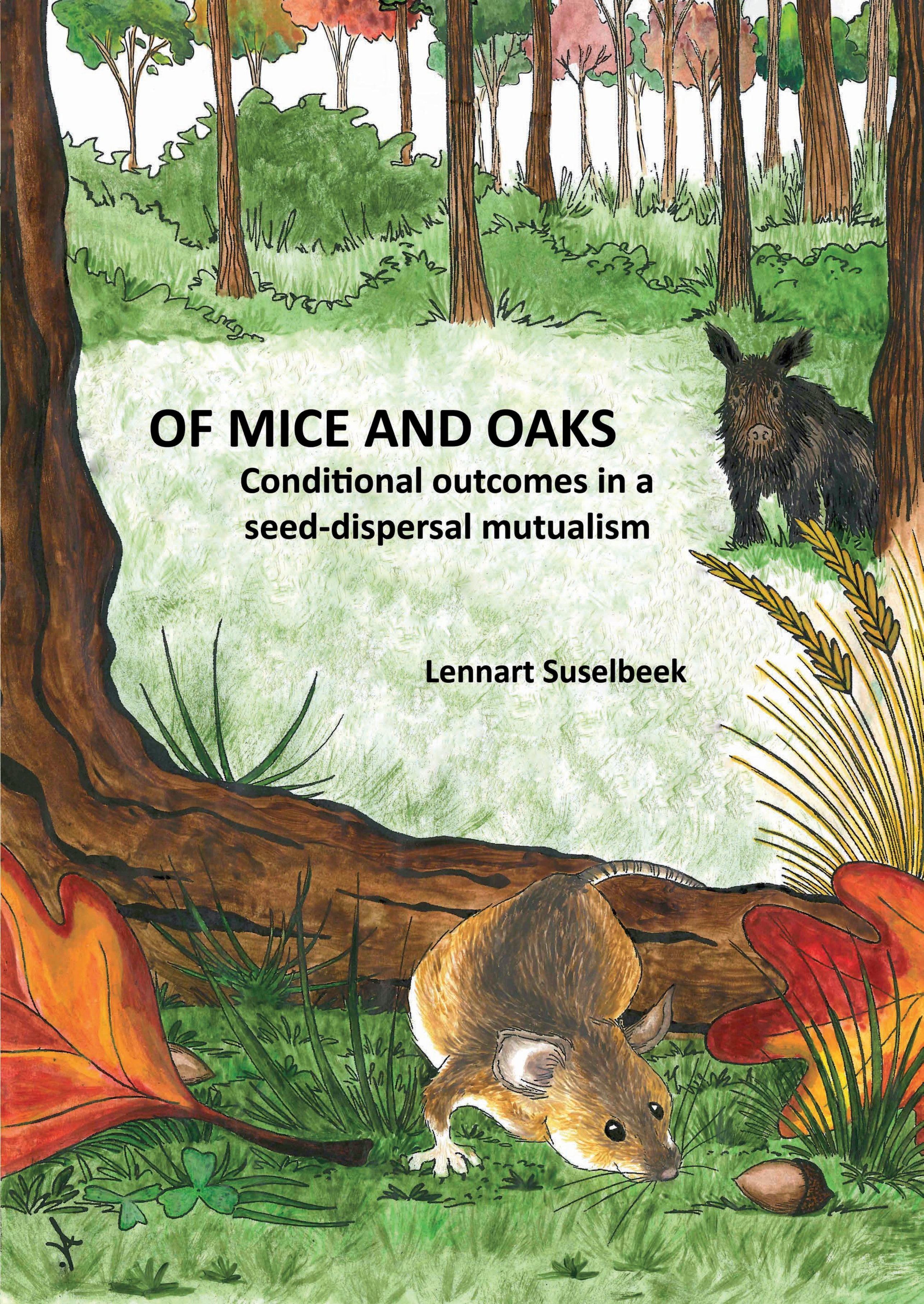

OF MICE AND OAKS

Conditional outcomes in a seed-dispersal mutualism

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Chapter 1

General introduction

Lennart Suselbeek

Background to the study of mutualisms

Mutualisms occur in habitats around the world and are considered fundamental to ecological and evolutionary processes (Tomback 1982; Howe 1984; Boucher 1985; Bond 1994; Bronstein 1994b; Ferriere *et al.* 2002; Herrera & Pellmyr 2002; Del-Claro 2004; Holland *et al.* 2004; Bronstein *et al.* 2006; Bascompte 2009; Bronstein 2009). In *The Origin of Species,* Darwin (1859) was one of the first to give substantial attention to mutualism but it was the Belgian zoologist Pierre Joseph van Beneden who introduced the term mutualism in biology. Van Beneden defined *mutualism* to apply to “mutually beneficial relationships between species” (Van Beneden 1876). A more formal definition of a mutualism is “an interaction between individual organisms in which the realized or potential genetic fitness of each participant is raised by the actions of the other” (Janzen 1985). Mutualisms may be roughly subdivided into four types: 1) pollination mutualisms, such as the pollination of flowers by bees (Kearns *et al.* 1998), 2) digestive mutualisms, such as the interaction between ruminants and microbes in their gut (Mackie *et al.* 1997), 3) protective mutualisms, such as plant protection by carnivorous insects (Wäckers & van Rijn 2005), and 4) seed-dispersal mutualisms, such as the interaction between frugivorous birds and fruit-producing plants (Howe 1986).

The concept of conditionality

Traditionally, interactions among species were thought to be either positive (+), negative (-), or neutral (0) for each species involved in the interaction. Consequently, mutualism (+,+) was one of six potential outcomes of interspecific interactions, the others being commensalism (+,0), agonism (+,-), competition (-,-), amensalism (-,0), and neutralism (0,0) (Malcolm 1966; Lewis 1985; Bronstein 1994a). In reality, the outcome of a given interaction may vary in space and time (Bronstein 2001; Bronstein *et al.* 2003), and can shift, for instance, from mutualistic to antagonistic (Bronstein 1994a; Herre *et al.* 1999; Del-Claro & Oliveira 2000; Siepielski & Benkman 2008). Therefore, it would be more appropriate to consider species interactions from a cost / benefit perspective, i.e., If either the costs or the benefits change in magnitude, the degree to which an association is mutually beneficial will change as well.

Variation in the outcome of species interactions as a function of the biotic and abiotic context in which the interaction occurs, commonly termed *conditionality* or *context dependence*, has been documented in several types of species interactions (reviewed in Chamberlain *et al.* 2014). However, despite substantial evidence that the magnitude and sign of species interactions is context-dependent, the ecological and evolutionary significance of conditionality and the main factors driving it have not been well characterised (Benedetti-Cecchi 2000; Agrawal *et al.* 2007; Chamberlain *et al.* 2014). Within the field of mutualisms, it has been predicted that conditional outcomes are most likely to occur if a) the mutualism is facultative rather than obligate, b) the densities of both interacting partners vary, or c) a third species is intimately involved in the interaction (Bronstein 1994a; Herre *et al.* 1999; Del-Claro & Oliveira 2000; Siepielski & Benkman 2008). These and other predictions regarding conditionality have received considerable theoretical and empirical attention in plant-pollinator-, digestive-, and plant-protection studies (reviewed in Beattie 1985; Cushman & Addicott 1991; Bronstein 1994a; Bronstein & Barbosa 2002; Bronstein *et al.* 2006; Chamberlain & Holland 2009; Chamberlain *et al.* 2014). However, theoretical exploration of the phenomenon in seed-dispersal mutualisms has only just begun (Theimer 2005; Holland & DeAngelis 2009; Schupp *et al.* 2010), and empirical evidence of conditionality in seed-dispersal mutualisms is still scarce (but see, Siepielski & Benkman 2008; Jorge & Howe 2009; Klinger & Rejmánek 2010; Liu *et al.* 2013). My study is an attempt to fill this gap by quantifying the role of seed-hoarding rodents as conditional mutualists of large-seeded trees in a multi-trophic system, and to explore the main factors driving conditionality in seed-dispersal mutualisms.

Seed-dispersal mutualisms

Seed-dispersal mutualisms are widespread and are considered of crucial importance for plant regeneration (Janzen 1970; Howe & Smallwood 1982; Boucher 1985; Howe 1989; Nathan & Muller-Landau 2000; Levey *et al.* 2002; Steele & Smallwood 2002; Vander Wall 2002; Forget *et al.* 2005). A seed-dispersal mutualism can be defined as an interaction between a plant and a seed disperser, resulting in resources for the disperser as well as seed dispersal for the plant, with both partners benefiting from the interaction in terms of fitness or reproductive growth (Janzen 1985). The process of seed dispersal includes the movement of seeds away from the parent plant and its siblings, but also away from other seeds, thereby reducing density- and distance-dependent mortality (Janzen 1970) and directed to sites that are more favourable for germination and establishment (Howe & Smallwood 1982; Briggs *et al.* 2009; Hirsch *et al.* 2012b). Although the ecological and evolutionary significance of seed-dispersal mutualisms have been recognized long ago, critical examination of the phenomenon has been hampered by the difficulty to non-invasively follow the seed-dispersal process from seed shedding to seedling establishment (Wang & Smith 2002; Forget *et al.* 2005; Vander Wall *et al.* 2005b).

Animals dispersing seeds range from species as small as ants (Christian & Stanton 2004) to large mammals like rhinoceros (Dinerstein & Wemmer 1988), and also the mechanism by which animals disperse seeds are diverse (Van der Pijl 1972). Many birds and herbivores disperse seeds by defecating seeds that are contained in fleshy fruits away from the host plant (Howe 1989), while several ant species transport seeds to the nest where they consume a nutritive elaiosome, attached by the plant to the seed, leaving the seed unharmed and protected in or just at the entrance of the ant's nest (Handel & Beattie 1990). Finally, many rodent species living in regions with alternating periods of food scarcity and food abundance, disperse seeds by storing them in underground caches (Howe & Smallwood 1982; Jensen & Nielsen 1986; Vander Wall 1990; Vander Wall *et al.* 2005b). In this process, called food hoarding, a substantial proportion of seeds handled is consumed or damaged by the hoarder, however, some cached seeds may be forgotten or may otherwise escape predation and are left to germinate and establish seedlings (Vander Wall 1990). Large-seeded trees are thought to rely heavily on seed-hoarding rodents for the dispersal of their seeds (Vander Wall 1990; Jansen & Forget 2001; Jansen *et al.* 2004; Forget & Jansen 2007).

Rodents as conditional mutualists of large-seeded trees

The interaction between plants and seed-hoarding rodents is likely to show conditionality, because seed-hoarding rodents play a dual role in plant regeneration. They consume large quantities of seeds, thereby reducing plant recruitment, but at the same time they store many seeds, potentially reducing the seed's risk of desiccation, consumption by strict seed predators, and distance- and density-dependent mortality below the parent plant and close to conspecifics (Janzen 1970, 1971; Jansen & Forget 2001; Vander Wall 2001; Zwolak & Crone 2012). In addition, seed dispersal is sometimes directed to places where seed- and seedling survival are relatively high (Howe & Smallwood 1982; Briggs *et al.* 2009; Hirsch *et al.* 2012b). However, whether the costs of seed predation outweigh the benefits of caching seems to be highly dependent on the hoarder's strategy of caching, which in turn is likely to vary with the circumstances under which hoarding takes place (Stapanian & Smith 1978; Vander Wall 1990; Theimer 2005; Zhang *et al.* 2011; Zwolak & Crone 2012).

The strategies that rodents use for storing seeds typically range between two extremes: scatter hoarding and larder hoarding (Vander Wall 1990). Larder hoarding involves the placement of many food items in one or few caches ('larders'), usually located in or near the nest, in underground burrows, or in tree cavities. Larders are relatively easy to create and recover, yet usually require active defence against other animals (Clarkson *et al.* 1986; Vander Wall 1990; Daly *et al.* 1992; Dally *et al.* 2006). Scatter hoarding, in contrast, involves spreading of food over many widely spaced caches with one or few food items each ('scatters') (Morris 1962; Clarkson *et al.* 1986; Vander Wall 1990, 2001). Scatters are not individually defended and involve higher energetic costs in terms of travel (food is spaced out more widely) and memory (more locations have to be remembered), as well as increased mortality risk due to longer exposure to predators and adverse environmental conditions (Stapanian & Smith 1978; Smith & Reichman 1984; Stapanian & Smith 1984; Jenkins *et al.* 1995; Dally *et al.* 2006). While larder hoarding generally precludes recruitment because large numbers of seeds are stored in deep burrows where germination and seedling establishment is highly unlikely, scatter hoarding may positively affect plant regeneration because individual seeds are buried in numerous spatially scattered, shallow soil or litter caches. Non-recovered scatter-hoarded seeds may thus be left in sites that are suitable for germination and seedling establishment: they are dispersed away from their parents, siblings, and associated parasites and pests, and they are planted at shallow depth but out of predators' immediate reach (Vander Wall 1990).

Conditionality in this type of interaction arises due to many rodent species following a mixed-hoarding strategy, in which seeds may either be individually scatter hoarded, be hoarded in small clusters, or be larder hoarded in underground burrows (Clarke & Kramer 1994; Den Ouden *et al.* 2005). The degree to which the scatter-hoarding strategy is followed – determining the effectiveness of hoarding as seed-dispersal mode – typically varies in space and time (Theimer 2005), but the drivers of this variation are largely unknown . One theory is that the pattern of seed hoarding employed by an animal is related to its ability to defend caches against competitors (hereafter, superiority) (Stapanian & Smith 1978). If the hoarder is superior over its competitors, larder hoarding would be an optimal strategy, since larders can be defended against inferior competitors. However, if the hoarder faces superior competitors, larders would not be safe from pilferage (i.e., cache predation by an individual other than the original hoarder of that cache) and thus the optimal strategy may be scatter hoarding. Under high food competition, especially with superior competitors present, scatter hoarding could thus yield a greater net reward than larder hoarding for the inferior hoarder. In other words, the benefits of decreased cache pilferage may outweigh the increased costs of scattering, remembering, and retrieving the caches (Stapanian & Smith 1978; Dally *et al.* 2006).

This study

The objective of my study was to investigate the role of seed-hoarding rodents as conditional mutualists of large-seeded trees in a multi-trophic system. I specifically aimed to examine whether and how intra- and interspecific food competition affects the hoarding patterns of rodents such that their role as mutualists of large-seeded trees changes. To do this, I studied the interactive effects of seed abundance, rodent abundance, and the presence of a third-species competitor on seed-hoarding patterns as produced by rodents, both in the field and in controlled experiments. The ultimate goal was to explore the broader ecological and evolutionary consequences of conditionality in seed-dispersal mutualisms.

I focused on the interaction between a large-seeded tree, Pedunculate oak (*Quercus robur*), two seed-hoarding rodents, Wood mouse (*Apodemus sylvaticus*) and Bank vole (*Myodes glareolus*), and a heterospecific seed predator, Wild boar (*Sus scrofa*). What distinguishes my study from other recent studies of conditionality in seed-dispersal mutualisms (Siepielski & Benkman 2008; Jorge & Howe 2009; Klinger & Rejmánek 2010; Liu *et al.* 2013), is the inclusion of a third-species competitor that is intimately involved in the interaction, and the use of a novel technique (i.e., PIT tagging) for non-invasively tracking individual seeds all the way up to the seedling establishment.

Design of the study

My study consisted of a combination of a large empirical field study and two controlled experiments. The field study involved tracking of over a thousand acorns in time and space to determine their ultimate position and fate, across twelve sites that varied in wild-boar presence and rodent abundance, but that were otherwise comparable (Fig. 1.1a). For this, I used Passive Integrated Transponder (PIT) tags, inserted into acorns (Fig. 1.1b). PIT tags are small (2x12 mm) uniquely coded glass transponders that can be tracked indefinitely and can be detected with antennae up to distances of 40 cm. The use of PIT tags allowed me to non-invasively and individually follow the seeds until they had either died or established seedlings. The results of this field study enabled me to evaluate whether and how seed-hoarding patterns of rodents were affected by wild-boar presence, rodent abundance, and seed abundance (this varied between years), and how this, in turn affected the mutualism between rodents and oaks.

The two controlled experiments were set up to disentangle the complex web of interactions encountered in the field. In the first controlled experiment eight captive wild boar were allowed to search for acorns that had been experimentally hidden according to different hoarding strategies. This experiment was used to evaluate whether differential seed-hoarding patterns actually affected the risk of seeds being found and pilfered by wild boar (Fig.1.1c). The second controlled experiment involved experimental caching of a fixed number of acorns by wood mice that varied in sex and weight and that had been wild-caught from sites with contrasting wild-boar presence (Fig. 1.1d). This enabled me to test whether variation in hoarding patterns could be explained by weight, sex, and/or provenance of the hoarder.

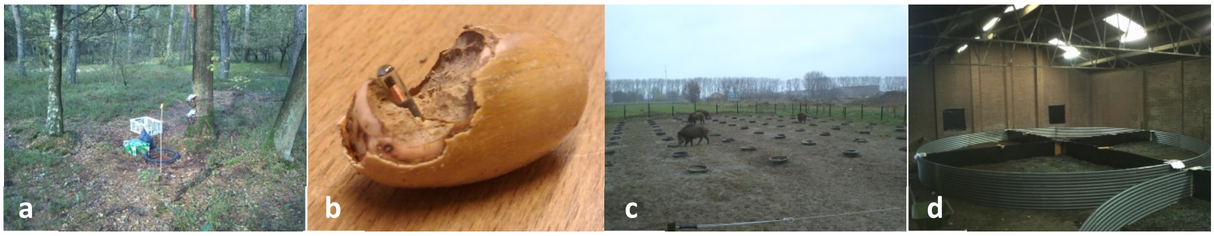

Fig. 1.1: (a) shows the experimental setup for monitoring seed removal as part of the field study described in **chapter three**. (b) shows a PIT-tagged acorn of the pedunculate oak that was partly consumed by rodent. This technique is further described in **chapter two**, and was used for tracking seed-dispersal in the field (**chapter three**) and in the experiment with captive rodents (**chapter five**). (c) shows the experimental setup of **chapter four**, and (d) shows the experimental setup of **chapter five**.

Study site

The fieldwork for this study took place between September 2010 and July 2012 in a forested area in the Netherlands, the Veluwe (52˚1'N, 5˚43'E). The area consists of sandy deposits and a spodosol soil type (Bloemers *et al.* 1980). Mean annual precipitation during 2010-2012 was 870 mm and average temperature ranged between 3 ˚C in January to 18 ˚C in July (Koninklijk Nederlands Meteorologisch Instituut (KNMI) 2014). Tree cover comprised a mixture of coniferous and deciduous species, dominated by Scots pine (*Pinus sylvestris*), Douglas fir (*Pseudotsuga menziesii*), Japanese larch (*Larix kaempferi*), European beech (*Fagus sylvatica*), Red oak (*Quercus rubra*), and Pedunculate oak. The understory was dominated by Bracken *(Pteridium aquilinum)*, Blueberry *(Vaccinium myrtillus)*, Bramble (*Rubus fruticosus*), and several species of grasses and sedges (Smit *et al.* 2001; Den Ouden *et al.* 2005; Jansen & Den Ouden 2005; Kramer *et al.* 2006).

The study area was effectively divided into two parts by a fenced four-lane motorway, established in 1956. Conveniently, wild boar occurred in relatively high densities north of the motorway (i.e., approximately 3-5 individuals km^{-2}) while they were not allowed, and thus absent, south of the motorway (Spek 2014). Apart from providing a stark contrast in wild-boar presence, the motorway also served as a barrier for mixing of rodent populations between both parts of the study area. This enabled testing for behavioural differences between rodents living in areas with a long history of wild-boar presence and rodents living in areas with a long history of wild-boar absence.

Study species

Pedunculate oak (Fig. 1.2) is a large-seeded deciduous tree species abundant in central and western Europe that exhibits alternate bearing, with periodic rich and poor mast years (Crawley & Long 1995). Seeds start to ripen in September-October and are shed between October and December (Watt 1919; Jones 1959; Bossema 1979; Crawley & Long 1995). In good seed-years, production averages about 50,000 seeds (Jones 1959; Crawley & Long 1995; Tyler 2008). The seeds of pedunculate oak (henceforth: acorns) are used by many animals to overcome food scarcity during winter, such as Eurasian jay (*Garrulus glandarius*), Wood pigeon (*Columba palumbus*), Red squirrel (*Sciurus vulgaris*), Red deer (*Cervus elaphus*), Roe deer (*Capreolus capreolus*), Bank vole, Wood mouse, and Wild boar. In return, pedunculate oak relies heavily on animals for the dispersal of its seeds, which is taken care of primarily by corvids, squirrels, and rodents (Jones 1959; Bossema 1979; Jensen & Nielsen 1986; Crawley & Long 1995; Gomez *et al.* 2003; Den Ouden *et al.* 2005; Gomez *et al.* 2008). Other than being eaten by seed predators, acorns are prone to die from drying, freezing, or rotting when left on the forest floor (Jones 1959).

Fig. 1.2: drawing of the large-seeded tree species used in my study, the pedunculate oak.

The two species of seed hoarders in my study system are both common European woodland rodents: the Wood mouse and the Bank vole (Fig. 1.3). The wood mouse measures 75-110 mm (excluding tail) and weighs between 14 and 35 g, whereas bank vole tends to be slightly larger, measuring 85-135 mm (excluding tail) and weighing between 12 and 40 g. They primarily breed between April and October, depending on temperature and food availability (Lange *et al.* 1994). Both species are largely nocturnal and are characterized by well-developed olfactory, visual, and auditory senses. They are typical granivores, with seeds constituting roughly 70% of the diet, particularly so in autumn and winter (Watts 1968; Flowerdew *et al.* 1985). Wood mice and bank voles play an important role as predator and disperser of acorns in Europe, by hoarding acorns in autumn to serve as a food reserve for winter (Vander Wall 1990; Den Ouden *et al.* 2005; Takahashi *et al.* 2007). They may function as mutualists of oak when scatter hoarding seeds, providing seed dispersal and protection against seed predators and environmental stress, but they function as seed predators when larder hoarding seeds in underground burrows (where recruitment is near-absent), when eating seeds without first hoarding them, r when recovering all caches for food (Den Ouden *et al.* 2005).

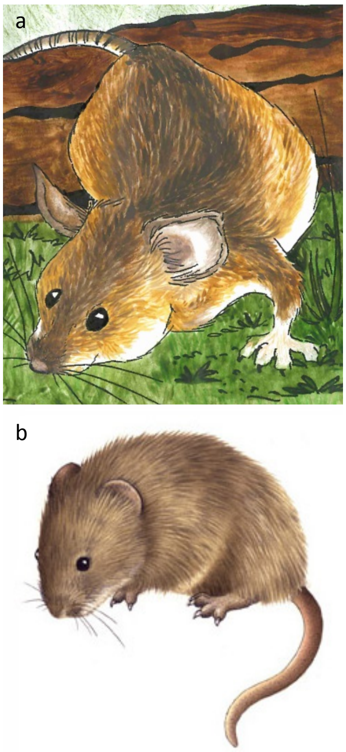

Fig. 1.3: drawings of the two species of seed-hoarding rodents used in my study. (a) shows a drawing of the wood mouse, and (b) shows a drawing of the bank vole.

The third species, believed to be intimately involved in the interaction between oaks and seed-hoarding rodents, is the Wild boar (Fig. 1.4). Wild boar is a medium-sized (50-200 kg) ungulate that is regarded as an important competitor to seed-hoarding rodents in forests and woodlands throughout its range of occurrence (Focardi *et al.* 2000; Massei & Genov 2004). Acorns are a dominant food source in autumn when they have freshly shed and are abundantly available on the forest floor (Massei & Genov 2004; Elston & Hewitt 2010), but also in mid- and late winter when very few acorns remain on the forest floor (Groot-Bruinderink *et al.* 1994; Den Ouden *et al.* 2005; Muñoz & Bonal 2007). The latter suggests that wild boar actively search for and pilfer acorn caches created by seed hoarders, such as wood mouse and bank vole (Borchert *et al.* 1989; Herrera 1995; Focardi *et al.* 2000; Gomez *et al.* 2003; Den Ouden *et al.* 2005). Wild boar have well-developed olfactory senses, which they may use for locating cached acorns and other hidden food items (Briedermann 1986; Focardi *et al.* 2000; Den Ouden *et al.* 2005).

Fig. 1.4: drawing of the third-species competitor used in my study, the wild boar.

Outline of the thesis

In chapter 2, I describe the PIT-tagging method that I used for tracking rodent-dispersed seeds. My study was the first to use PIT tags for tracking seeds and establishing seed fate. I explain how PIT tags work, what the pros and cons are of using this technique, and how PIT tagging compares to other frequently used techniques for tracking rodent-dispersed seeds, such as thread marking.

Chapter 3 deals with the large empirical field study, in which PIT-tagged acorns were presented to seed-hoarding rodents living in areas with a long history of either wild-boar presence or wild-boar absence. Seed-removal rate, seed-dispersal distance, cache spacing, and ultimate seed fate were recorded to test whether and how hoarding patterns varied with wild-boar presence, rodent abundance, and seed abundance, and how this, in turn, affected the mutualism between rodents and oaks.

Chapter 4 considers the risk of pilferage by wild boar, under different hoarding patterns. I designed this controlled experiment to mimic alternative outcomes of the hoard-size / number trade-off that hoarders face. Given a certain food supply (i.e., a fixed number of seeds or other food items) and available area (i.e., the home range), pilferage rates can be influenced only by changing the distribution of food items over the available patches and by varying the depth at which food items are cached. I tested this by distributing a fixed number of acorns (32) over a fixed number of patches (100) within a fixed area (750 m^2), but varying cache size and cache depth, thus mimicking alternative hoarding patterns. I then had a fixed number of wild boar searching for the hidden food items, to test whether pilferage rates were affected by the hoarding pattern.

Chapter 5 describes the second controlled experiment. Here, I placed wood mice, varying in sex and weight and wild-caught from areas with contrasting wild-boar presence, individually in large sand-filled arenas, where they were allowed to hoard a fixed number of acorns. This experiment was designed to evaluate the importance of individual characteristics of the hoarder (i.e., superiority) for explaining variation in seed-hoarding patterns. A test with a conspecific audience was later introduced to investigate whether and how pilferage risk by conspecifics affected seed-hoarding patterns.

Finally, in chapter 6, I present a synthesis of the main findings, also including some results of unpublished experiments. I discuss the phenomenon of conditionality in seed-dispersal mutualisms, and more specifically, how the presence of a third-species competitor can affect the outcome of seed-dispersal mutualisms. I evaluate the relative importance of inter- and intraspecific food competition on seed-hoarding patterns and on ultimate seed fate, and I propose some directions for future studies.

Chapter 2

Tracking rodent-dispersed large seeds with Passive Integrated Transponder (PIT) tags

Lennart Suselbeek, Patrick A. Jansen, Herbert H.T. Prins, Michael A. Steele

This chapter was modified from:

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Abstract

Seed dispersal, a critical phase in the life history of many plants, is poorly understood due to the difficulty of tracking and monitoring dispersing seeds until they reach their ultimate fate. Scatter-hoarding rodents play a substantial part in the seed dispersal process of many plant species, however, existing tracking methods do not allow seed monitoring without risk of influencing the hoarding process and seed fate. Here, we describe and test the use of Passive Integrated Transponders (PIT) tags inserted into seeds for the tracking and monitoring of large seeds dispersed by rodents. Unlike other tagging methods, PIT tagging combines the advantages of leaving no external cues and being readable without disturbance of caches. Rodents cannot remove these tags. We evaluated the performance of PIT tagging through a series of trials with *Quercus* acorns dispersed by rodents, both in North America and in Europe, with equipment from different manufacturers. We quantified effects of tagging on seed removal and caching, cache pilferage, and seed germination, by comparison between PIT-tagged and untagged acorns. We evaluated the detectability of buried tags to researchers. Minimal effects of PIT tagging on seed mass and seed germination were found. As with typical seed handling by rodents, seed removal of PIT-tagged seeds was instantaneous, seeds were either cached or consumed, and some caches were retrieved over time while others were not, suggesting that rodent behaviour was little influenced by PIT tagging. Buried PIT tags were retrieved with high reliability by naïve researchers, even at burial depths up to 30 cm. Identification codes could be read even when multiple tags were buried at a single location, as in larder hoarding. The method was successfully applied in two field studies of dispersal of *Quercus palustris* and *Q. rubra* acorns by Eastern gray squirrels *Sciurus carolinensis* in North America, and *Q. robur* acorns by Wood mice *Apodemus sylvaticus* in the Netherlands. The proportion of seeds recovered was comparable to that in studies using traditional thread tags. We conclude that PIT tagging is a particularly suitable method for tracking and monitoring of seeds dispersed by scatter-hoarding rodents. PIT tagging solves most of the main problems generally encountered when following the fate of rodent-dispersed seeds over time.

Keywords: *acorn, hoarding, passive integrated transponder, PIT tag,* Quercus*, rodents, seed dispersal, seed tracking*

Introduction

Seed dispersal is a major determinant for plant regeneration (Van der Pijl 1972; Lemke *et al.* 2009). The majority of large-seeded trees, such as oak, chestnut, hickory, pine, and many palm species, in temperate, sub-tropical and tropical forests rely on seed hoarding by granivorous mammals and birds for primary and/or secondary seed dispersal (Howe & Smallwood 1982; Jansen *et al.* 2004; Forget *et al.* 2005). These so-called 'scatter hoarders' store large numbers of seeds, individually or in small quantities throughout their home range, serving as food supplies during periods of food scarcity (Morris 1962). The shallow cache sites used by scatter hoarders are often ideal for both seed storage and germination. Thus, when animals fail to recover some of the cached seeds, the latter are likely to establish as seedlings (Vander Wall 1990; Jansen & Forget 2001; Steele & Smallwood 2002; Forget *et al.* 2005). Scatter hoarders can move large numbers of seeds in a relatively short time span, sometimes over considerable distances (Smith & Reichman 1984; Price & Jenkins 1986; Vander Wall 1990; Steele & Smallwood 2002).

Whereas many studies have attempted to quantify the role of scatter-hoarding animals in seed dispersal and tree regeneration, few have been able to actually estimate the proportion of scatter-hoarded seeds germinating and establishing. A major reason is that scatter-hoarded seeds are often repeatedly recovered and re-cached before they reach their ultimate fate (Vander Wall *et al.* 2005b; Jansen *et al.* 2012). Existing tagging methods, such as coloured threads or flagging tape, inserted magnets or metal objects, and radio isotopes (Forget & Wenny 2005) are not well suited for tracking and monitoring seeds beyond their initial cache, either until the seeds die or until they germinate and establish as seedlings. One major concern is that these methods present cues to rodents that can increase cache dynamics (e.g., increased re-caching, increased pilferage) and thus bias ultimate seed fate. Internal tags and radio isotopes typically require disturbance of the cache to identify the individual seed, thus producing digging traces that rodents use to locate and pilfer cached seeds (Murie 1977; Guimarães Jr *et al.* 2005). Likewise, external visual tags may also increase the likelihood of rodents locating cached seeds (Hirsch *et al.* 2012a). Thirdly, traditional tagging methods often result in a considerable increase in seed weight, while seed mass is known to affect seed removal and dispersal (e.g., Jansen *et al.* 2002; Jansen *et al.* 2004).

Here, we describe a new technique for tracking the movement of animal-dispersed seeds that is free of these constraints: internal tagging of seeds with Passive Integrated Transponder tags, henceforth PIT tags. PIT tags are widely used to individually tag animals in husbandry and wildlife conservation (e.g., Elbin & Burger 1994; Hewitt *et al.* 2010; Hoy *et al.* 2010). In ecology, PIT tags have been used to study the movement and behaviour of freshwater fish (e.g., Greenberg & Giller 2001; Cucherousset *et al.* 2005), and for animal monitoring and studies of population dynamics (reviewed in Gibbons & Andrews 2004). In this paper, we provide a technical description of the method, and summarize potential advantages and disadvantages for studying removal, dispersal, and caching of seeds. Then, we present results from greenhouse and field experiments that evaluate the suitability of the technique for tracking seed dispersal, using acorns (*Quercus* spp) in two different study systems. Results of a full field study using this method are presented in a companion paper (Steele *et al.* 2011).

Technical description

A PIT tag is an electronic microchip connected to an electric resonance circuit that acts as a receiving/transmitting antenna, encased in a biocompatible glass cylinder (Fig. 2.1) (Jansen & Eradus 1999). Each PIT tag is programmed with a unique alphanumeric code, permitting individual identification with a Radio Frequency Identification (RFID) transmitter-receiver (Gibbons & Andrews 2004). The RFID reading device generates a carrier radio wave, while an antenna system attached to the reader generates an electromagnetic field that prompts the transponder to send back its code, which is then received by the antenna and interpreted by the RFID reader (Bonter & Bridge 2011). The PIT tags do not require an internal power source, and can work indefinitely. PIT tags are available in various sizes, ranging from 4 x 34 mm down to as small as 1.5 x 7 mm, and weighing just 0.05 grams, small enough to be inserted into seeds. PIT tags typically have a read range of about 25-60 cm (Fuller *et al.* 2008), large enough to detect seeds hoarded underground. This range can be extended to 1-2 meters with customized antenna and tag designs (Cucherousset *et al.* 2005). Generally, larger tags will have larger detection ranges.

Fig. 2.1: Two sizes of PIT tags (in cm) (Trovan Ltd.)

Performance tests

We evaluated the performance of PIT tags for tracking seeds in two study systems: acorns of Pedunculate oak *Quercus robur* dispersed by Wood mice *Apodemus sylvaticus* in the Netherlands, and acorns of Red oak *Q. rubra* and Pin oak *Q. palustris* dispersed by Eastern gray squirrels *Sciurus carolinensis* in Pennsylvania, USA. We used the following criteria for this evaluation: (1) no effect on seed mass, which is important because individual dispersal distance and fate are affected by seed mass (e.g., Jansen *et al.* 2002; Jansen *et al.* 2004; Pons & Pausas 2007); (2) no effect on germination and seedling growth; (3) negligible effect on animal behaviour related to scent and modification of the seed, and; (4) high rates of recovery, i.e., a low chance of missing seeds within the search area.

In the Netherlands, we prepared experimental acorns by drilling a small hole of 2 x 12 mm in the basal half of each acorn (using a wood drill), inserting a single glass-encapsulated PIT tag (ID100A, 2 x 11.5 mm, 125 kHz, Dorset Identification, Netherlands), sealing the hole with scent-free wax (Entwas, Asepta BV, Netherlands) and polishing the seed with an odourless cloth to remove all traces of wax on the seed shell. We wore scent-free gloves during all seed handling. The entire tagging process takes less than a minute per seed. The reading equipment consisted of a high-performance handheld reader (GR-250, Dorset Identification, Netherlands) and a flat-panel antenna system (LID-650, Dorset Identification, Netherlands) optimized to work with these PIT tags. The reader continuously displays tag code data received by the antenna, and alerts the operator whenever a tag is detected (as in Cucherousset *et al.* 2005). This system allows the simultaneous reading of multiple tags, which is important when caches can contain more than one seed.

In Pennsylvania, we used similar-sized PIT tags (1.5 x 12.5 mm, 134.2 kHz; Model HPT12, Biomark Corporation, Idaho, USA) with a Destron-Fearing reader and hand-held loop antenna (Model FS2001F-ISO, Biomark Corporation, Idaho, USA). Acorns of pin oak and red oak were prepared and tagged in a similar manner to that described above, sealing the hole first with odourless wood filler (Elmer's Products Inc., Ohio, USA), allowing it to dry, and then disguising the sealed hole with a small patch of the filler similar in colour to the shell of the acorns (colour varies considerably with oak species). Careful closure is important in this system, because any indication that the shell of an acorn is compromised will likely result in selective consumption rather than caching of an acorn by rodents because the animals are highly sensitive to seed perishability (Hadj-Chikh *et al.* 1996; Steele *et al.* 1996).

