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# **No clear effect of odour repellents on roe deer behaviour in the vicinity of roads**

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Ungulate–vehicle collisions pose a traffic safety issue as well as wildlife-conservation issues in many countries. While fences are recommended as reliable safety measures for motorways and other high-traffic volume roads, no generally accepted measures of the same efficiency are available for secondary roads. Odour repellents are applied in many central European countries, but contradictory results are available concerning their efficiency. We tested the effect of odour repellents on both a crossing frequency and the presence near roads of six individuals of roe deer over a period of five months (April–August 2019). The odour repellents were installed along two secondary roads, and along two semi-open habitats (forest–meadow and forest–arable land) alternately, in several phases. Two hypotheses were tested. The first one focused on the change in animal presence close to the profiles where the odours were applied, while the second hypothesis concerned a change in the number of crossings of the same profiles. The results demonstrate that no clear effect of odour repellents on roe deer behaviour in both hypotheses were obtained. Apart from the obtained results, we discuss the importance of the methodology. We conclude that this kind of study design is extremely sensitive to a number of factors with a potentially negative influence on the course of the study design.

Keywords: animal, roadkill, study design, wildlife, wildlife–vehicle collisions

Preventing wildlife from crossing, or directing their movement across, the transportation infrastructure is a worldwide effort when mitigating wildlife–vehicle collisions (WVCs). While fencing of motorways and high-intensity primary roads is a generally recommended practice, no reliable and universally accepted measure exists for low-intensity secondary roads. Odour repellents represent a WVCs mitigation measure which should primarily focus on these low-intensity secondary roads. They have been extensively applied in central Europe in order to prevent or minimise the number of road casualties of ungulates on roads due to crashes with motor vehicles. Despite the fact that they have been used for a long time (Melchiors and Leslie 1985, Lebersorger 1993), a number of questions related to their effectiveness remain open.

Certain studies suggest that odour repellents (ORE) could be effective in reducing the number of collisions with animals.

Andreassen et al. 2005, showed a reduction in the frequency of moose crossing railways, Putman et al. (2004) observed a 30–80% reduction and Bíl et al. (2018) demonstrated a 26–43% roadkill decrease for roe deer and wild boar.

ORE (as a subgroup of other chemical repellents) are supposed to trigger avoidance behaviour via four mechanisms: 1) neophobia; 2) irritation; 3) conditioned aversion and 4) flavour modification (Kimball et al. 2009). ORE effectiveness further depends on the individual herbivore's motivation to cross the given road (Kušta et al. 2015). Many factors can further affect the efficacy of chemical repellents e.g.: seasonal food availability, weather, temperature, frequency of use and concentration of product (Wagner and Nolte 2001, Knapp et al. 2004, Diaz-Varela et al. 2011), and disturbances during the hunting season (Benhaiem et al. 2008). The efficiency of chemical repellents is also influenced by the way of application (Kimball et al. 2009, Elmeros et al. 2011). ORE are usually applied to adjacent vegetation around roads (Iuell et al. 2003) or to wooden sticks placed along roads (Bíl et al. 2018) or railways (Keken and Kušta 2017). A new kind of ORE installation, recently recommended by ORE producers, involves rolls of bast injected with the chemical substance. This kind of application should offer certain

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environmental benefits (bast is a natural product in contrast to artificial and synthetic foam) and therefore this kind of application was also utilised in this study.

Huijser et al. (2007) summarised several traffic safety measures related to WVCs. They concluded, based on a literature review, that the evidence for ORE effectiveness remains sparse and the effects of these measures are temporary at best. A number of studies contested the effectiveness of chemical repellents as often declared by the producers (Lutz 1994, Schlageter and Wackernagel 2012, Bíl et al. 2018). Other works warned that WVCs outside of the areas treated with chemical repellents can then increase (Lebersorger 1993, Iuell et al. 2003, Putman et al. 2004). Kinley et al. (2003) speculated about potential undesirable ORE effects such as attracting predators to the roadside and causing a panic reaction in ungulates. These studies also call for further information and more detailed analysis in the context of the use of ORE, including requirements for maintenance (Bíl et al. 2018) and costs effectiveness. Bíl et al. (2018) emphasised that repeated reapplication of the odour should be secured. To prevent habituation by animals, ORE should only be placed during critical periods (Iuell et al. 2003) and at critical sections of the road infrastructure (WVC hotspots).

