The potential of acquired tolerance of *Onthophagus taurus* to Abamectin LV and/or Combitak

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beetle species: *Onthophagus taurus*
trial dates: March 2022 – March 2023
population 1: Portland region (Vic) - exposed
population 2: Bungendore region (NSW) – naïve

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O. taurus (male)

1. Introduction

The Glenelg-Hopkins Catchment Management Authority comprises an area of around 2.6 million hectares with the dominant land use, accounting for over 2 million hectares, being grazing on dryland pastures. Meat and Livestock Association (MLA) figures show the region supports the nation's second largest second sheep flock (@4.8 million head) and the ninth largest cattle herd (@0.8 million head). Drenching to control internal parasites is a necessary and widespread activity but some of the chemicals in the drenches can have a negative impact on dung beetle fauna. This is especially true for the macrocyclic lactones (ML) in the avermectin subclass, such as ivermectin (Appendix A), that have been shown to be toxic to the larval stage of dung beetles in the subfamily *Scarabaeinae*,[1,2,3] which accounts for most of the economically important dung beetle species. Another ML in the milbemycin subclass, moxidectin (Appendix A), has proven safe for dung beetles.[3] Despite this known toxicity there are confounding observations of high abundances of dung beetles on properties with well documented and frequent drenching regimes employing MLs in the avermectin subclass.

We recognise that in any population of organisms, in this case dung beetles, there will be varying susceptibilities towards chemical toxicity. When exposed to a selection pressure those individuals with a higher tolerance will survive and pass on their genes to the next generation, thereby developing a resistant population. This process of selection is a mechanism by which a species can develop resistance to toxic chemicals that they are exposed to and is no different to the acquired resistance that intestinal nematodes develop towards the chemicals in drenches.

This prompted us to ask whether dung beetles, a non-target organism, have developed a tolerance to chemicals used to control parasites. This research project was designed to examine if populations of the dung beetle (*Onthophagus taurus*) collected around Portland (population 1), which had been exposed through multiple generations to dung from animals drenched to treat intestinal nematodes were more tolerant toward these chemicals than *Onthophagus taurus* dung beetles that were sourced from an organic farm near Canberra (population 2), where exposure to drenches through multiple generations had not occurred.

2.0 Materials and Methods

The dung beetle *Onthophagus taurus* (*Scarabaeidae, Scarabaeinae*) was first introduced into Australia by CSIRO as a part of the Australian Dung Beetle Project in February 1975 with strains selected from Spain, Italy, Greece and Turkey.[4] The species was selected for this study due to its widespread geographical occurrence across southern Australia including high abundance at Cashmore Park, near Portland, Victoria (Figure 1). The insect is active from late spring to early autumn and coincides well with periods of drenching on the property.

