrels have the cognitive resources to determine the most energetically profitable food item but may instead prefer less-profitable food in order to maximize their time spent in cover (Lima & Valone 1986).

4.3 Volatile compounds from acorns

When we compared the volatile profile of acorn seed parts (Paper II), there were 30 volatile compounds present in the shell headspace and 22 in the nut headspace. All compounds present in the nut headspace was also found in the shell headspace. There was, however, a distinct difference between the volatile profiles of nut and shell, which mainly could be attributed to the higher volatile emissions from the shell (Figure 6).

Two of the detected compounds, isoamyl alcohol (3-Methylbutan-1-ol) and acetoin (3-Hydroxybutan-2-one), have been found both in the diet of rats and also to attract rats (Jackson *et al.* 2018). Although isoamyl alcohol could be derived from terpenes (a group of plant secondary metabolites), it can also be derived from nutritive compounds such as sugars and amino acids (Myers *et al.* 1970; Nout & Bartelt 1998). Acetoin is found in many different foods and is also a major part of the butter flavour (Xiao & Lu 2014). We also found the aldehyde nonanal, which is a general oxidation product of ω-9 fatty acids such as oleic acid. Oleic acid is one of the most abundant fatty acids in acorns of *Q. robur* (Colville *et al.*, 2012, supplementary).

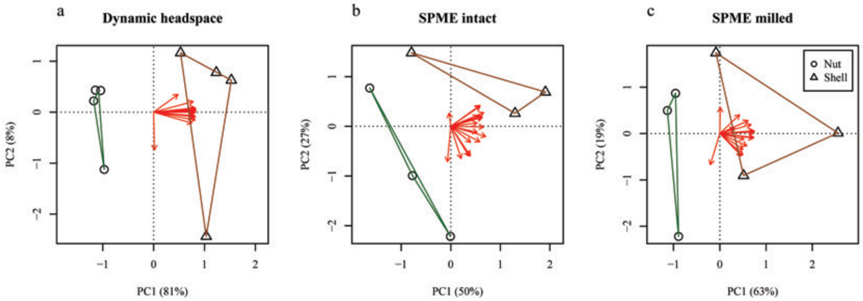

Figure 6. PCA on the volatile profile of the nut and the shell sampled using three different methods: dynamic headspace with Porapak as adsorbent (a), SPME with intact seed parts (b), and SPME with milled seed parts (c). Arrows represent the compounds found in the headspace and their direction the association with either nut or shell samples. Nut samples are connected with green lines and shell samples with brown lines (from paper II).

The majority of the compounds found in our volatile analysis (paper II & III) were monoterpenes, mostly hydrocarbons. As Plant secondary metabolites, the terpenes are not indicative of nutrient content, but can still be used as foraging cues. For example, the mammalian herbivore swamp wallaby (*Wallabia bicolor*) use the oxygenated terpene 1,8-cineole to locate suitable forage (Bedoya-Pérez *et al.* 2014). Also, several granivorous insects are attracted to terpenes present in the headspace of their host-seed (Rembold *et al.* 1989; Roseland *et al.* 1992; Bruce *et al.* 2011; Ndomo-Moualeu *et al.* 2016). Many of the terpene hydrocarbons, such as limonene, α- and β-pinene and terpinene found in the headspace of acorns are also present in oak leaves (Pearse *et al.* 2013), thus when these plant parts co-occur, rodents would have to distinguish acorns from leaves by quantitative differences of volatiles in the headspace.

Acorns and rodent chow were also quantitatively different, but first and foremost were they qualitatively different (paper III). Monoterpenes dominated the acorn extract while the rodent chow extract consisted mainly of alcohols and aldehydes. Only two compounds were found in both aeration extracts, limonene and α-pinene.

There were some differences in the volatile compounds collected using dynamic headspace (aeration extracts) between the studies (Paper II & III). Acorns have a high metabolic activity (Bonner & Vozzo 1987) and variation in moisture content as small as 2% will affect the volatile profile (Colville *et al.* 2012). Therefore, the differences in studies could be due to small differences in the moisture content of the acorns in the different studies. Also, in paper II we sampled the different seed parts and in paper III the whole acorns, which could lead to differences. A previous study using SPME found that the differences between intact acorns and acorns where the shell had been removed, only showed quantitative differences of the compounds present in the headspace (Yang *et al.* 2018), which would indicate that there should not be substantial qualitative differences between intact acorns and nuts. On the other hand, comparing dynamic headspace extraction and SPME (paper II) indicate that SPME is very good at concentrating compounds present at low amounts, which leads to little qualitative differences between nut and shell. In contrast, dynamic headspace extraction yielded more qualitative differences between the nut and shell which probably is reflected by that absorption of compounds to the aeration column is not as strongly affected by physical properties of the compounds. This is reflected by the different proportions of compounds in the headspace between nut and shell using Dynamic headspace and SPME (intact acorns) for the compounds o-xylene, 1-hexanol and isoamyl alcohol (but not limonene, β-phellandrene and acetoin). In other words, dynamic headspace may have failed to collect some compounds present in the headspace of the nut because they were present in very low amounts, while the more sensitive SPME collected and concentrated these compounds.

