NON-TARGET EFFECTS OF EXTENDED-RELEASE LONGRANGE® EPRINOMECTIN ON COPROPHILOUS INSECTS

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Abstract

I studied the effect of residues in dung voided by cattle treated with the novel parasiticide Extended-release LongRange® (LR) on dung breeding insects. Field and lab studies over two years showed that residues are excreted for at least 25 weeks post-treatment at levels sufficient to reduce survival of some taxa with detectable effects on total insect abundance, taxa richness, and diversity. Using pitfall traps baited with dung with and without LR residues, I detected an effect of residues on attraction of some species of insects to dung. Results showed no changes in abundance, taxa richness, or diversity, but significant changes for individual taxa, albeit contrasting between years. Taken together, these findings show that cattle treated with LR in the spring will defecate residues at high enough concentrations to significantly affect the dung insect community for the entire duration of the grazing season.

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List of Abbreviations

Symbol	Definition
%	Percent
±	Plus or minus
°	Degrees
°C	Degrees Celsius
ANOVA	Analysis of variance
BIC	Bayesian information criterion
cm	Centimeter
df	Degrees of freedom
e.g.	Exempli gratia (for example)
Et al	Et alia (and others)
EtOH	Ethanol
F0	Parent (first) generation
Fig	Figure
g	Gram
GLMM/GLIMMIX	Generalized linear mixed model
ha	Hectares
i.e.	Id est (that is)
km	Kilometers
L	Litre
lat	Latitude
lbs	Pounds
LeRDC	Lethbridge Research and Development Centre
long	Longitude
Log	Base 10 logarithm
LR	LongRange® eprinomectin
m	Meter
ML	Macrocyclic lactone
mL	Millilitre
n	Sample size
NE	North-east
p	p-value
PSGR	Purple Springs Grazing Reserve
SAS	Statistical Analytic Software
SE	Standard error
Sp	Species
SR	Sustained-release
TFA	Temporary field authorization
VOC	Volatile organic chemical
vs.	Versus

Chapter 1: Literature Review & Thesis Structure

1.1 The Dung Pat & Its Community

Cattle dung is a short-lived, spatially-heterogeneous resource that is frequently abundant on the pasture landscape. A fresh dung pat can contain more than 80% water but is otherwise nutrient-rich with organic material that was undigested by the animal[1,2]. Nitrogen is abundant, primarily in the form of bacteria, in addition to phosphorous, potassium, calcium, magnesium, and other trace elements[3–5]. Dung also contains a plethora of anaerobic microbiota passed from the gut microbiome of cattle and is later replaced by external, aerobic microbiota as the pat is aerated[6].

Although dung pats are not overly large in size, they harbour complex interactions among a diverse community of coprophilous (dung-loving) organisms[7,8]. These organisms include, but are not limited to, arthropods (insects and mites), fungi, microbes, and earthworms – all of which work in conjunction to degrade the pat[7,9]. The insects are mainly represented by the orders Diptera, Coleoptera, and Hymenoptera[7,8]. Floate estimates that there are approximately 300 insect species associated with cattle dung in Canada[7]. However, many of these are considered "visitors" to the pat, rather than "true" coprophagous (dung-feeding) species that require access to dung to complete their development[7]. Fungi and microbes assist in further decomposing organic material, while earthworms feed on the periphery of the pat from the underlying soil[10,11]. Occasionally, dung may also contain the larvae, eggs, or adults of parasitic nematodes, trematodes, or cestodes that are passed from an infected animal or otherwise introduced into the pat[7,12].

Coprophilous insects are attracted to fresh dung in response to the release of volatile organic compounds (VOCs)[13,14]. VOCs are the result of decomposing organic

material that is driven by the activity of microbiota and fungi[15]. Insects are then able to locate dung pats by following plumes of VOCs upwind during flight, even at relatively low concentrations[13]. Sladecek *et al*. identified up to 54 VOCs associated with dung, some of which were common, and others that were more specific to the diet of the animal and age of the pat[16]. Thus, VOCs may further provide information about the type, quality, and age of a dung pat to coprophilous insects, allowing for some choosy foraging behaviour[16–18]. Since the combination of VOCs and the amount released changes as the dung ages and forms a crust, this can influence the order of succession of colonizing coprophilous insects[14–16]. In brief, dipterans are mostly attracted to early stage VOCs (i.e., the first day after excretion) whereas coleopterans are typically attracted to with late stage VOCs (i.e., 2 or more days after excretion)[16].

Dipterans are amongst the first insects to colonize a dung pat, often within minutes of defecation[7,16]. Those that do breed in dung typically prefer a fresh pat for oviposition since the crust that forms on the pat within a few hours is difficult to penetrate[19,20]. A small number of dipteran species are pests of livestock; e.g., face flies (*Musca autumnalis*), stable flies (*Stomoxys calcitrans*), and horn flies (*Haematobia irritans*; Muscidae)[7,21]. However, most other species are beneficial or innocuous. For example, adults of the dung fly *Scathophaga stercoraria* (Scathophagidae) are predators of other dung-breeding insects, including pest flies[22,23]. Other flies may be predators as adults and (or) as larvae, or feed on microorganisms in the dung[7]. Flies common in dung include members of the families Anthomyiidae (flower flies), Calliphoridae (blow flies), Muscidae (muscid flies), Psychodidae (moth flies), Sphaeroceridae (lesser dung flies),

Sepsidae (black scavenger flies), Sarcophagidae (flesh flies) and Stratiomyidae (soldier flies)[7,8].

Coleopterans are also quick to arrive to a dung pat but typically do not reach peak numbers until a few days following defecation[8,24]. Beetles in the family Scarabaeidae (dung beetles) are arranged into different functional groups based on their life-history strategies and reproductive behaviour[9]. Rollers bury dung in which they have laid eggs (i.e., brood balls) in tunnels away from the pat[9]. Tunnellers bury brood balls in tunnels beneath the pat[9]. Dwellers breed and lay eggs directly in the pat in addition to overwintering[8,9,25]. A fourth group, the detritivores, may feed in fresh dung, but typically lay their eggs in organic-rich soils or heavily degraded dung pats[7]. Common species of dung beetles in Alberta include the rollers *Canthon pilularius* and *C. praticola*, the tunnellers *Colobopterus erraticus* and *Onthophagus nuchicornis*, the dweller *Aphodius pedellus*, and the detritivores *Calamosternus granarius* and *Chilothorax distinctus*[7,26,27]. This latter species is often recovered in dung-baited pitfall traps in large numbers late in the fall prior to the first cold snap of the year[28,29]. In Canada, common native species include *C. pilularius, C. praticola, Planolinus vittatus,* and *Pseudagolius coloradensis*[26,27]. Other common species are introduced from Europe[26,27].

Other common coprophilous beetles include species in the families Staphylinidae (rove beetles), Histeridae (clown beetles), and Hydrophilidae (water scavenger beetles)[7,8]. Members of these families are mostly predators of other insects, but some species of Hydrophilidae may additionally feed directly on the dung[7,30–32]. A number of other beetles are common in dung, but are often overlooked because of their small size; e.g., 1–5 mm in length. These include species in families Clambidae (minute beetles),

Cryptophagidae (silken fungus beetles), and Ptiliidae (feather-winged beetles), which are coprophagous or fungivorous[7,8].

Several hymenopteran species also are common in dung[7,33,34]. They are not coprophagous, but are most commonly parasitoids and lay their eggs in the immature stages (i.e., eggs, larvae, or pupae) of other dung-breeding species[33,35]. Numerous studies have investigated the role of these parasitoids wasps as natural enemies of pest flies[33–35]. Some of these parasitoids have even been commercialized as biocontrol agents[33].

1.2 Importance of Dung Insects

Dung insects provide important pasture ecosystem services that include dung degradation, nutrient cycling, and the control of livestock pest insects and parasites affecting livestock[30,36]. The tunnelling and feeding activity by larvae of dweller species of dung beetles promotes microbial activity to accelerate degradation and reduces the pat into small fragments[6,7]. Adults of the tunnelling species *O. nuchicornis* and the rolling species *C. pilularius* can relocate dung into the soil to a depth of 10-20 cm in the form of brood balls[37,38]. This burial activity returns nutrients to the soil and increases soil porosity, which promotes plant growth and water retention[39–41]. Dung burial and fragmentation also have the added benefits of drying out the pat to reduce its suitability as a breeding site for pests and parasites in addition to facilitating removal of the pat as a barrier to plant growth beneath the site of deposition[7,42-45,46].

Recognition of the importance of insect activity on dung degradation is illustrated by the dung beetle introduction program onto Australian rangelands. The native dung insect community was unable to process the excrement of introduced cattle, causing

considerable pasture fouling, nutrients sequestered in hardened dung pats, and an abundant habitat for pest flies[30,47]. This necessitated the introduction of exotic species of dung beetles that could efficiently degrade and process cattle dung[48,49]. In Alberta, similar results were reported by Floate who showed dung pats were mostly untouched after 340 days following exclusion of insects caused by treatment with the parasiticide ivermectin[50]. In comparison, untreated dung pats were mostly degraded after 80 days[50]. Thus, although other factors are involved in dung pat degradation (e.g., rainfall, freeze/thaw cycles, microbial activity), it occurs most efficiently in the presence of a diverse and abundant dung insect community[30,51,52].

Given the importance of coprophilous insects to pasture ecosystems, factors that may alter their ability to colonize and thrive in cattle dung are of particular interest; one such factor is the application of macrocyclic lactone parasiticides (endectocides) to livestock.

