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WILDLIFE RESEARCH

Factors affecting bait uptake by the grey squirrel (*Sciurus carolinensis*) and the future delivery of oral contraceptives

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ABSTRACT

Context. Invasive species negatively affect natural communities and human activities. The grey squirrel is an invasive species in the UK, causing damage to forestry and the decline of the native red squirrel. Oral contraceptives have the potential to reduce numbers of grey squirrels; however, to be effective a sufficient proportion of a population must consume a bait containing contraceptives. **Aims.** The objective of this study was to estimate the efficacy of delivering baits via feeders to grey squirrels and to determine the factors most important to bait uptake. **Methods.** Bait uptake was measured using the bait marker Rhodamine B mixed with 100% hazelnut butter and delivered to grey squirrels via purpose-designed feeders. Different concentrations of Rhodamine B were first trialled on captive grey squirrels for their palatability and detectability in the hair. Bait-uptake field trials were then conducted in 10 UK woodlands by using the preferred concentration. **Key results.** In captive trials, it was found that a concentration of 0.18% Rhodamine B mixed in hazelnut butter was palatable to grey squirrels and that individuals needed to consume only small amounts (<5 g) for it to be detected in the flank hair by using an ultraviolet microscope. It was possible to deliver bait to the majority of grey squirrels in 6 of 10 woods within 4 days. Season, feeder density and squirrel density were important factors affecting bait uptake, with more squirrels consuming bait in summer than in winter and from three feeders per hectare rather than from one per hectare. **Conclusions.** This study demonstrated that baits delivered via feeders can target the majority of grey squirrels in woodland environments. **Implications.** Oral contraceptives could offer a cost-effective tool to reduce numbers of grey squirrels across the UK landscape and mitigate the negative impacts they have on native wildlife and tree health.

Keywords: bait uptake, fertility control, invasive species, population control, Rhodamine B, wildlife management.

Introduction

Invasive species negatively affect natural communities and human activities. It is estimated that non-native species have contributed to 40% of the animal extinctions that have occurred in the past 400 years (Secretariat of the Convention on Biological Diversity 2006) and that they are responsible for a 5% loss in the world economy's production each year (Pimental 2002). The grey squirrel (*Sciurus carolinensis*) is a highly invasive species in the UK. The species was introduced to the UK in the 19th century and has since increased in numbers to approximately 2.5 million, distributed across the majority of the country (Croft *et al*. 2017; Mathews *et al*. 2018). The economic and environmental impacts of the species have been estimated to cost the UK in excess of £10 million per year (Derbridge *et al*. 2016) and more recent estimates have suggested that grey squirrels could cause up to £37 million of damage to forestry in England and Wales alone (The Royal Forestry Society 2021). The grey squirrel is also responsible for the decline of the native red squirrel (*Sciurus vulgaris*), through the transmission of the squirrel pox virus and

Collection: Fertility Control for Wildlife in the 21st Century competition for resources (Rushton et al. 2006; Gurnell et al. 2016), to the extent that the red squirrel is now classed as endangered in both England and Wales (The Mammal Society 2021).

Traditionally, culling has been used to reduce grey squirrel populations and mitigate their economic and environmental impacts; however, culling can be ineffective, inhumane, expensive or environmentally hazardous. Increased animal welfare and environmental protection legislation, such as the EU Biocides Regulation 528/2012(EU BPR), together with growing public opposition towards lethal methods (Fagerstone 2002; van Eeden et al. 2017), has meant that the number of available and acceptable lethal methods of wildlife control is declining.

