Novel edible coatings to improve longevity of rodent baits

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ABSTRACT
Rats and mice cause severe biodiversity impacts worldwide, including in New Zealand, where poisoning campaigns are undertaken on the mainland and offshore islands using cereal-based baits. However, bait stations are often irregularly serviced so wildlife managers require a bait that would remain palatable to rodents for at least 6 months in the field. One potential way to improve the field life of baits is by coating them in materials that reduce water uptake and subsequent mould growth. However, these coatings must be palatable to the rodents. This report investigates the palatability of captive Norway rats (Rattus norvegicus) and mice (Mus musculus) of both weathered and unworn rodent food pellets coated with various polymer solutions. When compared with uncoated alternatives, fresh baits coated with shellac or polyvinyl butyral (PVB) in ethanol solvent were preferred by the rats. The use of acetone rather than ethanol solvent did not affect bait palatability. A combination of shellac and PVB reduced palatability compared with shellac alone. Weathered shellac-coated baits remained more palatable than uncoated fresh pellets to rats for up to 6 months and outperformed the PVB and combination coatings. Overall, the palatability of fresh-coated baits was lower for mice. The PVB-coated baits had lower mean palatability scores than the shellac-coated baits but none of the bait types was significantly less palatable than uncoated baits. The shellac-coated baits remained palatable to mice for up to 4 months. Shellac is recommended as a coating for multi-species rodent baits.

Introduction
Because rats and mice are prolific breeders, and are highly adaptable, they are often successful colonisers (Meehan 1984; Ruscoe & Murphy 2005). Their populations can reach pestilential proportions very quickly. This makes them agricultural and conservation pests worldwide. In New Zealand they have a major impact on the native biota. Mice feed on a wide variety of native plant seedlings and seeds, invertebrates, birds’ eggs and smaller lizards (Ruscoe & Murphy 2005). Rats are more ferocious predators, killing adult birds and larger invertebrates (Amori & Clout 2003; Mulder et al. 2009). Offshore islands are a key component in the strategy to conserve at-risk New Zealand biota. Bird
populations that are susceptible to predation from terrestrial predators on the mainland are able to reproduce and sustain larger population densities when protected on predator-free islands (Towns et al. 2012). Because these islands are close to the New Zealand mainland, there is a constant risk of pest reinvasions. Norway rats (*Rattus norvegicus*) are strong swimmers, capable of crossing a body of water to reach offshore islands (Russell et al. 2010). Along with the self-reinvasion of swimming pests, other rodent species such as the ship rat (*Rattus rattus*) and the common house mouse (*Mus musculus*) are introduced through their interactions with human transport vessels. So not only does every last rodent need to be removed to turn an island into a wildlife sanctuary, but constant vigilance is needed to maintain the pest-free status. This requires highly effective rodent control strategies (Howald et al. 2007; Russell & Broome 2016).

In New Zealand, both mainland and island rodent populations are controlled largely with rodenticides in cereal bait form (O’Connor & Eason 2000). For sustained control, there is a need for a long-life, palatable rodent bait that will remain attractive to rodents following long periods of environmental exposure. Bait longevity is of particular concern on offshore islands. Their remote locations make them expensive to service, so baits are not regularly replaced. This leads to concerns for bait attractiveness and palatability. Once unattractive and unpalatable, confidence in invading rodents interacting with available bait decreases.

Currently used formulations of cereal baits can significantly deteriorate and become mouldy with environmental exposure, reducing acceptance. Morriss et al. (2008) found that despite weathering, palatability of a multi-species rodent bait (Contrac) remained relatively high for both ship rats and house mice for up to 12 months’ exposure in the field. Contrastingly, it was reported that Norway rats were more likely to avoid weathered, mouldy baits, resulting in lower witnessed mortality during toxicity trials (Morriss et al. 2008). These authors recommended that a long-life, multispecies bait that remains palatable to Norway rats following weathering needed to be developed.

Previous studies have documented the effect of long-life bait coatings on palatability amongst both wild and captive rodent subjects. Initial efforts to encase baits within tin foil and self-sealable polythene bags only increased the neophobic responses of Norway rats, thus decreasing bait palatability (Airey & O’Connor 2003). Traditional wax coatings have also resulted in a decrease of palatability for wild house mice (O’Connor & Eason 2000; O’Connor & Booth 2001; Dilks & Towns 2002), and Morriss et al. (2008) concluded that hard cereal baits outperformed wax-coated bait blocks in a toxin fatality trial where both baits were presented to Norway rats, ship rats and house mice.