1.3 Endectocides & Their Usage

Formulations of endectocides were developed to treat both the internal (endo) and external (ecto) load of parasites in livestock simultaneously[53,54]. To date, the active ingredients of endectocides are restricted to the avermectins (e.g., ivermectin, eprinomectin, and doramectin) and milbemycins (e.g., moxidectin), also known collectively as the macrocyclic lactones (MLs)[55]. MLs were discovered in the late 1960s and are chemical derivatives from fermenting fungi in the genus *Streptomyces*[56,57]. Following their discovery, formulations of endectocides began to enter the market in the early 1980s and are some of the most recent chemical products to be synthesized for use

as parasiticides. To date, endectocides make up the majority of modern parasiticides and are prevalent within the livestock market[58,59]. Studies in the United Kingdom and South Dakota, USA, indicate that most ranchers use pour-on formulations of ivermectin at least once or twice a year, typically in the spring and fall[58,59].

The list of MLs synthesized for use as active ingredients to date is extensive. However, only ivermectin, eprinomectin, doramectin, and moxidectin are registered for use in Canada[60]. The most commonly used formulations of endectocides are topical, also known as pour-ons[58,59]. They are applied externally and absorbed through the skin or ingested when the animal licks itself or other treated animals[61,62]. Boluses, although discontinued on the market, are inserted down the throat of the animal where it is deposited in the rumen for long-term active-ingredient release[62,63]. Finally, injectable formulations are delivered subcutaneously into the muscle (i.e., injected beneath the skin)[62].

The usage of endectocides likely became popularized worldwide due to their broad-spectrum efficacy at low dosages while maintaining low toxicity to mammals[64]. They are effective against a broad range of different species of nematodes, mites, ticks, and pest insects[53,57,65]. Furthermore, depending on the formulation, they can be effective for prolonged periods of time, ranging from weeks to months[66–68]. A sustained-release (SR) bolus formulation of ivermectin conferred protection for cattle up to 135 days from both internal and external parasites[63,69]. Although the SR bolus has been discontinued in the market, it has since been replaced by LongRange® (LR) eprinomectin, a dual-peak, extended-release, injectable formulation[67]. LR confers protection to cattle for up to 150

days post-treatment against pests, the typical duration of a grazing season in temperate zones[70,71].

Livestock treated with endectocides faecally excrete residues that consist primarily of the parent compound with little to no metabolism[54,68,72,73]. Depending on the formulation, animals may excrete residues for up to 6 months post-treatment[74,75]. Typical peak excretion of residues for topical and injectable formulations occurs within the first 7 days followed by a rapid decline in the following weeks[68,74]. In contrast, boluses can persist at higher concentrations in faeces for an extended period of time due to the SR nature of their formulation[63,69,74]. Similarly, chemical analyses of dung from cattle previously treated with extended-release LR documented residues detectable in dung up to at least 20 weeks, with peak excretion at 1 and 12 weeks post-treatment[75].

Faecal residues retain their insecticidal activity which suggests the additional benefit of residues controlling pest species of dung-breeding flies[73]. The results of early studies in the 1980s illustrated how ivermectin residues were adequate to suppress the numbers of horn fly, face fly, and stable fly breeding in the dung of cattle for up to 4 weeks post-treatment; depending on the formulation[73,76]. Further inquiries by Floate *et al*. confirmed pour-on formulations of ivermectin, in addition to doramectin and eprinomectin, were excreted in sufficient concentrations to suppress horn fly and stable fly for up to 5 weeks post-treatment; albeit moxidectin had little to no effect[77]. However, the insecticidal activity of ML residues also raises concerns about their effects on non-pest species[78].

1.4 Non-Target Effects on Dung Insects

The potential for faecal residues of MLs to affect coprophilous insects has been the topic of considerable study. In one of the earliest investigations, residues in dung of cattle treated with an SR bolus formulation of ivermectin exhibited fewer colonizing insects and slower degradation relative to dung from untreated cattle[79]. However, subsequent studies on dung degradation have shown mixed results of either no effect or delayed degradation[50,80–82]. In two studies conducted in Canada, one reported a negative effect of ivermectin residues while the other reported no effect of ivermectin residues on the rate of dung degradation[50,82]. In contrast, the effects of residues on insect activity in dung are much less ambiguous, and, for the purposes of this review, are broadly categorized as lethal, sublethal or altered colonization. Further in depth reviews on non-target effects are provided in Floate *et al*. and Jacobs *et al*.[21,83].

The lethal effects of ML residues are primarily of concern to insects whose larvae feed on dung, although cases may also be cross-trophic for predatory or parasitoid species[84]. Broadly, residues have been shown to be highly toxic to dung-breeding insects[83,85]. However, the strength of these effects and their duration is almost entirely dependent on the active ingredient (doramectin > eprinomectin ≅ ivermectin > moxidectin) and their formulation (bolus > injection > pour-on), respectively[82,83]. Furthermore, not all taxa of dung breeding insects are equally susceptible to residues. The most common example used in the literature, although perhaps over simplified, suggests that larvae in the dipteran suborder Cyclorrhapha are more susceptible to residues than those in the suborder Nematocera[50,86,87]. Even within more specific taxonomic groups, such as the Aphodiine beetles (Coleoptera: Scarabaeidae), individual species can vary in

their susceptibility[50,82,88,89]. In the case of predatory and parasitoid species, insects are feeding on the immature stages of coprophagous insects instead of the dung itself[7]. In the event that the abundance of the latter is reduced due to the toxicity of residues, the former will likely also exhibit a decrease; albeit not necessarily directly caused by residue toxicity[26,73,78]. Some studies have investigated the toxicity of residues on coprophagous adults although most conclude that residues are non-lethal to fully mature adults[90–92]. However, Wardhaugh *et al*. showed that newly emerged adults of *Onthophagus taurus* experienced a higher mortality rate when fed dung collected 3 days following treatment of cattle with a pour-on formulation of eprinomectin compared to untreated control dung[92].

Although juvenile insects may survive development in dung containing ML residues, sub-lethal effects can arise for the duration of all their life stages. Larvae that are reared, and adults which feed, on contaminated dung can experience effects such as slower development, reduced fecundity, and locomotory disorders; most of which are documented for species in the family Scarabaeidae[21]. Krüger & Scholtz showed the slower development of *Euoniticellus intermedius* and *Onitis alexis* reared in dung collected up to 4 and 3 weeks post-treatment with ivermectin, respectively[93]. Although no developmental delay occurred for *Musca nevelli* (Diptera: Muscidae), adults reared on dung collected 5 weeks post-treatment with ivermectin exhibited up to a 60% reduction in fecundity[94]. O'Hea *et al*. and Wardhaugh *et al*. showed similar effects on *Aphodius rufipes* and *O. taurus*, respectively, with slowed development in addition to reduced egg clutch size resulting from adults reared from, or fed dung containing residues[91,92]. Finally, in a recent study by Verdú *et al*., adults of *Scarabaeus cicatricosus* (Coleoptera:

Scarabaeidae) fed dung containing low concentrations of ivermectin residues exhibited reduced olfactory and locomotory function during foraging tasks[95]. Thus, although not always lethal, residues may have persisting effects on dung insects who consume them at any life stage.

Finally, the non-target effects of ML residues present in dung may indirectly alter rates of insect colonization, as caused by changes in attraction to dung. However, there is little consensus in the literature regarding the nature of the effects. Although many taxa of coprophilous insects have shown preferences for dung that does, or does not, contain residues, the results have been inconsistent relative to the active ingredients, the formulation, the region, the season, and even between consequent years[96,97]. In a study by Floate, different pour-on formulations containing one of four different MLs yielded contrasting results, even for trials using the same product in subsequent years[97]. For example, *Aphodius erraticus* (now *Colobopterus erraticus*) was repelled by dung containing residues of ivermectin in one year, but attracted to dung containing residues of the same product in the following year[97]. Furthermore, response can be altered by the concentration of residues excreted in dung, not just their presence or absence[69,96–98]. Although no studies have addressed the underlying mechanism of how residues alter the attractiveness of dung, VOCs are largely the topic of speculation as they have previously been shown to be responsible for dung insect attraction in cases where residues are not present[16,99].

1.5 Thesis Structure

In the following two chapters, I evaluate the non-target effects on coprophilous insects associated with the application of LongRange® eprinomectin (LR) to cattle. LR is a novel formulation of eprinomectin for which the non-target effects of residues in dung of treated animals are of particular concern. Animals treated with LR excrete a peak concentration of residue in their feces approximately 1 week post-treatment and a second, reduced peak approximately 12 weeks post-treatment[75]. Only Nieman *et al*. have investigated the non-target effects of faecal resides associated with use of this product on dung-breeding insects[75]. Although they reported high interspecific variability in susceptibility to LR residues, these chemicals tended to cause an overall suppression in dung insect emergence (i.e., reductions in abundance, richness, and diversity) up to 20 weeks post-treatment[75].

In Chapter 2, I examine the toxicity of LR residues on dung-breeding insects in both a field study and a companion lab study utilizing house fly larvae (*Musca domestica*). In the field study, I artificially molded pats using dung that was collected at specified intervals from cattle treated previously with LR. These pats were subsequently colonized naturally by local dung insects. This study was done in part to validate results by Nieman *et al*. but primarily to expand upon their findings[75]. Due to Nieman *et al*. reporting suppression of insects up to 20 weeks post-treatment, I extended this range up to 25 weeks post-treatment to better estimate how long effects may persist. In addition to the field study contributing results from a unique region, and thus a unique community of taxa, the experiment was conducted in each of two years; once in the spring of 2019 and once in the fall of 2020. This contributed further understanding of the susceptibility of

changing insect communities across different seasons. Finally, a companion lab bioassay was completed in one year utilizing known numbers of house fly larvae. This laboratory approach removed uncertainty surrounding unknown colonization rates in the field study and allowed results to be clearly associated with the toxicity of residues. Furthermore, no results of bioassays on non-target insects have been reported in the literature to date for LR. Thus, this report will serve as a foundation for future lab bioassays using LR.

