

RESEARCH ARTICLE

Human urine does not protect acorns against predation by the wood mouse (*Apodemus sylvaticus*): a field study with video recording

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Direct seeding is a revegetation method that can offer great advantages for forest restoration, but suffers the drawback of seed loss due to granivorous rodents. Thus, to make direct seeding a useful and scalable forest restoration method, we need to find ways to protect seeds against rodents. Scents produced by carnivorous or omnivorous animals that elicit a fear response on rodents are a promising group of substances that can be used to protect seeds and that have demonstrated effectiveness in a number of cases. Here, I test if human urine has a protective effect on Holm oak (*Quercus ilex*) acorns against predation by mice. Human urine would be much easier and cheaper to obtain than the urine of other animals, providing the possibility, if effective, to scale the application of a repellent substance. I set up a field experiment in an area with high wood mouse (*Apodemus sylvaticus*) abundance and used eight urine donors. Neither urine nor donor had any effect. Acorn removal was very fast and high, reaching 97.2% after 7 days. Video recording with camera traps showed that the wood mouse was the main predator, but the Eurasian jay also removed acorns. I conclude that human urine is not useful as a repellent against mice and suggest to focus efforts in finding other substances to repel seed predators.

Key words: direct seeding, fear response, forest restoration, repellent substances, revegetation, rodent predation

Implications for Practice

- Direct seeding of acorns can be an efficient method to restore oak forests, but for that, it is necessary to protect seeds against predation by rodents.
- Predator scents derived from excrements, urine, or glands can elicit a fear response in rodents and act as repellents to protect acorns.
- However, human urine did not provoke a fear response in the wood mouse, indicating its inefficiency in protecting acorns.
- The research on animal-derived scents to deter seed predators should focus on other substances and discard human urine as a candidate.

Introduction

The restoration of the planet's forests has become an activity of enormous relevance for the recovery of biodiversity, climate change mitigation, human well-being, and ultimately the health of a biosphere that has reached unprecedented levels of ecosystem destruction (Castro et al. 2021; FAO 2022). To achieve this on the spatial and temporal scales that are necessary today, we must find reforestation methods that are efficient both from an ecological and economic standpoint (Castro et al. 2021). One of the main aspects that determine cost and success is the choice of revegetation method (Lázaro-González et al. 2023). Direct

seeding, that is, the burial of the seed directly in the site where the adult plant should be, is currently a minority revegetation method for forest restoration compared to the most common outplanting of nursery-grown seedlings (Grossnickle & Ivetic 2017; Leverkus et al. 2021; Lázaro-González et al. 2023). However, direct seeding can offer relevant advantages over planting under certain circumstances, given that it reduces (or even eliminates) the risk of transferring diseases into natural ecosystems, allows a proper development of the root system (which may suffer permanent deformities in plants cultivated in forestry containers in nurseries), and has a minimum impact on the restored ecosystem (Castro et al. 2015; Grossnickle & Ivetic 2017; Leverkus et al. 2021). Direct seeding also has a substantially lower cost than outplanting seedlings (Löff et al. 2019; Pérez et al. 2019; Raupp et al. 2020). Moreover, seedling cultivation, transportation, and planting may not only be more costly, but also operationally cumbersome in remote areas or even in non-remote areas which are difficult to access (see reviews in Grossnickle & Ivetic 2017; Löff et al. 2019 for a summary of the advantages

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offered by direct seeding). Nonetheless, direct seeding suffers from the disadvantage of a greater loss of propagules, especially due to seed predation. This is particularly true for plant species that produce large seeds, such as oaks, whose seeds usually suffer from high predation rates by vertebrates because of their high energy and nutrient content and, in addition, are produced in smaller quantities given the trade-off between quantity and size, making seed supply for restoration more restricted. In fact, high predation rates are a major reason for discarding direct seeding as a revegetation method (Dey et al. 2008; Löf et al. 2019; Leverkus et al. 2021).