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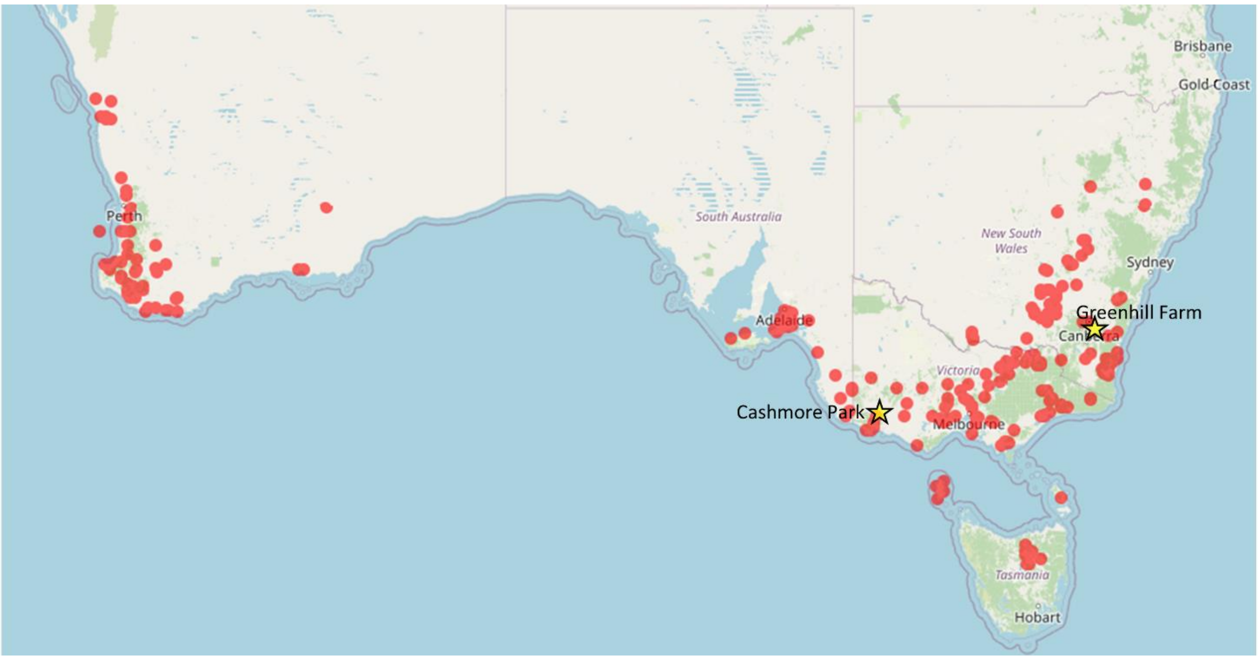

Figure 1: Range of the introduced dung beetle Onthophagus taurus (adapted from data sourced from the Atlas of Living Australia)

2.1 Drenching practice at Cashmore Park, Victoria.

Cashmore Park is a property of approximately 1650 Ha located in the Glenelg-Hopkins Catchment Management Authority region near Portland in south western Victoria. The property carries in excess of 20,000 dry sheep equivalents with a 95/5 sheep:cattle ratio and is well developed with 120 paddocks, extensive laneways and large under cover sheep handling facilities.

The drenching chemicals used in this study are those used in the drenching practices on the property and were sourced directly from the property. The regime employed used a 1:1 (v/v) mixture of Abatak LV and Combitak and has been used on the property for around 15 years. Abatak LV comprises 2 g/L of abamectin and 1 g/L of selenium. Rate of oral application is recommended at 1 mL/10 kg bodyweight. Combitak comprises 34.0 g/L of albendazole (present as albendazole oxide, 36.0 g/L) and 70.0 g/L of levamisole (present as levamisole hydrochloride 82.5 g/L). Rate of oral application for Combitak was also 1 mL/10 kg bodyweight. Adult ewes are drenched on a mob by mob basis at marking, typically mid-September to late October while ewe weaners are typically drenched in mid-November. A summer drench is routinely conducted with faecal egg counts in January determining the timing of the drench, which is often in late February. A final, tactical drench is conducted as required in May. Drenching is done using a 1:1 (v/v) combination of Abatak LV and Combitak at 1 mL/5 kg body weight, set to the heaviest sheep in the mob, with the fattest 5% left undrenched as refugia.[5]

In addition, a small number of cattle were on the property, and they were drenched with Combitak and ivermectin once to twice a year as per veterinary recommendations.

2.2 Mesocosms.

Mesocosms were constructed using plastic containers 250 mm (L) x 160 (W) x 120 mm (H) and were filled a with a moistened combination of sandy loam (3 parts) and grade 1 vermiculite (1 part) to a compressed depth of 100 mm (Figure 2). Lids were drilled with holes (30 x 2 mm) to allow ventilation while retaining beetles.

Figure 2: Example of a triplicate series of mesocosms used in the feeding trials.

2.3 Feeding.

All dung used in the feeding was obtained from cattle that had not been treated with any chemicals for at least 8 weeks prior to dung collection. Cattle dung represented a more convenient dung source and *O. taurus* are known to feed on both dung types. Initially beetles were acclimated in the mesocosm with clean dung for seven days, then over a period of two weeks were fed dung containing various mixtures and concentrations of the drench chemicals before once again being fed clean dung (Table 1, Appendix Tables B1 and B2).

