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Can cover crops reduce rabbit-induced damages in vineyards in southern Spain?

Isabel C. Barrio, Rafael Villafuerte & Francisco S. Tortosa

Damage caused by wildlife foraging can lead to significant agricultural losses and the problem can be further complicated if the damage-inducing animal is a valuable resource in its own right. Provision of alternative food sources such as cover crops might be a means of reducing the damage which appears to be linked to scarcity of alternative foods in intensively-managed agroecosystems. Cover crops may provide other benefits to agroecosystems, i.e. preventing soil erosion but can potentially have some undesired consequences, i.e. water competition with the cash crop. In our study, we tested the effectiveness of cover crops in reducing the damage caused by foraging European rabbit Oryctolagus cuniculus to vineyards in a semi-arid agroecosystem in southern Spain. Experimental treatments consisted of a combination of the presence/absence of sown cover crops (70% oat Avena sativa and 30% garden vetch Vicia sativa) with/without rabbit exclusion. In the 2009 growing season, we assessed rabbit-induced damage using a browsing index on vine shoots, rabbit use of plots was estimated based on faecal pellet counts and grapevine vield was measured at harvest. Rabbits ate the cover crops, and rabbit use was highest in the plots sown with the oat and vetch cover crop. However, the effect of the presence of the cover crop on the amount of damage caused by rabbits was limited and, moreover, the presence of the cover crop had a negative effect on grapevine yield. Exclosure fences effectively reduced rabbit damage by keeping rabbit densities close to zero, but even a low rabbit number (~ 1 rabbit/ ha) can cause significant damage. Although cover crops provided rabbits with an alternative food source, they acted as attractants for rabbits and were not effective in reducing the damage caused to vineyards by higher rabbit numbers. Therefore, adding cover crops might not be an effective measure in controlling rabbit-induced damage in semi-arid wine-growing regions.

Key words: browsing, European rabbit, intercropping, Oryctolagus cuniculus, pest control, Vitis vinifera, yield

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In intensively human-modified landscapes, game species can be agricultural pests whose foraging can lead to significant agricultural losses, especially in semiarid landscapes where agriculture and game-hunting industries coexist. In some semi-arid agroecosystems in southern Spain, where vineyards are one of the most important crops in terms of income, employment and environmental impact, these crops are compromised by the damage caused by foraging wild European rabbits *Oryctolagus cuniculus*. In the region, the damage caused by rabbits might be exacerbated by a reduction in the availability of alternative foods (e.g. arable weeds) caused by agricultural intensification (Barrio et al. 2010b).

In this sense, cover crops, i.e. sowing lines of herbaceous vegetation within valuable crops, might represent an alternative food source for the damaging pest and may thus provide a means of reducing wildlife-induced damage to crops (Barrio et al. 2010b). Cover cropping can be a means of reversing the loss of diversity in intensively managed agroecosystems by increasing plant diversity and landscape heterogeneity (Storkey & Westbury 2007). Besides, cover crops can be a benefit to agroecosystems by reducing soil erosion, improving nutrient and water retention in soils, and activating soil processes (Steenwerth & Belina 2008). Some studies have examined the effects of cover crops with an emphasis on invertebrate pest control (Nicholls et al. 2001, Thomson & Hoffman 2009), but less is known about the effects of these measures on vertebrate pests (Ingels et al. 2005).

Vineyard cover cropping is used widely in the world's winegrowing regions, particularly in areas that have summer rainfall or use irrigation as a means of controlling vigour and enhancing wine quality (Chaves et al. 2007). The benefits of intercropping in vineyards have been as well demonstrated in areas that experience low rainfall in summer and high evaporative water loss (Monteiro & Lopes 2007, Lopes et al. 2008, Steenwerth & Belina 2008), but winegrowers remain concerned about excessive competition for water between swards and vines (Celette et al. 2005). That said, the addition of alternative food sources such as cover crops over long periods to divert animals away from the cash crop might pose other problems. The addition of a concentrated supplemental food source might lead foraging animals to congregate in large numbers, which might cause the food supply to be temporarily exhausted and lead to an overall increase in the damage inflicted on the cash crop (Calenge et al. 2004, Sullivan & Sullivan 2004). The problem can become particularly acute when supplemental food is made available in winter or whenever natural foods are scarce (Conover 2001). In addition, some cover crops might provide refuge for the pest species (Ingels et al. 2005) and increase the potential for damage to vineyards (R. Villafuerte, pers.obs.).

