Mink excrement - a potential repellent to prevent bank vole consumption of direct seeded beech nuts and acorns

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Abstract

Granivorous rodents cause significant damage to direct-seeded regenerations of beech (*Fagus sylvatica*) and oak (*Quercus robur*) by consumption and removal of seeds. In the present study, the use of repellent-treated seeds to reduce such damage was evaluated under laboratory conditions. The potential of chilli (*Capsicum chinense*)/coconut fat, citronella (*Cymbopogon winterianus*)/rape oils, garlic (*Allium sativum*), mink (*Mustela vision*) excrement, neem (*Azadirachta indica*)/detergent, and sand coating, as bank vole (*Myodes glareolus*) repellents was investigated. Primary screening led to the exclusion of garlic and neem/detergent. The remaining four substances were applied to beech nuts and acorns, and tested against seeds soaked in water in a no-choice feeding trial. Over ten days, forty bank voles were individually given access to seeds at a rate of one treatment per day. Chilli/coconut fat, and mink excrement each reduced the consumption of beech nuts; a sand coating increased consumption of both beech nuts and acorns. In a germination test, chilli/coconut fat and citronella/rape oils reduced the germination rate of both beech nuts and acorns compared to the seeds soaked in water; mink excrement slightly reduced the germination rate of beech nuts. The remaining treatments did not differ significantly from the water soaked seeds. The results suggest that the application of mink excrement to seeds has the potential to deter rodents from consuming beech nuts and acorns in forest regenerations using direct seeding.

Keywords: beech, direct seeding, oak, pest control, rodents, seed predation

Introduction

In response to the threats of climate change and declining biodiversity (Anonymous 2007), a need has arisen to increase the amount of broadleaved forest in southern Sweden. However, although artificial forest regeneration by planting bare rooted seedlings is currently common practise, it is expensive. Less costly methods are therefore needed in order to encourage forest owners to undertake regeneration with broadleaves. One such method is direct seeding: however, a significant disadvantage when this is implemented on forest land is the high consumption rate of seeds by granivorous rodents (Buckley and Sharik 2002; Madsen and Löf 2005), especially the bank vole (*Myodes glareolus* Schreber), yellow-necked mouse (*Apodemus flavicollis* Melchior) (Jensen 1982; Heroldova et al. 2008) and wood mouse (*Apodemus sylvaticus* L.) (Hulme and Hunt 1999). In many areas, also squirrels (*Sciurus* sp., *Tamiasciurus* sp.) remove large amounts of seeds (Tanton 1965; Bellocq et al. 2005), but previous results (Birkedal et al. 2009) indicate that they are not the major post-sowing seed consumer in southern Scandinavia. Wild boars (Jedrzejewska et al. 1994) may also cause significant damage to areas regenerated with beech nuts and acorns, but such large animals can usually be reasonably kept out with fences. Through covering the seeds after sowing, interference by granivorous birds can be kept at a minimum since they seek food visually (Nystrand 1998).

Since the 1940's, pest rodents have mainly been controlled with poisons (Myllymäki 1975). However, because such methods have negative impacts on health and the environment, other solutions are now needed. Several ways of preventing rodents from removing tree seeds after direct seeding have previously been tested. For example, Sullivan (1979) spread sunflower seed and oats together with seeds of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco.) to reduce consumption of the fir seeds by dilution; Nilsson et al. (1996) planted Pedunculate oak (*Quercus robur* L.) acorns at different depths to decrease rodents' ability to find them. Although both studies reduced consumption, additional protection would be required to ensure satisfactory regeneration results.

Many repellents have been tested on different animal groups with varying results (Armour 1963). However, pesticide use in forestry is at present heavily restricted by legislation in Sweden and other European countries. Any potential repellent must not, therefore, be harmful to the seed-eater, to the seed itself, or to the environment. Furthermore, it needs to be easily applied to the seeds or to the regeneration area, and it must not compromise the economics or performance of the seeding operation. When evaluating the efficiency of methods to repel granivorous rodents, it is essential to take their behaviour into account. Being the main prey of several avian and terrestrial predators, rodents exhibit various anti-predator behaviours (e.g. Ylönen et al. 2003); for example forest-living rodents prefer habitats that provide shelter and food (Olsson et al. 2005; Fedriani and Boulay 2006) and they are sensitive to predator odours (Sullivan et al. 1988; Jedrzejewski et al. 1993). Having well developed olfactory senses rodents also use smell to locate food (Vander Wall 1998).

Mechanical protection of seeds has been successfully demonstrated with caged seeds (Hayes 1913; Shaw 1968), and less convincingly with plastic seeding tubes (Madsen and Löf 2005). However, these methods are costly in terms of the materials and labour required; a disadvantage that could be avoided by using a method of coating the seed. For example, the flexible sand coating "Conniflex" creates a mechanical barrier around conifer seedlings, that feeding pine weevils (*Hylobius abietis* L.) cannot penetrate (Nordlander et al. 2009).