Table 1: Dung – drench feeding combinations

	Additive 1	Additive 2	Additive 3
dung A	-	-	-
dung B	abamectin LV (1 ppm)	-	-
dung C	abamectin LV (1 ppm)	albendazole (1 ppm)	levamisole (2.3 ppm)
dung D	abamectin LV (5 ppm)	albendazole (5 ppm)	levamisole (11.5 ppm)

2.4 Experiments.

Each experiment detailed below was conducted using 10 young adult beetles with a sex distribution of seven females to three males, consistent with the sexual bias observed in the field. Relative age was determined by the lack of wear on the protibial teeth of each insect. Experiments were conducted over the summer of 2022/2023 in two cohorts as adults became available. Population 1 was sourced from Cashmore Park, Vic (-35.260412, 149.495348) (Figure 1) and represented beetles exposed to drench while population 2 represented naïve beetles and consisted of beetles trapped at Greenhill Farm, a certified biodynamic farm near Bungendore, NSW (-35.260412, 149.495348) (Figure 1). Each of the four experiments were performed in triplicate using two populations over 56 days. Throughout the experiment mesocosms were regularly examined to assess beetle health with minimal disturbance to the soil medium. After 56 days the mesocosms were emptied and examined for brood ball production, larvae and the number of adult dung beetles.

Population 1 – Cashmore Park - exposed beetles.
Experiment 1: 10 beetles (7 female and 3 male) selected from population 1 were placed in a mesocosm and fed dung A according to the schedule in Table B1 (see Appendix).

Experiment 2: 10 beetles (7 female and 3 male) selected from population 1 were placed in a mesocosm and fed dung A or B according to the schedule in Table B1 (see Appendix).

Experiment 3: 10 beetles (7 female and 3 male) selected from population 1 were placed in a mesocosm and fed dung A or C according to the schedule in Table B1 (see Appendix).

Experiment 4: 10 beetles (7 female and 3 male) selected from population 1 were placed in a mesocosm and fed dung A or D according to the schedule in Table B1 (see Appendix).

Population 2 – Greenhill Farm - naive beetles.
Experiment 1: 10 beetles (7 female and 3 male) selected from population 1 were placed in a mesocosm and fed dung A according to the schedule in Table B2 (see Appendix).

Experiment 2: 10 beetles (7 female and 3 male) selected from population 1 were placed in a mesocosm and fed dung A or B according to the schedule in Table B2 (see Appendix).

Experiment 3: 10 beetles (7 female and 3 male) selected from population 1 were placed in a mesocosm and fed dung A or C according to the schedule in Table B2 (see Appendix).

Experiment 4: 10 beetles (7 female and 3 male) selected from population 1 were placed in a mesocosm and fed dung A or D according to the schedule in Table B2 (see Appendix).

3.0 Discussion:

All experiments were run in triplicate and consisted of four treatments. These were a control, abamectin spiked dung (1 ppm), abamectin and albendazole/levamisole spiked dung (1 ppm)[§] and abamectin and albendazole/levamisole spiked dung (5 ppm)[**]. All dung was collected from a single collection and stored frozen until required. For calculations and assumptions around drench concentrations used in the experiments see Appendix C.

3.1 Population 1 – Cashmore Park - exposed beetles.

Experiment 1: The beetles continually processed dung (see images in Appendix D). Throughout the experiment during routine inspection did not find any dead beetles. Upon completion of the experiment examination of the mesocosms revealed 35 beetles, five beetles more than the 30 (3 x 10) that were used to commence the experiment. In addition, 11 brood balls were recovered, five of which had beetles eclose while the other 6 were empty.