Oral contraceptives are currently being developed for grey squirrel management (Massei 2018; Massei et al. 2020) and oral contraceptives delivered in baits have the potential to reduce population sizes of grey squirrels, reduce the rate of population recovery after culling and eradicate local populations (Croft et al. 2021). Fertility control offers a number of potential advantages to manage wildlife, particularly when compared with culling. These advantages include the following: (1) infertile animals remain in the population and, thus, potentially contribute to density-dependent feedback that constrains recruitment and survival, slowing population recovery; (2) fertility control could be particularly effective at maintaining populations at low densities after initial culling; (3) fertility control would be expected to cause less social perturbation than culling, thus decreasing risk of disease transmission; and (4) population control via contraceptives is more publicly acceptable than that by lethal methods, meaning it could be deployed on a greater scale than other methods (White et al. 1997; Prentice et al. 2019; Saunders et al. 2002; Merrill et al. 2003; Woodroffe et al. 2006; Dunn et al. 2018; Jacoblinnert et al. 2022).

For fertility control to be effective, a bait should be easily administered, species-specific and a sufficient proportion of the population must consume bait containing contraceptives with predominantly the females being targeted (Massei and Cowan 2014; Jacoblinnert et al. 2022). Bait uptake by wildlife can be affected by many factors such as sex (Whisson and Salmon 2009), the time of year at which bait is deployed (Robinson et al. 2017), bait density and distribution, the availability of alternative food sources (McRae et al. 2020), and the density of the target species (Haley et al. 2019). Understanding the relative importance of these factors for a target species is integral to the design of effective bait-delivery campaigns.

Rhodamine B (RB), commonly utilised as a bait marker, has been used to measure bait uptake by wildlife, including the proportion of a population that will consume a bait (Fisher 1999). Following ingestion of RB-treated baits, RB is incorporated in hairs and whiskers and can be observed as a fluorescent orange band under UV light. However, it is important to determine the useable concentration of RB in baits for each wildlife species, because RB may decrease the palatability of a bait (Fernandez and Rocke 2011).

The objectives of this study were to estimate the efficacy of delivering baits via feeders to woodland populations of grey squirrels and to determine the most important factors affecting population-level bait uptake. The specific aims were (1) in trials on captive grey squirrels, to determine useable concentrations of RB in bait, by assessing palatability, detectability and changes in detectability with bait age; and (2) to analyse the effect on bait uptake by male and female squirrels of season, feeder density, ratio of squirrels to feeders, and squirrel density. The study findings will ultimately be used to optimise the delivery methods of an oral contraceptive for grey squirrels.

Materials and methods

Captive trial 1: determining the concentration of Rhodamine B for bait

Two concentrations of RB (CAS number 81-88-9, Sigma-Aldrich), namely 0.12% and 0.18% weight/weight (w/w) mixed into 100% hazelnut butter (Bulk™) were tested for their comparative palatability to captive grey squirrels and detectability in squirrel hair. The 0.12% concentration was selected on the basis of the minimum amount of RB per bodyweight required for detection, i.e. 10 mg/kg, found by a study on prairie dogs (*Cynomys ludovicianus*; Fernandez and Rocke 2011). At 0.12%, it was calculated that a grey squirrel with a bodyweight of 522 g (the average weight recorded on a sample of 23 captive squirrels housed at APHA's Animal Unit) would have to eat at least 4 g of bait to ingest a sufficient dose of RB to mark its hair. This was deemed to be a reasonable minimum value because it is highly likely that, for a bait containing a contraceptive, more than 4 g would have to be consumed by a grey squirrel to ensure effectiveness. A previous trial conducted at APHA (Beatham, S. E., Massei, G. unpubl. data) found that 100% hazelnut butter was readily consumed by captive grey squirrels and 0.25% weight/weight (w/w) RB in hazelnut butter was detectable in the hair of squirrels that ingested it, but it was less palatable to the squirrels than hazelnut butter alone; therefore, 0.18% was selected as a midway concentration between 0.12% and 0.25%.

Two grey squirrel-specific bait feeders with integrated PIT-tag readers (Beatham et al. 2021) were placed in nine outdoor purpose-built pens (width = 2.7 m, length = 9.7 m, height = 2.4 m), each housing one male and two female grey squirrels. The feeders were placed opposite each other, on either side of the pen on 90 cm high wooden stands. Each squirrel had previously been fitted with an Identichip® PIT-tag subcutaneously in the scruff of the neck. Every time a squirrel entered a feeder, the date, time and ID of the squirrel was recorded by the feeder and saved onto an SD card.