In this study, we evaluated experimentally the effect of the presence of cover crops on the damage caused to vineyards by rabbits in a semi-arid agroecosystem in southern Spain. Specifically, we assessed the effect of the addition of cover crops on local rabbit use of plots and the extent of the damage

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they caused to vines. Secondly, at the level of the individual plant, we evaluated the effect of cover crops on the severity of the damage caused to vines and the impact on grapevine yield. If cover crops act as a food source for rabbits and damage is driven by scarcity of alternative foods, i.e. the Food-limitation Hypothesis (Barrio et al. 2010b), cover crops will reduce the damage to vineyards caused by rabbits; however, if cover crops primarily act as attractants for rabbits and increase their abundance locally, the result might be an increase in the extent of the damage caused to vineyards by rabbits.

Methods

Study area and experimental design

Our study was conducted in a semi-arid agricultural landscape in the province of Córdoba (Montilla-Moriles Winegrowing Region), southern Spain (37°33'N, 4°37'W). The region has a dry Mediterranean climate, with mean annual temperatures ranging between 8 and 26°C, and an average annual rainfall of 500 mm. Most of the precipitation occurs in autumn. The soils are calcareous and most of the arable land is devoted to intensive agriculture. The area is a mosaic of olive groves, vineyards, cereal crops and, to a small extent, patches of natural vegetation. Winegrowing represents a vast proportion of the arable lands ($\sim 40\%$; I.C. Barrio, unpubl. data) and is a major component of the local economy, with grapes being used mainly in commercial wine production. In addition, small-game hunting is of significant economic importance to the area (Vargas et al. 2007), and regionally, rabbits occur in moderately high numbers (Barrio et al. 2010a).

To conduct the experiment, we selected vineyards of 3-8 ha at three sites that were > 1 km apart. The vineyards contained grapevines *Vitis vinifera* of the variety Pedro Ximénez. Cultivation practices (e.g. crop protection treatments and fertilizers) might have differed slightly among the vineyards because they had different owners, but the differences were likely to be minor because all were regulated under the same Winegrowing Region. For the experiment, each vineyard was divided into four contiguous sections (hereafter 'plots') and an experimental treatment resulting from the combination of two management actions (presence/absence of sown cover crops and exclosure fencing) was assigned to each plot (N = 12). The size of plots was constant within sites but differed among sites owing to vineyard size, and in all cases, it was > 0.75 ha. In November 2008, a cover crop of 70% oats Avena sativa and 30% garden vetch Vicia sativa were sown in alternating rows parallel to the edge of the vineyard (seed density ~ 75 kg/ha). Seeds were sown in 50-cm wide strips and the area immediately under the vines was left unsown. To avoid excessive competition for water between vines and cover crops, the cover crops were mowed in late April 2009. To exclude rabbits from two of the plots in each vineyard, 1-m high fences were embedded 20 cm into the ground immediately after the sowing of the cover crops, and all rabbits were removed from these plots. To ensure that the abundance of rabbits within the fenced plots was close to zero, the integrity of the fences was checked each week. The unfenced plots (UF) mimicked the natural condition in the study area, where rabbits had unfettered access to the crops and the availability of natural foods was low. In the unfenced plots where cover crops were sown (UF+S), the abundance of food for rabbits was artificially increased by the sowing. Rabbits were excluded from fenced plots (F) and fenced plots sown with cover crops (F+S), which provided a basis for assessing the effects of the presence of cover crops on vineyard in the absence of the potentially confounding effect of rabbit-induced damage. At each of the sites, logistical constraints meant that the two fenced plots and the two sowed plots were always contiguous; otherwise, the allocation of experimental treatments to plots was random.