Experiment 2: The beetles clearly did not like the spiked dung fed to them during weeks 2 and 3 and while feeding occurred it was clearly reduced compared to the control (see images in Appendix D). Across the course of the experiment (8 weeks) 15 (50%) of the beetles died. Despite this high mortality rate 19 live beetles were recovered indicating the eclosure of four additional beetles during the experiment. Seven brood balls were recovered, four had beetles already eclosed and the remaining 3 were empty.

Experiment 3: The beetles did not like the spiked dung and feeding was substantially reduced and did not increase for 2 weeks after the spiked dung feeding had ceased (see images in Appendix D). Across the course

[§] 1 ppm in abamectin and 1 ppm in the albendazole oxide component of Combitak (2.3 ppm in levamisole hydrochloride).
[**] 5 ppm in abamectin and 5 ppm in the albendazole oxide component of Combitak (11.5 ppm in levamisole hydrochloride).

of the experiment 15 (50%) of the initial cohort of beetles died, the same number as occurred in experiment 2. The number of live beetles recovered was 22 indicating seven eclosures. In addition, 13 sealed brood balls were removed upon completion of the experiment, and these contained 11 live larvae, examples are shown in Figure 3. The balls were resealed with moist dung and development continued until beetles eclosed in 10 of the 13 instances.

Figure 3: Examples of larvae, showing various developmental stage, revealed by opening brood balls in experiment 3 - population 1.

Experiment 4: This represented the highest concentration of chemicals in dung and was higher than what a beetle would be exposed to under field conditions. Deterrence to feeding induced by the chemicals was similar to that observed for experiment 3 (see images in Appendix D). Once again across the course of the experiment 15 (50%) of the initial cohort of beetles died. The number of live beetles recovered was 20 indicating seven eclosures. In addition, 9 sealed brood balls were removed upon completion of the experiment, and these contained 5 live larvae. The brood balls were resealed with moist dung and development continued until beetles eclosed in 4 of the 5 instances.

Summary: Drenched dung is clearly detected by the beetles, and they reduce their feeding when it is the only dung present. In a field situation where clean dung may be available it is reasonable to assume beetles actively avoid contaminated dung. Where no choice is available reduced feeding may result in a lower mortality than would otherwise be expected. Experiments measuring the amount of dung processed in various scenarios would be worth undertaking. At no stage were dead larvae identified in any of the brood balls. In some instances, brood balls were empty and this may indicate an issue with egg laying ability but no sign of larvae that failed were observed. Curiously the control experiment produced 11 brood balls but all of them were empty wile Experiment 3 and 4 produced 13 and 9 brood balls respectively containing 9 and 5 larvae. This may indicate that rather than inhibiting breeding the stress caused by the chemicals is stimulating breeding in *O. taurus*.

3.2 Population 2 – Greenhill Farm - naive beetles.

Experiment 1: The beetles continually processed dung (see images in Appendix E). Throughout the experiment upon completion inspections did not find any dead beetles. Upon completion of the experiment examination of the mesocosms revealed 30 beetles. In addition, 17 brood balls were recovered but all of them were empty.

Experiment 2: As was observed in population 1, beetles did not like the spiked dung fed to them during weeks 2 and 3 and while feeding occurred it was clearly diminished compared to the control (see images in Appendix E). Across the course of the experiment (8 weeks) 20 (67%) of the beetles died compared to 15 (50%) in population 1 (Figure 4). There were no emergences during this experiment whereas in population 1 there were four. Eight brood balls were recovered, four were empty and the remaining four contained viable larvae.

Experiment 3: The beetles did not like the spiked dung and feeding was substantially diminished and did not increase for several weeks after the contaminated dung feeding was stopped (see images in Appendix E). Across the course of the experiment, 18 (60%) of the initial cohort of beetles died, slightly more than the 15 (50%) observed in experiment 3 employing beetles from population 1 (Figure 4). The number of live beetles recovered was 12 (40%) with no emergences having occurred from any of the 12 brood balls recovered from the experiment, only one of which was found to contain a live larva, the others being empty.