The above mentioned behavioural traits, as well as previous experiences with mechanical protection of regeneration material, were considered when choosing the following strategies to restrain rodents: predator odour; taste and smell repellents; and a physical barrier. Mink (*Mustela vision* Schreber) excrement sorted under the first strategy, while chilli (*Capsicum chinense* Jacq.)/coconut fat, citronella (*Cymbopogon winterianus* Jowitt)/rape oils, garlic (*Alllium sativum* L.) and neem (*Azadirachta indica* A. Juss)/detergent belonged to the second, and the final strategy was represented by a sand coating.

In the present study chilli/coconut fat, citronella/rape oil, garlic, mink excrement, neem/detergent and sand coating, were tested under laboratory conditions as potential rodent repellents for use in direct seeding regenerations of European beech (*Fagus sylvatica* L.) and Pedunculate oak. The ethical aspect when performing animal tests is important, and therefore an initial screening with the primary task to identify and exclude any substances demonstrating harmful effects to the rodents was conducted. Since none of the substances was detrimental to the rodents, the four substances which had produced the best preliminary results were chosen for further tests with bank voles in a no-choice feeding trial.

Because a no-choice feeding trial give a conservative measure of repellency (Clark 1997), it is appropriate to determine the potency of the repellents under study. However, because the previous diet of voles influences present food choice (Hansson 1993), using wild-caught animals with an unknown dietary history might bias results. Laboratory-bred animals, with identical previous experiences, are therefore preferred in these types of test. Furthermore, bank voles are easily maintained and handled in captivity, and since laboratory bred animals are accustomed to the study environment, they are less stressed by the experimental conditions than wild-caught animals.

The objectives of this study were: 1) to screen six substances with potential repellent effect on bank voles for possible detrimental effects on the animals 2) to evaluate, in comparison with a control of seeds soaked in water, the repellent effect on bank voles of four substances applied to beech nuts and acorns; and 3) to test the effect of these four substances on the germination capacity of beech nuts and acorns.

Materials and methods

Experimental design

In the initial screening test, 14 bank voles (6 males, 8 females) were each individually given access to batches of either ten beech nuts or five acorns, each batch having been treated with one of the repellents or soaked in water. Voles were confined singly to each batch of treated seeds and the screening trial was repeated on a second day.

The seed species used for trials were very different in size, i.e. volume and surface area, hence also amount of adhesive repellent that "contaminated" these food items in the perspective of the rodents. Therefore, to ensure that each rodent was subject to a sufficient amount of food and repellents, twice as many beech nuts (n = 10) compared to acorns (n = 5) were used in the trials.

The no-choice feeding trial was conducted over two, five-day sessions, during which four repellents were tested in parallel against seeds soaked in water. Forty bank voles were divided into two batches: one, comprising 12 males and 8 females were given access to treated beech nuts; the other comprising 8 males and 12 females were given access to treated acorns. Over the five days of each session, each vole was given access in turn to one of the five seed treatments. For each vole, days and treatments were arranged as a series of 5 × 5 Latin Squares in order to control for any possible influence of a previous day's treatment. Thus, each vole experienced a different sequence of treatments from all other voles during each session. However, each vole experienced the same sequence of treatments in session two as it had in session one. Each vole was subjected to two sessions in order to see if the voles changed their behaviour towards any of the substances the second time they encountered it. Therefore, session two was considered the main trial.

On an experimental screening or feeding trial day, single voles were transferred to clean cages, identical to those in which they were normally kept. The allocated treatment (i.e. ten beech nuts or five acorns, treated with one of the repellent substances or soaked in water) was placed on the bottom of the cage. No other food was available during the seven hours that the trials lasted.

Measurements

Seeds were weighed before and after each trial. The chilli/coconut fat and sand coating treatments had a tendency to be rubbed off the seeds during trials, which may have resulted in an over-estimation of seed consumption in these treatments. As much of the substances as possible was collected and weighed together with the seeds, and to further reduce the impact on the results, the comparisons between consumption in the different treatments were calculated as percentages. Since these two treatments had greater intrinsic weight, the gross effect of any substance falling off was lessened by presenting differences proportionally. However, to compare the consumption of the two seed species, actual weights had to be used in the calculations since the initial weight of acorns was higher than that of beech nuts.

If all seeds in a cage had been left completely untouched during a no-choice feeding trial that cage was recorded as 1. If some or all of the seeds had been moved but none had been eaten from, the cage was recorded as 0.5. Cages where any of the seeds had been eaten from were recorded as 0.

Samples of treated beech nuts and acorns were also weighed before and after having been left, in open glass jars, in the same room as the cages for the duration of the trial, in order to determine water loss from, or uptake by, the seeds.

Bank voles

In total, 26 male and 28 female bank voles were used in the screening test and the no-choice feeding trials. All animals were previously experimentally inexperienced and bred at the Swedish Institute for Infectious Disease Control, Stockholm, where the test was performed. All voles were at least two months old, and their initial weights ranged between 14.4 g and 26.7 g. The temperature in the room where the voles were kept was 19ºC and the air humidity was 88%. The light regime was 12 h daylight and 12 h darkness with "lunar" light, which was created by a diode with whitish/bluish light. The experiment was non-detrimental to the rodents and ethically approved (Dnr N104/07). Between trials, voles were kept in same-sex pairs, in cages ($60 \times 30 \times 40$ cm^3) with wood chips on the floor and environmentally enriched with a paper sheet. Voles had free access to pelleted mouse food (RM1, Special Diet Services, United Kingdom) between trials, and water was provided *ad libitum* at all times.