Throughout the trial, the squirrels were provided with a diet of maize, peanuts and mixed bird seed, along with environmental enrichment, including wool bedding, foliage, tubes, ropes, nest boxes and branches. The feeders in each pen were baited with 7 ± 0.5 g of 100% hazelnut butter once a day for 1 week. The six pens that had the most bait consumed were split into three groups, two pens per group. Each feeder was provided with 7 g ± 0.5 g of bait at the same time of day on four consecutive days and the bait weighed after 24 h. Group 1 was given 100% hazelnut butter, Group 2 was given 0.12% RB w/w in 100% hazelnut butter and Group 3 was given 0.18% RB w/w in 100% hazelnut butter. The RB baits were mixed fresh each day. Each time the bait was removed from the feeders, the area around the feeder was checked for bait spillage (the bait was bright pink) and the spillage weight was recorded, to the nearest gram.

Three weeks after RB bait deployment, the squirrels were caught, restrained and 10–20 hairs plucked from the flank and the tail and placed in a transparent plastic sample bag. Each hair sample was analysed in its bag by using a Leica DMLB ultra-violet microscope (Leica Microsystems UK Ltd) at ×4 magnification and the presence or absence of RB fluorescence was recorded. Because squirrel hair exhibits natural fluorescence, a control squirrel hair sample was used as a reference. The detectability of each RB dose was assessed by comparing the numbers of individuals in each treatment group that exhibited RB bands in the flank and tail hair with the relative amount of bait consumed by each individual squirrel. The latter was estimated from the comparative number of PIT-tag records for each squirrel over the four trial days, as a PIT-tag is more likely to be recorded for a visit where bait is consumed than one where it is not (Beatham *et al.* 2021). The relative palatability of the different baits was assessed from the total amount of bait consumed per pen per day for each group. During the bait trial, 7 ± 0.5 g of hazelnut butter was left in a feeder in an area with environmental characteristics (e.g. temperature and humidity) similar to the RB bait in the pens. This was weighed every day to ensure that there was no effect of moisture absorption or other environmental factors on the weight of the RB baits.

Captive trial 2: testing the viability of 14-day-old Rhodamine B bait

A concentration of 0.18% RB bait was mixed with 100% hazelnut butter and stored in a sealed plastic container, 14 days prior to use. This was deemed a practicable length of time in which bait could be mixed and transported to external practitioners, if required, ready for deployment in the field. Eight pens, containing one male and two female captive grey squirrels, were installed with two feeders per pen, as described in the first captive trial. Each feeder was pre-baited with approximately 7 g of 100% hazelnut butter per day for 4 days. The following week, the four pens that had the most bait consumed were provided with 7 ± 0.5 g RB bait per day and the amount of bait consumed weighed for each feeder each day. Each time the bait was removed from the feeders, the area around the feeder was checked for signs of bait spillage and any recorded, along with the spillage weight, to the nearest gram. PIT-tags are more likely to be recorded for visits where a squirrel feeds, than for those where they do not (Beatham *et al.* 2021); therefore, the relative proportion of bait taken by each squirrel in each pen was estimated on the basis of the relative number of PIT-tag records.

Two weeks following the final treatment day, male squirrels from each of the four treatment pens were caught as part of routine husbandry procedures. Hair from the flank and tail were taken and the samples analysed for RB fluorescence, as described above.

Field trial: measuring bait uptake in populations of grey squirrels

Field trials were conducted in 10 woods between December 2017 and September 2020. Woods were between 6 and 18 ha in area. The area of each wood was measured from a satellite base map by using a measure tool (Google My Maps 2017–2020). All woods were mature in age. Woods 1, 8, 9 and 10 contained broadleaf tree species only, Woods 2–6 were broadleaf–conifer mixed and Wood 7 was conifer only. Woods 1–8 and 10 were located in Yorkshire, England, and Wood 9 was located in Denbighshire, Wales. Woods 1–8 were relatively isolated from other woods, whereas Woods 9 and 10 were connected via wooded areas to adjacent woods of equal or greater size.