In each plot, to account for the possibility of an edge effect, from the edge of the crop inwards, 20 vines were marked every 5 m in two parallel rows that were 5 m apart. The starting point of these marked vine-rows was selected to be in the midpoint of one external side of the plot, so as to maximise distances between the marked rows between plots (in all cases > 200 m). In the 2009 growing season, the marked vines were monitored monthly from the time of the emergence of buds in early March until grape harvest in late August (N =5 visits). Only the vines (N = 221) that did not dry out or were not affected by disease were followed throughout the season and included in the analyses. Discarded vines represented a small proportion of the vines (7.9%), and therefore, this may not have a big effect on our results. We assessed the rabbitinduced damage to vineyards at two levels (Pietrzykowski et al. 2003): at the plot level, we measured the

extent of the damage affecting a number of plants in each plot, and at the plant level, we evaluated the severity of the damage inflicted on individual vines. We quantified the severity of the rabbit-induced damage by counting the number of buds and shoots bitten on each vine and expressed it as the proportion (in %) of all of the buds and shoots on a vine that were removed by rabbits. Other herbivores whose browsing damage may be confounded with that of rabbits', such as hares Lepus spp., occur in the area in very low numbers, so that damage can be safely assumed to be caused by rabbits only (I.C. Barrio, pers. obs). At the plot level, the extent of the damage caused by rabbits was expressed as the proportion (in %) of the vines in each plot that were damaged. At harvest, the grapevine yields of the marked vines were quantified in the field by weighing each of the grape clusters on each of the vines using an electronic kitchen scale (with a precision of ± 0.1 g).

To estimate rabbit use of the plots, each month, we counted the number of rabbit faecal pellets at fixed sampling points within each experimental plot (Taylor & Williams 1956), as per the grid design used to monitor vines. During March - July 2009, we counted pellets within 0.5-m² circular sampling points. After each count, the pellets were removed from the sampling units and the pellet counts from the first visit to each plot were excluded from the analyses. The number of pellets in each unit was counted, and for each month and sampling plot, a mean density of pellets per day and surface unit was calculated.

In addition, to evaluate whether the sown cover crops served as a food source for rabbits, we used a stratified random sampling. Within each of the plots sown with cover crops, we distinguished between edge (i.e. < 10 m from the border) and core (i.e. > 20 m into the crop) areas and, within each of these areas, once a month until mowing (N = 3 visits), we randomly set five sampling units. The sampling unit was a 25 × 25-cm square that had a 5 × 5 cm mesh grid. Using the Point Intercept Method, we recorded 25 observations per sampling unit, which were classified as bare ground, browsed sown cover crop or unbrowsed sown cover crop.

Data analysis

To evaluate the effects of the presence of cover crops on rabbit-induced damage to vineyards, we used Linear Mixed Models (LMM) for continuous response variables and binomial Generalised Linear Mixed Models (GLMM) with a binomial distribution and a logit link when response variables were expressed as proportions (Zuur et al. 2009). In the plot-level analysis, each plot was the experimental unit (N = 12) and 'sampling site' was treated as a random factor. In the plant-level analysis, the individual vine was the experimental unit (N = 221) and 'plot within each sampling site' and 'sampling site' were included as random factors in the model due to the nested design of the experiment. To control the effects of heterogeneity in residual spread, variance structures were added to the models (Pinheiro & Bates 2000), which allowed the residuals to have different spreads across the levels of a categorical variable; i.e. the variance covariate (sampling site in the plant-level analysis).

To evaluate the effect of cover crops on rabbit use of plots and the damage they cause at the plot level, we used two models in which the response variables were the mean rabbit use over the period of the study and the extent of rabbit-induced damage (highest proportion of vines damaged per plot throughout the sampling period), respectively. We log-transformed mean rabbit use of plots to achieve normality. In both of the models, the two management actions (rabbit exclosures and the sowing of cover crops) and their interaction were included as fixed effects.

To evaluate the effects of cover crops on rabbit damage and yield loss at the plant-level, the response variables in the two models were the maximum severity of the damage inflicted on each plant in the course of the study and grapevine yield, respectively. We square-root transformed grapevine yield to achieve normality. In addition to the two management actions and their interaction, we included the distance from each vine to the crop edge as fixed effect in both models. With grapevine yield, the severity of the damage was also included as an explanatory variable in the fixed component.

To illustrate differences among experimental treatments, i.e. combinations of rabbit exclosure levels and presence/absence of cover crops, additional LMM and GLMMs were built and post-hoc Tukey tests were conducted.