Seed material

Seeds of European beech (Haderslev F.692, Denmark, 2006), and Pedunculate oak (PL-RD 0346, Poland, 2006) were used both in the screening of potential repellents and in the no-choice feeding trial. The seeds were stored at nurseries in Denmark and Sweden, and the beech nuts were pre-treated to break seed dormancy at the nursery (Statsskovenes Planteavlsstation, Humlebæk, Denmark), prior to use. From the time of delivery until the start of experiments all seeds were stored at 4°C. The viability of beech nuts and acorns, according to a cut-test, was 80%. Seed batches (beech nuts – Skäralid 083, Sweden, 2006; acorns – Flakulla 138, Sweden, 2007) used for the germination test were stored at Ramlösa Plantskola, Helsingborg, Sweden. Beech nuts were pre-treated to break dormancy at the nursery before delivery and repellent application. All handling of seeds at the nurseries was conducted according to international guidelines (Anonymous 1993).

Preparation of seeds with repellents

The amounts and proportions of different ingredients used to prepare the seeds with repellent substances for the no-choice trial are given in Table 1. The proportions of the different ingredients were the same for the screening and the germination test. The methods of preparing the treatments were as follows. Water – seeds were soaked in water. Chilli/coconut fat – chopped pieces of habanero chilli peppers were put into melted coconut fat, and the seeds were dipped into the mixture as soon as it had cooled down, before it had solidified. Pieces of the chilli fruits (5.5 g for the screening; 6.7 g for the no-choice feeding trial; and 7.4 g for the germination test) were dried in 60ºC for 24h to determine dry weight. Citronella/rape oils – seeds were soaked in a mixture of citronella oil, Java, (3-15% citronella, 85-97% geraniol) and rape oil. Garlic – seeds were soaked in water with chopped pieces of garlic added. Pieces of garlic (7.4 g for the screening) were dried in 60ºC for 24h to determine dry weight. Mink excrement – seeds were soaked in water to which mink excrement was added. Neem/detergent – seeds were soaked in a mixture of neem seed oil (100% cold pressed), water and detergent. Sand coating – potato starch flour was boiled in water and stirred until it formed a thick paste. The paste was removed from the heat, sugar and sand were added, and the seeds coated by dipping into the cooled mixture. Chilli and citronella were mixed with fats, since the effective substances are not soluble in water; the detergent was added to the neem seed oil to make it disperse in water. Following treatment, substances were air-dried and the seeds refrigerated until required for use 1 to 11 days later, depending on which day in the no-choice feeding trial they were used.

Table 1. *Contents of repellent substances used for the no-choice trial (garlic and neem/detergent treatments show amounts used for the screening)*

Treatment	Content	Amount (proportion, %)
Water	water	3000 ml (100.0)
Chilli/	chilli habanero (dry wt)	19 g (1.3)
coconut fat	coconut fat	1500 g (98.7)
Citronella/	citronella oil, Java	45 ml (1.5)
rape oils	rape oil	3000 ml (97.5)
Garlic	garlic (dry wt)	38 g (3.7)
	water	1000 g (96.3)
Mink	mink excrement (wet wt)	400 g (11.8)
excrement	water	3000 g (88.2)
Neem/	neem seed oil	17 ml (1.7)
detergent	water	1000 ml (98.0)
	detergent	3 ml (0.3)
Sand	sand	2500 ml (55.8)
coating	water	1600 ml (35.7)
	potato starch flour	200 ml (4.5)
	sugar	180 ml (4.0)

Germination test

Ten samples of 200 seeds from each species–treatment combination, i.e. the two tree species by the five treatments (four repellents plus the water treatment) used in the no-choice feeding trial, were sent to the seed station in Humlebæk, Denmark for analysis of germination capacity. Beech nuts were kept in vermiculite at 5°C for one week, and thereafter at 5°C (8 h) / 15°C (16 h) until germination. After 14 weeks, seeds that had not germinated were cut to determine their viability status. Acorns were kept in sand at 4°C for three weeks and thereafter at 20°C (8 h) / 25°C (16 h) until germinants (the first pair of leaves) had developed. After 14 weeks, the status (living or dead) of the root was determined for germinated acorns where no germinant had yet developed.