Direct seeding has therefore the potential to be a promising method for forest restoration, but for this to occur, we must find methods that substantially reduce seed predation by vertebrates. Methods to protect seeds against predators are variable, from physical structures to chemical substances (Löf et al. 2019; Brown et al. 2021; Villalobos et al. 2023). In general, useful seed protectors should satisfy four conditions: be easy to implement, innocuous for the biota but efficient against seed predators, not negatively affect germination or seedling establishment, and cheap enough to be competitive with alternative procedures such as planting seedlings. In this sense, repellent substances that create an aversive reaction in seed predators could be one of the best solutions, as they can be cheap and easy to apply in comparison to physical, ecological, or silvicultural methods (Löf et al. 2019). One such group of substances with a high potential for use are scents from meat-eater animals (either obligate carnivores, mesocarnivores, or omnivorous animals) that prey on seed predators, such as the odor from excrements, urine, or anal glands. Numerous studies document how the scents produced by a wide range of predators, such as several species of canids (coyote, foxes, wolf, dog, etc.), felines (lynx, bobcat, lion, jaguar, cougar, etc.), mustelids (mongoose, stoat, mink, ferret, etc.) or omnivorous, like the brown bear, provoke an avoidance reaction in their prey, including mice and other small rodents like voles and pocket gophers (reviewed in Apfelbach et al. 2005; Murray et al. 2006; Parsons et al. 2007; Ruiz-Tagle et al. 2021). It seems that the aversive response in the prey is mediated by common cues related to the meat consumption of the predators, which produce a higher concentration in their excrements, urine, or secretions of sulfurous metabolites derived from the digestion of the meat with respect to animals with a non-carnivorous diet (Nolte et al. 1994; Sündermann et al. 2008). Herbivores and rodents will detect that the scent comes from an animal that consumes meat, therefore being a potential predator, which triggers a fear response. Finding substances of animal origin that could be easy to obtain and use and are cheap and efficient would help solve the problem of seed predation in direct seeding.

Predator scents tend to be more efficient in eliciting a fear response in their preys when both predator and prey have evolved in sympatry or, at least, have been sharing the habitat for a long time even if their evolutionary history differs (Blumstein et al. 2002; Apfelbach et al. 2005; Parsons et al. 2007). In this sense, human urine has the potential to be a useful substance to protect seeds against rodent predation. Although humans do not consume mice today, they might have

done in the paleolithic (e.g. Calatayud et al. 2020), and in any case their omnivorous diet might still trigger a response in these animals. Moreover, humans have been chasing rodents for millennia to prevent their own food sources (e.g. grains or other source of food that could be temporally stored and that could be consumed by rodents), an activity that very likely predates the neolithic period. Human urine is massively produced on a global scale and could be extraordinarily easy to obtain and handle for its use as a repellent at a price much below the price of other animal scents. Consider, for example, the ease with which human urine can be used compared to that of other species like lynx or foxes. However, the effect of human urine as a potential repellent against herbivores or seed predators has been, surprisingly, studied little, and the information we have to date is not only scarce but also inconclusive when considering the heterogeneity of results and methodological issues. For example, Rosell (2001) found no effect of human urine as a repellent of the gray squirrel (note also that this study is erroneously listed in a review work [Apfelbach et al. 2005] as the only one, to that date, with human urine having a repellent effect). Blumstein et al. (2002) also did not find an effect of human urine on wallabies, but the study was done with a urine donor with a vegan diet trying to emulate the urine of an herbivore, not of a predator. Parsons et al. (2007) also did not find an effect of human urine on gray kangaroos, but the animals were in semi-wild conditions, their food source was supplemented by humans, and the owner of the area even hand-reared some of the kangaroos, which may have created a positive attraction of the animals to human presence instead of a fear response. More specifically, in relation to mice, Rivard et al. (2014) found no response of the house mouse to human urine in a laboratory experiment, but the mice used were from a laboratory strain bred by humans from a century ago, with the risk of the loss of genetic response to human presence and socialization of the pups with caretakers. Swihart et al. (1991) found no effect of human urine on white-tailed deer herbivory when topically applied to Japanese yew saplings. Nonetheless, the data reported in this work (Fig. 2 of the study) suggest a potential effect of the human urine when compared to a control or to the urine of an herbivore (rabbit) that might not have been detected by the power of the nonparametric test used for statistical analysis. These are, to the best of my knowledge, all the peer-reviewed studies published on the effect of human urine as a repellent for herbivorous and granivorous mammals. As indicated, the heterogeneity of target species and methodological issues make it impossible to make conclusions on the potential effect of human urine as a repellent for rodents, even if all the studies listed above showed no effect. Besides, human scents or products (hair) have proven effective against animals like the Eurasian beaver or the wild hog (Rosell & Czech 2000; Kamsano et al. 2018), supporting the potential effect of human scents against mammals.