To evaluate effects of PIT tagging on seed mass, we randomly selected and numbered 60 Pedunculate oak acorns in the Netherlands, and 60 Pin oak and 59 Red oak acorns in Pennsylvania from composite samples from 3-5 trees of each species. We weighed each acorn with a precision balance, inserted a PIT tag, reweighed the acorn after drying of the glue and/or filler, and compared the weights with pairwise t-tests.

For Pedunculate oak in the Netherlands, we report results from a two-year field study where nearly 1,200 PIT-tagged acorns were offered to wood mice at twelve different locations in October 2010 and October 2011. Hoarded acorns were subsequently relocated and followed through time until July the following year, at which time caches were recovered and long-term seed fate was established. In addition, we experimentally tested the effects of PIT tagging on seed germination and seedling establishment, by comparing the proportion of acorns germinating and seedling growth between 60 tagged and 60 untagged acorns of Pin oak in Pennsylvania. Acorns were germinated in 1-liter plastic containers by filling the containers with dampened paper towels and nesting the acorns within the towels along the sides of the container so their germination progress could be regularly observed. This approach allows acorns to grow well up to 12 weeks or more and allows assessment of seed performance without the further confounding effects of soil nutrients. Equal numbers of pit-tagged and untagged acorns were alternately placed around the edge of each container. Paper towels were moistened daily. Seed germination was initiated on July 23, 2010 and all seedlings were harvested on August 13, 2010. For each seedling, we measured the radicle length, epicotyl length, and number of leaves. We tested for differences with Chi-squared tests (germination) and t-tests (seedling size).

We assessed effects on animal behaviour in the Netherlands, by presenting differently marked acorns to wood mice and recorded their handling and removal. We tested two wire-marking techniques; wire glued to the acorn, and wire stitched through the acorn, and one type of internal tag marking; a 5x3 mm tag inserted into the acorn (and the hole sealed with scent-free wax). Here, the tag was a magnet rather than a PIT tag, but the treatment was the same otherwise. We also report preliminary findings from a field study using PIT tags in the same area. In a separate study in Pennsylvania, we presented habituated, free-ranging Eastern gray squirrels in a semi-natural park setting with PIT-tagged and untagged acorns (Steele *et al.* 2011) to follow patterns of seed fate before and after cache owners were removed from the site.

To assess the accuracy of cache retrieval by researchers, we buried PIT-tagged acorns of Pedunculate oak in an open grassy field in the Netherlands and had a naïve researcher attempt to recover them. We individually buried 12 PIT-tagged acorns at each of 4 depths: 5 cm, 10 cm, 15 cm, and 30 cm, and determined what proportion was retrieved by the test person. We also buried 12 tagged acorns at 10 cm depth randomly scattered across a grassy field of 1000 m^2 and then had a test person, who was unaware of the cache locations, recover them. We also report here some data on cache retrieval for the long-term field study we performed in the Netherlands. Likewise, in Pennsylvania, we buried 12 PIT-tagged acorns of Red oak at each of 3 depths: 5 cm, 10 cm, and 15 cm, and had an observer recover them. Finally, we tested for the maximum detection range of both systems, measuring maximum distances at which 12 individual PIT-tagged acorns were detected when placed on the soil surface.

Results

Effects on seed mass

PIT tagging resulted in a significant, yet minor increase in seed mass in two of the three species (Fig. 2.2). Mean mass of individual acorns changed from 4.54 g (N = 60, SD = 0.70) to 4.56 g (SD = 0.70) after PIT-tag insertion in Pedunculate oak (Paired T-test; t = 16.25, df = 59, $p < 0.001$), from 5.56 g (N = 59, SD = 1.44) to 5.61 g (SD = 1.41) in Red oak (t = 5.32, df = 58, $p < 0.001$), and from 2.280 g (N = 60, SD = 0.251) to 2.281 g (SD = 0.243) in Pin oak (t = 0.10, df = 59, p = 0.9). However, seed mass did not significantly differ between randomly selected PIT-tagged and untagged acorns from the same source, neither in Pedunculate oak (Student's T-test: t = 0.013, df = 118, p = 0.99) nor in Red oak (t = 0.198, df = 116, p = 0.84).

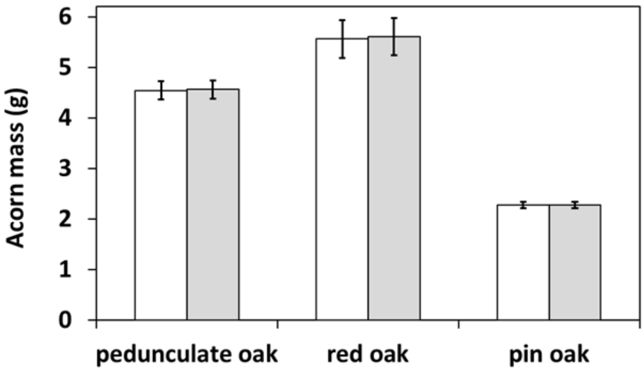

Fig. 2.2: Effects of PIT tag insertion on seed mass for pedunculate oak (N = 60), red oak (N = 59) and pin oak (N = 60). White bars represent untagged acorns and grey bars represent PIT-tagged acorns. Error bars represent 95% confidence intervals.

Effects on germination and seedling establishment

In Pedunculate oak, a field study showed that PIT-tagged acorns readily germinated and established as seedlings. Of nearly 1,200 PIT-tagged acorns offered to and hoarded by wood mice in October 2010 (N = 589) and October 2011 (N = 588), 833 (70.8%) were later retrieved within the search area. Of these, 114 (13.7%) still remained in July the following year. Of these last 114 acorns that were not recovered by the wood mice, only 5 (4.4%) had died from a fungal infection while all others had germinated (95.6%). Finally, 72 (63.2%) of the PIT-tagged acorns that had germinated, emerged and established as seedlings.

In Pin oak, a greenhouse experiment showed a slight effect of PIT tagging on germination but little effect on seedling growth. We observed initial germination (radicle growth > 1 cm) in 45 (75.0%) of the 60 tagged acorns and 55 (91.7%) of the 60 untagged acorns (χ^2 = 5.255, p = 0.02; Fig. 2.3a). Continued germination (plumule emergence) was observed in 39 (65.0%) and 53 (88.3%) of the tagged and untagged acorns, respectively (χ^2 = 8.382, p = 0.004; Fig. 2.3b). We noted that in these nearly 1-year-old acorns, which appeared otherwise sound, traces of fungus or mould were found in 36 of the tagged and 0 of the untagged acorns. This suggests that in older acorns, tagging may predispose them to lower germination success by allowing colonization by pathogens. Thus, care should be taken to use newly collected seeds and to minimize contamination when preparing tagged nuts. Under normal circumstances PIT tagging would occur at the time of acorn maturation and if the acorn is well sealed, mould and fungus are unlikely to penetrate the cotyledon.

Among the tagged and untagged Pin oak acorns successfully exhibiting aboveground seedling growth, we observed nearly identical measures of seedling performance six weeks after planting; including mean number of leaves (tagged (N = 39): mean ± SD; 6.2 ± 2.3; untagged (N = 53): 6.0 ± 2.7; Welch's t-test = 0.342, p = 0.73; Fig. 2.3c), mean epicotyl length (tagged: 17.6 ± 5.7 cm; untagged: 18.7 ± 5.8 cm; t = -.973, p = 0.33; Fig. 2.3d) and mean radicle length (tagged: 20.6 ± 6.3 cm; untagged: 20.7 ± 7.2; t = -0.083, p = 0.93; Fig. 2.3e).

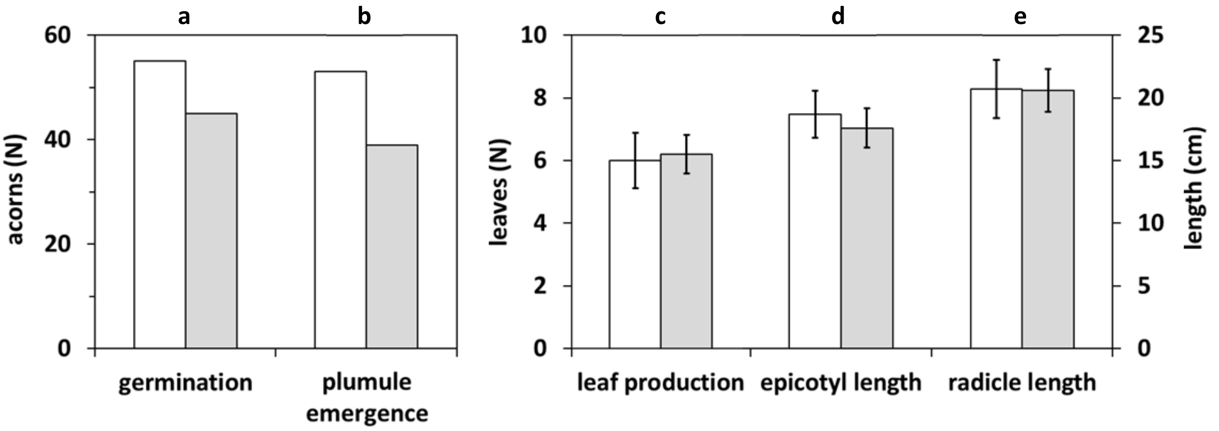

Fig. 2.3: Effects of PIT tagging on (a) germination (N= 60), (b) plumule emergence (N = 60), (c) number of leaves produced (N = 53 for untagged and N = 39 for PIT tagged), (d) mean epicotyl length (N = 53 for untagged and N = 39 for PIT tagged), and (e) mean radicle length (N = 53 for untagged and N = 39 for PIT tagged) for Pin oak acorns. White bars represent untagged acorns and grey bars represent PIT-tagged acorns. Error bars represent 95% confidence intervals.

Effects on animal behaviour

In the Netherlands, wire-marking techniques did not work well with wood mice. The majority of wire-marked acorns (55%, N = 76) was not removed from the plot at all by wood mice, and of the acorns that were handled by wood mice, 84% was gnawed off the wire and only the wire was retrieved. In contrast, all magnet-marked acorns (N=45) were removed from the seed plot within one night. Of these, 19 (42%) were retrieved within the search radius of 30 m, while the other acorns had probably been dispersed outside the search area. In 10 cases (53%), acorns were found intact while in the other 9 cases magnets were retrieved together with acorn shell remains, indicating consumption at the retrieval site. Our field study with PIT-tagged acorns (12 sites, 49 acorns / site, N=588) provided similar results to those from magnet-tagged acorns. All PIT-tagged acorns were removed within one to three nights and were often retrieved intact in shallow individual caches throughout the search area or as exposed PIT tags accompanied by acorn shells, indicating local consumption.

In Pennsylvania, animals never rejected tagged acorns but instead either ate or cached them within sight. After consumption, PIT tags were often dropped at the feeding site and easily recovered among the feeding debris. Immediately after the animal cached an acorn, we mapped the cache location and verified the presence of the acorn in the cache site. We were then able to revisit cache sites almost indefinitely and monitor the presence of the cache without disturbing the cache in any manner. When an acorn was removed, either by a pilfering conspecific or by the cache owner, an obvious pit was observed and the PIT tag was not detectable. When the acorn was eaten at the site, observers often recorded acorn shell fragments and the intact PIT tag. Steele *et al.* (2011) used this technique to follow the fate of acorn caches and assess pilfering rates of natural caches when cache owners were removed from their home ranges.

Retrieval success

Using the Trovan system in the Netherlands, all seeds artificially cached at 5, 10, and 15 cm depth were detected and read by the naïve researcher. At 30 cm depth, 90% of the acorns were detected and read. The test person also recovered all acorns that we artificially scatter hoarded (at 10 cm depth) within 6 minutes, without need to repeatedly search the same area. The maximum depth of detection for 12 tagged acorns ranged between 27.8 and 34.2 cm (31.43 ± 1.70), and the system was capable of detecting and reading up to 6 PIT-tagged acorns cached together, and of detecting caches with up to 16 PIT-tagged seeds. Results from a field study in a natural forest area in the Netherlands, where nearly 1,200 PIT-tagged acorns were offered to and hoarded by wood mice, showed a retrieval success of 70.8% (N = 833) within a search radius of 45 meters from the source location. Likewise, using the Biomark system in Pennsylvania, all PIT-tagged acorns were detected and read at all three depths. Maximum depth of detection for 12 tagged acorns of Red oak ranged between 18.0 and 31.5 cm (23.75 ± 4.77), which is enough to also detect seeds that have been taken into an average-size burrow system (Jennings 1975).

Discussion

Assessing the ultimate fate of seeds dispersed by rodents requires tracking and monitoring of seeds without animals severing the tags, and without tags presenting cues to pilferers, as the latter may accelerate or seriously alter cache dynamics (e.g., increased re-caching, increased movement of seeds and increased risk of predation on seeds) (Guimarães Jr *et al.* 2005; Hirsch *et al.* 2012a). Here, we describe how insertion of PIT tags allows the non-invasive measurement of removal, dispersal, and ultimate fate of seeds dispersed by scatter-hoarding rodents. We show that 1) insertion of PIT tags in acorns has a negligible effect on seed mass, 2) PIT tagging hardly influences acorn germination and seedling establishment probabilities, 3) PIT-tagged seeds are treated similarly by seed dispersers as untagged seeds and that the tags are not removed, and 4) experimenters' retrieval success of PIT-tagged acorns after dispersal is high.

Although a diverse range of seed-marking techniques has been described in literature (for a review, see Forget & Wenny 2005), none of them possesses the unique combination of advantages listed above. For example, metal- (e.g., Sork 1984), magnet- (e.g., Den Ouden *et al.* 2005), and radio-isotope labelling (e.g., Vander Wall 1994, 2000) also hardly affect seed mass, but do not allow individual identification of the seed, at least not without disturbing the cache and/or leaving a possible cue for cache detection (e.g., Forget 1990). Similarly, thread marks (e.g., Forget *et al.* 1994), wire tin-tags or plastic seed tags (e.g., Xiao *et al.* 2006), telemetric thread tags (Hirsch *et al.* 2012a), and VHF radio transmitters (e.g., Tamura 1994; Soné & Kohno 1996) also allow individual identification of seeds, but they may significantly increase seed mass, are frequently severed by rodents, can influence seed predation and germination rates if seeds are pierced for tag attachment, or present cues to cache pilferers (Hirsch *et al.* 2012a). In contrast, PIT-tagged seeds were readily removed from seed plots and cached or consumed by rodents without leaving cues for pilferers, both in the Netherlands and in Pennsylvania. And although a greenhouse experiment with 1-year old Pin oak acorns showed some effects of PIT-tagging on seed germination, it did not seem to affect seedling growth once the seedling had emerged. We in part selected pin oak acorns for these germination studies because their size is among the smallest for oaks, increasing the potential for a negative effect of the tag on the embryo. It should also be noted that the dormancy period (i.e., cold stratification) of red oak species (section: *Lobatae*) requires older acorns from the previous year to evaluate germination rates. Under normal circumstances, red oaks would be tagged and sealed a few weeks after maturation, and germination would begin about 5-6 months later, thus reducing the probability of rot.

PIT tags remain detectable virtually indefinitely, allowing to follow seedlings even after the acorns have rotten away. In Pennsylvania we have recovered PIT tags at the base of tree saplings more than four years after deployment in the field. Although PIT tagging involves piercing of the shell and removal of some cotyledon mass, the tags are entirely inside and the acorns are sealed after PIT-tag insertion. As a result, the long-term impact on seed rot, seedling establishment, and growth is likely no worse, and possibly far better, than with other tagging methods that involve piercing of the acorn shell. For example, the metal-tagging method first suggested by Sork (1984) and used extensively by Steele *et al.* (2001) and Moore *et al.* (2007) is generally assumed to have no negative effect on seedling establishment and seedling survival, despite the fact that these brad nails typically leave the acorn exposed to possible pathogen infestation. Moreover, oaks are known to use only a small percentage of the cotyledon biomass for seedling development up to autotrophy (e.g., Bossema 1979; Andersson & Frost 1996). This is also why cotyledon removal by jays after seedling emergence has no adverse effects on seedling growth or development (Bossema 1979; Sonesson 1994; García-Cebrián *et al.* 2003). Therefore, any negative effects of PIT tagging are likely to show during the early developmental stages of the seedling. That PIT tags do not seem to influence long-term seed fate is further suggested by our 2-year field study in the Netherlands, in which PIT tags did not markedly influence seed survival, seed germination, and seedling establishment. Germination and seedling establishment probabilities for PIT-tagged acorns were similar to probabilities reported for untagged acorns (90-100%; e.g., Shaw 1968). This low apparent impact, combined with the fact that PIT tags do not require internal batteries and thus function indefinitely, illustrates their great potential for studying ultimate seed fate and seedling establishment, particularly when seeds with delayed dormancy must be followed until germination and seedling emergence.

Another advantage of PIT tagging, not evaluated in this paper, is the possibility of wiring specific areas to record passage of individual seeds (and animals that carry PIT tags) over a data logger. This provides more robust data than monitoring with remote cameras (e.g., Jansen & Den Ouden 2005), and it requires far less effort. This is particularly useful for studying removal rates of seeds from a central location (e.g., a seed station) or for studying pilfering from a previously created cache. Moreover, PIT tags can be simultaneously used for disperser and seed identification, by not only individually marking seeds, but by also marking the animals that disperse the seeds. Such an approach can allow one to control for independence of individual dispersal events and study the interaction of conspecific scatter hoarders, such as pilfering behaviour in an experimental setting (see Vander Wall *et al.* 2008).

Prices for individual PIT tags vary between $ 2.50 and $ 5.50, depending on type and size of the tag. Tags used in this study cost € 2.20 (~ $ 2.75) per tag in the Netherlands and $ 5.00 per tag (for a purchase over 500 tags) in the U.S. Generally, readers and antennae cost around $ 500 – $ 1,500, again depending on brand, size and qualifications. The customized flat panel antenna system used in the Netherlands cost around € 1,050 (~ $ 1,350), while the handheld reader was € 700 (~ $ 900). The Biomark system used in Pennsylvania currently costs $ 3,125 for the advanced reader and loop-antenna system used in this study (cheaper systems < $ 1,000 are available). If compared to thread-, metal- or magnet marking the use of PIT tags is more expensive, however, if compared to telemetric thread tags or VHF radio transmitters this technique is considerably cheaper. Also, since PIT tags function indefinitely, they can be re-used in various studies, lowering the costs per study.

One disadvantage of PIT tags remains the need to closely approach buried tags for detection and scanning, as in tagging with magnets, metal and radio isotopes. In irregular terrain and dense vegetation, where it can be hard or even physically impossible to cover every spot of the search area, PIT tagging can be somewhat labour intensive (it generally takes one hour to search a forest patch of 25 x 25 m) and may result in more false negatives and lower recovery rates than tagging with thread tags or active radio transmitters, which can be detected from a greater distance. In the temperate forest study systems in which we used PIT tags, however, we achieved higher recovery rates than prior studies using other tags at the same sites, even though the retrieval success for our field study in the Netherlands was negatively influenced by the size of our search area. Our search area covered a radius of 45 meters around the seed station, but it is likely that some seeds were dispersed beyond this distance.

We conclude that PIT-tagging is an excellent technique for tracking seed removal, dispersal, re-caching and ultimate seed fate in a variety of field and laboratory situations. PIT tagging solves some of the main problems generally encountered when following the fate of animal-dispersed seeds over time. They provide a reliable, non-invasive and durable seed-marking technique particularly a) in systems where typical seed-dispersal distances are less than about 50 m or in experiments where dispersal is limited to a fixed area, b) in studies where the focus is not on initial dispersal but more on re-caching and ultimate fate of seeds, and c) in long-term monitoring projects in which seed germination and seedling establishment are followed.

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Chapter 3

Conditionality in seed-dispersal mutualisms: a field test with seed-hoarding rodents and large-seeded trees

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Abstract

Animal-mediated seed dispersal is of crucial importance for plant regeneration, particularly for large-seeded trees. Consequently, seed-dispersal interactions are generally classified as being mutualistic. However, depending on the conditions, the interaction outcome may vary. This so-called 'conditionality' can have large implications for the ecological and evolutionary significance of seed-dispersal interactions, yet it has received little attention so far. We conducted a field study in which we presented PIT-tagged acorns to seed-hoarding rodents living in sites with and without wild boar (*Sus scrofa*), a species known to compete with rodents for acorns. We tested whether and how seed-hoarding patterns were affected by acorn abundance, rodent abundance, and wild-boar presence, and whether and how this in turn affected germination of hoarded seeds. Seed-removal rate was negatively related to wild-boar presence and acorn abundance, while it increased strongly with rodent abundance. Seed-dispersal distance was positively related to acorn abundance and seed spacing was positively related to rodent abundance. Changes in these seed-hoarding patterns significantly affected germination of hoarded seeds. Generally, seed germination tended to increase with rodent abundance, except if wild boar were absent and acorn abundance was low. Seed germination tended to be highest if rodents were relatively abundant and acorn abundance was high, regardless of wild-boar presence. Interestingly, under these conditions, seed-removal rates were relatively high, and seed-dispersal distance and seed spacing were greatest. We conclude that seed-hoarding patterns are influenced by the intensity of intra- and interspecific competition for seeds, which in turn affects the likelihood of seed germination. The intensity of intra- and interspecific competition thus affects the interaction outcome of seed-dispersal mutualisms, confirming the occurrence of conditionality in seed-dispersal mutualisms and highlighting the importance of considering multi-species interactions in seed-dispersal studies.

Keywords: *hoarding; seed dispersal; rodents; conditionality; mutualism; species interactions.*

Introduction

Seed-dispersal interactions between plants and animals are traditionally described as mutualisms (reviewed in Bronstein 1994a). More recently, however, studies have shown that for many proposed mutualisms, the interaction outcome is not fixed, but dynamic depending on the conditions under which the interaction takes place (Bronstein & Barbosa 2002; Bronstein *et al.* 2003; Bronstein *et al.* 2006; Holland & Bronstein 2008; Bronstein 2009; Chamberlain *et al.* 2014). It has been predicted that conditionality in mutualistic interactions is most likely to occur when 1) the mutualism is facultative rather than obligate, 2) a third species is intimately involved in the interaction, and 3) the benefits involved with the interaction are a function of the abundance of its partners (Bronstein 1994a). Based on these predictions, Theimer (2005) argued that the scatter hoarder-plant interaction would be very likely to fall in the category of conditional mutualisms. Consequently, he developed a conceptual model of how the outcome of the scatter hoarder-plant interaction depends on both the relative abundance of each partner and the challenges to recruitment faced by the plant. Thus far, the predictions derived from Theimer's (2005) conceptual model have been empirically tested in a small number of studies, most notably by Siepielski and Benkman (2008), Jorge and Howe (2009), Klinger and Rejmánek (2010), and Liu *et al.* (2013). All these studies focused on scatter-hoarding rodents, rather than on rodents that may show variation in the hoarding patterns, and they investigated how specific conditions affected the availability of seeds to a hoarder (Siepielski & Benkman 2008), the decision to eat or cache seeds (Jorge & Howe 2009; Klinger & Rejmánek 2010), or the distance at which seeds were dispersed (Liu *et al.* 2013). However, given the general consensus that scatter hoarding potentially benefits plant recruitment while larder hoarding generally precludes plant recruitment (Vander Wall 1990), variation in the actual pattern of hoarding could shift the interaction between seed-hoarding rodents and large-seeded trees from mutualism to antagonism or vice versa. For example, the availability of seeds to an individual hoarder may affect its effort invested in hoarding these seeds, but also the intensity of competition with conspecifics and/or heterospecifics may affect the pattern of hoarding.

Under pressure of competition, hoarders are predicted to adopt a seed-hoarding pattern that minimizes pilferage risk. Depending on the search tactics used by the competitor, this may imply (a) an increase in seed-removal rates to quickly remove seeds from the soil surface, as this lowers the likelihood of detection, (b) the movement of seeds away from the parent tree (i.e., increased dispersal distances), as this lowers the likelihood of encounter and detection, and (c) movement of seeds away from siblings (i.e., increased seed spacing), as this lowers the likelihood of detection. From the tree's perspective, all this may have profound effects on the probability of seed survival and germination. It is generally assumed that seed-survival probabilities increase with the rate of removal, the distance of dispersal, and the spacing between individual seeds (Janzen 1970, 1971). For instance, increasing seed-dispersal distances lowers the likelihood of distance-dependent risk of seed predation, while increasing the spacing between individual seeds lowers the risk of density-dependent seed predation (Janzen 1970; Stapanian & Smith 1978; Clarkson *et al.* 1986; Jansen & Forget 2001).

Here, we tested whether the outcome of a potentially mutualistic seed-dispersal interaction between seed-hoarding rodents and large-seeded trees is conditional on the circumstances under which the interaction takes place (Bronstein 2009). We specifically included a large third-species seed competitor in our sturdy to test for its role in shaping the mutualism. We studied the interaction between a large-seeded tree, Pedunculate oak (*Quercus robur*), two seed-hoarding rodents, Wood mouse (*Apodemus sylvaticus*) and Bank vole (*Myodes glareolus*), and a large seed predator, Wild boar (*Sus scrofa*). Oak relies heavily on animals for the dispersal of its seeds (henceforth: acorns) (Den Ouden *et al.* 2005). Particularly wood mouse, but also bank vole, serve as potential seed dispersers for the oak as they collect large quantities of acorns in autumn, when the seeds are shed, and hoard them as winter food supply. Wild boar are predicted to play an important role in this system as they compete for acorns with rodents in autumn when the acorns are freshly shed (Groot-Bruinderink *et al.* 1994; Groot-Bruinderink & Hazebroek 1996; Focardi *et al.* 2000; Massei & Genov 2004; Elston & Hewitt 2010), but also in mid- and late winter when very few acorns remain on the forest floor (Groot-Bruinderink *et al.* 1994; Den Ouden *et al.* 2005; Muñoz *et al.* 2009). The latter suggests that wild boar actively searches for and pilfers acorn caches created by seed hoarders, such as wood mouse and bank vole (Borchert *et al.* 1989; Herrera 1995; Focardi *et al.* 2000; Gomez *et al.* 2003; Den Ouden *et al.* 2005).

We hypothesized that increased competition for acorns, either due to the presence of wild boar, due to low acorn abundance, or due to a high rodent abundance would drive seed-hoarding rodents to change their hoarding pattern, by (1) increasing seed-removal rate, (2) increasing seed-dispersal distance, and (3) increasing seed spacing. We predicted the effect of rodent abundance to be strongest in sites without wild boar and with low acorn abundance, while we predicted the effect of wild-boar presence to be strongest in sites with few rodents and with low acorn abundance. In other words, we predicted the effects of wild-boar presence and rodent abundance to be strongest if competition otherwise was low, while we predicted the effect of acorn abundance to be strongest if competition otherwise was high. We further hypothesized that (4) high seed-removal rate, large seed-dispersal distance, and large seed spacing favours germination of hoarded seeds. Successful seed germination would thus be most likely under high competition, but only up to a certain point. If competition were to become too high, the majority of the seeds would likely be consumed and survival to germination would again diminish.

Methods

Site and species

Fieldwork was conducted in the southwest Veluwe area, a temperate forest ecosystem in the centre of the Netherlands (52˚1'N, 5˚43'E). The area is a large push-moraine complex formed by the Saalien glacial during the Pleistocene era, consisting of sandy deposits and a spodosol soil type. Mean annual precipitation is 833 mm and the average maximum temperature between October and April ranges from 7 to 11 ˚C. Tree cover comprises of a mixture of coniferous and deciduous species, dominated by Scots pine (*Pinus sylvestris*), Douglas fir (*Pseudotsuga menziesii*), European larch (*Larix decidua*), European beech (*Fagus sylvatica*), Red oak (*Quercus* *rubra*), and Pedunculate oak. The understory is dominated by Bracken fern *(Pteridium aquilinum)*, Blueberry *(Vaccinium myrtillus)*, Bramble (*Rubus fruticosus*), and several species of grasses and sedges.

The study area is effectively divided into two parts by a fenced four-lane motorway, established in 1956. Wild boar occur in relatively high densities north of the motorway, and are absent south of the motorway, providing a stark contrast in wild-boar abundance. The motorway also serves as a barrier between rodent populations of both subareas. Twelve sites were selected; six north and six south of the motorway. This set-up allowed testing for behavioural differences between mice living in areas with a long history of wild-boar presence and mice living in areas with a long history of wild-boar absence. Each site consisted of one isolated pedunculate oak (henceforth, “focal tree”), within a forest stand dominated by scots pine. Focal trees were comparable across sites in height, crown size and diameter at breast height. Sites were selected based on similarity in crown openness, and understory vegetation and -structure. To ensure sampling of independent mouse populations, sites were located in distinct forest stands situated at least 500 m apart from each other.

The Pedunculate oak is a large-seeded deciduous tree species abundant in central and western Europe that exhibits alternate bearing, with periodic rich and poor mast years (Crawley & Long 1995). Seeds start to ripen in September-October and are shed between late October and early December (Watt 1919; Bossema 1979; Crawley & Long 1995). Seeds of pedunculate oak (i.e., acorns) form an important food source to overcome winter scarcity for many animals, such as Eurasian jay (*Garrulus glandarius*), Wood pigeon (*Columba palumbus*), Red deer (*Cervus elaphus*), Roe deer (*Capreolus capreolus*), Bank vole, Wood mouse, and Wild boar. In return, the pedunculate oak relies heavily on animals for the dispersal of its seeds, which is taken care of primarily by Eurasian jay, wood mouse, and bank vole (Bossema 1979; Gomez *et al.* 2003; Den Ouden *et al.* 2005; Gomez *et al.* 2008).

The Wood mouse and the Bank vole are both common rodents of European woodland, that weigh between 10-35 g and breed between April and October. Both species are largely nocturnal and characterized by well-developed olfactory, visual, and auditory senses. They are typical granivores, with seeds constituting roughly 70% of their diet, particularly so in autumn and winter (Watts 1968; Flowerdew *et al.* 1985). Wood mice and bank voles play an important role as predator and disperser of acorns in Europe. They hoard acorns in autumn, primarily through scatter hoarding, to serve as a food reserve for winter (Vander Wall 1990; Den Ouden *et al.* 2005; Takahashi *et al.* 2007).

The Wild boar is a medium-sized (50-200 kg) ungulate that is regarded as an important competitor to seed-hoarding rodents in forests and woodlands (Massei & Genov 2004). Acorns are a dominant food in autumn when they have freshly shed and are abundantly available on the forest floor (Massei & Genov 2004; Elston & Hewitt 2010), but also in mid- and late winter when very few acorns remain on the forest floor (Groot-Bruinderink *et al.* 1994; Den Ouden *et al.* 2005; Muñoz & Bonal 2007). The latter suggests that wild boar actively search for and pilfer acorn caches created by hoarders, such as wood mouse and bank vole (Borchert *et al.* 1989; Herrera 1995; Focardi *et al.* 2000; Gomez *et al.* 2003; Den Ouden *et al.* 2005).

Experimental design

In this study, PIT-tagged acorns were presented to free-ranging wood mice and bank voles in each of the twelve sites, and for two consecutive years. Prior to presenting the tagged acorns, we attempted to capture and individually mark all wood mice and bank voles present in each site to estimate rodent abundance. At that time, we also visually inspected the seed crop to estimate acorn abundance in each site. After the seeds had been presented, the exact time of removal for each seed was monitored using automated cameras. Once all seeds had been removed, we relocated the seeds using custom-made PIT-tag readers. For all relocated seeds, the seed-dispersal distance and angle from the source were recorded, which allowed us to later calculate the spacing between seeds. Finally, in July the next year, the final fate of all relocated seeds was established.

Wild-boar presence – Although wild boar were not allowed south of the motorway (Groot-Bruinderink & Spek 2001; Groot-Bruinderink *et al.* 2004; Spek 2014), we monitored all sites (north and south of the motorway) with motion-activated camera traps (Reconyx HC500 Hyperfire IR, Reconyx Inc., USA) and carefully checked each site for any traces of wild-boar presence, such as uprooted patches, faeces, and hairs. No wild boar were ever recorded on the cameras in sites south of the motorway, but they were regularly recorded on the cameras in sites north of the motorway. Also, no traces of wild-boar presence were found in the sites south of the motorway.

Rodent abundance – In each site, wood mice and bank voles were captured using 25 Longworth live traps (Penlon Ltd., UK) that were placed in a 5x5 grid with 5 m spacing between traps. Traps were run for four consecutive days per site during October 2010 and October 2011, just before the majority of acorns are shed from the trees (Watt 1919; Bossema 1979; Crawley & Long 1995). Traps were filled with fresh hay and baited with a mixture of peanut butter, rolled oats and apple, and were checked twice daily, just after sunrise and approximately five hours after sunset. During the day, traps were left open and then set to catch animals every afternoon at 5 PM. Consequently, the maximum amount of time that an animal could be in a trap was 7 h. Captured individuals were sexed, weighed, and marked by applying a distinctive fur-clipping pattern (in 2010) or by injecting a uniquely coded PIT tag (in 2011) at first capture, and then released again at the capture site. Fur-clipping and PIT-tag injection had no notable effects on animal locomotion or behaviour, based on personal observations during release and repeated recapture of the same individuals. All experimental handling procedures were approved by the Animal Experiments Committee of Wageningen University (2010: WUR-2010082.c, 2011: WUR-2011091.b). As our trapping design was identical across sites, the total number of individuals captured in each site could be directly compared and used as a measure of relative abundance of conspecifics.

Acorn abundance – In both years, natural acorn production was close to zero for all focal trees as well as for other oaks in the direct vicinity of each site. Therefore, in the second year, we supplemented each site with a total of 1,000 acorns, collected from mature trees of the same species in the surrounding areas of our field sites. Consequently, acorn abundance was highly comparable across sites and within years, but, at least locally, it varied substantially between years.

Seed-removal rate – Acorns were collected from mature oaks in the surrounding areas of our field sites during October 2010 and 2011, and selected based on a visual check of intactness, floatability, and mass. Only acorns ranging from 7 to 9 g fresh weight were used in order to minimize seed-size variation, as this is known to influence seed-dispersal patterns (Jansen *et al.* 2004), but was beyond the scope of this study. Acorns were stored in a refrigerator at 4 °C. Acorns were uniquely marked using PIT tags (2x12 mm Trovan ID100A Glass Encapsulated Transponder, Dorset ID, Netherlands). Acorns were tagged following the procedures described in Suselbeek *et al.* (2013). PIT-tagging large seeds seems to have no effect on seed removal by rodents, nor does it affect germination and growth of the seedling (Suselbeek *et al.* 2013).

In early November 2010 and 2011, 49 PIT-tagged acorns were placed on the forest floor in each site, approximately one meter south of the focal oak tree (henceforth: seed station). Acorns were placed in a 7x7 grid with 6 cm spacing between seeds. To prevent animals other than wood mouse and bank vole from removing PIT-tagged acorns, seed stations were covered with a large crate that was secured to the ground with stakes during the day. Two motion-activated camera traps (Reconyx HC500 Hyperfire IR) were positioned at different angles facing each seed station to monitor seed-removal times as well as the identity of the individual rodent removing each acorn. In addition to the two camera traps, a custom-made PIT-tag antenna ring with a stationary reader (LID572, Dorset ID, Netherlands) was placed around each seed station in 2011. This reader recorded time and identity of both PIT-tagged rodents and PIT-tagged acorns moving over the antenna ring, providing additional accurate records of when and by whom acorns were removed from the seed station.