In Chapter 3, I examine the effects of LR residues on the attraction of coprophilous insects to dung-baited pitfall traps. The results of this chapter provide the first test of the effects of LR residues on rates of dung insect colonization. To accomplish this, I compared catches of insects using dung-baited pitfall traps with high (1 week post-treatment), intermediate (8 and 12 weeks post-treatment), or low concentrations (16 weeks post-treatment) of LR residues compared to pitfall traps baited with control dung (untreated). Ideally, this would reveal trends of attraction or repellency to dung not only for absence vs. presence, but also trends related to LR concentrations. Similar to the field study, pitfall traps were run in each of two years, 2019 and 2020.

1.6 Literature Cited

1. Lysyk, T. J., Easton, E. R. & Evenson, P. D. Seasonal changes in nitrogen and moisture content of cattle manure in cool-season pastures. *J. Range Manag.* **38**, 251–254 (1985).

2. Lee, C. M. & Wall, R. Cow-dung colonization and decomposition following insect exclusion. *Bull. Entomol. Res.* **96**, 315–322 (2006).

3. Marsh, R. & Campling, R. C. Fouling of pastures by dung. *Herb. Abs* **40**, 123–130 (1970).

4. Gupta, K. K., Aneja, K. R. & Rana, D. Current status of cow dung as a bioresource for sustainable development. *Bioresour. Bioprocess.* **3**, 2–11 (2016).

5. Eghball, B., Wienhold, B. J., Gilley, J. E. & Eigenberd, R. A. Mineralization of manure nutrients. *J. Soil Water Conserv.* **57**, 470–473 (2002).

6. Yokoyama, K., Kai, H., Koga, T. & Aibe, T. Nitrogen mineralization and microbial populations in cow dung, dung balls, and underlying soil affected by paracoprid dung beetles. *Soil Biol. Biochem.* **23**, 649–653 (1991).

7. Floate, K. D. Arthropods in Cattle Dung on Canada's Grasslands. in *Arthropods of Canadian Grasslands (Volume 2): Inhabitants of a Changing Landscape* 71–88 (Ottawa: Biological Survey of Canada, 2011).

8. Mohr, C. O. Cattle droppings as ecological units. *Ecol. Monogr.* **13**, 275–298 (1943).

9. Hanski, I. & Cambefort, Y. *Dung Beetle Ecology*. (Princeton University Press, 1991).

10. Holter, P. Effect of dung-beetles (*Aphodius* spp.) and earthworms on the disappearance of cattle dung. *Oikos* **32**, 393–402 (1979).

11. Angel, K. (Sr) & Wicklow, D. T. Decomposition of rabbit faeces: An indication of the significance of the coprophilous microflora in energy flow schemes. *J. Ecol.* **62**, 429–437 (1974).

12. Laurence, B. R. The larval inhabitants of cow pats. *J. Anim. Ecol.* **23**, 234–260 (1954).

13. Stavert, J., Drayton, B., Beggs, J. & Gaskett, A. The volatile organic compounds of introduced and native dung and carrion and their role in dung beetle foraging behaviour. *Ecol. Entomol.* **39**, 556–565 (2014).

14. de Jongh, E. Navigation and orientation in Coleoptera: A review of strategies and mechanisms. *Anim. Cogn.* **24**, 1153–1164 (2021).

15. de Bruyne, M. & Baker, T. C. Odor detection in insects: Volatile codes. *J. Chem. Ecol.* **34**, 882–897 (2008).

16. Sladecek, F. X. J., Dötterl, S., Schäffler, I., Segar, S. T. & Konvicka, M. Succession of dung-inhabiting beetles and flies reflects the succession of dung-emitted volatile compounds. *J. Chem. Ecol.* **47**, 433–443 (2021).

17. Dormont, L., Epinat, G. & Lumaret, J. P. Trophic preferences mediated by olfactory cues in dung beetles colonizing cattle and horse dung. *Environ. Entomol.* **33**, 370–377 (2004).

18. Dormont, L., Jay-Robert, P., Bessière, J. M., Rapior, S. & Lumaret, J. P. Innate olfactory preferences in dung beetles. *J. Exp. Biol.* **213**, 3177–3186 (2010).

19. Hammer, O. Biological and ecologican investigations on flies associated with pasturing cattle and their excrement. *Vidensk. Medd. Fra. Dansk. naturh. Foren.* **105**, 1–257 (1941).

20. Erzinclioglu, Z. *Blowflies*. (The Richmond Publishing Co., 1996).

21. Floate, K. D., Wardhaugh, K. G., Boxall, A. B. A. & Sherratt, T. N. Fecal residues of veterinary parasiticides: Nontarget effects in the pasture environment. *Annu. Rev. Entomol.* **50**, 153–179 (2005).

22. Cotterell, G. S. The life history and habits of yellow dung-fly (*Scathophaga stercoraria*): A possible blow-fly check. *Proc. Zool. Soc. London* 629–647 (1920).

23. Blanckenhorn, W. U., Pemberton, A. J., Bussière, L. F., Roembke, J. & Floate, K. D. A review of the natural history and laboratory culture methods for the yellow dung fly, *Scathophaga stercoraria*. *J. Insect Sci.* **10**, 1–17 (2010).

24. Sladecek, F. X. J., Segar, S. T. & Konvicka, M. Early successional colonizers both facilitate and inhibit the late successional colonizers in communities of dung-inhabiting insects. *Eur. J. Entomol.* **118**, 240–249 (2021).

25. Finn, J. A. & Giller, P. S. Patch size and colonisation patterns: An experimental analysis using north temperate coprophagous dung beetles. *Ecography (Cop.).* **23**, 315–327 (2000).

26. Floate, K. D. & Gill, B. D. Seasonal activity of dung beetles (Coleoptera: Scarabaeidae) associated with cattle dung in southern Alberta and their geographical distribution in Canada. *Can. Entomol.* **130**, 131–151 (1998).

27. Kadiri, N., Lumaret, J. P. & Floate, K. D. Functional diversity and seasonal activity of dung beetles (Coleoptera: Scarabaeoidea) on native grasslands in southern Alberta, Canada. *Can. Entomol.* **146**, 291–305 (2014).

28. Seamans, H. L. *An Insect Weather Prophet*. (1934).

29. Gordon, R. D. & Skelley, P. E. *A Monograph of the Aphodiini Inhabitating the United States and Canada (Coleoptera: Scarabaeidae: Aphodiini).* (The American Entomological Institute, 2007).

30. Nichols, E. *et al.* Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. *Biol. Conserv.* **141**, 1461–1474 (2008).

31. Landin, B. O. *Ecological studies on dung-beetles:(Coleoptera: Scarabaeidae)*. (Entomologiska Sällskapet, 1961).

32. Bousequet, Y. & Laplante, S. *The Insects and Arachnids of Canada. Part 24. Coleoptera Histeridae.* (NRC Research Press, 2006).

33. Floate, K. D. & Gibson, G. A. P. Filth Fly Parasitoids (Hymenoptera: Pteromalidae) in North America. in *Encyclopedia of Entomology, Volume 2* (ed. Capinera, J. L.) 838–840 (Kluwer Academic Publishers, 2004).

34. Blume, R. R. A check-list, distributional record, and annotated bibliography of the insects associated with bovine droppings on pasture in America north of Mexico. *Southwest. Entomol.* **9**, 1–55 (1985).

35. Rueda, L. M. & Axtell, R. C. *Guide to Common Species of Pupal Parasites (Hymenoptera: Pteromaliidae) of the House Fly and Other Muscoid Flies Associated With Poultry and Livestock Manure. North Carolina Agricultural Research Service, Technical Bulletin 278* (Noth Carolina State University, 1985).

36. Beynon, S. A., Mann, D. J., Slade, E. M. & Lewis, O. T. Species-rich dung beetle communities buffer ecosystem services in perturbed agro-ecosystems. *J. Appl. Ecol.* **49**, 1365–1372 (2012).

37. Ritcher, P. O. *White Grubs and Their Allies, a Study of North American Scarabaeoid Larvae*. (Oregon State University Monograph Series, 1954).

38. von Lengerken, H. Die brutfürsorge- und brutflegeinstinkte der käfer. *2.,Aufl., Leipzig.* 207–235 (1954).

39. Macqueen, A. & Beirne, B. P. Effects of cattle and dung beetle activity on growth of beardless wheatgrass in British Columbia. *Can. J. Plant Sci.* **55**, 961–967 (1973).

40. Badenhorst, J., Dabrowski, J., Scholtz, C. H. & Truter, W. F. Dung beetle activity improves herbaceous plant growth and soil properties on confinements simulating reclaimed mined land in South Africa. *Appl. Soil Ecol.* **132**, 53–59 (2018).

41. Bang, H.-S. *et al.* Effects of paracoprid dung beetles (Coleoptera: Scarabaeidae) on the growth of pasture herbage and on the underlying soil. *Appl. Soil Ecol.* **29**, 165–171 (2005).

42. Fincher, G. T. Dung beetles as biological control agents for gastrointestinal parasites of livestock. *J. Parasitol.* **59**, 396–399 (1973).

43. Fincher, G. T. *Biological Control of Dung-Breeding Flies Affecting Pastured Cattle*. *Beef Cattle Handbook* (1914).

44. Sands, B. & Wall, R. Dung beetles reduce livestock gastrointestinal parasite availability on pasture. *J. Appl. Ecol.* **54**, 1180–1189 (2017).

45. Holter, P. Herbivore dung as food for dung beetles: Elementary coprology for entomologists. *Ecol. Entomol.* **41**, 367–377 (2016).

46. MacLusky, D. S. Some estimates of the areas of pasture fouled by the excreta of dairy cows. *Grass Forage Sci.* **15**, 181–188 (1960).

47. Doube, B. M. Ecosystem services provided by dung beetles in Australia. *Basic Appl. Ecol.* **26**, 35–49 (2018).

48. Bornemissza, G. F. The Australian dung beetle project 1965-1975. *Aust. Meat Res. Comm. Rev.* **30**, 1–30 (1976).

49. Hughes, R. D. Assessment of the burial of cattle dung by Australian dung beetles. *Aust. J. Entomol.* **14**, 129–134 (1975).