Experiment 4: This experiment represented the highest concentration of chemicals in dung and was higher than what a beetle would be exposed to under field conditions. Diminished feeding induced by the chemicals was observed (see images in Appendix E). Across the course of the experiment 25 (83%) of the initial cohort of beetles died compared with 12 (40%) of the beetles in experiment 4 that was conducted using beetles from population 1, that had a history of exposure to the chemicals (Figure 4). It is important to note that while this was a trend it was only significant in experiment 4. The differential mortality rate supports the development of an acquired tolerance in beetles from population 1 toward the drench chemicals. Of further interest, 19 brood balls were recovered from this experiment the highest amount recovered from any of the experiments in the trials, and these contained 15 viable larvae. This indicated that while the dung was more toxic and killed more beetles it may have also induced a stress response that resulted in increased breeding.

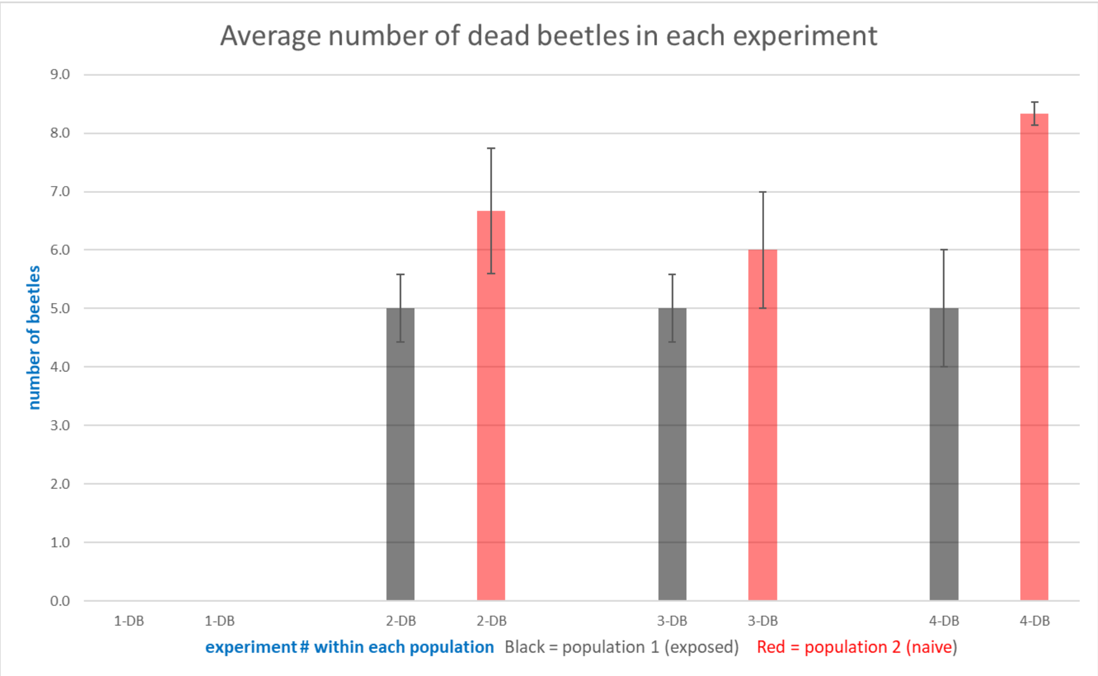

Figure 4: Average number of dead beetles in each of the 3 mesocosms of the four experiments for each population.

Summary: Drenched dung was able to be detected by the beetles and their feeding was diminished when it is the only dung present. In a field situation, where clean dung may be available, it is likely that beetles would actively avoid contaminated dung (see Dung Choice Experiment below).

At no stage, in any of the experiments conducted, were dead larvae identified in any of the brood balls. In many instances, brood balls were empty and this may indicate an issue with egg laying but no sign of larvae that failed to develop were observed. Curiously the control experiment in the naïve population (population 2) produced 11 empty brood balls, while Experiments 2, 3 and 4 produced 8, 13 and 9 brood balls containing 4, 9 and 5 live larvae respectively. This may indicate that rather than inhibiting breeding the stress caused by the chemicals being present in the dung is stimulating breeding in *O. taurus*.