If possible, the exact timing of removal for each acorn was recorded from either the camera traps or the antenna ring, however, in those instances where the actual removal event was neither recorded by the cameras nor by the antenna ring, the first instance at which a tagged acorn was no longer observed at the seed station, was taken as the removal time. In 2011, the cameras were set to take one picture every minute (regardless of motion detected) reducing this uncertainty interval to a maximum of one minute. For each acorn, the inter-acorn removal time, was calculated as the time between removal of that acorn and that of the previously removed acorn. The actual seed-removal rate (expressed in acorns h^{-1}) was then obtained by taking the inverse of the inter-acorn removal time.

Seed-dispersal distance – We used two customized PIT-tag detectors (LID650 stationary decoders with 40x40 cm ANT612 Panel antennae, Dorset ID, Netherlands) as well as a handheld reader (Trovan GR250, Dorset ID, Netherlands) to locate PIT-tagged acorns. Upon encounter of a tagged acorn, we recorded its status as (1) ‘cached’ – acorn stored by wood mouse or bank vole; (2) ‘consumed’ – only a PIT tag found; (3) ‘moved’ – acorn removed from previously located cache and not relocated; (4) ‘recached’ – acorn removed from previously located cache to a new location, and (5) ‘not retrieved’ – acorn not encountered since its removal from the seed station. For establishing the status of each tagged acorn, we did not disturb the cache or the seed but only visually inspected the cache location. Cache locations were non-invasively marked by placing a 30-cm bamboo stick at 30 cm from the cache, and away from the seed station. The angle and distance from the seed station as well as the number of acorns per cache were recorded for each retrieved acorn, using a precision compass (Suunto KB-77/360RL, Suunto, Finland) and an ultrasonic range finder (Haglöf DME 201 Cruiser, Haglöf Sweden AB, Sweden).

In 2010, seed-dispersal patterns were recorded at 3 and 10 days, and at 3, 5, 9, and 20 weeks after placement of the tagged acorns. During the first visit, PIT-tagged acorns were searched for in a radius of 25 m around the seed station, while during the other visits the search area encompassed a radius of 50 m around each seed station. Due to less-man-power and time-investment, seed-dispersal patterns were recorded only after 2 days and at 5 weeks in 2011. During the first visit, PIT-tagged acorns were searched for in a radius of 20 m around the seed station, while during the other visit the search area encompassed a radius of 35 m around each seed station. During each visit, previously located caches were revisited to check whether they were intact or depleted, and during all visits, caches were not physically disturbed but only the location of each PIT tag was recorded.

Seed spacing – Seed spacing was defined as the distance to the nearest neighbouring PIT-tagged acorn, and was calculated from the dispersal distance and angle for each acorn recorded in the field. Seed spacing could theoretically range from 0 to infinity, with 0 being the seed spacing between two tagged acorns stored in one cache. If two acorns would be found in two caches spaced 1 m apart, with no other tagged acorns in close vicinity, then the spacing between these acorns would be 1 m.

Seed fate – During July 2011 and July 2012 all sites were visited again and seed-dispersal patterns were recorded once more. At this time, all caches were opened to record final seed fate, as (1) ‘cached’ – acorn intact and seemingly viable in cache; (2) ‘consumed’ – only a PIT tag found; (3) ‘germinated’ – the acorn developed a root and was still alive; (4) ‘emerged’ – the acorn developed a root and a shoot and was still alive, and (5) ‘died’ – acorn remains still visible in cache, but clearly rotten, infected with a fungus, or otherwise severely damaged , but not consumed. Acorns that were ‘cached’, ‘germinated’, or ‘emerged’ at this census were left in the field for potential follow-up studies, while all other PIT-tags and seed remains were removed.

Data analysis

Seed-removal data were fitted with a Generalized Linear Mixed Model with a Gamma distribution (Ronnegard *et al.* 2010), as seed-removal rates were greater than 0, continuous, and non-normally distributed (right-skewed). We tested for main effects and for potential interaction effects between wild-boar presence, acorn abundance, and rodent abundance on seed-removal rate (N = 1136).

A Mixed Effects Cox Model analysis was used to test for effects of wild-boar presence, acorn abundance, and rodent abundance on seed-dispersal distance (Therneau 2012, 2013). A Cox model (i.e., a special type of survival analysis) was chosen to acknowledge the fact that we had observations that were censored at 50 m (i.e., acorns that had been removed from the seed station, but that were not retrieved within the search radius of 50 m) (Jansen *et al.* 2004; Hirsch *et al.* 2012c). We tested for main effects and for potential interaction effects between wild-boar presence, acorn abundance, and rodent abundance on seed-dispersal distance (N = 1177).

We fitted a Generalized Linear Mixed Effects Model with a Gamma distribution to test for effects of wild-boar presence, acorn abundance, and rodent abundance on seed spacing (Ronnegard *et al.* 2010). A Gamma distribution for the dependent variable was selected because the spacing data were all greater than 0 (i.e., after adjusting seed spacing for acorns cached in the same cache to 1 cm, rather than 0 cm), continuous, and non-normally distributed (right-skewed). We tested for main effects and for potential interaction effects between wild-boar presence, acorn abundance, and rodent abundance on seed spacing (N = 670).

A Generalized Linear Mixed Model with a Binomial distribution was fitted to the seed-fate data (Bates *et al.* 2013). A Binomial distribution was selected because seed fate was defined as the ratio between the number of successes and the number of failures, with successes representing acorns that had successfully germinated and/or emerged in July of the year following the placement of acorns, and failures representing all other acorns that were still followed at that time. Thus, all acorns that had never been retrieved or that had been lost over time, were excluded from the analysis. We tested for main effects and for potential interaction effects between wild-boar presence, acorn abundance, and rodent abundance on seed fate (N = 670).

For all analyses, a mixed-effects structure was selected because our individual acorn data originated from twelve different sites, with 49 tagged acorns being offered in each site, while we were not interested in a potential site-effect. We therefore included site as a random factor. All analyses were carried out in R3.0.2 (R Core Team 2013).

Results

In total, nearly 75% (N = 866) of all PIT-tagged acorns (N = 1177) were retrieved at some point in time during the study period, while 57% (N = 670) of all PIT-tagged acorns could be followed until July the next year, when final fate of the seeds was recorded. A summary of all retrieval results per year and per site is given in table 3.S1.

Seed-removal rate ranged among sites from 0.7 to 17.2 acorns per hour (N = 24, median = 3.2 acorns / hour). There was a significant interaction effect on seed-removal rate between acorn abundance and wild-boar presence (Generalized Linear Mixed Model: Wald $t_{1121} = 4.86$, $p < 0.001$; Fig. 3.1, Table 3.1). If acorn abundance was low, wild-boar presence had a weak negative effect on seed-removal rate (Wald $t_{1121} = 2.40$, $p = 0.0167$; Fig. 3.1b,d), while if acorn abundance was high, wild-boar presence had no effect on seed-removal rate (Wald $t_{1121} = 0.04$, $p = 0.96$; Fig. 3.1a,c). On the other hand, acorn abundance had a strong negative effect on seed-removal rate if wild boar were absent (Wald $t_{1121} = 5.90$, $p < 0.001$; Fig. 3.1a,b), and a weak negative effect on seed-removal rate if wild boar were present (Wald $t_{1121} = 2.42$, $p = 0.0155$; Fig. 3.1c,d). There was also a significant interaction effect on seed-removal rate between acorn abundance and rodent abundance (Wald $t_{1121} = 2.00$, $p = 0.0463$; Fig. 3.1, Table 3.1). The effect of rodent abundance on seed-removal rate was positively related to the abundance of acorns, such that the seed-removal rate increased more rapidly (16.2% increase per unit increase in rodent abundance) if acorn abundance was high than if acorn abundance was low (11.3% increase per unit increase in rodent abundance). In general, however, there was a strong positive relation between rodent abundance and seed-removal rate under low acorn abundance (Wald $t_{1121} = 8.25$, $p < 0.001$; Fig. 3.1b,d) and under high acorn abundance (Wald $t_{1121} = 6.87$, $p < 0.001$; Fig. 3.1a,c).

Table 3.1 Summary of the main and interactive effects of wild-boar presence, acorn abundance, and rodent abundance on seed-removal rate, seed-dispersal distance, seed spacing, and seed fate. In case of significant interaction effects, the p-value for the interaction is given and the direction and strength of the effect is specified for each combination of the interactive variables.

	Seed-removal rate	**Seed-dispersal distance**	**Seed spacing**	**Seed fate**
wild-boar presence (WBP)	low AA: - high AA: ***NS***	***NS***	***NS***	low AA: - high AA: ***NS***
acorn abundance (AA)	WB absent: - - - WB present: -	**+ + +**	***NS***	WB absent: ***NS*** WB present: **+**
rodent abundance (RA)	low AA: **+ + +** high AA: **+ + +**	***NS***	**+ + +**	low AA – WB absent: ***NS*** low AA – WB present: **+** high AA – WB absent: ***NS*** high AA – WB present: ***NS***
WBP x AA	**p < 0.01**	***NS***	***NS***	**p < 0.01**
WBP x RA	***NS***	***NS***	***NS***	low AA: **p < 0.05** high AA: ***NS***
AA x RA	**p < 0.05**	***NS***	***NS***	WB absent: ***NS*** WB present: ***NS***
WBP x AA x RA	***NS***	***NS***	***NS***	**p < 0.05**

- / + for p < 0.05, - - / + + for p < 0.01, - - - / + + + for p < 0.001, NS for p > 0.05

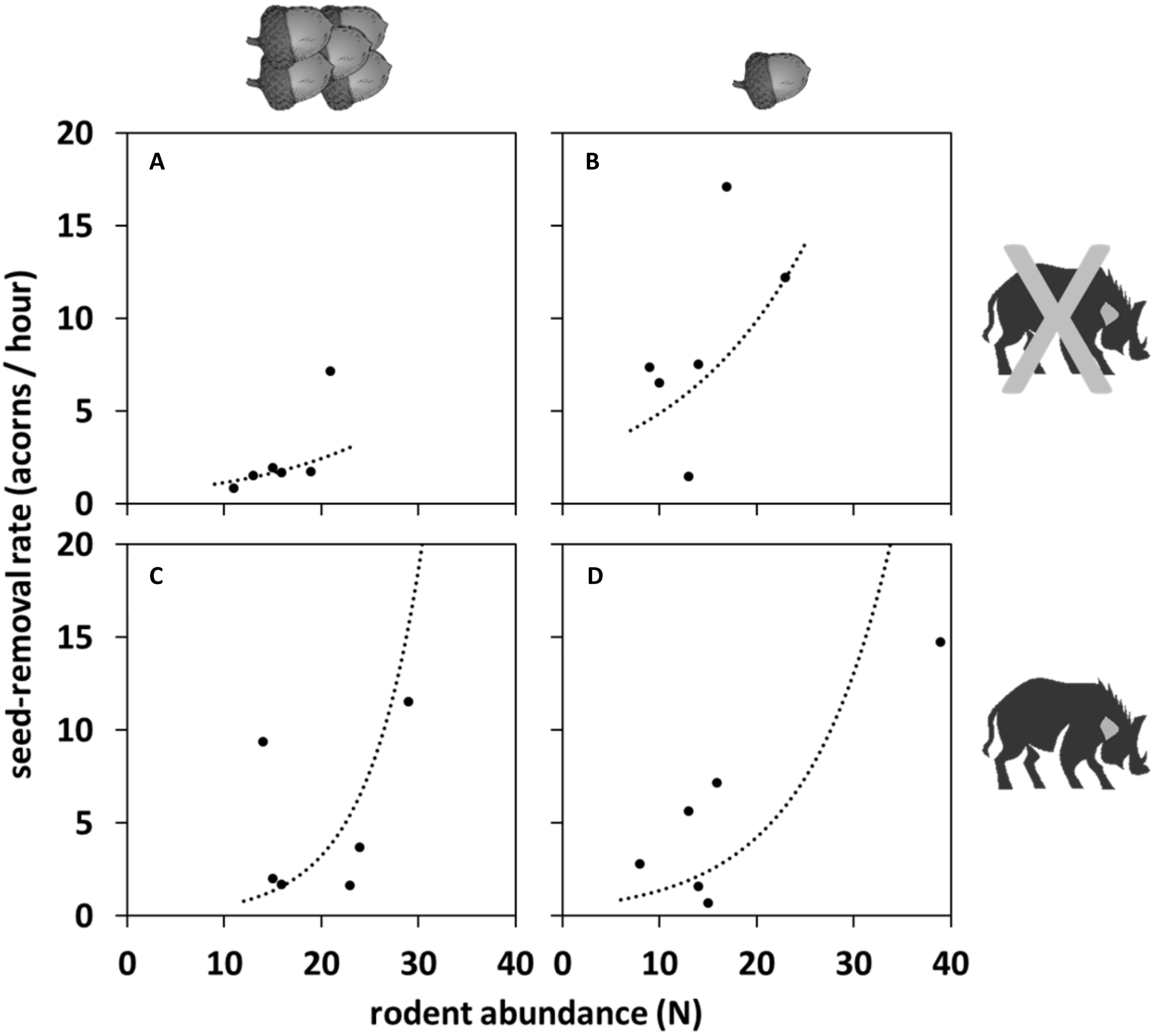

Fig. 3.1: Interactive effects of wild-boar presence, acorn abundance, and rodent abundance on seed-removal rate of acorns removed by wood mice and bank voles in a field study. The bullets depict the average seed-removal rate as measured in each replicate site, while the dashed curves represent the model's fitted relationship between rodent abundance and seed-removal rate. Panels (a) and (b) represent sites without wild boar, respectively under high or low acorn abundance, while panels (c) and (d) represent sites with wild boar, respectively under high or low acorn abundance.

Seeds were dispersed at distances ranging from 0.8 – 46.3 m (N = 1177, with 670 events and 507 censored observations, median = 18.8 m). Under high acorn abundance, median seed-dispersal distance was estimated to be a factor 1.44 higher (95% CI = 1.23 – 1.68) than under low acorn abundance (Cox Proportional Hazards Survival Analysis: Wald z = 4.61, p = 0.000004; Fig. 3.2, Table 3.1). Median seed-dispersal distance was estimated to be a factor 1.39 higher (95% CI = 0.89 – 2.16) if wild boar were present than if wild boar were absent, however, this effect was not significant (Wald z = 1.44, p = 0.15). Rodent abundance had not effect on median seed-dispersal distance (Wald z = 0.16, p = 0.87).

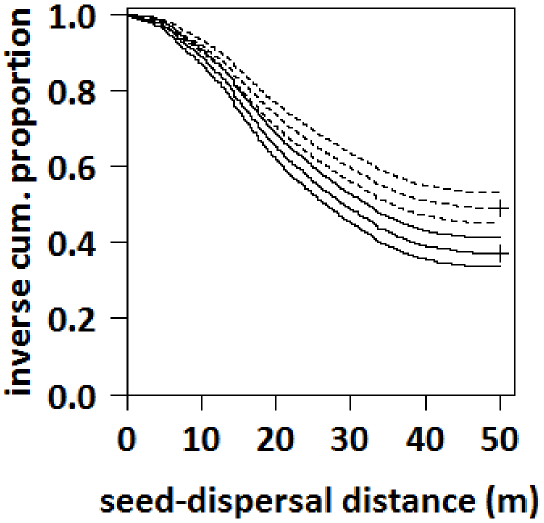

Fig. 3.2: Effects of acorn abundance on seed-dispersal distance of acorns dispersed by wood mice and bank voles in a field study. The panel shows fitted survival curves with 95% confidence interval envelopes, representing seed-dispersal distance in meters under low acorn abundance (solid curves) and under high acorn abundance (dashed curves).

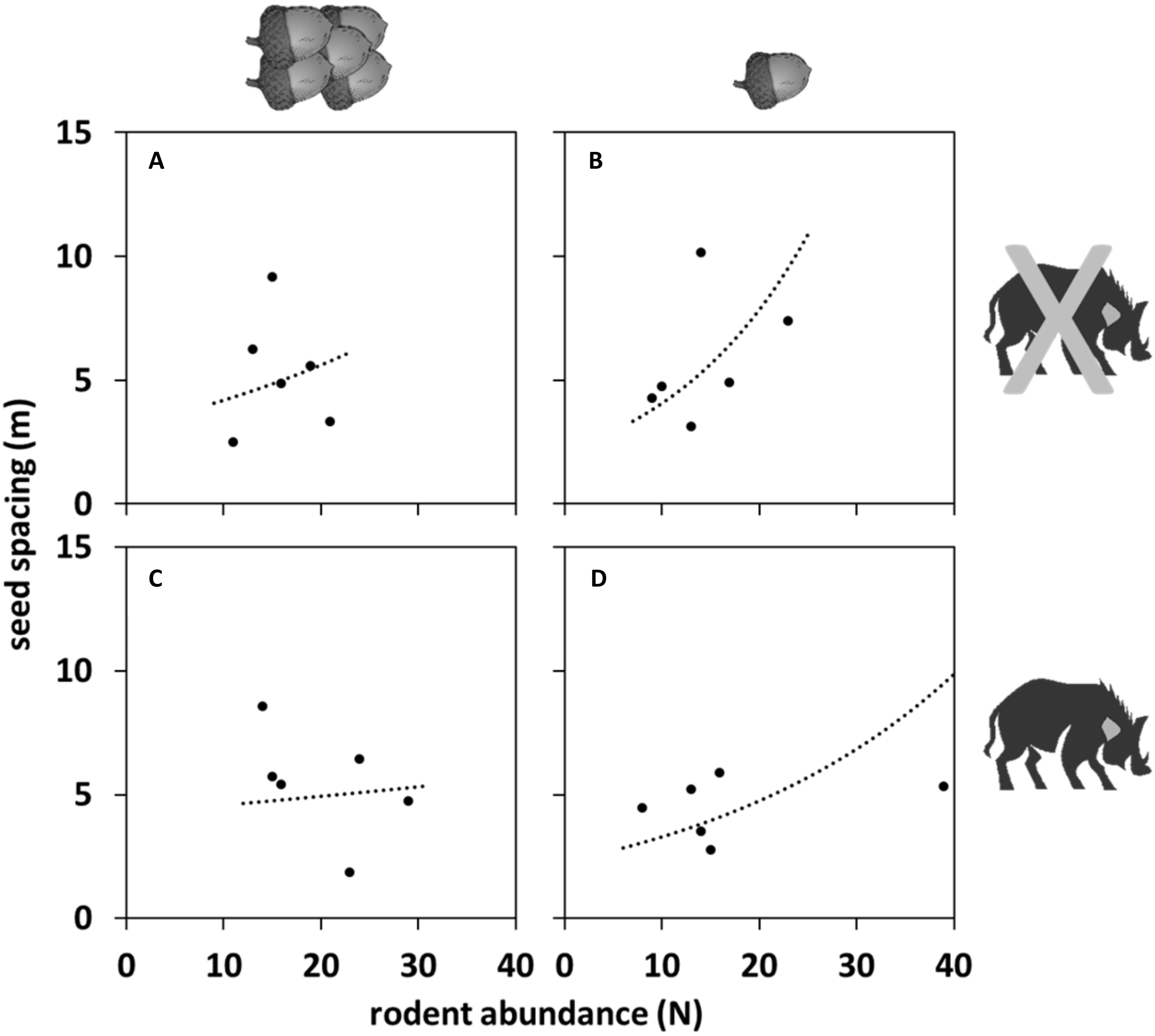

Fig. 3.3: Interactive effects of wild-boar presence, acorn abundance, and rodent abundance on seed spacing of acorns dispersed by wood mice and bank voles in a field study. The bullets depict the average seed spacing as measured in each replicate site, while the dashed curves represent the model's fitted relationship between rodent abundance and seed spacing. Panels (a) and (b) represent sites without wild boar, respectively under high or low acorn abundance, while panels (c) and (d) represent sites with wild boar, respectively under high or low acorn abundance.

Seed spacing ranged between 0 and 24.9 m (N = 670, median = 4.0 m). Seed spacing was not affected by wild-boar presence (Generalized Linear Mixed Model: Wald t_{659} = -1.10, p = 0.27; Fig. 3.3) or acorn abundance (Wald t_{659} = -0.79, p = 0.43; Fig. 3.3), but increased significantly with rodent abundance (Wald t_{659} = 3.62, p = 0.00032; Fig. 3.3, Table 3.1).

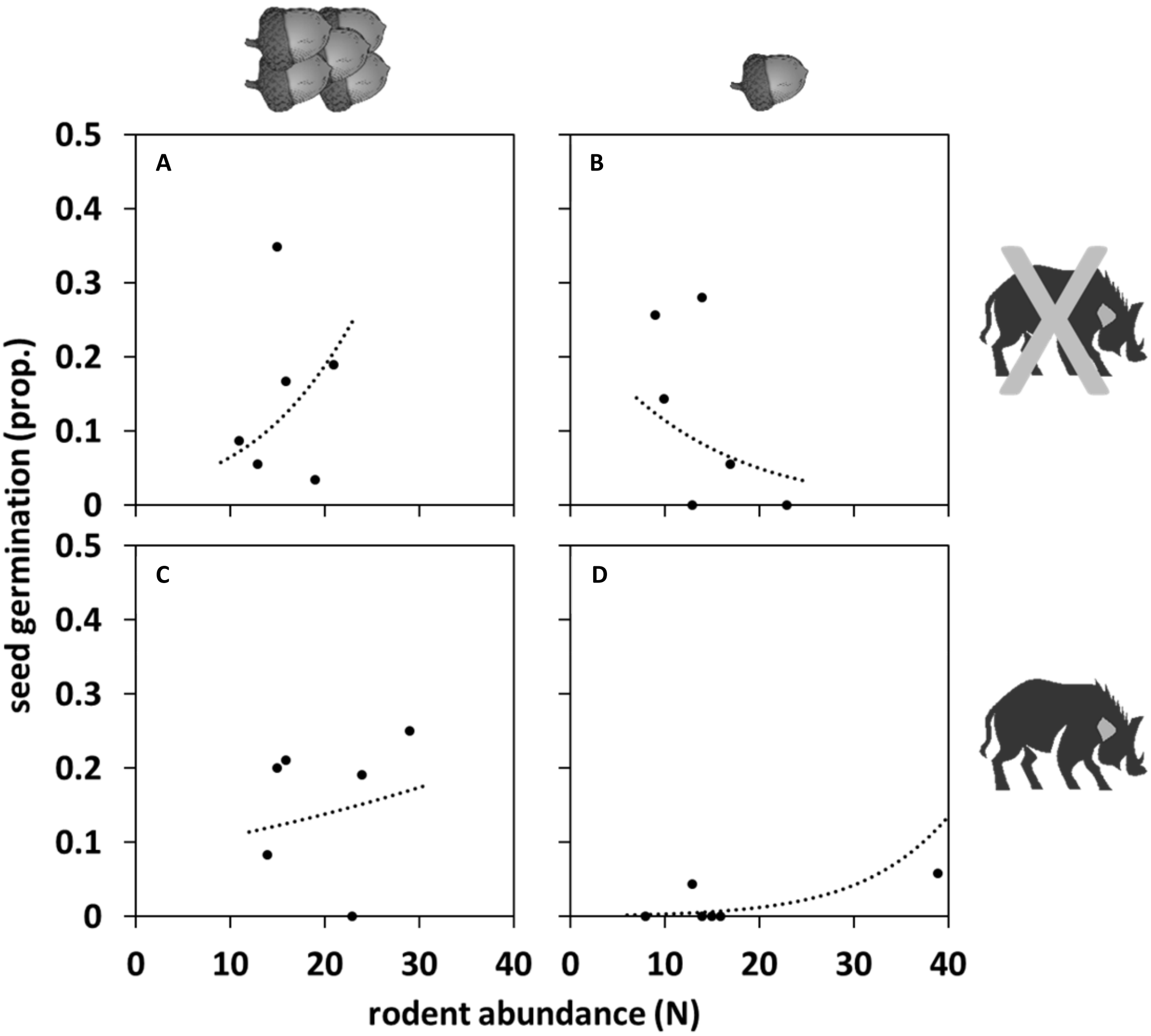

Fig. 3.4 Interactive effects of wild-boar presence, acorn abundance, and rodent abundance on seed fate of acorns dispersed by wood mice and bank voles in a field study. The bullets depict the proportion of germinated seeds as measured in each replicate site, while the dashed curves represent the model's fitted relationship between rodent abundance and seed germination. Panels (a) and (b) represent sites without wild boar, respectively under high or low acorn abundance, while panels (c) and (d) represent sites with wild boar, respectively under high or low acorn abundance.

Seed germination within sites ranged between 0 and 35% (N = 24, median = 7%). There was a significant three-way interaction effect on seed fate between wild-boar presence, rodent abundance, and acorn abundance (Generalized Linear Mixed Model: Wald z = 2.18, p = 0.02916), and significant two-way interaction effects between wild-boar presence and acorn abundance (Wald z = 2.66, p = 0.00789) and between wild-boar presence and rodent abundance (Wald z = 2.08, p = 0.03783; Fig. 3.4, Table 3.1). If acorn abundance was low, wild-boar presence negatively affected seed germination (Wald z = 2.82, p = 0.00508; Fig. 3.4b,d), while if acorn abundance was high, wild-boar presence had no effect on seed germination (Wald z = 0.70, p = 0.48; Fig. 3.4a,c). Similarly, if wild boar were present, acorn abundance negatively affected seed germination (Wald z = 2.08, p = 0.03764; Fig. 3.4c,d), while if wild boar were absent, acorn abundance had no effect on seed germination (Wald z = 1.65, p = 0.09984; Fig. 3.4a,b). If acorn abundance was low and wild boar were present, seed germination significantly increased with rodent abundance (Wald z = 2.31, p = 0.02102; Fig. 3.4b). In all other cases, however, rodent abundance did not affect seed germination although there was a general tendency for seed germination to increase with rodent abundance (Fig. 3.4a,c,d).

Discussion

In this study, we examined whether and how the abundance of seeds, the abundance of rodents, and the presence or absence of a large competitor, influences seed-hoarding patterns of rodents and whether and how this subsequently affects their role as mutualists of large-seeded trees. We found that seed-hoarding patterns (i.e., seed-removal rate, seed-dispersal distance, and seed spacing) of wood mice and bank voles were affected by the combined effects of wild-boar presence, rodent abundance, and acorn abundance. This, in turn, influenced the likelihood of seed germination and with that it shifted the outcome of the interaction between seed-hoarding rodents and large-seeded trees from being mutualistic to progressively more antagonistic.

Seed-removal rates varied strongly between sites. The factor best explaining these differences in removal rates was rodent abundance. Generally, the more rodent conspecifics there were, the higher the seed-removal rate was, a finding that is in line with those reported in several other studies (e.g., Bowers & Dooley 1993; Wang *et al.* 1999; Jansen *et al.* 2004; Jansen & Den Ouden 2005; Vander Wall *et al.* 2005b; Perez-Ramos *et al.* 2008; Perea *et al.* 2011). In contrast to our predictions, wild-boar presence had a weak negative effect on seed-removal rates, but only if acorn abundance was low. This may, however, be explained by the fact that wild boar also predate on rodents (Groot-Bruinderink & Hazebroek 1996; Focardi *et al.* 2000; Baubet *et al.* 2004; Gimenez-Anaya *et al.* 2008; Gomez & Hodar 2008; Bueno *et al.* 2009; Elston & Hewitt 2010). Hoarders may thus face a trade-off between quickly collecting and removing seeds upon encounter, or moving more cautiously while exposing themselves less to predators like wild boar (Bowers & Dooley 1993; Jansen & Den Ouden 2005). We found seed-removal rates to be negatively influenced by acorn abundance, which was in line with our and other's predictions (Jansen *et al.* 2004; Jansen & Den Ouden 2005; Vander Wall *et al.* 2005b; Perez-Ramos *et al.* 2008; Hirsch *et al.* 2012a). As expected, seed-removal rates were lowest if acorn abundance was high, wild boar were absent, and rodent abundance was low, that is, if competition for seeds was lowest.

Optimal cache spacing theory predicts that food items are stored at a density that balances the gains of reducing pilferage against the costs of spacing food items out further (Stapanian & Smith 1978, 1984; Clarkson *et al.* 1986; Dally *et al.* 2006; Gálvez *et al.* 2009). We found that seed-dispersal distance was strongly positively related to the abundance of acorns. This increase in seed-dispersal distance with acorn abundance confirms the optimal cache spacing theory in that rodents seem to move seeds further away from the source, if this source contains more seeds (Stapanian & Smith 1978, 1984). By moving seeds further away from the source, seed density is lowered, while seed spacing may remain constant. Similarly, we found that seed spacing was strongly positively affected by rodent abundance. Again, this is in line with optimal cache-spacing theory, as an increase in rodent abundance is likely to incur increased pilferage risk, and thus seed spacing should be increased to lower the density of seeds in a certain area (Stapanian & Smith 1978, 1984). Wild-boar presence had no effect on seed spacing, but seed-dispersal distance showed a tendency to increase with wild-boar presence. Although this was in contradiction with our prediction, we think that the potential effects of wild-boar presence may be overruled by the effects of acorn abundance and rodent abundance. That is, competition with conspecifics already requires a certain degree of seed spacing and transportation of seeds away from the source. However, a controlled experiment in which effects of rodent abundance and acorn abundance could be filtered out, would perhaps provide more insight into how and why wild boar presence affects seed-dispersal distance and seed spacing.

The changes that we observed in seed-removal rate, in seed-dispersal distance, and in seed spacing led to significant changes in seed fate. The direction and magnitude of the effect, however, varied strongly depending on the conditions. Due to this large variation, most of the effects were not significant. However, in line with our predictions, we found that the likelihood of seed germination was lowest if acorn abundance was low and wild boar were present. In this situation, it is likely that the majority, if not all, of the seeds are being consumed by seed predators (Crawley & Long 1995). However, if wild boar were absent, rodent abundance was low, and acorn abundance was low, the likelihood of seed germination was relatively high. Again, from a predator-satiation perspective, this is in line with predictions (Crawley & Long 1995). The highest likelihood of seed germination, however, was found when wild boar were absent, acorn abundance was high, and the abundance of rodents was intermediate. Interestingly, taking into account that seed-dispersal distance was strongly positively related to acorn abundance and that seed spacing was strongly positively related to rodent abundance, it seems that increased seed dispersal and increased seed spacing positively affect seed germination. This is in line with results from other studies and predicts the existence of density- and distance-dependent risk of predation (Janzen 1971; Howe & Smallwood 1982; Stapanian & Smith 1984; Jansen & Forget 2001).

We used some novel techniques to monitor seed removal and -dispersal. To accurately record who removed which acorn at what time, we used camera traps – to visually record which individual removed which seeds at what time – in combination with a custom-made antenna ring that recorded the exact time of a PIT-tagged acorn (and in 2011 also a PIT-tagged rodent) moving through the ring. This combined set-up provided us with an accurate removal time for each acorn. Then, we used PIT tags, inserted into each acorn to uniquely identify acorns and to be able to relocate them after they had been dispersed by the hoarders. PIT tagging large seeds is relatively cheap and it does not impact seed choice, seed removal, and seed-dispersal patterns by rodents, nor does it affect germination and growth of the seedling (Suselbeek *et al.* 2013). PIT tagging allowed us to accurately relocate the cache location, the approximate cache depth, the number of tagged acorns in a cache and the microhabitat surrounding the cache, without any physical disturbance of the cache. Nonetheless, PIT tagging has some potential drawbacks, most notably the effort needed to relocate PIT-tagged seeds. The maximum detection distance for PIT-tagged seeds in underground caches was about 35 cm. Relocating PIT-tagged seeds thus requires a meticulous search throughout the search area, which increases exponentially with distance from the source. Particularly if seed-dispersal distance generally exceeds 50 m, this method may become too demanding. Secondly, PIT tagging does not allow real-time following of seeds. This makes it hard to accurately record secondary-dispersal events (i.e., re-caching) and predation events by, for example, wild boar. Here, this led to a fair amount of seeds initially found but lost again over time Nevertheless, we were able to follow 57% of all presented acorns for a period of 10 months until their final fate was established; a proportion that is much higher for such a long post-dispersal period than achieved in most other seed-dispersal studies (Steele *et al.* 2001; Den Ouden *et al.* 2005; Forget *et al.* 2005; Xiao *et al.* 2006). For instance, in a study that actually compared two seed-tagging methods for assessing post-dispersal seed fate in rodent-dispersed trees, the proportion of *Quercus variabilis* seeds for which the fate could be established two months after seed placement was only 2% for thread-marked tags and 32% for wire tin-tagged seeds (Xiao *et al.* 2006). In another study, only 22% of all metal-tagged acorns that had been dispersed by small mammals in autumn 1994, were retrieved between December 1994 and June 1995, while 78% was never retrieved (Steele *et al.* 2001).

If one strives to understand the biological processes affecting tree recruitment, appreciating long-distance dispersal by animals is of particular importance. Ignoring the fact that some seeds are dispersed outside the search area (26% in this study) prevents a clear understanding and proper representation of dispersal distances. This study is among the first to use the censored-tail-reconstruction method (Jansen *et al.* 2004; Jansen *et al.* 2008; Hirsch *et al.* 2012c). Instead of omitting seeds that were not retrieved, they were assumed to be dispersed at least as far as the search radius, allowing us to obtain more accurate estimations of the seed-dispersal distance. We cannot rule out the possibility that some seeds were overlooked within the search area. If, however, some seeds were indeed overlooked, for instance due to inaccessibility of cache locations, we have no reason to assume that the risk of overlooking such seeds would vary between sites, and therefore it is unlikely to have influenced the comparison between sites.

In this field study, we determined whether and how the outcome of a seed-dispersal interaction between seed-hoarding rodents and large-seeded trees was influenced by the abundance of seeds and by the presence and abundance of both inter- and intraspecific competitors. We found that seed-hoarding rodents responded most strongly to a change in abundance of intraspecific competitors, but that there were significant interactions with wild-boar presence and acorn abundance. Generally, seed-removal rate, seed-dispersal distance, and seed spacing were increased in response to increased competition for seeds. These changes in seed-hoarding patterns resulted in significant differences in seed fate. The likelihood of successful seed germination was highest if wild boar were absent, and generally tended to increase with rodent abundance, but only if acorn abundance was sufficiently high to satiate the seed-predator / seed-disperser community. This suggests that seed-hoarding rodents aid seed germination so long as there is a surplus of seeds available to the hoarder (Theimer 2005). It also suggests that wild boar do not enhance the mutualism between seed-hoarding rodents and large-seeded trees. For instance, if wild boar were absent and acorn abundance was high, seed-hoarding patterns of rodents were more favorable for acorn germination than if wild boar were present and / or acorn abundance was low, resulting in higher levels of seed germination.

To conclude, we believe to have convincingly shown that the outcome of seed-dispersal interactions, in particular, those that intimately involve a third species, is very likely to be conditional on the circumstances under which the interaction takes place. Nevertheless, more controlled experiments would be needed to further disentangle the relative importance of specific conditions affecting the outcome of seed-dispersal interactions. For example, it would be very interesting to gain more insight on 1) the specific effects of intra- and interspecific competition on seed-hoarding patterns and 2) whether and how different hoarding patterns affect seed-germination rates and with that the outcome of seed-dispersal interactions. Rather than studying and reporting the outcomes of several direct interactions between two species independently, a more integrated approach should be taken in which broader ecological processes are studied as one entity. Such multi-species interaction studies will contribute to a solid scientific basis for forest and wildlife management and -conservation and enhance our understanding of conditionality in species interactions in general.