RB bait (0.18% RB mixed in 100% hazelnut butter) was deployed in each wood via the same design of squirrel feeders as used in the captive trials. Feeders were either deployed in summer (July/August) or winter (December/January) and were distributed approximately evenly across each wood, guided by accessibility to operators. In winter 2017, feeders were deployed at a density of three per hectare in two woods. In 2018, a balanced design was used to compare feeder densities of one per hectare and three per hectare for four woods in summer and two woods in winter. On the basis of the 2018 results, the feeder density was increased once more to three per hectare for the two woods in summer 2019 and summer 2020. To reduce access to bait by non-target animals, each feeder was mounted on a stand, approximately 1 m in height (Fig. 1). Feeders were pre-baited with 40 g of 100% hazelnut butter three times in one week. The following week, the feeders were baited with 40 g of fresh RB bait per day on four consecutive days and the amount of bait consumed was weighed after 24 h. The feeders were removed on the fifth day.

Within 3 weeks of the feeder removal, squirrel live-capture cage-traps were deployed, attached to 1 m high wooden stands evenly distributed throughout each wood at a density of three traps per hectare. The traps in each wood were wired open and pre-baited for 3–11 days, dependent on the availability of time and resources. Traps were then set and

Fig. 1. Grey squirrel using a feeder during a bait-uptake study, recorded with a remote camera. Feeders were fixed to 1 m high stands to help prevent access by non-target animals.

checked at least once every 24 h. For animal-welfare and health and safety reasons, traps were not set if heavy rain, snow, high winds or temperatures below 2°C were forecast. This meant that for winter surveys, traps were often set early morning and checked mid–late afternoon. Trapping was typically conducted over consecutive days, Monday to Friday, until squirrel capture rates were reduced to an average of less than one per day over three consecutive days.

Squirrels were trapped and humanely dispatched using a Home Office approved (Schedule 1) method by a trained and competent person. Each squirrel was given a unique ID, weighed, sexed, aged and health-checked, and at least 20 hairs were sampled from the flank. The hair was stored at room temperature in transparent, sealed plastic bags. The total number of squirrels trapped was used to estimate the squirrel population size and density in each wood. The flank hair and whiskers from each squirrel were analysed for RB fluorescence as described for the captive trials. The percentage of squirrels sampled from each wood that tested positive for RB was calculated and the most important factors affecting bait uptake for male and female squirrels, and female squirrels alone, as the target sex, were assessed using the global model (R Core Team 2019), as follows:

$$\begin{aligned}\mathrm{glm}(\mathrm{formula} = \mathrm{cbind}(&\mathrm{RB.positive},\ \mathrm{RB.negative})\\ &\sim \mathrm{season} + \mathrm{wood\ size} + \mathrm{feeder\ density}\\ &+ \mathrm{squirrel\ density},\ \mathrm{family} = \text{'binomial'},\\ &\mathrm{data} = \mathrm{dat},\ \mathrm{na.action} = \text{'na.fail'})\end{aligned}$$

For male and female squirrels and for female squirrels alone, 16 candidate models were assessed and the most important factors affecting bait uptake were selected on the basis of Akaike's information criterion corrected for small sample sizes (AICc), by using the method recommended by Richards (2008); models were selected that had an AICc within six units of the model with the lowest AICc; however, those that had simpler, nested models with a lower AICc were excluded. A second global model, which replaced feeder density and squirrel density with squirrels per feeder, was also assessed to see whether it described the data more parsimoniously.

Ethical statement

The study was conducted by trained Home Office Personal Licence holders under the advice of the Named Veterinary Surgeon and under a UK Home Office licence, in accordance with the *Animals* (*Scientific Procedures*) *Act 1986*. The study was approved by the Animal and Plant Health Agency's Animal Welfare and Ethical Review Body.