In this study, I seek to test if human urine produces an aversive response in the wood mouse (*Apodemus sylvaticus* L.), a widespread rodent species in Europe and one of the main post-dispersal acorn predators in this region (Mitchell-Jones et al. 1999; Sunyer et al. 2013). Oaks are a major component of Holarctic forest and a major group of species used to restore

forests in this area of the planet (Löff et al. 2019). However, acorn sowing is an impractical revegetation method unless the acorns are protected against rodents (Dey et al. 2008; Tobing et al. 2018; Löff et al. 2019). Here, I hypothesize that human urine will repel wood mice. I also hypothesize that mice will be the main agent of acorn removal. To test this, I conducted a field experiment exposing the acorns to the surface in fenced study plots, two conditions that facilitate the encounter of the mice with the acorns, therefore helping to focus on the effect of the experimental factor (urine). In addition, I used camera traps to ascertain the animals that provoked seed loss and to get a finer measure of the timing of seed removal or predation. Overall, this study seeks to find an easy to use, cheap, and universal substance to protect seeds against predators in forest restoration programs via seed sowing.

Methods

Study Area and Experimental Design

The study was carried out in Loma de los Panaderos (Trevenque site, Sierra Nevada National Park, SE Spain; 37°5'N, 3°28'W)

using three fenced areas of circa 3500 m² each that were built in 1997 for research purposes (Castro et al. 2002). I used fenced areas (plots, hereafter) to avoid the consumption of acorns by animals other than rodents such as wild boars, Spanish ibex, or domestic goats and sheep, that are common in the study site. In addition, it is common that the rodent population is higher in fenced areas as a result of the protection that these structures offer them against their own predators, such as carnivores or the wild boars. Plots were separated each other by circa 100 m (Fig. 1) and have a slope ranging between 5 and 15° approximately. The vegetation is composed mostly of shrubs and scattered pine trees (*Pinus sylvestris* L. and *P. nigra* Arnold), with a predominance of *Salvia lavandulifolia* Vahl (Lamiaceae), *Prunus ramburii* Boiss., *Crataegus granatensis* Boiss. (Rosaceae), *Berberis hispanica* Boiss. & Reuter (Berberidaceae), and *Cytisus scoparius* (L.) Link.

At each plot, I established 40 sampling points distributed randomly across the surface of the plots (Fig. 1), and at each sampling point, we placed three acorns of Holm oak (*Quercus ilex* L.) on the soil surface, with a 2–3 cm separation between each acorn. Half of the sampling points were ascribed to the Urine treatment, where 100 mL of human urine was poured on