It was notable that when the number of live beetles recovered was added to the number of live larvae recovered in each experiment, giving an indication of total population the exposed population always was always higher (Figure 5). This was significant for experiment 3 where population 1 produced an average of 11 while population 2 only produced 4.3 individuals (beetles + larvae) /experiment. The fact that the experiment 3 population in each mesocosm was equivalent to the control further supports the theory of an acquired tolerance.

3.3 Dung Choice Experiment.

Our observations that dung beetle feeding was diminished when the dung was spiked with the drench chemicals used in our study appeared to contrast with a previous report in which the authors observed increased attractiveness to dung containing avermectins used to bait pitfall traps.[6] The authors indicated that the increased attractiveness was apparent in the higher catch numbers in baited pitfall traps when spiked dung was used and was more pronounced for cattle than for sheep. However, we contend that attractiveness does not necessarily equate to palatability and while interest may be enhanced resulting in a greater number of trapped beetles the question to be asked is, was the dung more palatable?

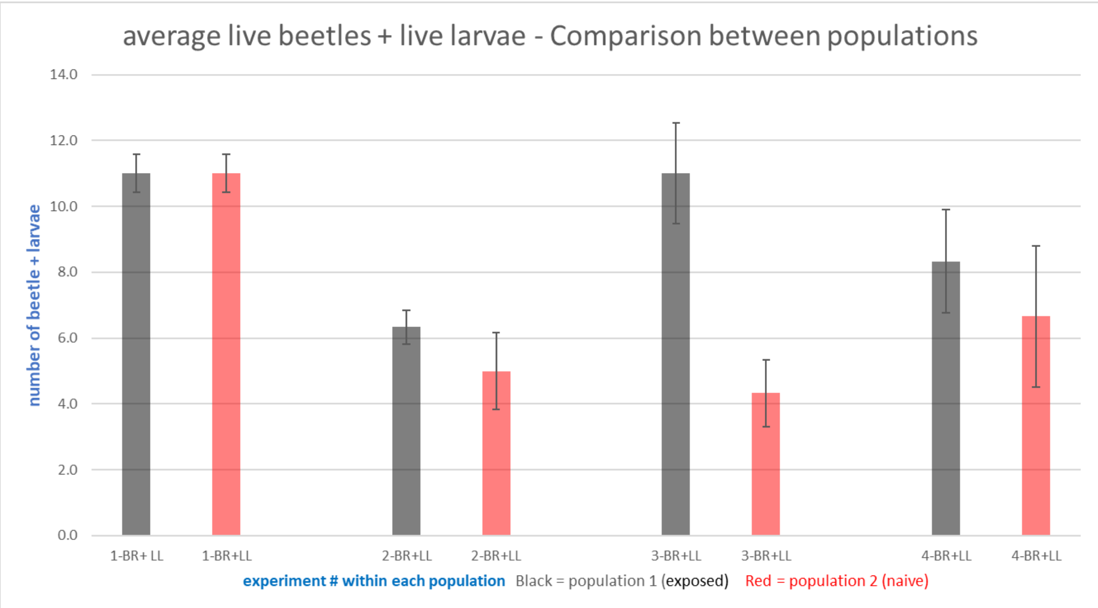

Figure 5: Average number of live beetles plus live larvae in each of the 3 mesocosms of the four experiments.

We presented a simple choice experiment to a cohort of 20 *Onthophagus taurus* dung beetles in which the captive beetles were presented with equal masses of dung differing only in the presence of drench (abamectin, albendazole at 1 ppm and levamisole at 2.3 ppm). The side-by-side choice test was definitive in showing diminished feeding by *O. taurus* on spiked dung with a clear preference toward the clean dung (Figure 6). The experiment was performed in triplicate using beetles from both population 1 and population 2, with identical observations. While not a primary objective of this study the observation has raised the possibility that dung beetles, given a choice, will avoid feeding on dung containing drenches and this phenomenon should be quantitively investigated in a future program and assessed as a potential complement to current dung beetle management strategies on properties where drenching is routine.