Results

Captive trial 1: determining the optimum concentration of Rhodamine B for bait

There was no evidence that the higher concentration of RB bait was less palatable than the lower concentration or the control bait (Table 1). The two control pens consumed 103 g of hazelnut butter over the 4-day treatment period, compared with 101 g for the 0.12% RB treatment group and 108 g from the 0.18% RB treatment group. The blank feeder in pre-bait week gained 1.6 g, while in the treatment week it did not differ in weight by more than 0.1 g on any day. It was likely that water absorption was the cause for the increase. Bait spillage was recorded on 5 of the 48 checks made (in each case <1 g) in the treatment pens and was typically recorded as pink staining or smears of bait found at the feeder entrance.

RB was detected in both the tail and flank hair of two of six squirrels in the 0.12% treatment group and four of six squirrels in the 0.18% treatment group (Table 1). The only PIT-tags recorded by the feeders were from squirrels that tested positive for RB. In Pen 4, one squirrel tested positive for RB, despite having a low number of visits to the feeder (four PIT-tag records). From the total amount of bait taken and the representative number of PIT-tag records, it is estimated that this squirrel took less than 5 g. A high level of natural fluorescence was found in two tail-hair samples from pens where no bait was taken.

Captive study 2: testing the viability of 14-day-old Rhodamine B bait

Between 45.5 and 52.7 g of RB bait was consumed from each of the four pens over the four trial days (Table 2), and in total over 80% of the bait provided via the feeders in each pen. All four males sampled exhibited RB fluorescence in either the bulb or shaft of the flank hair. Only one male did not exhibit RB fluorescence in the tail hair; that squirrel had the lowest number of PIT-tag records and was estimated to have eaten less than 5 g of

Table 1. The amount of bait containing 0.12% or 0.18% w/w of Rhodamine B consumed from feeders over 4 days by captive grey squirrels housed as one male and two females per pen and the presence of Rhodamine B in flank and tail hair samples.

Treatment group	Pen	Bait consumed pre-bait period (g)	Bait consumed treatment period (g)	Squirrel	Pit-tag records	RB fluorescence in hair	
						Flank	Tail
0.12%	1	50.8	51.8	M1	0	N	N
				F1	112	Y	Y
				F2	0	N	N
	2	49.8	49.1	M2	29	Y	Y
				F3	0	N	N
				F4	0	N	N
0.18%	3	47.6	52.7	M3	36	Y	Y
				F5	13	Y	Y
				F6	0	N	N
	4	51.3	54.8	M4	4	Y	Y
				F7	92	Y	Y
				F8	0	N	N
Control	1	54.7	50.4				
	2	53.6	52.8				

The number of PIT-tags recorded by the feeders for each squirrel was used as a measure of the relative amount of the bait eaten by an animal.
Y, yes; N, no.

bait. No bait spillage was recorded around any of the feeders during baiting.

Field trial: measuring bait uptake in populations of grey squirrels

In total, between 20% and 64% of the total amount of bait deployed in each wood was removed from the feeders. Data gathered from camera traps monitoring the feeders suggested that this was achieved entirely by grey squirrels. In total, 613 squirrels (320 females, 293 males) were trapped and sampled from 10 study woods and over half of the squirrels trapped and sampled in 6 of 10 woods tested positive for RB (Table 3) and, in five of these woods, over half of the females tested positive. For 9 of 10 woods, 3 weeks of trapping and removing squirrels proved sufficient to reduce numbers caught to less than one squirrel per day over three consecutive days. For Wood 10, after 13 trap-days, limited human resources meant that the trapping was stopped at 1.3 squirrels per day for the last 3 days.

There was evidence from the trial at Wood 10 that minor modifications made to the feeders to incorporate a bait-weighing device may have reduced the accessibility of the bait to the squirrels, so that the majority of squirrels did not enter the feeders during pre-bait and even fewer during the deployment of RB bait (88 squirrels in this study were microchipped prior to feeder deployment and their feeder access was monitored). In addition, 24 of the 45 feeders deployed showed an increase in RB bait weights between 1 and 4 g on at least one trial day, which had not been observed to the same extent in other trials. The most likely cause was the absorption of water because

Table 2. The amount of 14-day-old bait, containing 0.18% w/w of Rhodamine B, consumed over 4 days by four captive male grey squirrels and the presence of Rhodamine B in flank and tail hair samples.

