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Efficacy of Olfactory Repellents on the Aversive Behaviour of Captive Red Deer (*Cervus elaphus*), Based on Field Experiments in Scotland

Abstract

Browsing behaviours exhibited by red deer (*Cervus elaphus*) can pose a challenge for woodland regeneration and management. Repellents are an alternative solution to tackling the problem of overbrowsing by deer in protected areas, where woodland regeneration plans are currently being implemented. In areas where deer fences have been erected in an effort to relieve this problem, vulnerable woodland grouse species have been found to suffer high mortality rates as a result of fence collisions, making this method redundant in sensitive areas. Although other methods exist, limited research has been conducted in Scotland on the effects and benefits of area olfactory wind dispersal repellents, which have had proven success in North America. For the purposes of this study, two herds of captive red deer housed at separate study sites, the Scottish Deer Centre and Atholl Estates were observed, and Beecraigs Country Park utilised as a repellent holder trial site. Three sulphur emitting odour based repellents (rotten eggs, pig's blood and wolf faeces) were used through area wind dispersal, to assess their efficacy in deterring deer from a given area. A significant increase in alert behaviours was observed with the presence of pig's blood, when compared with behaviour observed on control days, or the rotten eggs and wolf faeces repellent. The spatial use of the enclosure by the deer was also observed to be significantly reduced in the area where the repellent was placed for both the Scottish Deer Centre and Atholl Estates. The results are in contrast with previous findings in Scotland and indicate that area olfactory wind dispersal repellents are an effective form of deterrence, meaning they may be a cost-effective and easily accessible method of influencing deer behaviour, when used in conjunction with other management strategies.

Keywords

Alert behaviours, area olfactory wind dispersal, pig's blood, repellent, rotten eggs, spatial use, wolf faeces.

Introduction

Forests were once a dominating feature across the Scottish landscape yet today the woodlands account for a mere 17% of total Scottish land cover (Scottish Natural Heritage, 2017), with native pinewoods forming only 1% (Puplett, 2017). The emergence of agriculture in Scotland approximately 3,900 years ago (Puplett, 2017) and the resulting demand for grazing land, signalled the decline of its woodlands (Scottish Natural Heritage, 2017). Over the course of the following centuries, grazing pressures, poor farming management techniques and the continued practice of burning land to encourage the new growth of heather for livestock, adversely affected woodland regeneration (Puplett, 2017). The demand for timber and fuel in later years caused even further deforestation (Scottish Natural Heritage, 2017), and in more recent times an increase in grazing pressures, climate change and an increase in housing and industrial developments have become some of the more critical threats to woodland regeneration (Natural Scotland, 2017).

The benefits of woodlands are today widely acknowledged, with recent studies showing their importance not only to the Scottish economy through the sale of timber, wood-fuel and the increasing popularity of ecotourism and outdoor pursuits (Bryden, Westbrook, Burns, Taylor & Anderson, 2010), but also benefiting mental and physical health (MIND, 2013; Phillips & Mutsatsa, 2014; Woodland Trust, 2013). In addition, it is recognised that forests play a pivotal role in reducing the risk of flood damage (Willis et al., 2003) and reducing increased climate changing carbon dioxide (CO^2) emissions into the atmosphere through carbon sequestration (Forestry Commission, 2014). The native woodlands of Scotland also provide a valuable habitat for many diverse flora and fauna species, including pine marten (*Martes martes*), red squirrel (*Sciurus vulgaris*), twinflower (*Linnaea borealis),* capercaillie (*Tetrao urogallus*), crested tit (*Lophophanes cristatus*), Scottish crossbill (*Loxia scotica*) and black grouse (*Tetrao tetrix*) (Scottish Natural Heritage, 2013).

Given the many threats facing Scottish woodlands, the Scottish Government, along with Non-Governmental Organisations (NGOs) have implemented action plans with a view to increasing and expanding Scottish woodlands (Forestry Commission Scotland, 2014). In order for these to be successful, management strategies must be implemented efficiently to ensure optimum forest regeneration and reduce the effect of negative impacts on biodiversity (Natural Scotland, 2017).

Research has shown that one of the main threats facing woodland establishment and regeneration in Scotland today, is the high density of the red deer (*Cervus elaphus*) population and their propensity for bark-stripping and browsing of trees and saplings. This behaviour has been shown to inhibit the growth of saplings, increase their vulnerability to disease and result in a loss of vegetation (Månsson & Jarnemo, 2013; Nolte, 1998). With an estimated Scottish population of between 360,000 and 400,000 red deer (Edwards & Kenyon, 2013) and with no natural predators to maintain a sustainable population (Edwards & Kenyon, 2013; Jayakody, Sibbald, Gordon & Lambin, 2008), the damaging effects of red deer on new and recovering woodlands continues to be a significant problem (Scottish Natural Heritage, 2016). A variety of management practices have therefore been implemented in an attempt to overcome this problem. Examples of some of these are provided below.

Fences were erected in Scotland to deter deer from habitats containing new seedlings or woodland which were of particular importance in sustaining rare and vulnerable species, including capercaillie and black grouse. Whilst the fences have signified a positive management aid in the deterrence of deer from sensitive areas, they are not without their problems and cannot be relied upon as a permanent solution (Forestry Commission Scotland, 2014). Fences can be expensive (Armstrong, Gill, Mayle & Trout, 2003; Elmeros, Winbladh, Andersen, Madsen & Christensen, 2011; Forestry Commission Scotland, 2014; Miller et al., 2008; Trout & Brunt, 2014), with a limited lifespan (Forestry Commission Scotland, 2014; Scottish Natural Heritage, 2016), and in extreme weather conditions such as heavy snowfall, are no longer functional (Forestry Commission Scotland, 2014). Studies into woodland grouse species have indicated high mortality rates related to fence collisions (Baines & Andrew, 2003; Baines & Summers, 1997) and whilst marking the fences appears to be a successful strategy in the reduction of fatal collision incidences (Baines & Andrew, 2003), a significant level of mortality rates is still evidenced (Royal Society for the Protection of Birds, 2017). The erection of fences may also prove to be detrimental to the welfare of deer through preventing access to shelter (Scottish Natural Heritage, 2016).

Further to this, the culling of deer is a common population management strategy in Scotland (Baines & Andrew, 2003; Baines & Summers, 1997), with approximately 68,064 red deer culled in 2014/15 (Scottish Natural Heritage, 2016). As well as being an effective method of reducing deer numbers, this is a necessary management practice for maintaining a healthy and sustainable deer population. However, it is evident that even with the use of culls to control the population, high deer numbers remain, indicating that the negative impact upon vulnerable areas through overbrowsing and grazing is still being observed (Scottish Natural Heritage, 2016).

Alternative methods of deterrent, such as chemical deer repellents, should be considered (Nolte, 1998) for where fences have been removed or marked. Chemical repellents tend to operate within four modes: fear (odours which emit sulphurous compounds mimicking predator activity or visual and sonic repellents), conditioned aversion (the deer form an association between the repellent and illness), pain (repellent contains compounds which cause irritation to the sensitive skin, mainly of the mouth and nose of the deer) and taste (repellent contains bitter agents when ingested by the deer) (Kimball, Taylor & Perry, 2009; Miller et al., 2008; Wagner & Nolte, 2001). The primary aim of the repellent is to deter deer from the treated area. Many studies in Europe and North America have examined the efficacy of repellents as a deer deterrent to protect trees susceptible to browsing damage (Miller et al., 2008; Nolte, 1998; Renaud, Verheyden-Tixier & Dumont, 2003). However, few of these studies have been conducted in Scotland (Armstrong & Robertson, 2013; Armstrong et al., 2003; Hodge & Pepper, 1998; Putman, 2004) where there is a lack of available approved chemical repellents (Armstrong & Robertson, 2013).

Studies researching the efficacy of taste repellents found there to be no significant correlation between deer avoidance and the treated area (Elmeros et al., 2011; Nolte, 1998; Wagner & Nolte, 2001). Repellents causing conditioned aversion were also proven to be ineffective against deer (Kimball et al., 2009), as were the visual and sonic fear repellents (D'Angelo et al., 2006; Mayle, 1999; Perry, 2017; Putman, 2004; Ujvari, Baagoe & Madsen, 2004). The repellent which caused irritation was shown to be an effective tool in deterring deer (Armstrong et al., 2003; Hodge & Pepper, 1998; Armstrong & Robertson, 2013). However, under the Chemicals Regulation Directorate (Armstrong & Robertson, 2013), this deterrent mode cannot be used in Scotland as it does not have the required approval (Armstrong & Robertson, 2013). Fear inducing olfactory repellents have proven to be the most effective form of deterrent (Miller et al., 2008; Nolte, 1998; Wagner & Nolte, 2001) as deer depend on their sense of smell to identify predator presence (Elmeros et al., 2011). The odour repellents which contain compounds producing sulphurous scents, such as egg (Bullard, Leiker, Peterson & Kilburn, 1978; Miller et al., 2008; Trent, Nolte & Wagner, 2001; Wagner & Nolte, 2001) and blood or predatory animal faecal matter (Trent et al., 2001) were found to be the most effective barrier to deer (Wagner & Nolte, 2001). These can be applied via two methods; topical application which is sprayed directly onto the plant, but is susceptible to climatic conditions making it ineffective after heavy rainfall or snow (Perry, 2017) and area application which is normally presented in dried sachet form (Kimball et al., 2009; Wagner & Nolte, 2001).

It should be noted that a number of factors can determine the efficacy of the repellent: the palatability of food present (Armstrong & Robertson, 2013; Nolte, 1998;

Trent et al., 2001), the level of hunger in the animals (Miller et al., 2008) and the length of time taken for the deer to become habituated to the repellent (Elmeros et al., 2010). Therefore at present, no repellent is considered to be 100% effective, particularly in the context of large areas where they have most benefit (Trent et al., 2001). Recommendations from earlier studies suggest that all repellents perform better as a short term emergency solution (Trout & Brunt, 2014) for when saplings are at their most vulnerable (Nolte, 1998), and used in conjunction with alternative management techniques, including the provision of alternative feeding stations and shelter (Armstrong & Robertson, 2013; Armstrong et al., 2003; Elmeros et al., 2011; Nolte, 1998; Trent et al., 2001). However, providing alternative feeding stations may inadvertently attract deer to sensitive areas and whilst supplementary food is supplied, the risk of the deer browsing on the protected, palatable plants remains high. Supplying additional forage also enables a higher number of deer to be sustained within that environment (Armstrong & Robertson, 2013). It is therefore considered that this technique is not a practical approach to deterring deer from protected areas.