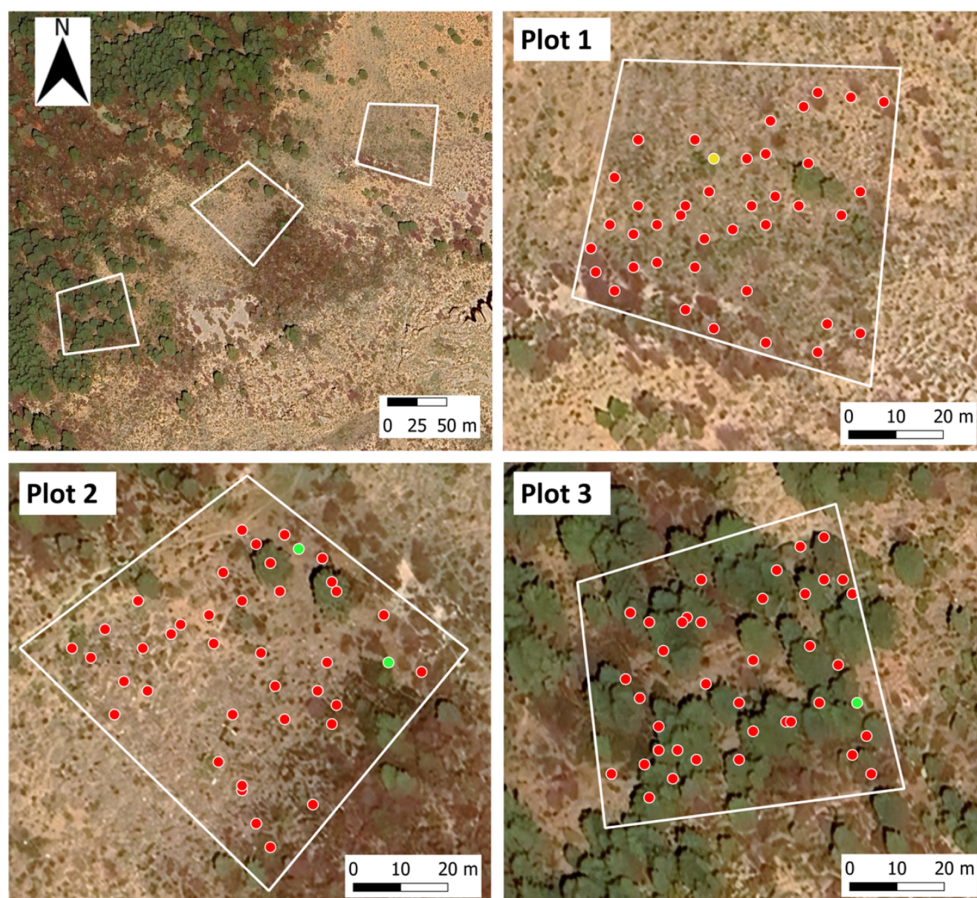


Figure 1. Study plots. The white lines mark the fence boundaries of each plot. The dots mark the sampling points: red, points where all three acorns were depredated; yellow, point where one acorn was depredated; green, points where no acorn was depredated. The area is 0.38, 0.37, and 0.33 ha for plots 1, 2, and 3, respectively.

top of the acorns once they were placed on the ground, and the other half were ascribed to a Control treatment, where a similar amount of tap water was poured on top of the acorns. Note that the amount of urine added is much higher than that used in previous experiments (e.g. 10 mL in Blumstein et al. 2002; 12 mL in Parsons et al. 2007; 3 mL in Rivard et al. 2014), seeking to create ideal odor conditions for testing the study hypotheses. The whole experiment therefore contained 180 acorns per urine treatment (Control vs. Urine; 3 plots \times 20 sampling points \times 3 acorns per sampling point; 360 acorns in total). Sampling points of each urine level were intermingled over the surface of each experimental plot and were usually at least 10 m from each other (Fig. 1). Human urine was collected in the week prior to the experiment, and stored in 100 mL plastic bottles frozen at -20°C . The bottles were taken out of the freezer the night before the experimental setup. The urine samples came from eight people (factor "Donor," hereafter) with an omnivorous (six) or vegetarian (two) diet and with a similar number (seven to eight) of bottles per person. The different donors were distributed evenly across the three plots. The experiment was set up on February 16, 2023, from 10:00 to 15:30 hours. The acorns used were collected in November 2022 from 10 different maternal trees with a similar number of acorns per maternal tree and stored in the refrigerator at 4°C until the conduction of the study. Prior to the experimental setup, nonviable acorns (empty or preyed upon by insects) were eliminated by floating them in water. From that moment onward, the acorns were manipulated with disposable latex gloves to avoid human odor. The fresh weight of the acorns was 9.02 ± 0.38 g ($n = 50$). The study site was selected given that I knew from previous studies that the rodent population is usually high in the area. In addition, I could confirm a high density of mice tracks after it snowed a few days before the experimental setup.

Acorn Removal Monitoring

Acorn removal was monitored by two complementary procedures: (1) in situ sampling and (2) video recording using camera traps (see below). In situ sampling was done by noting the number of acorns that were missing in each sampling point on day 4 and day 7 after the experiment was mounted. Acorn removal by day 7 was almost total (see Section 3) and therefore, the experiment was considered finished by that time. In some points, there were remains of acorn predation (debris of acorn shells). Nonetheless, with this sampling, I cannot ascertain if any particular acorn was removed or consumed, and therefore all acorns were categorized as "removed" in the in situ sampling. Whether the acorn was consumed later, abandoned, or cached by the animal is something that I cannot determine with this experiment.