Pen	Bait consumed over 4 days (g)	Squirrel ID	Number of pit-tag records	RB fluorescence in hair?	
				Flank	Tail
5	45.4	M5	13	Y	N
6	52.7	M6	79	Y	Y
7	49.6	M7	16	Y	Y
8	49.2	M8	44	Y	Y

The number of PIT-tag records for each squirrel was used estimate the amount of the bait eaten by an animal.
Y, yes; N, no.

Table 3. The percentage of grey squirrels that consumed bait containing Rhodamine B from feeders, placed in woodlands at a density of one or three per hectare in winter and summer, from the numbers trapped and removed until the rate of captures per day was less than one over three consecutive days.

Wood ID	Year	Season	Feeder density (n/ha)	Size (ha)	Number of trap days	Number of squirrels trapped		Squirrels/ feeder	Squirrel density (n/ha)	% RB positive	
						Females	Total			Females	Total
1	2017	Winter	3	8	8	15	31	1.29	3.88	53.3	54.8
2				8	7	20	38	1.58	4.75	60.0	65.8
8	2018			10	6	22	39	1.39	3.90	45.5	43.6
7			1	14	15	105	207	14.79	14.79	8.6	8.7
5	2018	Summer	3	6	7	12	22	1.22	3.67	75.0	77.3
6				10	9	8	17	0.57	1.70	62.5	88.2
9	2019			14	10	34	62	1.48	4.43	67.7	71.0
10	2020			15	13	53	105	2.33	7.00	22.6	19.1
3	2018		1	18	15	43	75	4.17	4.17	27.9	34.7
4				9	6	8	17	1.89	1.89	37.5	58.8

of high environmental humidity, which may reduce bait palatability by causing the RB to become more concentrated in parts of the bait.

In several respects, including the higher final trap rate and lower feeder acceptance by squirrels, Wood 10 was an outlier. For that reason, the analysis of factors affecting uptake was conducted twice, namely, once with Wood 10 included and once with Wood 10 excluded. For both males and females combined, and females alone, a model including just feeder density and season had the lowest AICc value for explaining bait uptake in nine populations of grey squirrels (Table 4; Fig. 2). A model including feeder density and squirrel density performed similarly well (ΔAICc $\leq$ 1.26), suggesting that all three factors (squirrel density, feeder density and season) may play some role in determining bait uptake. The results were qualitatively similar when Wood 10 was included for the male and female analysis, although season was not selected as an important predictor. Despite its strong similarity and lower parameter burden, a model including squirrels per feeder did not explain variation in squirrel bait uptake as well as did a model containing both feeder density and squirrel density (Table 4).

Discussion

The objectives of this study were to estimate the efficacy of delivering baits via feeders to woodland populations of grey squirrels and to determine the most important factors affecting population-level bait uptake, so as to develop an effective strategy to deliver an oral contraceptive to reduce numbers of grey squirrels.

This study found that RB is a suitable bait marker to measure bait uptake in populations of grey squirrels. The results of the captive-squirrel trials showed that, on the basis of the amount of bait consumed, a concentration of 0.18% RB mixed in hazelnut butter was as palatable to squirrels as hazelnut butter alone and that an individual needed to consume only small amounts of bait (<5 g) for it to be detected in the flank hair. This is comparable to the results

Table 4. GLM results from 16 candidate models based on Global model 1 (season + wood size + feeder density + squirrel density) and eight candidate models based on Global model 2 (season + wood size + squirrels/feeder) testing factors affecting bait uptake in nine woodland populations of grey squirrels.