Studies conducted in North America showed significant results with egg and blood based odour repellents, proving their effectiveness against the browsing damage caused by white-tailed deer (*Odocoileus virginianus*) and black-tailed deer (*Odocoileus hemionus*) (Chelsea, 2013; Kimball et al., 2009; Mattern, 2017; Nolte, 1998; Perry, 2017; Wagner & Nolte; 2001). Studies in Scotland focused only on aluminium ammonium sulphate based repellents, which have proven to be ineffective (Armstrong & Robertson, 2013; Hodge & Pepper, 1998; Mayle, 1999; Trout & Brunt, 2014). The chemical repellents which are commercially available in Scotland are therefore limited in both their effectiveness and variety. Additionally, no research appears to have studied the successful application of egg and blood based compounds in deer deterrence in Scotland (Armstrong & Robertson, 2013). In view of this, this study proposes to examine the effectiveness of egg and blood based repellents, together with wolf faeces, to determine if this form of repellent will prove to be as successful with the red deer in Scotland as it has with the white-tailed and black-tailed deer in North America. The use of area repellents, presented in liquid form and using the wind for dispersal will be trialled in this study. The repellents such as rotten eggs, are composed of organic matter only and therefore not subject to approval by the Chemicals Regulation Directorate (Armstrong & Robertson, 2013), thus allowing managers easy access to an inexpensive and legislation free deterrent.

This study aims to promote effective cooperative forest management for small scale and sensitive woodland regeneration areas where vulnerable species, such as woodland grouse, are prone to fence collision, resulting in a high mortality rate. The use of deer fences will still be an effective management tool in deer deterrence, but only in areas which do not impact upon vulnerable woodland grouse and where it does not impede the Land Reform (Scotland) Act 2003, which allows the public the right to roam upon any land within Scotland (Scottish Outdoor Access Code, 2017). Until such time as the reintroduction of natural predators in Scotland effectively controlling the red deer numbers, culls are an important management strategy to ensure sustainable populations are maintained within the carrying capacity limits of the land. Tree guards can be a more cost-effective solution in smaller areas (Hodge & Pepper, 1998; Trout & Brunt, 2014) and would be a useful tool when used in conjunction with an area olfactory wind dispersal repellent. It is considered that this study will prove the effectiveness of organic based area odour repellents as a viable alternative to the ineffective topical repellents currently available in Scotland. When combined with other management strategies such as tree guards, they will provide a significant and inexpensive forest management tool in repelling problem deer from vulnerable and protected areas.

The object of this study was to examine the efficacy of three olfactory repellents to determine their effectiveness on two captive herds of red deer at the Scottish Deer Centre, Fife and Atholl Estates, Perthshire. A third site, Beecraigs Country Park, West Lothian was used as a trial site for the experimentation of the deterrent holder to determine the most suitable casing for the deterrent and furthermore, for it to withstand any interactions from the red deer. The repellents, which consisted of pig's blood, rotten eggs and wolf faeces, were used to ascertain any impacts on deer spatial or alert behaviours within the enclosure. From this, the effectiveness of the repellent could be determined.

It has been found that alert behaviours in prey species increases in the presence of predators (Jayakody et al., 2008). Therefore, it was reasoned that these behaviours would increase in the presence of the repellents, as each of the compounds signalled a level of predator activity.

This study predicted the following hypotheses, with H_0 representing the null hypothesis and H_p representing the predicted hypothesis:

Hypothesis One

H_0 - Alert behaviours displayed in red deer will not increase with the presence of a repellent

H_p - Alert behaviours displayed in red deer will increase with the presence of a repellent

Hypothesis Two

H_0 - Spatial use of the enclosure by the red deer will not be less frequent in the area where the repellent is present

H_p - Spatial use of the enclosure by the red deer will be less frequent in the area where the repellent is present

Materials and Methods

Study Sites

Captive red deer housed at three separate sites across Scotland: Beecraigs Country Park, West Lothian, the Scottish Deer Centre, Fife and Atholl Estates, Perthshire were used for the purpose of this study. They were observed between the period of 30th May until 6th July 2017, with 10 day observational studies conducted at each site.

The studied deer at Beecraigs consisted of 57 one year old animals, with a mix ratio of males and females. Although Beecraigs was open to the public, there was no access to any fence line of the enclosure and the deer therefore had little human interaction. The enclosure provided all their required food through grazing, and supplementary feed was not offered during this study time.

The Scottish Deer Centre housed 15 adult deer with a ratio of 12 female and three male individuals. Calves born during the study period were also observed; two on day 1 of the study, four calves on day 2, five on days 3 to 6 and six calves on days 6 to 10. This centre was also open to the public who had access to the deer via the east fence line of the enclosure. This allowed the public to both interact with the deer and to feed them, although no additional feed was provided by the centre as they were able to graze within their enclosure. An enclosure adjacent to the south side of the deer housed three adult wolves which, although visible to the deer, did not appear to affect their behaviour or spatial use of the enclosure.

The observed deer herd at Atholl Estates consisted of 12 animals; nine female and three male, plus five calves, making a total of 17 deer altogether. The estate was open to the public and they were able to access the north east and south east fence lines of the enclosure. From days 1 to 7 no supplementary feed was provided, however on days 8 to 10 a small amount of food was given to them by estate staff during a tour given for the public, who were then able to observe the deer at close quarters. This food was placed in the same section (B3) of the enclosure on each day.

The initial implementation and experimentation of the holder at Beecraigs Country Park was not successful and a new holder had to be designed to ensure it would withstand any probable interaction from the deer. Beecraigs was therefore excluded in the study of behavioural and spatial observations and instead utilised as a trial site to determine the most suitable and functional holder.

The enclosures at the remaining two study sites were visually divided into equal grid sections and labelled accordingly. The Scottish Deer Centre was sectioned into nine areas (see Appendix One) and identified as A1, A2, A3, B1, B2, B3, C1, C2 and C3. Atholl Estates was divided into 12 segments (see Appendix Two), as an additional field adjoined the main enclosure but was separated from it by a fence. Access to this field was via two entrances situated at each end of the enclosure and thus D1, D2 and D3 were added to the grid system.

Repellents

Compounds which represent predator presence through either their origination from the predator itself, i.e. faeces, or through their emission of sulphurous odours, i.e. egg and blood, have been demonstrated as the most effective form of area repellents against deer browsing damage in North America (Trent et al., 2001; Wagner & Nolte, 2001). Little research has been conducted in Scotland into the effectiveness of these products, and it is for this reason they were selected for this study in order to determine their efficacy on red deer. The selected repellents, such as rotten eggs, are composed of organic matter only and therefore not subject to approval by the Chemicals Regulation Directorate (Armstrong & Robertson, 2013). Fresh wolf faeces supplied by the Scottish Deer Centre, fresh pig's blood supplied by Shotts Abattoir and rotten eggs were the three repellent compounds used in this study. The wolf faeces were mixed with a small amount of water to produce a more fluid consistency and to facilitate the application of the deterrent. The mixture was kept at room temperature throughout the study. The pig's blood was stored in a cool dark room to forestall its coagulation and additionally, was stirred at regular intervals throughout the study period. The rotten egg mixture consisted of 10 fresh eggs beaten together and left in a clear container in a greenhouse for 14 days prior to its use in the study, to enable the putrefaction process. Two batches of putrid eggs were made to ensure equal replication at the Scottish Deer Centre trial and the Atholl Estates study.

A large sponge was halved and together with two pieces of flannel was immersed in the repellent to be tested and allowed to soak for 36 hours prior to its use. Sponges were used to ensure optimal absorption and containment of the liquid repellent whilst the flannels also provided maximum intake of the deterrent. After its immersion in the repellent, each piece of flannel was wrapped around a sponge and inserted into a large fatball holder, designed for use in bird feeding. Ultimately, the fatball holder was placed in an outer casing before its placement within the enclosure. A small plastic bowl was attached to the bottom of the fatball holder to collect any runoff from the repellents, particularly the pig's blood. This ensured no spillage was allowed to contaminate the ground. It was also reasoned that any leakage of a repellent from the container could adversely affect the results of the study. Each fatball holder was sterilised after use in the field, and again prior to its use at the next site. This reduced the threat of possible contamination, or the risk of disease, to the observer and the deer whilst in use. It also removed any repellent scent which could influence the deer at the subsequent study site.

Personal protective equipment, specifically face mask, latex gloves and overalls, were used when applying each of the repellents. This was to prevent direct contact, ingestion or inhalation of the deterrents, which could cause irritation or illness.

Holder

The outer casing to house the fatball holder was constructed from two semi circular, pvc coated metal frames, which, when positioned together formed an open cylinder (see Appendix Three). The two halves were securely bound together with 1mm wiring fastened at regular intervals around the casing. The finished height was 91cm, the diameter 22cm and comprised 11cm rectangular gaps through which all the elements could easily pass. Four metal poles of height 110cm and diameter 2cm, were placed inside the encasement and hammered firmly into the ground to a depth of 30cm, and anchored to the casing with heavy duty duct tape. Six metal tent pegs were hammered into the base to ensure optimal stability against possible deer scrutiny, and thereby reducing the likelihood of the whole contraption being toppled over, causing spillage in the area and probable contamination. Finally, to complete the assembly of this sturdy structure, a section from a pvc coated wire hanger was cut to size and folded in the centre to fashion a 20cm V-shaped tool, from which the fatball holder would be suspended by its handle. Each of the ends were similarly bent to a length of 7.5cm and inserted in the tops of two of the four metal poles. The whole device was secured in place with the use of duct tape. The completed configuration was a sturdy production which allowed the wind to pass through unimpeded and facilitated the dispersal of the repellent odour into all areas of the enclosure.

