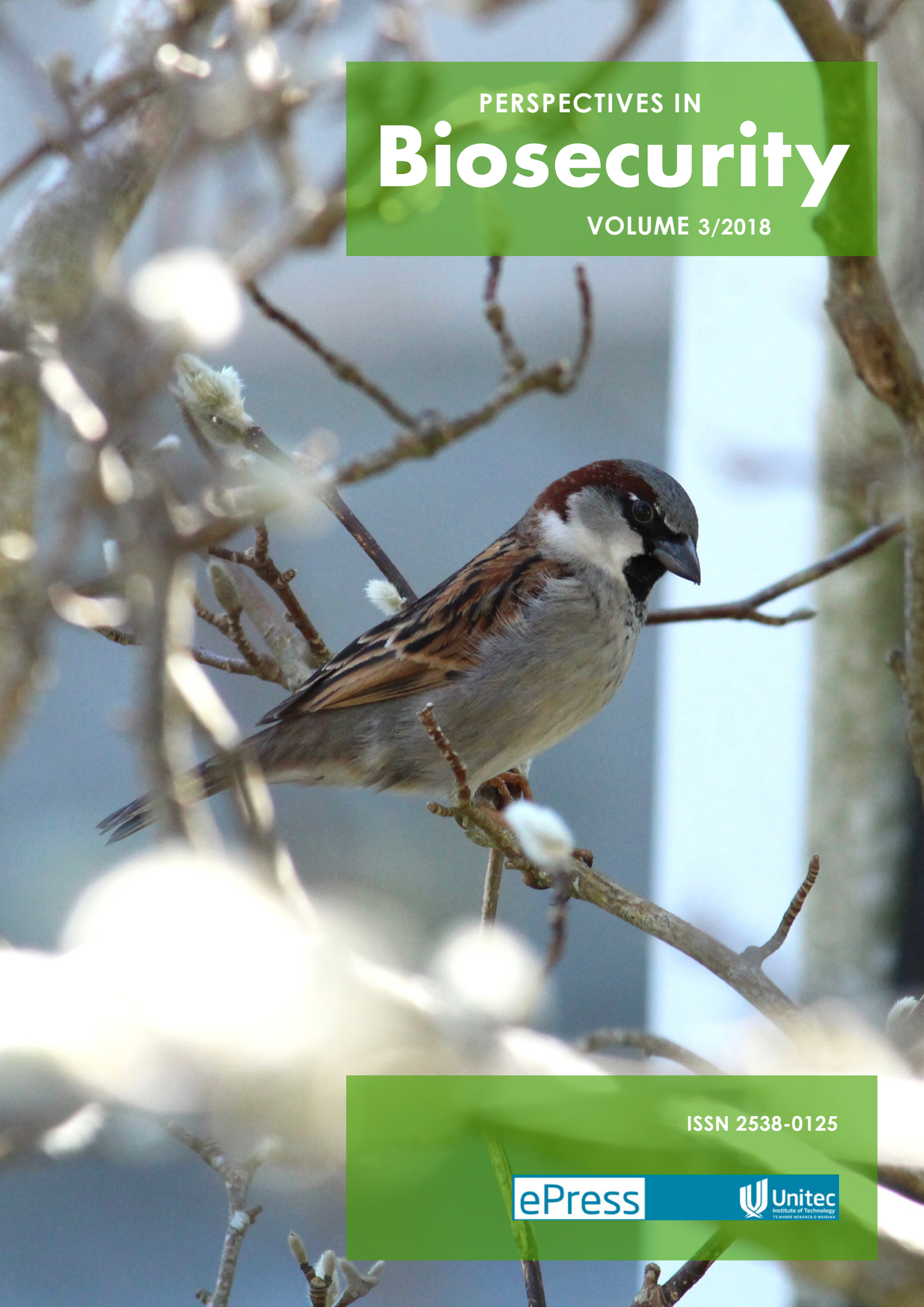

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Contact
epress@unitec.ac.nz

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epress@unitec.ac.nz
www.unitec.ac.nz/epress/
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EDITORIAL

Biosecurity in 2018 – research is key

New Zealand is currently battling with a range of biosecurity threats to agriculture and its native flora and fauna:

- Kauri dieback (*Phytophthora agathidicida*) continues to spread throughout most of the natural range of kauri in the upper North Island. While some treatment options may be available, research on other control methods continues;
- *Mycoplasma bovis*, a bacterial disease of cattle, probably arrived in New Zealand in 2015/16. The disease has significant animal welfare implications and has already caused significant losses to dairy and cattle producers. The New Zealand Government has committed to a plan of nation-wide eradication;
- The effects of myrtle rust (*Austropuccinia psidii*) did not manifest as feared over the 2017/18 summer. Currently it is unknown if this is because the New Zealand climate is not optimal for myrtle rust, or if the pathogen is in a lag phase of establishment with the true impact yet to come. But the pathogen is still here, and has been recorded from wide areas of the North Island and the top of the South. So far, the main impact appears to be to *Lophomyrtus* (ramarama) species.

The situation with these species exemplifies the uncertainties associated with the invasion of an alien species into a novel environment – until the species arrives, establishes and expands, we can only speculate about the ecological implications. And when incursions are detected, can we respond fast enough to address the impacts? Research will support evidence-based management decisions in these situations.

Well-funded research is the key to managing biosecurity threats and preventing the arrival of new invasive species. It is critical that we understand the ecology and life history of invasive species, but it also very important that we understand the place of human attitudes and behaviour when creating management plans. The recent debate around the use of 1080 is a good example of this. A multi-agency, interdisciplinary approach that involves the community and iwi is the most effective way forward. Fortunately, funding for biosecurity research is increasing. The Auckland Council recently introduced a Natural Environment Targeted Rate: dedicated funding to support initiatives including management of kauri dieback and possum control, which will also support research. Council employees consider the targeted rate to be a 'game changer' for the management of our natural environment in the Auckland region. In addition, in November 2018 the New Zealand Government announced a funding increase of NZ$13.75 million over three years for research on kauri dieback and myrtle rust, through the BioHeritage Science Challenge.

Most biosecurity issues that attain a high public profile are those that have actual or potential impacts on people or GDP at the national level. As we have seen, such issues may invariably generate funding for high-level research or social marketing. However, for biosecurity issues of a lower profile, the need for basic research to determine the ecology of invasive species and explore management options remains crucial. *Perspectives in Biosecurity* was established to provide an avenue for the peer-reviewed publication of research of this nature, with the scope to encompass the multi-dimensions of biosecurity. We look forward to publishing future research (and short notes, opinions, reviews and other outputs) to add to the growing knowledge base of biosecurity.

Dan Blanchon and Mel Galbraith, Editors

Measuring the Efficacy of Repellent on House Sparrows (*Passer domesticus*)

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Contact:
epress@unitec.ac.nz
www.unitec.ac.nz/epress/
Unitec Institute of Technology
Private Bag 92025, Victoria Street West
Auckland 1142
New Zealand

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Measuring the Efficacy of Repellent on House Sparrows (*Passer domesticus*)

Kristie Cameron, Roxanne Wassenaar, Ayellet Panapasa, Kelsey Brown, Angela Halliday, Kaitlyn Lodge-Osborn, Emily Robson, Joanne Aley, Graham Jones, Jodi Salinsky, Diane Fraser and Nigel Adams

Simple Summary

House sparrows (*Passer domesticus*) are vectors for diseases transmittable to humans and animals, therefore effort is made to deter sparrows from roosting and feeding in urban areas such as cafés and private buildings. In this experiment, four methods of measuring sparrow avoidance of a commercially available avian repellent were trialed in aviaries and in the field. The methods were designed to detect repellency at differing levels of sensitivity. Experiments attempted to measure changes in the use of an aviary in relation to the presence of the repellent and the effect of proximity of the repellent on feeding in both an aviary setting and in the field where alternative food was available. We were consistently unable to detect any repellent effect of this commercially available product, indicating birds were insensitive to any intended aversive properties of its odour or visual appearance. The formulation of effective repellents based on visual and olfactory signals alone is likely to be very challenging.

Abstract

Behavioural analytic techniques were used to assess the efficacy of a repellent to the house sparrow (*Passer domesticus*). The repellent, using a combination of olfactory and visual cues, is aimed at deterring birds from roosting sites where faecal contamination may result in disease transmission to humans and animals, and damage to public and private property. In this experiment, four methods of measuring avoidance by sparrows to a commercially available avian repellent were trialed in aviaries and in the field. In initial experiments, the number of sparrows was recorded in predetermined zones across an aviary, and faecal counts were measured as the position of the repellent varied. In further experiments, food removal was recorded when repellent was placed at varied distances from food sources to test the effect of proximity on sparrow feeding behaviour in the aviaries and in the field. There was no apparent repellent effect of this commercially available product, indicating birds were insensitive to any intended aversive properties of its odour or visual appearance. Therefore, as formulated, the product is unlikely to be of any use in a practical setting. Development of chemical repellents based primarily on olfactory cues might be challenging and require additional aversive stimuli.

Introduction

House sparrows (*Passer domesticus*) are potential vectors for a variety of pathogens (Benskin, Wilson, Jones, & Hartley, 2009) including Campylobacter spp. identified in faecal samples taken from wood, concrete, soil, bark, plastic and grass surfaces (Abdollahpour, Zendehbad, Alipour, & Khayatzadeh, 2014). Increasing the risk of zoonotic disease transfer to humans is the tendency for house sparrows to be closely associated with urban settings, including nesting in the roof cavities of buildings (Shaw, Chamberlain, & Evans, 2008) and feeding on discarded food (Gavett & Wakeley, 1986) available in places such as outdoor café areas. At high concentrations the faeces may also cause damage to property (Whiley, van den Akker, Giglio, & Bentham, 2013). Approaches to deterring wild birds from utilising sites in urban areas for nesting and foraging include the use of physical barriers or exclusion methods such as nets or sharp projections (Alderson & Greene, 1995; Steiger, Fidler, Valcu, & Kemnpenaers, 2008). However, these approaches are not always suitable and can themselves become anchors for sparrow nests (Alderson & Greene, 1995). Another approach is the use

of chemical repellents (Alderson & Greene, 1995; Smith, 2014).

Avian chemical repellents have been used and evaluated in situations where the objective is to deter birds from consuming what may be potential food. For example chemical repellents have been used to protect agricultural crops (Avery, 2002; Clapperton, Porter, Day, Waas, & Matthews, 2012) and explored as an approach for deterring birds from consuming baits containing poisons developed for controlling mammal pests (Clapperton et al., 2012; Cowan, Booth, & Crowell, 2015). These approaches involve developing an aversion to the potential food. Primary repellents invoke an immediate aversive response through an unpleasant smell or taste. Secondary repellents invoke a delayed post-ingestion illness or discomfort, resulting in a learned aversion (Avery, 2002). Visual cues, such as colour, for example, blue or green, can enhance avoidance behaviour (Clapperton et al., 2012). Therefore, avian chemical repellents often combine visual and olfactory deterrent mechanisms with secondary repellents, which have delayed physiological effects, to provide effective deterrent (Clapperton et al., 2012).

Numerous chemical compounds have been used within bird repellents as primary and secondary deterrents (Avery, 2002). For example, primary repellents have been used to stop sparrows feeding on food sources by treating the food with tannic acid, which has a bitter taste (Greig-Smith & Rowney, 1987), whereas Optamint® and d-pulegone both use peppermint extracts with associated olfactory and taste cues for repelling birds (Avery, 2002). Secondary repellents include anthraquinone and cinnaminide, which cause discomfort or distress after ingestion (Clapperton et al., 2012; Greig-Smith & Rowney, 1987; Porter, 1995).