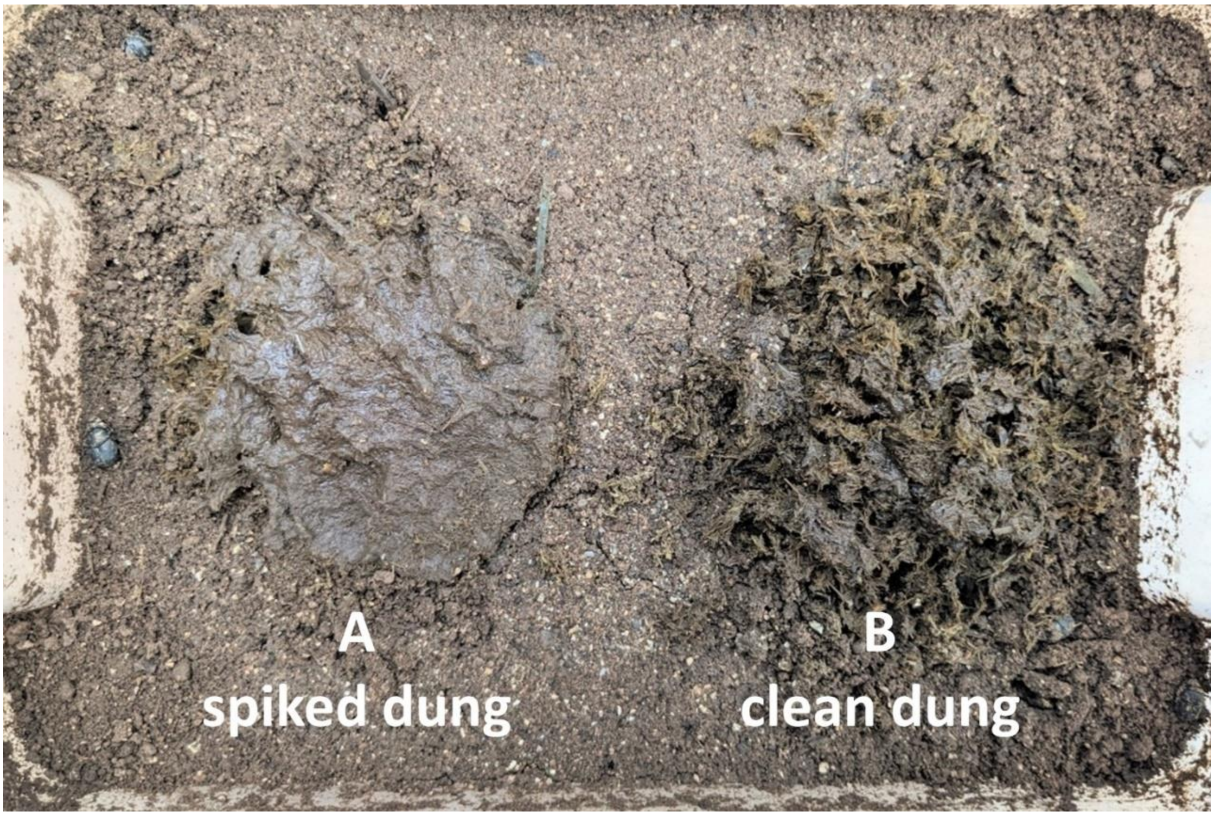

Figure 6: Choice experiment in which 20 beetles were offered 10 g of spiked dung containing *abamectin + Combitak (1 ppm) (A) and 10g of clean dung (B). After 2 days the clean dung had been heavily procressed with minimal feeding on the spiked dung.*

4. Conclusion

The primary objective of this pilot study was to ascertain if there was evidence to support the existence of acquired tolerance towards chemicals used in drenches among a population of *Onthophagus taurus* dung beetles that had been routinely exposed to the chemicals used in this study over a 15-year period (see section 2.1). The single most compelling statistic indicating that drench exposed beetles derived from Cashmore Park (population 1) were more tolerant to contaminated dung is the overall survival of adult beetles.