Subject	Global model	Terms	d.f.	AICc	ΔAICc	Weight	R^2
Males and females	1	Feeder density + season	3	60.1	0	0.451	0.802
	2	Squirrels/feeder	2	60.9	0.8	0.302	0.724
	1	Feeder density + squirrel density	3	61.3	1.2	0.247	0.764
Females	1	Feeder density + season	3	44.0	0.0	0.646	0.898
		Feeder density + squirrel density	3	45.2	1.2	0.354	0.838

Models with an AICc within six units of the model with the lowest AICc were selected for male and female squirrels and for female squirrels alone. Models that had simpler nested models with a lower AICc were excluded.

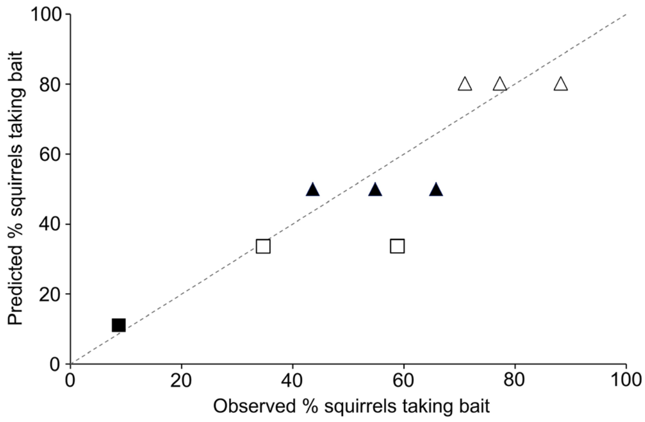

Fig. 2. The percentage bait uptake recorded in nine woods for male and female grey squirrels in summer (white) and winter (black) and for three feeders per hectare (triangle) and one feeder per hectare (square). Observed values are plotted against values predicted from the most parismonious model (feeder density and season).

of a study by Fernandez and Rocke (2011), who found that concentrations of 0.05–0.30% of RB in bait were readily accepted by prairie dogs and that RB concentrations of 12 mg/kg bodyweight were sufficient for detection in whiskers under UV light. The grey squirrels in the captive studies were not individually housed, so the exact amount of bait consumed by each squirrel is not known. However, on the basis of average captive squirrel bodyweights, the total amount of bait consumed in each pen and the relative number of PIT-tag records recorded for each squirrel by the feeders, it is estimated that RB detection was achieved at less than 17 mg/kg bodyweight.

Squirrel flank hair appeared to be more reliable than tail hair for RB detection, because tail hair was found to have more natural fluorescence, that made lower levels of RB fluorescence harder to identify. In previous studies, hair samples for RB analysis have been washed and mounted on slides in preparation for analysis (Weerakoon *et al.* 2013). In this study, hair samples were analysed effectively while inside the transparent plastic sample bags that they were placed in when taken in the field; thus, the process was much more time efficient and less susceptible to labelling errors or sample contamination.

Baits deployed for wildlife management purposes have the potential to affect non-target species (Shore and Coeurdassier 2018) and an oral contraceptive is unlikely to be species specific, therefore it is important that specificity is achieved via the bait-delivery method (Massei and Cowan 2014). The feeders used in this study have an outer metal case, have a weighted door on the entrance and were fixed to 1 m high stands, to reduce the number of non-target species that can access the bait (Beatham *et al.* 2021). The minimal spillage recorded during the captive and field trials suggests that squirrels that did not enter feeders are unlikely to have been able to access the RB bait. For contraceptive delivery, baits of similar properties deployed via feeders of a similar design should be available only to animals that can access the feeder bait compartment.

The feeders proved very effective at delivering bait to grey squirrel populations, with the majority of squirrels in 6 of 10 woods consuming bait within 4 days. In three woods surveyed in summer using three feeders per hectare, over 70% of squirrels consumed bait from the feeders. Season, feeder density and squirrel density were the most important factors affecting bait uptake, with a larger proportion of squirrels consuming bait in summer than in winter and with three feeders rather than one feeder per hectare. Fitted models based on these factors explained large proportions of the variance in the proportions of grey squirrels that consumed bait from feeders deployed in woods of between 6 ha and 18 ha in size and with varied tree composition and degrees of connectivity to other woodland.