Observations

An ethogram (see Appendix Four) was designed prior to field observations to determine the behaviour categories that the deer would display. Each site was studied over a 10 day period with interval scan behavioural observations (Dawkins, 2007; Martin & Bateson, 2007) and spatial observations being recorded every 10 minutes within the hour long study times throughout the day. Each treatment day was preceded by a control day, where the holder remained in the enclosure but lacked the containment of any repellent. The first day of study at each site was utilised to gauge the spatial use within the enclosure of the deer i.e. which part of the enclosure did they preference and spend most of their time. This data provided the requisite information for the placement of the holder for the following control day. This process was continued throughout the study to formulate which grid area the holder should be placed into for the subsequent day of study. This information was analysed to determine whether or not the deer avoided, or ignored, the presence of the holder, with or without the existence of the repellent, and therefore the efficacy of the repellent used and if any significant differences were apparent between the three repellents. It was predicted that the deer would show avoidance of the areas where the holder containing repellent was placed on the repellent days, whereas the control days with only the holder present in the enclosure would result in no significant difference in their spatial use. With regards to the behavioural observations, it was predicted that the deer would display a lower frequency of alert behaviours on the control days over the repellent days, with a higher frequency of rest behaviours observed on the control days over the repellent days. The calves present at both the Scottish Deer Centre and Atholl Estates were included in the data collection for both behaviour and spatial use. Although there was an absence of recordings of calves during the live data collection, due to their instinct to hide in the long grass in order to hide from, and be protected from, predators (Wass, Pollard and Littlejohn, 2004), when they were visible their behaviours and spatial use showed no difference from that of the adults. However, the out of sight behaviour, although recognised by the observer was not included in the data analysis as it applied only to the calves and could not be verified visually.

Three camera traps (Bushnell Trophy Cam HD 14MP Aggressor No-Glow Trail Camera) were placed in the enclosure and positioned to focus on the grid area which housed the holder. They were used to record spatial data within the enclosure of the deer over a 24 hour period, and in particular during the times when the deer were most active and live data was unable to be collected. The cameras were programmed to take pictures every 15 minutes. The behaviours that each deer displayed within the holder area combined with their spatial use were then recorded. Unfortunately, all behavioural data, apart from holder interaction, were lost from the Scottish Deer Centre site, due to the malfunction of the external drive and therefore this information could not be used in the analysis. It should be noted that prior to the live collection of data, a habituation period of half an hour was allowed, to ensure the settlement of the deer after their disturbance during the placement of the holder into the grid area, and the repositioning of the three camera traps.

Data Analysis

The behaviours analysed were compartmented into the following categories; alert, feeding, holder interaction, locomotive, rest and standing. Drinking, grooming, interaction with deer, object and people, out of sight and suckling behaviours were omitted from the analysis process as they were not behaviours that would have been influenced by the presence of the repellents and were displayed too infrequently to allow for reliable statistical analysis.

The analysis of variance (ANOVA) test was performed, to determine if any significant differences ($P<0.050$) would be found between the alert behaviours displayed on the control days, and those displayed on the repellent days. The ANOVA test allows for analysis to be conducted on an explanatory variable with more than two categories (Dawkins, 2007; Martin & Bateson, 2007), and this study contained four categories of explanatory variable: control, wolf faeces, rotten eggs and pig's blood. If ANOVA determined a significant result was present in the data, a Tukey test was performed (see Appendix Five) to examine which of the pair-wise differences between the categories were significant. The standard errors of the significant pair-wise differences were calculated using the formula

$$(\text{difference - lower value}) / 2.$$

Finally, r^2 was calculated, using the formula

$$r^2 = 1 - RSS_{MODEL} / RSS_{NULL}$$

with RSS_{MODEL} representing the residual sum of the squares of the model and RSS_{NULL} representing the residual sum of the squares of the null model. This result determined the amount of the difference in variation between the response variable (observed behaviours) and the explanatory variable (repellents and control data). A Shapiro-Wilk test determined whether the residuals were normally distributed ($P\geq 0.050$), to ascertain whether the ANOVA result could be accepted as reliable. If the residuals were found to be normally distributed, with a non significant ANOVA, then the result would be accepted and no further tests performed, therefore accepting the null hypothesis. If however, they were found to be not normally distributed ($P<0.050$), the data was square root transformed to see if this action would now reject the null hypothesis after further ANOVA testing. If the ANOVA results again found no significant difference in the data, a non-parametric Kruskal-Wallis (see Appendix Six) test would be performed to draw a final conclusion as to whether a significant result could be achieved or not. If the resulting P-value was found to be not significant ($P\geq 0.050$), then it could be assumed that there were no overall significant differences between the increase in observed behaviours and the presence of a repellent, thereby accepting the null hypothesis. All of the above tests were performed in RStudio (2016).

A chi-square test of independence (X^2) (see Appendix Seven) was used to determine if there was a significant association between the presence of a repellent and the spatial use within the enclosure by the deer (Ling, 2008). The formula used was

$$X^2 = \sum [(O - E)^2 / E]$$

where O is the observed data and E is the expected observed data. The observed data represented the total number of deer observed within the area containing the holder for that relevant repellent day. The expected data represented a proportion of the deer observed in the holder area on the total control days divided by the total sum of observed deer for each control day

E (%) = (Total control in H / Total control in H + N) x Total treatment in H + N.

The critical value of X^2 was found to be 3.84 after determining the degrees of freedom to be 1. Here the formula

$$(r - 1) \times (c - 1)$$

was performed, where r represents the number of rows within the table, and c represents the number of columns present. If the resultant data showed a value higher than this critical value, the null hypothesis of the repellent not affecting the spatial use within the enclosure of the red deer was rejected. A final P-value for each test was calculated and reported using the Excel (2007) CHITEST function.

Results

Spatial Data

Live observational recordings showed the frequency of distribution by the deer within their enclosures, was significantly reduced at the Scottish Deer Centre when the rotten eggs repellent (see Figure 1.), pig's blood repellent (see Figure 2.) and wolf faeces repellent (see Figure 3.) were present. This observation was replicated at Atholl Estates for each of the repellents (see Figures 4 - 6). The control days had no significant effect on the deer's spatial use of the enclosure.

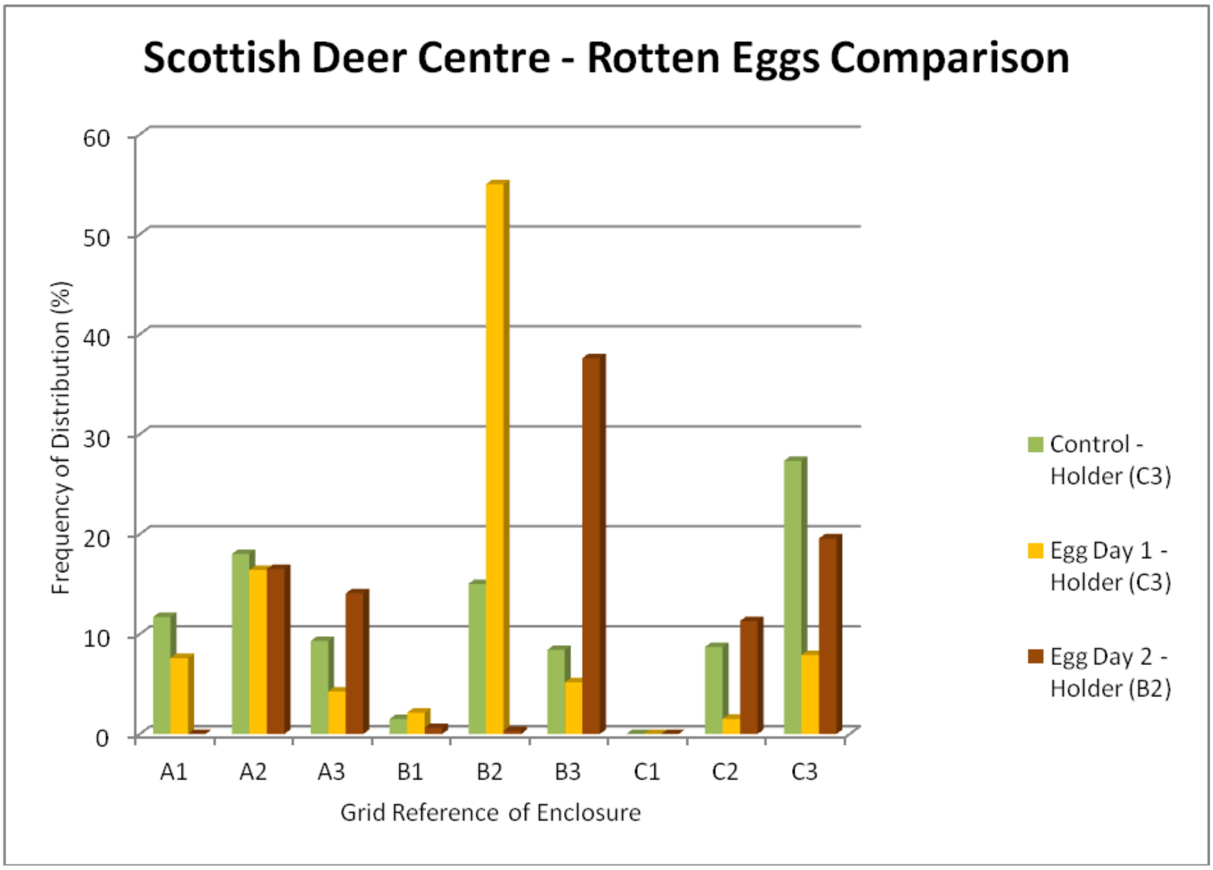

Figure 1. A bar graph showing the comparison of the red deer's frequency of distribution (%) in their enclosure on the control days with those when the rotten egg repellent was present at the Scottish Deer Centre. The grid area where the holder was placed for each day is shown in brackets. Placement of the holder within the enclosure was determined by which grid area had shown to house the highest number of deer on the previous day.