Calculations and statistical analysis

Seed weight was corrected for any weight change due to a change in water content, before the daily consumption of seeds was calculated. Small differences in water uptake occurred in some cases between the seeds in the cages and the seeds in the glass jars, which resulted in an apparent negative consumption, i.e. the weight of seeds increased during the trial. Weight

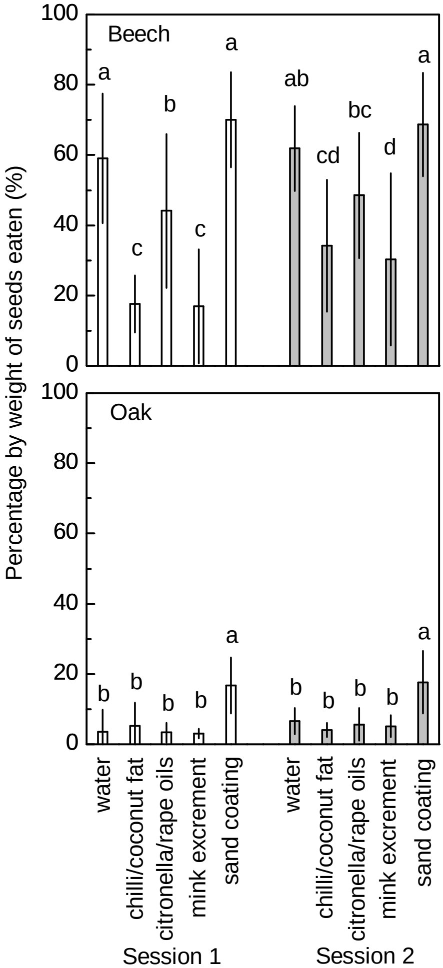

Figure 1. Bank vole consumption (% of weight) of seeds ± SD. Letters above bars indicate statistically significant differences ($P < 0.05$), within sessions.

increase only occurred in eleven samples of four hundred during the no-choice trials and was never more than 2.4 g (10%) in acorns and 0.1 g (4%) in beech nuts. During the no-choice feeding trial, two male voles died after fighting with their respective cage companions, and one male vole escaped during a trial and was found the following morning. These individuals were treated as missing values in the analysis.

The general linear model (GLM) procedure for the analysis of variance was used to perform statistical tests on treatment effect on the proportion of seeds eaten, with 'day' and 'vole' as factors in the Latin square model (Minitab Inc., Pennsylvania, USA). GLM was also used to compare: 1) consumption of the two seed species, with 'vole' as a factor in the model; 2) the difference in consumption between the two sessions, with 'vole' as a factor in the model; and 3) the difference in germination capacity between the treatments. Tukey tests were used to separate the effects of 'treatment', 'day', 'vole', 'seed species' and 'session'. All effects with $P < 0.05$ were considered significant. Assumptions about normality, equal variances and independent residuals were met, except for the mink excrement treatment, session 1, in the comparison between beech nuts and acorns, where no statistical test was performed.

Results

None of the substances tested in the screening had detrimental effects on the voles. Therefore the substances for continued evaluation in the no-choice feeding trial were chosen guided by consumption reductions indicated by the screening test. When results from beech nuts and acorns were combined, the application of mink excrement most consistently reduced the consumption of seeds by bank voles. The effects of the other treatments were not conclusive; but the consumption of garlic treated seeds was relatively high for both seed species and the consumption of neem/detergent treated seeds was high for beech nuts and stable from day one to day two for both seed species. Thus garlic and neem/detergent were excluded from further tests.

In the second no-choice feeding trial session, the consumption of beech nuts treated with chilli/coconut fat and mink excrement was 34% and 30% respectively, which was significantly ($P < 0.001$) lower than the seeds soaked in water at 62% (Fig. 1). This was an increase ($P < 0.01$) in consumption of both chilli/coconut fat and mink excrement treated beech nuts from 18% and 17% in the first session. The consumption of citronella/rape oils treated beech nuts decreased ($P < 0.01$) compared to the seeds soaked in water in the first session (44%) only. The consumption of sand coated beech nuts did not differ from the consumption of the water soaked ones. However, acorns coated with sand were consumed at a significantly higher ($P < 0.001$) rate (17% and 18% in session 1 and 2 respectively), compared with the water soaked seeds (4%; 7%). This was the only substance that gave a significantly different consumption rate compared to the water soaked seeds, when applied to acorns. Consumption increased ($P < 0.05$) from 3% to 6% and from 3% to 5% from the first to the second session for the citronella/rape oils and mink excrement treated acorns, respectively.

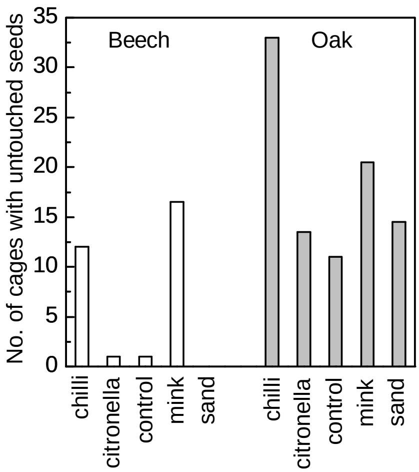

Figure 2. Number of cages with seeds untouched by bank voles; data combined from both sessions. Cages where seeds were completely untouched were recorded as 1 and 0.5 if seeds had been moved, but not eaten.

In the first session, a significantly smaller (*P* < 0.05; data not shown) proportion of beech nuts was eaten on the first day than on the other days. Significant differences in consumption by individual voles (data not shown) could be seen in the first session for beech nuts (*P* < 0.05), and in the second session for acorns (*P* < 0.01). Furthermore, differences in consumption between sessions for individual voles (data not shown) occurred for mink excrement treated beech nuts (*P* < 0.01), and acorns treated with water (*P* < 0.05) and citronella/rape oils (*P* < 0.01).