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Appendix

Table 3.S1: Summary of the number of PIT-tagged acorns placed in each site in autumn, the number of PIT-tagged acorns retrieved at any point in time between autumn and July the next year, and the number of PIT-tagged acorns for which final fate was recorded in July. The proportion of seeds relative to the total number of seeds presented in each row is given in brackets.

			total presented	**total retrieved**	**total with final fate recorded in July**
low acorn abundance (2010)	**total**		**589**	**448 (76.1%)**	**378 (64.2%)**
	wild boar present	**total**	**295**	**221 (74.9%)**	**174 (59.0%)**
		1	49	34 (69.4%)	23 (46.9%)
		2	49	45 (91.8%)	35 (71.4%)
		3	49	32 (65.3%)	23 (46.9%)
		4	49	39 (79.6%)	35 (71.4%)
		5	49	25 (51.0%)	18 (36.7%)
		6	50	46 (92.0%)	40 (80.0%)
	wild boar absent	**total**	**294**	**227 (77.2%)**	**204 (69.4%)**
		1	49	48 (98.0%)	42 (85.7%)
		2	49	40 (81.6%)	39 (79.6%)
		3	49	37 (75.5%)	36 (73.5%)
		4	49	29 (59.2%)	26 (53.1%)
		5	49	42 (85.7%)	36 (73.5%)
		6	49	31 (63.3%)	25 (51.0%)
high acorn abundance (2011)	**total**		**588**	**418 (71.1%)**	**292 (49.7%)**
	wild boar present	**total**	**294**	**194 (66.0%)**	**126 (42.9%)**
		1	49	32 (65.3%)	19 (38.8%)
		2	49	26 (53.1%)	24 (49.0%)
		3	49	30 (61.2%)	20 (40.8%)
		4	49	46 (93.9%)	30 (61.2%
		5	49	26 (53.1%)	12 (24.5%)
		6	49	34 (69.4%)	21 (42.9%)
	wild boar absent	**total**	**294**	**224 (76.2%)**	**166 (56.5%)**
		1	49	45 (91.8%)	36 (73.5%)
		2	49	27 (55.1%)	23 (46.9%)
		3	49	48 (98.0%)	37 (75.5%)
		4	49	32 (65.3%)	29 (59.2%)
		5	49	31 (63.3%)	18 (36.7%)
		6	49	41 (83.7%)	23 (46.9%)
GRAND TOTAL			**1177**	**866 (73.6%)**	**670 (56.9%)**

Chapter 4

Scatter hoarding and cache pilferage by superior competitors: an experiment with wild boar (*Sus scrofa*)

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Abstract

Food-hoarding patterns range between larder hoarding (few large caches) and scatter hoarding (many small caches), and are, in essence, the outcome of a hoard-size – number trade-off in pilferage risk. Animals that scatter hoard are believed to do so, despite higher costs, to reduce loss of cached food to competitors against which they cannot defend their food reserves (henceforth: superior competitors). We tested the underlying assumption that the cost of having more caches under scatter hoarding, thus increasing the likelihood of cache encounter by superior competitors, is outweighed by the benefit of having small caches, that are less likely to be detected upon encounter by superior competitors. We carried out a controlled experiment in which we distributed a fixed number of acorns over a fixed number of patches within a fixed area, varying cache size and cache depth, thus mimicking alternative hoarding patterns. We then recorded cache pilferage by a fixed number of wild boar (*Sus scrofa*), a well-known pilferer of acorn caches. The time wild boar needed to pilfer the first cache was shortest for scatter hoarding, but the time needed to pilfer all caches was slightly longer for scatter hoarding than for larder hoarding. Overall, however, the rate of pilferage did not differ between scatter hoarding and larder hoarding, and was not affected by cache depth. We conclude that the effects of alternative hoarding patterns on reducing cache pilferage by wild boar were smaller than expected, and that superior competitors may thus not be important drivers of scatter hoarding. Instead, other factors, such as conspecific pilferage or the risk of cross-contamination of food items in large caches, which can also cause catastrophic loss of food reserves, may be more important drivers of scatter hoarding.

Keywords: *competition; caching; seed dispersal; theft; olfaction; cache defence; rodents; oaks; acorns; seeds.*

Introduction

Many seed-eating animals cache seeds at times of high food availability and rely on these reserves during periods of food scarcity (Vander Wall 1990). The spatial pattern at which seeds are cached ranges between larder hoarding and scatter hoarding (Vander Wall 1990). Larder hoarding involves the placement of food items in one or few caches ('larders'), usually located in or near the nest, in burrows, or in tree cavities, that are often actively defended. Scatter hoarding, in contrast, involves spreading of food over many widely spaced caches with one or few items each ('scatters'), scattered throughout the hoarder's territory, usually created and accessed from the soil surface (Morris 1962; Clarkson *et al.* 1986; Vander Wall 1990, 2001).

Both hoarding strategies have their own set of advantages and disadvantages. Larders are relatively easy to create and recover, yet usually require active defence against other animals, which costs time and energy, involves risk of injury, and is successful only if the hoarder is superior to its competitors (Clarkson *et al.* 1986; Vander Wall 1990; Daly *et al.* 1992; Dally *et al.* 2006). Moreover, larders emit stronger odours, hence for competitors larders may be easier to detect by olfaction than small caches. Scatter hoarding, on the other hand, involves higher energetic costs in terms of travel (food is spaced out more widely) and memory (more locations have to be remembered), as well as increased mortality risk (longer exposure to predators and adverse environmental conditions) (Stapanian & Smith 1978, 1984; Dally *et al.* 2006), but scatter hoards are not usually defended individually (Smith & Reichman 1984; Jenkins *et al.* 1995).

Given a certain number of food items to be hoarded, and a limited number of patches suitable for hoarding within a limited area (i.e., the home range), the hoarder's options are constrained, because reducing the number of food items per cache comes with increasing the number of caches and vice versa (Vander Wall & Jenkins 2003; Dally *et al.* 2006). Thus, in essence, the decision about how to hoard a given number of food items is the outcome of a hoard-size – number trade-off, in which hoarders optimally balance the risks associated with having few large caches (i.e., larder hoarding) against the risks associated with having many small caches (i.e., scatter hoarding) (Alpern *et al.* 2012; Hirsch *et al.* 2013). For many rodent and bird species, this balance is shifted towards scatter hoarding, presumably because they have competitors against which they cannot defend larders (henceforth: superior competitors) (e.g., Stapanian & Smith 1978; Hurly & Robertson 1987; Daly *et al.* 1992; Brodin *et al.* 2001; Vander Wall & Jenkins 2003; Dally *et al.* 2006). Whereas for some species, this may be a flexible decision made by individuals, depending on the context under which hoarding takes place, for other species changes in hoarding patterns may have evolved over time. That is, a population of hoarders that has been exposed to superior competitors for a long period of time, may have evolved different hoarding patterns than hoarders of the same species that have not been exposed to superior competitors (e.g., Andersson & Krebs 1978; Stapanian & Smith 1978; Smith & Reichman 1984; Vander Wall 2001; Levey *et al.* 2002; Vander Wall & Jenkins 2003; Dally *et al.* 2006; Siepielski & Benkman 2008; Brodin 2010).

There are two hypotheses regarding the mechanisms by which scatter hoarding could reduce the risk of cache pilferage (Dally *et al.* 2006). The assumption underlying the first hypothesis (here termed the 'risk spreading' hypothesis) is that potential pilferers use random search or explorative search to find hidden food items, and that larder hoarding involves the catastrophic risk of instantaneously losing the entire food supply (i.e., complete pilferage), imperilling the animal's survival (Wauters *et al.* 1995). Scattering food items over many small caches spreads the risk of pilferage and thus makes complete pilferage less likely (e.g., Kraus 1983; Jacobs 1992; Macdonald 1997; Devenport *et al.* 2000; Dally *et al.* 2006). For instance, if we consider 100 repeated trials of a pilferer randomly visiting a fixed number of 50 patches (and potentially revisiting some) out of a total of 100 patches, the probability of this pilferer encountering all caches is approximately 80% if the total number of caches is 2, while it is only 7% if the total number of caches is 32. In other words, simply increasing the number of caches should reduce the risk of complete pilferage if the pilferer operates by means of random search or explorative digging (Vander Wall 1990; Dally *et al.* 2006; Gálvez *et al.* 2009). The assumption underlying the second hypothesis (here termed the 'cue-reduction' hypothesis) is that potential pilferers use special cues to locate hidden food items. It reasons that scattered food is more difficult to detect and pilfer, for example, because smaller caches are less easily detected by olfactory cues (e.g., Reichman & Oberstein 1977; Vander Wall 1993b, 1998, 2000, 2003; Geluso 2005), or because it is more difficult for a pilferer to spy on a hoarder's caching events if these are swift and numerous, as happens with scatter hoarding (reviewed in Dally *et al.* 2006).

Many field and experimental studies in a variety of systems have shown that food-hoarding animals indeed change their hoarding patterns in response to superior competitors, often by scattering the food items more widely. For example, in North America, Merriam's kangaroo rats (*Dipodomys merriami*) change their hoarding patterns in response to cache pilferage by the more aggressive and dominant Chisel-toothed kangaroo rat (*D. microps*) (Preston & Jacobs 2005), and in Spain, spacing patterns of acorn caches created by Wood mice (*Apodemus sylvaticus*) differed between areas with and without wild boar (*Sus scrofa*) (Muñoz & Bonal 2007; Puerta-Piñero *et al.* 2010). No study, however, has yet tested experimentally how cache characteristics resulting from alternative hoarding patterns affect the risk of cache pilferage, while taking into account the hoard-size – number trade-off.

We carried out a controlled experiment to determine how hoarding patterns affected the rate at which superior competitors pilfered the hidden food supply. The experiment involved an arena in which we distributed a fixed number of pedunculate oak acorns (*Quercus robur*) over a fixed number of patches, but in varying hoard-size – number configurations, ranging from few caches with many acorns (larder hoarding) to many caches with a single acorn (scatter hoarding). We then measured the rate at which the cached food was pilfered by wild boar, a keen-scented ungulate (e.g., Briedermann 1986) with a strong preference for acorns (e.g., Groot-Bruinderink *et al.* 1994; Focardi *et al.* 2000; Schley & Roper 2003; Herrero *et al.* 2006), that intensely competes for acorns with food-hoarding animals such as wood mice (e.g., Gomez *et al.* 2003; Muñoz & Bonal 2007; Perez-Ramos & Maranon 2008). The interaction between wood mice, wild boar, and oaks is interesting, as it is known from previous studies that hoarding patterns of wood mice can vary between individuals and between populations (e.g., Jennings 1975; Jensen & Nielsen 1986; Clarke & Kramer 1994; Jenkins & Breck 1998; Den Ouden *et al.* 2005; Lu & Zhang 2005, 2008), and that they can vary depending on wild-boar presence (e.g., Focardi *et al.* 2000; Muñoz & Bonal 2007; Muñoz *et al.* 2009; Puerta-Piñero *et al.* 2010).

We tested contrasting predictions derived from the cue-reduction hypothesis and the risk-spreading hypothesis: (1) Scattering decreases the rate of pilferage for the first cache(s) and for the last remaining cache(s), because scattered caches emit weaker olfactory cues and are therefore much harder to detect than larders. Thus, the overall rate of pilferage is lower for scatter hoarding than for larder hoarding (cue-reduction hypothesis). Alternatively, (2) scattering increases the rate of pilferage for the first cache(s) because there are simply more caches to be encountered, but decreases the rate of pilferage for the last remaining cache(s) because it takes more time to encounter many small caches than few large caches, and therefore the overall rate of pilferage is lower for scatter hoarding than for larder hoarding (risk-spreading hypothesis). To further assess the role of cue reduction in avoiding cache pilferage, we added a scenario in which seeds were buried at greater depth. The prediction was that (3) pilferage rates are lower for deep caches than for shallow caches, due to weaker olfactory cues emanating from deep caches than from shallow caches (cf Vander Wall 1993a).

Materials and methods

Study system

The wild boar is a medium-sized (50-200 kg) ungulate that is regarded as an important competitor to seed-hoarding rodents in forests and woodlands. The wild boar is one of the most widespread ungulates in the world. Its natural range extends over most of Europe and Asia and is still expanding rapidly (Massei & Genov 2004). Female wild boar generally live in family groups of 6 to 30 individuals, while males are mostly solitary (Poteaux *et al.* 2009). Their natural diet consists of 80-90% plants, but they also feed opportunistically on live and dead animal matter. Being mono-gastric ungulates, wild boar cannot efficiently extract carbohydrates from cellulose, and therefore rely on high-energy food items, such as mast of large-seeded trees, and nowadays, agricultural crops (Groot-Bruinderink *et al.* 1994; Schley & Roper 2003; Massei & Genov 2004).Acorns are a particularly dominant food in autumn when they have been freshly shed and are abundantly available on the forest floor (Massei & Genov 2004; Elston & Hewitt 2010), but also in mid- and late winter when very few acorns remain on the forest floor (Groot-Bruinderink *et al.* 1994; Den Ouden *et al.* 2005; Muñoz & Bonal 2007). This suggests that in winter, wild boar actively search for and pilfer acorn caches created by hoarders such as the wood mouse (Borchert *et al.* 1989; Herrera 1995; Focardi *et al.* 2000; Gomez *et al.* 2003; Den Ouden *et al.* 2005). Wood mice play an important role as seed predators and dispersers of acorns in Europe. They hoard acorns in autumn as a food reserve for the winter. Food hoarding patterns of the wood mouse range from almost exclusive scatter hoarding to a mix of scatter- and larder-hoarded caches (e.g., Vander Wall 1990; Den Ouden *et al.* 2005; Lu & Zhang 2005; Takahashi *et al.* 2007). The majority of food items, however, is typically scattered throughout the home range of the wood mouse (e.g., Jennings 1975; Jensen & Nielsen 1986;

Shimada 2001; Den Ouden *et al.* 2005; Suselbeek *et al.* 2013) with the degree of scattering being larger in the presence of wild boar than in their absence (Puerta-Piñero *et al.* 2010).

Experimental design

Experimental trials were conducted from 16 February to 25 March 2011, with eight wild boar (five females, three males). Wild boar were taken as six-month old piglets from “Natuurpark Lelystad”, the Netherlands, where they had been living with a social group of twelve adult individuals under semi-wild conditions in a 7-ha fenced natural area. To capture the wild boar from their living area, a trap of 5x5 m was built on 10 August 2010, which had a 0.2x0.4 m lockable entrance, to ensure exclusive access for piglets to the trap. The animals were then fed daily in and around the trap to habituate them to it. On 14 September 2010, the animals were fed in the trap at 7 a.m. and the entrance was closed during feeding, while eight piglets were inside. A team of five experienced caretakers from the park positioned a wildlife trailer so that the piglets could be swiftly guided through the trap entrance and into the trailer. A pre-built mobile fence was used to reduce the size of the trap and to direct the piglets (without physical contact) to its entrance and into the trailer. The entire capturing procedure took less than 10 minutes and was done without administering any drugs to the piglets. The wildlife trailer was not compartmentalized and had an 8 m^2 floor lined with a 20-cm layer of fresh hay. The trailer was approximately 1.9 m high and had roof hatches at the top of the back door which were all opened to ensure sufficient ventilation during transportation. As soon as the animals were in the trailer, they were transported to the experimental facility, a journey that lasted approximately one hour. Upon arrival, the trailer was again positioned so that the animals could walk out of the trailer without help or physical contact from the caretakers, and into the 1,200 m^2 field enclosure at the experimental facility “De Haar” in Wageningen, The Netherlands. No adverse effects of capturing and transportation were detected. Inside the field enclosure, the wild boar had access to four 8 m^2 shelter boxes with a layer of fresh hay. The shelter boxes had 1.2-m high walls made of concrete plywood, and had a roof covering approximately half of each shelter box. Shelter boxes could be entered by a 0.5x1 m opening in the wall at the front. Shelter boxes could be entered and exited by the wild boar at all times.

Wild boar had *ad libitum* access to water and were fed twice daily with fresh and dried plant material supplemented with sow pellets. Every week, a large pile of fresh stems, branches, and twigs was placed in the middle of the field enclosure, below an observation tower, to allow natural sheltering and to provide natural food to the wild boar. Throughout the study, wild boar were not physically contacted, but they were habituated to the presence of the experimenters and the regular caretakers. At the end of the study, the wild boar were culled by a professional wildlife hunter, who is also responsible for the yearly culling of wild boar in “Natuurpark Lelystad”.

Experimental trials took place in an outdoor arena of 26 x 30 m, consisting of bare clay soil with little or no vegetation. In this arena, a 10x10 grid of 100 sand-filled buckets with a diameter of 40 cm were dug into the earth, at regular intervals of 2 m, representing 100 distinct potential cache patches. A 3-m buffer zone was maintained around the grid to avoid edge effects (Fig. 4.1). Experimental acorns were collected from oaks in the surrounding area and then mixed and stored under low temperatures in large storage trays. Wild boar were habituated to the living area and to the experimental arena for the entire period between arrival (10 Sep 2010) and the start of experimental trials (16 Feb 2011). For each trial, a fixed number of 32 experimental acorns was randomly selected from the storage trays, and distributed over the patches. Preliminary results from a complementary field study showed that a density of 32 acorns spread out over an area of approximately 800 m^2 (i.e., the size of our experimental arena) corresponded well with natural wood mouse hoarding patterns. In this field study, 49 PIT-tagged acorns were offered in 12 different field sites. In each site, 20-40 tagged acorns were relocated after hoarding by wood mice within a radius of 15 m (i.e., an area of 780 m^2) around the seed station. This set-up, with a fixed number of acorns distributed over a fixed number of suitable patches within a fixed area, mimics the options available to hoarders for adjusting their hoarding patterns to the risk of pilferage. This level of control would be impossible to achieve in a field study.

Fig. 4.1: Overview of the experimental arena and the experimental setup with sand-filled buckets in which acorns were cached.

We compared three different spacing patterns of 32 acorns over the 100 patches, so as to simulate alternative hoarding patterns: 1) larder hoarding, with two randomly selected patches containing 16 acorns each, 2) intermediate hoarding, with eight patches containing four acorns each, and 3) scatter hoarding, with 32 patches containing one acorn each. All caches were created 5-8 cm below the soil surface. These depths approximate the depths reported for seeds hoarded by wood mice (Jennings 1975; Mallorie & Flowerdew 1994; Den Ouden *et al.* 2005). In addition, we ran a fourth treatment (“deep larder hoarding”), in which seeds were larder hoarded as above (treatment 1), but at greater depth: 15-20 cm below the soil surface. All treatments were randomly assigned to trials.

Prior to each trial, soil moisture content (%) was measured (Theta Probe soil moisture sensor ML2x, Delta-T Devices ltd, UK) in three randomly selected patches, with three measurements per patch, as soil moisture is known to affect olfactory cues and with that the detectability of hidden food items (reviewed in Vander Wall 2003). To avoid potential effects of human scent on cache detection, we wore surgical gloves during all acorn handling, and we raked the soil in all patches prior to each trial, while placing seeds in only some of the patches. During the night, and on days without trials, patches were covered with 50x50 cm plastic container lids to avoid large fluctuations in soil moisture content within the patches due to rainfall. Trials were also only conducted in dry weather.

Each trial started with allowing four selected individuals inside the arena to search for the cached acorns. This set-up with four animals simultaneously searching and competing for cached acorns corresponds to the natural situation, where wild boar live and forage together in groups and thus also compete for cached resources. During each trial, two observers continuously recorded the order in which all individuals visited the patches in the arena. In addition, all trials were recorded using two video cameras (Panasonic SDR-S50, Panasonic Corp., USA), positioned so that the entire arena fell inside the combined field of view. Trials ended two minutes after all caches had been found, or when all animals stopped searching for more than five minutes. Trials ended with examination of caches for non-discovered acorns. All experimental handling procedures were approved by the Animal Experiments Committee of Wageningen University (WUR-2010088.C).

The exact timing and order of cache encounter and pilferage were obtained from the combination of observer data and video recordings. The video recordings were prepared for further analysis using a custom-made program modelled in AutoHotkey (Mallet 2009), that enabled clips from the two cameras to be viewed simultaneously.

Data analysis

We used Cox Proportional Hazards (CPH) models (Therneau 2013) with moisture content as covariate, to test for differences in time until pilferage of the first and the last remaining cache between the alternative hoarding patterns (N = 16 for scatter- and intermediate hoarding and N = 15 for shallow larder- and deep larder hoarding). CPH models are frequently used when comparing survival times across treatment levels, as these models can deal well with censored observations and covariates. We included a frailty term (i.e., a random factor, here: ID_group) in the survival analysis to account for repeated tests of the same group of wild boar under the same hoarding pattern (Therneau 2013). We also used CPH models (Therneau 2013) with moisture content as covariate to test for differences in the overall rate of cache pilferage between the alternative hoarding patterns. All acorn retrieval events from the individual trials were lumped per treatment level, resulting in 512 events for scatter hoarding (32 events x 16 trials), 128 events for intermediate hoarding (8 events x 16 trials), and 30 events for both shallow and deep larder hoarding (2 events x 15 trials). We again included a frailty term (here: ID_trial) in the survival analysis but this time to account for multiple (non-independent) events within each trial (Therneau 2013). We used One-sample T-tests to compare expected and observed encounter probabilities of caches across treatment levels. Expected encounter probabilities were defined based on the ratio of empty patches to cache-patches, for each hoarding pattern. For example, in the case of scatter hoarding, 32 out of 100 available patches contained a cache, resulting in a 32% probability that a randomly encountered patch contained a cache. Thus, these were fixed probabilities for each treatment level (i.e., 0.32 for scatter hoarding, 0.08 for intermediate hoarding, and 0.02 for larder hoarding). These expected encounter probabilities were then compared to the true observed encounter probabilities, which were based on the actual ratio of empty-patch to cache-patch visitation by one prior-defined focal individual for each trial. It may have been that group composition influenced the response of the focal individual, but since we had repeated measurements on the same focal individual under the same treatment level, but with different companion animals in the group, we first averaged the observed visitation rates for each individual and then used these in the analysis. All analyses were carried out in R3.0.2 (R Core Team 2013).

Results

A total of 62 trials were run, with 21 replicate groups of wild boar (i.e., groups that consisted of a unique combination of individual wild boar). Altogether, 16 trials (12 replicate groups tested) were run for scatter hoarding, 16 trials (11 replicate groups tested) for intermediate hoarding, 15 trials (11 replicate groups tested) for shallow larder hoarding and 15 trials (15 replicate groups tested) for deep larder hoarding. Summaries of a) the number of times that each wild boar was tested within each treatment level, and b) the number of times that each group of wild boar was tested within each treatment level can be respectively found in table 4.S1 and table 4.S2. Soil moisture content ranged among trials from 1.6 to 7.2% (Mean 4.2%, SD = 1.3), but did not differ among treatments (One-way ANOVA: $F_{3,58} = 0.19$, $p = 0.9$).

Hoarding pattern significantly influenced the time it took a group of wild boar to pilfer the first cache (Cox Proportional Hazards Model: Wald $\chi^2 = 21.0$, $p = 0.00003$; Fig. 4.2a), and the last remaining cache of a trial (Cox Proportional Hazards Model: Wald $\chi^2 = 16.5$, $p = 0.003$; Fig. 4.2b). In line with our second prediction, the time until pilferage of the first cache was significantly shorter under scatter hoarding (mean = 6.2 s, 95% CI = 4.6 – 8.5) than under intermediate (12.9 s, 8.2 – 20.4) or larder hoarding (41.0 s, 19.7 – 85.0), while pilferage of the last remaining cache took significantly more time under scatter hoarding (mean = 567 s, 95% CI = 415 – 774), than under intermediate (389 s, 278 – 545) or larder hoarding (221 s, 122 – 400). Soil moisture content did not explain any variation in the time it took to pilfer the first cache (Wald $\chi^2 = 0.6$, $p = 0.44$), however, regardless of the hoarding pattern, an increase in soil moisture content reduced the time it took a group of wild boar to pilfer the last remaining cache (Wald $\chi^2 = 10.0$, $p = 0.002$).

Contrary to our predictions, hoarding pattern did not affect the overall rate at which acorns were pilfered by a group of wild boar (Cox Proportional Hazards Model: Wald $\chi^2 = 9.1$, $p = 0.996$; Fig. 4.3a). The average proportion of caches pilfered after 5 minutes of wild-boar searching did not significantly differ between scatter hoarding (86.5%, N = 16) and larder hoarding (73.3%, N = 15) (Mann-Whitney U test: U = 114, p = 0.81), and the probability that all caches had been pilfered after 10 minutes of searching also did not significantly differ between scatter hoarding (62.5%, N = 16) and larder hoarding (86.7%, N = 15) (Pearson $\chi^2 = 2.36$, $p = 0.12$). Regardless of the hoarding pattern, the overall rate of pilferage significantly increased with soil moisture content (Wald $\chi^2 = 7.5$, $p = 0.006$).

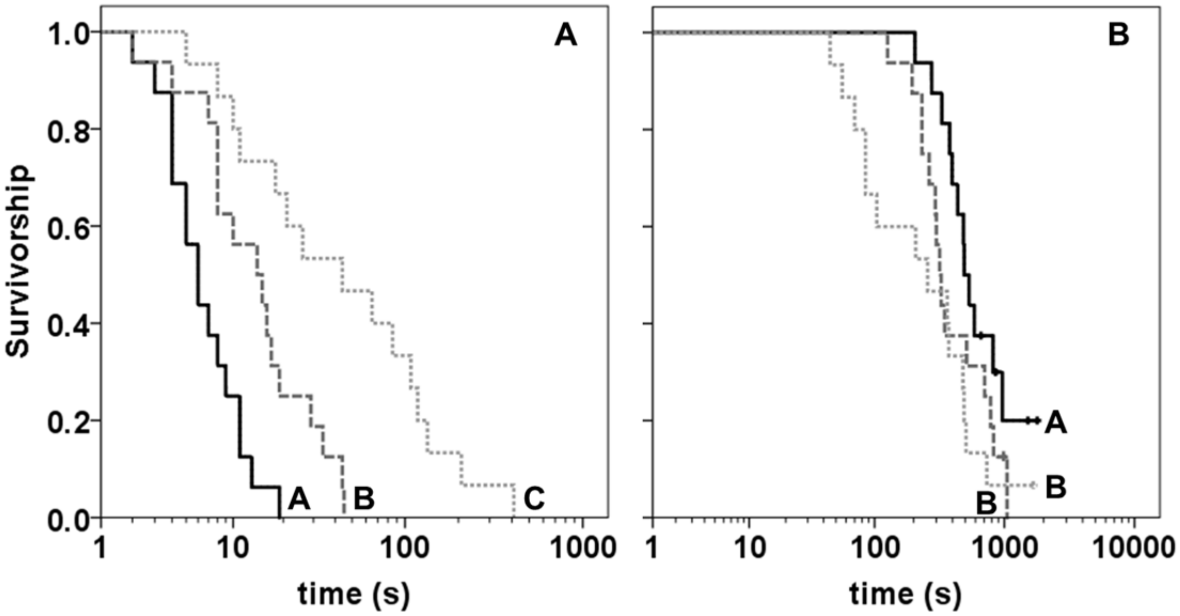

Fig. 4.2: Effect of hoarding pattern on the rate of cache pilferage by wild boar in a controlled experiment. (a) and (b) show survivorship curves indicating time-to-pilferage for (a) the first cache, and (b) the last remaining cache, for scatter hoarding (solid line), intermediate hoarding (dashed line) and larder hoarding (dotted line). Crosses in curves represent censored observations.

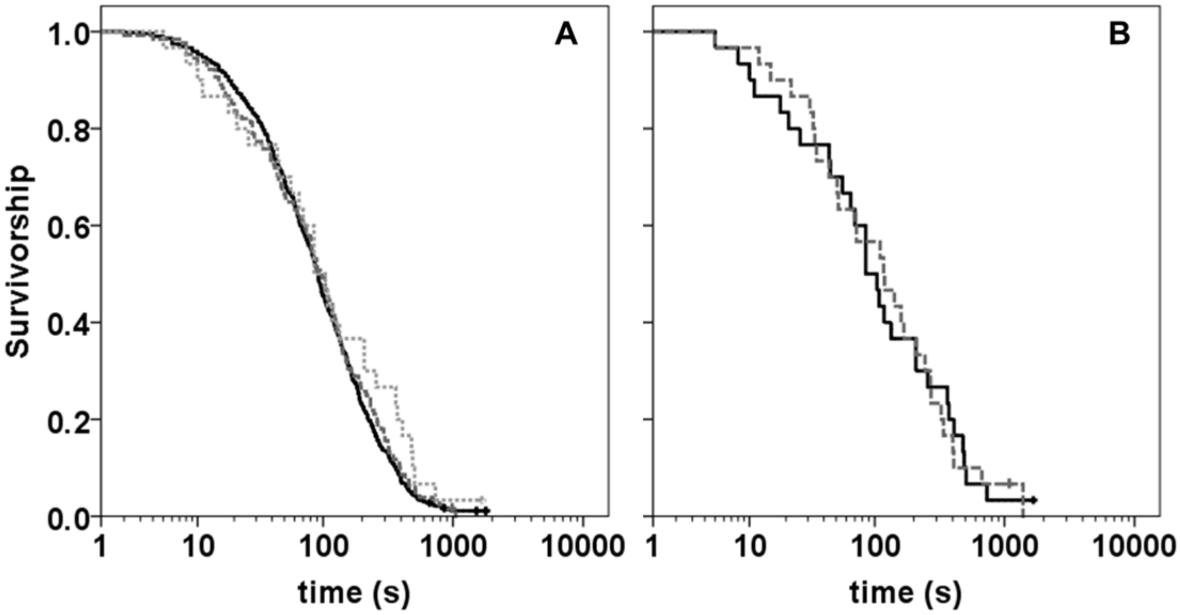

Fig. 4.3: Effects of hoarding pattern and cache depth on the overall rate of acorn pilferage by wild boar for all trials, lumped per treatment. Panel (a) shows survivorship curves for scatter hoarding (solid line), intermediate hoarding (dashed line) and larder hoarding (dotted line), and panel (b) shows survivorship curves for shallow larder hoarding (solid line) and deep larder hoarding (dashed line). Crosses in curves represent censored observations.

Contrary to our third prediction, cache depth did not influence the overall rate of cache pilferage by wild boar (Wald $\chi^2 = 0$, $p = 0.988$; Fig. 4.3b). Cache depth also did not affect the time it took a group of wild boar to pilfer the first cache (Wald $\chi^2 = 0.12$, $p = 0.73$; Fig. 4.4a) or the last remaining cache (Wald $\chi^2 = 0.06$, $p = 0.81$; Fig. 4.4b).

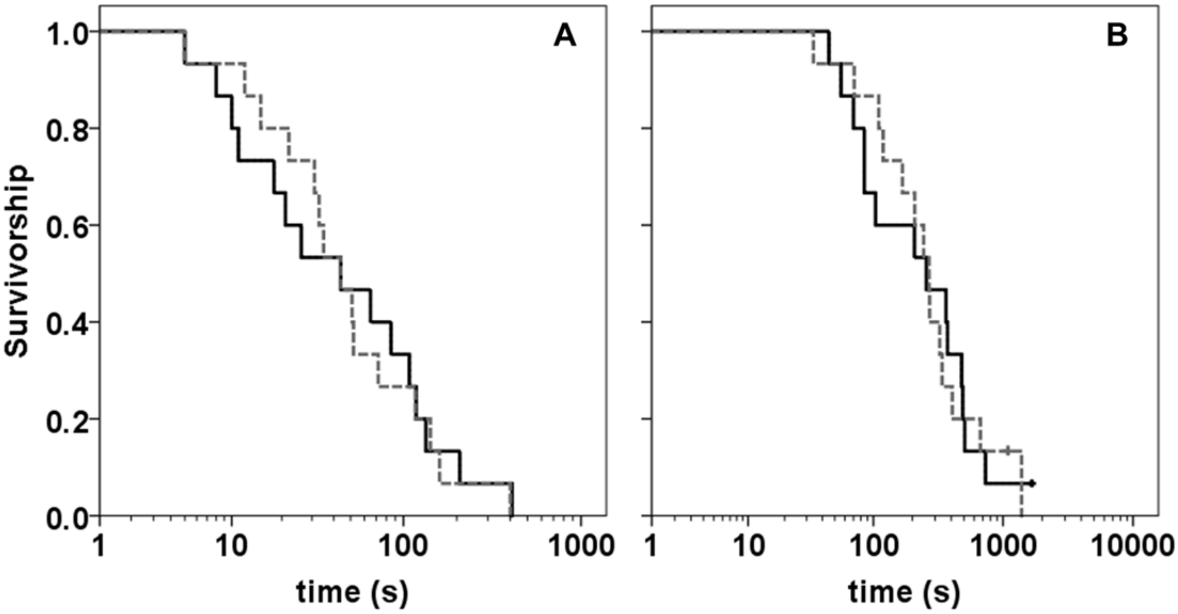

Fig. 4.4: Effect of cache depth on the rate of cache pilferage by wild boar in a controlled experiment. (a) and (b) show survivorship curves indicating time-to-pilferage for (a) the first cache, and (b) the last remaining cache, for shallow larder hoarding (solid line) and deep larder hoarding (dashed line). Crosses in curves represent censored observations.

The above results suggest that wild boar visited patches more or less randomly, regardless of whether they contained a cache. We verified this possibility by comparing the random encounter probability of patches that contained acorns with the observed encounter rate of such patches. Observed encounter rates for patches that contained a cache did not differ from expected encounter rates based on random patch visitation, irrespective of the hoarding pattern (One-Sample t-tests: all p-values > 0.05; Table 4.1).

Table 4.1: One-Sample T-test results of the comparison between mean observed and mean expected cache encounter probabilities for eight individual wild boar, summarized for each alternative hoarding pattern.

treatment	mean expected	mean observed	median observed	T (*df*)	P	min 95% CI	max 95% CI
scatter (32 caches)	0.320	0.309	0.319	-0.634 (7)	0.55	0.269	0.350
intermediate (8 caches)	0.080	0.074	0.077	-1.036 (7)	0.33	0.059	0.088
shallow larder (2 caches)	0.020	0.029	0.030	0.949 (7)	0.37	0.007	0.051
deep larder (2 caches)	0.020	0.029	0.026	0.871 (7)	0.41	0.005	0.053

Discussion

We conducted a controlled experiment to determine how alternative hoarding patterns influenced pilferage of cached acorns by a large non-hoarding food competitor, the wild boar. We found that scatter hoarding, as expected, reduced survival time for the first cache and slightly increased survival time for the last remaining cache. The overall rate of pilferage by wild boar did not, however, differ between scatter- and larder hoarding, and the overall rate of pilferage was also not influenced by cache depth. This suggests that wild boar were either unable to pick up olfactory cues from acorn caches, e.g., because these were too weak, or were not using olfactory cues to locate cached acorns. It also suggests that risk spreading, rather than cue reduction, may be the dominant mechanism by which acorn pilferage by wild boar is minimized.