There are few studies that isolate bird aversion to odour in repellents other than when combined with a secondary repellent such as anthraquinone. Most birds were thought to rely primarily on visual and auditory inputs to evaluate their surrounding environment, however, there is increasing evidence that olfaction is also an essential sense and this extends beyond its previously recognised importance to groups such as seabirds and other specialist nocturnal bird groups (Steiger et al., 2008). Accordingly, odour-based aversion maybe another option in the design of avian repellents. Stock and Haag-Wackernagel (2013) recorded pigeon behaviour when an 'optically-aversive' and odorous gel was placed in the loft of a church known to house wild pigeons. This repellent thus acts as a primary repellent. Landing and approach behaviour on two shelves with or without contact with the gel decreased over 26 days, but time spent on the shelves with containers of gel increased after four days of exposure, suggesting that the repelling effect on pigeons decreased with time.

The aim of this study was to determine the effectiveness of a commercially available bird repellent presumed to have its effect through its odour and visual characteristics. Initially, tests were conducted, to determine whether placement of repellent at specific locations in the aviary affected the spatial use of the aviary by sparrows, by direct observation of birds and determining the distribution of bird faeces on the floor of the aviary as an indicator of spatial use. The spatial use of the aviary was compared with and without repellent present. It was predicted that birds would avoid areas close to the repellent source. Further experiments tested whether feeding and interaction with food in the aviary were affected by the distance between the repellent and food source. It was predicted that increasingly smaller amounts of food would be removed from food containers as the distance between the repellent and food containers reduced. A final set of experiments tested whether feeding or food interaction by free-living birds in the field would similarly decrease as the distance between the experimentally provided food source and repellent was reduced. These birds would have alternative food sources available in the environment that were outside the range of an odorous repellent. This series of experiments represented a gradient in the ability to detect a possible repellent effect.

Method

Subjects

Wild-caught sparrows served in Experiments 1-5 with six naïve sparrows used in each experiment, and wild sparrows (of unknown number) were exposed to the experimental treatment in Experiments 6-7. Ethical approval for this study (approval notice 001605) was obtained from The University of Auckland Animal Ethics Committee. Consistent with our ethical approval notice, wild sparrows were held captive for a maximum of 26 days. All experiments were conducted in autumn (April-May in New Zealand), Experiments 1-3 in 2015, and Experiments 4-7 in 2016.

Repellent

The gel repellent is commercially available and advertised as a deterrent for pigeons from roosting sites (Bird Free®, ingredients: polysiobutylene 68%, grease 22%, peppermint and cinnamon oils 10%. Jeonjinbio Co. Ltd, Daegu, Korea). It is the colour and consistency of caramel and considered a non-toxic food-grade gel. It is described as providing a visually aversive stimulus detectable within the ultraviolet visual range of birds as flames (Jeonjinbio Co. Ltd, 2017). In addition, the repellent is described as a deterrent based on smell, touch and taste (Jeonjinbio Co. Ltd, 2017). The repellent was presented in small circular trays (5 cm in diameter and 0.5 cm in depth). The manufacturer's instructions suggest the repellent is suitable for preventing birds roosting and utilising possible nesting sites, and multiple trays need to be placed between 15 and 25 cm apart. We utilised single plastic trays of gel and manipulated the distance of the tray from a food source from directly adjacent to the gel (0 cm) to 120 cm, depending on the experiment.

To avoid birds coming into direct contact with the sticky gel and fouling their feathers, a fine plastic mesh was placed over the tray. The mesh did not decrease the repugnance of the gel to the human nose and it is stated by the manufacturer that a single dose of the gel is effective for periods up to four years (BirdFree, 2017); however, we refreshed the gel trays daily to maintain constant volatility of the substance during the experiment.

Aviary Apparatus

Experiments 1-5 were conducted in wooden-framed aviaries 2.4 m deep, 2.4 m wide and 2.4 m high, located at the Unitec campus in Auckland, New Zealand. For Experiments 1-3 the aviaries were placed under an open-walled structure that provided protection from direct rainfall but was otherwise open to the environment. To provide a visual barrier but allow airflow, the aviaries were wrapped in shade cloth on all sides. Due to possible effects of disturbance caused by foot traffic, for Experiments 4 and 5 the aviaries were moved to a concrete pad within a large free-range chicken enclosure to further minimise any external disruption to the animals, including that of the chickens housed in the enclosure, none of which had access to the aviaries.

The aviary contained three perches placed 1.5 m above the floor, running parallel to the entrance. Ledges at the end of the perches allowed for placement of food and the test apparatus (Figure 1). Sparrows were fed a commercially available budgie mix (Animates®). Water was available ad libitum in containers attached to the opposite aviary walls. Cleaning, feeding and observations were completed daily between 12 p.m. and 2.30 p.m.

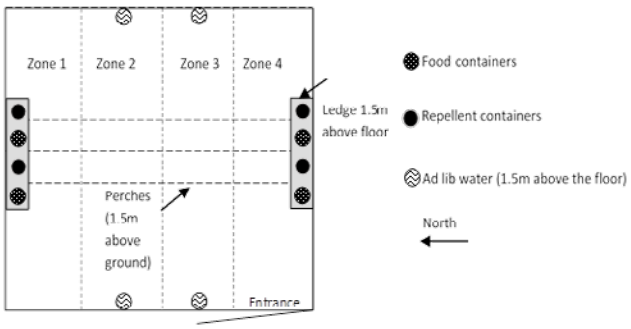

Figure 1. Aviary set up for Experiments 1 and 2 (not to scale). The aviary was delineated into four zones 0.6 m apart. Food was available in Zones 1 and 4 at all times and the presence of repellent containers on shelves was varied with treatment. (Trt 1 = no repellent, Trt 2 = repellent in Zone 1, Trt 3 = repellent in Zone 4; Trt 4 = repellent in Zones 1 and 4).

Experiments 1 and 2: Experiments 1 and 2 were designed to test the effect of the presence or absence of the repellent beside a food source and spatial use of the aviary. The sparrows in the aviary were exposed to four 'treatments' after a week of habituation to the aviary. In Treatment 1 only control containers (empty of repellent) were placed next to the food dishes (Figure 1). In Treatment 2, two controls were replaced by containers of repellent in Zone 1. Treatment 3 was a similar manipulation but containers of repellent were placed in Zone 4. In Treatment 4, the containers of repellent were placed in Zone 1 and in Zone 4. Food was available in Zones 1 and 4 at all times.

Experimental Procedure

Experiment 1, behaviour sampling: A video camera (Panasonic HC-V700 full HD camera on a tripod) was set up to capture bird activity for data analysis. After replenishing the food containers and repellent, and changing the position of the repellent for the next condition, behavioural recordings were conducted between 1.30 and 2.30 p.m., a consistent time slot each day for a total of 16 days. Instantaneous behaviour sampling was undertaken every 30 seconds, recording the position of each bird in the aviary. This provided 120 data points per bird per day.

Experiment 2, faecal deposit: The number of faecal

deposits accumulated on the plastic-covered floor of the aviary across a 24-hour period in each of the four zones was recorded. Each day the black plastic was removed and replaced by another sheet marked with Zones 1-4. Data were recorded for a total of 16 days, with four repetitions of each treatment.

Experiments 3-5: Experiments 3-5 were designed to assess whether the distance between the repellent and the food source influenced food removal. Feeding bowls containing bird-seed were placed 30 cm apart at one side of the aviary. Control containers empty of the repellent gel were placed at varying distances from the food containers in two lines, depending on the experiment (Figure 2). Repellent containers were placed inside the control container at the same position in each of the two lines, at a specified distance, each day. Food removal from the food containers over a 24-hour period was determined by weighing the containers between 12 p.m. and 2 p.m. Thereafter the seed in the containers was replenished and re-weighed. The location of the paired repellent containers was set for the next day, following a randomised schedule.

Experiment 3: The control containers were secured to the plastic-covered floor at 30 cm, 60 cm, 90 cm and 120 cm in two rows centred between and extending from three food containers (Figure 3). Food containers were not provided on shelves as in Experiments 1 and 2. Repellent was located at each distance for two days (one day in each direction). Preliminary analysis indicated food removal did not vary with the position of the repellent container. Accordingly, additional trials in which repellent was placed immediately adjacent to the food source at 0 cm and 15 cm were conducted at one end of the aviary for two further experimental sessions (two days) each as post hoc additions. Data were recorded for 12 days.

Experiment 4: The control containers were secured at 0 cm, 15 cm, 30 cm and 45 cm in two rows extending from two food containers. A tray was placed underneath the food containers to catch food 'spillage' (mass of food in container – mass of food spilled = mass of food removed). After two days of habituation the position of the repellent was randomised at each distance for five days each, in addition to five days where no repellent was present. Data were recorded over a 25-day period.

Experiment 5: Repellent was located in one row at each distance each day to measure the effect of the controls on food removal and food spillage. The repellent was located at each distance for five days each, randomised over a 24-day period after two days of habituation. The repellent was located in Line A only for the first 12 days and in Line B for the second 12 days. There were no post-habituation-period days where the aviary was free of repellent, due to the 26-day limit on sparrow captivity.

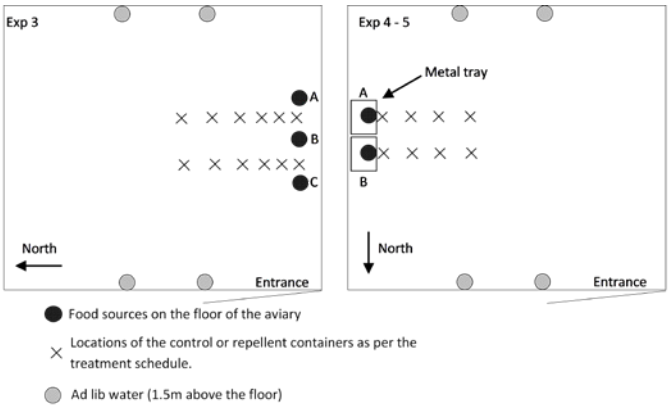

Figure 2. Aviary set up for Experiments 3 and 4-5 (not to scale), showing the locations of the repellent and control containers, and the food. In Experiment 3, the control containers were placed at 0 cm, 15 cm, 30 cm, 60 cm, 90 cm and 120 cm with the food containers on the right side of the aviary and control containers extended to the centre of the aviary. The set up was then repeated from the opposite side for all but 0 cm and 15 cm distances as these were added post hoc. In Experiments 4-5, two rows of control containers extended from two food containers from the left side of the aviary only at 0 cm, 15 cm, 30 cm and 45 cm.

Field Study Apparatus

Experiments 6 and 7: The field study was conducted using a similar methodology to Experiments 4 and 5 to measure the effect of the repellent on wild sparrows that had alternative food sources. The experiment was conducted in the cordoned-off northeast section of a 14.7 m x 18 m free-range hen enclosure where sparrows were known to forage for surplus chicken food. The experiment could not be accessed by the domestic hens (*Gallus gallus domesticus*) and spotted doves (*Streptopelia chinensis*). The experimental food was potentially accessed by green finches (*Carduelis chloris*) and goldfinches (*Carduelis carduelis*), however, the populations of these birds were small compared to sparrows. Black plastic sheeting (2 m long x 1.5 m wide) was placed over an area of grass. Hanging bird-seed feeders were suspended over the centre of the black plastic 50 cm apart and 50 cm from the ground, and were filled with budgie seed. Metal trays (25 cm x 30 cm)

were placed underneath the feeders. Control containers were glued in two lines (A and B) starting from directly under the hanging feeders and at distances of 15 cm, 30 cm and 45 cm from the feeders. In Experiment 6, repellent was located in both lines at a particular distance each day, and in Experiment 7 repellent was located in one line. The position of the repellent-containing tray was determined on a randomised schedule whereby the same distance was not repeated on successive days.

Experimental Procedure

The amount of food spillage and the amount removed from feeders were determined as described for Experiments 4 and 5. It was necessary to correct seed weights, due to exposure to rain in the field setting, by correcting the measurement of wet seed to dry matter. The correction factor of 0.5 was determined by the drying of wet seed over 48 hours and determining the fractional mass gain.