More recently, both naturally occurring and artificially produced human food preservatives have been recommended as possible coatings for rodent baits (Blackie et al. 2014). These preservative coatings allow for the natural characteristics of the baits relating to smell and taste to remain, whilst protecting against the development of mould and decay.

Based on the above, this research trial had two key objectives. The first was to establish whether rodent food pellets coated with various preservative coatings remained palatable when compared with their uncoated equivalents. The second was to determine whether the coated pellets remained palatable to rodents after an extended period of environmental exposure, as to make them suitable for long-term use in bait stations deployed on offshore island sanctuaries or in remote areas on the New Zealand mainland.
Methods

We followed established methods for determining the relative palatability of a bait within a controlled environment (Buckle & Kaukeinen 1988; O’Connor & Eason 2000). The methods involved a choice test in which caged Norway rats and mice at Lincoln University’s Johnson Memorial Laboratory were presented with a selection of two food sources. First, we determined the palatability of four different bait coatings compared with a non-coated control bait. Second, a weathering trial was performed to determine whether the coated baits remained palatable to target rodent species after an extended period of environmental exposure.

Bait preparation

Four potential bait coatings were produced at the New Zealand Institute for Plant & Food Research Ltd, Lincoln, New Zealand. These coatings were applied to standard commercial rodent feed pellets (approximately 10 mm × 16 mm × 25 mm and 4 g in weight supplied by Weston Animal Nutrition, Rangiora, New Zealand). Standard food pellets were used in this trial because previous research has indicated that both wild and captive rats can exhibit initial neophobia to novel food (Modlinska et al. 2015). Accordingly, to accurately assess the response of the rats to the coating we coated their existing food pellets which they had already been exposed to during acclimatisation (see below). The coating materials were selected to have low odour and low likelihood of tainting the flavour of the pellets. This was done by choosing materials that are either foodstuff grade or food contact safe. Shellac (Resene Orange flake Shellac) and polyvinyl butyral (PVB; Mowital B30HH grade, Kuraray Ltd) were used as supplied. Shellac is a natural product derived from secretions from the lac bug (Kerria lacca) and PVB is a synthetic polymer made by the transesterification of polyvinyl alcohol. Shellac has the advantage of being completely biodegradable. Table 1 provides a description of the four bait coatings used.

The solvent choice was dictated by considerations of processability and palatability. Acetone is highly volatile, and therefore will allow a faster manufacturing process time. Ethanol is slower to evaporate, but may have a beneficial effect on bait palatability because it has been noted that alcohol vapour (as from fermenting fruit) is used for ‘olfactory localization of transient food sources’ (Dudley 2004). In either case the evaporated solvent can be recovered during the drying process and reused. This is achieved through a machine attachment that collects the solvent vapours and condenses them back to the liquid form to be reused, enhancing production efficiency. The pellets were dip coated by immersion, and then spread on trays to dry overnight under ambient conditions. This produced a transparent coating no thicker than 500 µm.

<table>
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<th>Table 1. Bait coatings, common uses and solvent used.</th>
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<td>Identification code</td>
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Animal husbandry

The Otago University School of Medicine in Christchurch supplied laboratory-bred animals. Norway rats (*Rattus norvegicus*; Wistar albino) and house mice (*Mus musculus*) were housed individually in polycarbonate cages (40 × 20 × 15 cm high for mice and 44.5 × 28 × 20 cm high for rats) at the Johnson Memorial Laboratory, Lincoln, New Zealand. Before commencing the two-choice trials, the caged animals were all given a 2 week acclimatisation period to adapt to the new environment and only animals with stable body weights were used. During the 2 week acclimatisation period, water and a diet of feed pellets and seed/grain mixture was available ad lib. For the bait trials (see below) we coated the animals’ standard feed pellets. This was done due to concerns of neophobia, particularly for the Norway rats, and the researchers wanted to ensure that all animals were consuming the feed pellets prior to exposure to coated feed pellets. Because of laboratory space limitations, the different coatings were tested on separate dates, as were the rat and mice trials. For all trials, the researchers sought to use naive animals for each trial; however, this was not always possible and some animals were used in multiple trials. Test subjects for each palatability trial were selected out of a total pool of 88 adult rats and 65 adult mice with a targeted aim of a 50:50 male to female ratio. Sample sizes in each test ranged from 10 to 23 for rats and 7 to 21 for mice.