The effectiveness of odour repellents can be determined by monitoring ungulate mortality before and after the application of this measure along roads (Bíl et al. 2018) or by evaluating ungulate behaviour on various stimuli using GPS telemetry (Kröschel et al. 2017, Hofman et al. 2019). These stimuli include a possible change in the behaviour of ungulates when exposed to the odour of predators (Ratikainen et al. 2007), which has not always been, however, proven (Sarno et al. 2008).

WVCs are common in the Czech Republic. More than 11% of all traffic crashes were collisions with wildlife in 2017 (Bíl et al. 2018) and these numbers are still on the rise (as much as 15% of WVCs were registered in 2019). It is estimated that approximately 75% of WVCs involve roe deer (Bíl et al. 2017). Roe deer ranks among the most successful and most abundant ungulate across Europe (Apollonio et al. 2010, Kušta et al. 2017). Therefore, the WVCs caused by roe deer are a common phenomenon in many European countries such as Austria (Steiner et al. 2014), Belgium (Morelle et al. 2013), Croatia (Vrkljan et al. 2020), Finland (Niemi et al. 2015), Germany (Hothorn et al. 2015, Seidel et al. 2018), the United Kingdom (Langbein and Putman 2006), Hungary (Cserkész and Farkas 2015), Italy (Favilli et al. 2018), Lithuania (Ignatavičius and Valskys 2018, Kučas and Balčiauskas 2020), Poland (Tajchman et al. 2017), Slovenia (Pokorny 2006), Spain (Rodríguez-Morales et al. 2013, Colino-Rabanal and Peris 2016) and Sweden (Seiler 2004, Olsson et al. 2007). This was the reason why roe deer was also selected as a target species in this study.

Various traffic safety measures are used in the Czech Republic to decrease the high number of incidents with roe deer. ORE are broadly used and often applied even to primary roads with high traffic flows. ORE are applied by both road administrators and hunters. The aim of this study is to evaluate ORE effectiveness. We focused on roe deer and, using controlled field experiments, we tested whether or not their behaviour changes in terms of both the presence near selected ORE profiles and the number of their crossings with and without ORE applied.

# **Data and methods**

### **Study location**

The study area was located in the northern part of the Czech Republic (Fig. 1), south of the town of Nový Bor. The area where 100% of GPS positions were located is about 350 ha. The area is situated at elevations 268–323 m a. s. l. Forest (43.4%), grassland (22.7%) and arable land (16%) are the most represented land cover categories there (Table 1).

A primary road no. I/9 (14 068 annual average daily traffic, AADT) divides the area into two parts, the secondary road no. III/26850 (approx. 600–700 AADT) connects the village of Chotovice. We estimated AADT for both roads on the basis of data from a statistical radar which was installed there over two weeks in 2019. Data concerning the traffic flow on the second secondary road (to the town of Nový Bor) were not available. We estimate AADT there to be approximately the same as the III/26850. No traffic, except infrequent forest machineries, occur on two additional ORE profiles (Fig. 1A–B).

#### **Roe deer capture and tracking**

On average, 50 roe deer per 1000 ha are hunted in the study area and the overall roe deer population is estimated (according to hunting area users) to be 130 ind. per 1000 ha. As many as 25 roe deer were killed by cars on roads in this area in 2019 (on the basis of data from <www.srazenazver.cz>). Ten roe deer (Table 2) were caught during two winter seasons between 2017 and 2019 into wooden box traps in the surrounding of public roads (max. distance trap-road 300 m). This rather short distance from the primary road should increase the probability that captured animals will cross roads.