We assessed the development of sown cover crops and their use as a food source by rabbits by calculating the proportion of all point intercepts that were bare ground in each sampling square, and the proportion of non-bare ground intercepts that were covered by browsed plants in sown experimental plots. To identify the main factors that influenced these indices, we generated binomial Generalised Linear Mixed Models in which the 'sampling site' was treated as a random factor due to the sampling design. Initial models included the effects of month, location relative to the crop border and the presence/absence of exclosure fences

In all cases, model selection was based on Akaike's Information Criterion (AIC) by comparing nested models following a backwards procedure

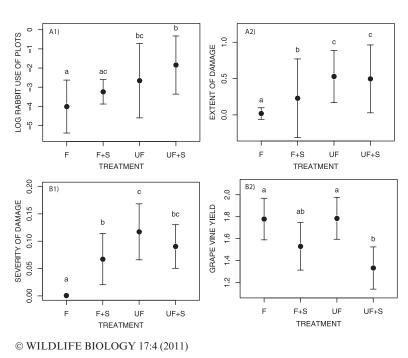


Figure 1. Effects of experimental treatment levels at a plot level (A) regarding rabbit use of plots (pellets/m²/day; A1) and the percentage of damaged vines per plot (A2), and at a plant level (B1 and 2), showing the damage exerted to each vine plant measured as the percentage of browsed shoots (B1) and the effect on grapevine yield (in kg; B2). Small case letters indicate significant differences (P = 0.05) between groups, as assessed using post-hoc Tukey tests. Mean values and 95% confidence intervals are shown. F = fenced plots, F+S = sown fenced plots, UF: unfenced plots and UF+S: sown unfenced plots.

(Zuur et al. 2009). For GLMMs, we report the χ^2 statistics of the likelihood-ratio tests between models with and without a certain fixed term (Bolker et al. 2009). In all of the models, the assumptions of normality, homogeneity and independence in the residuals were met (Zuur et al. 2009). Statistical analyses were performed using R 2.10.1 (R Development Core Team 2009) and the packages nlme and lme4.

Results

Effects of cover crops at the plot level

Rabbit use of plots differed significantly among the four experimental treatments (F = 7.883, df = 3,6, P = 0.0167; Fig. 1.A1). The presence of exclosure

fences and cover crops (Table 1) had significant effects on the use of plots by rabbits (mean = 0.071 pellets/m²/day, SD = 0.080; range: 0.00-0.25), but the interaction between these two factors was not significant (LRT=0.009; P=0.925). Use by rabbits of unfenced plots sown with cover crops was relatively high (mean \pm SD = 0.167 \pm 0.091 pellets/m²/day).

The mean proportion of the vines in each plot that were affected by rabbit-induced damage was 31.78% (SD = 25.75; range: 0.00-68.42) and was highest in the unfenced plots, whether sown with cover crops or not (mean extent of damage in unfenced plots = 51.18%, SD = 15.07; range: 29.41-68.42). We found a significant effect of the interaction between the two management actions, indicating that the effect of cover crops in reducing the

Table 1. Final models for the effects of cover crops at the plot level: on rabbit use of plots (A1) and the extent of rabbit damage (A2), and at the individual plant level (B): on the severity of damage (B1) and on grapevine yield (B2). Linear Mixed Models (LMM) were used for continuous response variables and binomial Generalised Linear Mixed Models (GLMM) for response variables expressed as proportions. Δ AIC indicates the improvement in model fit of the final model compared with the full model; Δ AIC equals zero when the final model is the same as the full model.

A1. LMM for rabbit use of plots; Response: rabbit use of plots (log transformed); Random factor: study site; $\Delta AIC = 10.178$				
	Estimate (± SE)	Df	t-value	Significance
Intercept	-2.650 (± 0.281)	7	-9.441	0.000
Fence	-1.371 (± 0.302)	7	-4.544	0.003
Sowing	0.793 (± 0.302)	7	2.628	0.034

A2. Binomial GLMM for the extent of damage

Response: proportion of damaged vines; Random factor: study site; $\Delta AIC = 0.000$

	Estimate (± SE)	Z-value	Significance
Intercept	0.123 (± 0.384)	0.321	0.748
Fence	-4.246 (± 1.059)	-4.009	0.000
Sowing	-0.172 (± 0.394)	-0.436	0.663
Fence*Sowing	-3.029 (± 1.142)	2.653	0.008

B1. Binomial GLMM for the severity of damage

Response: proportion of shoots damaged on each vine; Random factor: treatment plot within study site; $\Delta AIC = 0.000$

	Estimate (± SE)	Z-value	Significance
Intercept	-1.787 (± 0.606)	-2.947	0.003
Distance to edge	-0.019 (± 0.004)	-4.810	0.000
Fence	-5.209 (± 0.960)	-5.425	0.000
Sowing	-0.339 (± 0.523)	-0.649	0.516
Fence*Sowing	4.046 (± 1.103)	3.668	0.000