Video Recording of Animal Activity

Animal activity was directly monitored using 14 camera traps with both day and night vision (Moultrie M-990i [13 cameras] and Moultrie M-990i Gen2 [1 camera], Moultrie Products, AL, U.S.A.). This allowed us to sample with high precision and detail the removal of a fraction of the acorns, as well as identify

the animals that handled them. Cameras were installed on 75 cm tall, 3×3 cm wide wooden poles, at circa 1.20 m from the sampling points, half in urine-treated sampling points and half in control sampling points, and evenly distributed between the three plots. Night recordings lasted 20 or 30 seconds, whereas daylight recordings usually lasted 60 or 90 seconds. A first trial of video recordings was set up just after the experimental setup, lasting 4 days. On day 4 (coinciding with the moment of in situ sampling of acorn removal), I changed the cards of the cameras and kept recording for 3 more days. At that moment, the cameras of the sampling points where all acorns were removed (12 in total) were installed in new sampling points, still having the maximum number of acorns as possible (10 replaced in points with 3 acorns, 1 in a point with 2 acorns, and 1 in a point with 1 acorn), seeking to optimize the recording of events of animal activity. This renders a total of 26 sampling points monitored with camera traps. The videos were later visualized in the lab noting the following variables for each of them:

- (1) Species identity and number of individuals per video.
- (2) Period of animal activity, that was categorized as (1) day or (2) night according to the vision used by the camera (that matched correctly with what could be considered day and night by the human eye).
- (3) Animal handling of the acorns, that was categorized as (1) removal (if the animal took the acorn and disappeared from the recording area transporting it) or (2) consumed, if the animal consumed the acorn in situ (either totally or partially).
- (4) Time needed for the first acorn handling by the animal (Time for first handling). For that, I established a common time for all the cameras as a baseline, that was 17:00 hours of the day of the experimental setup, thus 1.5 hours after we left the study area with the full experiment mounted. For example, if the first acorn of one sampling station was removed at 2:00 hours on day 17, the time for first handling would be 9 hours from then. This variable provides information on how much time is needed for the mice to exploit the sampling points. Although the number of sampling points recorded raised to 26, some of them did not have three acorns when the camera was installed. Altogether, this reduced the number of sampling points with appropriate conditions for the analysis of the Time for first handling to 23.
- (5) Time elapsed for second or third encounter (Time since first encounter). This variable was used as a measure of the time spent by mice to remove a second or a third acorn from the sampling points once the first one was removed. For that, I took as baseline the time of the first removal and measured the time elapsed (in minutes) for the removal of subsequent acorns.

Data Analysis

Acorn removal from direct in situ sampling was analyzed with a generalized linear model (GLM) considering Plot and Urine as fixed factors with a binomial distribution and a logit link

function. The response variable was the probability of having acorns removed versus the probability of acorns remaining for each sampling point. The effect of the donor was analyzed separately in another GLM considering it as a fixed factor using the same distribution. Urine donor might be considered a random factor, but the strong imbalance of data precluded the construction of a full, generalized mixed effects model (only 3 out of 120 points had no predation). I report the data for day 4 and day 7, although the analyses here presented were restricted to day 7 (end of the experiment) for simplicity and considering that the patterns were similar through time. Data from video cameras were analyzed on a per-video basis, discarding all the videos that did not record any animal activity. Specific analysis for some of the parameters measured is indicated in Results. Analyses were done with package Stats of R version 4.1.2.

Results

Acorn Removal from In Situ Sampling

Acorn predation was very high, reaching 84.2% by day 4 and 97.2% by day 7. Non-removed acorns (10 in total) were concentrated in three sampling points where rodents did not remove any of them plus a sampling point where they left a single acorn (see the dots of different colors in Fig. 1 for removal rate in the sampling points). Neither Plot or Urine treatment had a significant effect on removal rate after 7 days (Table 1), and the donor

Table 1. Summary of the results of the generalized linear model for testing the effect of Urine and Plot on the probability of having acorns removed versus the probability of acorns remaining after 7 days. Level *Plot 1* from the factor Plot, and level *Urine [No]* from the factor Urine are included in the intercept.