Our finding that scatter hoarding increased the rate of pilferage of the first cache(s), but reduced the rate of pilferage of the last remaining cache(s) is in agreement with the risk-spreading hypothesis, which argues that scattering of food increases the probability of a pilferer randomly encountering some caches (as simply more patches contain a cache) but also increases the effort needed to locate all caches, and that, as a consequence, the probability of at least some caches escaping pilferage is higher (Kraus 1983; Jacobs 1992; Macdonald 1997; Devenport *et al.* 2000; Dally *et al.* 2006). These findings are not, however, in line with the cue-reduction hypothesis, under which scatter hoarding should reduce the rate of pilferage for both the first and the last remaining cache(s) due to reduced olfactory cues emanating from scatter hoards as compared to larder hoards (as a consequence of scatters being smaller) (e.g., Reichman & Oberstein 1977; Vander Wall 1993a, 1998, 2003; Geluso 2005). Similarly, we found no effect of cache depth on pilferage by wild boar, even though several studies have shown that shallow caches emanate stronger olfactory cues than do deep caches (reviewed in Vander Wall 2003). This, together with the results of the comparison between expected and observed cache encounter probabilities, suggests that cue reduction is not the mechanism by which acorn pilferage by wild boar is avoided. This could be because olfactory cues emanating from acorn caches were too weak to be picked up by wild boar, or because they simply do not use olfactory cues for cache finding but perhaps use systematic or random searches instead.

The finding that wild boar may not be able to pick up olfactory cues from buried acorns or may not be using them for cache finding was unexpected, as wild boar are well-known for their sensitive olfactory senses. Much of the literature's acclaim of the wild boar's sense of smell is, however, based on their capacity to locate black truffles (see Briedermann 1986). Black truffles and all other hypogeous fungi have evolved to produce their fruiting bodies below ground and thus they rely, for having their spores dispersed, on being found and dug up by animals (e.g., Bellina-Agostinone *et al.* 1987; Bruns *et al.* 1989; Talou *et al.* 1990; Johnson 1996; Pyare & Longland 2001). Acorns, on the other hand, are the fruiting bodies of oak trees that require transportation by scatter-hoarding animals to reach a location for germination and establishment, and so it is unlikely that there has been a selective advantage for oaks to produce acorns that can be found and destroyed by wild boar. On the contrary, selection pressure may have favoured acorns that emanate weak olfactory cues that cannot be picked up efficiently by seed predators such as the wild boar. Consequently, wild boar may only be able to easily locate cached acorns by opportunistically foraging and rooting at locations likely to have acorns, such as directly below and around adult oak trees. This would also provide an explanation for high concentrations of rooting found below and directly around adult oak trees (e.g., Groot-Bruinderink & Hazebroek 1996; Welander 2000; Fig. 4.5). If wild boar were able to pinpoint the location of cached acorns by means of olfaction, one would expect to see many small rooting patches rather than a few large rooting patches. Nevertheless, a useful follow-up experiment could focus on the wild boar's ability to detect (hidden) acorns on the basis of olfaction, and perhaps to test at which spatial scale these olfactory cues could be picked up.

Fig. 5 A typical acorn hotspot in the Veluwe, The Netherlands; an adult oak tree, with the soil below and around the tree uprooted by wild boar.

Pilferage rates of cached acorns by wild boar did not differ between scatter- and larder hoarding. The reason for this may be that under scatter hoarding, the initial rate of pilferage was higher, but this decreased with depletion of caches, while under larder hoarding the rate of pilferage was more constantly low over time. Thus the overall rate of pilferage ended up being very similar for scatter- and larder hoarding. The question that thus arises is: why do many animals primarily scatter hoard their food items? First, and perhaps most importantly, scatter hoarding is assumed (and was found in our study) to be a strategy to lower the risk of a catastrophic loss (i.e., instantaneously losing the entire food supply) (e.g., Smith & Reichman 1984; Vander Wall 1990; Dally *et al.* 2006). While several studies have suggested that complete pilferage by superior competitors poses the largest threat of catastrophic loss (e.g., Macdonald 1997; Preston & Jacobs 2001; Zhang *et al.* 2011), there may be several other factors that could result in such catastrophic loss. For example, many large seeds are prone to fungal infections due to their high natural moisture content. Caching large quantities of such seeds together may thus pose a large threat of catastrophic loss due to fungal cross-contamination (e.g., Reichman *et al.* 1985; Edelman 2011). Similarly, physical disturbance of the cache (through e.g., windthrow) as well as density-responsive seed predators (Janzen 1970) may pose a large threat of catastrophic loss. Another possibility is that scatter hoarding by wood mice is a response to competition with conspecifics and that hoarding patterns depend on the individual characteristics of the hoarder, in terms of its dominance over conspecific competitors (Clarke & Kramer 1994), a response that has also been shown for other species (e.g., Sanchez & Reichman 1987; Daly *et al.* 1992; Preston & Jacobs 2001; Leaver 2004; Zhang *et al.* 2011). Finally, while scatter hoarding does not seem to have a large effect on pilferage risk by wild boar, this effect may be different for other species, depending on the cues provided by the caches and by the hoarder itself, and depending on the search tactics applied by the pilferer. For instance, hedgehogs (*Erinaceus europaeus*) and red foxes (*Vulpes vulpes*), which use olfaction to locate buried eggs, have been shown to be able to detect (by smell) eggs buried at 3 cm depth up to a distance of respectively 50 cm and 3 m (Tinbergen 1965). After retrieval of a buried egg, hedgehogs only searched in an approximately 1 m^2 area around the cache location, suggesting that scattering individual food items beyond the olfactory detection distance of the pilferer reduces pilferage risk.

One aspect that should not be overlooked here is the direct risk of predation imposed upon wood mice by wild boar. Wild boar are known to have a very diverse diet, that certainly includes animal matter, ranging from insects and worms to mice and voles, and they even scavenge around the carcasses of larger animals (Briedermann 1986). It is thus not unlikely that wild boar form a direct threat to the survival of wood mice, and as a consequence, wood mice might have adjusted their hoarding patterns so that risk of predation by wild boar, rather than risk of pilferage, is minimized (Focardi *et al.* 2000; Muñoz & Bonal 2007; Puerta-Piñero *et al.* 2010). Generally, larder hoards are connected to the underground burrow system of the hoarder and are usually located near the hoarder’s nest, while scatter hoards are created from the soil surface, and are usually not connected to the burrow system of the hoarder (Jennings 1975; Vander Wall 1990). The scatter-hoarding set-up, where food is detached from the burrow system, is likely to reduce the risk of wild boar encountering the nest or hiding place of a hoarder while it is searching for buried food items (Vander Wall 1990, 2001).

Our experiment (in which we distributed a fixed number of acorns over a fixed number of potential cache locations within a fixed area) was designed to mimic alternative outcomes of the hoard-size – number trade-off that hoarders face. Given a certain food supply (i.e., a fixed number of seeds or other food items) and available area (i.e., the home range), pilferage rates can be influenced only by changing the distribution of food items over the available patches and by varying the depth at which food items are cached. In our experiment, we controlled for the number of food items and the number of available patches as well as for pilferer pressure (i.e., the number of wild boar in the area). Nevertheless, two aspects of our study differ slightly from the natural situation; 1) we used a fixed number of distinct patches, rather than a continuous area in which caches could be created, and 2) wild boar could have been aware that hidden food items were present in the experimental arena during each trial. These aspects may have facilitated exploration by wild boar and may thus have inflated the rate with which caches were pilfered, as compared to a truly natural situation. Since these conditions were similar across different treatments, however, this will not have influenced our final conclusion. Finally, in a natural situation, wild boar may be using cues other than those assumed in this study. For instance, they may use visual or olfactory cues to locate the entrance of a hoarder's burrow system and accordingly uproot the entire burrow system (Focardi *et al.* 2000). If this were true, it would particularly favour scatter hoarding.

The results from this study suggest that the overall rate of cache pilferage by wild boar, and with that, the risk of catastrophic loss of food reserves, is little affected by the hoarding pattern applied by the food hoarder. This suggests that superior competitors are not driving the decision of a hoarder to scatter hoard. Although scatter hoarding may have the potential to lower the rate at which competitors pilfer cached food, this probably depends on the cues provided by the caches and by the hoarder, and on the search tactics used by the pilferer. There may also be other factors, such as conspecific pilferage, physical cache disturbance, or cross-contamination of food items, that could lead to catastrophic loss of food reserves and as such could be more important drivers of the decisions of animals to scatter hoard the majority of their food reserves.

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Appendix

Table 4.S1: Summary of the number of times that each of the eight individual wild boar was part of a team that was tested in each of the four treatment levels. Column totals represent the total number of individuals tested in each treatment level.

		scatter hoarding	intermediate hoarding	shallow larder hoarding	deep larder hoarding
1	F1_ALF	5	6	6	6
2	F2_BIL	8	8	7	9
3	F3_MAT	6	6	6	7
4	F4_OSW	7	6	6	3
5	F5_SOP	9	11	10	8
6	M1_BIG	10	10	10	10
7	M2_CHA	8	7	6	7
8	M3_HAZ	11	10	9	10
	TOTAL	**64**	**64**	**60**	**60**

Table 4.S2: Summary of the number of times that each of the 21 unique teams of wild boar was tested within each of the four treatment levels. Column totals represent the total number of trials for each treatment level.

					scatter hoarding	intermediate hoarding	shallow larder hoarding	deep larder hoarding
1	F1_ALF	M1_BIG	F2_BIL	M2_CHA				1
2	F1_ALF	M1_BIG	F2_BIL	M3_HAZ	1	1	1	1
3	F1_ALF	M1_BIG	F2_BIL	F5_SOP		1		
4	F1_ALF	M1_BIG	M2_CHA	M3_HAZ	1	1	1	1
5	F1_ALF	M1_BIG	M3_HAZ	F5_SOP			1	
6	F1_ALF	F2_BIL	F3_MAT	F5_SOP				1
7	F1_ALF	M2_CHA	M3_HAZ	F3_MAT				1
8	F1_ALF	F3_MAT	F4_OSW	F5_SOP	3	3	3	1
9	M1_BIG	F2_BIL	M2_CHA	M3_HAZ	3	2	2	1
10	M1_BIG	F2_BIL	M2_CHA	F5_SOP			1	
11	M1_BIG	F2_BIL	M3_HAZ	F3_MAT				1
12	M1_BIG	F2_BIL	M3_HAZ	F5_SOP	1	1	1	1
13	M1_BIG	F2_BIL	F3_MAT	F5_SOP				1
14	M1_BIG	F2_BIL	F4_OSW	F5_SOP	1			
15	M1_BIG	M2_CHA	M3_HAZ	F3_MAT	1	1	1	1
16	M1_BIG	M2_CHA	M3_HAZ	F5_SOP	1	2	1	1
17	M1_BIG	M3_HAZ	F4_OSW	F5_SOP	1	1	1	1
18	F2_BIL	M2_CHA	M3_HAZ	F4_OSW	1			
19	F2_BIL	M2_CHA	M3_HAZ	F5_SOP		1		1
20	F2_BIL	F3_MAT	F4_OSW	F5_SOP	1	2	2	1
21	M2_CHA	M3_HAZ	F3_MAT	F5_SOP	1			
	TOTAL				**16**	**16**	**15**	**15**

Chapter 5

Intraspecific variation in hoarding patterns of the wood mouse (*Apodemus sylvaticus*)

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Abstract

Hoarding patterns vary from larder hoarding to scatter hoarding, both between and within species, and this variation is generally ascribed to differences in pilferage risk. We experimentally tested whether within-species variation in hoarding patterns is more driven by conspecific or by heterospecific competition, and whether dominance of the hoarder over its competitors plays a role. We compared acorn hoarding between male and female wood mice (*Apodemus sylvaticus*) of known body sizes, and from areas with and without wild boar (*Sus scrofa*), a reputed pilferer of acorn caches. Mice were allowed to hoard acorns in indoor arenas, with acorn removal recorded using camera traps. After each trial, distance and angle of dispersal as well as the proportion of acorns cached were recorded. All individuals predominantly scatter-hoarded acorns, however, females cached significantly more seeds than males did. Also, females from areas with wild boar scattered seeds more widely than did females from areas without wild boar. The introduction of a conspecific audience mouse did not significantly affect hoarding patterns in the wood mouse, however, in females their tended to be a negative relationship between seed scattering and body size in response to a conspecific audience. Our results show that dominance status of the hoarder in relation to its competitors, regardless of the type of competitor (conspecific or heterospecific), leads to variation in hoarding patterns between individuals, but not to the extent that hoarding patterns shift from larder hoarding to scatter hoarding or vice versa. Other factors may thus be driving the decision to scatter- or larder hoard.

Keywords: *rodents, acorns, hoarding, competition, seed dispersal, dominance, seeds, wild boar*

Introduction

Food-hoarding animals cache food items at times of high food availability and rely on these reserves during periods of food scarcity (Vander Wall 1990). The spatial pattern at which food items are cached ranges between larder hoarding and scatter hoarding. Larder hoarding involves the placement of many food items in one or few caches ('larders'), usually located in or near the nest, in underground burrows, or in tree cavities. Larders are relatively easy to create and recover, yet usually require active defence against other animals (Clarkson *et al.* 1986; Vander Wall 1990; Daly *et al.* 1992; Dally *et al.* 2006). Scatter hoarding, in contrast, involves spreading of food over many widely spaced caches with one or few items each ('scatters') (Morris 1962; Clarkson *et al.* 1986; Vander Wall 1990, 2001). Scatters are not individually defended but involve higher energetic costs in terms of travel (food is spaced out more widely) and memory (more locations are to be remembered), as well as increased mortality risk due to longer exposure to predators and adverse environmental conditions (Stapanian & Smith 1978; Smith & Reichman 1984; Stapanian & Smith 1984; Jenkins *et al.* 1995; Dally *et al.* 2006).

Variation in hoarding patterns occurs between species but also within species (Roberts 1979; Clarkson *et al.* 1986; Vander Wall 1990; Dally *et al.* 2006). Numerous field and experimental studies in a variety of systems have shown that between-species variation in hoarding patterns is largely explained by differences in pilferage risk (e.g., Vander Wall & Jenkins 2003; Vander Wall *et al.* 2005a; Dally *et al.* 2006). For instance, larger-bodied species are generally better able to defend reserves against potential pilferers, and therefore more prone to larder hoard (Roberts 1979). However, the large majority of these studies considered hoarding patterns to be fixed within species, which is why within-species variation in hoarding patterns has received much less attention up to now. Those studies that reported within-species variation in hoarding patterns, almost exclusively focused on responses to conspecific pilferage rather than to heterospecific pilferage. For example, Korean field mice (*Apodemus peninsulae*) and Merriam's kangaroo rats (*Dipodomys merriami*) shift almost entirely from scatter hoarding to larder hoarding in response to cache pilferage by a conspecific (e.g., Preston & Jacobs 2001; Zhang *et al.* 2011). We could find only one study in which the role of heterospecific competitors in explaining within-species differences in hoarding patterns was investigated (Zhang *et al.* 2013), and no study that was focused on the combined effect of conspecific and heterospecific competition on hoarding patterns, or on the relative contribution of both types of competition to variation in hoarding patterns.

The mechanism of pilferage is likely to differ between conspecific and heterospecific pilferers. For example, pilferage by conspecifics generally results in a steady decrease in hoarded food items (sieve-effect), an act to which a hoarder could respond by redistributing the remainder of the food items, such that further pilferage is prevented. In contrast, pilferage by heterospecifics, particularly if they are large, is often a sudden and catastrophic event resulting in loss of the entire hoarded food supply (catastrophe-effect). Such a sudden act of complete pilferage cannot be counteracted by the hoarder and the risk of this happening should thus be minimized. Therefore, it is likely that the hoarder's response to conspecific pilferage is instantaneous, while the hoarder's response to heterospecific pilferage has evolved over time and is only apparent in hoarder populations that have a long history of being subjected to this heterospecific competitor, but not in other populations of the same hoarder.

Apart from responses to conspecific and/or heterospecific competitors, individual characteristics, such as sex and body size, may help explaining within-species variation in hoarding patterns, as this may affect the ability of the hoarder to defend its caches (Clarke & Kramer 1994; Jenkins 2011; Zhang *et al.* 2011). For example, small-sized individuals may be more inclined to scatter hoard, because they are less able to defend their caches against larger-sized individuals of the same species (Dally *et al.* 2006; Price & Mittler 2006; Swartz *et al.* 2010; Zhang *et al.* 2011).

Here, we used the study system of a species that hoards seeds in both larder- and scatter forms, the Wood mouse (*Apodemus sylvaticus*), a superior heterospecific competitor, the Wild boar (*Sus scrofa*), and their communal food source, acorns of the Pedunculate oak (*Quercus robur*), to experimentally compare hoarding patterns between male and female wood mice that differed in body size, and were taken from areas with a long history (nearly 60 years) of either wild-boar presence or wild-boar absence. Our experiment was designed to mimic alternative outcomes of the hoard-size – number trade-off that hoarders face (Vander Wall & Jenkins 2003; Dally *et al.* 2006; Alpern *et al.* 2012; Hirsch *et al.* 2013). Given a certain food supply (i.e., a fixed number of seeds or other food items) and available area (i.e., the home range), hoarding patterns can be varied by changing the speed at which food items are removed and secured in safe locations, by changing the detectability of the food items to others (e.g., by hiding the food items below ground), and by changing the number of food items per cache (and with that the total number of caches). Our experiment was performed in large indoor arenas, and was controlled for the number of food items presented, the size of the hoarding area, the structural diversity of the ground cover, as well as for pilferer pressure. Our experiment consisted of two sub-experiments; the first experiment tested seed-hoarding patterns of individuals that varied in sex, body size, and provenance, while the second experiment was a test for the response of individual hoarders to the presence of a conspecific audience. Here, treatment mice, all taken from similar areas, were first tested without and then with an audience mouse to further investigate how individual differences in sex and body size affected seed-hoarding patterns.

With respect to the first experiment, we predicted that 1) seed-removal rates, the number of seeds cached, and the spatial extent of seed scattering would be greater for females, small-sized individuals, and individuals from areas with wild boar, than for males, large-sized individuals, and individuals from areas without wild boar. With regards to the second experiment, we predicted that 2) females and small-sized individuals would respond to the presence of a conspecific audience by increasing the spatial extent of seed scattering, increasing caching and increasing the rate of seed removal, while males and large-sized individuals would not change hoarding patterns in response to the presence of a conspecific audience.

Materials and methods

Study system

The wood mouse is a common European woodland rodent that weighs between 10-30 g and breeds between April and October. Wood mice are nocturnal and characterized by well-developed olfactory, visual, and auditory senses. The wood mouse is a typical granivore, with seeds constituting roughly 70% of their diet (Watts 1968; Flowerdew *et al.* 1985). Wood mice play an important role as seed predators and dispersers of acorns in Europe. They hoard acorns in autumn, primarily through a combination of scatter- and larder hoarding, as food reserve for winter (Vander Wall 1990; Den Ouden *et al.* 2005; Takahashi *et al.* 2007).

The wild boar is a medium-sized (50-200 kg) ungulate that is regarded as an important competitor to seed-hoarding rodents in forests and woodlands (Massei & Genov 2004). Acorns are a dominant food in autumn when freshly shed and abundantly available on the forest floor (Massei & Genov 2004; Elston & Hewitt 2010), but also in mid- and late winter when very few acorns remain on the forest floor (Groot-Bruinderink *et al.* 1994; Den Ouden *et al.* 2005; Muñoz & Bonal 2007). The latter suggests that wild boar actively search for and pilfer acorn caches created by hoarders, such as the wood mouse (Borchert *et al.* 1989; Herrera 1995; Focardi *et al.* 2000; Gomez *et al.* 2003; Den Ouden *et al.* 2005).

Experimental design

Experimental trials were conducted from 9 November 2011 to 24 February 2012 at the experimental facility “De Ossenkampen” in Wageningen, the Netherlands. Wood mice were taken from mixed-forest stands in the Southern Veluwe, the Netherlands. This area is crossed by a motorway (A12) that was established in 1956 and since then effectively divides the study area into a northern part with wild boar and a southern part without wild boar. Pedunculate oak and Scots pine (*Pinus sylvestris*) are dominant trees in the area. Besides the wood mouse, potential acorn hoarders present in the area were Eurasian jay (*Garrulus glandarius*), red squirrel (*Sciurus vulgaris*) and bank vole (*Myodes glareolus*). In total, 112 wood mice were captured and used in the experiment. Of these, 59 were males (46 from areas without wild boar and 13 from areas with wild boar) and 53 were females (42 from areas without wild boar and 11 from areas with wild boar). Body size of wood mice used in the study ranged from 11 to 27 g for males (mean = 19.2 g) and from 10 to 32 g for females (mean = 18.6 g). Wood mice were captured using Longworth live traps (Penlon Ltd., Oxford, U.K.) that were pre-baited with a mixture of peanut butter, rolled oats and fresh apple for two nights. Traps were set at 11 PM and checked at 7 AM the following morning. Wood mice in apparent healthy condition were taken to the experimental facility, where they were weighed, sexed and individually marked by means of fur-clipping (cf. Clarke & Kramer 1994). Pregnant or lactating individuals were excluded from the experiment and immediately released at the capture site. Experimental acorns were collected from the same areas as the mice and were stored at 4 °C in a refrigerator at the experimental facility.

At the experimental facility, wood mice were housed individually in 42x26x19 cm living cages, with a 5-cm layer of natural forest soil and a 10-cm layer of fresh hay as bedding and enrichment. Animals had *ad libitum* access to fresh apple (for water) and a mixture of peanut butter with rolled oats. Living cages were placed in a 4x6 m room inside an isolated building at the experimental facility. The room had windows but no lights, thus having a natural light : dark cycle. Temperature and humidity inside the room fluctuated with outside weather conditions, but less extreme.

Experimental trials took place in a large 15x30 m barn, adjacent to the building where experimental mice were kept. Poor isolation of the barn made the indoor climate very similar to the outdoor climate, except for the absence of precipitation. Two rings of corrugated iron (11 m diameter, 1 m high) were placed in the barn. Experimental arenas were created by subdividing each ring into four quadrants by using 1-m high wooden frames covered with black silage film. The smoothness of silage film prevented mice from gnawing through and/or climbing over the arena walls. This resulted in 8 pie-shaped arenas of approximately 24 m^2. Arenas were filled with a layer of approx. 25 cm of natural forest soil (Hooijer Renkum B.V., the Netherlands). Half of the surface area of each arena was covered with a camouflage netting that had a leaf-like textile structure knitted on top (Seyntex N.V., Belgium), to resemble a natural leaf layer.

Experimental trials started with the introduction of one experimental mouse into each arena, between 3 and 4 p.m. on day one. Each mouse was then allowed to acclimatize to the arena and had access to a mixture of peanut butter and rolled oats, some fresh hay to serve as nesting material, and a bottle cap filled with water. The next day, between 2 and 3 p.m., experimental acorns, that had been soaked in cold water for 24 hours, were PIT-tagged (Trovan ID100A, 11.5 x 2.0 mm, Dorset ID, Netherlands) and weighed on a precision balance. These electronic tags do not influence removal rates or dispersal distances and can be re-used indefinitely, while allowing individual identification of buried acorns (Suselbeek *et al.* 2013). Then, at approximately 3 p.m. on day two, 6 PIT-tagged acorns were placed in a 2x3 grid in the seed station of each arena. Acorns were slid through a 2.5-m PVC pipe, allowing placement away from the outside edges of the arena and adjacent to the camouflage netting, without the need for the experimenter to enter the arena. The mouse was then allowed to hoard acorns until approximately 10 a.m. in the morning of day three. During each trial, a motion-sensitive camera trap (HC500; Reconyx Inc., Wisconsin, USA) was used to monitor acorn removal from the seed station.

Trials ended with digging out the mouse from its burrow, if it had one, and guiding it back into its living cage using cardboard sheets that narrowed towards the cage entrance. This capture procedure generally took less than two minutes and had no visible adverse effects on the mice. If a mouse was scheduled to be tested in a second trial, it was returned to the living-cage room and kept there for 6 days, until the start of the second trial for that mouse. Otherwise, the mouse was released the same day at the location of capture. Consequently, mice were in captivity for a maximum of 12 days. All experimental handling procedures were approved by the Animal Experiments Committee of Wageningen University (WUR-2011092.f).

After the trial had ended, acorns were relocated using a handheld PIT-tag reader (Trovan GR-250, Dorset ID, Netherlands). For each acorn, its hoarding status (i.e., cached or exposed), as well as the distance and angle from the seed station, were recorded. Once all acorns had been relocated, the top soil layer of the arena was thoroughly raked and mixed, to remove or at least reduce any mouse- or human traces of the preceding trial as much as possible.

To quantify seed spacing, Minimum Spanning Trees (MST) were obtained by mapping the spatial distribution of cached acorns in ArcGIS 10 (ESRI, California, USA). However, the estimate of MST in itself does not always ‘capture’ the true extent of seed scattering. for instance, if 5 acorns are clumped together and the sixth acorn is placed in another cache at 5 m distance the MST will be 5 m, while if all acorns are cached individually with a regular spacing between caches of 1 meter, the MST will still be 5 m (Fig. 5.1). Therefore, we defined the spatial extent of seed scattering as a combination of the MST and the total number of caches created, relative to the maximum number of caches possible and to the maximum MST within the arena. The spatial extent of seed scattering was thus obtained by multiplying the MST of a trial by the number of caches created in that trial, and dividing this over the theoretical maximum of the MST (i.e., 15 m) multiplied by the theoretical maximum of the number of caches (i.e., 6). This resulted in a measure of scattering that fell between 0 (all seeds clumped together) and 1 (widest possible scattering).

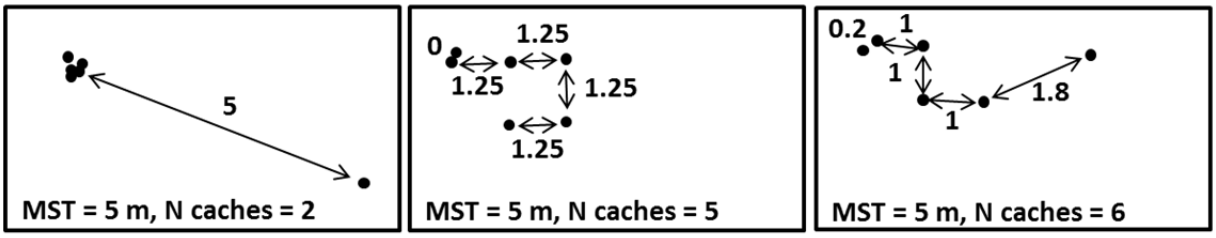

Fig. 5.1: Panels illustrate the relationship between minimum spanning tree (MST) and the number of caches created. MST equals 5 m across all panels, however, the number of caches varies and thus the spatial extent of seed scattering also differs.

Data analysis

We used Linear Mixed Models to test for differences in seed-removal rate and for differences in the extent of seed scattering between males and females of different body size and from areas with and without wild boar. We used a Generalized Linear Mixed Model with a binomial error distribution to test for differences in the proportion of seeds cached between males and females of different body size and from areas with and without wild boar. We included “individual” and “arena” as random effects in all models to account for repeated use of the same individual and the same arena.

We used Repeated-Measures ANOVA to test for a change in seed removal rate, proportion of seeds cached, and extent of seed scattering after the introduction of a conspecific audience, for males and females of different body size from areas without wild boar. Preliminary data from a complementary field study suggest that wood mouse activity is influenced by ambient temperature. At temperatures well below freezing, wood mice decreased their activity. Thus, to account for possible effects of temperature on hoarding patterns, we included the daily minimum ambient temperature, obtained from a professional weather station located 1 km away from the experimental facility, as covariate in all models. All analyses were carried out using the packages *lme4* (Bates *et al.* 2013), *lmerTest* (Kuznetsova *et al.* 2013), and *stats,* in R3.0.2 (R Core Team 2013).

Results

Single individual experiment

Seed-removal rate did not differ between males and females (Linear Mixed Model: p = 0.44; Fig. 5.2a), nor between individuals from areas with or without wild boar (p = 0.56), or with body size (p = 0.69). However, the proportion of seeds cached, as opposed to seeds that were left exposed, was significantly higher for females (mean = 0.98, 95% CI = 0.95-0.99) than for males (mean = 0.93, 95% CI = 0.80-0.98) (Generalized Linear Mixed Model: χ^2_1 = 5.19, p = 0.02267; Fig. 5.2b), but did not differ between individuals from areas with or without wild boar (p = 0.29), and was not affected by body size (p = 0.95).

Individuals from areas with wild boar tended to scatter seeds more widely (mean = 0.12, 95% CI = 0.05-0.27) than individuals from areas without wild boar (mean = 0.07, 95% CI = 0.04-0.11) (Linear Mixed Model: χ^2_1 = 5.08, p = 0.07887), however, this provenance effect was only apparent in females, as indicated by a significant interaction effect between sex and provenance (χ^2_1 = 4.78, p = 0.02881; Fig. 5.2c). Females from areas with wild boar scattered seeds more widely (mean = 0.24, 95% CI = 0.07-0.57) than females from areas without wild boar (mean = 0.05, 95% CI = 0.02-0.10), while the extent of seed scattering was similar for males from areas with wild boar (mean = 0.06, 95% CI = 0.02-0.19) and for males from areas without wild boar (mean = 0.10, 95% CI = 0.05-0.18). The extent of seed scattering was not influenced by body size (p = 0.67).

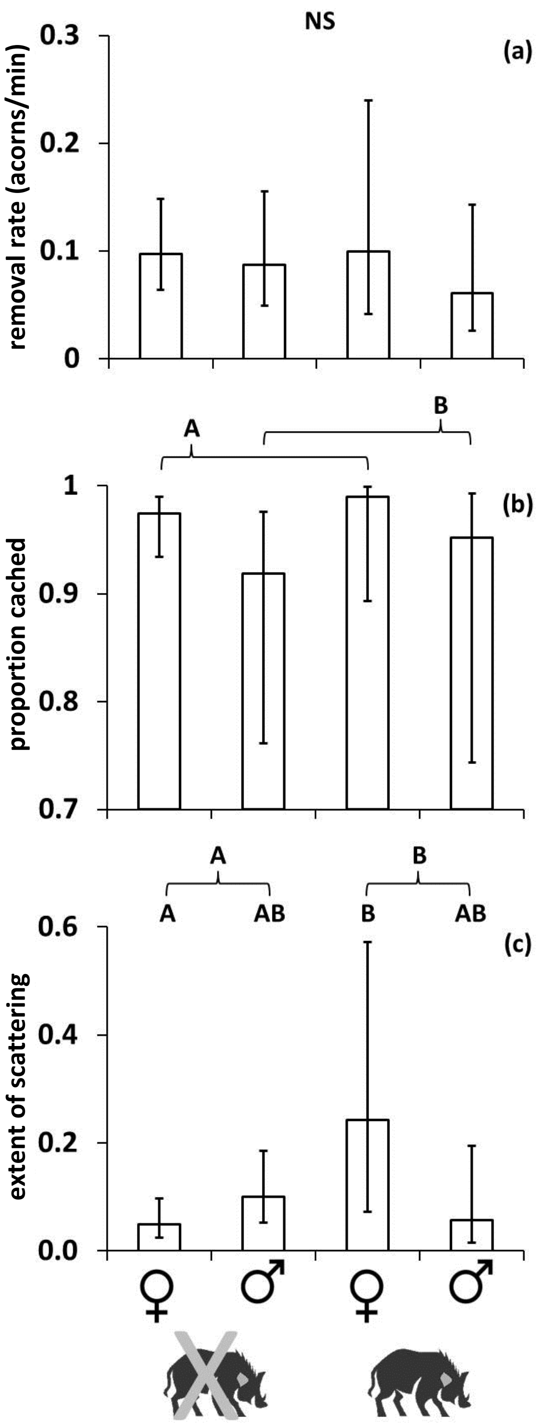

Fig. 5.2: Effects of sex and provenance on (a) the average seed removal rate (acorns/min), (b) the average proportion of seeds cached, and (c) the average extent of seed scattering. Letters indicate significant (interaction) effects. Error bars represent 95% confidence intervals.

Conspecific audience experiment

The conspecific-audience experiment revealed that for the control treatment there were no effects of sex and / or body size on seed-hoarding patterns. This led us to assume that any difference detected in the audience treatment resulted from the addition of a conspecific-audience mouse. On average, the presence of a conspecific audience led to an increase in seed-removal rate for males by a factor 1.8 (95% CI = 0.7-2.8), while it increased seed-removal rate in females by a factor 1.4 (95% CI = 0.4-2.4). Due to large variation, however, these effects were not significant for males (Repeated-measures ANOVA: $F_{1,11} = 0.05$, p = 0.8; Fig. 5.3a), nor for females (p = 0.8). There was no effect of body size on the response to the presence of a conspecific audience, in terms of seed-removal rate (p = 0.6).

In males, the introduction of a conspecific audience led to a 3.7% decrease in the proportion of seeds cached (95% CI = 18% decrease – 11% increase), while in females, it led to a 1.5% increase in the proportion of seeds cached (95% CI = 13% decrease – 16% increase). These changes were, however, not significant ($F_{1,22} = 0.25$, p = 0.6; Fig. 5.3b). The change in proportion of seeds cached after introduction of a conspecific audience was also not affected by body size (p = 0.7).

The extent of seed scattering did not change with the introduction of a conspecific audience in males (mean change = -0.08, 95% CI = -0.21 – 0.05) or in females (mean change = -0.04, 95% CI = -0.18 – 0.09) ($F_{1,22} = 0.19$, p = 0.7; Fig. 5.3c), and was not influenced by body size in general (p = 0.3). However, in females seeds tended to be scattered less in response to the presence of a conspecific audience as the hoarding individual was larger ($F_{1,10} = 3.58$, p = 0.0876).