All research was conducted with the approval of the Lincoln University Animal Ethics Committee under AEC #399 and 432.

Assessment of the fresh pellets

Paired trays containing equal amounts of both coated and uncoated food pellets (30–40 g of each for rats, 10–20 g each for mice) were presented to each of the caged individuals for a 24 h period with water provided ad lib throughout. Trays were placed in opposite corners of the cages during acclimatisation and the pellet type put in each tray randomised. Each pellet type was weighed before and at the conclusion of the 24 h period, to determine the amounts consumed and overall differences in palatability between the coated and uncoated pellets. The percentage palatability was calculated in accordance with similar literature (Brown et al. 2012; Jokić et al. 2013) and defined as coated bait consumed (g)/total bait consumed (g) × 100. A percentage palatability over 50% therefore indicates a preference for coated (treatment) pellet, whilst a percentage palatability under 50% indicates a preference for uncoated (control) bait. For this research, where 95% confidence intervals of coated pellet palatability included the 50% figure these were said to be ‘as palatable’ as uncoated pellets.

Assessment of the weathered pellets

A second trial was then set up at the same time of the year (May) to determine whether the coated pellets maintained palatability after an extended period in the field. Four plastic Kilmore rodent bait stations (2 kg capacity; Pest Control Research Ltd) containing samples of one of the four coated pellet types were attached to wooden stakes approximately 30 cm off the ground. Each bait station faced north next to a shelter belt allowing exposure to the weather, as they would be set up in the field. These Kilmore stations were
also encased with aluminium insect mesh to avoid animal interference. The bait stations
then remained in the field for a 6 month period (May to October). Roughly every 4 weeks a
small sample of 'weathered' pellet was retrieved from each station, to be used in a choice
test with caged rats and mice (in identical conditions to the first two-choice trials). In
general, once the weathered bait became unpalatable then that coating was no longer
tested. Caged individuals were again presented with a choice between fresh uncoated
pellets and the weathered coated pellets, for a period of 24 h. Weathered control pellets
were not tested. Percentage palatability was calculated as above and analysed as below.

**Statistical analysis**

The amount of coated pellet consumed was analysed with a generalized linear model
(McCullagh & Nelder 1989) with binomial errors and logit link, using the total amount
(coated + uncoated) consumed as the binomial total. The explanatory variables included
in the model were coating (PA, PE, PSE and SE; see Table 1), animal type (rat or
mouse), a coating-by-animal type interaction term and duration of weathering. Fresh
pellets were treated as having 0 months of weathering and modelled together with the
weathered bait. To account for the fact that different weathering durations were used
for each coating (and for each of rats and mice within the PSE coating), a separate duration
factor was used for each group and nested within the coating-by-animal component of the
model. Other possible explanatory variables (such as sex, bodyweight, weathering station
position, etc.) were not reliably collected and not formally assessed. Over-dispersion was
apparent in the data and this was accounted for in the model by allowing the dispersion
parameter to be estimated from the residual deviance. Diagnostic residual plots showed no
major violations of model assumptions. The results are presented as means and associated
95% confidence limits. These were obtained on the transformed (logit) scale, and back-
transformed for presentation. *P*-values presented below are the probabilities for *t*-statistics
testing whether each parameter differs significantly from zero (on the logit scale, or 50% 
when back-transformed), keeping the other parameters fixed. The analysis was carried out
with GenStat 17th Edition (GenStat Committee 2014). Graphs were created in R (R Core
Team 2014) using the ggplot2 package (Wickham 2009). A 95% confidence interval
excluding 50% represents a statistically significant preference either way. Coatings are
described as palatable when the 95% confidence interval does not lie entirely below the
50% line.

**Results**

**Palatability of the fresh pellets**

Rat and mouse mean preferences amongst the fresh pellet coatings are presented in
Figure 1. Rats preferred both PE- and SE-coated pellets over uncoated pellets (*P* = 0.018
and *P* < 0.001, respectively), whilst the confidence intervals presented in Figure 1 indicate
that all four coatings were palatable prior to weathering. Although mice did find all coated
pellets to be palatable, only the SE pellets were significantly preferred (*P* = 0.048) over
uncoated fresh pellets. These results suggest that the SE coating is the most promising
coating tested for multispecies baits.
Figure 1. Predicted mean percentage palatability of fresh pellets by coating and animal type (error bars represent 95% confidence intervals). Non-overlapping error bar with 50% value indicates statistical significance ($P < 0.05$).