The failure of the acorn aeration extract to attract bank vole (paper III) could be due to that the attractive compounds were present in very low amounts (Hollander *et al.* 2012) or that the extraction procedure resulted in too high or low concentrations to be attractive (Bedoya-Pérez *et al.* 2014). Another solvent could also have been more appropriate, as the polarity of the solvent has shown to affect which compounds are extracted from a plant (Eriksson *et al.* 2008).

The bank voles had never experienced acorns, and the odour was not similar to rodent chow. Although the sampling of the volatile headspace (aeration extracts) possibly failed to collect some crucial odorants, it is still likely that bank voles are innately attracted to acorns since several rodents species exhibit acorn-specific behaviours although being lab-bred or living in areas where oaks have for long been locally extinct (Steele *et al.* 2006; Xiao & Zhang 2012).

4.4 Measuring odour preference

In paper III, we found that quantification of two behaviours, approaching (time spent in odour preference zone) and investigation (numbers of nose pokes in odour inlets), resulted in similar response profiles to odours. Bank voles were attracted both to the odour of acorns and their standard laboratory food, but not to the extract. Sensing an odour can be done from a distance, and it is therefore not necessary for an animal to enter a spatial zone although it is attracted to it (Slotnick & Schellinck 2002; Doty 2003). Therefore, odour attraction measured by approach could lead to accepting the null hypothesis, although it is false (type II error). However, since the result of approaching was similar to investigation, a behaviour that cannot be performed from a distance, it is clear that rodents indeed moved into the zone of the preferred odour.

Short time intervals for the approaching behaviour yielded higher effect sizes (Figure 7a), which probably is related to that test animals usually lose interest in test odours quickly (Slotnick & Schellinck 2002). The shorter time intervals did not just have a larger effect size, but also greatly reduced the time for the experiment (Figure 7b). Using 15 s intervals reduced measurement time with 50 % compared to 60s intervals and with almost 20% compared to 30s intervals. Although no comparisons of intervals were made for the investigation behaviour, our observation was that several animals showed low activity at the beginning of the trials. Therefore reducing the time interval for this behaviour could result in less odour preference data.

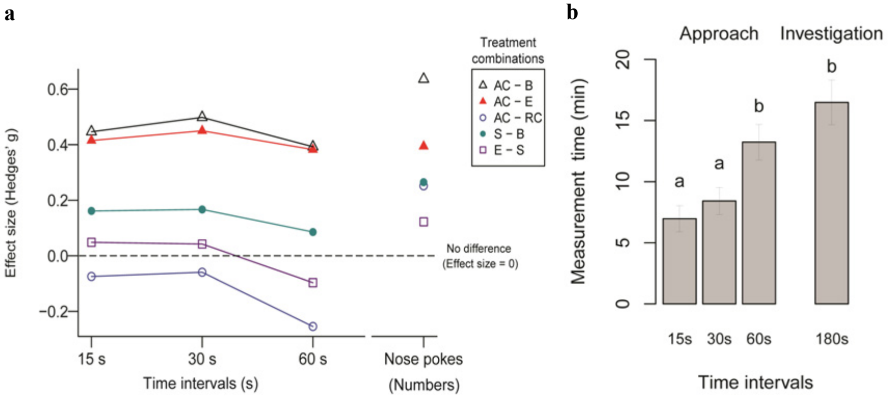

Figure 7. a) Effect size of the treatment combinations acorn – blank (open triangles), acorn – extract (solid triangles), solvent – blank (solid circles), extract – solvent (open square), acorn – rodent chow (open circles). b) The time to complete one behavioural trial depending on which behaviour and interval used to quantify odour preference (from paper III).

5 Conclusions and future perspectives

The overall goal of this thesis was to investigate how small rodents use smell to find buried tree seeds, and thereby provide a basis for how this knowledge can be used during direct seeding of oaks.