Predictors	Odds ratios	p
Intercept	84.08	<0.001
Plot (2)	0.16	0.091
Plot (3)	0.33	0.336
Urine (yes)	2.41	0.210

Table 2. Summary of the species that were recorded by the camera traps. Percentages in the column “#Videos” represent the percentage of videos where each species was recorded from those videos where animal activity was present (thus removing videos without animals).

Species	# Videos	Time of presence (seconds)	#Acorns removed	#Acorns predated	#Videos daylight	#Videos night
Wood mouse (<i>Apodemus sylvaticus</i>)	218 (78.9%)	3158	48	6	0	218
Rock bunting (<i>Emberiza cia</i>)	24 (8.7%)	403	0	0	24	0
Vole (likely <i>Pitymys duodecimcostatus</i>)	7 (2.5%)	261	0	0	4	3
Ring ouzel (<i>Turdus torquatus</i>)	6 (2.1%)	124	0	0	6	0
Eurasian jay (<i>Garrulus glandarius</i>)	5 (1.8%)	155	3	0	5	0
Eastern subalpine warbler (<i>Sylvia cantillans</i>)	5 (1.8%)	45	0	0	5	0
Common Blackbird (<i>Turdus merula</i>)	4 (1.4%)	44	0	0	4	0
Lizards (unidentified)	4 (1.4%)	106	0	0	4	0
Fox (<i>Vulpes vulpes</i>)	2 (0.7%)	6	0	0	1	1
Alpine accentor (<i>Prunella collaris</i>)	1 (0.4%)	18	0	0	1	0
Non-identified	1 (0.4%)	1	0	0	0	1

had no effect either ($p > 0.05$ for all the donors with respect to the intercept [Control]). Interestingly, by day 4 the proportion of acorns removed or predated in sampling points with camera traps was higher (3.0 ± 0.0 acorns per sampling point, that means, 100% of acorns) than in sampling points without camera traps (2.46 ± 0.11 acorns; 82% of the acorns), although differences were not statistically significant ($p = 0.4762$, GLM with exponential distribution and reciprocal link function).

Animal Activity From Video Recordings

A total of 1440 videos were recorded (1377 minutes in total). Animal activity was registered in 276 videos (19.18% of the videos; the rest shuttled by other factors, likely wind-moved vegetation), 54 during the daytime and 222 during the night time. Of those, the most abundant animal recorded was the wood mouse (recorded in 78.9% of the videos, always at night), whereas a total of nine additional species (two mammals, six birds, and one reptile) were recorded in the rest of the videos (Table 2). The number of individuals recorded per video was one, except in six cases, where two birds (four videos) or two mice (two videos) appeared in the same recording. Unexpectedly, a fox was recorded two times, indicating that this predator could cross the fence. Nonetheless, no ungulates like goats or wild boars that may consume acorns were recorded at any moment.

Acorns handled (removed or consumed) by animals were recorded in 57 occasions, either by the wood mouse (54 acorns; 94.7%) or the Eurasian jay (3 acorns; 5.3%; Fig. 2). Of the 54 acorns handled by the wood mouse, 48 (88.9%) were removed and transported to an unknown site outside the visual frame of the camera, whereas 6 (11.1%) were consumed in situ. In the case of the Eurasian jay, the three acorns were removed; all the three acorns belonged to the same sampling point. Thus, overall, of the 57 events recorded of animals handling the acorns, 6 (10.5%) were direct predation, whereas the remaining 89.5% should be considered removed but with an unknown fate (likely immediately consumed in other location, abandoned, or cached).



Figure 2. Eurasian jay (*Garrulus glandarius*) and wood mouse (*Apodemus sylvaticus*) removing acorns. These were the two species that handled (removed or predated) the acorns in the study area. Note that the study was conducted inside fenced plots, blocking the access of larger acorn predators like wild boars or goats. In the case of the mouse, we can observe how the animal remove the second acorn (center image) and the third (last) acorn of the same point (right image).