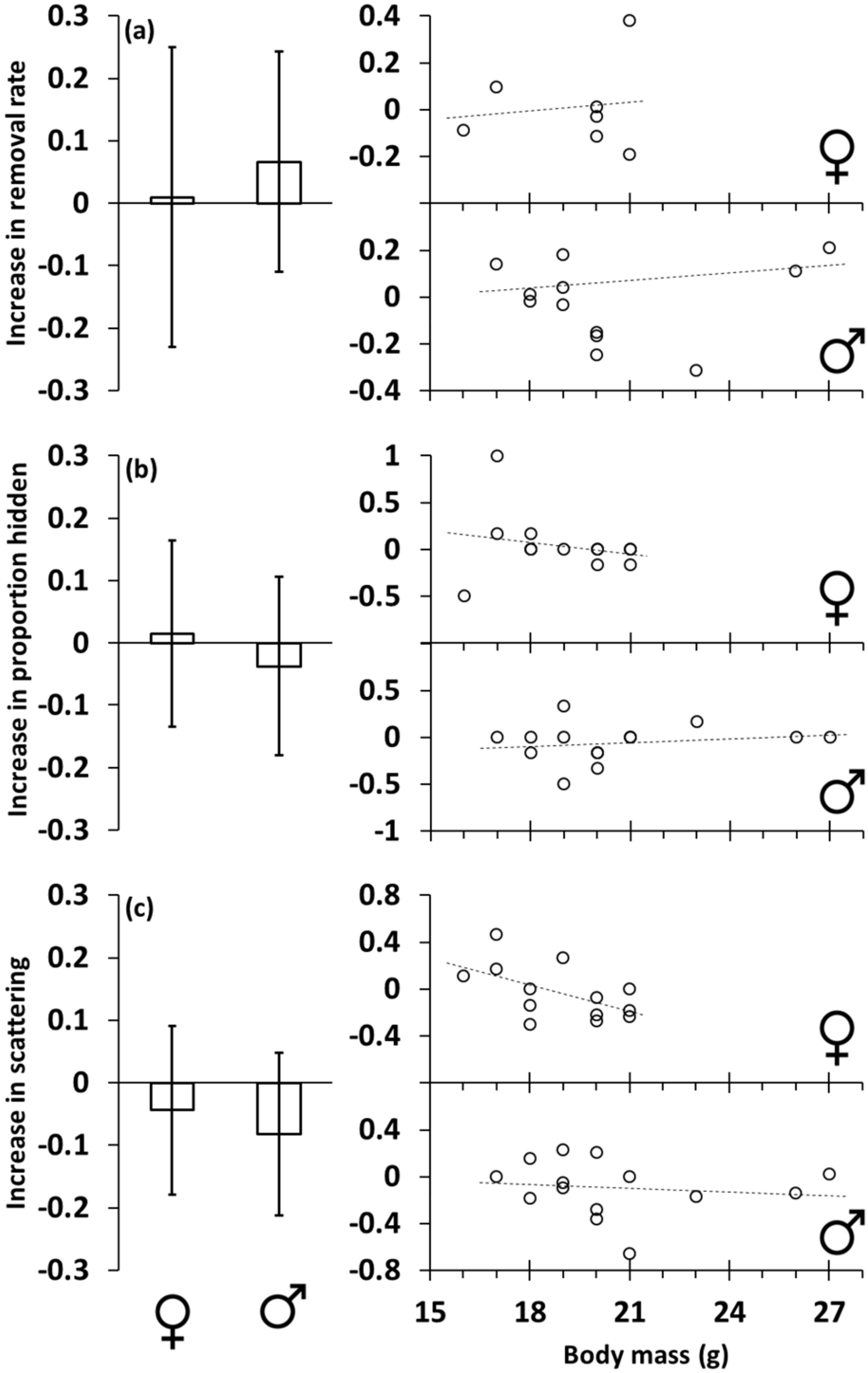

Fig. 5.3: Hoarding responses of wood mice to the introduction of a conspecific audience in an experimental arena. Panels show the change in (a) average seed removal rate (acorns/min), (b) average proportion of seeds cached, and (c) average extent of seed scattering, after the introduction of a conspecific audience. For each panel, scatterplots show the average change for males and females and the relationship between body mass and change for males and females. Error bars represent 95% confidence intervals and dashed lines represent non-significant trend lines.

Discussion

Food-hoarding patterns range from larder- to scatter hoarding between species but sometimes also within species. Little is known, however, about the drivers of within-species variation in hoarding patterns, even though the decision to scatter- or larder hoard may have profound implications the role of seed-hoarding rodents as effective seed dispersers for trees. We experimentally compared hoarding patterns between male and female wood mice of known body sizes, and from areas with a long history of either wild boar presence or absence, to investigate the effect of conspecific and heterospecific pilferage as well as the effect of individual dominance characteristics, such as sex and body size, on hoarding patterns. We found that females hid significantly more seeds (as opposed to leaving them exposed) than did males, and that females from areas with wild boar scattered seeds more widely than did females from areas without wild boar. These findings are in line with the hypothesis that individual dominance characteristics cause within-species variation in hoarding patterns (as suggested by Clarke & Kramer 1994; Dally *et al.* 2006; Jenkins 2011; Zhang *et al.* 2011).

The introduction of a conspecific audience mouse led to a slight but not significant increase in scatter hoarding by small-sized females, while large-sized females actually tended to increase larder hoarding. Although this effect was not significant, the tendency was in line with the second hypothesis that subordinate individuals (i.e., females and / or small-sized individuals) would change their hoarding patterns in response to a conspecific audience by increasing scatter hoarding, while dominant individuals (i.e., males and / or large-sized individuals) were expected to maintain their existing hoarding patterns or to even increase larder hoarding to be able to defend the food supply from competitors. We did not find any other effect of the introduction of a conspecific audience on seed-hoarding patterns. This again, is in line with our hypothesis that dominant individuals may not respond to a conspecific audience by changing their hoarding patterns, but it is in contrast with the prediction that subordinate individuals would change their hoarding patterns in response to a conspecific competitor. Interestingly, several other studies have found mixed results with respect to the response of a hoarder to a conspecific audience (e.g., Jenkins *et al.* 1995; Preston & Jacobs 2005; Dally *et al.* 2006; Leaver *et al.* 2007; Zhang *et al.* 2011; Tong *et al.* 2012). For instance, Preston and Jacobs (2001) found that Merriam's kangaroo rats (*Dipodomys merriami*) did not respond to the presence of a conspecific audience (as in our study), but that they did change their hoarding patterns in response to actual pilferage by conspecifics (which we did not test for here), while in another study, Zhang *et al.* (2011) found that Korean field mice (*Apodemus peninsulae*) and Chinese white-bellied rats (*Niviventer confucianus*) responded to the presence of a conspecific audience by increasing seed-hoarding intensity and seed-removal rate, and that Korean field mice shifted from scatter- to larder hoarding in response to the mere presence of a conspecific audience.

The effects that we found were all related to individual dominance characteristics. For instance, we found differences in hoarding patterns between males and females, with females showing more pilferage-avoidance behaviour than males, which is in line with several other studies (e.g., Macdonald 1976; Vander Wall & Jenkins 2003; Dally *et al.* 2006; Leaver *et al.* 2007; Steele *et al.* 2008). Similarly, only in females did we find an effect of wild boar presence, suggesting that females respond more strongly to interspecific competition than males do.

Lastly, we found that smaller females responded more strongly to the presence of a conspecific audience than larger females, while males, regardless of their size, showed very little tendency to respond to the presence of a conspecific audience. One possible reason for the absence of a strong response to the presence of a conspecific audience may be that we attempted to match the characteristics of the hoarder as much as possible with those of the conspecific audience. That is, if the hoarder was a 15-g female, we made sure that the conspecific audience mouse was also a female of comparable body size. If we had instead chosen to contrast the characteristics of the hoarder with those of the audience mouse, we might have found stronger responses of the hoarder. For instance, a small-sized female may respond more strongly to a large-sized male audience than to a similarly-sized female audience (Zhang *et al.* 2011; Tong *et al.* 2012).

These findings suggest that there is some response of wood mice to conspecific and / or heterospecific competitors, however, the effect was weak. Hence, pilferage risk is unlikely to be the main driver of the widespread within-species variation in hoarding patterns found in natural systems. But what then drives some individuals to scatter hoard and others to larder hoard? Most importantly, scatter hoarding is assumed to be a strategy to lower the risk of catastrophic loss (i.e., instantaneous loss of the entire food supply) (e.g., Smith & Reichman 1984; Vander Wall 1990; Dally *et al.* 2006). While several studies have suggested that complete pilferage by superior competitors (i.e., competitors against which caches cannot be defended) poses the largest threat of catastrophic loss (e.g., Macdonald 1997; Preston & Jacobs 2001; Zhang *et al.* 2011), other factors could also result in catastrophic loss. For instance, many seeds are prone to fungal infections due to high natural moisture contents, so that caching large quantities of seeds together may pose a threat of catastrophic loss due to fungal cross-contamination (e.g., Reichman *et al.* 1985; Edelman 2011). Similarly, physical disturbance of the cache (e.g., windthrow) as well as density-responsive seed predators (Janzen 1970) may pose a threat of catastrophic loss. Finally, although a hoarder may be dominant over its current competitors, at any time a more superior competitor may arrive. The risk of this happening, albeit it very small, will likely have fatal consequences for a larder-hoarding animals, and this in itself may be sufficient reason for most animals to scatter hoard. Thus, although pilferage and dominance may play a substantial role in explaining variation in hoarding patterns, other factors may be equally important. The importance of such other factors as drivers of within-species variation in hoarding patterns begs for further investigation.

Our experiment (in which we offered a fixed number of acorns to one hoarder within a fixed area) was designed to mimic alternative outcomes of the hoard-size – number trade-off that hoarders face. Given a certain food supply (i.e., a fixed number of seeds or other food items) and available area (i.e., the home range), hoarding patterns can be varied by changing the speed at which food items are removed and secured in safe locations, by changing the detectability of the food items to others (e.g., by hiding the food items below ground), and by changing the number of food items per cache (and with that the total number of caches). Our experiment was unique in fully controlling for the number of food items, the size of the hoarding area, and the structural diversity of the ground cover, as well as for pilferer pressure (i.e., by testing mice individually, or by including one audience mouse in the arena). Nevertheless, three aspects of our study differ from the natural situation; 1) The arenas had a surface area that was smaller than the average wood mouse home range, 2) wood mice were alone in the arena and could have learned this during the acclimation period, and 3) the audience mouse and the focal mouse were able to see and smell each other, but the audience mouse was unable to actually pilfer food items from the other individual's caches. It may be that the responses of the tested individual therefore were less extreme then to be expected in a natural situation.

Our results suggest that dominance of the hoarder over its competitors, regardless of the type of competitor (heterospecific or conspecific), explains some variation in hoarding patterns. However, responses found were too weak to explain the widespread variation in hoarding patterns found under natural conditions. We therefore conclude that other factors, such as cross-contamination of food items, may be more important in driving variation in hoarding patterns.

Acknowledgements

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Chapter 6

General discussion

Lennart Suselbeek

Introduction

The main objective of this thesis was to gain insight in the role of seed-hoarding rodents as conditional mutualists of large-seeded trees in a multi-trophic system. I specifically aimed to examine whether and how intra- and interspecific food competition affects the hoarding patterns of rodents such that their role as mutualists of large-seeded trees changes.

In **chapter two**, I introduced PIT tagging as a novel seed-tracking method to follow individual seeds from initial dispersal all the way up to seedling establishment. I showed that PIT tagging provides a reliable, non-invasive and durable seed-marking technique, solving some of the main problems generally encountered when following the fate of animal-dispersed seeds over time. Then, in **chapter three**, I used this technique in a large field study to investigate whether and how acorn abundance, rodent abundance, and wild-boar presence, affected seed-hoarding patterns of rodents, and how this in turn influenced the likelihood of seed germination. Rodents almost exclusively scatter hoarded seeds, but the extent to which this strategy was applied was related to food competition. Successful seed germination was most likely when competition was low. In the following chapters, I experimentally disentangled the factors potentially influencing seed-hoarding patterns of rodents. In **chapter four**, I investigated the likelihood of cache pilferage by wild boar under various hoarding strategies, while in **chapter five**, I compared seed-hoarding patterns displayed by wild-caught rodents of different sex, weight, and provenance (i.e., captured in areas with or without wild boar). Variation in seed-hoarding patterns did not affect cache-pilferage rates by wild boar, but part of the variation in hoarding patterns displayed by rodents could be explained by individual differences in dominance status of the hoarder in relation to potential pilferers.

In this synthesis, I will discuss how these results relate to each other and what new perspectives they bring in relation to conditionality in seed-dispersal mutualisms and in species interactions in general.

The concept of conditionality in mutualistic interactions

Variation in the outcome of interspecific interactions is common in nature. This phenomenon is commonly described as conditionality or context dependency, i.e., the sign or magnitude of the effect on fitness changes as a function of the biotic or abiotic context in which the interaction occurs (Chamberlain *et al.* 2014). Thompson (1988) was among the first to review the evidence for variation in interspecific interactions, and many studies since then have documented conditional outcomes in species interactions (reviewed in Chamberlain *et al.* 2014).

Particularly in the field of mutualisms, the concept of conditionality has been studied extensively (Thompson 1988; Bronstein 1994a). For example, many studies focusing on ant-plant-protection mutualisms have shown that the benefits of this interaction largely depend on the abundance of enemies for the plants. With very few or no enemies in the area, ants benefit from food rewards produced by the plant, but plants do not actually benefit from protection offered by the ants, as there is nothing to be protected from (reviewed in Cushman & Addicott 1991). Similarly, several studies have investigated the role of insects robbing nectar in pollination mutualisms. These so-called 'larcenists' may enter flowers as genuine pollinators do, collecting nectar from the plants, but not always transferring pollen from one flower to the next (reviewed in Irwin *et al.* 2001). In her review on conditional outcomes in mutualistic interactions, Bronstein (1994a) predicted that conditionality was most likely to be found in mutualisms 1) that are facultative rather than obligate, 2) in which a third species is intimately involved, and 3) in which the benefits for both partners are a function of the abundance of these partners.

Interestingly, as of yet, the concept of conditionality has not been extensively studied in seed-dispersal mutualisms. This is perhaps due to the difficulty to non-invasively follow the seed-dispersal process from seed shedding to seedling establishment (Wang & Smith 2002; Forget *et al.* 2005; Vander Wall *et al.* 2005b). In box 6.1, I provide more background information on the typical issues involved with marking seeds and tracking seed dispersal, and I also discuss the technique that I used to overcome most of the typical seed-tracking issues in my study. Studies that did test for conditional outcomes in seed-dispersal mutualisms gathered convincing evidence that conditionality seems to be a widespread phenomenon in seed-dispersal mutualisms (e.g., Brathen *et al.* 2007; Schupp 2007; Schupp *et al.* 2010; Zwolak & Crone 2012; Perea *et al.* 2013). Of all types of seed-dispersal mutualisms, that of seed-hoarding rodents and large-seeded trees has probably received most attention with respect to the phenomenon of conditionality in recent years, most likely due to a theoretical exploration of conditionality in this type of interaction by Theimer (2005). Theimer predicted the interaction between scatter-hoarding rodents and large-seeded trees to be a likely case of conditional mutualism. More specifically, he argued that the outcome of this type of interaction would depend on a) the seed : seed-hoarder ratio, and on b) the recruitment of seedlings from seeds that are not handled by seed hoarders versus the recruitment of seedlings from seeds that are handled by seed hoarders, of which both are likely to vary across space and time (Theimer 2005). Not surprisingly, the theory that was postulated by Theimer (2005), combined with Bronstein's (1994a) review on conditional outcomes in mutualistic interactions, formed a solid theoretical basis for my study.

Rodents as conditional mutualists of large-seeded trees

Many rodent species act as seed dispersers by moving seeds away from the parent plant and conspecifics and storing them in underground caches, a process called food hoarding (Jensen & Nielsen 1986; Price & Jenkins 1986; Vander Wall 1990; Jansen & Forget 2001). Patterns of food hoarding typically vary between species, ranging between scatter hoarding and larder hoarding (Stapanian & Smith 1978; Vander Wall 1990; Clarke & Kramer 1994). Larder hoarding involves the placement of many food items in one or few caches ('larders'), usually located in or near the nest, in underground burrows, or in tree cavities (Vander Wall 1990). Scatter hoarding, in contrast, involves spreading of food over many widely spaced shallow caches with one or few food items each ('scatters') (Morris 1962; Vander Wall 1990, 2001). While larder hoarding generally precludes plant recruitment because large numbers of seeds are stored in deep burrows where germination and seedling establishment is highly unlikely, scatter hoarding may positively affect plant recruitment because individual seeds are buried in numerous spatially scattered, shallow soil or litter caches, and often in sites that are suitable for germination and seedling establishment (Vander Wall 1990).

Box 6.1: The difficulty of studying animal-mediated seed dispersal

One of the main problems hampering seed-dispersal studies is the difficulty to track the fate of seeds from the moment they are being released by the trees until they have reached their final location (Forget *et al.* 2005; Dennis *et al.* 2007). This may also be one of the reasons as to why studies on seed-dispersal mutualisms have been lagging behind as compared to studies on other types of mutualistic interactions (Chamberlain *et al.* 2014).

Although several techniques have been proposed to mark seeds, either collectively or individually, and reliably follow their removal, dispersal, and ultimate fate, no current technique can do this without influencing the seed-dispersal process (Forget & Wenny 2005). For instance, seeds can be marked using external tags, attached to the seeds by a thread or wire, with dispersal and final fate being established by visually retracing the tags in the field, however retrieval of the tags is time-consuming and therefore often limited to a confined area. Also, tags are often cut off by the dispersal agent upon encounter, making it impossible to establish the fate of the seeds. Apart from the difficulty in retrieving all or at least most of the tagged seeds, the actual tagging influences their detectability to potential pilferers, thus affecting the fate of the seeds (Jansen *et al.* 2012). Other existing techniques, such as isotope labelling, radioactive marking, and telemetric tagging may overcome some of the specific issues mentioned above, however, they have their own set of disadvantages to cope with (Forget & Wenny 2005).

In this thesis, I described a novel technique for tracking the fate of individual seeds, namely PIT tagging (**chapter two**). PIT tagging involves the insertion of a 2 x 12 mm Passive Integrated Transponder (PIT) tag into a seed. For seeds that are large enough to have the tag inserted, I showed that this method allows for non-invasive marking of individual seeds all the way up to seedling establishment (Suselbeek *et al.* 2013). With PIT tagging, the characteristics of the seeds as observed by seed-dispersal agents are not altered, and also their detectability to potential pilferers is not influenced. In addition, since PIT tags do not have an internal battery they last indefinitely, making them very suitable for tracking long-term seed-dispersal processes, e.g., in seeds that have a long dormancy period.

This study has shown the potential for using PIT tags to track seed dispersal and seed fate, not only in the field (**chapter three**), but also and perhaps even more so in controlled experiments **(chapter five)**. The only drawback of tracking seed dispersal by means of PIT tagging, is the fact that tags used in this study could only be detected up to distances of 40 cm (Suselbeek *et al.* 2013). The effort involved with this type of tracking increases exponentially with the dispersal distance of the seeds, and although tag detection within the search area is very reliable if searched meticulously, there is a risk of missing tagged seeds that are cached at depths larger than 40 cm or immediately below a very large adult tree. Nevertheless, I believe that PIT tagging is one of the most promising new methods for non-invasively tracking individual seeds. Considering the fact that current commercial development of PIT tags is aimed towards decreasing size and increasing reading distances, the applicability of this technique for tracking seed dispersal may extend further in the near future.

Those species that exclusively larder hoard seeds, can thus be confidently described as antagonists of the seed-producing trees, while species that exclusively scatter hoard seeds are at least likely to be mutualists of the trees that produce the seeds. In reality, however, many species follow a mixed-hoarding strategy and show variation in the extent to which a certain strategy is followed. Based on this, Theimer (2005) argued that the interaction between seed-hoarding rodents and large-seeded trees is likely to be a conditional mutualism, as the effectiveness of seed-hoarding rodents as mutualists of trees seems to be determined by the extent to which they follow the scatter-hoarding strategy. The interaction between rodents and large-seeded trees is also likely to be a conditional mutualism because seed-hoarding rodents play a dual role in tree regeneration; they are both seed predator and seed disperser. Their effectiveness as seed predator thus influences the likelihood of seeds surviving and establishing seedlings.

Thus far, the predictions postulated by Theimer (2005) have been empirically tested in a handful of studies, most notably by Siepielski and Benkman (2008), Jorge and Howe (2009), Klinger and Rejmánek (2010), and Liu *et al.* (2013). Interestingly, all studies testing Theimer's (2005) predictions focused on true scatter-hoarding rodents, rather than on rodents that may show variation in the patterns of hoarding itself, and investigated how specific conditions affected the availability of seeds to a specific hoarder (Siepielski & Benkman 2008), the decision to eat or cache seeds (Jorge & Howe 2009; Klinger & Rejmánek 2010), or the distance at which seeds were dispersed (Liu *et al.* 2013). However, given the general consensus that scatter hoarding potentially benefits plant recruitment while larder hoarding generally precludes plant recruitment (Vander Wall 1990), variation in the actual pattern of hoarding could shift the outcome of the interaction between seed-hoarding rodents and large-seeded trees from mutualism to antagonism or vice versa. For example, the availability of seeds to an individual hoarder may affect its effort invested in hoarding these seeds, but also the intensity of competition with conspecifics and/or heterospecifics may affect the pattern of hoarding. My study was an attempt to disentangle the factors involved with the decision-making process of rodents during seed hoarding.

INTERACTIONS BETWEEN OAKS, RODENTS AND WILD BOAR

In my study, I focused on the interaction between a large-seeded tree, Pedunculate oak, two seed-hoarding rodents, Wood mouse and Bank vole, and a third-species food competitor, Wild boar. Wood mice and bank voles are known to show variation in seed-hoarding patterns between and perhaps even within individuals (Flowerdew *et al.* 1985; Vander Wall 1990; Den Ouden *et al.* 2005). They may function as mutualists of oak when scatter hoarding seeds as winter food supplies – providing seed dispersal and protection against seed predators and environmental stress – but they function as seed predators when larder hoarding seeds in underground burrows, when consuming seeds rather than hoarding them, or when recovering all hoarded seeds for food. I predicted that the pattern of hoarding, the ratio of consumed seeds:hoarded seeds, and the proportion of seeds recovered from caches may all be conditional on a) the abundance of wood mice and bank voles, b) the abundance of seeds, and c) the abundance of wild boar. Before synthesizing the results obtained from the various experiments described in this thesis, I first shortly reflect on the rationale for focusing on oaks, wood mice, and wild boar.

Rationale for focusing on oak as large-seeded tree

The genus *Quercus* is a member of the family Fagaceae (excluding *Nothofagus*), which also includes *Fagus* (beeches) and *Castanea* (the true chestnuts). The genus *Quercus* is one of the most important clades of woody angiosperms in the northern hemisphere in terms of species diversity, ecological dominance, and economic value. Oaks are dominant members of a wide variety of habitats, including temperate deciduous forest, temperate and subtropical evergreen forest, subtropical and tropical savannah, and tropical montane forest (Nixon 2006). Various *Quercus* species are sources of high-quality timber, and it is the preferred firewood in many areas. Because of the dominance of oak in many forests, it is the subject of a vast number of ecological studies (Kappelle 2006), and its evolutionary and ecological significance in forest ecosystems is well acknowledged (e.g., Watt 1919; Jones 1959; Bonner & Vozzo 1987; Sork 1993; Niven & Liddle 1994; Crawley & Long 1995; Nixon 2006; Reif & Gärtner 2007). All species of oak produce (relatively) large seeds (acorns), which are consumed by a very broad range of species. However, some of these species actually also disperse acorns in the process of handling, e.g., when they store acorns as food supplies for periods of food scarcity. It is these types of animals that oaks rely heavily on for the dispersal of their seeds to new sites for establishment (Jones 1959; Crawley & Long 1995). Examples of seed predators that may also function as seed dispersers include several species of corvids (e.g., *Cyanocitta cristata* – Johnson and Webb (1989), *Garrulus glandarius* – Bossema (1979), *Corvus frugilegus* and *Pica pica* – Waite (1985)), and rodents from several genera (e.g., *Sciurus* – Steele *et al.* (1996), *Apodemus* – Jensen and Nielsen (1986), and *Peromyscus* – Vander Wall (1990)). In North-western Europe, my study area, two main species of oaks occur, namely Pedunculate oak (*Quercus robur*) and Sessile oak (*Quercus petraea*). In terms of biology, both species are very comparable and they are even known to hybridize in some areas. In my specific study area, however, pedunculate oak is the dominant species and therefore I selected this species as my study species (Fig. 6.1a).

Rationale for focusing on wood mouse and bank vole as seed-hoarding rodents

In my study area, acorns are primarily dispersed by Eurasian jay (e.g., Bossema 1979; Den Ouden *et al.* 2005), Eurasian red squirrel (e.g., Wauters *et al.* 1995), Wood mouse (e.g., Jensen & Nielsen 1986; Den Ouden *et al.* 2005), and Bank vole (e.g., Jensen & Nielsen 1986). All above species disperse acorns in the process of creating food stores (hoards) for periods of scarcity. While Eurasian jay is more important for long-distance dispersal of seeds to sites outside forest stands, small rodents are more involved with seed dispersal on the small scale, often within forest stands (Steele & Smallwood 2002). From a methodological perspective, following seed dispersal by birds is more difficult than following seed dispersal by ground-dwelling rodents, for one because average seed-dispersal distances are generally much longer for bird-dispersed seeds. In addition, both Eurasian jay and Eurasian red squirrel have been shown to exclusively scatter hoard seeds (Stapanian & Smith 1978; Wauters *et al.* 1992), while wood mouse and bank vole are known to show variation in hoarding patterns between individuals and perhaps even within individuals (e.g., Jennings 1975; Jensen & Nielsen 1986; Vander Wall 1990; Clarke & Kramer 1994; Jenkins & Breck 1998; Steele & Smallwood 2002; Den Ouden *et al.* 2005; Lu & Zhang 2005, 2008). This, combined with the fact that the natural distribution of wood mouse and bank vole overlaps largely with that of pedunculate oak (Fig. 6.1a-c), and that they are easily captured (using life traps) and individually marked, made them very suitable candidates for inclusion in this study.

Rationale for focusing on wild boar as a third-species food competitor

The Wild boar is one of the most widespread ungulates of the world; its natural range extends over most of Europe and Asia (Fig. 6.1d), and is still expanding rapidly (Massei & Genov 2004). Female wild boar generally live in family groups of 6 to 30 individuals, while males are mostly solitary (Poteaux *et al.* 2009). Their natural diet consists for 80-90% of plants but they also opportunistically feed on live and dead animal matter. Acorns are a staple food in autumn when they have freshly shed and are abundantly available on the forest floor (Massei & Genov 2004; Elston & Hewitt 2010), but also in mid- and late winter when very few acorns remain on the forest floor (Groot-Bruinderink *et al.* 1994; Den Ouden *et al.* 2005; Muñoz & Bonal 2007). The latter suggests that wild boar actively searches for and pilfers acorn caches created by other species (Borchert *et al.* 1989; Herrera 1995; Focardi *et al.* 2000; Gomez *et al.* 2003; Den Ouden *et al.* 2005). In this process, wild boar is thought to rely on its keen sense of smell for locating the hidden food items (e.g., Briedermann 1986). Wild boar may affect the interaction between oaks and seed-hoarding rodents in several ways; 1) wild boar may directly compete with small rodents for freshly shed acorns, thereby reducing the abundance of acorns (e.g., Groot-Bruinderink & Hazebroek 1996; Muñoz *et al.* 2009), 2) wild boar may pilfer rodent-made caches, thereby a) altering the spatial pattern of hoarding, and b) imposing a large risk of losing the entire food supply on rodents (e.g., Focardi *et al.* 2000; Muñoz *et al.* 2009), 3) wild boar may actively predate on rodents, thereby potentially altering space use and abundance of rodents (e.g., Focardi *et al.* 2000; Muñoz & Bonal 2007; Muñoz *et al.* 2009), 4) wild boar may directly change microhabitat conditions, thereby reducing the availability of a) suitable hoarding sites for acorns and b) suitable hiding places for rodents (e.g., Muñoz & Bonal 2007; Muñoz *et al.* 2009), and 5) wild boar may promote directed dispersal of acorns to places that are not easily uprooted (e.g., below large objects or at the base of large trees) (e.g., Muñoz *et al.* 2009). These aspects, combined with the fact that small rodents are unable to defend their stores against wild boar, made wild boar a very interesting species to include in my study as a third-species competitor.

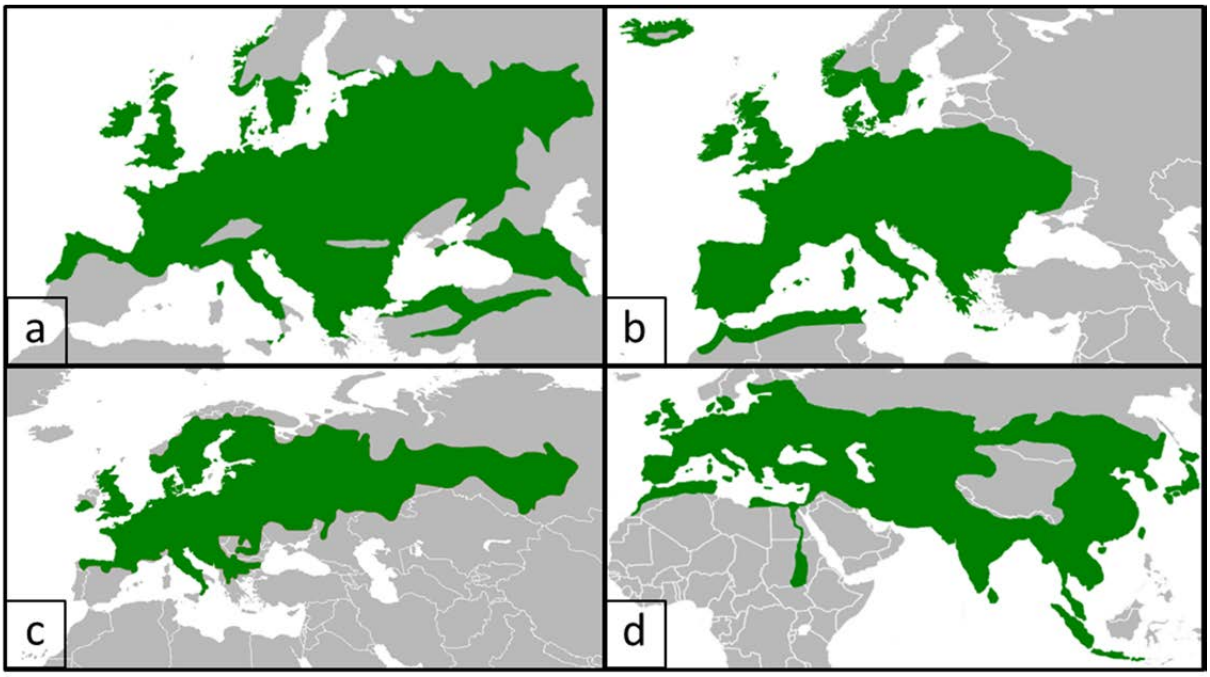

Figure 6.1: Species distribution maps for (a) pedunculate oak, (b) wood mouse, (c) bank vole, and (d) wild boar.

Seed hoarding by rodents: the benefits for pedunculate oak

While for rodents the benefits involved with seed hoarding are easily identified as providing a source of food – if carefully stored – can help them overcome periods of food scarcity, the benefits for oaks of having seeds hoarded by rodents are less easily identified. To conclude anything about the added value of seed hoarding for oaks, one needs to study the biology of the species, and particularly the biology of acorns.

Pedunculate oak exhibits alternate bearing, which means that seed production is rich in some years and poor in others according to a certain periodic pattern (Crawley & Long 1995). It is thought that periodic synchronous seed production is an adaptation which allows satiation of seed-feeding animals, and hence increases the probability of seedling recruitment following years of peak seed production (Janzen 1971; Silvertown 1980; Crawley & Long 1995). In rich years, seed production averages around 50,000 seeds per adult tree – with peaks of up to 90,000 seeds per tree – while in poor years seed production can be close to zero (Jones 1959).

Generally, pedunculate oak acorns weigh between 2 and 15 grams, but this varies dramatically between acorns of one tree, between trees, and between years. Acorns start to ripen in September and are shed between half October and early December. After shedding, they readily germinate, forming a long taproot. Seedling emergence, however, generally occurs no sooner than May-June of the year following shedding. Basically, acorns face four major threats precluding germination and / or establishment as seedlings, namely; death by drying, death by freezing, death by rotting, and death by predation (Jones 1959). All threats are linked to specific aspects of the biology of acorns. With respect to death by drying, fresh acorns have to maintain a relatively high moisture content of about 40-50%. A loss of 5% moisture can be tolerated, but further drying will lower acorn quality. Viability will be completely lost when moisture content drops to about 25 percent (Jones 1959; Bonner & Vozzo 1987). Due to their high moisture content, acorns are also sensitive to frost. Temperatures below -10 °C are lethal for most acorns (Jones 1959). With respect to rotting, acorns are most prone to infection by the fungus *Ciboria batschiana*, which causes “black rot” in acorns (Jones 1959; Delatour *et al.* 1977; Delatour & Morelet 1979; Bonner & Vozzo 1987). Infection with this fungus can take place while the acorn is still on the tree, or after it has shed. Black rot very easily spreads from one seed to the other if they are close to each other, e.g., directly below the parent tree (Bonner & Vozzo 1987). Finally, predation is one of the most obvious causes of death in acorns. Just in the United States, already over 150 species of animals have been recognized as predators of *Quercus* acorns (Van Dersal 1940). Seed predation takes place while the acorns are still attached to the tree, but also after they are shed. Most seed predators, however, occur in the direct vicinity of seed-producing oaks.

Thus, for seed hoarding to be beneficial to oaks, it is suggested that the acorns are handled by the hoarder in such a way that 1) the moisture content of acorns is maintained, 2) the acorns are protected from adverse weather conditions, such as frost, 3) the acorns are transported away from the parent plant and away from other seeds a) to minimize the risk of fungal infections, such as with black rot, and b) to minimize the risk of being found and eaten by seed predators.

To see which one of the above aspects was most important for successful germination of acorns, I set up an experiment where I placed 1664 pedunculate oak acorns in the field in autumn and monitored their fate over time. Seeds were either placed directly below adult oaks or 50 m away from the nearest adult oak, they were either placed on the surface or buried 5-8 cm below the soil surface (i.e., at typical scatter-hoarding depth, Jennings 1975; Mallorie & Flowerdew 1994; Den Ouden *et al.* 2005), and they were either placed as a clump of 16 acorns or as individual acorns that were spaced at least one meter apart from each other. In all cases, however, metal wire cages were used to protect the seeds from vertebrate predation or dispersal. The results from this experiment are not included in this thesis, but I will shortly describe the main results here.

Just over 40% of all acorns germinated, while 32% established a seedling. The odds of successful germination were by far the highest for seeds that were buried (Fig. 6.2a). Of the seeds that were buried, the highest probability of germination was observed in seeds that were placed individually, as compared to clumped seeds. The probability of germination seemed to be little affected by movement away from the parent plant. Of the acorns that had germinated, the highest probability for establishing a seedling was again observed for seeds that were buried, but also for seeds that were placed away from the parent plant (Fig. 6.2b). It thus seems that burial is thus most important in all stages of development, as it protects seeds from adverse weather conditions, such as large temperature fluctuations (drying and freezing) and excessive rainfall (rotting), but it also protects seeds from predation by reducing visual and olfactory cues to seed predators. Interestingly, movement away from the parent plant seemed to be most important once the seedling had emerged aboveground. This is likely due to seed- and seedling predators that are attracted to adult oaks and then visually orient towards seedlings.

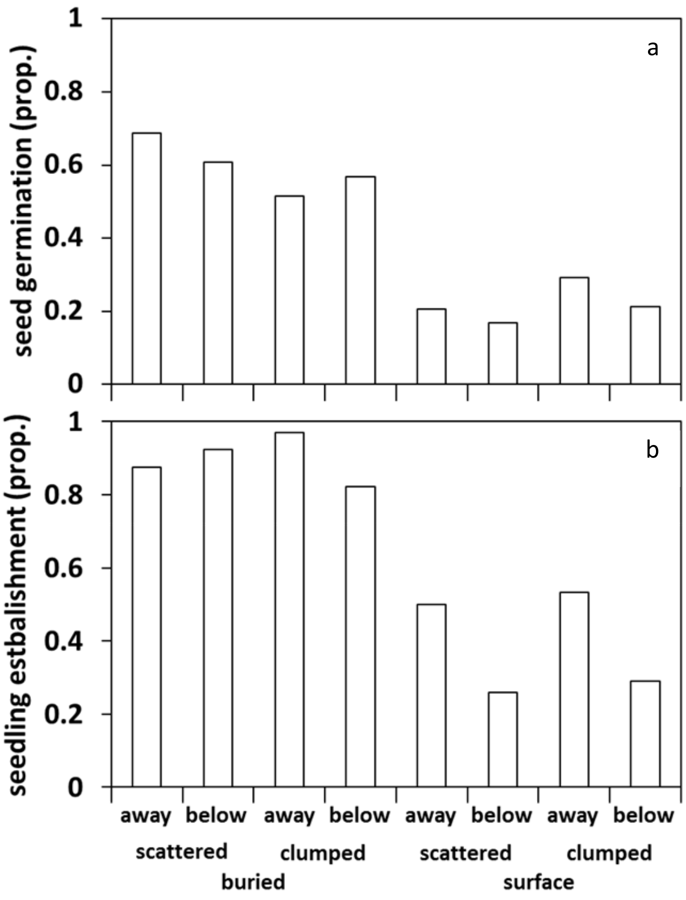

Figure 6.2: (a) Proportion of seeds germinating and (b) proportion of germinated seeds establishing seedlings, under different experimental conditions but always protected from vertebrates.