There where more cages with acorns untouched by bank voles than there were cages with beech nuts untouched (Fig. 2). Chilli/coconut fat and mink excrement treated seeds of both species were handled less than the sand coated or citronella/rape oils treated seeds, or the ones soaked in water. The consumed weight of water soaked acorns was significantly lower (*P* < 0.05) than the consumed weight of beech nuts soaked in water during both sessions (Fig. 3).

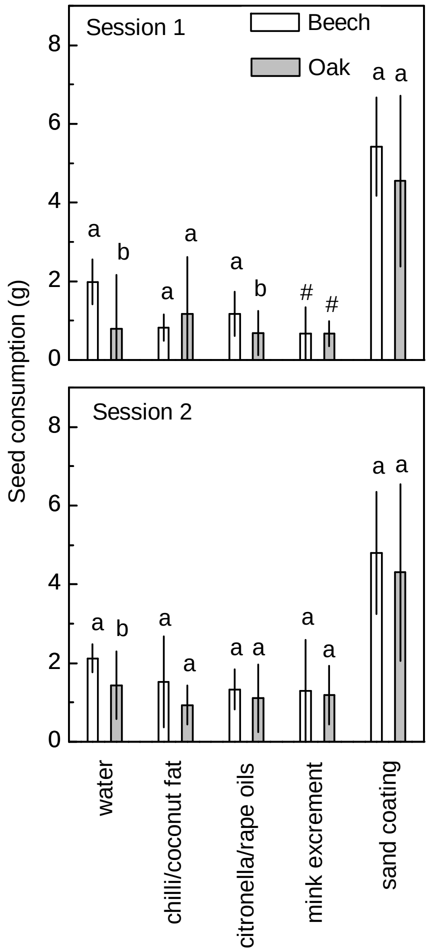

Figure 3. Bank vole consumption (g) of seeds ± SD. Letters above bars indicate statistically significant differences (*P* < 0.05) between consumption of different seed species, within session and treatment. (#) No statistical test was performed since data were not normally distributed.

The germination capacity of beech nuts was significantly impaired (*P* < 0.001) by chilli/coconut fat and citronella/rape oils treatments compared to the water, and significantly, but to a lesser extent, by mink excrement (*P* = 0.042) compared to the water treatment (Table 2). In general, the formation of beech germinants was low, since the root seemed to dry out after germination. Therefore, the beech nut test was stopped after germination. After 14 weeks few of the beech nuts that had not germinated were viable. Acorn germination and capacity to form germinants was reduced ($P < 0.05$) by the chilli/coconut fat and citronella/rape oils treatments, while application of mink excrement or a sand coating did not reduce the ability of the seeds either to germinate or to produce germinants. After 14 weeks most of the germinated acorns that were still alive had developed a germinant.

Table 2. *Results of the germination test after 14 weeks. Sample size was 200 seeds per tree species and treatment. Treatments followed by different letters are significantly different, P < 0.05, within tree species.*

Seed species	Treatment	Germinated[a] (%)	Germinants (%)	Viable at cut-test[b] (%)
Beech	Water	79.0 a	-	2
	Chilli/coconut fat	18.0 c	-	2
	Citronella/rape oils	5.5 c	-	4
	Mink excrement	61.0 b	-	1
	Sand coating	70.5 ab	-	1
				Live root[c] (%)
Oak	Water	73.5 a	61.5 a	3
	Chilli/coconut fat	51.5 b	36.0 bc	6
	Citronella/rape oils	51.5 b	27.5 b	8
	Mink excrement	70.5 ab	55.0 ac	3
	Sand coating	62.5 ab	49.0 abc	3

[a]Including seeds with germinants
[b]Viable but still ungerminated after 14 weeks
[c]Germinated and with living root but no germinant formed after 14 weeks

Discussion

Of those strategies to deter bank voles from consuming beech nuts and acorns, tested in the present study, the most promising was the use of predator odour on the seeds. Application of mink excrement to beech nuts significantly reduced seed consumption by bank voles; a similar trend, although not statistically significant, was found for acorns. Seeds of both species were also handled less by the bank voles when they were treated with mink excrement. The effects of various predator odours on many species of rodents are previously described (Apfelbach et al. 2005 and references therein), but the effect on consumption when applied to seeds has – to the best of our knowledge - not formerly been documented; neither has the influence of predator excrements on germination capacity been reported in the literature. In this study, a slight reduction in germination capacity of beech nuts was demonstrated, but no significant reduction was found for acorns. However, more tests are needed to verify the consistency of these results. Germination capacity should also be tested in a field trial, since seeds in a larger volume of substrate may be less affected by the repellent, which might be leached away to a greater extent under more natural conditions (Gosling and Baker 2004).