Statistical Analyses

The data for Experiments 1 and 2 was aggregated across treatment and zone. Repeated measures ANOVAs were used to compare the effect of the repellent in each treatment with the number of sparrows (Experiment 1) and faecal count (Experiment 2) recorded within each zone. The data for Experiments 3-5 were aggregated for each distance, and food removal and spillage was standardised for graphing due to the escape of individual sparrows during the experiment. For Experiments 6 and 7 total removal and spillage amount was used because the number of wild sparrows in the area was unknown. Repeated measures ANOVAs were used to compare the effect of distance between the food and repellent on food removal and spillage.

Results

The gel maintained its strong odour throughout the course of the experiments with no apparent change in appearance.

Experiment 1: Behavioural sampling

The frequency at which sparrows were recorded averaged over all days of the experiment was highest in Zone 1 across all treatments, irrespective of the placement of the repellent (Figure 3a). A repeated measures ANOVA revealed a significant main effect of zone on the number of sparrows observed in each zone [F (3, 9) = 1014.63, p <.001, η_p^2 = 1.0]. Pairwise comparisons, with significance levels adjusted using the Bonferroni correction, showed the number of sparrows was highest in Zone 1 compared to the other zones (all ps <.001) and higher in Zone 2 compared to Zone 3 (p =.036). In contrast, there was no significant effect of treatment on the number of sparrows observed in each zone [F (3, 9) = 1.02, p = .427, η_p^2 = 0.25] or the interaction between zone and treatment type [F (9, 27) = 1.42, p = .229, η_p^2 = 0.32].

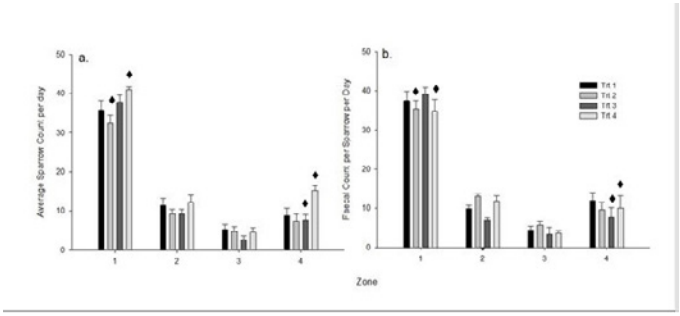

Figure 3. Average counts of sparrows (a, Exp 1) and faecal deposit (b, Exp 2) counts were recorded in each observational period in each zone across Treatments 1-4 and across all experimental days. Standard error bars are shown. The diamond symbol signifies the location of the repellent during each treatment. Trt 1 = no repellent, Trt 2 = repellent in Zone 1, Trt 3 = repellent in Zone 4; Trt 4 = repellent in Zones 1 and 4.

Experiment 2: Faecal deposit count

The number of faecal deposits was highest in Zone 1 irrespective of treatment (Figure 3b). A repeated measures ANOVA revealed a significant main effect of zone on the number of faecal deposits [F (3, 9) = 216.00, p <.001, η_p^2 = 0.99]. Pairwise comparisons, with significance levels adjusted using the Bonferroni correction, showed the number of faecal deposits was highest in Zone 1 compared to the other zones (all ps < .007) and higher in Zone 4 compared to Zone 3 (p = .037). There was a small but significant effect of treatment on the number of faecal deposits found in each zone [F (3, 9) = 7.11, p = .009, η_p^2 = 0.70]; and an interaction effect trending towards being significant between zone and treatment type [F (9, 27) = 2.14, p = .062, η_p^2 = 0.42].

The strongly favored use of Zone 1 by sparrows across all treatments in Experiments 1 and 2 suggests there were factors affecting the behavior of the sparrows that potentially obscured any repellent effects. Surrounding

activities included a nearby building renovation closest to Zone 4 and the frequent use of the area surrounding the aviaries by students of the institute.

Experiment 3: Proximity effects

Food removal was expressed as per sparrow per day because during the experiment one sparrow escaped, requiring standardisation of the food removal measure. Food removal decreased as the distance between the food source and repellent increased (Figure 4). A repeated measures ANOVA revealed a significant difference in food removal when the distance between the food and repellent was varied [F (5, 25) = 8.62, p < .001, η_p^2 = 0.66]. Pairwise comparisons, with significance levels adjusted using the Bonferroni correction, showed that significantly more food was removed when the repellent was immediately adjacent to food (0 cm) compared to when the repellent was placed 30 cm away (p = .004) and significantly more food was removed at 15 cm compared to 30 cm (p = .026). There was, however, more variability, measured using the standard error of the mean, in food removal when the repellent was located at distances further from the repellent. Variability in food removal was greater when food was more distant from the repellent [60 cm (SE = 1.10 g) and 120 cm (SE = 1.29g)] than when in close proximity [0 cm (SE = 0.62g) and 15 cm (SE = 0.59g)]. This increased variability in food removal and increased spillage when food was in containers more distant from the repellent may reflect a difference in actual feeding behavior associated with proximity to the repellent.

Paired t-tests revealed a significant difference in food removal between food (containers) A (M = 3.5g, SE = 0.16g) and B [M = 3.2g, SE = 0.12g; t (11) = 3.03, p = .012, d = 0.69]; and between food sources B (M = 3.2g, SE = 0.12g) and C [M = 3.5g, SE = 0.13g; t (11) = 3.57, p = .004, d = 0.63]. There was no difference in food removal between food sources A (M = 3.5g, SE = 0.16g) and C (M = 3.5g, SE = 0.13g), [t (11) = 0.62, p = .546, d = 0.10], but removal was low at B compared with A and C. This suggests that the direction of the experimental set-up (closest to a building renovation or the entrance to the aviary) did not cause differential food removal from the food containers based on location.

Experiment 4: Proximity effects

Food removal was expressed as removal per bird per day, as during the experiment one sparrow escaped. Food removal within a particular line was similar, irrespective of the distance between the food container and the repellent [F (4, 16) = 2.17, p = .120, η_p^2 = 0.35]

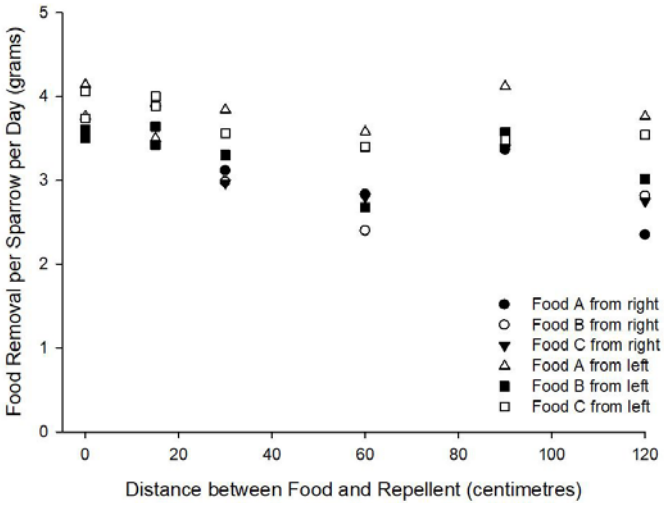

Figure 4. Food removal per sparrow per day (grams) as distance (cm) between repellent and food containers A, B and C was varied (Exp 3). Food removal in both the initial (from right) and reverse (from left) directions are shown.

but differed between the two lines of repellent [F (1, 4) = 185.48, p < .001, η_p^2 = 0.98] (Figure 5a). Food removal was significantly higher in Line A compared to Line B across, irrespective of the position of the repellent. There was no interaction effect between line or distance on food removal [F (4, 16) = 0.57, p = .688, η_p^2 = 0.13].

There was significantly greater food spillage in Line B compared to Line A across distances [F (1, 4) = 21.63, p = .010, η_p^2 = 0.84; Figure 5b]. There was no significant effect of distance on food spillage [F (4, 16) = 1.56, p = .232, η_p^2 = 0.28] or interaction between line and distance on food removal [F (4, 16) = 0.50, p = .734, η_p^2 = 0.11].

Experiment 5: Proximity effects (single repellent line)

In Experiment 5, the repellent was present in either Line A or Line B only each day (Figure 6a). There were no significant differences in food removal between Lines A and B across distances [F (1, 2) = 0.14, p = .744, η_p^2 = 0.07], or in repellent versus non-repellent lines [F (1, 2) = 1.46, p = .346, η_p^2 = 0.42] or across distances [F (3, 6) = 0.88, p = .502, η_p^2 = 0.31]. Similarly, there were no interaction effects between each of the variables: Lines A and B, presence of the repellent and the distances between food and the repellent (all ps > .05).

There were no significant differences in food spillage (Figure 6b) between Lines A and B [F (1, 2) = 5.87, p = .136, η_p^2 = 0.75], or when repellent was

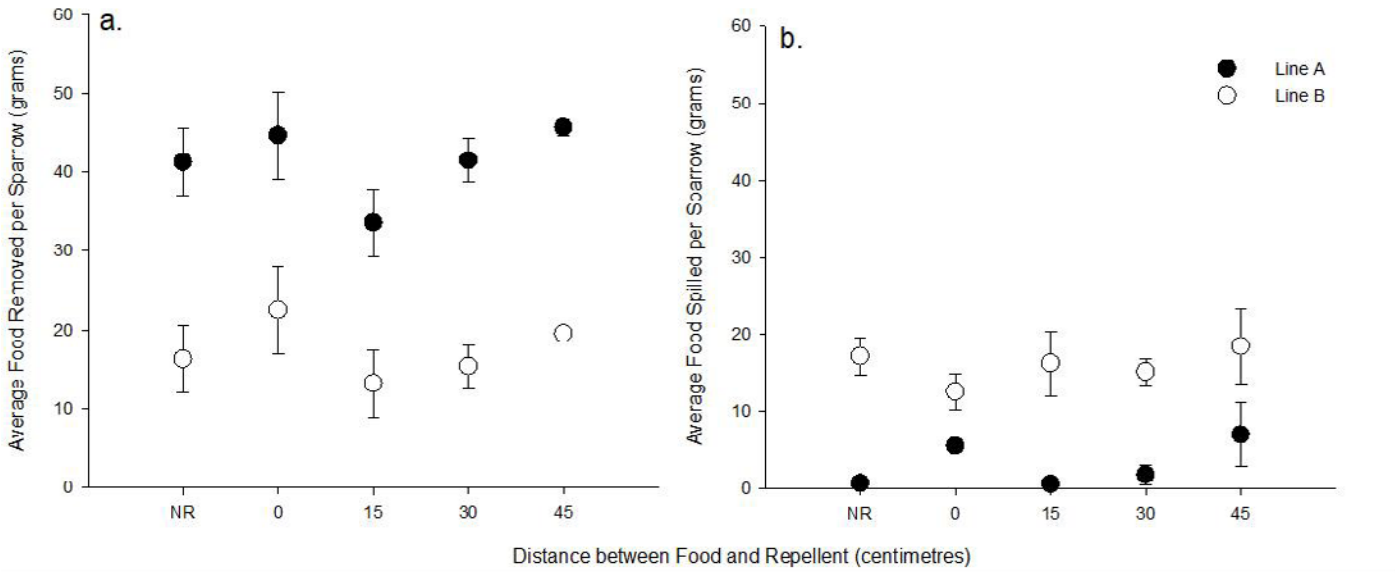

Figure 5. Average food removed (a) and spilled (b) per sparrow (grams) as the distances (cm) between the food and the repellent were varied in Lines A (solid circles) & B (open circles). Standard error bars are shown. (NR = no repellent).

present or not [F (1, 2) = 0.89, p = .794, η_p^2 = 0.42], or across distances [F (3, 6) = 0.56, p = .660, η_p^2 = 0.22]. Similarly, there were no interaction effects between each of the variables Lines A and B, presence of the repellent and the distances between food and the repellent (all ps > .05). An interaction between the line, presence of repellent and distance that approached significance (p = .060), reflected a generally higher food spillage per sparrow in repellent Line A compared to non-repellent Line B across distances and a generally higher food spillage in non-repellent Line A compared to repellent Line B across distances.