50. Floate, K. D. Off-target effects of ivermectin on insects and on dung degradation in southern Alberta, Canada. *Bull. Entomol. Res.* **88**, 25–35 (1998).

51. Pecenka, J. R. & Lundgren, J. G. The importance of dung beetles and arthropod communities on degradation of cattle dung pats in eastern South Dakota. *PeerJ* **6**, e5220 (2018).

52. Anderson, J. R., Merritt, R. W. & Loomis, E. C. The insect-free cattle dropping and its relationship to increased dung fouling of rangeland pastures. *J. Econ. Ecol.* **77**, 133–141 (1984).

53. Hotson, I. K. The avermectins: A new family of antiparasitic agents. *J. South African Vet. Assoc.* **53**, 87–90 (1982).

54. Steel, J. W. Pharmacokinetics and metabolism of avermectins in livestock. *Vet. Parasitol.* **48**, 45–57 (1993).

55. Vercuysse, J. & Claerebout, E. Macrocylic Lactones. *Merck Manual; Veterinary Manual* https://www.merckvetmanual.com/pharmacology/anthelmintics/macrocyclic-lactones (2014).

56. Burg, R. W. *et al.* Avermectins, new family of potent anthelmintic agents: Isolation and chromatographic properties. *Antimicrob. Agents Chemother.* **15**, 368–371 (1979).

57. Campbell, W. C., Fisher, M. H., Stapley, E. O., Albers-Schönberg, G. & Jacob, T. A. Ivermectin : A potent new antiparasitic agent. *Science.* **221**, 823–828 (1983).

58. Ulrich-Schad, J. D. *et al.* What and why: South Dakota rangeland livestock producers' usage of parasiticides. *Rangel. Ecol. Manag.* **79**, 190–200 (2021).

59. Barton, C. H. J., Dale, E. F., Dixon, C. & Coles, G. C. Survey of parasite control on beef farms in south-west England. *Vet. Rec.* **159**, 682–684 (2006).

60. Health Canada. Drug Product Database. https://www.canada.ca/en/health-canada/services/drugs-health-products/drug-products/drug-product-database.html.

61. Bousquet-Melou, A., Mercadier, S., Alvinerie, M. & Toutain, P.-L. Endectocide exchanges between grazing cattle after pour-on administration of doramectin, ivermectin and moxidectin. *Int. J. Parasitol.* **34**, 1299–1307 (2004).

62. Floate, K. D. Endectocide use in cattle and fecal residues: Environmental effects in Canada. *Can. J. Vet. Res.* **70**, 1–10 (2006).

63. Alvinerie, M. *et al.* Persistence of ivermectin in plasma and faeces following administration of a sustained-release bolus to cattle. *Res. Vet. Sci.* **66**, 57–61 (1999).

64. Forbes, A. B. A review of regional and temporal uses of avermectins in cattle and horses worldwide. *Vet. Parasitol.* **48**, 19–28 (1993).

65. Sutherland, I. H. Veterinary use of ivermectin. *Acta Leiden.* **59**, 211–216 (1990).

66. Hamel, D. *et al.* Eprinomectin pour-on (Eprinex® pour-on, Merial): Efficacy against gastrointestinal and pulmonary nematodes and pharmacokinetics in sheep. *BMC Vet. Res.* **13**, 1–12 (2017).

67. Forbes, A. B. LongRange™ (eprinomectin 5%) extended-release injection parasiticide and the utility of extended-activity antiparasitics in cattle. *Vet. Parasitol.* **192**, 308–312 (2013).

68. Aksit, D., Korkut, O., Aksoz, E. & Gokbulut, C. Plasma disposition and faecal excretion of eprinomectin following topical and subcutaneous administration in non-lactating dairy cattle. *N. Z. Vet. J.* **64**, 207–211 (2016).

69. Errouissi, F. & Lumaret, J. P. Field effects of faecal residues from ivermectin slow-release boluses on the attractiveness of cattle dung to dung beetles. *Med. Vet. Entomol.* **24**, 433–440 (2010).

70. Soll, M. D. *et al.* An eprinomectin extended-release injection formulation providing nematode control in cattle for up to 150 days. *Vet. Parasitol.* **192**, 313–320 (2013).

71. CVMP. *Final CVMP Assessment Report for LongRange*. (2018).

72. González Canga, A. *et al.* The pharmacokinetics and metabolism of ivermectin in domestic animal species. *Vet. J.* **179**, 25–37 (2009).

73. Schmidt, C. D. Activity of an avermectin against selected insects in aging manure. *Environ. Entomol.* **12**, 455–457 (1983).

74. Herd, R. P., Sams, R. A. & Ashcraft, S. M. Persistence of ivermectin in plasma and faeces following treatment of cows with ivermectin sustained-release, pour-on or injectable formulations. *Int. J. Parasitol.* **26**, 1087–1093 (1996).

75. Nieman, C. C. *et al.* Eprinomectin from a sustained release formulation adversely affected dung breeding insects. *PLoS One* **13**, 1–11 (2018).

76. Miller, J. A., Kunz, S. E., Oehler, D. D. & Miller, R. W. Larvicidal activity of Merck MK-933, an avermectin, against the horn fly, stable fly, face fly, and house Fly. *J. Econ. Entomol.* **74**, 608–611 (1981).

77. Floate, K. D., Spooner, R. W. & Colwell, D. D. Larvicidal activity of endectocides against pest flies in the dung of treated cattle. *Med. Vet. Entomol.* **15**, 117–120 (2001).

78. Fincher, G. T. Injectable ivermectin for cattle: Effects on some dung-inhabiting insects. *Environ. Entomol.* **21**, 871–876 (1992).

79. Wall, R. & Strong, L. Environmental consequences of treating cattle with the antiparasitic drug ivermectin. *Nature* **327**, 418–421 (1987).

80. Suárez, V. H., Lifschitz, A. L., Sallovitz, J. M. & Lanusse, C. E. Effects of faecal residues of moxidectin and doramectin on the activity of arthropods in cattle dung. *Ecotoxicol. Environ. Saf.* **127**, 481–488 (2003).

81. Dadour, I. R., Cook, D. F. & Neesam, C. Dispersal of dung containing ivermectin in the field by *Onthophagus taurus* (Coleoptera: Scarabaeidae). *Bull. Entomol. Res.* **89**, 119–123 (1999).

82. Floate, K. D., Colwell, D. D. & Fox, A. S. Reductions of non-pest insects in dung of cattle treated with endectocides: A comparison of four products. *Bull. Entomol. Res.* **92**, 471–481 (2002).

83. Jacobs, C. T. & Scholtz, C. H. A review on the effect of macrocyclic lactones on dung-dwelling insects: Toxicity of macrocyclic lactones to dung beetles. *Onderstepoort J. Vet. Res.* **82**, 1–8 (2015).

84. Floate, K. D. & Fox, A. S. Indirect effects of ivermectin residues across trophic levels: *Musca domestica* (Diptera: Muscidae) and *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae). *Bull. Entomol. Res.* **89**, 225–229 (1999).

85. Junco, M. *et al.* Effect of macrocyclic lactones on nontarget coprophilic organisms: a review. *Parasitol. Res.* **120**, 773–783 (2021).

86. Madsen, M. *et al.* Treating cattle with ivermectin: Effects on the fauna and decompsition of dung pats. *J. Appl. Ecol.* **27**, 1–15 (1990).

87. Jochmann, R., Lipkow, E. & Blanckenhorn, W. U. A field test of the effect of spiked ivermectin concentrations on the biodiversity of coprophagous dung insects in Switzerland. *Environ. Toxicol. Chem.* **35**, 1947–1952 (2016).

88. Manning, P., Lewis, O. T. & Beynon, S. A. Effects of the veterinary anthelmintic moxidectin on dung beetle survival and dung removal. *Entomol. Exp. Appl.* **166**, 810–817 (2018).

89. Ishikawa, I. & Iwasa, M. Toxicological effect of ivermectin on the survival, reproduction, and feeding activity of four species of dung beetles (Coleoptera: Scarabaeidae and Geotrupidae) in Japan. *Bull. Entomol. Res.* 1–9 (2019).

90. Lumaret, J. P. *et al.* Field effects of ivermectin residues on dung beetles. *Appl. Ecol.* **30**, 428–436 (1993).

91. O'Hea, N. M., Kirwan, L., Giller, P. S. & Finn, J. A. Lethal and sub-lethal effects of ivermectin on north temperate dung beetles, *Aphodius ater* and *Aphodius rufipes* (Coleoptera: Scarabaeidae). *Insect Conserv. Divers.* **3**, 24–33 (2010).

92. Wardhaugh, K. G., Longstaff, B. C. & Morton, R. A comparison of the development and survival of the dung beetle, *Onthophagus taurus* (Schreb.) when fed on the faeces of cattle treated with pour-on formulations of eprinomectin or moxidectin. *Vet. Parasitol.* **99**, 155–168 (2001).

93. Krüger, K. & Scholtz, C. H. Lethal and sublethal effects of ivermectin on the dung-breeding beetles *Euoniticellus intermedius* (Reiche) and *Onitis alexis* Klug (Coleoptera, Scarabaeidae). *Agric. Ecosyst. Environ.* **61**, 123–131 (1997).

94. Krüger, K. & Scholtz, C. H. The effect of ivermectin on the development and reproduction of the dung-breeding fly *Musca nevilli* Kleynhans (Diptera, Muscidae). *Agric. Ecosyst. Environ.* **53**, 13–18 (1995).

95. Verdú, J. R. *et al.* Low doses of ivermectin cause sensory and locomotor disorders in dung beetles. *Sci. Rep.* **5**, 1–10 (2015).