Capsaicin in peppers creates a burning sensation in mammals' mouths (Szolcsanyi 1990). Nolte and Barnett (2000) tested capsaicin treatment of seeds of long-leaf pine (*Pinus palustris* Mill.) for its repellent effect on rodents (*Mus musculus* L., *Peromyscus maniculatus* Wagner) and found it to be effective against *M. musculus*. This is in agreement with the findings of Jensen et al. (2003), who studied the effect of capsaicin against *Rattus norvegicus* (Berk.) and *M. musculus* applied to poultry feed, and found it to be an effective repellent for both species. In the present study, treatment with chilli pepper and coconut fat reduced bank vole consumption of beech nuts, but no effect was seen on the consumption of acorns. However, although the consumption of chilli/coconut fat treated acorns did not decrease significantly, the handling of seeds was reduced for both seed species. Because the seeds were coated with pepper using coconut fat as a carrier, further work is required to determine whether the repellent effect was due mainly to the chilli or the fat. The effect of capsaicin on germination has been tested previously (Barnett 1998; Gosling and Baker 2004) with varying results depending on concentration (Barnett 1998). In the present study there was a negative effect on germination, hence, it can not, as applied here, be recommended as a repellent to deter rodents. However, it is possible that the reduced germination rate results from the coconut fat negatively affecting the permeability of the seed coat to oxygen and water (Zelawski 1960; Lamond and Levert 1980). In addition, since seeds in the chilli/coconut fat treatment were not soaked in a water-based mixture, the poor germination rate in these seeds could be linked to a lower initial level of re-hydration than occurred in other treatments.

Citronella has a strong odour that may affect the possibility of the rodents to find seeds with the assistance of their olfactory senses. In the first session of the no-choice feeding trial in the present study, the citronella/rape oils treatment significantly decreased consumption of beech nuts compared to the water treatment. This trend, although not statistically significant, was repeated in the second session, and recorded in both sessions for acorns. The seeds in this study could be located visually, and it is possible that substances working mainly through their strong smell would be more efficient on buried seeds. If the effect of the repellent is caused only by a severely unpleasant odour it would deter rodents from consuming seeds on the ground as well as buried ones. On the other hand, if the effect is caused by concealing the scent of the seeds it would work better on buried seeds, unless the rodents learn to associate the smell with food. In that case the repellent could actually help them to find buried seeds more easily. Nevertheless, regardless of the effectiveness of citronella/rape oils as a repellent, it cannot be recommended for use on beech nuts and acorns, since it impaired the germination of these seeds. However, as in the chilli treatment, citronella was applied in a fatty carrier, rather than water and the poor germination may, again, result from less re-hydration of the seed or the creation of anaerobic conditions. Biswas and Biswas (2006) reported a slight reduction in germination rate of rice seed when mixed with citronella (*Cymbopogon nardus* (L.) Rendle) oil. To be able to separate the effects of the fatty carriers from effects of the intended repellent substances further germination tests, where seeds are treated with coconut fat or rape oil only, are required.

Coating living tissue with sand has previously been used as a way to reduce feeding on seedling shoots by roe deer (*Capreolus capreolus* L.) (Bergquist and Örlander 1996) and on seedling root collars by pine weevils (Nordlander et al. 2009). The possibility of using a similar technique against seed consuming rodents was examined in the present study, but the result was unfavourable since the consumption of coated seeds increased. This could be because the sand did not create the desired barrier; the application of the coating was unsatisfactory; or the paste holding the sand contained palatable and nutritious ingredients, namely potato starch flour and sugar.

More consistent application and determination of repellent concentrations would have been facilitated by the use of pure efficient substances, e.g. capsaicin. Since the use of pesticides in forestry is strictly regulated in Sweden as well as many other countries the intention in this study was to test as ‘natural’ ingredients as possible. Furthermore, several of the substances under study needed a carrier either to remain around the seed or to spread the effect over the entire seed. However, for further studies on the substances tested in the present experiment controls for the carriers are needed to separate the effects of the carriers from the intended repellents.

In the no-choice feeding trial, each vole was twice exposed to each treatment, in order to document whether they changed their behaviour towards any of the substances after having previously encountered it, and thereby gaining experience. An increase in consumption, from the first to the second encounter, was found for chilli/coconut fat and mink excrement treated beech nuts, and acorns treated with citronella/rape oils or mink excrement, which may indicate that the effect of these substances is, at least partly, due to neophobia. In contrast, Epple et al. (1993) found no sign of habituation to coyote (*Canis latrans* Say) urine by mountain beavers

(*Aplodontia rufa* Rafinesque) over a five day period, and Nolte and Barnett (2000) reported no habituation to capsicum and thiram by *P. maniculatus* during a four-day trial. The consumption of beech nuts was lower during the first day of the experiment, which does suggest that the voles were subject to some degree of neophobia. After day two, no continued increase in consumption occurred, hence any habituation can be assumed to have been rapid. No such day-one effect was detected for acorns.

Acorns were left untouched in the cages more often than beech nuts, and the consumption of water soaked acorns was lower than that of beech nuts soaked in water. This supports previous observations that bank voles prefer beech nuts to acorns (Jensen 1985). An average bank vole requires an energy intake of approximately 54 kJ per day during the summer (Grodzinski 1985), which corresponds to about 19 beech nuts or 2 acorns (Jensen 1985). This may explain, at least partly, why a much greater proportion of the beech nuts was consumed, since the bank voles in this study had access to more acorns, but fewer beech nuts, than they needed to eat in one day.