We found that, indeed, are rodent predation on buried acorns the major issue during direct seeding of oak (Paper I). The predation issue had led to a flora of research on how acorns can be protected from rodents. Removal of rodent habitat and physical barriers have been proven to reduce rodent predation, but these methods have implications. Removal of rodent habitats may be in contrast to biodiversity goals such as the increase of deadwood, it can be costly and can facilitate predation on acorns by other mammals such as large ungulates. Physical barriers are costly to produce and handle, and no suitable biodegradable product has been developed. Thus, the barriers have to be collected after seedling establishment. As rodenticides are not allowed to use in forestry, much effort has been put into researching effective repellents with no adverse effects on ecosystems. Rodents use olfaction to detect the numerous predators which have rodents as their primary source of food. Therefore, often predator odour in the form of urine, faeces or gland secretions have been applied. These have at present provided little efficacy in the field. However, little attention has been given to the actual mechanism of odour-guided foraging in rodents. Such knowledge could, for example, be used to develop seed camouflage or a better understanding of when during the germination process seeds are most vulnerable.

Both the nut and the shell was used by rodents to locate food (Paper II). However, cues from the nut were used to a higher degree by males and in habitats with more cover. We proposed that rodents reduce their foraging accuracy when predation risk is higher. This implies that odour-based foraging disruptors are more effective in risky habitats than in safe habitats. Most compounds were emitted in lower concentrations from the nut than the shell, which is in line with recent observations that lower concentrations of volatile compounds are more attractive to rodents. When rodents approach close to the location of a buried seed, they use odour gradients to locate the seed and possibly amplification of odours could disturb rodent foraging.

When measuring the time spent in an odour preference zone, shorter time intervals were better and more time-efficient in order to identify attractive odours to bank voles in a Y-maze (Paper III). Nose pokes into an odour source may be a more ethological relevant measurement but also increase the time of the assay, which can have large implications when screening natural foods which emit a wide variety of volatile compounds.

The measured volatile profile of acorns *Q. robur* varies with its' metabolic state and the sampling method (Paper II & III). Use of dynamic headspace methods give better quantitative estimates while SPME is a more sensitive technique that may, therefore, collect volatile compounds which are released in low amounts. Many seeds have evolved to emit less of attractive compounds in order to reduce predation. Simultaneously, rodents can be more attracted to low amounts of odours. Therefore, SPME may be a more suitable method to identify attractive compounds.

Finally, identifying the behaviourally attractive compounds emitted from acorn nuts in the laboratory and verifying their importance for foraging in the field can be used to disrupt rodent foraging but also open up a new field of research of how seed odour has evolved both to facilitate seed dispersal and to escape predation.

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Populärvetenskaplig sammanfattning

Skogsekosystem förser oss med en rad livsviktiga tjänster, som rening av vatten och luft, produktion av träråvara samt rekreationella värden. Skogarna innehåller också en stor del av den landlevande biologiska mångfalden och genom trädens upptag av koldioxid dämpas klimateffekten. Över 50 % av jordens ursprungliga skogar har försvunnit vilket har lett till flera globala initiativ att restaurera skog. I dessa initiativ, såsom Bonn-utmaningen och New York Forest Declaration, har regeringar, NGOs och privata aktörer lovat att återföra miljoner hektar till skog.

En av de stora utmaningarna med storskalig restaurering av skogsmark är de höga kostnaderna varav plantering och plantmaterial är en stor utgiftspost. En möjlighet är att sänka kostnaderna genom sådd istället för plantering. Ek-släktet består av ca 600 arter spridda över hela jorden och är särskilt lämpliga att så då de har näringsrika frön, ekollon, som förser den groende plantan med växtkraft.

Dock används sällan sådd för att föryngra ek på grund av att gnagare är mycket duktiga på att hitta och konsumera de nedgrävda ekollonen. Vanligtvis försvinner nästan alla ekollon inom fem månader. Trots att det länge varit känt att gnagare använder sina känsliga doftsinnen för att hitta frön i marken så är det fortfarande okänt hur de går tillväga för att hitta fröna, samt vilka doftämnen de attraheras av. Dofter består vanligtvis av flera doftämnen. Dessa tas upp av luktsinnet i nosen som skickar signaler till hjärnan. I hjärnan tolkas dessa signaler om till dofter. Genom att ta reda på vilka doftämnen som gnagarnas luktsinnen känner av, kan dessa ämnen syntetiseras och användas för att störa gnagarnas jakt på ekollon. Till exempel kan en syntetisk ollondoft spridas ut över en eksådd för att på så vis kamouflera ekollonen.