96. Floate, K. D. Does a repellent effect contribute to reduced levels of insect activity in dung from cattle treated with ivermectin? *Bull. Entomol. Res.* **88**, 291–297 (1998).

97. Floate, K. D. Endectocide residues affect insect attraction to dung from treated cattle: Implications for toxicity tests. *Med. Vet. Entomol.* **21**, 312–322 (2007).

98. Römbke, J. *et al.* Effects of the parasiticide ivermectin on the structure and function of dung and soil invertebrate communities in the field (Madrid, Spain). *Appl. Soil Ecol.* **45**, 284–292 (2010).

99. Frank, K., Brückner, A., Blüthgen, N. & Schmitt, T. In search of cues: Dung beetle attraction and the significance of volatile composition of dung. *Chemoecology* **28**, 145–152 (2018).

Chapter 2: Extended-release LongRange® eprinomectin residues reduce dung-breeding insect abundance and species richness in dung of treated cattle

2.1 Abstract

The goal of endectocide use is to treat the internal and external parasites of livestock but may have unintended adverse effects on coprophilous insects during juvenile stages. Following treatment, active ingredient residues are excreted in dung where a diverse community of insects feed and breed. Here I conduct research to assess the insecticidal activity of residues in dung from cattle treated with LongRange® eprinomectin, a novel formulation of parasiticide for which the non-target effects are little known. In a field study replicated in two years, the number of insects developing in dung collected from treated cattle (1, 2, 4, 8, 12, 16, 20, 24/25 weeks post-treatment) were compared to the number of insects developing in dung from untreated cattle (control). Results varied between years, but showed suppression of insect abundance and taxa richness in dung from cattle treated up to and including 25 weeks previously, and suppression of diversity in dung from cattle treated between 4 and 8 weeks previously. Results for individual taxa indicated either no effect of treatment or suppression of development in dung from cattle treated up to and including 25 weeks post-treatment. Thus, cattle treated with LR excrete dung containing residues toxic enough to suppress adult emergence of certain taxa for the entire pasture season. In a lab bioassay utilizing the house fly, *Musca domestica* (Diptera: Muscidae), fewer flies survived to emerge as adults from dung of cattle treated 1 week previously versus dung from untreated cattle (control) or from cattle treated 8, 12 and 16 weeks previously.

2.2 Introduction

Endectocides are a class of chemicals applied to livestock to control both internal (endo) and external (ecto) parasites. More formally known as macrocyclic lactones (MLs), members of this class include the avermectins (ivermectin, eprinomectin, and doramectin), and milbemycins (moxidectin)[1]. These active ingredients maintain low toxicity to mammals with the prescribed dosage and are highly effective against their target parasites which include different species of nematodes, mites, ticks and insects[2–4]. Animals treated with endectocides excrete residues in their dung comprised mostly of unmetabolized active ingredient[5–7].

Concerns have been raised about the potential non-target effects of residues on dung-breeding insects due to the benefits of their presence in pasture ecosystems. This community of insects consists largely of species of beetles, flies, and parasitoid wasps, very few of which are considered pests of livestock[8–11]. Of those pest insects, the most notable are dipteran species including horn fly (*Haematobia irritans*), face fly (*Musca autumnalis*), and stable fly (*Stomoxys calcitrans*)[11]. Other, beneficial, species provide important ecosystem services primarily through contributions to livestock pasture health via rapid dung burial and promoting the activity of other beneficial fauna and flora in dung pats and associated soils[12–16]. Feeding and tunneling by dung insects allow for aeration of the dung pat while burrowing and fragmentation of dung allow for essential nutrients to be returned to the soil and removed from the surface of pastures[14,17]. Additionally, beneficial dung insect activity can reduce the incidence of pest dung insects through competition for resources, predation, and parasitism[18–22]. When dung insect

activity is limited, pastures can become fouled by dung, which reduces their suitability for grazing and limits the growth of new foliage[17,23,24].

Previous studies have examined the consequences of faecal residues and identify a number of non-target effects to dung-breeding insects (reviews in Junco *et al*., Floate, and Jacobs)[25–27]. In brief, residues are primarily known to have insecticidal activity as fresh dung from cattle treated weeks to months previously can reduce insect emergence. Furthermore, there are effects on dung-insects that are sub-lethal, including delayed development, effects on higher trophic levels, breeding deficiencies, effects on locomotion, and altered attraction to dung[28–31]. However, the effects also depend on the taxa of insect being studied as some are more susceptible to residues in dung compared to others. For example, Conforti *et al*. showed that susceptibility to residues of ivermectin varied even between species of flies in the same genus; i.e., Sepsis[32]. Finally, the strength and duration of the effects are highly dependent on the formulation and active ingredient (ML) used[25,26].

The formulation in which the endectocide is applied affects the concentration of residue in the dung of the treated animal[33]. When applied topically or via subcutaneous injection, peak faecal concentrations occur within a few days post-treatment followed by a rapid decline until the product has completely vacated the animal[5,33]. When the endectocide is applied to the animal in a sustained-release (SR) bolus formulation, residues increase during the first 1-2 weeks before declining to reach a steady concentration for approximately 16 weeks[34]. Residues in dung of cattle treated with an SR bolus formulation of ivermectin have been shown to suppress development of the dung beetle *Aphodius constans* in dung deposited up to 157 days post-treatment of

cattle[35]. Although the SR bolus formulation is no longer on the market, it has since been replaced by the extended release formulation, LongRange®.

LongRange® (LR) is a relatively novel extended-release formulation of eprinomectin that is applied to the animal as a subcutaneous injection. Whereas previous topical or injectable formulations of endectocides provide protection against nematode parasites for 14-42 days post-treatment, LR confers protection for an advertised period of up to 150 days post-injection[36,37]. Animals treated with this product faecally excrete a peak concentration of residue approximately one week post-treatment and a second, smaller peak approximately 12 weeks post-treatment with a decrease in concentration in between peaks[38,39]. The bimodal peak of excretion following treatment with LR has been documented for cattle in both blood plasma and in dung[36,39].

To date, only Nieman *et al*. have investigated the non-target effects of faecal residues associated with use of this product on dung-breeding insects[39]. They found that residues significantly reduced the overall abundance and richness of the insect community developing in dung deposited by cattle treated up to and including 20 weeks previously, beyond which residue effects were not assessed[39]. Furthermore, the diversity of this community was significantly reduced in dung of cattle treated between 12 and 16 weeks previously[39]. For cases of individual taxa, residues either had no detectable effect or suppressed development for the entire period of assessment (i.e., 20 weeks)[39].

The goal of the current study was to combine field-based and lab experiments to test the effects of LR residues on the abundance, richness, and diversity of the dung-breeding insect community by expanding upon the findings of Nieman *et al*.[39]. Whereas they examined the effect of residues in dung of cattle treated with LongRange® up to 20

weeks in the field, I extended this period of assessment for up to 25 weeks. Furthermore, Niemen *et al*. reported results for a single experiment whereas I repeated this experiment twice, once in June (2019) and once in September (2020). By conducting my experiments in southern Alberta, Canada, we expected to obtain data on insect taxa, in two different seasons, that were not assessed in the previous study, which was conducted in Wisconsin, United States of America. Finally, I performed a lab bioassay using larvae of house fly (*Musca domestica*) to assess the effects of LR faecal residues in a more controlled setting (e.g., a known number of individuals, optimal conditions, reduced mortality factors, etc.).

2.3 Materials & Methods

2.3.1 Cattle Treatment & Dung Collection

As a source of dung for use in experiments, cattle were housed in pens at the Lethbridge Research and Development Centre (LeRDC). All cattle were cared for in accordance with the guidelines of the Canadian Council for Animal Care and with the approval of the LeRDC Animal Care Committee (Protocols 1826, 1916 and 1929). Cattle were maintained on a diet of barley silage for the duration of dung collections to exclude a change in diet as a potential confounding factor affecting treatment.

Two sets of cattle were treated with LR following standards set by the manufacturer's label (1 mL/110 lbs). One set of cattle (n = 10) was treated in November 2018 with dung collected immediately prior to treatment to act as a control (week 0) and subsequent collections of dung 1, 2, 4, 8, 12, 16, 20, and 24 weeks post-treatment. A second set of cattle (n = 10) was treated in July 2019 with fresh dung collected immediately prior to treatment to act as a control (week 0) and subsequent collections of

fresh dung 1, 2, 4, 8, 12, 16 and 25 weeks post-treatment. Prior to the LR treatment, the animals had not previously been treated with parasiticides. The absence of a week 20 collection and the collection at week 25, instead of week 24, were unavoidable consequences of federal government COVID-19 restrictions in place at the time. Due to the necessity of assessing all treatments concurrently, dung was bulked in buckets (11 L), mixed by hand, and frozen (-20C°) until use. Because of the stability of active ingredient residues in dung, freezing was not expected to alter their composition appreciably[40]. This is supported by Nieman *et al*., who chemically analyzed eprinomectin residues in dung following a freezing period of approximately 2 years[39].

Subsamples of dung from each collection date in both years were set aside to chemically measure residues. However, due to unforeseen circumstances, these analyses were not completed. Thus, concentrations of residues in dung were presumed to closely follow analyses completed by Nieman *et al*. with peaks at 1 and 12 weeks post-treatment, a low point in between peaks at week 8, and a steady decrease following the second peak[39].

2.3.2 Field Toxicity Study

To test the effect of eprinomectin residues on the ability of insects to develop in dung, a study was set up in June 2019 (Year 1) using dung from cattle treated in 2018, and a second replicate study set up in September 2020 (Year 2) using dung from cattle treated in 2019. For these studies, buckets of frozen dung were thawed at room temperature, the dung mixed, and then formed into pats of standard size and shape using a circular mold (500 g, n = 12 pats per collection week). Each pat was then placed on

damp sand and a Styrofoam™ plate (Fig. 2.1). The sand helped to weigh down the plate and retain moisture in the pat during the exposure period outdoors.