Experiment 6: Field study

In Experiment 6, repellent was placed in both Lines A and B simultaneously at each distance (Figure 7a). There was a significant difference in food removal between Line A and Line B [F (1, 4) = 9.98, p = .034, η_p^2 = 0.71]; however, there was no significant main effect of distance on food removal [F (4, 16) = 0.23, p = .919, η_p^2 = 0.05] or significant interaction between line and distance on food removal [F (4, 16) = 0.45, p = .768, η_p^2 = 0.10].

There was no main effect of line on food spillage, [F (1, 4) = 0.14, p = .911, η_p^2 = 0.004] or distance on food spillage [F (4, 16) = 0.97, p = .450, η_p^2 = 0.20] and no interaction between line and distance on food spillage [F (4, 16) = 1.37, p = .288, η_p^2 = 0.26 (Figure 7b)].

Experiment 7: Proximity effects (single repellent line) field study

There were no significant differences in food removal between Lines A and B [F (1, 2) = 5.80, p = .138, η_p^2 = 0.74], between repellent and non-repellent lines [F (1, 2) = 0.90, p = .444, η_p^2 = 0.31] or across distances [F (3, 6) = 1.47, p = .313, η_p^2 = 0.42; Figure 8a]. An interaction effect between line and the presence of repellent was trending towards significance [F (1, 2) = 17.32, p = .053, η_p^2 = 0.90].

There were no significant differences in food spillage between Lines A and B [F (1, 2) = 6.98, p = .118, η_p^2 = 0.78, or in repellent and non-repellent lines [F (1, 2) = 1.30, p = .373, η_p^2 = 0.39] or across distances [F (3, 6) = 1.08, p = .426, η_p^2 = 0.35; Figure 8b]. There were no interaction effects between each of the variables in spillage across Lines A and B, presence of the repellent and the distances between food and the repellent (all ps > .05).

General Discussion

This series of experiments aimed to measure the efficacy of a commercially available repellent for deterring sparrows. These progressed through experiments that tested whether the presence of repellent altered the spatial use of an aviary through to more sensitive indicators of repellency based on levels of food removal

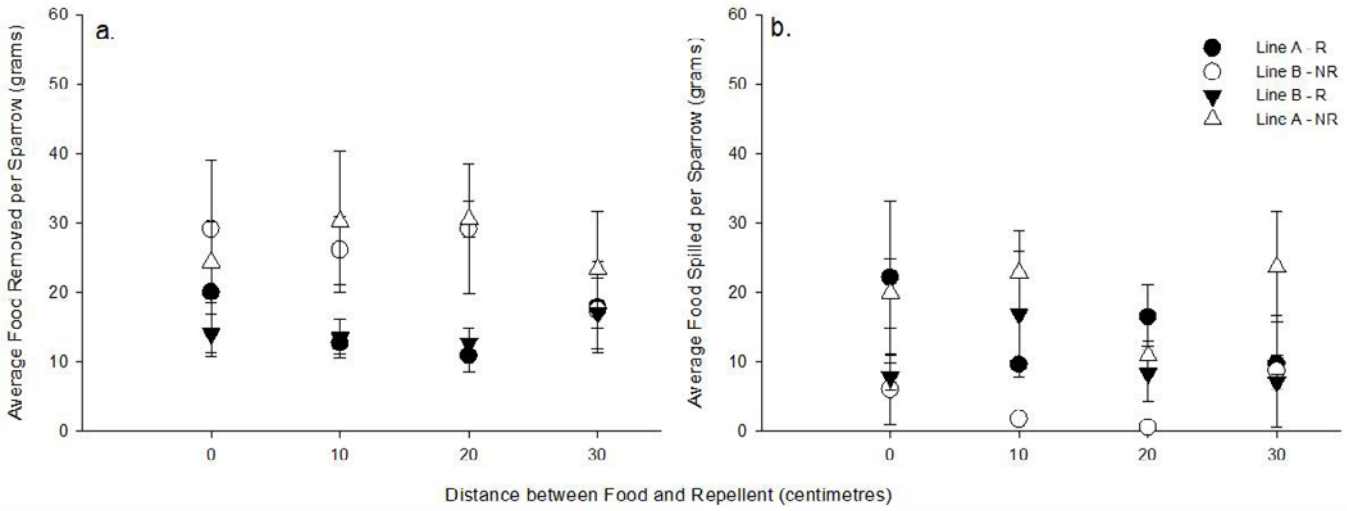

Figure 6. Average food removed (a) and spilled (b) per day (grams) as distances (cm) between food and repellent were varied in Lines A & B. Line A (repellent R: solid circles; no repellent NR: solid triangles) or Line B (repellent R: open triangle; no repellent NR: open circles). Standard error bars are shown.

and spillage from containers at varying distances from the repellent. In addition, in the field experiments, birds could make a choice of where to feed; whether at the feeders closer to or further from repellent or at alternate food sources. None of these experiments provided evidence of a repellent effect of the gel on sparrows.

Based on the position of captive sparrows (Experiment 1) and locations of faecal accumulation (Experiment 2) in the aviary, the birds preferred a particular area within the enclosure. However, this preference was independent of the location of the repellent and suggests that an environmental factor, such as an area more disrupted by foot traffic closest to Zone 4, was causing birds to focus their activity in Zone 1 of the aviary. In addition, there was the possibility that the aviary door and repeated human entry affected feeding behaviour; however, there was no difference in food removal in Experiment 3 when the set up was reversed (Figure 4) and experimenters entered the aviary once per day for a short period to replenish food, thus it was concluded that there was minimal disruption. The results of the latter experiments support the conclusion of lack of effect of the aviary door; in Experiment 4 more food was removed from the container furthest from the door, and in Experiment 5 more food was removed from the container closest to the door.

We did demonstrate some significant differences in food removal rates in relation to distance from the gel. However, contrary to expectations, food removal increased when the repellent was close to the food (Experiment 3). In other experiments, we demonstrated some significant differences in food removal and spillage between food sources but that did not differ between distances from the gel (Experiments 4 and 6). As with the earlier experiments, the differences in feeding between the food sources suggest some spatial bias in the feeding which appears unrelated to the presence of the repellent. We did note differences in the variability of the amount of food removal related to distance of the food bowl from the repellent, which may suggest some differences in the way birds interact with the food in relation to proximity to the repellent.

The properties of the gel used in the current series of experiments were based on it having aversive smell, optical or visual properties acting as a primary repellent. Olfactory repellents are assumed to target aspects of the animals' chemosensory systems, eliciting irritation as a defense mechanism (Stevens & Clark, 1988). For example, airborne delivery of methyl anthranilate (MA) may act as potent avian irritant stimulating the nociceptive system associated with the mucosa of the noses and eyes, and by being detected orally (Stevens & Clark, 1988). Our results suggest that the volatile substances within the tested gel had no such effects. The visual, apparently aversive, signal from the repellent is described by the manufacturer to be detectable within the ultraviolet visual range of birds as flames (Jeonjinbio Co. Ltd, 2017). While birds can detect ultraviolet light, a study on pigeons found no evidence of a visual repelling effect (Day et al., 2003).

Consistent with its likely use in field settings, one of the major bases of the design of the current

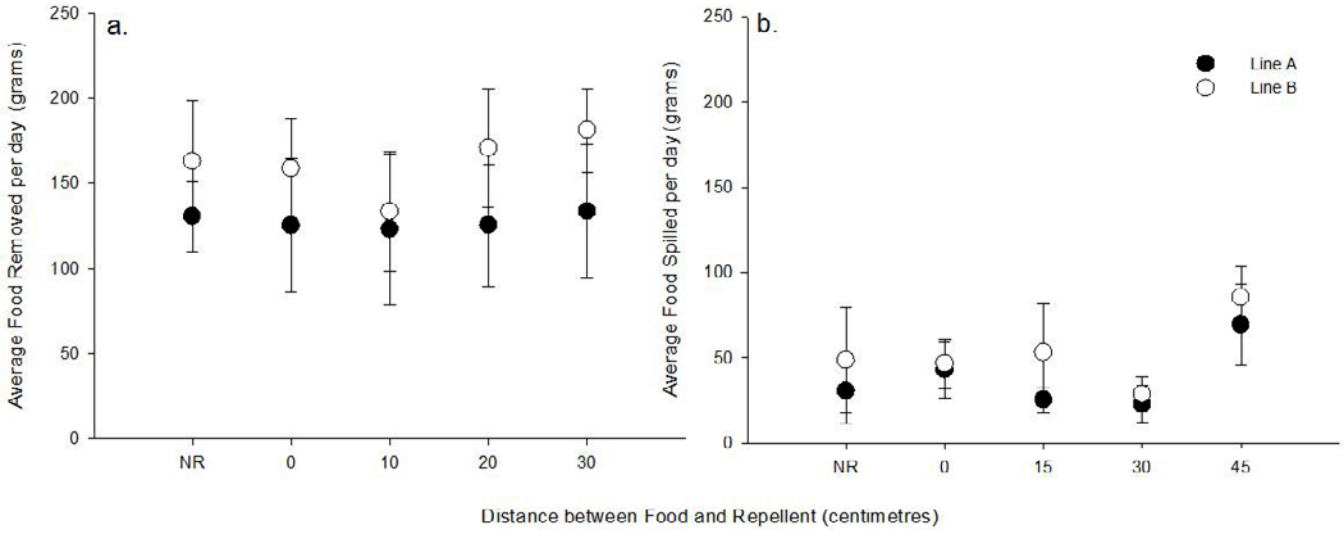

Figure 7. Average food removed (a) and spilled (b) per day (grams) as distances (cm) between food and repellent was varied for Line A (solid circles) and Line B (open circles). Standard error bars are shown. (NR = no repellent).

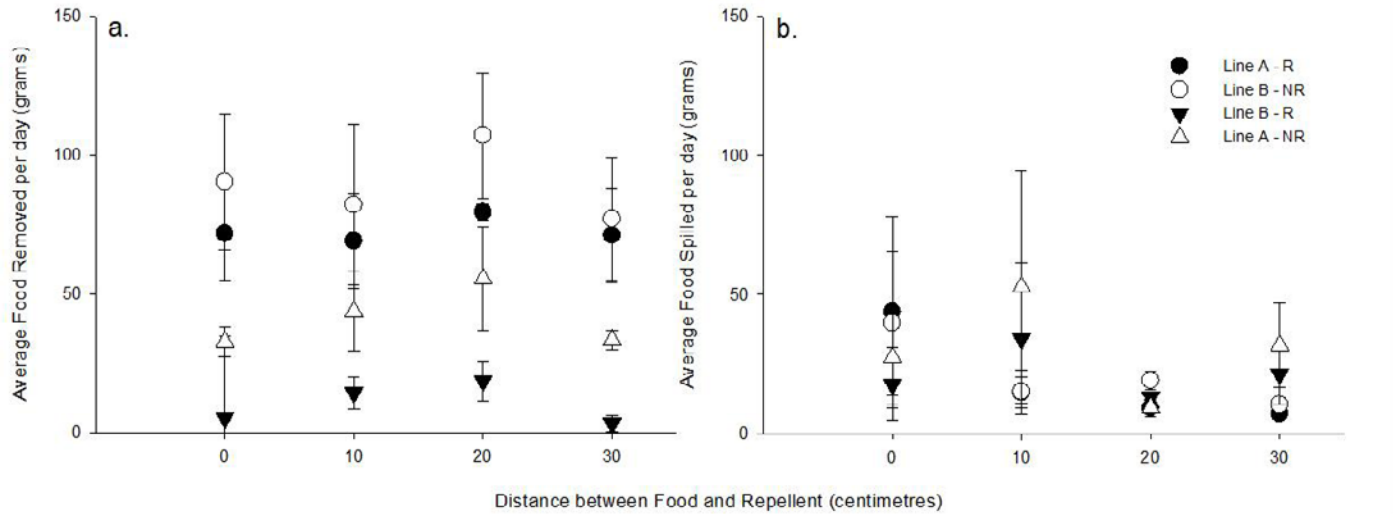

Figure 8. Average food removed (a) and spilled (b) per day (grams) as distances (cm) between food and repellent were varied in Lines A or B. Line A (repellent R: solid circles; no repellent NR: solid triangles) or Line B (repellent R: open triangle; no repellent NR: open circles). Standard error bars are shown.

experiment was to test the effects of the repellent over an extended period of up to 26 days. It is possible that such a design may have hidden an early repellent effect that was lost because birds habituated to the smell that was acting as a primary repellent with no physiological effects or consequences. Post hoc analyses, however, comparing the initial and final series of replications within each experiment showed no habituation in the form of increased food removal in each experiment. Many repellents are intended to have an effect beyond their immediate location, known as common hazing (Cook, Rushton, Allan, & Baxter, 2008). These include scarecrows, model predators and bird distress calls. There is a reduced effectiveness of such repellents, particularly if presented continually or on a predictable schedule (Cook et al., 2008). Primary repellents used over more extended periods are frequently ineffective, as an early learned avoidance of a mildly unpleasant sensation decays rapidly (Day et al., 2003), especially where the outcome is access to a valued resource such as food.