All the deer, equipped with a tracking neck collar, manifested good health. All the capturing, tagging and monitoring protocols were approved by the animal welfare and hunting administration of the Czech Republic (Czech University of Life Sciences, number 63479/2016-MZE-17214). The collars (type collar 1D) were made by e-obs GmbH (Munich, Germany), weighed 370 g and, thus, reached maximally 2.5% of the body mass of the tagged deer as suggested in the literature (Kenward 2001). The collar itself was made of leather and hosted a GPS sensor, an acceleration sensor and a UHF transmitter in a case on top and one battery. The data were downloaded via a UHF terminal.

These collars provide GPS position data and acceleration data. A balance between the capacity of the internal battery and the internal memory on the one hand and the number of records which can be obtained (between data downloading) on the other hand had to be maintained. Therefore, we only measured the acceleration at the beginning of each minute (and not continuously). GPS was activated when the variance of five consecutive acceleration bursts of the z axis

 $\mathfrak{D}$ 



Figure 1. Home ranges (estimated as a convex hull of 99% collected GPS positions closest to the estimated centre of the respective home range) of seven roe deer individuals before the application of ORE. Red dots indicate places where roe deer were trapped. Thick dashed lines stand for the home ranges of selected six roe deer whose behaviour was studied. Letters A–D indicate respective test profiles along which ORE were applied.

reached a threshold (Fig. 2, active mode). This feature was also utilised when accurate road crossings were determined (see later). GPS records were also obtained, irrespective of the acceleration threshold exceedance, every half hour (inactive mode).

#### **Test profile selection**

In the phase of ORE application (April 2019), we already had information about roe deer home ranges available (Fig. 1). No ORE was eventually applied along the primary road as the collared animals were crossing this road only occasionally. The rather low number of animals and the fact that only two roe deer had been crossing secondary roads on a regular basis influenced the selected methods for the analysis of ORE effectiveness. Herbert and Zdislav were crossing the secondary roads frequently, whereas another five animals remained in the forest. We therefore also applied ORE along

Table 1. Land cover categories within the study area of Novy Bor, Czech Republic.



a forest/arable land border (Fig. 1, profile B) and along a forest/meadow border (profile A). The control profiles were selected in the vicinity of the case profiles. We eventually worked with six individuals. Míla died before ORE application (probably as a result of a crash with a car). Dan also died from an unknown case (probably killed by another roe deer). The GPS collar attached to Dianka lost UHF connection due to the low energy available and roe deer Julinka did not cross any ORE profile (Fig. 3).

#### **Hypotheses tested**

We used the word 'behaviour' throughout this work in the sense of two hypotheses, not from the viewpoint of all the aspects of animal behaviour. The first hypothesis studied the

Table 2. Roe deer individuals and their collar IDs (see Fig. 3 for comparison).

Collar ID	Capture date	Name	Sex	Age (year) $*$	Weight $(kg)^{+}$
5464	04.01.2018	Dan	M	$4 - 5$	30
5465	29.01.2019	Zdislav	M	$4 - 5$	30
5466	05.01.2018	Dianka	F	6 months	16
5467	15.03.2018	Herbert	M	$6 - 7$	28
5468	24.01.2018	Tim	M	$1 - 2$	22
5469	22.01.2018	Slávka	F	$3 - 4$	28
5470	19.03.2018	Pepík	M	$1 - 2$	22
5471	07.03.2018	Julinka	F	$1 - 2$	22
5472	25.01.2018	Míla	F	$4 - 5$	18
5473	13.01.2019	Edita	F	6 months	16

\* The age of individuals older than two years was estimated on the basis of teeth abrasion (Hrabe and Koubek 1987), † Estimated.



Figure 2. Active and inactive modes of GPS collars based on acceleration threshold exceedance.

influence of ORE to the time which the animals spent in the vicinity of a testing profile (case profile). We defined two areas representing the vicinity, a close vicinity as 20 m from the profile centreline and a more distant one as 50 m from the profile centreline. The hypothesis was tested for both ranges separately (Fig. 4). The null hypothesis was formulated as follows – H1: ORE do not affect the presence of roe deer in the vicinity of a profile. The second null hypothesis concerned the possible influence of ORE on the frequency of crossing a profile. H2: ORE does not influence the frequency of roe deer crossing a profile. Vigilance behaviour, based on acceleration data which could determine more detailed movement of animals, was not investigated due to the collar settings which preferred battery longevity.