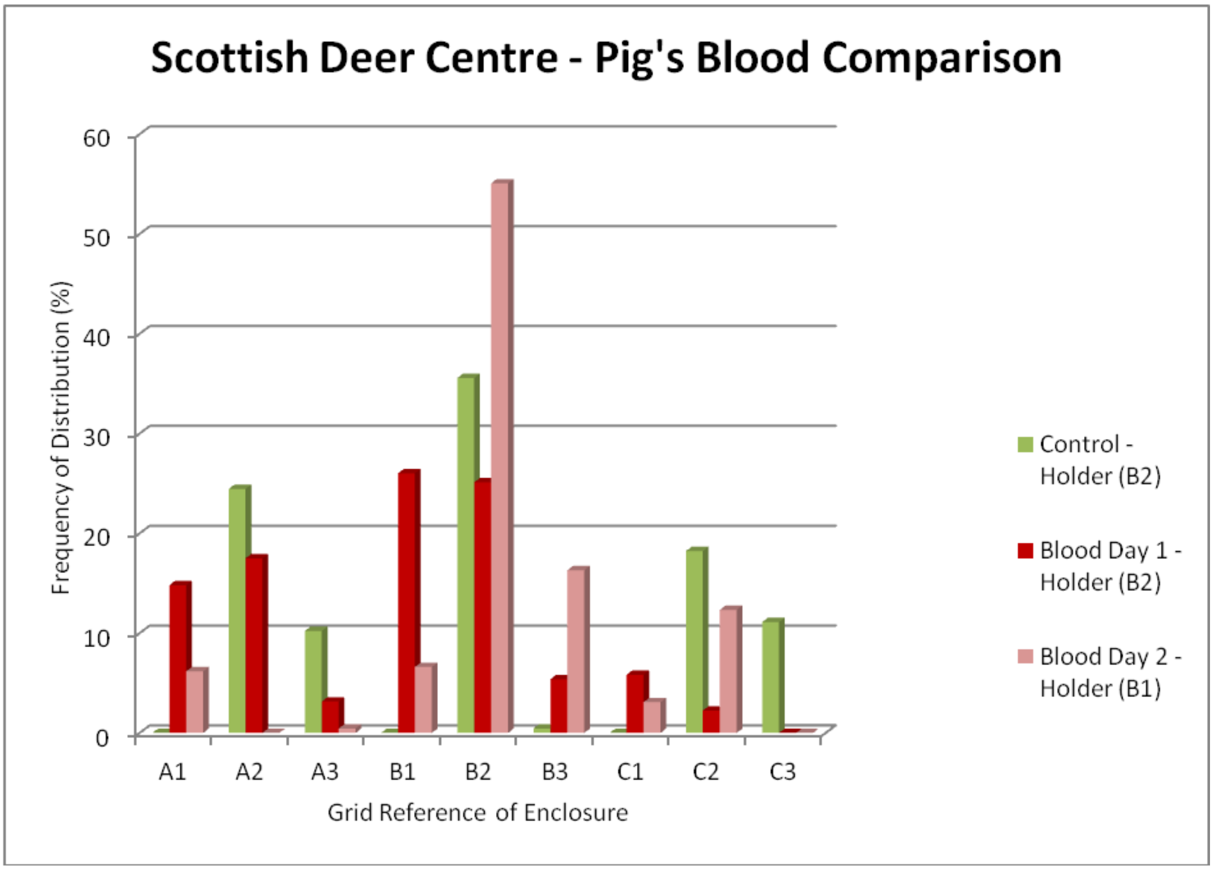

Figure 2. A bar graph showing the comparison of the red deer's frequency of distribution (%) in their enclosure on the control days with those when the pig's blood repellent was present at the Scottish Deer Centre. The grid area where the holder was placed for each day is shown in brackets. Placement of the holder within the enclosure was determined by which grid area had shown to house the highest number of deer on the previous day.

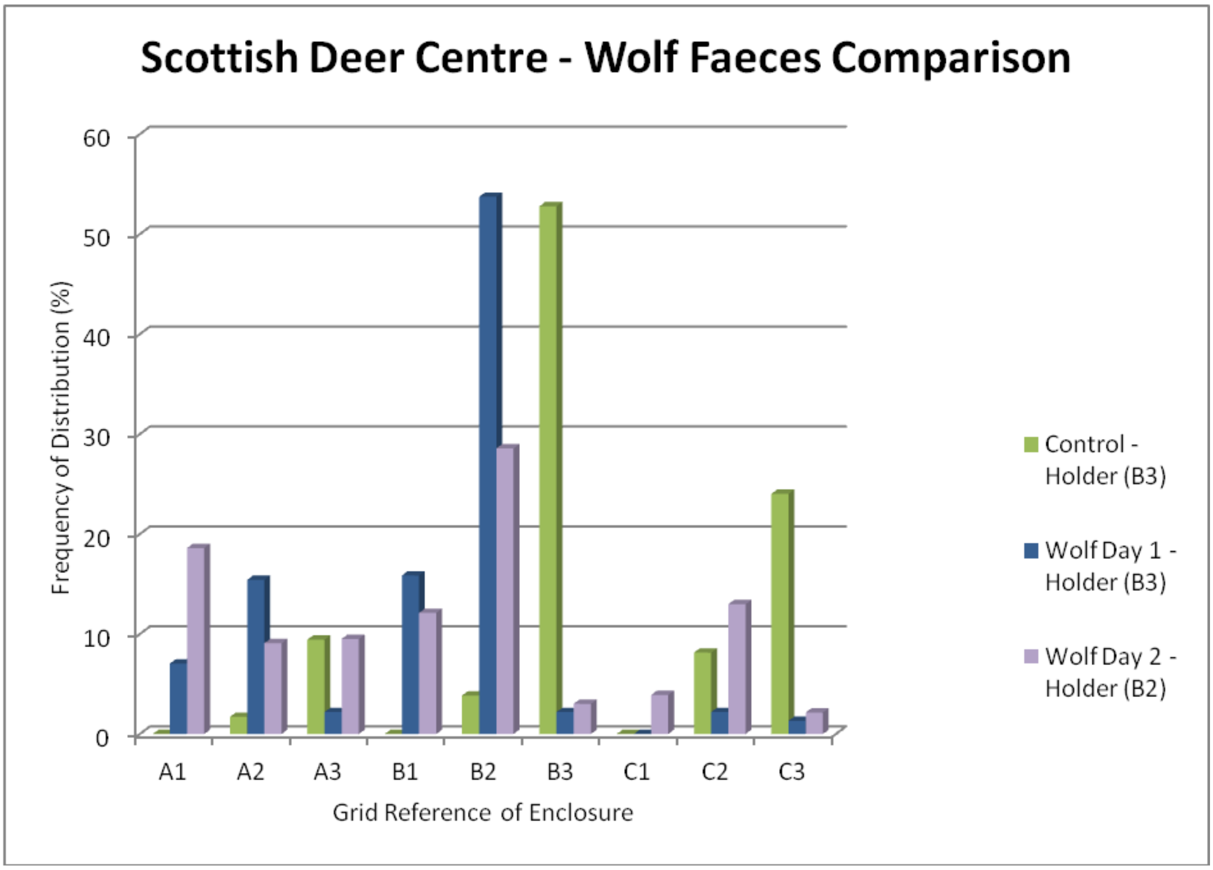

Figure 3. A bar graph showing the comparison of the red deer's frequency of distribution (%) in their enclosure on the control days with those when the wolf faeces repellent was present at the Scottish Deer Centre. The grid area where the holder was placed for each day is shown in brackets. Placement of the holder within the enclosure was determined by which grid area had shown to house the highest number of deer on the previous day.

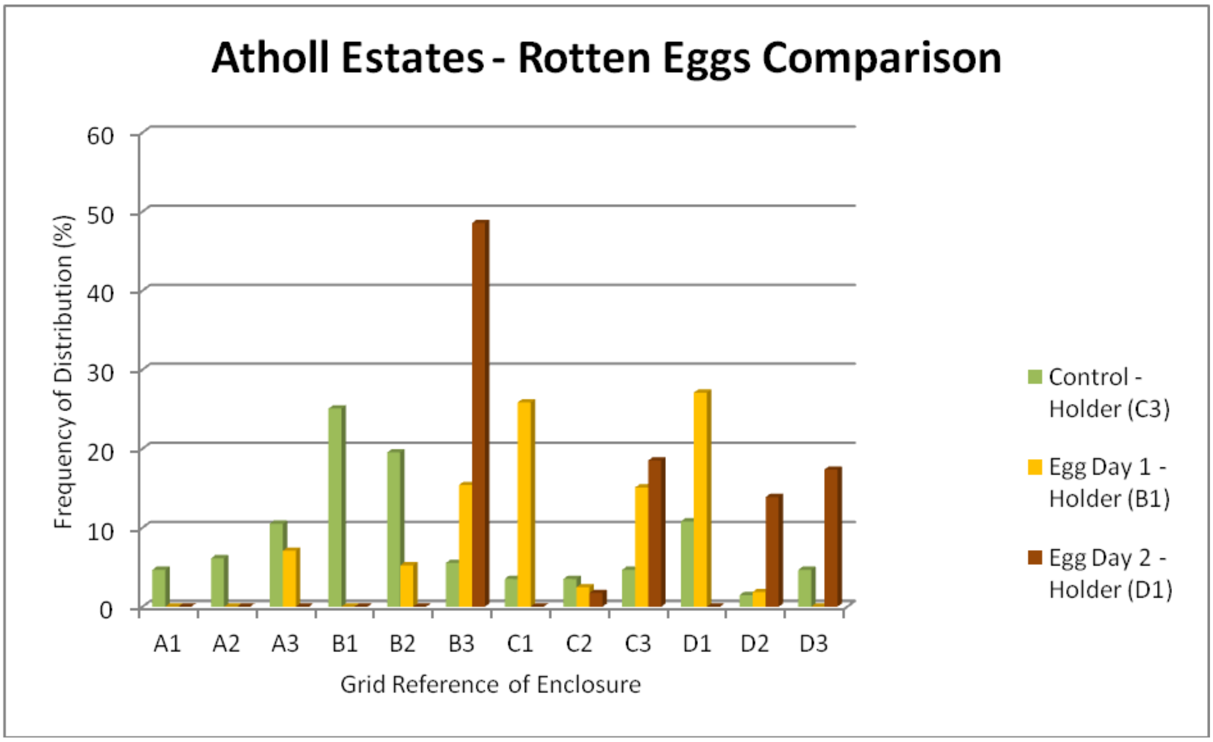

Figure 4. A bar graph showing the comparison of the red deer's frequency of distribution (%) in their enclosure on the control days with those when the rotten egg repellent was present at Atholl Estates. The grid area where the holder was placed for each day is shown in brackets. Placement of the holder within the enclosure was determined by which grid area had shown to house the highest number of deer on the previous day.