It is possible that we may not have been able to

detect a very mild repellent effect using the approaches described because provision of a high-value resource such as food masked any repelling effect. Such low repellence may only be detected when tested in situations where birds need to make a choice between utilising a low-value superabundant resource associated with a repellent, and another without. However, such a low repellent effect is unlikely to be of any use in a practical setting.

Approaches to increase the efficacy of a non-ingestive repellent and possibly decrease habituation include the addition and combining of other stimulus dimensions such as a different olfactory signal (Clapperton et al., 2012), altering the location of the repellent more frequently and randomly, and providing a consequence of an aversive event rather than simply 'simulating risk' (Bishop, McKay, Parrott, & Allan, 2003; Gill et al., 1998), as with scarecrows. The formulation of effective repellents based on visual and olfactory signals alone is likely to be very challenging, therefore future measurement of these types of repellent might be effective with additional aversive stimuli.

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Authors

Kristie E. Cameron, Graham Jones, Diane L. Fraser and Nigel J. Adams are Lecturers in Environmental and Animal Sciences at Unitec Institute of Technology, Auckland, New Zealand. Jodi R. Salinsky was a Lecturer at Unitec at the time of the study, but now works for the University of Auckland. Roxanne J. Wassenaar, Ayellet Panapasa, Kelsey J. Brown, Angela D. Halliday, Kaitlyn R. Lodge-Osborn, Emily A. Robson, and Joanne P. Aley are all former students in this department.

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Contact:
epress@unitec.ac.nz
www.unitec.ac.nz/epress/
Unitec Institute of Technology
Private Bag 92025, Victoria Street West
Auckland 1142
New Zealand

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The Ant Fauna of Rakitu (Arid Island), New Zealand

Andrew Veale, Thomas Bodey, Erin Doyle, Jo Peace, James Russell

Abstract

Monitoring the fauna of an island before ecological restoration work begins provides a baseline against which changes to that environment can be quantified. Ants are a diverse and ecologically important group of insects, and many are extremely successful invasive species. In this study we provide the first description of the ant fauna of Rakitu (Arid Island), a small island in the outer Hauraki Gulf of New Zealand. We used a combination of unbaited pitfall traps, baited stations (rat traps with peanut butter) and hand searching conducted in habitats across the island. Using morphological classification and genetic barcoding we detected seven species of ant: four New Zealand endemics (*Austroponera* sp., *Heteroponera brouni*, *Monomorium antarcticum*, and *Monomorium antipodum*) and three introduced (*Iridomyrmex suchieri*, *Ochetellus glaber*, and *Tetramorium grassii*). While the ecological effects of these introduced species are currently unquantified, none of them have previously been highlighted as likely ecological threats. Our results provide a baseline for future biosecurity monitoring of the island, and can be used to help assess changes in the environment related to the forthcoming removal of invasive rat species from Rakitu.

Introduction

Understanding the initial fauna and flora of an island before ecological restoration actions begin, such as invasive species eradication, is critical in order to understand the long-term effects of these programmes (Jones et al., 2016). The invertebrate fauna are often neglected in these ecological monitoring programmes, however, quantifying them is critical to understanding ecological function (Rosenberg, Danks, & Lehmkuhl, 1986; Sheehan, Székely, & Hilton, 2011; Sinclair et al., 2005). One specific area that requires addressing in these ecological monitoring programmes is assessing the presence of other invasive species – enabling these existing species to be managed if required, and providing a baseline knowledge of the fauna so that novel incursions can be identified rapidly before their populations expand.

Many ants are extremely successful invasive species with serious agricultural, social and environmental impacts throughout the world (Holway, Lach, Suarez, Tsutsui, & Case, 2002; Williams, 1994). New Zealand's native ant community is composed of eleven endemic species, along with a total of 37 introduced species (Don, 2007), including the invasive Argentine ant *Linepithema humile* (Ward et al., 2010). Understanding the spread and ecological effects of invasive ants in New Zealand is critical for both biosecurity and ecological restoration (Harris & Baker, 2007; Lester, 2005; Ward, Beggs, Clout, Harris, & O'Connor, 2006).

Ecological restoration programmes involving mammalian eradication are increasingly common on islands in the Hauraki Gulf, and these islands experience ever-increasing challenges to their biosecurity (Bassett, Cook, Buchanan, & Russell, 2016). A good example of this is Rakitu (Arid Island), a 328-hectare island in the outer Hauraki Gulf lying 2.5 kilometres off the east coast of Aotea (Great Barrier Island). It is a highly modified island, with areas of thick rank grass, scrub and patches of mature coastal broad-leaf (Cameron & Wright, 1982). Rakitu presents great potential for ecological restoration if invasive species can be eliminated and if biosecurity systems can be implemented to prevent their reintroduction. The last major terrestrial ecological survey was undertaken in January 1981 by the Offshore Island Research Group, and was published in Volume 28 of the University of Auckland journal *Tane* (Hayward, 1982). While many taxa were surveyed during this trip, invertebrates were not surveyed, and very little is known about this fauna.

The management history of Rakitu has meant that

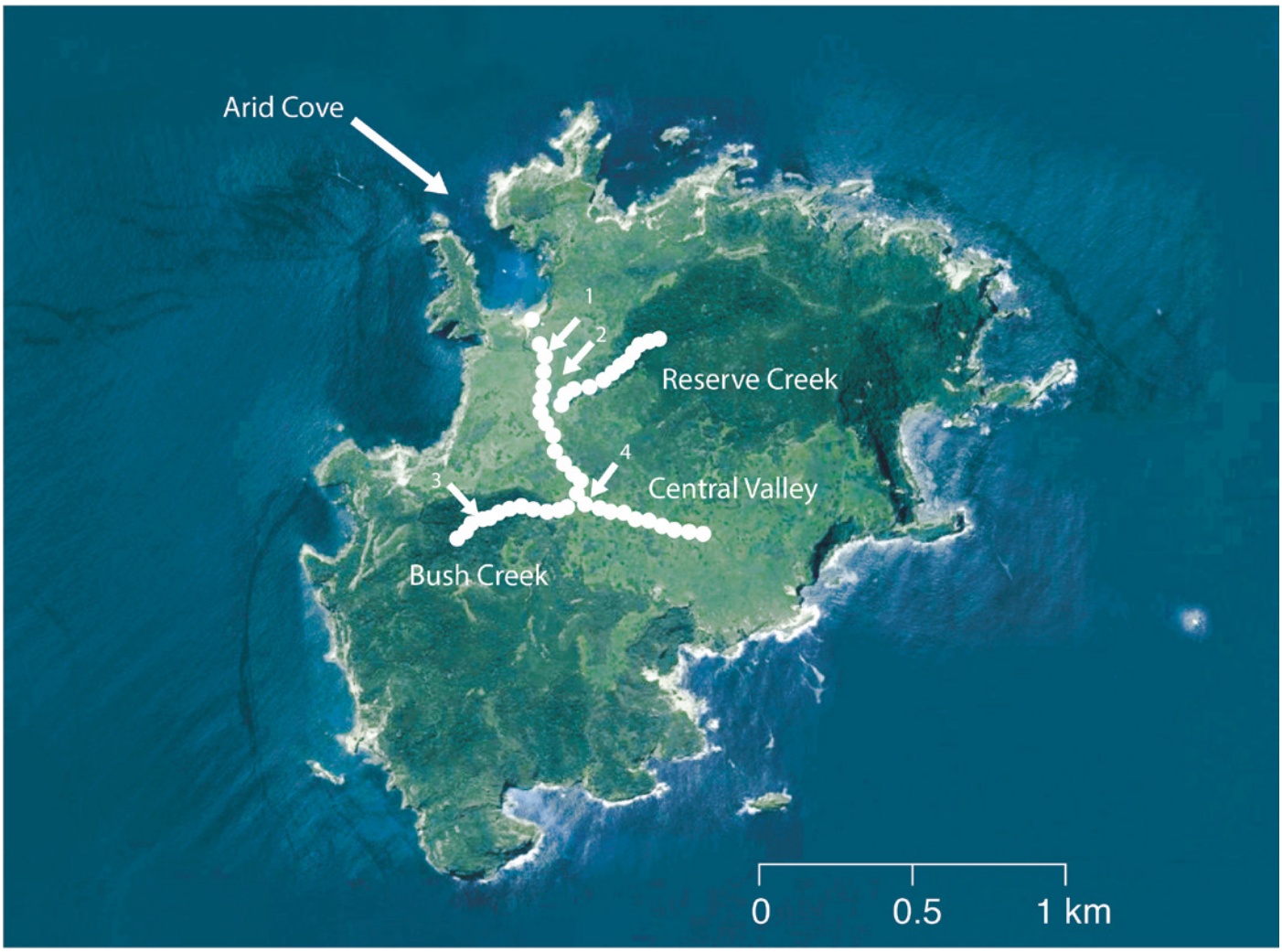

Figure 1: White circles indicate the Rakitu Island monitoring stations. The Reserve Creek and Bush Creek lines had both rat traps and pitfall traps, the Central Valley line only had rat traps. The numbers correspond to the single locations where four of the ant species were detected. 1: *Ochetellus glaber*; 2: *Iridomyrmex suchieri*; 3: *Heteroponera brouni*; 4: *Monomorium antarcticum*.

it is likely that many invertebrate species have been accidentally introduced; farm equipment and building supplies were brought to the island with minimal biosecurity awareness over the last century, and until recently there have been no formal biosecurity protocols for landing there. Since 1994 it has been in government ownership and managed as a Department of Conservation scenic reserve, allowing unrestricted public access. Arid Cove (Figure 1) is a popular sheltered anchorage, with public vessels regularly landing on the island – again, often with no knowledge of or consideration towards biosecurity and the prevention of novel insect establishment. A rat eradication operation is currently underway for Rakitu Island with the hope of restoring the island's indigenous fauna and re-establishing a seabird-driven ecosystem. Invasive ants have the potential to significantly impact New Zealand ecosystems, and specifically the ecology of seabird islands (Fukami et al., 2006; Plentovich, Hebshi, & Conant, 2009); therefore assessing the current ant fauna of these islands and monitoring for new invasive ants should be prioritised. It has previously been demonstrated that some invasive ant species (in this instance yellow crazy ants) have benefitted from rodent eradication operations (Feare, 1999). The specific mechanisms behind the interactions between invasive rodents and ants are yet to be properly studied, with a multitude of changes in the seed and seedling density, and increasing densities of large insect fauna likely to occur due to rodent removal (Watts, Armstrong, Innes, & Thornburrow, 2011).