**Palatability of the weathered pellets**

It was found that the preference described above for the fresh (unweathered) SE-coated pellets extended for the entire 6 month sampling period (Figure 2). Rat preference for weathered PE-coated pellets lasted for 4 months, and these pellets were still palatable after 6 months. Palatability of weathered PA and PSE pellets dropped off at 2–3 and 2–4 months, respectively.

Mice subjects displayed a more selective palate for weathered pellets. Palatability and preference for PE-coated pellets varied throughout the sampling period, as shown in Figure 2; however, palatability was lost altogether between 3 and 6 months. SE-coated pellets performed slightly better, and were found to be palatable after 4 months of weathering. Again, palatability to mice was lost between the 4 and 6 month sampling events. Mice palatability of weathered PA and PSE pellets was inadequate for a long-life coating.

**Discussion**

The results of this trial suggest that SE could be pursued as a suitable coating for rodenticide baits to increase their palatability and field longevity. Shellac dissolved in ethanol is commonly applied to improve the shelf life of human food products because of its ability to retain moisture content and delay/prevent mould development (Ziegler et al. 2004; Musa et al. 2013). The results presented in this trial suggest that the addition of a SE coating did not reduce pellet palatability, and in fact potentially increased pellet acceptance by both species when used on fresh pellets. O'Connor & Eason (2000) describe the ability of the bait formulation to mask certain flavour compounds within either a toxin or a bait coating as an important factor in determining final bait palatability (see Clapperton 2006 for a more exhaustive discussion of these factors). It would appear that the SE coating not only allowed the feed pellet to retain its natural attractiveness, but potentially increased initial rodent acceptance. The result of SE coating on bait
Figure 2. Predicted mean percentage palatability of both fresh and weathered pellets by coating and animal type (error bars are 95% confidence intervals). Non-overlapping error bar with 50% value indicates statistical significance ($P < 0.05$).

Palatability longevity is also encouraging, particularly for rats where SE-coated pellets remained palatable after 6 months of environmental exposure. It may be an effective means of prolonging palatability to Norway rats that are averse to degraded bait. The reason for the more rapid reduction in palatability of the SE-coated pellets for mice may be explained by the feeding habits of the two species. Mice are often described as erratic and light feeders, taking food from a wide range of different sources during a single feeding event (Rowe 1973). This behaviour might explain why we witnessed more varied response for mice when compared with the Norway rats.

Two issues with the study design are that we used captive animals and we did not always use naive animals for each weathered bait trial. Several authors have detailed the marked differences in feeding behaviour between captive and wild rodents (reviewed by Clapperton 2006). The naturally neophobic response of wild rats towards foreign food
sources is often lost amongst captive individuals, meaning that captive rats could respond more positively towards coated baits than could be expected from naive wild populations; however, recent research suggests that this behaviour is influenced by environmental stability (Modlinska & Stryjek 2016). Clapperton (2006) suggests that the effect of domestication on mice appears to be less influential on their feeding habits. Moreover, some of the rodents used in the weathering trial had previous exposure to coated pellets. Responses by naive rats and mice at time point 0 for the SE-coated pellets seemed to remain consistent at later time points and we did not observe marked differences in consumption between naive and conditioned animals. However, the results may have been different if purely naive animals were used at each time point, as has been done in other long-term captive palatability studies (Morriss et al. 2008). Accordingly, before SE-coated baits are tested in the field it would be prudent to trial the palatability of SE-coated, non-toxic, novel rodenticide baits compared with normal diet food items using naive animal at each time point.

The efficacy of coated baits under varying field conditions also needs to be assessed. The trial in this study was conducted over a winter/spring season in Canterbury. The effects of weathering may be greater in areas of New Zealand with a higher annual rainfall, or during different seasons, and thus these also need to be investigated. The SE coating also needs to be tested using toxic rodenticide baits, in the field, where the wild rodents have a range of alternative foods available, and varying levels of hunger.

In conclusion, those wishing to target rodent populations with rodenticide baits should consider the use of SE-coated baits, as these were shown to be more palatable than fresh uncoated pellets and remained palatable to both mice and rats for 4–6 months, respectively, under simulated field conditions. Accordingly, SE coating shows potential as a means to increase both initial palatability and bait longevity of rodent cereal baits.

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Disclosure statement

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