B2. LMM for grapevine yield

Response: grapevine yield (kg; square root transformed); Random factor: treatment plot within study site; Variance structure: different standard deviations per zone; $\Delta AIC = 34.116$

	Estimate (± SE)	Df	t-value	Significance
Intercept	1.778 (± 0.211)	208	8.438	0.000
Sowing	-0.349 (± 0.126)	8	-2.777	0.024

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extent of rabbit-induced damage, varied between fenced and unfenced plots (see Table 1); sowing significantly increased the extent of damage in fenced plots (Tukey contrast for multiple comparisons of means; z-value=2.666, P=0.033), whereas it had no effect in unfenced plots (Tukey contrast for multiple comparisons of means; z-value=-0.436, P=0.970).

Effects of cover crops at the plant level

At the plant level, the severity of the damage caused by rabbits varied widely, and was highest in the unfenced plots (mean damage = 11.72%, SD = 19.29; range: 0-91.3%). Experimental treatments differed significantly ($\chi^2 = 20.885$, df = 3, P = 0.000; see Fig. 1.B1), and the interaction between management actions had a significant effect on the severity of damage (see Table 1). In addition, distance to crop edge had a negative effect on the severity of damage, indicating that vines further into the field are less prone to rabbit damage (see Table 1).

In the three study vineyards, average grapevine yield was 3.16 kg/vine (SD = 2.70; range: 0.0-15.9). Grapevine yield was negatively affected by the presence of cover crops (see Table 1), but not significantly affected by the severity of the damage to vines (LRT=0.576; P=0.448), distance to crop edge (LRT=0.420; P=0.517) fencing (LRT=1.161; P= 0.281), or by the interaction between fencing and sowing (LRT = 1.496; P = 0.221). Plots that were sown with cover crops yielded 28.84% fewer grapes than did the plots not sown with cover crops (sown: mean=2.62 kg, SD=2.63; unsown: mean=3.67 kg, SD = 2.67).

Development and use of cover crops

The location of cover crops relative to the crop edge (i.e. edge or core position) did not have a significant effect on the proportion of the ground that was bare or the proportion of plants that were browsed (bare ground: $\chi^2 = 0.789$, df = 1, P = 0.374; browsed plants: $\chi^2 = 0.332$, df = 1, P = 0.565). The proportion of bare ground and browsed plants were influenced by month (Table 2), decreasing and increasing, respectively, along the study. The presence of fences decreased the proportion of both, i.e. bare ground and plants that were browsed (see Table 2).

Discussion

As predicted by the Food-limitation Hypothesis, in experimental plots within three vineyards in south-

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Table 2. Final models for the development of cover crops, as measured through the proportion of bare ground (A) and the proportion of browsed cover crops (B). Binomial Generalised Linear Mixed Models (GLMM) were used and Log-likelihood Ratio Tests (χ^2) for the comparisons of nested models are given. AIC indicates the improve in model fit of the final model from the full model; Δ AIC equals zero when the final model is the same as the full model.

A. Bare ground				
Response: proportion of bare ground; Random factor: study site: $\Delta AIC = 1.2$				
	χ^2	Df	Significance	
Month	11.493	2	0.000	
Fence	19.067	1	0.000	
B. Browsed co	over crops			
Response: pro study site; Δ	*	ed cover cro	ps; Random factor:	
	χ^2	Df	Significance	
Month	37.515	2	0.000	
Fence	360.240	1	0.000	

ern Spain, wild rabbits used cover crops as food, as suggested by browsing evidence on sown cover crops during the sampling period and by the higher use by rabbits of unfenced plots that were sown with cover crops. Interestingly, the intense use of those plots by rabbits did not result in greater damage to vines, whether measured at the level of the plot or the individual plant, which might have been because the rabbits used the cover crops as an alternative food, which attracted rabbits to sown plots but was not overwhelmed by feeding rabbits.