Our aim in this study was to identify the ant fauna on Rakitu Island, and to provide a baseline for future biosecurity and ecological monitoring studies. This study was undertaken opportunistically while conducting

a range of monitoring as part of the pre-rat-eradication biodiversity survey.

Methods

Three methods were used to detect ants across the island over a four-day surveying trip in January 2018: pitfall traps, baited traps and hand searching. The 24 pitfall traps were laid out in two transect lines, each starting in the kikuyu grass in the central valley, proceeding through the scrub and up into the surrounding remnant coastal broadleaf forests of Bush Creek and Reserve Creek (Figure 1). Two transects consisting of a total of 36 Victor Professional rat snap traps spaced every 50 metres, baited with peanut butter (Eta), were set to monitor rodent presence across the island. One of these transects (24 traps) followed the path along the central valley primarily through kikuyu grass and scrub habitat, while the other (12 traps) followed the pitfall traps through the forest of Reserve Creek, with traps located ~5 metres from pitfall traps. During the course of the rat-trapping work over two nights, ants were gathered on the peanut butter food resource on the traps. These ants were then shaken off the traps into plastic bags and stored in 70% ethanol. Hand searching was also conducted opportunistically at selected locations around the buildings in Arid Cove to detect potentially recently arrived ants.

Species identification

The ants sampled were identified morphologically to species level using the New Zealand ant key (available online from the Landcare Research website). DNA from the head, thorax and legs of representatives of each species was extracted using a DNeasy Blood & Tissue Kit (Qiagen). We used the standard universal COI primers LCO1490 and HC02198 (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994) to amplify the barcoding region of COI, and sequenced these PCR products in both directions using a BigDye Terminator Cycle Sequencing Kit, analysed on a 3130xL Genetic Analyser (Applied Biosystems). The reads obtained were then aligned and concatenated in Geneious 9.1.8 (Biomatters).

Results

We identified seven species of ant on the island: four native and three introduced (Table 1, Figure 2). Note that the single *Iridomyrmex suchieri* specimen is a queen (no workers were found of this species) therefore the size comparison to the other species is somewhat

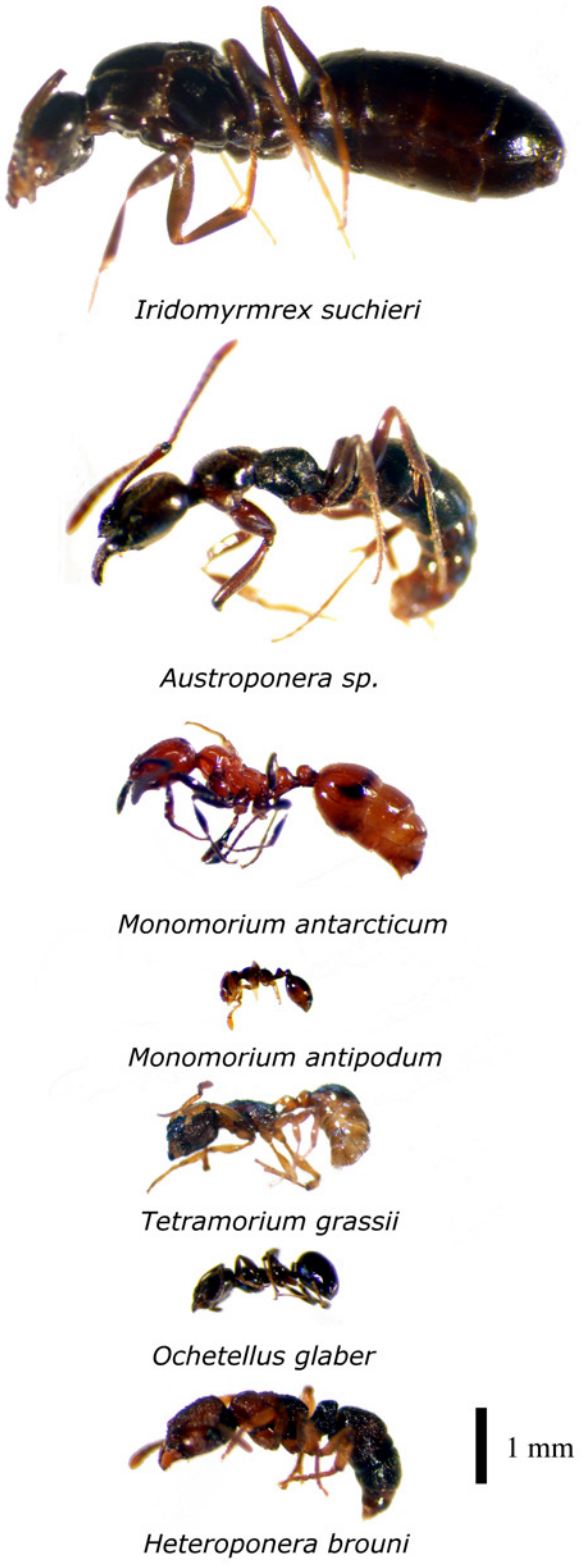

Figure 2: Ant species recorded on Rakitu. Note the pictured *Iridomyrmex suchieri* individual is a queen rather than a worker as for the other species.

Species	Status	Locations	GenBank #
Austroponera sp.	Endemic	9/20 pitfall traps, also occasionally on rat traps	MH539773
Heteroponera brouni	Endemic	1/20 pitfall traps, not recorded on rat traps	NA
Monomorium antarcticum	Endemic	10/20 pitfall traps, very common on rat traps	MH539776
Monomorium antipodum	Endemic	0/20 pitfall traps, 1 rat trap	MH539775
Iridomyrmex suchieri	Introduced	0/20 pitfall traps, 1 individual found in woodpile by house	MH539774
Ochetellus glaber	Introduced	0/20 pitfall traps, 1 rat trap	MH539777
Tetramorium grassii	Introduced	16/20 pitfall traps, very common on rat traps	MH539778

Table 1. Ant species present on Rakitu Island

misleading. We successfully obtained COI sequences from most of these species: GenBank accession numbers in Table 1. The one exception that was not sequenced was *Heteroponera brouni*, where the DNA from the two individuals caught appeared too degraded to amplify.

Two species (*Monomorium antarcticum* and *Tetramorium grassii*) were common across the island, dominating both the rat traps and pitfall traps across grass, scrub and forest habitats. One other species (*Austroponera* sp.) was also common across all habitats, but it was less abundant than the previous two. The other four species were only found in one location each: *M. antipodeum* was recorded at one rat trap in the central valley among thick kikuyu grass, *Ochetellus glaber* was only recorded at one rat trap in scrub, and *Heteroponera brouni* was only recorded in one pitfall trap in forest in thick leaf litter. The single *Iridomyrmex suchieri* specimen was found by hand searching in a wood pile below one of the houses. On most of the rat traps, only one species of ant was recorded.

Discussion

Most restoration islands (and mainland sanctuaries) in New Zealand have not had ant surveys published, and the composition of the general invertebrate fauna on these islands is rarely described – with a few exceptions, e.g. Elliott, Greene, Nathan, & Russell, 2015; Russell, 2012; Russell, Horn, Harper, & McClelland, 2018; Sinclair et al., 2005. This means that changes over time in the invertebrate fauna composition are difficult to quantify, and detecting novel invasive invertebrate species is similarly neglected. We need to move towards more complete faunal surveys for restoration islands, so that we can better monitor the outcomes of interventions such as mammalian eradication operations, and to have better baseline data to help detect novel incursions.

This study is the first to examine the ant fauna of Rakitu, and it was conducted six months before rat eradication in winter 2018. The native ant species present on Rakitu are all common species naturally occurring in northern New Zealand. *Monomorium antarcticum* is New Zealand's most ubiquitous native ant species, found throughout the North, South, Stewart, and on many

other offshore islands (Don, 2007). This species is highly variable in size and colour, and comparative analyses of poison gland alkaloids suggest that this is potentially a species complex of at least four species (Don & Jones, 1993; Jones, Stahly, Don, & Blum, 1988). *M. antipodum* is a small non-aggressive species that is probably endemic to New Zealand, and it is widely distributed in the northern South Island and across the North Island. *H. brouni* is a New Zealand endemic, primarily found in the northern North Island and on nearby offshore islands including Great Barrier Island (Don, 2007). Its ecology is poorly known, but it is a native forest dweller nesting in soil under leaf litter and in rotting logs. The *Austroponera* sp. found on Rakitu Island was not identified to species level, as there is considerable morphological overlap between *A. castanea* and *A. castaneicolour* (Don, 2007). The COI sequence published here will potentially help elucidate the identity of this species once more genetic resources become available for this genus. Both of these *Austroponera* species are common across the northern South Island, the North Island, and on many offshore islands, and are both endemic to New Zealand.

None of the introduced species are known to be major pests – though the ecological effects of all of these species have yet to be properly described. Importantly, we found no evidence of the invasive Argentine ant (*Linepithema humile*), though as populations of this species can be highly localised we cannot fully rule out its presence somewhere on the island. The most common introduced ant on Rakitu was *T. grassii*, which originally came from South Africa and was first recorded in Auckland in 1958 (Brown, 1958). It is now very common across Auckland and Northland. Don (2007) describes it as "a mild-mannered ant similar in appearance and behaviour to *M. antarcticum*". Currently these two ant species appear to co-exist on Rakitu Island, despite a probable similar niche. *Ochetellus glaber* is an Australian import, and though its impacts on native habitats are unknown it is not considered a major pest. It is commonly collected along the margins of forest and scrub around the northern North Island – particularly around Auckland (Don, 2007). *Iridomyrmex suchieri* is an Australian endemic which prefers open ground, and it may have the potential to displace other native ants due to its aggressive nature. Again, little is known about the potential impact it could have on native habitats and ant species assemblages.

Both *Iridomyrmex suchieri* and *Ochetellus glaber* were only found in one location each, and both of these locations were close to the buildings in Arid Cove indicating a possible recent invasion. In particular the *Iridomyrmex suchieri* was associated with recent human activity, being found among imported wood below the occupied house. This pattern means that both of these species may have yet to spread to their potential range on the island. Further monitoring of their populations and spread may be warranted.

The barcoding COI sequences generated in this study will be useful for future ant surveys in New Zealand. *Monomorium antarcticum*, *M. antipodum* and *Ochetellus glaber* already had published COI sequences on GenBank (with vouchers most closely matching our sequences: 94-99% identity). Of these three ant species, *M. antarcticum* was the only one that had an identity to the published sequence of less than 98% – it was 94%. Generally ant species have >98% identity at COI within the species. This result therefore reinforces the belief that there are multiple cryptic species within the current circumscription, therefore this species requires further taxonomic work. The COI sequence available on GenBank for *M. antarcticum* (GenBank # KJ847471.1) is from a PhD thesis that does not give the location, a picture of the specimen, or description of the morphology of the individual sequenced (Sparks, 2015). The author does however note the high probability of multiple species within the currently circumscription of the species as suggested by Don & Jones, 1993, and Jones et al., 1988. Sequences closely matching (99% identity) our *Austroponera* sp. sequence have been recorded from the nearby island of Te Hauturu-o-toi (Little Barrier Island) in eDNA samples of soil insects (which had not been identified to species level but are likely to be the same species) (Drummond et al., 2015). There were no published COI barcode sequences for *Tetramorium grassi* or *Iridomyrmex suchieri*, though sequences from close relatives (*Iridomyrmex anceps* and *Tetramorium humbloti*) were available to help confirm genus identification.