Season is an important factor to consider when planning the deployment of an oral contraceptive. Winter might be considered the best season to deliver oral contraceptives in baits because it coincides with the main breeding season of grey squirrels (Hayssen 2016) and, because of the relatively low availability of natural food resources, squirrels are more likely to feed on baits (Steele and Wauters 2016). Conversely, squirrels are more active for longer during the day in summer than in winter (Thompson 1977) and it is generally more practical to deliver bait in summer because of more favourable weather conditions and longer daylight hours.

These preliminary results suggest that the relationship between bait uptake and the density of squirrels relative to the density of feeders should remain constant across woodlands of different size and tree composition. The results from this study also suggest that natural food availability was not a significant factor affecting bait uptake from feeders by grey squirrels in summer or winter; therefore, if a vaccine required multiple doses within a year to be effective, provided that the numbers of squirrels per feeder was low enough, it should be possible to deliver bait to the majority of male and female squirrels in most woods within these seasons. It is particularly important that females are targeted, because, like many other mammal species, the grey squirrel exhibits polyandry, and fertility control models for other mammal species have demonstrated that a high proportion of females in a population need to be rendered infertile for fertility control to be effective (Massei and Cowan 2014).

The three woods with the lowest bait uptake (8.7–34.7%) were the only woods where the squirrel to feeder ratio was higher than 2 and the ratio of squirrels to feeders has been found to be an important factor determining bait uptake (Croft *et al* 2021). Our analyses found slightly equivocal support for this parameter but it was clear that both feeder density and squirrel density are likely to play some role in determining bait uptake. It is possible that when there are too few feeders per squirrel, some individuals monopolise the feeders, because the grey squirrel is known to have a hierarchy of dominance, particularly at bait stations (Lawton et al. 2016).

There was evidence from the captive and field trials that the RB bait had the potential to absorb water under some environmental conditions. The conditions during the bait trial in Wood 10 were very humid and this may have caused the RB to become concentrated in parts of the bait, decreasing its palatability. Any oral contraceptive will have to be designed to remain palatable under a range of environmental conditions.

Estimates of bait uptake recorded in this study are conservative, as bait was deployed for 4 days only and the number of squirrels removed might have included some animals that had moved into the woods after baiting ceased. The population size of each wood was measured from the number of squirrels trapped and removed until fewer than one per day were caught over three consecutive days. There will be some error in this estimate, as squirrels will begin moving into an area soon after removal begins. For example, in two 12 ha woods where squirrels had been completely removed, squirrel numbers returned to pre-cull levels in less than 10 weeks (Lawton and Rochford 2007).

During the trapping and removal of squirrels, a population estimate must ideally be taken before the last few, more-difficult-to-catch squirrels (Croft et al. 2021), are outnumbered by the squirrels moving into the area. This is more likely to be achieved in woods that are reasonably isolated, as was the case with 8 of 10 woods studied. The movement of squirrels into the study woods was likely to be minimal because of the short time period (less than 3 weeks) between the deployment of Rhodamine B-treated baits and the start of squirrel removal.

Conclusions

This study demonstrated that, using feeders, it is possible to deliver baits to the majority of grey squirrels in woodland environments and the same methods could be used to employ oral contraceptives to reduce grey squirrel numbers in the future. To understand how effective oral contraceptives could be for grey squirrel control, further research is required to estimate the frequency of feeding visits and the amount of bait consumed by individual squirrels per visit to a feeder, the contraceptive dose rate required to render an individual squirrel infertile and the percentage of squirrels in a population that would need to be rendered infertile to achieve eradication within a reasonable timescale. Future work should also explore bait uptake in larger woods, with different natural food availability and methodological refinements, such as different distributions and densities of feeders and the timing, frequency and longevity of bait deployment. This information could then be used to maximise bait uptake by squirrels and the cost effectiveness of population control via contraception.

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