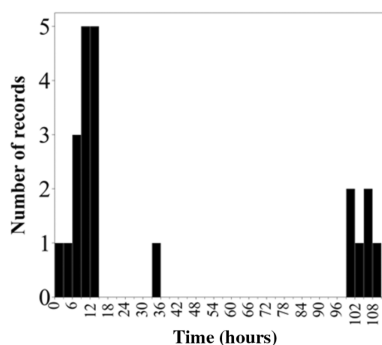


Figure 3. Temporal pattern for the handling of the first acorn by the wood mouse in the sampling points where camera traps were installed (points with urine or without urine pooled). There were a total of 22 sampling points where records were obtained. The bimodal distribution coincides with the time when camera traps were moved from points without acorns to new points that still had acorns by day 5 (see text for further explanations).

The time required for the handling of the first acorn by mice was recorded in 22 of the sampling points and showed a bimodal distribution (Fig. 3). In 68.2% of the cases, the first removal occurred within the first 24 hours (actually, during the first night after experimental setup), whereas in 27.3% of the sampling points, the first acorn was handled on night 5, coinciding with the day of the change of the cameras to these positions (Fig. 3).

There were marginally significant differences in the time required for the first handling by mice between urine-treated and control acorns (one-way analysis of variance, $F = 4.21$, $df = 1$, $p = 0.0533$; log-transformed data), although the time was shorter for urine-treated acorns (mean of 20.1 ± 9.1 hours) than for control acorns (53.6 ± 14.6 hours). Once the first acorn was handled, the following were handled quickly, with an average of 50.4 ± 21.9 minutes needed to remove or predated the other two acorns of the sampling point (data pooled for timing needed to handle the second or the third acorn with respect to the first one). In the case of the jay, this species removed the acorns of the single point exploited in day 5 (113.9 hours after experimental setup), and it took 6 minutes to remove the three acorns once the first was removed.

Discussion

The results show that, contrary to the initial hypothesis, the human urine had no effect as a repellent against the wood mouse, a scatter-hoarder rodent whose acorn predation activity, alone or in combination with other mice species, is one of the main limiting factors for the success of oak forest restoration via direct seeding (Birkedal et al. 2009; Leverkus et al. 2013; Löf et al. 2019). We must be aware that in most cases, the acorns were removed from the site but not consumed in situ, a common pattern in this and other species of rodents (e.g. Perea et al. 2012; Sunyer et al. 2013). A fraction of the removed acorns could be cached and not consumed immediately (e.g. Perea et al. 2012; Nóbrega et al. 2023) still allowing for possible seedling emergence. Nonetheless, the survival of removed acorns by the wood mouse and other scatter-hoarder mice is generally low (Gómez et al. 2003; Nóbrega et al. 2023), and in any case the conclusion from a restoration point of view remains identical, that is, a lack of effect of human urine to protect the acorns for direct seeding operations.

The results also confirm with high precision that, at least in a situation of high rodent abundance, these animals are the main acorn predators, that their activity is very fast, and that they handle a very high proportion of the seeds (Birkedal et al. 2009; Perea et al. 2012; Leverkus et al. 2013). The video recordings show that circa 70% of the acorns were predated or removed within the first night after the experimental setup (Perea et al. 2012; Villalobos et al. 2023 for similar removal rates within the first days). The in situ field sampling conducted after 4 days showed that, by that time, 84% of the acorns were removed or predated, a number that rose to 97% in only 1 week. Moreover, once the first acorn was handled, it took the rodent less than 1 hour to remove the remaining acorns in that specific point. This suggests a very philopatric behavior of the wood mouse that will consistently visit the same sampling point once it is found and will remove all its acorns. A similar pattern was found for the only point visited by the Eurasian jay, that removed the three acorns in 6 minutes. This provides information that can be useful for restoration planning. A common practice in direct seeding operations is to seed more than one acorn per sowing point to increase the changes of seedling recruitment

per point. Our results suggest that this might be useful from the point of view of the hazards related to seedling survival but has little sense from the point of view of seed predation as it is very likely that the predators will remove all the acorns once the sampling point is discovered. Thus, in this sense, it might be more recommendable to increase the number of sowing points with a single acorn than to put the same number of acorns in a lower number of sowing points. We have to consider, nonetheless, that in this study the acorns were exposed on the surface. Acorn burial, as will be the case in most direct seeding operations, reduce seed predation by rodents (e.g. Pérez-Ramos & Marañón 2008; Perea et al. 2012; Tobing et al. 2018), which still may support the practice of seeding more than one acorn per point.