This ant faunal survey should be regarded as preliminary, and it is likely more species occur on the island than were documented. Four of the recorded species were only recorded from one location each – highlighting the low chance of detection and/or patchy distribution for many species. Ant species can competitively exclude each other from food resources, therefore only the most common or dominant species may be recorded at baited stations (Stringer, Suckling, Mattson, & Peacock, 2010). This may have occurred in our study, with *Tetramorium grassii*, *Monomorium antarcticum* and *Austroponera* sp. all potentially excluding other species from the baited

rat traps. While these three species dominated the rat-trap sampling, they also dominated the pitfall trapping, increasing the evidence that they are among the most common ground-dwelling ants on the island.

Ideally, standardised sampling using hand searches and litter sampling with Tullgren funnel extraction would have complemented the bait stations and pitfall traps. However, as this study was undertaken opportunistically while focusing on other biodiversity monitoring, this more thorough survey was not conducted. Future studies on the ant fauna of Rakitu should employ these more defined survey techniques.

With the imminent eradication of rats from Rakitu, biosecurity measures must be implemented and maintained in order to prevent mammalian reinvasion. This increased biosecurity should extend to invasive invertebrate species in order to maximise the potential to restore the island's ecosystems. Along with better biosecurity at ports of origin, detection devices should be maintained in Arid Cove and around the buildings in order to detect novel ant species incursions, enabling these to be eradicated before they spread.

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Authors

Andrew Veale is a Senior Lecturer in the Environmental and Animal Sciences pathway at the Unitec Institute of Technology, Auckland, New Zealand.

Thomas Bodey is an honorary academic in the School of Biological Sciences, University of Auckland, and a postdoctoral researcher in Biosciences at the University of Exeter, UK.

Erin Doyle is a Research Associate in the Applied Molecular Ecology Laboratory in the Environmental and Animal Sciences pathway at the Unitec Institute of Technology.

Jo Peace is an Ecological Technician in the School of Biological Sciences, University of Auckland.

James Russell is an Associate Professor in the School of Biological Sciences and Department of Statistics at the University of Auckland.

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TE WHARE WĀNANGA O WAIRAKA

Some like it hot, but moth plant does not: The effect of commercial composting on moth plant (*Araujia hortorum*) seed viability

Sarah Killick and Dan Blanchon

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Contact:
epress@unitec.ac.nz
www.unitec.ac.nz/epress/
Unitec Institute of Technology
Private Bag 92025, Victoria Street West
Auckland 1142
New Zealand

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Some like it hot, but moth plant does not: The effect of commercial composting on moth plant (*Araujia hortorum*) seed viability

Sarah Killick and Dan Blanchon

Abstract

Invasive plants threaten native biodiversity and ecosystem structure and function. Although the removal of invasive plant material is important for the conservation of native plant communities, the disposal of live seeds and propagative material can assist the spread of the invader. Commercial-scale composting windrows can reach temperatures sufficient to render weed seeds unviable, but research has shown that results vary intraspecifically. Here we examine the effects of commercial composting on the viability of the invasive vine moth plant (*Araujia hortorum*). Moth plant seeds were subject to preliminary viability tests to evaluate background viability and to allow post-composting comparison. Mature pods were then buried in a commercial composting windrow for 33 to 99 days, and assessed for viability by tetrazolium assay and germination trials. We further examined the minimum temperature and exposure time required to kill seeds using incubation and water-bath experiments. Background seed viability was estimated at 99%. After composting in a windrow with a mean temperature of 59°C, seeds were no longer viable. Exposure to temperatures of at least 55°C was lethal to hydrated moth plant seeds in laboratory experiments; however, dry-incubated seeds were substantially more resilient. Overall the findings of this study suggest that large-scale composting windrows maintained above 55°C are an effective and reliable method for the disposal of moth plant pods.

Introduction

Invasive plants are a major threat to native biodiversity (van der Wal et al., 2008; Wardle & Peltzer, 2017), especially on islands and isolated habitats where native taxa are less resilient to change (Sala et al., 2000). Invasive plant establishment can affect plant community composition and structure, native animal populations, soil characteristics and fire regimes (Brooks et al., 2011; Pyšek et al., 2012). These changes are typically difficult to reverse and increase ecosystem vulnerability to further invasion, creating a positive-feedback cycle (Gaertner et al., 2014).

Inappropriate disposal of invasive plant material in garden and other green waste is a key human-mediated dispersal mechanism (Gill & Williams, 1996; McWilliam, Eagles, Seasons, & Brown, 2010). Dumped green waste can contain whole plants, plant fragments and seeds (Esler, 1988), all of which can establish along the edge of forests and wetlands (Sullivan, Timmins, & Williams, 2005; Foxcroft, Richardson, & Wilson, 2007; McWilliam et al., 2010). However, to effectively dispose of pest plants from ecological restoration sites, parks and residential gardens, it is necessary to have somewhere for the plant material and propagules to be contained and ideally destroyed. Burial nearby or transport to a landfill for deep burial are common practices (Kollmann, Brink-Jensen, Frandsen, & Hansen, 2011; Hansford, 2015), but with both of these methods there is a risk of the plant material being disturbed and spread to form new invasion sites (Kollmann, et al., 2011; Plaza, Speziale, & Lambertucci, 2018), or spread during transport (Gill, Graham, Cross, & Taylor, 2018).

Composting provides an attractive alternative to landfill disposal. In compost windrows, heat-tolerant microbes break down organic matter and release heat as a metabolic by-product. The high temperatures

Figure 1. Dehisced moth plant pod showing seeds.

and microbial activity transform green waste into a commercially tradeable garden medium. Windrows maintained at 55-60˚C are lethal to most seeds and propagative material (Grundy, Green, & Lennartsson, 1998; Meier, Waliczek, & Abbott, 2014), allowing some weeds to be composted without risk of further spread in contaminated compost. However, responses vary greatly interspecifically; for example, *Setaria faberi* R.A.W.Herrm. seeds are rendered unviable in compost at ≤ 45°C (Eghball & Lesoing, 2000), whereas the minimum lethal temperature for *Polygonum scabrum* Moench is 66.3°C (Larney & Blackshaw, 2003). In another study, water hyacinth (*Eichhornia crassipes* (Mart.) Solms) seeds retained 100% viability after 6.5 months at 40-57°C (Pérez et al., 2015).

Here, we investigate the response of moth plant (*Araujia hortorum* E.Fourn.) to composting conditions. Moth plant is a perennial climbing vine native to Southeast America (Coombs & Peter, 2010), introduced to New Zealand as an ornamental in the 1880s (Webb, Sykes, & Garnock-Jones, 1988). Moth plant has become fully naturalised in the North Island (Hill & Gourlay, 2011), and is now listed on the National Pest Plant Accord (NPPA) as an Unwanted Organism (Department of Conservation, 2001; NPPA 2010). In its exotic range, moth plant is ecologically harmful: the vine is known to climb and smother native vegetation (Environmental Protection Authority, 2015; Hill & Gourlay, 2011), and the abundant trumpet-shape flowers trap and kill pollinating invertebrates (Coombs & Peter, 2010). Moth plant produces a large number of windborne seeds (Elliott et al., 2009) (Figure 1), which are toxic to birdlife (Hart, 1940). Viability analysis of a closely related moth plant in Australia (*Araujia sericifera* Brot.) suggests very high (99.5%) seed viability (Vivian-Smith & Panetta, 2005). The purpose of this study is to determine whether moth plant seed viability is eliminated under temperatures experienced in a large-scale compost windrow, and to investigate the value of commercial composting as a disposal method for this weed.

Figure 2. Windrow at Living Earth.

Methods

Mature *Araujia hortorum* fruit were collected from a minimum of 18 plants at Mount Albert (36°53'03"S, 174°42'56"E), Henderson (36°52'27"S, 174°37'40"E) and Mangere (36°58'10"S, 174°47'37"E) in Auckland during May 2016 for the compost treatment trial and in August 2018 for the *ex-situ* laboratory trials. Background viability levels were estimated using a 1% 2,3,5-triphenyl-tetrazolium chloride (TTC) assay as described by Baskin and Baskin (2001).

Compost windrow experiment

This trial was conducted at Living Earth composting facility on Puketutu Island, Auckland (36°58'07"S, 174°44'57"E), from July to October, 2016. Windrows measuring 100-150 m in length were constructed with a variety of plant matter ('greenwaste'), including grass clippings, small branches, leaves and shrubs. The windrows were regularly monitored for temperature, moisture content and oxygen content during the 100-day composting process, and turned when necessary to maintain temperatures over 55°C.

Breathable mesh bags containing 15 mature whole fruit were buried shallowly (c. 30 cm) in a newly formed compost windrow using methods similar to Tompkins, Chaw and Abiola (1998) and Van Rossum & Renz (2015) (Figure 2). To measure viability loss over this process, bags were retrieved from the windrow at 33, 6, and 99 days from windrow formation. Upon retrieval, 200 seeds were assayed for viability using a 1% TTC assay. The remaining seed material was sown into seed-raising mix and maintained at 20°C under growth lights (CFL 150W 6500K blue bulbs) to monitor germination success, determined by cotyledon emergence.

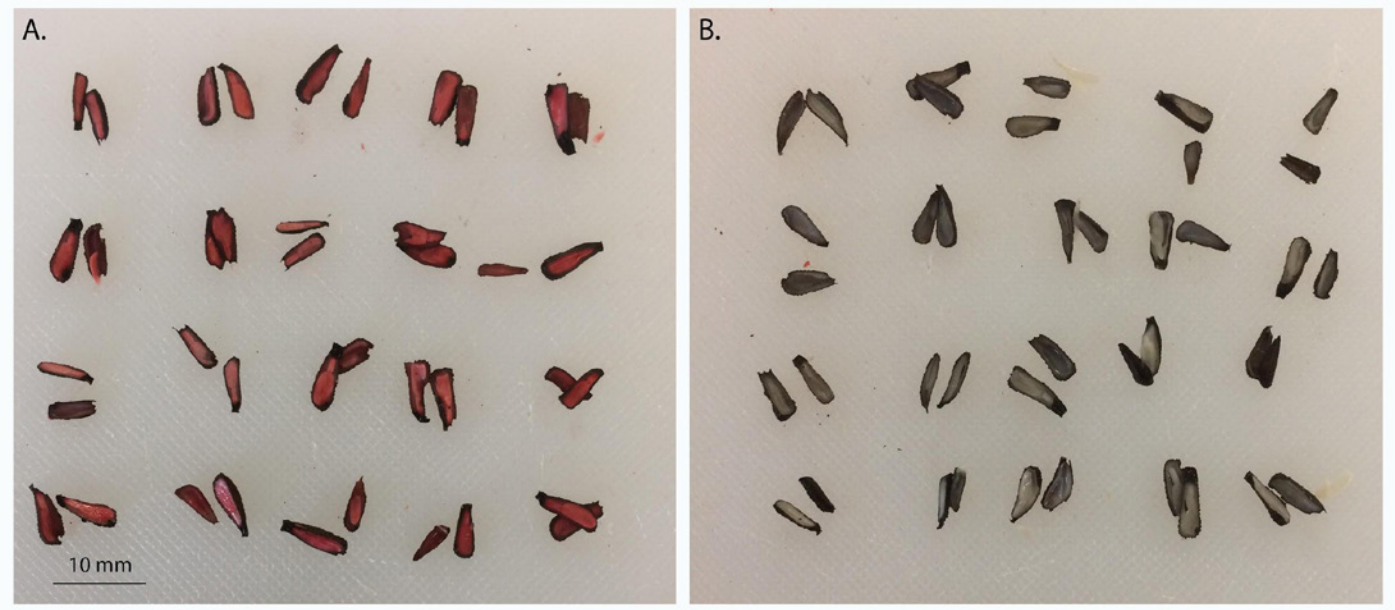

Figure 3. Results of tetrazolium trial: (A) Moth plant seeds after one hour of incubation at 45°C, (B) moth plant seeds after four hours of incubation at 55°C. Viable seeds are stained red.