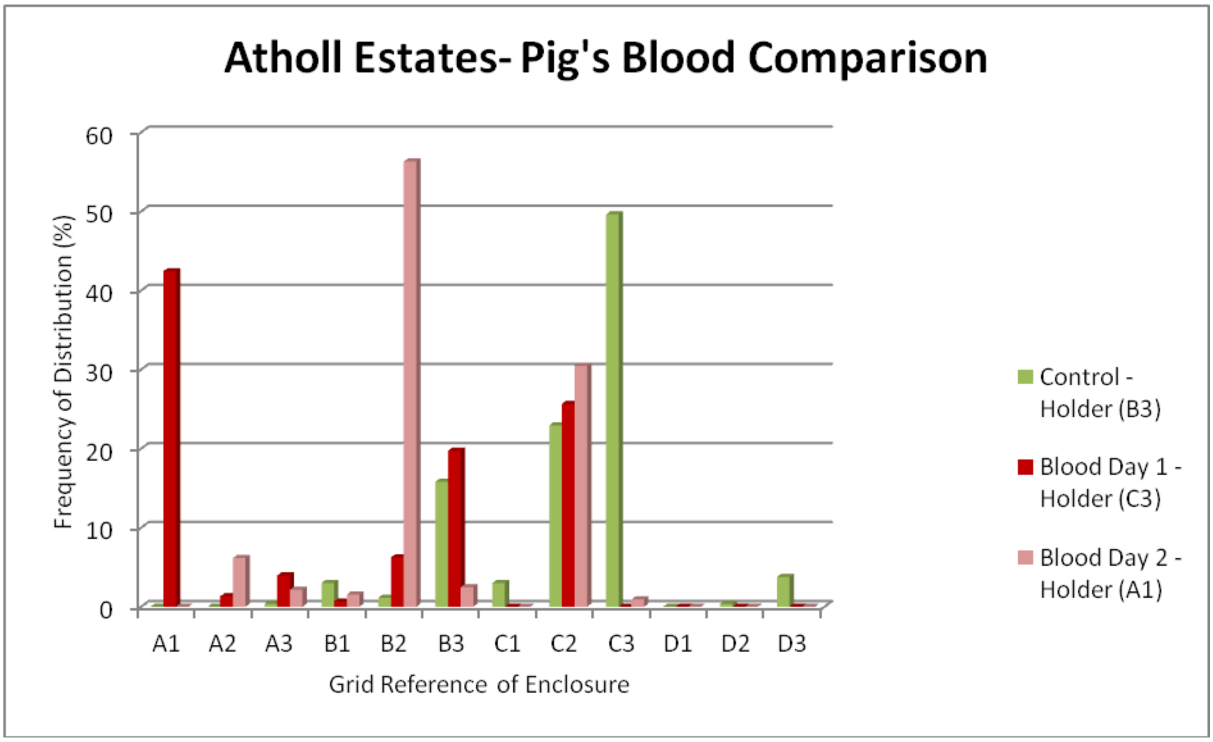

Figure 5. A bar graph showing the comparison of the red deer's frequency of distribution (%) in their enclosure on the control days with those when the pig's blood repellent was present at Atholl Estates. The grid area where the holder was placed for each day is shown in brackets. Placement of the holder within the enclosure was determined by which grid area had shown to house the highest number of deer on the previous day.

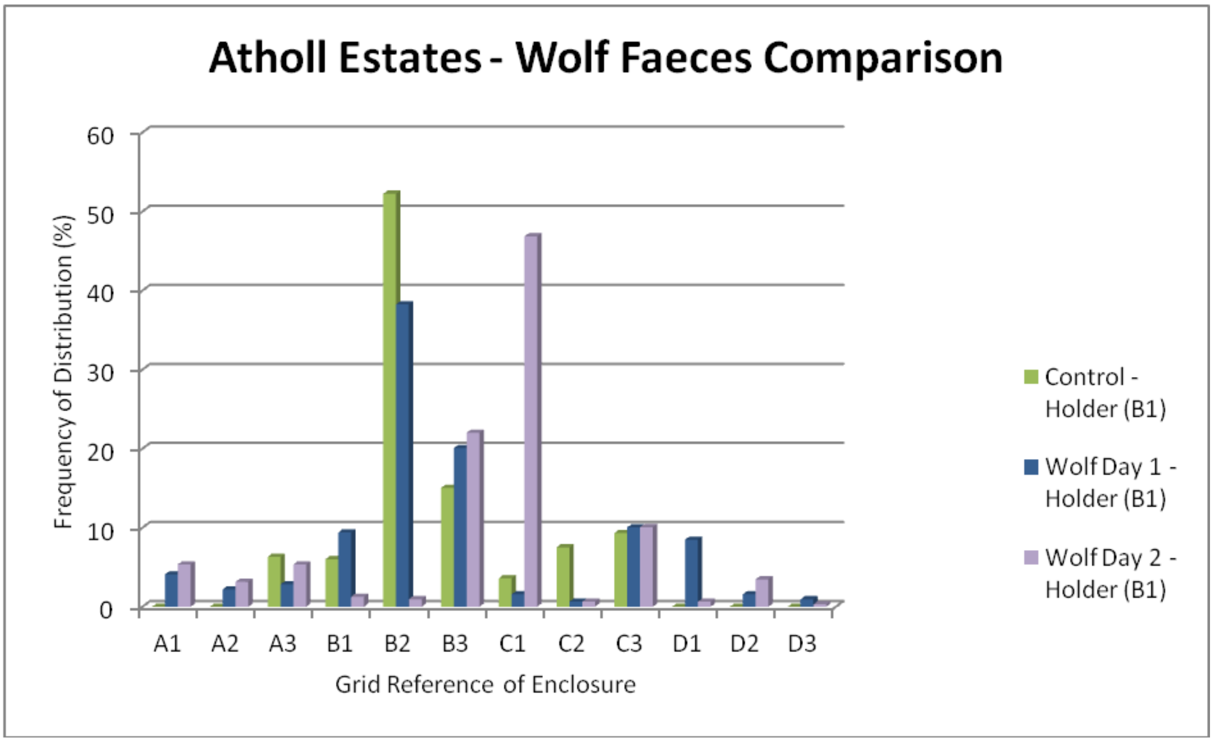

Figure 6. A bar graph showing the comparison of the red deer's frequency of distribution (%) in their enclosure on the control days with those when the wolf faeces repellent was present at Atholl Estates. The grid area where the holder was placed for each day is shown in brackets. Placement of the holder within the enclosure was determined by which grid area had shown to house the highest number of deer on the previous day.

Scottish Deer Centre

As shown in Table 1. the rotten egg repellent was found to have had significantly reduced the number of deer within the grid area where the repellent was placed. The pig's blood and wolf faeces repellent also signified a decreased frequency of the deer in the areas where they were placed within the enclosure, though not with as much effect as the rotten egg repellent.

Table 1. Results showing the Chi-square analysis and P-value on the spatial use within the enclosure of the red deer at the Scottish Deer Centre, during the treatment periods where repellent was present. Data presented is from live observations and camera trap observations.

	Live Data				**Camera Trap Data**			
	X^2	**df**	**N**	**P-Value**	**X^2**	**df**	**N**	**P-Value**
Rotten Eggs	304.32*	1	656	3.772×10^{-68}**	76.03*	1	3889	2.794×10^{-18}**
Pig's Blood	86.95*	1	450	1.113×10^{-20}**	38.73*	1	4130	4.867×10^{-10}**
Wolf Faeces	90.81*	1	458	1.582×10^{-21}**	39.18*	1	4019	3.865×10^{-10}**

df	degrees of freedom
N	total count of deer on observed day
*	critical chi-square statistic value $\geq$ 3.84
**	significant at P-value P<0.050

Atholl Estates

Table 2. shows the pig's blood repellent to have had the most significant effect on the deer's spatial use, by reducing their frequency in the area where the repellent was sited. Whilst the wolf faeces also showed a strong significant reduction during data collection during the camera trap observations, only a slight effect was noted during the live data collection. The rotten egg repellent was shown to be a significantly effective deterrent in reducing the deer's frequency within the deterrent area.

Table 2. Results showing the Chi-square analysis and P-value on the spatial use within the enclosure of the red deer at Atholl Estates, during the treatment periods where repellent was present. Data presented is from live observations and camera trap observations.

	Live Data				Camera Trap Data			
	X^2	df	N	P-Value	X^2	df	N	P-Value
Rotten Eggs	166.00*	1	498	5.532×10^{-38}**	40.37*	1	3264	2.101×10^{-10}**
Pig's Blood	209.67*	1	629	1.621×10^{-47}**	52.95*	1	3264	3.421×10^{-13}**
Wolf Faeces	9.83*	1	637	0.002**	240.31*	1	3264	3.366×10^{-54}**

df degrees of freedom
N total count of deer on observed day
* critical chi-square statistic value $\geq$ 3.84
** significant at P-value $P<0.050$

Behavioural Data

Scottish Deer Centre - Live Data Collection

No significant difference was found between any of the repellent days and the control days with locomotive behaviour (F=1.57,d,f=3,17,P=0.233), standing behaviour (F=1.27,d,f=3,17,P=0.317), feeding behaviour (F=0.62,d,f=3,17,P=0.612) and rest behaviour (F=0.15,d,f=3,17,P=0.925) after performing an ANOVA test. Holder interaction behaviour also showed no significant difference after the square root data transformation and conduction of a Kruskal-Wallis test (X^2=5.07,d,f=3,P=0.167). Significant differences in alert behaviour were found between the observed repellent days and the observed control days (F=5.92,d,f=3,17,P=0.006). A post hoc Tukey test showed that the mean score for the presence of the pig's blood repellent was significantly higher than the presence of the
wolf faeces repellent (difference=-6.28$\pm$2.48SE,P=0.011),
rotten egg repellent (difference=-5.27$\pm$2.26SE,P=0.019) and the control days (difference=-5.76$\pm$2.20SE,P=0.008). A 51% variation in displayed alert behaviours was explained by the variation with the presence of pig's blood (r^2=0.51).