In all cases the beetles that had been previously exposed to drench chemicals fared better than the naïve population. This is most readily seen when comparing the average number of live beetles recovered between populations which displayed a >60% average overall survival rate compared to a 30% average overall survival rate for naïve beetles (Figure 7). Curiously, the highest rate of drench application (5 ppm) in experiment 4 did not result in a significant increase in mortality with average overall survival remaining relatively constant.

A factor that could contribute to the unexpectedly high population of dung beetles from a property that had continually exposed dung beetles to toxic chemicals is the observation that there appears to be a stress response amongst beetles that stimulates breeding. This phenomenon was most noticeable in experiments 3 and 4 but was sporadic in nature across mesocosms involved in the trials. Future research could design a series of experiments focussed on this question to develop the hypothesis that drench chemicals induce a stress response that increases breeding. Such a study should also examine the beetles that emerge from breeding using drench tainted dung. A previous study using ivermectin indicated that newly emerged *O.*

taurus showed signs of delayed sexual maturation.[7] The current study did not explore sexual maturation of the newly eclosed beetles.

A final finding that arose through observations made in the current study was diminished feeding in both beetle populations when drench contaminated dung was the only food source on offer. This observation was conclusively demonstrated in a simple choice test (Figure 6) and warrants further investigation due the potential to modify grazing management practices to benefit dung beetles.

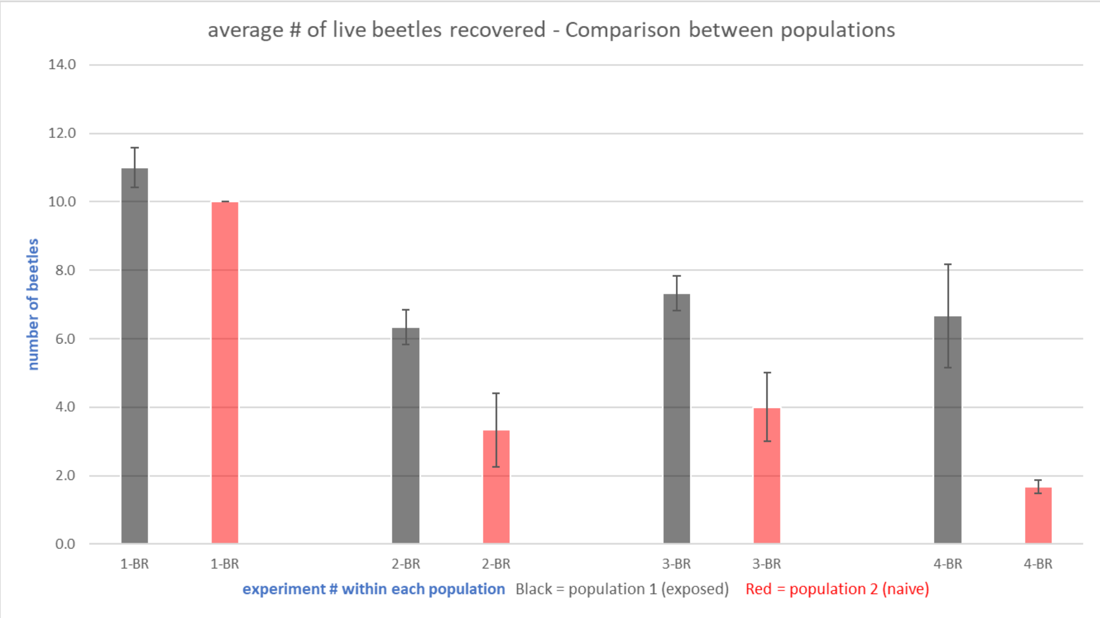

Figure 7: Overall survival of dung beetles. Comparisons between populations in each experiment.

Acknowledgment

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