I den här avhandlingen har jag sammanställt kunskap om gnagares påverkan på eksådd samt undersökt hur olika dofter påverkar gnagarnas födosök. I en sammanfattning av forskningsläget (studie I) fann vi att gnagares konsumtion av ekollon är den enskilt största faktorn som påverkar sådd av ek. Därför har också en rad olika metoder för att skydda ekollon testats. Det absolut bästa skyddet har uppnåtts med hjälp av olika fysiska skydd, till exempel gallerburar. Men dessa är dyra att producera, sätta ut och ta bort när plantan väl etablerat sig. Försök med att olika växt- och rovdjursbaserade repellenter har visat sig ineffektiva då de antingen skadar plantans tillväxt eller så vänjer sig gnagarna efter en tid. Den enda studien som nämnde att dofter kunde maskera ekollon hade hittat ett samband med mindre ollonpredation i marker med harris, författarna spekulerade då om att det kunde vara orsakat av harrisets speciella doft.

I en fältstudie (studie II) så undersökte jag om gnagare i ekskog använde sig av den ätliga nöten eller det oätliga skalet när de letade efter ekollon. Studien utfördes i två ekskogar, varav en var gammal med ett rikt buskskikt och den andra var en ungskog helt utan buskar. Slagfällor betades med antingen skal eller nöt från ekollon. De båda betena stoppades i vita tepåsar för att gnagarna inte skulle kunna använda synen i jakten på ollonen. I fällorna fångades skogssork samt större och mindre skogsmus. Alla arterna gick både i fällor betade med nöt och i de betade med skal, vilket visar att de kan använda sig av både doft från nöten och skalet för att hitta ekollon. Dock skilde sig resultatet mellan de två olika lokalerna. På lokalen med mycket buskar och annan undervegetation, som gnagarna kan gömma sig bland, så använde de sig av nöt-doften i högre utsträckningen än doften från skal. Vi tror att gnagare som letar mat i skydd av buskar kan göra mer noggranna val av vilka dofter de ska följa, medan när det finns få buskar så prioriterar gnagarna snabba och slarviga val för att minska tiden de är utsatta för fara från rovdjur. Alla arter visade samma mönster, men det var främst hanar som valde nöt-doften. Detta kan bero på att hanar är mer benägna att ta risker och därför spenderar längre tid utan skydd för att göra mer noggranna val. Tidigare forskning visar också att just hanar är överrepresenterade i rovfåglars diet. En analys av vilka flyktiga ämnen som nöt och skal avger fann att de avger till stor del samma ämnen, vilket kan förklara varför båda ekollon-delarna var attraktiva. Skalet avgav fler ämnen och ofta i större mängd, främst olika monoterpener. Ett ämne som avgavs ungefär lika mycket från både nöt och skal var acetoin, ett ämne som har en karaktäristisk smör-doft och dessutom funnits ha en attraktiv effekt på råttor.

I den tredje studien utvecklade jag en metod för att mäta hur attraktiv en doft är för skogssorkar, en av de vanligaste ekollonpredatorerna. Arbetet utfördes på Jagellonska universitetet i Krakow. Skogssorkarna sattes i en arena formad som ett Y, där två olika dofter pumpades in i varsin arm. Det gick både att mäta doftpreferensen hos skogssork med hur lång tid de spenderade i vardera arm i arenan samt med hur många gånger de stoppade in sin nos i det hål där dofterna kom ifrån. Det snabbaste sättet att mäta doftpreferensen var att under kort tid (15 s) mäta den tid skogssorken spenderade i armarna. Snabbheten har stor betydelse för det fortsatta arbetet med att bestämma vilka doftämnen som attraherar skogssorkar då jag genom kemiska analyser fann att ekollonen avger ca 30 olika ämnen. Skogssorkarna som var uppfödda i en laboratoriemiljö var även attraherad av ekollon-doft, trots att de aldrig ätit ekollon, vilket tyder på att skogssorken har en medfödd attraktion till ekollon.

Sammantaget visar mina studier att gnagare är väl anpassade för att hitta ekollon som är nedgrävda. Ekollon avger en mängd olika flyktiga ämnen varav flera har potential som doftämnen. Dessa skulle kunna användas för att maskera ekollonen, vilket i kombination med frånvaro av strukturer såsom buskar skulle kunna skydda ekollon från gnagarpredation.

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Rodent predation on seeds is the main problem during reforestation of oak using direct seeding. This thesis focuses on how rodents use odours to locate acorns. Rodents were attracted to both edible and non-edible parts of the acorn. Combined gas chromatography and mass spectrometry showed several volatile compounds from acorns that potentially can be used by rodents to locate acorns. Since rodents use olfaction to locate buried seeds, odour manipulations have the potential to provide cost-efficient protection to direct-seeded acorns.

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