Atholl Estates - Live Data Collection

An ANOVA test found no significant difference between rest behaviour (F=1.16,d,f=3,22,P=0.346), feeding behaviour (F=0.38,d,f=3,22,P=0.765), standing behaviour (F=0.79,d,f=3,22,P=0.512) and locomotive behaviour (F=2.71,d,f=3,22,P=0.070) and the presence of repellents in the enclosure. There was also no significant difference shown after the square root data transformation and conduction of a Kruskal-Wallis test in alert behaviour (X^2=3.43,d,f=3,P=0.330) and holder interaction behaviour (X^2=1.89,d,f=3,P=0.596).

Scottish Deer Centre - Camera Trap Data Collection

There was no significant difference observed between holder interaction behaviour displayed in the deer and the presence of the different repellents (F=0.95,d,f=3,5,P=0.482).

Atholl Estates - Camera Trap Data Collection

No significant difference was found between the holder interaction behaviour (F=0.69,d,f=3,5,P=0.595), alert behaviour (F=0.36,d,f=3,5,P=0.785), standing behaviour (F=0.54,d,f=3,5,P=0.676) and locomotive behaviour (F=4.69,d,f=3,5,P=0.065). Rest behaviour also showed no significant difference after square root data transformation and performance of a Kruskal-Wallis test (X^2=5.04,d,f=3,P=0.169). A significant difference however, was found in the display of feeding behaviour and the presence of repellents (F=7.04,d,f=3,5,P=0.030). A post hoc Tukey test showed that the mean score for the presence of the rotten egg repellent was significantly higher than the presence of wolf faeces (difference=-59.27$\pm$58.60,P=0.048). An 81% variation in displayed feeding behaviour was explained by the variation with the presence of the rotten egg repellent rather than with the wolf faeces repellent (r^2=0.81).

Discussion

The aim of this study was to determine whether alternative methods of repellent could be used as an effective deterrence against red deer from negative browsing in Scotland (Armstrong & Robertson, 2013; Hodge & Pepper, 1998; Mayle, 1999; Trout & Brunt, 2014). It questioned whether these repellents would result in an increase in alert behaviours in the deer, therefore indicating a conscious avoidance to perceived predator presence (Jayakody et al., 2008). With previous studies preferring topical application of repellents, this study investigated the use of wind dispersal, utilising liquid presentation rather than the dried sachet format (Kimball et al., 2009; Wagner & Nolte, 2001). It considered the benefits of utilising organic based compounds, such as rotten eggs, which would not need to adhere to the Chemicals Regulation Directorate (Armstrong & Robertson, 2013).

The results of this study reached a similar conclusion to Miller et al. (2008), Nolte (1998), Trent, Nolte and Wagner (2001) and Wagner and Nolte (2001) that a fear inducing olfactory repellent would successfully deter deer from a specific area. However, Armstrong and Robertson (2013), Hodge and Pepper (1998) Mayle (1999) and Trout and Brunt (2014) found no evidence to support this. The use of an egg based compound together with a blood mixture and animal faeces, proved to be an effective deterrent against deer, as a result of their resemblance to predator presence (Trent et al., 2001 and Wagner & Nolte, 2001). This contradicts however, the findings of Elmeros, Winbladh, Andersen, Madsen and Christensen (2011) and Kimball et al. (2009) who did not reach this conclusion during their research. This study found a significant result with the effectiveness of a wind based application of an area repellent. This finding is in contrast with earlier studies which have found a topical repellent a more effective method of deterrence (Armstrong & Robertson, 2013; Elmeros et al., 2011; Hodge & Pepper, 1998; Kimball et al., 2009; Trent et al., 2001; Trout & Brunt, 2014; Wagner & Nolte, 2001). However, as this particular method of application was not tested during this study, no comparison can be drawn. It had been considered by Hodge and Pepper (1998) during their research, that the use of repellents would be costly, but these findings were not evidenced in this study. In concurred instead, with Miller et al. (2008) that the rotten egg compound appeared to effectuate the most significant impact upon the deer in deterring them from a given area. Whilst the repellents used in this study were effective, easily attainable and inexpensive, it is considered they would provide a short term solution only (Armstrong et al., 2003; Hodge & Pepper, 1998; Nolte, 1998) and would require the use of supplemental deterrent strategies (Armstrong & Robertson, 2013; Armstrong et al, 2003; Elmeros et al., 2011; Nolte, 1998; Trent et al., 2001).

As with many prey species, red deer have adapted their behaviours to evade predation and ensure their survival (Jayakody et al., 2008). Repellents, with their predatory mimicking odours (Wagner & Nolte, 2001), could be perceived as a threat by the deer, but it is evinced that the animals will quickly habituate to the new stimulus (D'Angleo et al., 2006; Elmeros et al., 2011; Kimball et al., 2009; Trent et al., 2001; Ujvari et al., 2004) when they sense there is no danger to them. The length of time taken for the deer to habituate to each repellent present could not be measured in this study due to the relatively short time frame. The design fault of the initial holder, and its subsequent failure, meant Beecraigs had to be eliminated from the study. Whilst this resulted in a reduced observational study sample size and therefore possibly weakened the overall reliability and independence of the statistical analysis (Dawkins, 2007; Martin & Bateson, 2007), it was concluded that the data analysed from the other two studied sites was reliable and independent. Credible data was also lost from the camera traps sited at the Scottish Deer Centre, as previously explained, further reducing the sample size of observed alert behaviours to analyse. However, with the loss of Beecraigs as a study site, it was concluded that sufficient data had been retrieved from Atholl Estates through live and camera trap observations, for the results to not be considered as happening randomly. This study would have benefited from the presence of an independent party to observe the camera trap data. This would have minimised any observer bias from the possible misinterpretation of spatial use within the enclosures (Caravaggi et al., 2006), which would have increased the reliability of the results. A different outcome could have been affected through climatic conditions and also the time of year, in relation to the behaviour of the deer and their spatial use of the enclosure when the repellents were in situ (Wagner & Nolte, 2001). Although this study was conducted on herds of captive deer, which are supplied with supplementary food throughout the winter months, it is not considered that this would have made any difference to the final outcome. Whilst there is provision of grazing and additional food within the observed sites, it is considered that the repellents may vary in their degree of effectiveness with their counterparts living in the wild, if food availability is scarce and the palatability of the desired protected plants is high (Armstrong & Robertson, 2013; Nolte, 1998; Trent et al., 2001; Wagner & Nolte, 2001). A final factor to be taken into consideration, is the effect the repellents could have upon the existing wildlife in areas where the repellents would be sited. For example, questions could be raised as to whether the use of repellents would be counterproductive if being used to aid the conservation of woodland grouse species, as they may attract predatory animals (Bullard et al., 1978) to the site or deter the woodland grouse species from their habitats. This factor could not be researched within this study as it focused on herds of captive deer, but should be considered when developing woodland conservation and management plans.

The majority of the observed behaviours from both the Scottish Deer Centre and Atholl Estates, showed no significant difference between the presence of repellents and the control days. However, a significant difference was recorded in the alert behaviours when the pig's blood repellent was present in the live observational data collected from the Scottish Deer Centre. However, due to the loss of the camera trap data, these results cannot be conclusively accepted as being reliable, as it cannot be verified that these alert behaviours continued during the non daylight hours, when it was not possible to perform live observations. The camera trap recordings from Atholl Estates showed a significant difference in the feeding behaviour of the deer, with the presence of the rotten egg repellent having a greater effect than the wolf faeces repellent. Feeding behaviour in animals is considered to be non vigilant as they are unlikely to detect predation threats when their heads are lowered (Jayakody et al., 2008; Lima & Bednekoff, 1998). However, as the deer were grouped in a herd, fewer animals needed to be alert, as only a small number of vigilant deer are required, allowing more deer to feed in relative safety (Jayakody et al., 2008; Krebs & Davies, 1987; Lima & Bednekoff, 1998). The interpretation of this could therefore be that the animals displayed less vigilance with the rotten egg mixture, due to the reduced need for all the deer to display alert behaviour. The spatial use of the enclosure by the deer, demonstrated a significant avoidance of the area where the holder was located, when each of the repellents was present. The rotten egg repellent induced the most significant reaction of deer avoidance to the grid area where the repellent was placed. This finding corresponds with the results of Bullard et al. (1978), Miller et al. (2008), Trent et al. (2001) and Wagner and Nolte (2001) who also concluded that the egg based repellent was the most effective in deterring deer. The pig's blood and wolf faeces repellents showed similar significant results in their ability to deter deer from the areas where they were present. Wolf faeces showed a significant deterrence with the deer housed at the Scottish Deer Centre. This result was surprising, as the deer enclosure here is sited adjacent to the wolf enclosure, from where the faeces were obtained. It had been considered that there would be little effect displayed with the presence of this repellent, but it possibly demonstrated the instinctive avoidance behaviour towards potential predators when placed in close proximity (Elmeros et al., 2011; Kimball et al., 2009; Miller et al., 2008).

The results of this study conclude that area sulphur emitting odour repellents could be of benefit to small scale areas, which require protection for saplings and vulnerable species, particularly in areas where fences have been removed. Each of the repellents demonstrated significant and effective deterrence of deer from the requisite area. The repellent holder proved to be durable, easy to maintain and inexpensive to construct. It was also simple to dismantle and relocate to another site, making it reusable and therefore economically viable to use. The repellents themselves were cost-effective and easily accessible. An additional benefit of the most successful deterrent, the rotten egg mixture, is the fact that it does not require regulation through the Chemicals Regulation Directorate (Armstrong & Robertson, 2013). With no manufacturing required for the rotten egg repellent, overhead costs would be minimal. Staff responsible for deer management and conservation could produce the repellent in situ, thus removing the need for third party involvement. The organic composition of the egg repellent also diminishes the risk of contamination to the area or to any wildlife with which it may come into contact. Additionally, it poses a minimal health and safety risk to employees working with the deterrent.