However, browsing indices on vines suggested that the presence of cover crops did not have a strong effect in reducing rabbit-induced damage. This might have been a consequence of selective grazing by the rabbits (Diaz 2000, Martin et al. 2007) as they may actively search for the highly nutritious vine buds, even when other palatable foods are available, especially during periods of high energetic demand, such as during the breeding season (Gonçalves et al. 2002). Thus, cover crops such as those used in our experiment might provide an adequate food source but are not attractive enough to deter rabbits from damaging vines. At the level of the plot and the level of the vine, damage was most strongly influenced by the presence of fences, which minimised the abundance of rabbits within plots. Rabbit abundance and damage to vines can be positively correlated (Barrio et al. 2010b), and even at low densities, rabbits can cause significant

damage to crops (Bell et al. 1999, Dendy et al. 2003). Based on a daily defecation rate of 370 pellets/day (González-Redondo 2009), average rabbit densities in our study plots were about 2 rabbits/ha, which is within the wide range of densities reported in rabbit populations in Europe (Marchandeau et al. 2006) and corresponds to low-moderate densities. The significant effect of the interaction between fencing and sowing on the extent and severity of damage, with significantly higher damage in fenced plots sown with cover crops when compared to fenced plots without cover crops, might have been a result of the inadvertent presence of rabbits in one of the sown fenced plots on one of our visits. Even at the low (~ 1 rabbit/ha) local densities that occurred accidentally in this plot, rabbit-induced damage occurred, which indicated the sensitivity of the vineyards to rabbits. Thus, fencing appeared to be effective in reducing rabbit-induced damage to vines by keeping rabbit densities close to zero within the plots (McKillop et al. 1998), but it can be an expensive and time-consuming measure, especially in the area of our study where fences are erected at the beginning of each growing season. In addition, the failure to exclude all rabbits from a vineyard means that some damage can still occur and, therefore, the integrity of the fences must be exhaustively checked.

According to our expectations, there was an edge effect on the severity of rabbit-induced damage. However, this effect was not detected in rabbit browsing on sowings. Peripheral wildlife damage to crops has been widely described (Calenge et al. 2004, Barrio et al. 2010b). In our study, however, the presence of palatable crops within the vineyard might have made the vineyards more attractive to rabbits allowing them to penetrate farther into the field, given the greater and spatially uniform use of the plots sown with cover crops. This change in the spatial use of crops might have further implications, especially where intercropping provides shelter, rather than food, which allows rabbits to penetrate farther into the crop. In our study, this was not the case because rabbit browsing constrained the height of the cover crops. Research is needed on the impact of such management practices on the spatial distribution of rabbit-induced damage on a regional scale because food availability at a broader level might influence the use of space by rabbits (I.C. Barrio, unpubl. data).

In the experimental plots in the vineyards of southern Spain, the presence of cover crops had a negative effect on grapevine yield. Plots that were sown with cover crops were up to 30% less productive than plots that were not. Similar reductions in grapevine yield (Morlat & Jacquet 2003, Tesic et al. 2007) can be associated with competition for water and other resources, particularly nitrogen (Celette et al. 2009). The negative effects of the presence of cover crops on grapevine yield observed in our study might have been compounded by rabbit-induced damage. Rabbit browsing on vines mainly affects buds and young tissues, which can lead to a significant reduction in foliar mass in sub sequent vegetative growth. Defoliation leads to reduced yield, although it might provide some benefits to berry ripening by improving microclimate conditions at the site of the cluster (Chaves et al. 2007). Indeed, despite a significant yield reduction in grapevine yield, some studies have observed improved berry composition and must quality under cover cropping systems (Rodríguez-Lovelle et al. 2000, Morlat & Jacquet 2003), and therefore, other factors associated with wine production should be examined in detail before a definitive management strategy is achieved.

The management of wild rabbit populations is challenging in areas where they have value as a game species, but make up an agricultural pest. In intensively human-modified landscapes, the sowing of cover crops might serve as an alternative food source for rabbits, but it does not effectively reduce the damage caused to vineyards by foraging rabbits. In southern Spain, however, the addition of cover crops to vineyard plots attracted rabbits and the potential of using them to modify the use of space by rabbits warrants further study, for example by locating cover crops far from sensitive crops such as vineyards. However, the potential consequences of adding these supplemental food sources to rabbit population dynamics should be investigated in the long term as population increases linked to enhanced food availability might be expected. Although exclosure fencing kept rabbit densities near zero and was the most effective way to reduce the damage caused by rabbits, the inadvertent presence of even a few rabbits within the fenced plots led to damage, and inadequate surveillance will limit the effectiveness of this measure in protecting vineyards against the damage caused by rabbits.

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