The likelihood of habituation would be better studied in a type of trial where rodents are exposed to each substance over a longer period than in the present study. However, for animal welfare reasons a no-choice trial is not suitable for tests over long periods. For that purpose a cafeteria trial where rodents have access to several types of food would be preferable. A cafeteria trial may also simulate natural conditions better, since wild-living rodents have access to many different food sources. On the other hand, a no-choice trial gives strong indications on the potency of a substance since the rodent only have two options – handle the substance or go hungry. Therefore, to develop a repellent for use on beech nuts and acorns for direct seeding, further studies are needed both to better test the likelihood of habituation and the effect of the substance under field conditions.

The desired effect of a repellent for direct seeding is that it completely prevents rodents from interfering with the seeds. If it only stops them from consuming seeds, not from removing and caching them, the usefulness of the repellent could be completely lost, depending on where and how the rodents store the seeds. In general, rodents do not transport seeds to caches farther away than approximately 50 m, and in most cases even closer than that (Wang and Chen 2008). Those short dispersal distances indicate that the seeds do not leave the regeneration area, and that caching therefore is not a major problem. However, seedling distribution will be patchy, and caches are often located under bushes or fallen logs (Takahashi et al. 2006), which is not a favourable environment for newly germinated seedlings. In addition, few of the cached seeds survive long enough to produce a seedling (Takahashi et al. 2006).

Conclusion

In this study the formula of the substances using chilli, citronella and sand as active ingredients, complicates the interpretation of the rodent consumption and seed germination results. Therefore, controls for the carriers or alternative ways of application are recommended for further studies on these potential repellents. The mink excrement treatment led to a halving of losses of beech nuts compared to the ones soaked in water; but this still amounts to a loss of about 30% of sown seeds to rodents. In practical forestry this is not an acceptable level, and therefore to reduce rodent impact on regeneration a repellent would have to be used in combination with other measures, e.g. sowing in large open areas (Johnson and Krinard 1985), or covering the seeds with soil (Johnson 1981; Nilsson et al. 1996). Mink excrement has in the present study shown potential as a repellent against bank voles for use in direct seeding of beech and oak. However, further studies to determine the best way of application, as well as the efficiency under field conditions, are needed before definitive recommendations can be given to forest managers.

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References

Anonymous (1993) International seed testing association. International rules for seed testing. Seed Sci Technol 21, 334 pp.

Anonymous (2007) Miljömålen – i ett internationellt perspektiv, de facto 2007. Miljömålrådets uppföljning av Sveriges 16 miljömål. Naturvårdsverket, Stockholm.

Apfelbach R, Blanchard CD, Blanchard RJ, Hayes RA, McGregor IS (2005) The effects of predator odors in mammalian prey species: A review of field and laboratory studies. Neurosci Biobehav Rev 29:1123-1144.

Armour CJ (1963) The use of repellents for preventing mammal and bird damage to trees and seed: a revision. For Abstr 24:27-38.

Barnett J (1998) Oleoresin capsicum has potential as a rodent repellent in direct seeding longleaf pine. General Technical Report – Southern Research Station, USDA Forest Service 20:326-328.

Bellocq MI, Jones C, Dey DC, Turgeon JJ (2005) Does the shelterwood method to regenerate oak forests affect acorn production and predation? For Ecol Manage 205:311-323.

Bergquist J, Örlander G (1996) Browsing deterrent and phytotoxic effects of roe deer repellents on Pinus sylvestris amd Picea abies seedlings. Scand J For Res 11:145-152.

Birkedal M, Fischer A, Karlsson M, Löf M, Madsen P (2009) Rodent impact on establishment of direct-seeded *Fagus sylvatica*, *Quercus robur* and *Quercus petraea* on forest land. Scand J For Res 24:298-307.

Biswas NP, Biswas AK (2006) Use of non-edible oils as grain protectant against rice weevil (*Sitophilus oryzae* L.) and their subsequent effect on germination. Adv Plant Sci 19:653-656.

Buckley DS, Sharik TL (2002) Effect of overstory and understory vegetation treatments on removal of planted northern red oak acorns by rodents. North J Appl For 19:88-92.

Clark L (1997) A review of the bird repellent effects of 117 carbocyclic compounds. In: Mason JR (ed) Repellents in wildlife management: proceedings of a symposium. National Wildlife Research Center, Fort Collins, Colorado, pp. 343-352.

Epple GJ, Mason R, Nolte D, Campbell D (1993) Effects of predator odors on feeding in the mountain beaver (*Aplodontia rufa*). J Mammal 74:715-722.

Fedriani JM, Boulay R (2006) Foraging by fearful frugivores: combined effect of fruit ripening and predation risk. Funct Ecol 20:1070-1079.