The fact that the pattern of acorn handling recorded with video cameras followed a bimodal distribution coincident with camera re-ubication suggests that the mice could be using some cue from the researchers to find the sampling points. This is also supported by the higher percentage (actually, total, 100%) of removed or predated acorns by day 4 in sampling points where camera traps were present versus a lower proportion in sampling points without camera traps (82%), despite differences being nonsignificant (a result very likely motivated by the extremely unbalance of the data). A plausible explanation is that we left a strongest human scent in these points that could attract the wood mice, as the installation of the camera traps took around 15 minutes each. If the mice do not perceive humans as a danger, our scent could actually have the opposite effect than expected (Duncan et al. 2002). In addition, another nonexclusive explanation could be related to the camera traps themselves. The model used is cataloged by the manufacturer as no-glow, a kind of camera that uses invisible light for mammals during night recordings. However, in previous studies with this batch of cameras (e.g. Castro et al. 2017, 2022) we could observe a very weak red light that switches on while recording at night, which could attract the attention of the mice. In the case of this study, I am confident that this does not preclude the conclusions about the effect of human urine on wood mouse behavior, as the effect of the cameras would be the same for sampling points with or without urine and, in addition, the predation rate among urine levels did not differ in the whole dataset, which includes much more sampling points than those with video cameras. Nonetheless, the results support that the type of cameras used should be carefully considered when planning studies with video recording mammal activity at night.

The results also show that acorn removal was also conducted by the Eurasian jay, and not only by rodents, an observation that has been rarely reported (but see Perea et al. 2012). The role of jays is small in this case, as they removed the acorns from only one point, totaling 5.4% of the acorns removed versus the 94.6% removed by the wood mouse (percentage referred to acorns whose removal agent was recorded; see Perea et al. 2012 for similar values). However, a bigger role for jays cannot be discarded in a more general context. The experimental setup ended in the afternoon, and until this time, the researchers were moving around the area, making it difficult for there to be any animal activity. Given that the mice removed or preyed on circa 70%

of the acorns in the first night (percentage referred to those recorded by video cameras), the potential activity of jays is strongly reduced after the first night, when they might begin their foraging activity. In addition, the study area clearly seems to have a high population of mice according to the video records and previous observations of tracks in the snow, but rodent population may fluctuate and be lower in other sites or moments, where perhaps jays could have a stronger presence and effect on the study. However, the studies on post-dispersal acorn predation that relies on animal marks but without direct observations tend to ascribe seed losses to rodents and to a lesser extent to other mammals such as wild or domestic goats, deer, or wild boars (Gómez et al. 2003; Puerta-Piñero et al. 2010; van Ginkel et al. 2013), whereas the potential role of jays as acorn removers in this context has been virtually neglected. I advocate that further research would be worthy to ascertain the role of the Eurasian jay as a potential vector of acorn removal in studies of post-dispersal acorn predation, a task that is nowadays facilitated with the use of camera traps.

The studies about the effect of human urine as a potential substance to repel herbivores and granivorous rodents are very scarce and, as indicated above, are very heterogeneous in terms of methodological issues regarding the target species, experimental design (e.g. lab vs. field studies) or even the consideration of humans as a source of fear (e.g. Blumstein et al. 2002 used the urine of a vegan person trying to emulate the urine of an herbivore; see references in Introduction). In this work, I test the effect of urine with an experimental setup that somehow forces the conditions to detect a potential signal from this substance. The experiment was conducted under field conditions in an area with a high density of mice; it used eight urine donors who included or did not include meat in their diets, and it used an unusually large amount of urine per sampling point. Moreover, the spatial scale of the study, conducted in three plots of circa 3500 m² each and separated by around 100 m from each other, ensured that a large number of mice were involved in seed handling, as the normal density of the wood mouse in Mediterranean ecosystems is around several dozen per hectare (e.g. Rosalino et al. 2011). This, together with the almost complete removal of the acorns, rules out potential confounding effects derived from individual personality (Feldman et al. 2019; Boone et al. 2022) and makes the results generalizable for the study system. Under all these circumstances that simulate more realistic conditions than previous studies, the results still show no effect of human urine. I therefore suggest the conclusion that human urine is not useful to protect acorns from mice predation and that we may focus our efforts in finding alternative repellents.

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