# **Odour repellent application**

We used approximately 700 ORE in the form of rolls of bast (Fig. 5, Table 3). This kind of application is both environmentally friendly and time-effective in contrast to the standard placing of scented synthetic foam to wooden sticks (which have to be installed first). The rolls of bast were injected by 'Pacholek' olfactory repellents produced by the company EKOPLANT. The substance should imitate, according to the producer, the smell of humans, bear and lynx. It specifically contains the following substances in the respective ratios: isovaleric acid (30–40%), oleic acid (25–35%), isopropyl alcohol (15–25%), acetone (10–15%), lavender oil (0.2%).



Figure 3. Identification of the period of odour repellent application (dashed lines, where S represents the beginning of the study and E the end, see Fig. 6 for comparison). Only six animals were finally involved in the study. The grey lines indicate individuals who died (Míla and Dan), collar battery failure (Dianka) or moved eventually outside the profiles (Julinka). The long-time interval before the ORE effectiveness testing was needed in order to define home ranges and select crossing profiles.

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Figure 4. A sketch of the two hypotheses.  $H_1$  was tested for two distances (20 and 50 m). Red lines indicate case and green lines control profiles.

The rollers were placed on the ground, 5 m apart, on each side of the selected case profile (Table 3). The first line of rollers was installed approximately 0.5–1.0 m from the edge of the case profile and the second line 9 m beyond this first line of rollers (as usually recommended by the producers). The rolls of bast were collected (if they were detectable) during the last day of the application periods (see later for period delimitations).

#### **Testing**

We applied a modified before–after–control–impact design (BACI, Rytwinski et al. 2016, Bíl et al. 2018) in our study. The standard BACI approach compares two time periods: 'Before' and 'After' of an application of any tested measure. We decided to repeat this approach several times in order to eliminate possible roe deer habituation to odour. The application of ORE repeated two (profile A) and three times (profiles B–D) as depicted in Fig. 6. Data were then grouped into four categories according to the profile (case/control) and period (with ORE/without ORE) and analysed as a standard  $2 \times 2$  contingency table. Both hypotheses were evaluated using odds ratio (OR, Simon 2001). OR is defined as a fraction: OR=(*bc*)/(*ad*) (Table 4). Values significantly lower or greater than 1.0 mean that ORE influenced the behaviour of a roe deer. All the computations were performed in R Software (<www.r-project.org>).

## **Profile crossings determination**

The GPS collar provides information about the precise locations of the animals. This information was not, however, continuously available over the entire time span of the testing. GPS only provided information on the position every 5 min. The attached accelerometer allowed for additional GPS activation when the threshold of acceleration was reached. The GPS position in shorter time intervals than 5 min were also then available. Despite the existence of this accurate data, we were not always able to determine the exact time and place of the profile crossings. We therefore defined two kinds of crossings (Fig. 7, 8):

- 1. 'Accurate crossing' defined according to the exact GPS position located as a sequence of points placed close and across the profile. These records clearly defined the time and exact places, but they were not unfortunately as frequent (Table 5).
- 2. We also therefore defined 'Rough crossings' based on intersections of profiles with lines connecting two consecutive GPS burst positions.

Not all GPS data available were used for the analyses as they were cleaned from erroneous values first (see also Kämmerle et al. 2017). Each GPS module provides information about the estimated horizontal accuracy. We filtered out those with an error above 15 m. We then eliminated



Figure 5. The odour in the form of a spray concentrate was applied to rolls of bast. They were then laid down on the terrain or attached with a rope to trees (where possible).