As stated earlier, previous studies in Scotland have focused on the efficacy of aluminium ammonium based topical products (Armstrong & Robertson, 2013; Hodge & Pepper, 1998; Mayle, 1999; Trout & Brunt, 2014) and have disregarded the effectiveness of area egg, blood and predator based topical repellents as researched in North America (Chelsea, 2013; Kimball et al., 2009; Mattern, 2017; Nolte, 1998; Perry, 2017; Wagner & Nolte; 2001). This study has demonstrated the effectiveness of egg, blood and predatory faecal matter as repellents, and their successful ability to deter browsing red deer in Scotland. It has additionally proved that this can be achieved through an area wind dispersal application, rather than the topical application which has been widely researched in other studies, conducted in Scotland and further afield (Hodge & Pepper, 1998; Kimball et al., 2009; Miller et al., 2008; Trent et al., 2001; Trout & Brunt, 2014; Wagner & Nolte, 2001). Area repellents used in previous studies have tended to present them in the dried format, normally contained in a sachet (Kimball et al., 2009; Miller et al., 2008; Trent et al., 2001; Wagner & Nolte, 2001), but these have proven to be relatively ineffective. This study presented the repellents in liquid format, but still relied on the use of wind to disperse the odour throughout the area. The liquid form is more pungent than the dried format and will have a longer-lasting shelf life. It would therefore, not have to be replaced as frequently. With the rotten eggs, pig's blood and wolf faeces repellents all producing significant impacts on the deer's spatial use, the repellents could be rotated to reduce the probability of habituation occurring and therefore reducing the effectiveness and viability of the deterrents.

This study was conducted with the aim of providing successful and cost-effective repellents that could be used anywhere in Scotland where the protection of saplings for woodland regeneration was necessary. It also considered the impact of deterrents on habitats housing vulnerable woodland grouse species, whose numbers have declined sharply, due in part to collisions with deer fences. Where these fences have consequently been removed, deer damage is still evident and problematic. Alert behaviours were not significantly enhanced with the presence of the repellents at either of the study sites. However, alert behaviour was more significant at the Scottish Deer Centre with the presence of the pig's blood repellent but this finding could not be accepted as reliable, due to the small sample size. Therefore, it is concluded that alert behaviours are not increased with the presence of the tested repellents. The deer's spatial use within the enclosures however, was shown to be significantly affected when each of the repellents was present at both the Scottish Deer Centre and Atholl Estates. This finding was apparent for both live observational data collection and camera trap data collection. It can therefore be concluded that the frequency of the deer in the area where the repellent was sited was significantly reduced. In addition to the use of other management strategies such as tree guards, fences and culling, egg based repellents would act as an effective tool in deterring deer from vulnerable sites and thus protect the saplings which are necessary for the regeneration of woodland habitats in Scotland.

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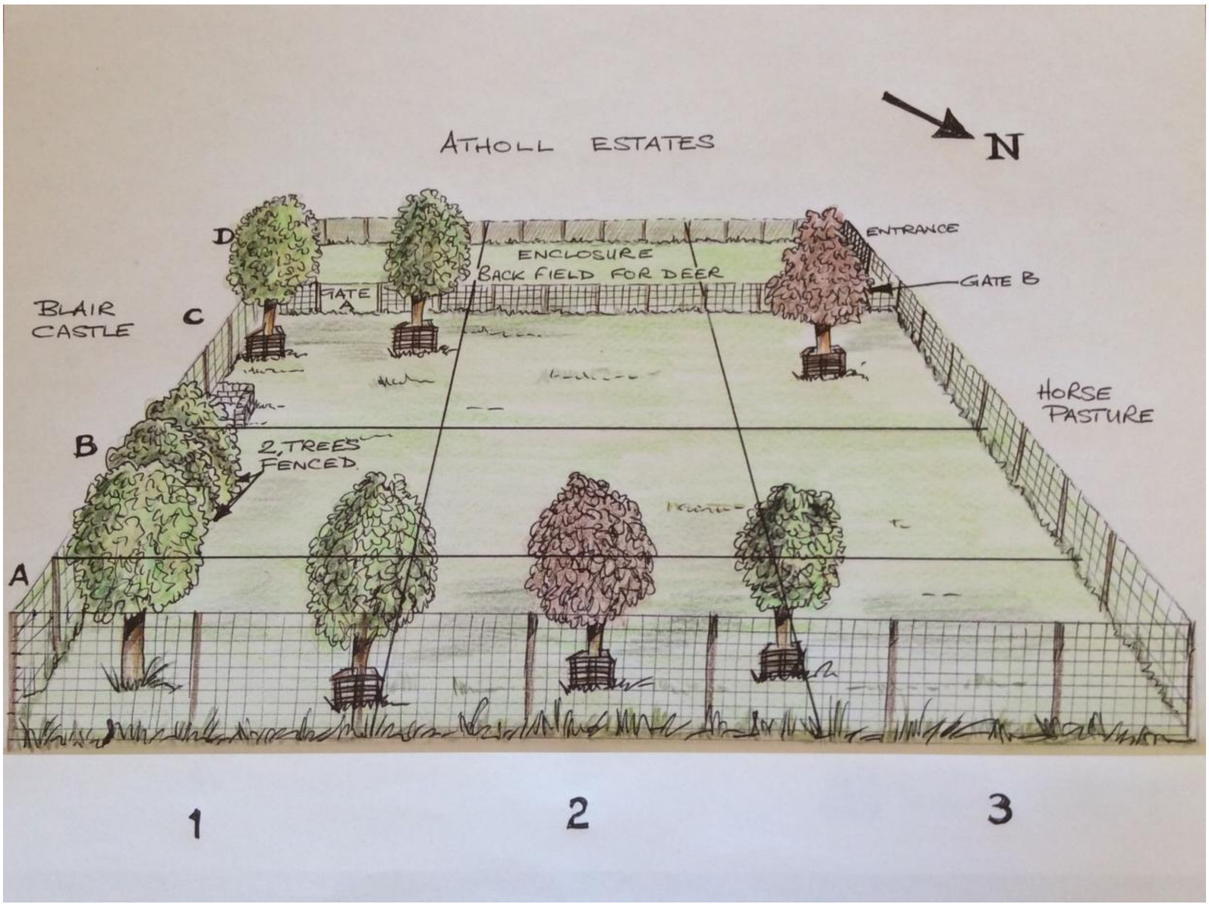
Appendices

Appendix One

Scottish Deer Centre enclosure. This design includes the grid division used during the collection of both live and camera trap observational data to determine the red deer's spatial use. Acknowledgement goes to E.J. McCallin for producing this illustration from my plan.

Appendix Two

Atholl Estates enclosure. This design includes the grid division used during the collection of both live and camera trap observational data to determine the red deer's spatial use. Acknowledgement goes to E.J. McCallin for producing this illustration from my plan.



Appendix Three

The holder design, containing the fatball holder for repellent containment, used in this study.

Appendix Four

Ethogram for the red deer (*Cervus elaphus*), including the definitions for each behaviour that could be displayed.

Behaviour	Abbreviation	Description
Non-Alert		
Standing	ST	Animal standing on all four limbs, ears positioned either to the side or backwards and may be twitching back and forth. May be chewing and licking nose with tongue.
Lying Down	LD	Animal lying down on ground, eyes open, ears positioned either to the side or backwards and may be twitching back and forth. May be chewing.
Sleeping	SL	Animal lying down on ground, eyes closed, ears positioned either to the side or backwards, head placed on the ground or along the body.
Walking	WA	Animal moving with a slow gait, ears positioned either to the side or backwards and may be twitching back and forth. May be chewing.
Running	RU	Animal moving with a quick gait, ears positioned either to the side or backwards and may be twitching back and forth. Head and neck may be outstretched.
Grooming	GR	Animal licking or scratching body with tongue, limb or antler.
Feeding	FE	Animal either standing or lying down, head placed towards the ground, ingesting food or standing on back limbs to browse. Ears positioned either to the side or backwards and may be twitching back and forth. May be chewing.
Drinking	DR	Animal standing, head placed in water trough, ingesting water, ears positioned either to the side or backwards and may be twitching back and forth.
Suckling	SU	Calf feeding from mother.
Interaction with Deer	ID	Interaction between two or more individual animals. Includes aggression, parental care, play fighting and sexual displays.
Interaction with People	IP	Interaction between animal and person, either through feeding or petting from person.
Interaction with Object	IO	Interaction between animal and an object, including logs, trees, camera traps.
Interaction with Deterrent Holder	IDH	Interaction between animal and deterrent holder. Includes physical touching, licking, biting, pawing with limbs, head butting.
Out of Sight	OS	Calf is not visible, lying down in long grass.
Alert		
Startle	SA	Animal jumps, produces sudden movement that can lead to either an alert or non-alert behaviour.

Alert Standing	AST	Animal standing on all four limbs, ears positioned forward and still, nose twitching and sniffing air, not chewing, head and neck erect and still.
Alert Lying Down	ALD	Animal lying down on ground, eyes are open, ears positioned forward and still, nose twitching and sniffing air, not chewing, head and neck erect and still.
Alert Walking	AWA	Animal moving with a slow gait, ears positioned forward and still, nose twitching and sniffing air, not chewing, head and neck erect and still.
Alert Running	ARU	Animal moving with a quick gait, ears positioned forward and still, nose twitching and sniffing air, not chewing, head and neck erect and still.

Appendix Five

Tukey test outcome for alert behaviours at the Scottish Deer Centre from the live observational data collection. The outcome includes the analysis of variance (ANOVA) test and the Shapiro-Wilk test for normality in annotated format.