Gosling PG, Baker C (2004) Six chemicals with animal repellent or insecticide properties are screened for phytotoxic effects on the

germination and viability of ash, birch, Corsican pine and sycamore seeds. Forestry 77:397-403.
Grodzinski W (1985) Ecological energetics of bank voles and wood mice. In: Flowerdew JR, Gurnell J, Gipps JHW (eds) The ecology of woodland rodents bank voles and wood mice. Oxford Science Publications, Oxford, pp. 169-192.
Hansson L (1993) Food preferences of voles related to post-weaning nutrition. Oikos 68:132-138.
Hayes WD (1913) Influence of birds and rodents in reforestation. Rev For Serv Invest 2:86-92.
Heroldova M, Suchomel J, Purchart L, Homolka M (2008) The role of granivorous rodents in beech forest regeneration in the Beskydy Mts. (Czech Republic). Beskydy 1:131-134.
Hulme PE, Hunt MK (1999) Rodent post-dispersal seed predation in deciduous woodland: predator response to absolute and relative abundance of prey. J Anim Ecol 68:417-428.
Jedrzejewska B, Okarma H, Jedrzejewski W, Milkowski L (1994) Effects of exploitation and protection on forest structure, ungulate density and wolf predatio in Bialowieza primeval forest, Poland. J Appl Ecol 31:664-676.
Jedrzejewski W, Rychlik L, Jedrzejewska B (1993) Responses of bank voles to odours of seven species of predators: experimental data and their relevance to natural predator-vole relationships. Oikos 68:251-257.
Jensen PG, Curtis PD, Dunn JA, Austic RE, Richmond ME (2003). Field evaluation of capsaicin as a rodent aversion agent for poultry feed. Pest Manage Sci 59:1007-1015.
Jensen TS (1982) Seed production and outbreaks of non-cyclic rodent populations in deciduous forest. Oecologia 54:184-192.
Jensen TS (1985) Seed-seed predator interactions of European beech, Fagus silvatica and forest rodents, Clethrionomys glareolus and Apodemus flavicollis. Oikos 44:149-156.
Johnson RL (1981) Oak seeding – it can work. South J Appl For 5:28-33.
Johnson RL, Krinard RM (1985) Oak regeneration by direct seeding. Alabama's Treasured For 4:12-15.
Lamond M, Levert J (1980) Influence des envelopes séminales sur l'imbibition des glands de chêne pédonculé (*Q. robur* L.) Ann Sci Forest 37:73-83.
Madsen P, Löf M (2005) Reforestation in southern Scandinavia using direct seeding of oak (*Quercus robur* L.). Forestry 78:55-64.
Myllymäki A (1975) Conventional control of field rodents and other harmful small mammals. In: Hansson L, Nilsson B (eds) Ecological

Bulletins - Biocontrol of rodents. NFR Editorial Service, Stockholm, pp. 113-128.

Nilsson U, Gemmel P, Löf M, Welander T (1996) Germination and early growth of sown *Quercus robur* L. in relation to soil preparation, sowing depths and prevention against predation. New For 12:69-86.

Nolte DL, Barnett JP (2000) A repellent to reduce mouse damage to longleaf pine seed. Int Biodeterior Biodegrad 45:169-174.

Nordlander G, Nordenhem H, Hellqvist C (2009) A flexible sand coating (Conniflex) for the protection of conifer seedlings against damage by the pine weevil *Hylobius abietis*. Agric For Entomol 11:91-100.

Nystrand O (1998). Post-dispersal predation on conifer seeds and juvenile seedlings in boreal forests. (Doctoral dissertation, Swedish University of Agricultural Sciences, 1998), 103 p. (ISBN: 91-576-5345-3)

Olsson GE, White N, Hjälten J, Ahlm C (2005) Habitat factors associated with bank voles (*Clethrionomys glareolus*) and concomitant hantavirus in northern Sweden. Vector-Borne Zoonotic Dis 5:315-323.

Shaw MW (1968) Factors affecting the natural regeneration of sessile oak (*Quercus petrea*) in north Wales – II. Acorn losses and germination under field conditions. J Ecol 56:647-660.

Sullivan TP (1979) The use of alternative foods to reduce conifer seed predation by the deer mouse, (*Peromyscus maniculatus*). J Appl Ecol 16:475-495.

Sullivan TP, Crump DR, Sullivan DS (1988) Use of predator odors as repellents to reduce feeding damage by herbivores. J Chem Ecol 14:363-377.

Szolcsanyi J (1990) Capsaicin, irritation, and desensitization – neurophysiological basis and future perspectives. In: Green BG, Mason JR, Kare MR (eds) Chemical senses Vol 2 Irritation. Marcel Dekker Inc., New York, pp. 141-168.

Takahashi K, Sato K, Washitani I (2006) The role of the wood mouse in Quercus serrata acorn dispersal in abandoned cut-over land. For Ecol Manage 229:120-127.

Tanton MT (1965) Acorn destruction potential of small mammals and birds in British woodlands. Quart J For 59:230-234.

Vander Wall SB (1998) Foraging success of granivorous rodents: effects of variation in seed and soil water on olfaction. Ecology 79:233-241.

Wang B, Chen J (2008) Tannin concentration enhances seed caching by scatter-hoarding rodents: An experiment using artificial 'seeds'. Acta Oecol 34:379-385.

Ylönen H, Pech R, Davis S (2003) Heterogeneous landscapes and the role of refuge on the population dynamics of a specialist predator and its prey. Evolut Ecol 17:349-369.

Zelawski, W (1960) Przyczynek do poznania roli owocni i łupiny nasiennej w wymianie gazowej nasion buka. Sylwan 104:69-78.