Pats, with their plates, were placed in a recently mowed field (lat: 49.690°, long: -112.774°) located at the LeRDC adjacent to a pasture with grazing cattle. This proximity to cattle increased the likelihood of dung insects laying eggs in the experimental pats. Pats were laid out in a grid with approximately 1 m between columns and 2 m between rows (Fig. 2.2). Treatments were randomized within the grid to reduce the chance of a location bias affecting experimental results. Grids contained 108 pats (9 dung collections x 12 replicates) and 96 pats (8 dung collections x 12 replicates) in Years 1 and 2, respectively.

Pats were individually covered with a pegged-down chicken wire dome to prevent interference by other animals (Fig. 2.1). They were then left undisturbed in the field for a total of 7 days in Year 1, and 10 days in Year 2 to allow time for insects to colonize the pats and lay eggs. Pats with their associated plates were then moved into emergence cages (11 L buckets with mesh sleeves fitted to the top) and placed in a controlled environment room (Fig. 2.3). In Year 1, conditions were set at 22 °C and 16 hours of light per day with 50 % relative humidity. In Year 2, due to a technical problem with the controlled environment room, pats were placed in the same room but with no controlled conditions. Thus, they were left at room temperature (~17.5 °C) and in the dark for the duration of the insect collection period.

Collection of adult insects that emerged into the buckets occurred every other week. During collections, the sand was re-dampened with 30 mL of tap water to maintain moisture in the pat and sand to prevent desiccation of developing insects. Dung pats were

gently lifted to check for insects underneath and all adult insects were collected from the bottom of the bucket. Collected insects were then stored in 70% EtOH until they could be identified and counted. Upon conclusion of adult insect collections, dung pats were broken apart and sand was sifted to search for any remaining insects. All insects collected were summed per replicate, per year, regardless of the number of collections that occurred. In Year 1, collection of adult insects concluded 8.5 weeks and 9 weeks in Year 1 and Year 2, respectively, after pats were removed from the field and adult insects stopped emerging.

Adult insects were identified to the lowest taxonomic level possible, ideally genus and species. The only group that was not identified beyond order were the parasitoid hymenopterans. Identifications of coleopterans were made using pinned reference material (LeRDC, Floate lab) and taxonomic keys[41–43]. Dipterans were also identified using taxonomic keys[44,45]. Coleopterans that were recovered in pails within the first three weeks were considered colonizers as they likely did not develop in the pat due to their typically long development times[46]. Instead, they were assumed part of the F0 (parent) generation and brought in as incidentals when the pats were moved indoors. Therefore, they were subsequently removed from emergence analyses. No dipterans nor hymenopterans were considered colonizers because most adults likely vacated the pats when they were removed from the field to be placed in buckets.

2.3.3 House Fly Bioassay

To test the insecticidal activity of residues under more controlled conditions, a lab bioassay was done using dung seeded with house fly larvae. Initially, a small-scale proof-

of-concept experiment was performed to confirm if house flies could successfully develop in cattle dung using control (week 0) dung from Year 1. Three 100 mL plastic cups were filled (1/3 full) with sand and approximately 30 g of dung was placed on top with space around the edges to allow fly larvae access to the sand for pupation. House fly larvae (n = 30 first instars) were placed on damp filter paper and overturned on top of the dung in each cup. Cups secured with lids, with minute holes to allow for excess gas to escape, were then placed in a controlled environment room at 25 °C and left undisturbed for 3 weeks until the flies emerged as adults (Fig. 2.4), at which time the adults were then counted.

Following the success of this initial study, the insecticidal activity of residues was tested using dung from cattle treated in 2018 and collected at 0, 1, 8, 12 and 16 weeks post-treatment. This selection provided a control (0 weeks) and treatments with expected high (1 week post-treatment), intermediate (8 and 12 weeks post-treatment) and low (16 weeks post-treatment) concentrations of residue. For each dung collection used, 12 cups (60 total) were made and populated with 25 house fly larvae each. This time, 250 mL cups were used containing 120 g of sand and 70 g of dung, but methods were otherwise the same as the initial study.

2.4 Statistical Analyses

2.4.1 Field Toxicity Study

To assess the suppression effect of residues at the level of the community, data analyses were performed to compare the effect of treatment on abundance (total individuals for all taxa combined), richness (number of taxa), and diversity (Hill's D

diversity = exp[Shannon's H]). The effect of treatment was also examined for the abundance of individual taxa. To avoid type II errors (false negatives), individual taxa with less than 12 individuals emerging from the control pats (average of one individual per pat) were excluded from consideration.

Because most of the data were non-normally distributed (Shapiro-Wilks test, $p < 0.05$) and the variance showed significant heterogeneity (Levene's test, $p < 0.05$) for measures of abundance and taxa richness, the data were analyzed using generalized linear mixed models (GLMMs). This was done to avoid the use of rank transformations, which can decrease statistical power[47]. The GLMMs assessed the fixed effects of treatment (weeks post-treatment) and the random effects of replicate. Modelled analyses were completed using the GLIMMIX procedure using SAS® studio software[48]. The distributions for the models (Poisson, negative binomial, or geometric) were chosen based on best fit as determined by the Bayesian information criterion (BIC) after 1000 iterations. If none of these distributions converged, a shifted-*t* distribution was used. Finally, the replicate number was used as part of the covariance structure following the same criteria as above (i.e., if the model converged after 1000 iterations and the BIC was lower than the initial model).

Initial tests for a significant difference were based on Type III Tests of Fixed Effects (Wald *F*). In the event of a statistically significant outcome ($p < 0.05$), a one-sided post-hoc Dunnett's test was then used to determine if any of the weeks post-treatment were significantly suppressed compared to the control treatment (0 weeks). Additional information for the models used in Year 1 and Year 2 are outlined in Appendices 2.1 and 2.2, respectively.

Hill's D diversity data were normally distributed (Shapiro-Wilks test, $p > 0.05$) and variance showed no significant heterogeneity (Levene's test, $p > 0.05$). Therefore, diversity data were assessed using a one-way ANOVA followed by a post-hoc Dunnett's test in the event of a statistically significant result ($p < 0.05$) to test for significant suppression in the treatments compared to the control treatment (0 weeks). Hill's D diversity was calculated in R Studio using the Vegan package[49,50]. Statistical analyses were completed in R Studio using base R and the DescTools package[51].

2.4.2 House Fly Bioassay

Adult abundance data were normally distributed (Shapiro-Wilks test, $p > 0.05$) and variance exhibited no heterogeneity (Levene's test, $p > 0.05$). Therefore, lab bioassay data were assessed using a one-way ANOVA followed by a post-hoc Dunnett's test in the event of a statistically significant result ($p < 0.05$) to test for significant suppression in treatments compared to the control (week 0). Statistical analyses for abundance were completed in R Studio using base R and the DescTools package[51].

2.5 Results

2.5.1 Field Toxicity Study

In Year 1, a total of 10,696 adult insects were recovered from 108 pats. After the removal of insect colonizers, 6,767 individuals remained for statistical analyses. The majority of colonizers were comprised of the beetles *Otophorus haemorrhoidalis*, Aleocharinae, and *Calamosternus granarius*. All remaining taxa (15 taxa of coleopterans, 12 taxa of dipterans, and the hymenopterans) and individuals were counted towards the

total abundance, richness, and diversity. Samples were comprised primarily of coleopterans (81%). Of the 9 taxa identified for individual abundance analyses (i.e., those represented by at least 12 individuals in the control treatment), Ptiliidae and Aleocharinae were the most common groups (31% and 24% of total individuals respectively), followed by *O. haemorrhoidalis* (13%). Of the dipterans (18% of total individuals), nearly half belonged to the family Sphaeroceridae.

In Year 2, a total of 6,391 adult insects were recovered from 96 pats. After the removal of colonizers, 4,237 remained for statistical analyses. The majority of colonizers were comprised of *Aphodius pedellus*, Aleocharinae, and *Cryptopleurum* sp. Similar to Year 1, all remaining taxa (12 taxa of coleopterans, 10 taxa of dipterans, and the hymenopterans) and individuals counted towards the total abundance, richness, and diversity. Coleopterans comprised a majority of the samples (64%). However, dipterans comprised a much larger portion of the insects in Year 2 (35%) compared to Year 1. Of the 15 taxa identified for individual abundance analyses (i.e., those represented by at least 12 individuals in the control treatment), *Cryptopleurum* sp. and Ptiliidae were the most common groups of the coleopterans (18% and 13% of all individuals, respectively), closely followed by *Aphodius pedellus* (11%). *Sepsis* sp. not only accounted for more than half of the dipterans, but was also the most abundant group overall (19%) despite almost exclusively emerging from control pats.

In Year 1, an effect of treatment was detected on total insect emergence (Type III Tests of Fixed Effects, $F_{8, 88} = 18.58$, $p < 0.0001$). Subsequent post-hoc Dunnett's tests identified fewer insects were recovered from dung collected 1 week post-treatment up to and including dung collected 12 weeks post-treatment, relative to that recovered from the

control (Fig. 2.5A). Similarly, in Year 2, an effect of treatment was detected on total insect emergence (Type III Tests of Fixed Effects, $F_{7, 77} = 21.83$, $p < 0.0001$). However, in contrast to Year 1, subsequent post-hoc Dunnett's tests identified fewer insects were recovered from all treatment groups for up to and including dung collected 25 weeks post-treatment (Fig. 2.5B).