Scottish Deer Centre - Alert Behaviour

```
# READ IN THE DATA
alertsdc<-read.csv(file=file.choose())

# PRODUCE BOX-AND-WHISKER PLOT TO VISUALISE HYPOTHESIS (WHERE
RESPONSE VARIABLE IS CONTINUOUS AND EXPLANATORY VARIABLE IS
CATEGORICAL)
plot(formula=alertsdc$frequency~alertsdc$treatment,xlab="Treat
ment",ylab="Frequency of Observed 'Alert' Behaviour
(%)",main="Scottish Deer Centre - Alert Behaviour")

# COMPARE NULL HYPOTHESIS WITH PREDICTED HYPOTHESIS USING THE
F-STATISTIC TO DETERMINE IF SIGNIFICANT (P<0.050) AND REJECT
NULL HYPOTHESIS
anova(object=glm(formula=alertsdc$frequency~alertsdc$treatment
),test="F")

Analysis of Deviance Table

Model: gaussian, link: identity

Response: alertsdc$frequency

Terms added sequentially (first to last)


                   Df Deviance Resid. Df Resid. Dev      F
Pr(>F)
NULL                                 20     211.19
alertsdc$treatment  3   107.91       17     103.28 5.9211
0.005883 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

- P-value is significant (P<0.050)

```
# READ IN TESTED HYPOTHESIS AS ".MODEL" FOR EASE OF INPUT TO R
alertsdc.model<-
glm(formula=alertsdc$frequency~alertsdc$treatment)

# PRODUCE A HISTOGRAM TO VISUALISE NORMALITY OF RESIDUAL
DISTRIBUTION
hist(x=residuals(object=alertsdc.model))
```

```
# PRODUCE SHAPIRO-WILK TEST TO DETERMINE NORMALITY OF RESIDUAL
DISTRIBUTION (P≥0.050) AND ACCEPT ANOVA AS RELIABLE
shapiro.test(x=residuals(object=alertsdc.model))

        Shapiro-Wilk normality test

data:  residuals(object = alertsdc.model)
W = 0.9137, p-value = 0.065
```

- Residuals are normally distributed (P≥0.050)

```
# PERFORM A TUKEY TEST TO DETERMINE WHICH PAIRWISE DIFFERENCES
WERE SIGNIFICANT (P<0.050) AND THEIR STANDARD ERRORS
TukeyHSD(x=aov(formula=alertsdc$frequency~alertsdc$treatment))

  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = alertsdc$frequency ~ alertsdc$treatment)

$`alertsdc$treatment`
                    diff        lwr        upr     p adj
control-blood -5.7553571 -10.146723 -1.3639910 0.0082685
egg-blood     -5.2725000  -9.794980 -0.7500195 0.0194107
wolf-blood    -6.2750000 -11.229129 -1.3208708 0.0107339
egg-control    0.4828571  -3.415031  4.3807451 0.9845011
wolf-control  -0.5196429  -4.911009  3.8717233 0.9864265
wolf-egg      -1.0025000  -5.524980  3.5199805 0.9209131
```

Standard Errors:

(diff - lwr) / 2

Comparison	Difference	Lower Value	Standard Error
Control - blood	-5.76	-10.15	2.20
Egg - blood	-5.27	-9.79	2.26
Wolf - blood	-6.28	-11.23	2.48

control - blood (difference = -5.76 ± 2.20 SE, P = 0.008)
egg - blood (difference = -5.27 ± 2.26 SE, P = 0.019)
wolf - blood (difference = -6.28 ± 2.48 SE, P = 0.011)

Calculate r^2 to find the variation difference between the response variable and the explanatory variable

$r^2 = 1 - RSS_{MODEL} / RSS_{NULL}$

1 - 103.28 / 211.19
1 - 0.49
$r^2 = 0.51$ (51%)

Appendix Six

Kruskal-Wallis test outcome for alert behaviours at Atholl Estates from the live observational data collection. The outcome includes the analysis of variance (ANOVA) test and the Shapiro-Wilk test for normality in annotated format.

Atholl Estates - Alert Behaviour

```
# READ IN THE DATA
alertba<-read.csv(file=file.choose())

# PRODUCE BOX-AND-WHISKER PLOT TO VISUALISE HYPOTHESIS (WHERE
RESPONSE VARIABLE IS CONTINUOUS AND EXPLANATORY VARIABLE IS
CATEGORICAL)
plot(formula=alertba$frequency~alertba$treatment,xlab="Treatme
nt",ylab="Frequency of Observed 'Alert' Behaviour
(%)",main="Atholl Estates - Alert Behaviour")

# COMPARE NULL HYPOTHESIS WITH PREDICTED HYPOTHESIS USING THE
F-STATISTIC TO DETERMINE IF SIGNIFICANT (P<0.050) AND REJECT
NULL HYPOTHESIS
anova(object=glm(formula=alertba$frequency~alertba$treatment),
test="F")
Analysis of Deviance Table

Model: gaussian, link: identity

Response: alertba$frequency

Terms added sequentially (first to last)


                  Df Deviance Resid. Df Resid. Dev      F
Pr(>F)
NULL                                 25     771.19
alertba$treatment  3   112.64        22     658.56 1.2542
0.3143
```

- P-value is not significant (P≥0.050)

```
# READ IN TESTED HYPOTHESIS AS ".MODEL" FOR EASE OF INPUT TO R
alertba.model<-
glm(formula=alertba$frequency~alertba$treatment)

# PRODUCE A HISTOGRAM TO VISUALISE NORMALITY OF RESIDUAL
DISTRIBUTION
hist(x=residuals(object=alertba.model))

# PRODUCE SHAPIRO-WILK TEST TO DETERMINE NORMALITY OF RESIDUAL
DISTRIBUTION (P≥0.050) AND ACCEPT ANOVA AS RELIABLE
shapiro.test(x=residuals(object=alertba.model))
```

```
        Shapiro-Wilk normality test

data:  residuals(object = alertba.model)
W = 0.85809, p-value = 0.00205
```

- Residuals are not normally distributed (P<0.050)

```
# RESIDUALS NOT NORMALLY DISTRIBUTED THEREFORE DATA SQUARE
ROOT TRANSFORMATED ('0' PRESENT IN ORIGINAL DATA)
sqrt(x=alertba$frequency)
 [1] 4.3000000 2.8982753 3.6674242 0.9165151 0.0000000
1.2961481 0.9165151
 [8] 0.0000000        NA 0.9165151 0.0000000 0.0000000
0.0000000 0.0000000
[15] 2.5922963 1.2961481 0.0000000 0.0000000 0.9165151
0.0000000 0.0000000
[22] 4.2011903 2.7495454 2.7495454 1.2961481 0.9165151
0.0000000

# PRODUCE BOX-AND-WHISKER PLOT TO VISUALISE HYPOTHESIS (WHERE
RESPONSE VARIABLE IS CONTINUOUS AND EXPLANATORY VARIABLE IS
CATEGORICAL)AFTER SQUARE ROOT TRANSFORMATION OF DATA
plot(formula=sqrt(alertba$frequency)~alertba$treatment,xlab="T
reatment",ylab="Frequency of Observed 'Alert'
Behaviour",main="Atholl Estates - Alert Behaviour (Square-Root
Transformed Data)")

# COMPARE NULL HYPOTHESIS WITH PREDICTED HYPOTHESIS USING THE
F-STATISTIC TO DETERMINE IF SIGNIFICANT (P<0.050) AND REJECT
NULL HYPOTHESIS AFTER SQUARE ROOT TRANSFORMATION OF DATA
anova(object=glm(formula=sqrt(alertba$frequency)~alertba$treat
ment),test="F")
Analysis of Deviance Table

Model: gaussian, link: identity

Response: sqrt(alertba$frequency)

Terms added sequentially (first to last)


                  Df Deviance Resid. Df Resid. Dev      F
Pr(>F)
NULL                                25     50.593
alertba$treatment  3   7.5715        22     43.021 1.2906
0.3024
```

- P-value is not significant (P≥0.050)

```
# READ IN TESTED HYPOTHESIS AS ".MODEL" FOR EASE OF INPUT TO R
AFTER SQUARE ROOT TRANSFORMATION OF DATA
alertba.model<-
glm(formula=sqrt(alertba$frequency)~alertba$treatment)
```

```
# PRODUCE A HISTOGRAM TO VISUALISE NORMALITY OF RESIDUAL
DISTRIBUTION AFTER SQUARE ROOT TRANSFORMATION OF DATA
hist(x=residuals(object=alertba.model))

# PRODUCE SHAPIRO-WILK TEST TO DETERMINE NORMALITY OF RESIDUAL
DISTRIBUTION (P≥0.050) AFTER SQUARE ROOT TRANSFORMATION AND
ACCEPT ANOVA AS RELIABLE
shapiro.test(x=residuals(object=alertba.model))

        Shapiro-Wilk normality test

data:  residuals(object = alertba.model)
W = 0.91897, p-value = 0.04253
```

- Residuals are not normally distributed (P<0.050)

```
# PRODUCE A KRUSKAL-WALLIS NON PARAMETRIC TEST (EXPLANATROY
VARIABLE WITH MORE THAN TWO LEVELS) ON ORIGINAL DATA TO
DETERMINE IF A SIGNIFICANT P-VALUE IS GIVEN (P<0.050)
kruskal.test(formula=alertba$frequency~alertba$treatment)

        Kruskal-Wallis rank sum test

data:  alertba$frequency by alertba$treatment
Kruskal-Wallis chi-squared = 3.4281, df = 3, p-value = 0.3302
```

- P-value is not significant (P≥0.050)

Appendix Seven

Example of Chi-square (X^2) calculation using the rotten eggs repellent at Atholl Estates.

Calculation of Expected data:

E (%) = (Total control in H / Total control in H + N) x Total treatment in H + N

EH = (232 / 942) x 498 = 124.50

EN = (710 / 942) x 498 = 373.50

	Holder area (**H**)	No Holder area (**N**)	Total
Observed data (**O**)	0	498	498
Expected data (**E**)	124.50	373.50	

Calculation of X^2:

$$X^2 = \sum [(O - E)^2 / E]$$

O = $[(0 - -124.50)^2 / -124.50] = 124.50$

E = $[(498 - 373.50)^2 / 373.50] = 41.50$

O + E = 124.50 + 41.50 = 166

	H	**N**
O	0	498
E	124.50	373.50
O - E	-124.50	124.50
$(O - E)^2$	15500.25	15500.25
$(O - E)^2$ / E	124.50	41.50

Calculation of degrees of freedom (df):

$$(r - 1) \times (c - 1)$$

r = 2

c = 2

df = (2 - 1) x (2 - 1) = 1

Critical value of X^2 from table:

3.84

- If X^2 value **more** than X^2 critical value - **reject** null hypothesis
  - accept predicted hypothesis
- If X^2 value **less** than X^2 critical value - **accept** null hypothesis
  - reject predicted hypothesis

Final findings:

$$X^2 (1, N = 498) = 166, P = 5.532 \times 10^{-38}$$