Table 3. ORE case profiles and the related number of rolls of bast applied

Profile type	Profile name	<b>AADT</b>	Roe deer name	Case profile length	No. of rolls/app.	ORE app.	No. of rolls
Meadow/forest border			Slavka	130	52		104
Forest/arable land border	B	$\qquad \qquad -$	Tim, Pepik, Edita	133	53		159
Secondary road III/26850		650	Herbert	227	90		270
Secondary road to Nový Bor		650	Zdislav	130	52		156

all the records which indicated a velocity above 30 km h−<sup>1</sup> (according to the distances of two consecutive points). These two steps helped us eliminate approximately 5% of GPS positions which in all probability indicated erroneous data.

# **Results**

A general description of data is provided below in Table 5. Table 6 provides information about the number of GPS records in the 50 m areas along ORE profiles.

It is evident that Herbert spent the highest portion (2547/5438, 46.84%) of the observation period within 50 m of the profile whereas Pepik, Tim and Zdislav only infrequently visited the respective 50 m-wide areas along their profiles. Thus, their data frequencies are rather low and nonsignificant results, due to wide confidence intervals, can be expected (Table 7). The same was valid regarding the number of their road crossings (Table 8).

#### **Results of two hypotheses for six animals**

There were only four (out of 12) statistically significant results indicating that ORE could deter roe deer (Table 7). Another result (for Slavka within the 50 m buffer along the profile) indicated the opposite.

Only one statistically significant result was reached in the case of H2 (Table 8). In the case of combined (both accurate and rough crossings) data, ORE increased the frequency of crossings of roe deer Edita.

# **Discussion**

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In previous works, e.g. by Bíl et al. (2018), ORE effectiveness was studied on the basis of the number of roe deer (and wild boar) carcasses recorded along the roads. They found that ORE could be effective in reducing the number of roadkill between 26 and 43%. The advantage of such kinds of studies, which are only based on a roadkill number comparison,

is evident. The more challenging studies, in both relevant data gathering and their interpretations, are based on animal behaviour as in this example.

The results of this study, based on the two hypotheses, suggest that ORE had no clear effect on roe deer presence near the profiles and it did not significantly change the number of profile crossings. The results were either negative or of an opposite meaning. The results for Edita were interesting. While H1 indicated that ORE should deter this animal, the results for H2 (also statistically significant) indicated that ORE installation increased the number of profile crossings.

Any statistical evaluation of animal behaviour in relation to odour repellent effectiveness is influenced by many factors. The way of application and the frequency of ORE reapplication rank among the most important. ORE are usually applied to wooden rods along roads. We are of the opinion that the rods located at road verge complicate both road verge and adjacent land management. This can be documented by destroyed rods by road maintenance crews or parcel owners. Coordination with road administrators, local hunters and parcel owners is therefore necessary before any ORE installation on poles.

We chose a new system of ORE application which was based on rolls of bast sprayed with the chemical substance. The reasons were both environmental and also included application effectiveness. The bast continuously disintegrated during the 14-day period which we took as representing ORE effectiveness (as declared by the producer on their website for this kind of ORE application). Although the rolls were collected (if they were detectable) during the last day of the ORE application periods, we did not measure the presence of the substance in rolls.

Chemical substances injected into foam or into rolls of bast, as used in this study, continuously evaporate over time. Logically, their (supposed) efficiency should also decrease from the time of their application. This issue is being treated with recommended regular ORE reapplication. The frequency of this maintenance varies, however, according to producers' recommendations and the odour carrier used. It usually spans from 14 days up to several months. Moreover,







current weather also strongly influences ORE efficiency (it is not recommended to use them during winters). We therefore shortened the period of (supposed) ORE efficiency to 14 days in order to limit the issue related to ORE intensity (i.e. the lowered efficiency due to evaporation) and expected roe deer habituation to ORE presence. This rather short interval of ORE reapplication will inevitably increase the overall costs in contrast to other measures which do not need to be maintained as frequently.

The substances, usually used in these kinds of repellents, imitate both human and predator's (bear and lynx) odour. While humans live nearby and regularly visit this area, only a few wolf individuals were occasionally observed there. They in all probability only visited this area from their stable territories which are located 30 km to the NW and did not interfere with our animals. No lynx and bear live here. The roe deer in this area can therefore be assumed to be naïve about the odour of their usual predators.