In Year 1, an effect of treatment was detected on taxa richness (Type III Tests of Fixed Effects, $F_{8, 88} = 12.53$, $p < 0.0001$). Subsequent post-hoc Dunnett's tests identified fewer taxa were recovered from dung collected 1 week post-treatment up to and including dung collected 12 weeks post-treatment relative to the control. The exception was from dung collected 8 weeks post-treatment which had no suppression relative to the taxa recovered from the control (Fig. 2.6A). Similarly, in Year 2, an effect of treatment was detected on taxa richness (Type III Tests of Fixed Effects, $F_{7, 77} = 14.46$, $p < 0.0001$). However, in contrast to Year 1, subsequent post-hoc Dunnett's tests identified fewer taxa were recovered from all treatment groups for up to and including dung collected 25 weeks post-treatment (Fig. 2.6B).

In Year 1, an effect of treatment was detected on Hill's D diversity (ANOVA, $F_{8, 99} = 3.89$, $p = 0.0005$). Subsequent post-hoc Dunnett's tests identified decreased diversity from dung collected 1 week post-treatment up to and including dung collected 12 weeks post-treatment, with the exception of dung collected 8 weeks post-treatment, relative to the diversity from the control (Fig. 2.7A). In Year 2, an effect of treatment was detected on Hill's D diversity (ANOVA, $F_{7, 88} = 3.03$, $p = 0.0067$). However, subsequent post-hoc Dunnett's tests identified no reductions compared to the control (Fig 2.7B).

In Year 1, out of the 27 unique taxa, individual abundance analyses were run on 9 of them (i.e., those represented by at least 12 individuals in the control treatment) including 4 taxa of coleopterans, 4 taxa of dipterans, and the hymenopterans. Some taxa were unaffected by treatment, whereas several taxa were affected by residues in dung deposited 12-16 weeks post-treatment (Table 2.1). In Year 2, out of 23 unique taxa, individual abundance analyses were run on 13 of them (i.e., those represented by at least 12 individuals in the control treatment) including 6 taxa of coleopterans, 6 taxa of dipterans, and the hymenopterans. Some taxa were unaffected by residues, whereas 6 taxa had numbers suppressed up to and including dung deposited by cattle treated 25 weeks previously (Table 2.2).

2.5.2 House Fly Bioassay

An effect of treatment was detected on adult fly emergence ($F_{4, 55} = 6.676$, $p < 0.001$). Subsequent post-hoc Dunnett's tests revealed a reduction in survival of flies was observed only in dung collected 1 week post-treatment of cattle (Fig. 2.8). Whereas an average (± SE) of 7.6 ± 0.90 flies were recovered in this treatment, 14.7 ± 1.9 flies were recovered in the control treatment. The maximum number of adult flies to successfully emerge from a cup was 23 out of the total possible 25. Two of these cups occurred within the control treatment and one in the 8 weeks post-treatment cups.

2.6 Discussion

2.6.1 Field Toxicity Study

In the current study, reductions of insect abundance and taxa richness were detected in dung from cattle treated up to and including 25 weeks post-treatment, with reductions in diversity detected in dung from cattle treated between 4 and 8 weeks post-treatment. Suppression strength decreasing in dung collected after 12 weeks post-treatment, and a statistically insignificant suppression of taxa richness and diversity in dung collected 8 weeks post-treatment, is consistent with previous reports of concentration curves from LR treated cattle[38,39]. The community level results are generally consistent with those of Nieman *et al*., which is the only other study to assess the insecticidal activity of LR eprinomectin residues in dung of treated cattle[39]. Nieman *et al*. reported reductions of insect abundance and taxa richness in dung from cattle treated up to and including 20 weeks post-treatment, and reductions of species diversity in dung from cattle treated between 12 and 16 weeks post-treatment[39].

However, some of the results of the current study were inconsistent between years or with those of Nieman *et al*.[39]. In Year 1, the duration of suppression of abundance and taxa richness was not as extensive compared to Nieman *et al*. or Year 2[39]. This is likely due to the presence of different taxa in geographic regions and insect activity in different seasons, respectively. Nieman *et al*. reported large numbers of Fanniidae and Stratiomyidae, both of which were shown to be highly susceptible to residues present in dung, but were absent in the current study[39]. Thus, I speculate that a higher proportion of residue tolerant coleopterans in Year 1 contributed to the absence of an extended effect in the spring. In contrast, in Year 2, a higher proportion of susceptible taxa, namely *Sepsis*

sp., likely contributed to significant results for dung collected later post-treatment. This is consistent with changing insect activity between seasons, i.e., June and September in the current study[8–10]. Finally, no suppression of diversity occurred in any dung post-treatment in Year 2, whereas there was suppression in dung collected from 12 to 16 weeks post-treatment in Year 1. Floate *et al*. suggest that Hill's D diversity is unlikely to accurately show effects of residues on insect communities regardless of the effects on abundance or taxa richness[52]. I speculate that increased evenness (i.e., equally abundant taxa) in the treatment groups compared to the control group could be masking the toxicity effect on diversity. Overall, variations in region and season between these two studies highlight the importance of replication in different geographic regions and seasons with unique insect assemblages to capture the effect of residues in dung.

Recovery of individual taxa of Scarabaeidae in the current study are variable across species, but they are overall consistent with previous reports in the literature. A meta-analysis by Finch *et al*. suggests, broadly, aphodiine beetles are susceptible to eprinomectin residues at high concentrations[53]. Furthermore, Nieman *et al*. reported an overall effect on this family in dung collected up to 12 weeks post-treatment of cattle with LR, which is consistent with our result of *O. haemorrhoidalis* in Year 1[39]. Although *Planolinus vittatus* showed no statistically significant reductions in any collection week post-treatment, no individuals were recovered in dung collected 1 week post-treatment, and abundance was considerably lower in dung collected 2 and 4 weeks post-treatment compared to control levels. This suggests that a biologically relevant level of suppression is occurring, albeit not one that is statistically detectable with the current sample size. Additionally, a treatment effect would be consistent with reports by Floate and Floate *et*

al. who showed that *P. vittatus* (as *Aphodius vittatus*) was suppressed in high concentrations of dung treated with ivermectin[46,54]. Alternatively, a lack of suppression may be supported as Floate found no suppression of *P. vittatus* in dung containing residues of eprinomectin[54]. Finally, *A. pedellus* showed no significant suppression of abundance in any collection week post-treatment with the exception of 8 weeks, although this is not considered to be a direct effect of residues. Although there is a possibility that it is highly resistant to LR residues, the data from the current study are inconsistent with previous reports. Floate *et al*. reported suppression of *A. pedellus* (as *Aphodius fimetarius*) in dung collected at least one week post-treatment of cattle with ivermectin[46]. Alternatively, insignificant results of *A. pedellus* could be due to beetles entering the pat to overwinter, as first observed by Mohr[55]. In other words, recovered *A. pedellus* in the current study may largely be comprised of the F0 generation of colonizers, as opposed to beetles who developed in the pat.

Similarly, results in the current study for the two taxa of Hydrophilidae are mostly consistent with reports in the literature. Nieman *et al*. report suppression of Hydrophilidae for dung collected up to 20 weeks post-treatment of cattle which is consistent with our report of *Cercyon* sp. being suppressed up to and including dung collected 25 weeks post-treatment in Year 2[39]. Reports from Floate and Jochmann *et al*. also support the suppression of *Cercyon* sp. and Hydrophilidae, respectively, in dung containing ivermectin residues[46,56]. In contrast, *Cryptopleurum* sp. only showed statistically significant levels of suppression in the current study from dung collected 4 and 8 weeks post-treatment. However, mean abundance in dung collected 1 and 2 weeks post-treatment was considerably reduced and is considered to be biologically meaningful

despite the lack of statistical significance for two reasons. First, *Cryptopleurum* is a closely related genus to *Cercyon*. Second, reports of *Cryptopleurum* and Hydrophilidae in the literature consistently show suppression of this family[46,52,57]. Therefore, lack of statistical significance is likely the result of highly variable means and an artefact of the methods chosen for statistical analyses.

Recovery of Aleocharinae, a subfamily of Staphylinidae, contrasted between Year 1 and Year 2. Although Aleocharinae is clearly suppressed in dung collected up to 12 weeks post-treatment in Year 1, in Year 2 there was no suppression in any treatment week. Results in Year 1 were consistent with the findings of Nieman *et al*., who showed suppression of Staphylinidae up to and including dung collected up to 12 weeks post-treatment of cattle[39]. Floate additionally showed suppression of species of Aleocharinae in high concentrations of ivermectin residues[46]. Thus, I speculate that contrasting findings between years could be caused by variation in the species composition of the group. For example, Floate *et al*. showed individual species of Aleocharinae had variable responses to residues and, when grouped together as a subfamily, showed no significant suppression in any treatment group[54]. Thus, an effect of season (i.e., spring vs. fall) may have contributed to differences in species composition of which more taxa were susceptible during the spring. Furthermore, Aleocharinae were recovered more frequently in Year 1 compared to Year 2, despite there being a large number of Aleocharinae colonizers present in Year 2. Thus, it is possible that a reduced number of Aleocharinae in the fall in combination with less susceptible species could be masking an effect of residues.

Similarly, an effect on Staphylininae, another subfamily of Staphylinidae, could also be masked due to low recovery numbers in Year 2. However, insufficient numbers

were recovered in Year 1 to make a comparison. Although a statistically significant suppression of Staphylininae was reported for Year 2 in week 8, I consider this effect to be artefact and unrelated to the toxicity of residues due to a reduced number of colonizers in this dung, albeit not a statistically significant one. Although species of Staphylininae are infrequently reported in the literature, Floate reported suppression of *Philonthus cruentatus* in high concentrations of dung containing ivermectin[46]. Similarly, Fincher also reported suppression of *P. flavolimbatus* in high concentrations of ivermectin in a bioassay[58]. Thus, the literature broadly supports suppression of this subfamily, although it is unclear how extensively it may impact this group beyond specific species.