Seed thermal tolerance

In a follow-up experiment, the minimum lethal exposure time to temperatures recorded in the compost windrow was assessed under controlled laboratory conditions. Seeds were removed from their pods prior to the heat treatment due to equipment size limitations. To test whether the wall thickness of whole pods, as used in the compost windrow experiment, protects seeds from thermal damage, whole fruit were submerged in 61.5°C (±.1°) water for one hour. Upon retrieval, internal temperature was immediately recorded using a probe-style digital thermometer. Individual fruit length, diameter and thickness was measured, and compared with internal temperature readings. Before analysis, temperature data were assessed for normality with a Shapiro-Wilk test. The relationship between fruit size and the internal fruit temperature was assessed by Pearson correlation.

Rate of seed viability loss under moist or dry conditions was assessed with an incubation experiment. Moth plant seeds were placed in glass beakers, either alone or with moist potting mix, and covered with aluminium foil to prevent moisture escape. The seeds were then incubated at the mean temperature recorded in the windrow experiment (59°C) for up to five hours, with one beaker of each treatment type retrieved every 30 minutes. Seeds were then tested for viability with a TTC assay. A Pearson's correlation test was computed for dry and hydrated seeds to estimate and compare the strength of relationship between seed viability and incubation time under both treatments.

Seed tolerance to lower temperatures (40-55°C) was assessed to estimate minimum lethal temperature. Watertight bags containing twenty *A. hortorum* seeds in moist potting mix were submerged in water baths, rather than an incubator, to allow for four different temperatures to be tested simultaneously. One bag was removed from each water bath per hour for five hours. Following removal, seeds were assessed for viability using a TTC assay. The relationship between seed viability, temperature and time spent in the treatment was estimated with a two-way ANOVA, and single relationships were measured with a Pearson product-moment correlation test.

All statistical analyses were undertaken using R version 3.4.1 (R Development Team, 2017).

Results

Background seed viability

Fresh *A. hortorum* seed viability was very high (99%, n = 250); all non-viable seeds lacked embryo and endosperm presence, and were likely aborted due to pollination failure.

Compost windrow experiment

Onsite windrow monitoring conducted by Living Earth recorded temperature fluctuations between 40-70°C, with a mean temperature of 59°C (SEM = 0.85). Fruit

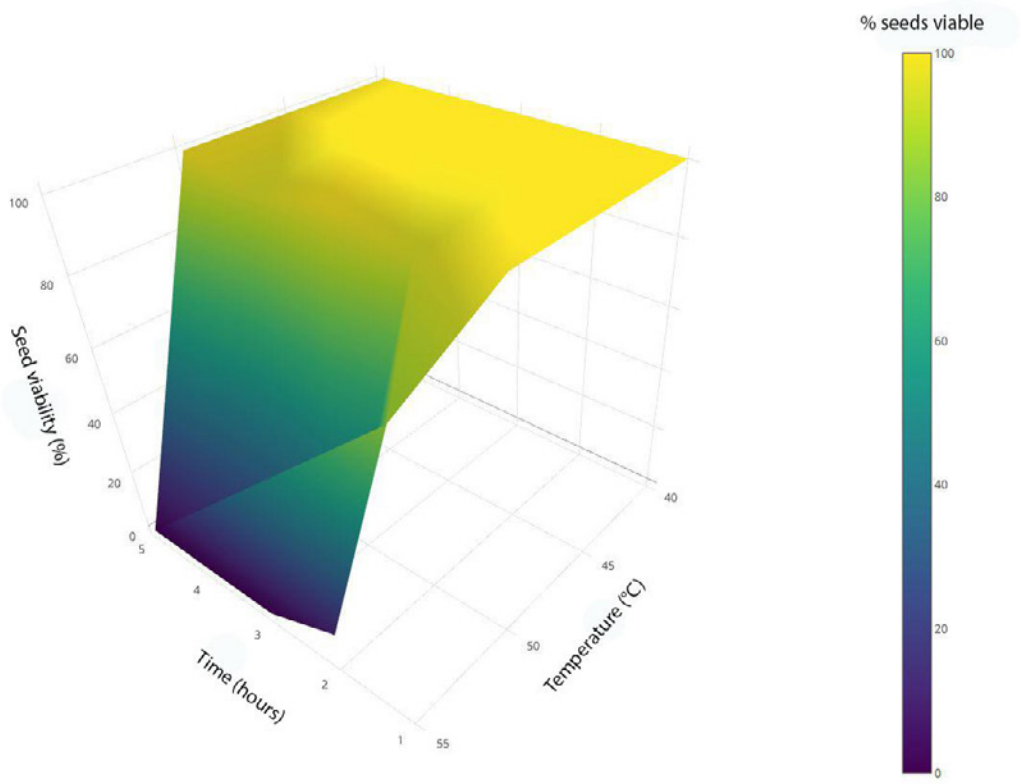

Figure 4. Moth plant seed viability (%) after exposure to 40-55°C temperatures over time.

retrieved at 33 days had disintegrated substantially. The seeds were found to be 3% viable by the tetrazolium assay, although none germinated. No viable or germinable seeds were identified after 66 or 99 days' composting.

Seed thermal tolerance

Moth plant pods submersed in 61.3-61.6°C water had an average internal fruit temperature of 59.3°C after one hour. The temperature data were normally distributed (W = 0.8), and no correlation was identified between internal temperature and fruit length (r = .227, p = .528), diameter (r = .231, p = .521) or thickness (r = .016, p = .965). It was therefore considered acceptable to continue the subsequent experiments on moth plant seeds removed from their pods.

The primary incubation trial subjected moth plant seeds to moist or dry incubation at 59°C for up to five hours. A moderate but statistically significant relationship between incubation time and seed viability was observed in the dry incubated seeds (r = -.68, p = .03); however, over 70% of dry seeds retained viability at all test periods throughout the five-hour period. Seeds incubated in moist soil had a strong and significant negative relationship (r = -.86, p = .001), and had lost 90-100% viability within 2.5 hours. Seeds were consistently unviable after five hours of 59°C moist incubation.

The minimum lethal temperature was recorded at 55°C amongst seeds exposed to three or more hours in the water-bath experiment. Seed viability was moderately but inconsistently reduced in the 50°C treatment group (85% viability after two and four hours). No effect on viability was observed over the five-hour period from submersion in 45°C. A two-way ANOVA confirmed strong and significant coupling between temperature and seed viability (p = < .001), but not between incubation time and seed viability (p = .334). Among the 55°C treatment group only, a Pearson correlation identified a strong but insignificant negative correlation between incubation time and seed viability (r = -.7661, p = .131) (Figures 3 and 4).

Discussion

In this study we examined the effect of commercial composting on the viability of moth plant seeds. Our *in situ* composting experiment demonstrated that commercial composting can effectively kill moth plant seeds over a one-to-two-month period. Under controlled laboratory conditions, the critical lethal temperature for hydrated moth plant seeds was identified as 55°C. Seeds exposed to the same temperatures without moisture remained viable, suggesting that *ex situ* heat-tolerance experiments also need to simulate compost moisture levels to accurately predict seed compostability. Although our laboratory trials used only seeds removed from the fruit, a small water-bath experiment indicated that moth plant fruit does not protect the seeds from external heat. This also suggests that entire pods may be composted safely without splitting.

To our knowledge, this study is the first to report the effects of commercial composting on moth plant seeds; however, our results are consistent with those reported for other weedy taxa. Eghball and Lesoing (2000) noted that composting systems that reach at least 60°C rapidly kill weed seeds of northern temperate herbaceous annuals (Nebraska, USA), and that lower temperatures can also be lethal under moist conditions, possible due to 'compost phytotoxins'. Larney & Blackshaw (2003) found that in windrows of barley straw and cattle manure, temperatures lethal to weed seeds (grass and broadleaf weeds from Alberta, Canada) ranged from 39° to > 60°C. Similarly Tompkins et al. (1998) found that a range of weed seeds of twelve herbaceous weeds from Alberta lost viability after four weeks at temperatures of 55-65°C in a composting system composed of cattle manure and bedding. Conversely, Thompson, Jones and Blair (1997) suggest that compost holding a temperature of 65°C is not likely to be an effective method of weed control as *Rumex obtusifolius* L. seeds retained viability after exposure to 65-81°C. However, as Grundy et al. (1998) argue, seed survival in the Thompson et al. (1997) trial may be attributable to a lack of moisture in the heat treatment. This was also evident in our study, where moist incubated seeds were substantially less resistant to high temperatures, likely due to the greater thermal conductivity of water relative to air (Hillel, 2004). Dahlquist, Prather and Stapleton (2010) found that in laboratory experiments on the seeds of barnyard grass, London rocket, common purslane, black nightshade, annual sowthistle and tumble pigweed, temperatures of 50°C were lethal, but the critical lethal temperature for some of the species varied from 39-50°C. Meier et al. (2014) tested the effectiveness of composting on killing weed seeds of a range of aquatic/wetland plants in Texas (Giant reed, *Arundo donax* L.; hydrilla, *Hydrilla verticillata* (L. f.) Royle; water hyacinth, *Eichhornia crassipes*; water lettuce, *Pistia stratiotes* L.) and found that temperatures of 57.2°C were required to effectively kill all the plant material (including seeds). Similar results (57°C) for water hyacinth were reported by Montoya et al. (2013).

Windrow temperature can vary depending on the ambient temperature (Tirado & Michel, 2010), the size of the windrow (Tirado & Michel, 2010), the composition of the composting material (Van Herk et al., 2004), and whether the compost has recently been turned (Joshua, Macauley, & Mitchell, 1998). Joshua et al. (1998) found that in an Australian windrow composting system temperatures ranged from 17.6-72.8°C, but 55-70°C was more normal in the inner zone of the windrow, particularly after turning. Home composting systems can achieve 55°C, but there can be difficulty in reaching or maintaining this temperature due to their smaller mass, which is particularly problematic in colder climates (Arrigoni, Paladino, Garibaldi, & Laos, 2018). In Palmerston North, New Zealand, Mensah (2017) reported temperatures of up to 53°C in domestic composting systems, but observed that most compost units failed to exceed 45°C. This suggests that home composting systems may not be appropriate disposal solutions for moth plant pods without pre-treatment. One possible solution is to treat moth plant pods with immersion in hot water at above 55°C for four or more hours prior to composting.

For future studies, it is interesting to note the discrepancy between the TTC results and germination success rate during the compost windrow experiment. The TTC assay is widely used to estimate seed viability, and is accepted to be in close agreement with germination response (Soares, Elias, Gadotti, Garay, & Villela, 2016). The solution reacts with dehydrogenase enzymes found in respiring tissue to produce formazan, which stains the respiring tissues red. TTC solution has also been successfully used to detect bacterial activity (Moussa, Tayel, Al-Hassan, & Farouk, 2013), but is a poor detector of fungal colonies (Praveen-Kumar & Tarafdar, 2003). We suggest that our initial compost windrow TTC result was a false-positive result, as evidenced by the negative germination response, and offer bacterial activity as a potential explanation.

Conclusions

Overall, this study demonstrates potential value in commercial-scale composting as a disposal tool for moth plant pods. It is important to note that the field portion of this study was conducted at a single large-scale composting facility, and the laboratory experiments were designed to measure seed viability loss over short periods of time. Further research is needed to determine whether longer exposure to temperatures below 55°C is fatal to moth plant seeds before generalised recommendations on the use of smaller-scale domestic composting systems at lower temperatures can be made. For this reason it is only recommended that moth plant pods are disposed of at compost facilities meeting the New Zealand Composting Standard (NZS4454), which requires windrow temperature to be maintained at ≥55°C for ≥15 days.

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Authors

Sarah Killick is a doctoral candidate in the School of Environment, University of Auckland, and a Research Assistant in the Herbarium at Unitec Institute of Technology, Auckland, New Zealand.

Dan Blanchon is a botanist and Head of Environmental and Animal Sciences, Unitec Institute of Technology.

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