Variation in seed-hoarding patterns by rodents in response to competition

In **chapter three** and **chapter five** I examined whether and how seed-hoarding patterns varied depending on the circumstances under which hoarding took place. In **chapter three**, I did this in a large field experiment, while in **chapter five** I set up a controlled experiment with wild-caught wood mice. Specifically, I tested for variation in seed-removal rate, seed-dispersal distance, and seed spacing as these can be varied by rodents in the process of hoarding and these factors are most relevant from the perspective of dispersal for acorns. For instance, a given rodent can decide to speed up the seed-removal process to more quickly place all available seeds out of view of its competitors, or it may store seeds at larger distances from the source in order to reduce the likelihood of detection by competitors. For seeds, the likelihood of escaping predation is suggested to be highest away from the parent plant (i.e., dispersal distance) and away from other seeds (i.e., seed spacing) (Janzen 1971). The more quickly this movement takes place (i.e., seed-removal rate), the higher the odds for seeds to escape predation.

I hypothesized seed-removal rate, seed-dispersal distance, and seed spacing to be positively related to the intensity of competition, both with conspecifics and with heterospecifics. More specifically, I predicted seed-removal rate, seed-dispersal distance, and seed spacing to decrease with a) the abundance of acorns and to increase with b) the abundance of rodents, and c) the presence of wild boar. Results from the field study are summarized in table 6.1, but a more detailed elaboration on each aspect of seed hoarding is given below.

Table 6.1: Summary of the main and interactive effects of wild-boar presence, acorn abundance, and rodent abundance on seed-removal rate, seed-dispersal distance, seed spacing, and seed fate. In case of significant interaction effects, the p-value for the interaction is given and the direction and strength of the effect is specified for each combination of the interactive variables.

	Seed-removal rate		**Seed-dispersal distance**	**Seed spacing**	**Seed fate**	
wild-boar presence (WBP)	low AA: high AA:	- ***NS***	***NS***	***NS***	low AA: high AA:	- ***NS***
acorn abundance (AA)	WB absent: WB present:	- - - -	+ + +	***NS***	WB absent: WB present:	***NS*** +
rodent abundance (RA)	low AA: high AA:	+ + + + + +	***NS***	+ + +	low AA – WB absent: low AA – WB present: high AA – WB absent: high AA – WB present:	***NS*** + ***NS*** ***NS***
WBP x AA	**p < 0.01**		***NS***	***NS***	**p < 0.01**	
WBP x RA	***NS***		***NS***	***NS***	low AA: high AA:	**p < 0.05** ***NS***
AA x RA	**p < 0.05**		***NS***	***NS***	WB absent: WB present:	***NS*** ***NS***
WBP x AA x RA	***NS***		***NS***	***NS***	**p < 0.05**	

- / + for p < 0.05, - - / + + for p < 0.01, - - - / + + + for p < 0.001, NS for p > 0.05

Seed-removal rate – With respect to seed-removal rate, I found substantial variation in the field (**chapter three**) and in the controlled experiment (**chapter five)**. The factor best explaining variation in seed-removal rate was the abundance of rodents (i.e., wood mice and bank voles). Generally, if rodent abundance – and thus competition – increased in the field, seed-removal rate also strongly increased. Seed-removal rate was also significantly higher if a conspecific audience was present in the controlled experiment. These findings are in line with those reported in other studies (e.g., Bowers & Dooley 1993; Wang *et al.* 1999; Jansen *et al.* 2004; Jansen & Den Ouden 2005; Vander Wall *et al.* 2005b; Perez-Ramos *et al.* 2008; Perea *et al.* 2011; Zhang *et al.* 2011; Tong *et al.* 2012). Again in accordance with my predictions, seed-removal rate was negatively related to acorn abundance. In other words, if acorn abundance was low – and thus competition was high – seed-removal rate increased, suggesting that rodents invested more in quickly removing seeds from the source to secure sufficient food resources for winter (Jansen *et al.* 2004; Jansen & Den Ouden 2005; Vander Wall *et al.* 2005b; Perez-Ramos *et al.* 2008; Hirsch *et al.* 2012a). This effect of acorn abundance on seed-removal rate, however, seemed to be overruled by the effect of rodent abundance, such that under high rodent abundance seed-removal rate was always high. I found no support for the prediction that seed-removal rate would be higher under wild-boar presence. Instead, in the field, I found seed-removal rate to be lower if wild boar were present than if wild boar were absent (**chapter three**), while in the controlled experiment, seed-removal rates were similar for wood mice from areas with and without wild boar (**chapter five**). These findings may be explained by the fact that wild boar also predate directly on rodents (e.g., Briedermann 1986; Groot-Bruinderink &

Hazebroek 1996; Focardi *et al.* 2000; Baubet *et al.* 2004; Gimenez-Anaya *et al.* 2008; Gomez & Hodar 2008; Bueno *et al.* 2009; Elston & Hewitt 2010), who thus may be moving more cautiously if wild boar are present and therefore take more time to encounter and remove seeds (Bowers & Dooley 1993; Jansen & Den Ouden 2005). Finally, although seed-removal rate was strongly affected by rodent abundance, the change in seed-removal rate was not affected by individual characteristics of the hoarder, such as sex or weight of the individual. It thus seems that intra-specific competition always leads to higher seed-removal rates, even though the individual removing the seeds may be dominant over its direct conspecific competitor(s). This result is in line with results reported in other recent studies (e.g., Zhang *et al.* 2011), and may be explained by the assumption that seeds are available to anyone so long as the seeds lie on the forest floor below the parent tree, and can be protected by means of active defence only if competitors can be excluded entirely from the area. It is unlikely that any individual would be able to exclude all competitors from a certain area, and thus seed-removal rate should be increased by all individuals in response to increased competition.

Seed-dispersal distance – I found that wood mice and bank voles dispersed seeds at a median distance of 20 m away from the source, which corresponds with median distances of seed dispersal by rodents reported in several other studies (e.g., Sork 1984; Jensen & Nielsen 1986; Iida 1996). However, as with seed-removal rate, seed-dispersal distance was quite variable depending on the context. This substantial variation in seed-dispersal distance, however, lead to wide confidence intervals and thus few significant effects. Nevertheless, seed-dispersal distance was shown to strongly increase with acorn abundance. Wild-boar presence and rodent abundance did not affect seed-dispersal distance, although wild-boar presence tended to lead to an increase in seed-dispersal distance (**chapter three**). This result is at least partly in line with optimal cache spacing theory, which predicts that food hoarders must space out their caches to a degree that optimally balances the benefits of reduced risk of cache pilferage and the costs of carrying food items to caches (Stapanian & Smith 1978, 1984; Clarkson *et al.* 1986; Dally *et al.* 2006; Gálvez *et al.* 2009). Namely, the finding that seed hoarders responded to increased abundance of acorns by increasing seed-dispersal distance, suggests that seed hoarders attempted to maintain a certain density of seeds and were thus forced to transport seeds over larger distances if the abundance of seeds was higher. Interestingly, rodent abundance had no effect at all on seed-dispersal distance, suggesting that the decision to transport seeds further away from the source is not affected by competition with conspecifics, perhaps because the reduction in pilferage risk does not weigh up to the increased cost of transportation. Biologically, it makes sense that there is an optimal or even a maximum for the median seed-dispersal distance, as rodents are restricted in their movements by a) territory size, and/or b) physical endurance, and that they may instead decide to space seeds further apart within the same dispersal area (e.g., Kikkawa 1964; Flowerdew *et al.* 1985; Korn 1986; Benhamou 1990; Telleria *et al.* 1991; Akbar & Gorman 1996; Corp *et al.* 1997; Vukicevic-Radic *et al.* 2006; Buesching *et al.* 2008).

Seed spacing – The extent to which animals scatter hoard seeds can be quantified by measuring seed spacing. The more seeds are spaced out, the more scattered they are. I found that the median seed spacing by rodents in the field study was 4.2 m (**chapter three**). Interestingly, however, rodents almost exclusively scatter hoarded seeds and only very incidentally created a larder hoard. For instance, in the field study, I only encountered two true larder hoards. One consisted of 7 acorns cached by a 17-g male wood mouse in a site without wild boar and under low acorn abundance, while the other larder consisted of 13 acorns and was created by an unknown bank vole (i.e., the individual had not been caught during the live-trapping session prior to presenting the tagged acorns) in a site with wild boar and under high acorn abundance. In other words, little could be concluded from these events with regards to the most likely conditions for larder hoarding to take place. However, even though scatter hoarding was the dominant pattern of hoarding displayed by rodents in my study, the extent to which this strategy was applied (as quantified by the extent of seed spacing) varied substantially between individuals and within individuals. Most importantly, seed spacing was strongly positively related to rodent abundance. This is again in line with my predictions and with optimal cache spacing theory, which suggests that increased risk of pilferage imposed on hoarders by the increased abundance of conspecifics may be countered by increasing the spacing between seeds. Wild-boar presence had no significant effect on seed spacing, which suggests that spacing seeds further apart does not lower pilferage risk by wild boar, or that it at least does not weigh up to the increased costs of transportation. Taking into account the mobility of wild boar, seed spacing may need to be increased too much to substantially lower the risk of pilferage. Finally, acorn abundance also did not affect seed spacing. This may be because acorn abundance was already shown to result in increased seed-dispersal distance. By doing so, seed hoarders are capable of maintaining a certain seed density, while spacing seeds out further within the same area does not lower seed density but does involve more costs of transportation. Finally, while results from the field study (**chapter three**) already showed a strong relation between seed spacing and rodent abundance, the results from the controlled experiment (**chapter five**) showed more detail about the effects of individual characteristics of the hoarder in relation to its competitors. For instance, females from areas with wild boar scattered seeds to a larger extent than females from areas without wild boar, and seed spacing tended to be negatively related with body size in females. These findings are interesting, because they suggest that dominance of the hoarder over its competitors may influence its hoarding patterns, such that subordinate individuals (i.e., females and / or small individuals) are more inclined to scatter hoard than dominant individuals (Clarke & Kramer 1994; Dally *et al.* 2006; Tong *et al.* 2012).

Linking seed-hoarding patterns to germination and seedling establishment

Not only did I find that seed-hoarding patterns varied substantially depending on acorn abundance, rodent abundance, and wild-boar presence, but also that germination rates of differentially hoarded seeds varied widely. Specifically, results from the field study showed that the proportion of seeds germinating within a certain site and year varied from 0% to 35% (**chapter three)**. Successful seed germination was most likely when acorn abundance was high and rodent abundance was relatively high, regardless of wild-boar presence. In this situation, seeds were removed rapidly, they were dispersed over large distances, and were spaced out widely. However, seed germination was also relatively high if acorn- and rodent abundance were low, and wild boar were absent. This suggests that if there are very few seed predators around, seed germination may also be relatively high. An increase in rodent abundance or wild-boar presence, however, rapidly reduced seed germination. Generally, however, so long as sufficient seeds were abundantly available, seed germination increased with rodent abundance, suggesting that rodents indeed play an important role as seed disperser for oaks.

How do wild boar search for hoarded acorns?

The results described in **chapter three** and **chapter five** give rise to the impression that wild-boar presence had little effect on seed-hoarding behaviour of rodents and on seed fate of acorns cached by rodents. That is, seed-removal rate was significantly lower if wild boar were present, however, only if acorn abundance was low, while seed-dispersal distance and seed spacing were not affected by wild-boar presence. It thus may be that the hoarding patterns displayed by rodents in this study reduce pilferage risk by wild boar even though the decision to apply this hoarding strategy is not affected by the presence of wild boar. However, it may also be that wild boar simply have difficulty locating cached acorns in general, regardless of how these are hoarded. To be able to draw conclusions on the role of wild boar as seed predator and competitor of rodents, I thus performed another controlled experiment. This experiment, described in **chapter four**, was set up to investigate how wild boar actually search for hidden acorns, and whether the rate of pilferage by wild boar could actually be influenced by changing seed-hoarding patterns. Specifically, I tested how changes in a) the number of seeds per cache, b) the total number of caches, and c) the depth of hoarding, affected the rate of pilferage by wild boar. I assumed that wild boar would use their keen sense of smell during their search for hidden acorns (reviewed in Briedermann 1986). Given that large and shallow caches emanate stronger olfactory cues than small and deep caches (reviewed in Vander Wall 2003), I predicted that wild boar would have much difficulty detecting scatter-hoarded seeds (i.e., small caches) and seeds that were cached at large depth (i.e., deep caches), while I predicted them to quickly detect larder hoarded seeds (i.e., large caches), particularly if these were cached just below the soil surface (i.e., shallow caches).

The time wild boar took to detect and pilfer the first cache was shortest for scatter hoarding, but the time they took to pilfer all caches was slightly longer for scatter hoarding than for larder hoarding. Overall, however, the rate of pilferage did not differ between scatter hoarding and larder hoarding, and also was not affected by cache depth. In light of my predictions, which were based on wild boar using olfactory cues for detecting hidden acorns, these findings were truly unexpected. However, if one would rethink the assumption that wild boar would use olfactory cues emanating from the caches to locate them, there may be good reasons to suggest that wild boar are either unable to pick up olfactory cues from hidden acorns, or that they do not use such cues for cache finding. For instance, it is well-acknowledged that searching behaviour in general is costly, but particularly searching on the basis of olfaction is demanding (e.g., Gazit & Terkel 2003). It may thus well be that searching by means of olfaction incurs a success rate in terms of food detected that is lower than the costs involved with this type of searching.

Alternatively, it may be that wild boar were unable to pick up the olfactory cues emanating from hidden acorns, in contrast to their proven ability to locate hidden truffles which largely forms the basis of the literature's acclaim of the wild boar's sense of smell (Briedermann 1986). An explanation for this would be that black truffles (*Tuber melanosporum*) and all other hypogeous fungi have evolved to produce their fruiting bodies belowground and have been selected to be found and dug up by animals to have their spores dispersed (e.g., Bellina-Agostinone *et al.* 1987; Bruns *et al.* 1989; Talou *et al.* 1990; Johnson 1996; Pyare & Longland 2001). In contrast, acorns are the fruiting bodies of oaks that require transportation to a location for germination and establishment but that do not require to be found by seed predators, and so it is unlikely that there has been a selective advantage for oaks to produce acorns that can be found and destroyed by wild boar or other seed predators. To the contrary, selection pressure may have favoured acorns that are visually conspicuous but that do not emanate olfactory cues.

Now, assuming that olfaction indeed is not the mechanism by which hidden acorns are being detected and pilfered by wild boar, what other mechanism may be used? Given the fact that the first caches were quickly encountered and pilfered under scatter hoarding, but that the time until pilferage of the first cache under larder hoarding was much more variable, I propose that wild boar were using random or systematic search for cache finding. Under scatter hoarding, 32% of all potential cache locations actually contained a cache, while under larder hoarding only 2% of all potential cache locations actually contained a cache. Thus, if one would randomly or systematically visit potential cache locations the odds would be very high that detection of the first cache would be faster under scatter hoarding than under larder hoarding. However, it is likely that every now and then, one of the first patches visited under larder hoarding actually contained a cache while in other cases it may be that the last few patches visited actually contained all caches, resulting in a large variation in the time it took until pilferage of the first cache. Still assuming that wild boar used random or systematic search, one would predict that it simply takes a certain amount of time to visit all patches, and that there thus may be little difference in the overall rate of pilferage between scatter and larder hoarding, even though one would again predict the variation in pilferage rate to be higher under larder hoarding, due to chance effects of quickly finding both caches. Finally, we found no differences in pilferage rates of shallow and deep caches, even though olfactory cues emanating from deep caches are weaker than those emanating from shallow caches (Vander Wall 2003).

All arguments raised above point in the direction that wild boar, at least in my experiment, relied on random or systematic search to locate hidden acorns. From the perspective of the rodents hoarding acorns, this implies that risk of pilferage by wild boar may be relatively low, so long as the seeds are being removed from the surface (to avoid visual detection by wild boar), and taken away from places where acorns are generally likely to be found (i.e., below or directly around adult oaks). In other words, to avoid wild-boar pilferage it may be most important for rodents to move seeds away from the source and to bury them, but it may be less important to scatter the seeds. Interestingly, this corresponds well with the responses that rodents showed in terms of seed-dispersal distance – i.e., seeds were always dispersed away from the parent tree – and seed spacing – i.e., seed spacing was not affected by wild-boar presence (**chapter three** and **chapter five**).

IN CONCLUSION

This study on conditional outcomes in seed-dispersal mutualisms has been the first to focus on rodents that are known to show within-species variation in hoarding patterns and that were likely to show within-individual variation in hoarding patterns. It was also the first to examine both individual and combined effects of hoarder abundance, seed abundance, and presence of a third-species food competitor – wild boar – on seed-hoarding patterns (i.e. on seed-removal rate, seed-dispersal distance, and seed spacing), and how this in turn affected the outcome of the mutualism between seed-hoarding rodents and large-seeded trees.

The results from the large field study, combined with findings obtained in two complementary controlled experiments, convincingly show that there is a great deal of variation in hoarding patterns both within and between individual rodents. This variation could at least be partly explained by the intensity of competition, as predicted prior to the start of my study. I had expected, however, to find more extreme shifts along the continuum from perfect larder hoarding to perfect scatter hoarding. In reality, I found rodents to primarily scatter hoard rather than larder hoard acorns, albeit to a varying extent depending on the context. Secondly, I had assumed that wild boar would be playing an important role in shaping the mutualism between rodents and oaks, by using their excellent sense of smell to actively search for and pilfer the caches that had been so carefully created by rodents. But again, in reality wild boar seemed to have much difficulty in locating hidden acorns, and they did not seem to use olfaction to locate cached acorns. Most likely, however, free-ranging wild boar use a combination of tactics, including visual orientation, memory, and olfaction, to find hidden food items. On the large scale, they may be using memory (e.g., to recall that distant oak stand several kilometres away), while on a small scale, they may be using visual orientation (e.g., to locate acorns lying on the forest floor below adult oaks), while on a tiny scale, they might use their sensitive nose for prodding in the soil and for sniffing out edible food items in the top soil layer. If this were indeed true, the most important aspects of successful hoarding behaviour (in terms of avoiding pilferage) would consist of at least removing the seeds from the soil surface – to prevent visual detection by pilferers – and moving them away from adult oaks – to reduce the chance of olfactory or tactile detection by pilferers. However, this would not necessarily imply that seeds would need to be scatter hoarded rather than larder hoarded.

If indeed the risk of pilferage by superior competitors (i.e., competitors against which caches cannot be defended) is not the most important driver of scatter hoarding, then why would animals like the rodents in my study primarily scatter hoard their food supplies? I believe that there are several alternative factors – other than risk of pilferage by superior competitors – that may be involved with the decision of animals to either scatter- or larder hoard seeds. Most importantly, scatter hoarding is a strategy to prevent catastrophic loss of food supplies (i.e., instantaneously losing the entire food supply) (e.g., Smith & Reichman 1984; Vander Wall 1990; Daly *et al.* 1992; Dally *et al.* 2006). While several studies have suggested that complete pilferage by superior competitors poses the largest threat of catastrophic loss (e.g., Macdonald 1997; Preston & Jacobs 2001; Zhang *et al.* 2011), there may be several other factors resulting in catastrophic loss of food supplies. For instance, many large seeds are prone to fungal infections or other types of diseases. Caching large quantities of such seeds together may pose a large threat of catastrophic loss due to cross-contamination of seeds (e.g., Reichman *et al.* 1985; Edelman 2011). Similarly, natural disturbances such as windthrow or flooding may instantly destroy a cache and its contents. Lastly, although the absolute risk of catastrophic loss due to whichever factor may be very small, the consequences for the hoarder are disastrous and in most cases lead to death of the hoarder by starvation. Thus, only those species that a) are able to defend their caches against competitors, that b) cache food items that are not sensitive to cross-contamination of diseases, and that c) store seeds in places where the risk of catastrophic loss is negligible, or species for which the consequences of instantaneously losing the entire food supply are not detrimental, may be expected to larder hoard.

Reflecting on the theories on conditional outcomes in mutualistic interactions in general (Bronstein 1994a), and on seed-hoarding rodents as conditional mutualists of large-seeded trees in specific (Theimer 2005), I believe that this study clearly confirms the existence and importance of conditionality in seed-dispersal interactions. It also confirms the predictions postulated by Theimer (2005), in that successful seed germination tended to increase with rodent abundance (at least up to a certain abundance or rodents) but not if acorn abundance was low. In that case, it is likely that the large majority of seeds were consumed by the rodents, either before or after they had been cached as winter food supply. There thus seems to be an optimum in the seed : seed-hoarder ratio, resulting in maximum seed germination. Also, the results from the unpublished seed-germination experiment, described above (Fig. 6.3), suggest that seed handling by rodents significantly improves the likelihood of successful germination of acorns. It is interesting to note that burial and movement away from the parent tree already significantly increase seed germination, even though the seeds were protected from all vertebrate pilferers. Given the wide array of vertebrate species predating on acorns, the differences in seed germination would likely have been even larger if the caches had not been protected. Nevertheless, the results from this experiment suggest that the likelihood of seed germination without handling by seed-hoarding rodents would be close to zero. Any handling of acorns by seed-hoarding rodents is thus likely to increase their likelihood of germination, at least so long as the abundance of acorns satiates the demand for food of the rodents (Crawley & Long 1995; Theimer 2005). With respect to Theimer's (2005) conceptual relationship between the potential recruitment of seedlings from seeds that are not handled by seed-hoarding rodents versus recruitment when seeds are handled by seed-hoarding rodents (Fig. 6.3), I thus predict that shifts in the probability of cache survival in response to different conditions may show a similar pattern as displayed in figure 6.3b, but that the curve representing potential recruitment of acorns in the absence of seed-hoarding rodents (Fig. 6.3b, curve D) would in most cases be lower than the other curves.

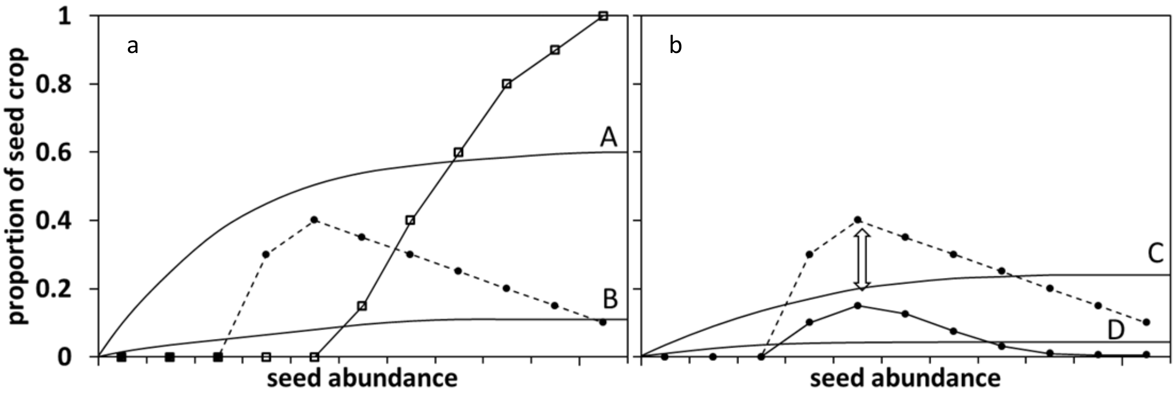

Figure 6.3: Panel (a) shows a hypothetical relationship between the proportion of the seed crop that successfully establishes seedlings from caches created by rodents (solid circles) and the proportion that escapes handling by rodents (open boxes) versus crop size. The potential recruitment of the same plant from seeds that were not handled by rodents could be higher than for seeds hoarded by rodents (curve A) or lower than that of seeds hoarded by rodents (curve B). Panel (b) shows the relationship between the probability of seedling recruitment in the absence and in the presence of seed-hoarding rodents. If curve C is the probability of seedling recruitment in the absence of seed-hoarding rodents, factors that shift the probability of cache survival from the solid line (solid circles) to the dashed line (solid circles) will cause a shift in the role of seed-hoarding rodents from antagonists to mutualists. Curve D in panel (b) represents my prediction of the potential recruitment of pedunculate oak in absence of wood mice and bank voles, which predicts seed-hoarding rodents to perform as mutualists under most conditions. *(figure adapted from Theimer (2005)).*

In conclusion, what I believe to be essential to take away from this study, is the need to move beyond the pairwise perspective of seed-dispersal interactions, i.e., the interaction between a single seed producer and a single seed disperser, and instead to focus on the network of species involved in the interaction. This does not only account for seed-dispersal interactions, however, but for species interactions at large. Certainly now that the body of literature acknowledging the widespread generality of conditional outcomes in species interactions is growing rapidly, we should encompass the phenomenon of conditionality and focus our studies on broader ecosystem- and community interactions. Only then will we advance our understanding of ecosystem functioning, and can we accurately judge the importance of conserving certain species within the larger communities.

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Summary

Of Mice and Oaks: Conditional outcomes in a seed-dispersal mutualism

Mutualisms are widespread and are considered fundamental to ecological and evolutionary processes. Mutualisms may be grouped into four types: 1) pollination mutualisms (e.g., pollination of flowers by bees), 2) digestive mutualisms (e.g., the interaction between ruminants and microbes in their gut), 3) protective mutualisms (e.g., plant protection by carnivorous insects), and 4) seed-dispersal mutualisms (e.g., the interaction between frugivorous animals and fruit-bearing plants). Traditionally, interactions among species were thought to be either positive, negative, or neutral for each species involved in the interaction. In reality, however, the outcome of a given interaction often varies in time and space, and can shift, for instance, from mutualistic to antagonistic. Variation in the outcome of species interactions, commonly termed *conditionality* or *context dependence*, has been documented in several types of species interactions. Within the field of mutualisms, conditional outcomes are predicted to be most likely if a) the mutualism is facultative rather than obligate, b) the densities of both interacting partners vary, or c) a third species is intimately involved in the interaction. These and other predictions regarding conditionality have received considerable theoretical and empirical attention in plant-pollinator-, digestive-, and plant-protection studies. However, theoretical exploration of the phenomenon in seed-dispersal mutualisms has only just begun, and empirical evidence of conditionality in seed-dispersal mutualisms is still scarce. Furthermore, the main factors driving conditionality in seed-dispersal mutualisms have not been well characterised. This study was an attempt to fill this gap by quantifying the role of seed-hoarding rodents as conditional mutualists of large-seeded trees in a multi-trophic system, and to explore the factors that drive conditionality in seed-dispersal mutualisms.

This study focused on a large seeded tree, the Pedunculate oak (*Quercus robur*), two seed-hoarding rodents, the Wood mouse (*Apodemus sylvaticus*) and the Bank vole (*Myodes glareolus*), and a third species food competitor, the Wild boar (*Sus scrofa*), to test whether and how the outcome of the seed-dispersal interaction between rodents and oaks varied as a function of the context in which the interaction occurs. Pedunculate oak is a large-seeded deciduous tree species abundant in central and western Europe that produces periodic rich and poor mast years. Seeds are shed between October and December, and seed crop averages about 50,000 seeds in good years. The seeds of pedunculate oak (henceforth: acorns) are used by many animals to overcome food scarcity during winter. In return, pedunculate oak relies largely on animals for the dispersal of its seeds, of which the wood mouse and the bank vole are good examples. Wood mouse and bank vole are both common rodents of European woodland. The wood mouse tends to be slightly smaller than the bank vole, but generally behaves more aggressively. Both species are largely nocturnal and are characterized by well-developed olfactory, visual, and auditory senses. They are typical granivores, and as such they play an important role as predator and disperser of acorns in Europe by hoarding acorns in autumn to serve as a food reserve for winter. They may function as mutualists of oak when scatter hoarding seeds, providing seed dispersal and protection against seed predators and environmental stress, but they function as seed predators when larder hoarding seeds in underground burrows (where recruitment is near-absent), when eating seeds without first hoarding

them, or when recovering all caches for food. The Wild boar is a medium-sized (50-200 kg) ungulate that is regarded as an important competitor to seed-hoarding rodents in forests and woodlands throughout its range. Wild boar consume large quantities of acorns in autumn when they are abundantly available on the forest floor, but also in mid- and late winter when very few acorns remain on the forest floor, suggesting that wild boar actively search for and pilfer acorn caches created by wood mouse and bank vole. Wild boar have well-developed olfactory senses, which they are thought to use for locating cached acorns and other hidden food items.

The interaction between pedunculate oak and seed-hoarding rodents was likely to show conditionality, for two main reasons. First, wood mice and bank voles play a dual role in plant regeneration. On the one hand, they consume large quantities of seeds, thereby reducing plant recruitment. On the other hand, they store many seeds, thereby potentially aiding the species' range expansion, and reducing the seed's risk of desiccation, consumption by strict seed predators, and distance- and density-dependent mortality below the parent plant and close to other seeds of the same species. Secondly, the patterns of seed hoarding in the wood mouse and the bank vole often shows variation between and sometimes even within individuals, and this in turn affects the balance between the costs of seed predation and the benefits of seed caching for the oak. The strategies that rodents use for hoarding seeds typically range between larder hoarding and scatter hoarding, where larder hoarding involves the placement of many food items in one or few caches that are often actively defended by the hoarder, while scatter hoarding involves spreading of food over many widely spaced caches with one or few food items each that are not generally defended by the hoarder. While larder hoarding generally precludes recruitment because large numbers of seeds are stored in deep burrows where germination and seedling establishment is highly unlikely, scatter hoarding is more likely to positively affect plant regeneration because individual seeds are buried in numerous spatially scattered, shallow soil or litter caches.

This study consisted of a combination of a large empirical field study and two controlled experiments. The field study took place across twelve sites in a forested area, centrally located in The Netherlands, and involved tracking of over a thousand acorns in time and space to record their ultimate position and fate. Passive Integrated Transponder (PIT) tags, inserted into acorns, were used to track the removal and dispersal of the seeds by seed-hoarding rodents. The results of the field study enabled me to evaluate whether and how seed-hoarding patterns of rodents were affected by wild-boar presence, rodent abundance, and seed abundance, and how this in turn affected the role of seed-hoarding rodents as mutualists of oaks. The two controlled experiments were set up to answer specific questions that could not be well-studied in the field. The first controlled experiment involved captive wild boar that were allowed to search for acorns that had been experimentally hidden according to different hoarding strategies. This experiment was used to evaluate the mechanism used by wild boar for cache finding, and whether differential seed-hoarding patterns actually affected the risk of seeds being found and pilfered by wild boar. The second controlled experiment involved wild-caught wood mice varying in sex, weight, and provenance (i.e., they had been captured in sites with contrasting wild-boar presence), that were allowed to first individually hoard a fixed number of seeds within a fixed area, and then hoard the same number of seeds in the same fixed area but then in presence of a conspecific audience mouse. This enabled me to test whether variation in hoarding patterns could be explained by weight, sex, and/or provenance of the hoarder, and whether the actual presence of a competitor influenced the patterns of hoarding.

In **chapter two**, the technique of using PIT tags inserted into seeds for the tracking and monitoring of seed dispersal by rodents was introduced and tested. The performance of PIT tagging was tested through a series of trials with *Quercus* acorns dispersed by rodents, both in North America and in Europe, with equipment from different manufacturers. Minimal effects of PIT tagging on seed mass and seed germination were found. Buried PIT tags were retrieved with high reliability by naïve researchers, even at burial depths up to 30 cm. Identification codes could be read even when multiple tags were buried at a single location, as in larder hoarding. It was shown that, unlike other tagging methods, PIT tagging combines the advantages of leaving no external cues, being readable without disturbance of caches, and of rodents being unable to remove the tags from the seeds.

In **chapter three**, the results from the field study using PIT tags were presented. We tested whether and how seed-hoarding patterns were affected by acorn abundance, rodent abundance, and wild-boar presence, and how this in turn affected seed germination. It was shown that seed-removal rate was negatively related to wild-boar presence and acorn abundance, while it increased strongly with rodent abundance. Interestingly, seed-dispersal distance was positively related to acorn abundance but was not related to rodent abundance, while seed spacing was positively related to rodent abundance, but was not related to acorn abundance. These results suggested that seed-hoarding rodents changed their hoarding patterns so that a certain optimal cache spacing was maintained. Generally, seed germination increased with rodent abundance, except if wild boar were absent and acorn abundance was low. In that situation, it was best not to have any seed predator at all, as they would not be easily satiated and all seeds would thus likely be consumed by the seed predators. Seed germination was highest, however, if rodents were relatively abundant and acorn abundance was high, regardless of wild-boar presence. Perhaps not surprisingly, seed-removal rate was relatively high, and seed-dispersal distance and seed spacing were greatest under these conditions, suggesting that these changes in hoarding patterns indeed affected seed germination as predicted.

In the study system of this thesis, wild boar compete strongly with seed-hoarding rodents and it is clear that rodents are unable to defend their reserves against wild boar. Generally, animals that are unable to defend their food reserves against competitors, are believed to scatter hoard food items to reduce the loss of cached food to such superior competitors. In **chapter four**, the underlying assumption was tested that the cost of having more caches under scatter hoarding is outweighed by the benefit of having small caches. A controlled experiment was carried out in which a fixed number of acorns was distributed over a fixed number of patches within a fixed area. Only cache size and cache depth was varied to mimic alternative hoarding patterns of rodents. A fixed number of wild boar was then allowed into the area to search for the hidden food supplies. It was shown that the overall rate of pilferage did not differ between scatter hoarding and larder hoarding, and that it was not affected by cache depth. These results suggested that wild boar may not be able to efficiently pilfer caches of acorns created by rodents. The results further hint towards the suggestion that wild boar did not actually use olfaction for cache detection, even though their keen sense of smell is generally acknowledged. If wild boar indeed make little use of olfaction for cache detection, but rather rely on visual cues or memory, it may imply that the movement of seeds away from places where acorn abundance is expected to be high, is most crucial in avoiding pilferage by wild boar.

The second controlled experiment of this study was described in **chapter five**. In this chapter, an attempt was made to disentangle the role of individual characteristics of the hoarder in explaining variation in seed-hoarding patterns. Wood mice that varied in sex and weight and were wild-caught in areas with and without wild boar were allowed into an indoor arena that had a uniform layer of natural forest soil. Wood mice were first individually allowed into the arena to hoard a fixed number of acorns, while in a follow-up experiment wood mice were allowed into an arena to hoard the same fixed number of acorns, but now in presence of a conspecific audience. If wood mice were alone in the arena, all individuals predominantly scatter-hoarded acorns. However, females cached significantly more seeds than males did, and females from areas with wild boar scattered seeds more widely than did females from areas without wild boar. The introduction of a conspecific audience mouse did not significantly affect hoarding patterns in the wood mouse. However, small females tended to scatter seeds more widely in response to a conspecific audience, while large females tended to clump seeds more in response to a conspecific audience. Altogether, however, the responses to the conspecific audience were weak and the variation between individuals was large. One possible explanation for this would be that we attempted to match the individual characteristics of the hoarder with those of the audience mouse. As a consequence, the difference in dominance rank between the hoarder and the audience may have been too small to effectuate an extreme response by the hoarder.