While studies which determine ORE effectiveness on the basis of roadkill records only rely on the number of carcass data, the behavioural studies need to provide detailed description of animal movements. GPS telemetry is therefore a useful tool for studying animal presence near the transportation infrastructure. GPS records need to be checked before any further analyses as false GPS fixes may occur and have to be removed first (see also Kämmerle et al. 2017, Hofman et al. 2019).

We only utilised the location (GPS) data even though this type of collar also contains the accelerometer and thus enables the application of continuous recording of acceleration which can be utilised in vigilance behaviour determination (Kröschel et al. 2017). Such a setting is extremely demanding in terms of internal battery capacity and internal memory. Therefore, the acceleration data in our study were only used to determine between the modes (Fig. 2). Our setting was selected in order to allow us to determine the home ranges thus we were then able to decide on an appropriate design for ORE profile placing. We only had information about animals' location within each 30-min interval during the ORE testing phase.

Our collars only allowed data transmission via UHF. This method of data transmission is reliable but has another limitation as the animals have to be traced and then approached closely enough (between 200 and 500 m in our cases). Moreover, the utilisation of UHF also requires frequent visits to the area of interest (Hofman et al. 2019).

#### **Study limitations and further recommendations**

We would like to point out certain constraints, related to this study, which can also be utilised by other researchers when designing similar studies in the future. We assumed that roe deer captured at a close distance to the primary road will also cross the road during the following year. Unfortunately, the primary road with AADT 14 068 seemed to present a barrier to regular daily movement of roe deer. The issue of the animal movement barrier, caused by roads with relatively high AADT, was mentioned before (Seiler



Figure 7. Examples of both accurate and rough crossing determination (roe deer Herbert, rough crossing 3 August 2019 and accurate on 5 May 2019). Whereas accurate crossing utilised the frequent GPS position launched by accelerometer threshold exceedance, the estimation of the rough crossing place was only determined as an intersection between the profile and a line based on two GPS points placed 5 min away from one another.



Figure 8. A visualisation of crossing patterns for all six individuals according to the crossing type (a) accurately determined crossing and (b) the roughly estimated crossings.

2005, Bíl et al. 2020). This particular road did not pose, however, an impermeable barrier. It increased roadkill risk as documented with two individuals (Mila and Tim), who crossed it more frequently. They were killed after 2 months (i.e. before ORE installation) and 17 months (53 and 44 successful crosses), respectively (Fig. 3). The remaining roe deer crossed this road only infrequently. Apparently, they used this road as their home range boundary. The same was also previously observed by Kämmerle et al. (2017) who studied roe deer crossing behaviour along roads. We therefore only studied ORE effectiveness on two secondary roads and along two profiles (a forest/arable land border and a forest/meadow border) road with negligible traffic (it was only occasionally used by agriculture machines).

This kind of study, as was mentioned above, is both time-consuming and without guaranteed success as many factors can influence the course of the study. Collared animals, despite the fact that they were caught close to a road, can also remain alongside the road with no effort or need to cross the road. The use of the GPS collars, even when accompanied with an accelerometer, is also apparently not the optimal technical solution for this kind of research when both detailed (frequent) and precise data are needed. Battery life and limited memory capacity should be increased





\* Tim was killed by a car 73 days from the beginning of the test.

\*\* Figure 6 for visual delimitation of the periods, which were aggregated here. Profile A, where Slavka was crossing, was maintained in a different setting.

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Table 6. Description of the subset of raw GPS data (number of records) which were used in the analyses.



\* Indicates the number of bursts derived from regular GPS bursts (Table 5).

in order to be successfully applied when vigilance behaviour should also be investigated. Additionally, the animals who cross roads with rather high traffic are frequently at high risk of roadkill. During the study, or even at the beginning of the study, such animals can therefore be killed. This complicates the overall process.

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Table 7. Results of hypothesis H1 in the form of odds ratio for six animals. Bold numbers represent statistically significant results.

Table 8. Results of the hypothesis H2 in the form of the odds ratio for six animals. Bold numbers represent statistically significant results.





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