This study on conditional outcomes in seed-dispersal mutualisms has been the first to focus on rodents that are known to show within-species variation in hoarding patterns and that were likely to show within-individual variation in hoarding patterns. It was also the first to examine both individual and combined effects of hoarder abundance, seed abundance, and presence of a third-species food competitor – wild boar – on seed-hoarding patterns (i.e. on seed-removal rate, seed-dispersal distance, and seed spacing), and how this in turn affected the outcome of the mutualism between seed-hoarding rodents and large-seeded trees. Generally, the results from **chapters three, four, and five** convincingly show that there is a great deal of variation in hoarding patterns both within and between individual rodents. This variation could at least be partly explained by the intensity of competition, although more by competition with conspecifics than with heterospecifics. Actually, the role of wild boar in shaping the mutualism between rodents and oaks turned out to be smaller than expected on beforehand. This expectation, however, was primarily based on the assumption that wild boar would be using their excellent sense of smell to search for and pilfer rodent-cached acorns. It may well be, however, that acorns have been selected to provide as little cues to seed predators as possible, while still being found and dispersed by seed dispersal agents. Most likely, free-ranging wild boar use a combination of tactics, including visual orientation, memory, and olfaction, to find hidden food items. This may lead them to acorn hotspots, i.e., places where acorn abundance is generally high (e.g., below adult oaks). If this were true, seed-hoarding patterns that involve the movement of seeds away from such hotspots may be sufficient in largely avoiding pilferage by wild boar. In that sense, scatter hoarding and larder hoarding may both reduce pilferage risk by wild boar, so long as the caches are being created away from acorn hotspots. The absence of a strong response to wild-boar presence may thus be (partly) explained by the suggestion that all types of seed hoarding already sufficiently reduce the risk of pilferage by wild boar. Several other factors are thus believed to be involved with the decision of animals to either scatter- or larder hoard seeds. Most importantly, scatter hoarding is a strategy to prevent catastrophic loss of food supplies (i.e., instantaneously losing the entire food supply), and there may be several other factors playing a role here. For instance, caching large quantities of such seeds together may pose a large threat of catastrophic loss due to cross-contamination of seeds. Similarly, natural disturbances such as windthrow or flooding may instantly destroy a cache and its contents. Lastly, although the absolute risk of catastrophic loss may be very small, the direct consequences of catastrophic loss for the hoarder may be fatal.

To conclude, this study has shown that seed-hoarding rodents play an important role as seed dispersers of large-seeded trees, and that any handling of acorns by wood mice and bank voles in this study is thus likely to increase their likelihood of seed germination in oaks, at least so long as the abundance of acorns satiates the demand for food of the rodents. The study, however, has also shown that the patterns of hoarding are flexible and depend on the broader community of species and their abundances. These findings highlight the necessity to move beyond the pairwise perspective of seed-dispersal interactions, i.e., the interaction between a single seed producer and a single seed disperser, and instead to focus on the network of species involved in the interaction. Certainly now that the body of literature acknowledging the widespread generality of conditional outcomes in species interactions is growing rapidly, the phenomenon of conditionality should be embraced and future studies should thus focus on broader ecosystem- and community interactions. Only then will we advance our understanding of ecosystem functioning, and can we accurately judge the importance of conserving species within the larger communities.

Samenvatting

Over Muizen en Eiken: conditionele uitkomsten in een zaadverspreidingsmutualisme

Mutualismen zijn wijdverspreid en worden als fundamenteel beschouwd voor ecologische en evolutionaire processen. Mutualismen kunnen worden ingedeeld in vier hoofdgroepen: 1) bestuivingsmutualismen (v.b. bestuiving van bloemen door bijen), 2) verteringsmutualismen (v.b. de interactie tussen herkauwers en microben in hun verteringsstelsel), 3) beschermingsmutualismen (v.b. de bescherming van planten door vleesetende insecten), en 4) zaadverspreidingsmutualismen (v.b. de interactie tussen fruiteters en fruitdragende planten). Traditioneel werden interacties tussen soorten geclassificeerd als zijnde positief, negatief of neutraal voor ieder van de betrokken soorten. In werkelijkheid varieert de uitkomst van een willekeurige interactie echter dikwijls in zowel ruimte als tijd, en kan de uitkomst omslaan van, bijvoorbeeld, mutualistisch naar antagonistisch. Variatie in de uitkomst van interacties tussen soorten, veelal *conditionaliteit* of *context-afhankelijkheid* genoemd, is gedocumenteerd in verscheidene typen interacties. Binnen het veld van de mutualismen is voorspeld dat conditionele uitkomsten het meest voor de hand liggend zijn als a) het mutualisme facultatief, in plaats van obligaat, is, als b) de dichtheden van beide interacterende partners variëren, of als c) een derde soort direct betrokken is bij de interactie. Deze, en andere voorspellingen met betrekking tot conditionaliteit, hebben ruimschoots theoretische en empirische aandacht gekregen in plant-bestuivers-, verterings- en plant-beschermers studies. Echter, de theoretische verdieping van het fenomeen conditionaliteit in zaadverspreidingsmutualismen is pas kort geleden begonnen, en empirisch bewijs van het voortkomen van conditionaliteit in zaadverspreidingsmutualismen is nog schaars. Daarnaast is het zo dat de bepalende factoren voor conditionaliteit in zaadverspreidingsmutualismen nog niet goed gekarakteriseerd zijn. Dit onderzoek was een poging dit gat te dichten, door de rol van zaad-hamsterende knaagdieren als conditioneel mutualisten van zaaddragende boomsoorten in een multitroof systeem te kwantificeren, en door de bepalende factoren voor conditionaliteit in zaadverspreidingsmutualismen in kaart te brengen.

Deze studie richtte zich op een boomsoort die grote zaden produceert, de zomereik (*Quercus robur*), twee zaad-hamsterende knaagdieren, de bosmuis (*Apodemus sylvaticus*) en de rosse woelmuis (*Myodes glareolus*), en een derde soort die met de knaagdieren concurreert om de zaden, het wild zwijn (*Sus scrofa*). Het doel was om te testen óf en hoe de uitkomst van de zaaverspreidingsinteractie tussen knaagdieren en eiken varieert als functie van de context waarbinnen de interactie plaats heeft. De zomereik is een veel voorkomende loofboomsoort in Centraal- en West-Europa, die in afwisselende jaren veel en weinig zaden produceert. De zaden van de eik vallen voornamelijk in de periode oktober-december van de boom en in een goede jaren worden ongeveer 50.000 zaden geproduceerd. De zaden van de zomereik (hierna: eikels) worden door veel dieren gegeten en gebruikt om de winterperiode van voedselschaarste te overkomen. Aan de andere kant is de zomereik sterk afhankelijk van dieren voor de verspreiding van zijn zaden, bijvoorbeeld door bosmuizen en rosse woelmuizen. Bosmuizen en rosse woelmuizen zijn beiden veel voorkomende knaagdieren in Europese bosgebieden. De bosmuis is iets smaller dan rosse woelmuis maar gedraagt zich over het algemeen meer agressief. Beide soorten zijn voornamelijk nachtactief en hebben een sterk ontwikkeld zicht, gehoor en reukzin.

Ze zijn typische zaadeters en daardoor spelen ze een belangrijke rol als predator en als verspreider van eikels in Europa, die ze hamsteren en verstoppen in de herfst, om zo de winter te overleven. Ze zouden kunnen fungeren als mutualisten van de eik als ze de eikels in een verspreid patroon verstoppen, waardoor ze zorgen voor zaadverspreiding en voor bescherming van de zaden tegen zaadpredatoren en omgevingsfactoren, maar ze zouden fungeren als zaadpredatoren als ze de eikels in grote hoeveelheden bij elkaar zouden verstoppen in hun diepe holen (waar ontkieming en ontwikkeling van de zaden bijna onmogelijk is), of wanneer ze de zaden meteen opeten zonder ze te verstoppen voor later, of wanneer ze alle verstopte zaden terugvinden en alsnog opeten. Het wild zwijn is een hoefdier van gemiddelde grootte (50-200 kg) die wordt gezien als een belangrijke voedselconcurrent van zaadhamsterende knaagdieren in bossen en struwelen in zijn gehele leefgebied. Wilde zwijnen eten grote hoeveelheden eikels in de herfst, wanneer de zaden in grote getalen op de grond onder de bomen liggen, maar ook midden en laat in de winter wanneer bijna alle zaden al van de grond zijn verdwenen. Dit suggereert dat wilde zwijnen actief op zoek gaan naar de voorraden van bosmuizen en rosse woelmuizen en deze plunderen. Wilde zwijnen hebben een sterk ontwikkeld reukvermogen en dit gebruiken ze waarschijnlijk voor het lokaliseren van de verstopte eikels en ander eten.

Er waren twee redenen waarom het aannemelijk was dat de interactie tussen de zomereik en hamsterende knaagdieren conditioneel zou zijn. Ten eerste spelen bosmuizen en rosse woelmuizen een dubbele rol in het regeneratieproces van planten. Aan de ene kant eten ze grote hoeveelheden zaden waardoor ze het regeneratieproces van planten negatief beïnvloeden. Aan de andere kant slaan ze grote hoeveelheden zaden op, waarbij ze de verspreiding van de plantensoort in ieder geval in potentie helpen, en waarbij ze het risico op uitdroging van de zaden verkleinen, het risico op consumptie door pure zaadpredatoren verkleinen, en afstands- en dichtheidsafhankelijke sterfte onder de ouderboom en dicht bij soortgenoten verkleinen. Ten tweede varieert de ruimtelijke wijze waarop bosmuizen en rosse woelmuizen hun voorraad aanleggen vaak van individu tot individu en soms zelfs binnen het individu. De verschillende vormen van ruimtelijke spreiding in het aanleggen van de voorraden hebben invloed op de balans tussen kosten van zaadpredatie en de baten van zaadverspreiding. De strategieën die knaagdieren gebruiken voor het verstoppen van zaden varieert van zogenaamd “larder hoarding” tot “scatter hoarding”, waar larder hoarding inhoudt dat alle zaden in één of enkele voorraadkamers verstopt worden en dat deze voorraadkamers vervolgens actief verdedigd worden door de eigenaar, terwijl scatter hoarding inhoudt dat alle zaden individueel of in kleine groepjes worden verstopt en dat deze individuele zaden zo ver mogelijk uit elkaar verstopt worden en vervolgens ook niet actief verdedigd worden door de eigenaar. Hoewel de kansen op succesvolle kieming van de zaden bij larder hoarding over het algemeen bijna nihil zijn omdat de zaden in grote hoeveelheden diep onder de grond verstopt liggen, zijn de kansen hierop bij scatter hoarding veel groter, omdat de zaden voornamelijk individueel verstopt liggen in holletjes die vlak onder de oppervlakte liggen en vaak ver uit elkaar gespreid zijn.

Deze studie bestond uit een combinatie van een grootschalige veldstudie en twee gecontroleerde experimenten. De veldstudie vond plaats in twaalf daarvoor geselecteerde plekken in bosgebieden in het midden van Nederland. In het kader van deze veldstudie werden meer dan duizend eikels in ruimte en tijd gevolgd om zo hun uiteindelijke locatie en lot vast te stellen. Er werd gebruik gemaakt van Passive Integrated Transponder (PIT) tags, welke in eikels werden geplaatst, om het wegnemen en verstoppen van de eikels door hamsterende knaagdieren in kaart te brengen. De resultaten van de veldstudie gaven mij de kans om te evalueren óf en hoe de zaadverspreidingspatronen van knaagdieren beïnvloed werden door de aanwezigheid van wilde zwijnen, de abundantie van knaagdieren en zaden, en hoe dit op zijn beurt weer invloed zou hebben op de rol van knaagdieren als mutualisten van de eik. De twee gecontroleerde experimenten werden opgezet op antwoord te kunnen geven op enkele specifieke vragen welke niet goed konden worden bestudeerd in het veld. Bij het eerste gecontroleerde experiment waren wilde zwijnen betrokken die in een speciale arena op zoek gingen naar door ons in verschillende patronen verstopte eikels. Dit experiment was bedoeld om inzicht te krijgen in het mechanisme dat door wilde zwijnen gebruikt wordt om verstopte eikels te lokaliseren, en om te evalueren óf verschillende zaadverspreidingspatronen leidden tot verschillen in het risico om gedetecteerd en geplunderd te worden door de zwijnen. Bij het tweede experiment waren bosmuizen betrokken die in het wild gevangen waren uit verschillende gebieden (mét en zonder wilde zwijnen) en die varieerden in gewicht en geslacht. De bosmuizen werd de kans geboden om eerst alleen en later met publiek een bepaald aantal eikels te verstoppen in een bepaald gebied. Dit bood mij de kans om te testen of variatie in zaadverspreidingspatronen verklaard konden worden door verschillen in geslacht, gewicht, en/of afkomst van de zaadverspreider, en of de aanwezigheid van een concurrent van dezelfde soort invloed had op de zaadverspreidingspatronen.

In **hoofdstuk twee** werd het gebruik van PIT tags als techniek voor het volgen en monitoren van zaadverspreiding door knaagdieren geïntroduceerd en getest. De prestaties van PIT tagging werden getest middels een serie tests met *Quercus* eikels verspreid door knaagdieren in Noord Amerika en in Europa, met apparatuur van verschillende fabrikanten. Minimale effecten van PIT tagging op zaadverspreiding, verstopgedrag, plundering, en kieming werden gevonden. Begraven PIT tags konden met hoge nauwkeurigheid worden teruggevonden door naïeve onderzoekers, zelfs tot op 30 cm diepte. De unieke identificatiecodes konden zelfs worden teruggelezen als er meerdere tags bij elkaar verstopt lagen, zoals in larder hoarding het geval is. Het experiment liet zien dat PIT tagging, in tegenstelling tot andere markeringstechnieken, de voordelen van het niet zichtbaar zijn, het op afstand afleesbaar zijn, en het niet verwijderbaar zijn door knaagdieren combineert.

In **hoofdstuk drie** werden de resultaten van de veldstudie, waarin PIT tags gebruikt werden. gepresenteerd. We testen óf en hoe zaadverspreidingspatronen beïnvloed werden door eikelabundantie, knaagdierabundantie, en aanwezigheid van wilde zwijnen, en hoe dit op zijn beurt invloed had op kieming van de zaden. De studie liet zien dat de snelheid van het wegnemen van de zaden negatief gerelateerd was aan de aanwezigheid van wilde zwijnen en aan eikelabundantie, terwijl de snelheid sterk toenam met knaagdierabundantie. Interessant genoeg was de zaadverspreidingsafstand positief gerelateerd aan eikelabundantie, maar was zij niet gerelateerd aan knaagdierabundantie, terwijl de spreiding van individuele zaden positief gerelateerd was aan knaagdierabundantie maar juist niet gerelateerd was aan eikelabundantie. Deze resultaten suggereren dat zaadhamsterende knaagdieren hun zaadverspreidingspatronen aanpassen zodat een bepaalde optimale spreiding tussen de zaden behouden bleef. Over het algemeen nam de kans op kieming voor de zaden toe met knaagdierabundantie, behalve wanneer wilde zwijnen afwezig waren en de eikelabundantie laag was. In die specifieke situatie was het het beste om geen enkele zaadpredator in het systeem te hebben, daar zijn niet snel verzadigd zouden zijn en dus waarschijnlijk alle aanwezige zaden zouden consumeren. De kans op kieming van de zaden was het hoogst als knaagdieren in relatief grote aantallen aanwezig waren en als de eikelabundantie hoog was. De aan- of afwezigheid van wilde zwijnen had onder die omstandigheden geen invloed op de kiemingskans. Wellicht niet verrassend was onder deze omstandigheden de snelheid van het wegnemen van de zaden relatief hoog, en waren de zaadverspreidingsafstand en de spreiding tussen individuele zaden het grootst, wat suggereert dat deze aanpassingen in de zaadverspreidingspatronen inderdaad invloed hebben op de kiemingskans van de zaden.

In dit studie system is er een sterke concurrentie tussen wilde zwijnen en zaadhamsterende knaagdieren en het is duidelijk dat de knaagdieren niet bij machte zijn om hun voorraden te verdedigen tegen wilde zwijnen. Over het algemeen wordt verondersteld dat dieren die niet bij machte zijn om hun voedselvoorraden tegen concurrenten te verdedigen hun voorraden volgens de strategie van scatter hoarding verstoppen, om zo de kans op plundering door sterkere voedselconcurrenten te minimaliseren. In **hoofdstuk vier** werd de onderliggende aanname getest dat de kosten van het aanleggen en onderhouden van een groot aantal verstopplaatsen onder scatter hoarding teniet gedaan worden door de voordelen van kleine verstopplaatsen. Een gecontroleerd experiment werd uitgevoerd waarbij een bepaalde hoeveelheid eikels verstopt werd over een bepaald aantal potentiële verstopplaatsen binnen een vast gebied. Alleen het aantal eikels per verstopplaats en de diepte waarop de eikels verstopt werden, werd gevarieerd om zo verschillende zaadverspreidingsstrategieën van knaagdieren na te bootsen. Vervolgens kreeg een vast aantal wilde zwijnen de kans om binnen het gebied te zoeken naar de verstopte eikels. Uit het experiment werd duidelijk dat de totale snelheid van plunderen niet beïnvloed werd door de strategie van verstoppen, en dat het ook niet beïnvloed werd door de diepte van de verstopplaatsen. Deze resultaten suggereren dat wilde zwijnen misschien niet in staat zijn om de door kaagdieren verstopte eikels efficiënt te plunderen. De resultaten wekken verder de suggestie dat wilde zwijnen hun reukvermogen niet gebruikten bij het lokaliseren van de verstopte eikels, ook al wordt algemeen beweerd dat hun reukvermogen zo sterk ontwikkeld is. Als wilde zwijnen inderdaad weinig gebruik maken van hun reukvermogen voor het lokaliseren van verstopte eikels, maar bijvoorbeeld van visuele hints of van hun geheugen, dan zou dat kunnen betekenen dat het simpele wegdragen van de eikels van plekken waar ze normaliter verwacht worden (bijvoorbeeld onder de ouderboom) het meest cruciaal is ter voorkoming van plundering door wilde zwijnen.

Het tweede gecontroleerde experiment van deze studie werd beschreven in **hoofdstuk vijf**. In dit hoofdstuk werd een poging gedaan om te ontrafelen wat de rol is van bepaalde individuele eigenschappen van de hamsteraar in het verklaren van variatie in zaadverspreidingspatronen. Bosmuizen die varieerden in gewicht en geslacht, en die gevangen waren in dezelfde gebieden als waar de veldstudie had plaats gevonden, kregen de kans om een bepaald aantal eikels te verstoppen in een speciale overdekte arena waarin een laag bosgrond was aangelegd. Eerst deden de bosmuizen dit alleen, maar in een opvolgend experiment werd een extra muis als publiek toegevoegd aan de arena. Als de muizen alleen in de arena waren deden ze vooral aan scatter hoarding. Echter, vrouwelijke individuen verstopte significant meer zaden dan mannetjes en vrouwtjes van gebieden met wilde zwijnen spreiden de eikels ook verder uit dan vrouwtjes uit gebieden zonder zwijnen. De introductie van de publieksmuis had geen significant effect op de zaagverspreidingspatronen van de bosmuis. Echter, kleine vrouwtjes hadden de neiging om eikels meer uitgespreid te verstoppen in reactie op de publieksmuis, terwijl grote vrouwtjes de neiging hadden om eikels juist meer bij elkaar te verstoppen in reactie op de publieksmuis. Alles bij elkaar bezien, was de response op de publieksmuis echter zwak en was de variatie tussen de individuen groot. Een mogelijke verklaring hiervoor zou zijn dat wij als onderzoekers gepoogd hebben om de testmuis zoveel mogelijk te matchen aan de publieksmuis (qua gewicht en geslacht), waardoor de verschillen tussen beide individuen klein waren en dus was het misschien voor de individuen niet meteen duidelijk wie hoger of lager in rang zou staan.

Dit is de eerste studie van conditioneel mutualisme in zaadverspreidingsmutualismen geweest die zich richtte op knaagdieren waarvan bekend was dat ze variatie in zaadverspreidingspatronen tussen de individuen vertonen, en waarvan voorspeld werd dat ze ook variatie in zaadverspreidingspatronen binnen het individu vertonen. Dit was ook de eerste studie die zowel de individuele als de gecombineerde effecten van knaagdierabundantie, abundantie van zaden, en aanwezigheid van een derde soort als voedselconcurrent – het wild zwijn – op zaadverspreidingspatronen (dat is, de snelheid van het wegdragen van zaden, de zaadverspreidingsafstand, en de spreiding tussen individuele zaden) onderzocht. Tegelijkertijd werd gekeken hoe deze effecten invloed konden hebben op de kiemingskans van de zaden en daarmee op de uitkomst van de interactie tussen de zaadhamsterende knaagdieren en de zaadproducerende bomen. Over het algemeen laten de resultaten van de **hoofdstukken drie, vier, en vijf** overtuigend zien dat er veel variatie in zaadverspreidingspatronen is tussen en binnen de individuele knaagdieren. Deze variatie kon ten minste voor een deel verklaard worden door de intensiteit van voedselconcurrentie, hoewel concurrentie tussen soortgenoten belangrijk was dan concurrentie met de derde soort. Sterker nog, de rol van wilde zwijnen als sturende factor van het mutualisme tussen knaagdieren en eiken bleek kleiner dan verwacht. Deze verwachting was echter voornamelijk gestaafd op de aanname dat wilde zwijnen hun sterk ontwikkelde reukvermogen zouden gebruiken om de door knaagdieren verstopte eikels te lokaliseren en plunderen. Het is echter goed mogelijk dat door natuurlijke selectie de eikels zo geselecteerd zijn dat ze zo min mogelijk signalen afgeven aan potentiële predatoren, terwijl ze toch nog wel gevonden en verspreid worden door echte zaadverspreiders. Het ligt daarom voor de hand dat wilde zwijnen, in de natuur een combinatie van tactieken gebruiken, inclusief visuele oriëntatie, geheugen, én reukzin, om verstopte zaden te lokaliseren. Dit zou hen kunnen leiden naar eikel hotspot, d.w.z. plekken waar de aanwezigheid van eikels over het algemeen verwacht kan worden (bijvoorbeeld onder ouderbomen). Als dit inderdaad het geval is, dan zou het misschien al voldoende zijn als de zaden weggedragen zouden worden van deze plekken tijdens het zaadverspreidingsproces, om zo de kans op plundering door wilde zwijnen te voorkomen. In dat geval zou zowel larder hoarding als scatter hoarding kunnen leiden tot een afname in het risico op plundering door wilde zwijnen, zo lang de verstopplaatsen maar weg van de hotspots gecreëerd worden. De afwezigheid van een sterke reactie op de aanwezigheid van wilde zwijnen zou dus (deels) verklaard kunnen worden door de suggestie dat alle verspreiding van zaden al bijdraagt aan het verkleinen van het plunderingsrisico. Verscheidene andere factoren hebben naar verwachting invloed op de beslissing van dieren om zaden te scatter hoarden of te larder hoarden. Het is vooral belangrijk dat scatter hoarding een strategie is om het risico op het catastrofale verlies van voorraden (d.w.z. het ineens verliezen van de complete voorraad) te voorkomen. Er zijn verscheidene andere factoren te noemen die hier een rol bij zouden kunnen spelen. Zo zou het kunnen dat het gegroepeerd verstoppen van grote aantallen zaden de kans kruisbesmetting met schimmels vergroot waardoor de kans op catastrofaal verlies groter wordt. Ook natuurlijke verstoringen, zoals een storm of overstroming zouden in en klap de gehele voorraad kunnen vernietigen. Tot slot is het zo dat hoewel het risico op catastrofaal verlies van voorraden klein kan zijn, de gevolgen vaak groot of zelfs fataal zijn voor de getroffen hamsteraar.

Ter conclusie, deze studie heeft aangetoond dat zaadhamsterende knaagdieren een belangrijke rol spelen als zaadverspreider voor bomen met grote zaden, en dat het hanteren van eikels door bosmuizen en rosse woelmuizen dus waarschijnlijk bijdraagt aan de kans op kieming in eiken, in ieder geval zo lang het aanbod van de eikels voldoende is om de hamsteraars te verzadigen. De studie heeft echter ook laten zien dat de patronen van zaadverspreiding flexibel zijn en afhankelijk zijn van de samenstelling van de leefgemeenschap en hun abundantie. Deze bevindingen onderstrepen de noodzaak om verder te kijken dan de één-op-één studies van zaadverspreidingsinteracties, d.w.z., de interactie tussen een zaadproducerende plant en een zaadverspreidend dier, en om zich in plaats daarvan te richten op het netwerk van soorten dat direct of indirect betrokken is bij de interactie. Zeker nu het aantal studies dat de algemeenheid van conditionele uitkomsten van interacties tussen soorten onderkent sterk toeneemt, zou het fenomeen conditionaliteit omarmd moeten worden en dus zouden de toekomstige studies zich moeten richten op bredere ecosysteeminteracties. Alleen dan zullen we ons begrip van het functioneren van ecosystemen verder kunnen uitbreiden en kunnen we goed beoordelen wat het belang is van het behouden van bepaalde soorten voor het functioneren van de bredere gemeenschap.

Acknowledgements

My PhD journey started on the first of October 2009. I had just finished my MSc theses, when my thesis supervisor, Patrick Jansen, hinted me towards the opening of a PhD position, under his supervision, focusing on seed dispersal by rodents in The Netherlands. I am still grateful to Patrick for suggesting me to apply for this PhD position! The project was embedded within two chair groups of Wageningen University, the Forest Ecology and Forest Management Group (FEM) and the Resource Ecology Group (REG). Of both groups, a co-promotor and a promotor were linked to the project, and with these four people I have spent the last five years working intensely on my PhD project. Now, I would like to take the opportunity to thank them for all their efforts in helping me develop into an independent scientist and in making my PhD a success. First, I thank my co-promotor Sip van Wieren for all his help, particularly with selecting proper field sites for my project, and with building my network of specialists and forest managers on the Veluwe. Then, I'd like to thank both my promotors, Herbert Prins and Frans Bongers. You have both been instrumental in making me think critically and in stimulating me to act and work independently but under continuous guidance and steering, when needed. Of the supervisory team, I received most support from Patrick Jansen, my second co-promotor and daily supervisor. We spent many hours working out and discussing the details of the project, setting up the experiments, planning the fieldwork. Patrick also provided a wealth of feedback and suggestions during the writing process of my thesis chapters and publications, much improving the final product. Thanks Patrick, Frans, Herbert, and Sip for being so supportive!

Being part of two chair groups isn't always easy. It means making choices with respect to joining certain activities while skipping others, as it was simply impossible to join all coffee breaks, lunch breaks, seminars, MSc thesis presentations, parties, defences, and drinks. I feel that in this respect I have somewhat neglected my colleagues of the FEM group as I spent most of my time in the REG group. I would like to stress, however, that I always felt very much at home and welcome in the FEM group and that I very much enjoyed the company, discussions, suggestions, advice, help, and laughter with all members of FEM. I have met many of you, and would like to particularly thank Britta, Catarina, Corneille, Danaë, Ellen, Emiru, Estela, Frank, Frits, Geovana, Gustavo, Jan, Jean, Joke, Kwame, Lars, Leo, Lourens, Lu, Lucy, Madelon, Mart, Marielos, Marisol, Marlene, Masha, Mathieu, Merel, Michiel, Monique, Motuma, Paul, Peter Groenendijk, Peter Schippers, Peter van der Sleen, Pieter, Tefera, Tiep, Ute, and Vency. Of these people, a special word of thanks goes to Jan den Ouden, who so generously provided feedback, suggestions, and critical comments on all aspects of the thesis. Then, of course I would like to express my gratitude to all members of the Resource Ecology Group (REG) as well. This group has really been my home during my PhD and I have received the most inspiring, motivating, stimulating, and challenging questions, answers, and suggestions from the REG-people. Hoping not to forget anyone, I would like to thank Alfred, Anil, Anne-Marie, Audrie, Bas, Benson, Cornelis, Daniël, David, Dorit, Edson, Eduardo, Edward, Emmanuel, Farshid, Frank, Fred, Gerda, Helen, Henjo, Herman, Ignas, Iris, Jasper, Jente, Joost, Kyle, Mariaan, Martijn, Mikhail, Milena, Ntuthuko, Patricia, Pim, Priya, Qiong, Ralf, Robert, Ron, Rudy, Sintayehu, Tessema, Tibor, Tim, Tom, Tsewang, Vincent, Xavier, Yolanda, Yong, Yorick, Yussuf, Zheng. A

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Since the first of January 2013, I started to work three days a week on my PhD project and two days a week for the graduate school PE&RC. Working at PE&RC has been and still is the best choice I could have made in terms of my career path. I very much enjoy helping other PhD candidates setting up a successful PhD trajectory and facilitating their progress. I am sure that a great deal of the joy I get from working at PE&RC is due to the PE&RC team: Theo, Claudius, Anja, Maartje, Lijbert, and Simona, you are a wonderful group of people and I hope to be part of the team for a long time still!!

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Curriculum Vitae

Biography

Lennart Suselbeek was born on December 25, 1984 in Ruinen, the Netherlands. He attended VWO secondary education at OSG De Groene Driehoek in Hoogeveen, from which he graduated in 2003. He subsequently enrolled in the Bachelor study Animal Management at the Van Hall Instituut in Leeuwarden. During this study, he also obtained a MBO degree as para-veterinary assistant, and did an internship at the Department of Conservation at the New Zealand government. Although originally, he planned to pursue a career as a veterinarian, he discovered the beauty and richness of natural wildlife in New Zealand and decided to be better off studying and working with healthy plants and animals. Consequently, he enrolled in the MSc study Forest & Nature Conservation at Wageningen University in September 2007.

Lennart did two MSc theses as part of his study, both in the tropical forests of Barro Colorado Island, Panama. His first thesis was with the Forest Ecology and Forest Management group of Wageningen University, and focused on activity patterns of the Central American agouti in relation to food availability and predation risk. His second thesis was with the Community and Conservation Ecology group of the University of Groningen, and focused on a new method to estimate the density of wild animal populations from camera trapping data. Both theses were supervised by his future PhD supervisor Patrick Jansen. In August 2009 he received his MSc degree.

In October 2009, he started the PhD research that led to this thesis at the Resource Ecology Group and at the Forest Ecology & Forest Management group of Wageningen University. During his PhD research, Lennart participated in several PhD Discussion Groups and was an active member and chair of the PE&RC PhD Council (PPC). He attended several national and international workshops, PhD courses, and conferences. As of January 2013, Lennart started working two days a week for the graduate school for Production Ecology and Resource Conservation (PE&RC), as PhD programme coordinator. Now, five years later, his PhD has come to an end and Lennart has managed to have his position at PE&RC expanded to four days a week. He also runs his own company “Wildlife Monitoring Solutions”.

Publications

1. **L. Suselbeek**, V.M.A.P. Adamczyk, F. Bongers, B.A. Nolet, H.H.T. Prins, S.E. van Wieren, and P.A. Jansen. **2014**. Scatter hoarding and cache pilferage by superior competitors: an experiment with wild boar (*Sus scrofa*). *Animal Behaviour (in press)*.
2. **L. Suselbeek**, W.-J. Emsens, B.T. Hirsch, R. Kays, J.M. Rowcliffe, V. Zamora-Gutierrez, and P.A. Jansen. **2014**. Food acquisition and predator avoidance in a Neotropical rodent. *Animal Behaviour 88: 41-48*.
3. **L. Suselbeek**, P.A. Jansen, H.H.T. Prins, and M.A. Steele. **2013**. Tracking rodent-dispersed large seeds with Passive Integrated Transponder (PIT) tags. *Methods in Ecology and Evolution 4(6): 513-519*.
4. W.-J. Emsens, **L. Suselbeek**, B.T. Hirsch, R. Kays, A.J.S. Winkelhagen, and P.A. Jansen. **2013**. Effects of food availability on space and refuge use by a Neotropical scatter-hoarding rodent. *Biotropica 45(1): 88-93*.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities).

Review of literature (6 ECTS)

- Conditionality in seed-dispersal interactions

Writing of project proposal (1.5 ECTS)

- Context-dependence in the outcome of a seed-dispersal mutualism: interactions between Wood mouse, Wild boar and Pedunculate oak

Post-graduate courses (6.8 ECTS)

- Consumer resource interactions; PE&RC, RSEE, SENSE (2010)
- Spatial ecology; PE&RC, RSEE, SENSE (2011)
- Survival analysis; PE&RC (2011)
- Linear models; PE&RC (2011)
- Generalized linear models; PE&RC (2011)
- Mixed linear models; PE&RC (2011)
- Introduction to R for statistical analysis; PE&RC (2011)

Invited review of (unpublished) journal manuscript (2 ECTS)

- Acta Theriologica: scatter-hoarding seeds by rodents (2010)
- Basic and applied Ecology: acorn dispersal by rodents (2011)
- Journal of Natural History: seed dispersal and oak regeneration (2013)

Competence strengthening / skills courses (2.3 ECTS)

- PhD competence assessment; WGS (2010)
- Project and time management; WGS (2013)
- Data management; WGS (2013)
- Mini-symposium: how to write a world-class paper; Wageningen UR Library, Elsevier (2013)
- Last stretch of the PhD programme; WGS (2013)

PE&RC Annual meetings, seminars and the PE&RC weekend (3 ECTS)

- PE&RC Weekend first year (2009)
- PE&RC Day (2009-2013)
- PE&RC Weekend last year (2012)

Discussion groups / local seminars / other scientific meetings (7.5 ECTS)

- 16th Benelux Congress of Zoology; Wageningen, The Netherlands (2009)
- Ecology Theory and Application (2009-2013)
- International Symposium for Seed Dispersal and Frugivory; Montpellier, France (2010)
- WEES Seminars (2010-2013)
- Netherlands Annual Ecology Meeting; Lunteren, The Netherlands (2010-2014)
- Het varken als landschapsontwikkelaar; Garderen, The Netherlands (2011)
- Biodiversity merger symposium; Wageningen, The Netherlands (2012)
- R Discussion Group (2012-2013)
- Current Themes in Ecology; Amsterdam, The Netherlands (2013)

International symposia, workshops and conferences (5 ECTS)

- ATBC; oral presentation; Marburg, Germany (2009)
- Wild Boar symposium; oral presentation; Hannover, Germany (2012)

Lecturing / supervision of practical‘s / tutorials (3 ECTS)

- Ecology (2010)
- Strategic planning in forest and nature conservation (2011, 2012)
- Animal ecology (2011, 2012)

Supervision of MSc students

- Daan Jacobs: effects of wild-boar presence on seed removal by wood mice (2011)
- Simone van Santen de Hoog: how resource competition affects seed dispersal in rodents (2011)
- Vena Adamczyk: effects of hoard distribution and depth on cache pilferage by wild boar (2011)
- Marco Snijder: microhabitat conditions of rodent-dispersed acorns: indications for directional hoarding? (2012)
- Malou van Meer: the mutualism between rodents and oak: effects of wild boar and masting on seed dispersal (2012)
- José van der Bijl: responses of seed-caching rodents to intra- and interspecific competition (2013)
- Anneke Valk: caching decisions of wood mouse: effects of pilferage risk by conspecifics and wild boar (2012)
- Madieke Gehem: the advantages of scatter hoarding for seeds: an experiment with oak in the Netherlands (2014)

Cover drawing by Anneke Valk.

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