Suppression of the dipterans was mostly consistent with previous reports that show, broadly, that nematocerous flies tend to be less susceptible to residues compared to cyclorrhaphous flies[46,59]. More specifically, the nematoceran families Cecidomyiidae, Chironomidae, and Scatopsidae showed no statistically significant suppression in any dung collection week post-treatment whereas the cyclorrhaphan families Sepsidae and Sphaeroceridae were heavily suppressed. However, there were exceptions for the nematocerous flies, namely families Psychodidae and Ceratopogonidae, both of which showed significant suppression for up to and including dung collected 24 or 25 weeks post-treatment in at least one of two years. Suppression of Psychodidae in the current study is consistent with previous reports in Floate and Nieman *et al*.[39,46]. Albeit results were not statistically significant in the former report, suppression of Psychodidae appears to be at least biologically meaningful. Furthermore, although there was no statistically significant suppression of Chironomidae in this study, they were absent from dung collected 1 week post-treatment, suggesting the variation in control treatments may be

too large to detect an effect. Reports of Ceratopogonidae and Chironomidae are infrequent in the literature, as are other small species of flies, likely due to the overall low abundance of these groups emerging from dung pats. Alternatively, reports could be limited due to the style of emergence cage used, with open air cages placed directly over the pat on the ground causing recovery to be challenging[52]. However, lack of suppression for both families is supported by Floate *et al*.[52].

Sepsis sp. and Sphaeroceridae were the most susceptible taxa recovered from the pats which is consistent with previous reports in the literature. Although insubstantial numbers of *Sepsis* sp. were collected in Year 1 for analyses, results in Year 2 showed heavy suppression for up to and including dung collected 25 weeks post-treatment. This finding is consistent with a study conducted by Wolf *et al*. in which sepsid flies were highly susceptible to ivermectin residues and showed little to no discrimination between control or dung containing residues for oviposition[60]. Conforti *et al*. similarly showed results supporting a lack of discrimination, amongst other sub-lethal effects[32]. Extensive suppression of sepsid flies in all concentration of residues is also supported in other studies reporting on their susceptibility and suitability as bioindicators[26,46,57]. Sphaeroceridae showed similar levels of suppression in both years, from dung collected 16-20 weeks up to and including dung collected 25 weeks post-treatment in Year 1 and Year 2, respectively. These findings are similarly consistent with previous literature that report on this family[46,52,61].

In some cases, similar to some groups of Coleopterans, I questioned statistical results showing a non-intuitive absence of treatment effects. For Ceratopogonidae, numbers in Year 1 were suppressed in all treatments relative to the control, except for

dung collected at 4 weeks post-treatment. This lack of suppression was attributed to an unknown factor unrelated to the presence of residue for two reasons. First, suppression was detected in other treatments for Year 1 with presumably lower residue levels; e.g., dung collected at 12, 16 and 24 weeks post-treatment. Second, results from Year 2 suggested suppression of Ceratopogonidae (albeit not significant) in all treatment groups excluding dung collected 25 weeks post-treatment. For Hymenoptera, numbers in Year 1 appeared to be suppressed in dung collected for a period of 16 to 20 weeks post-treatment, although significant suppression was not detected for dung collected 1 and 4 weeks post-treatment. The lack of suppression for these latter two treatments was attributed to a statistical artefact of highly variable data for three reasons. First, Hymenoptera were suppressed in Year 1 for treatments with lower levels of residue. Second, Hymenoptera were suppressed in Year 2 for all treatments up to and including dung collected 25 weeks post-treatment. Third, Hymenoptera have previously been shown to be highly sensitive to endectocide residues[28,46,54,57].

2.6.2 House Fly Bioassay

Results of the bioassay were consistent with previous lab bioassay findings that show house flies to be a residue tolerant taxon, even in high concentrations of residue. Floate *et al*. similarly reported a formulation of eprinomectin suppressing house flies for dung collected up to 1 week post-treatment of cattle, with a return to control levels in dung collected 2 weeks post-treatment[62]. Another similar study by Wardhaugh *et al*. reported suppression in dung collected in the first week post-treatment of cattle with a formulation of ivermectin[63]. Therefore, results of the current study, in combination with

previous reports, suggest that house flies, and perhaps closely related pest species such as *Musca autumnalis*, are highly residue tolerant. Thus, the added benefit of pest fly control using LR may not be practical given the negative impact on other beneficial insects. Additionally, house flies are likely not a good model insect for testing non-target effects.

2.7 Conclusion

Results of the current study indicate that, when cattle are treated with LR in the spring, they will faecally excrete residues at high enough concentrations to suppress dung-breeding insects for the entirety of a pasture season in Canda. However, the consequences of suppression may vary with the insect taxon. For example, although some dung insects are only susceptible in high concentrations of residues, if they only produce one generation per year and coincidentally breed in highly toxic dung in the spring, effects could persist for the entire season regardless of their tolerance for lower concentrations of residues. This has further implications for the ability to acquire resistance, as insects with fewer generations will do so more slowly, especially compared to pest insects or parasites with high fecundity. Finally, although it's plausible that some taxa would be suppressed for beyond 25 weeks post-treatment, the data in the current study is extensive enough to cover an entire pasture season and further investigation into duration of effect is not relevant, at least in the temperate climate of Canada.

Ambrožová *et al*. suggest that loss of functional groups of dung-breeding insects may have consequences on pasture ecosystem services[64]. The results of the current study, and many previously, support the potential for a reduction of: dung degradation on pasture, biocontrol of pest insects, and the return of nutrients to the soil due to reduced

insect activity; all of which could be costly to maintain if not managed naturally by beneficial dung insects. Not all dung insects play an equally important role in ecosystem services on pasture, and thus future studies should carefully consider the role of each dung insect and their breeding habits to better understand the potential long-term effects, i.e., those persisting beyond one or multiple years of treatment, of which to date is largely unknown and only speculated upon.

2.8 Literature Cited

1. Vercuysse, J. & Claerebout, E. Macrocylic Lactones. *Merck Manual; Veterinary Manual* https://www.merckvetmanual.com/pharmacology/anthelmintics/macrocyclic-lactones (2014).

2. Hotson, I. K. The avermectins: A new family of antiparasitic agents. *J. South African Vet. Assoc.* **53**, 87–90 (1982).

3. Burg, R. W. *et al.* Avermectins, new family of potent anthelmintic agents: Isolation and chromatographic properties. *Antimicrob. Agents Chemother.* **15**, 368–371 (1979).

4. Sutherland, I. H. Veterinary use of ivermectin. *Acta Leiden.* **59**, 211–216 (1990).

5. Aksit, D., Korkut, O., Aksoz, E. & Gokbulut, C. Plasma disposition and faecal excretion of eprinomectin following topical and subcutaneous administration in non-lactating dairy cattle. *N. Z. Vet. J.* **64**, 207–211 (2016).

6. González Canga, A. *et al.* The pharmacokinetics and metabolism of ivermectin in domestic animal species. *Vet. J.* **179**, 25–37 (2009).

7. Steel, J. W. Pharmacokinetics and metabolism of avermectins in livestock. *Vet. Parasitol.* **48**, 45–57 (1993).

8. Kadiri, N., Lumaret, J. P. & Floate, K. D. Functional diversity and seasonal activity of dung beetles (Coleoptera: Scarabaeoidea) on native grasslands in southern Alberta, Canada. *Can. Entomol.* **146**, 291–305 (2014).

9. Floate, K. D. & Gill, B. D. Seasonal activity of dung beetles (Coleoptera: Scarabaeidae) associated with cattle dung in southern Alberta and their geographical distribution in Canada. *Can. Entomol.* **130**, 131–151 (1998).

10. Bertone, M. *et al.* Seasonal activity and species composition of dung beetles (Coleoptera: Scarabaeidae and Geotrupidae) inhabiting cattle pastures in North Carolina. *Ann. Entomol. Soc. Am.* **98**, 309–321 (2006).

11. Floate, K. D. Arthropods in Cattle Dung on Canada's Grasslands. in *Arthropods of Canadian Grasslands (Volume 2): Inhabitants of a Changing Landscape* 71–88 (Ottawa: Biological Survey of Canada, 2011).

12. Nichols, E. *et al.* Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. *Biol. Conserv.* **141**, 1461–1474 (2008).

13. Manning, P., Slade, E. M., Beynon, S. A. & Lewis, O. T. Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. *Agric. Ecosyst. Environ.* **218**, 87–94 (2016).

14. Pecenka, J. R. & Lundgren, J. G. The importance of dung beetles and arthropod communities on degradation of cattle dung pats in eastern South Dakota. *PeerJ* **6**, e5220 (2018).

15. Lee, C. M. & Wall, R. Cow-dung colonization and decomposition following insect exclusion. *Bull. Entomol. Res.* **96**, 315–322 (2006).

16. Doube, B. M. Ecosystem services provided by dung beetles in Australia. *Basic Appl. Ecol.* **26**, 35–49 (2018).

17. Bang, H.-S. *et al.* Effects of paracoprid dung beetles (Coleoptera: Scarabaeidae) on the growth of pasture herbage and on the underlying soil. *Appl. Soil Ecol.* **29**, 165–171 (2005).

18. Fincher, G. T. *Biological Control of Dung-Breeding Flies Affecting Pastured Cattle*. *Beef Cattle Handbook* (1914).

19. Fincher, G. T. Dung beetles as biological control agents for gastrointestinal parasites of livestock. *J. Parasitol.* **59**, 396–399 (1973).

20. Fincher, G. T. Effects of dung beetle activity on the number of nematode parasites acquired by grazing cattle. *J. Parasitol.* **61**, 759–762 (1975).

21. Markin, G. & Yoshioka, E. Biological control of the horn fly, *Haematobia irritans L.*, in Hawai'i (Diptera: Muscidae). *Proc. Hawaiian Entomol. Soc.* 43–50 (1998).

22. Sands, B. & Wall, R. Dung beetles reduce livestock gastrointestinal parasite availability on pasture. *J. Appl. Ecol.* **54**, 1180–1189 (2017).

23. Anderson, J. R., Merritt, R. W. & Loomis, E. C. The insect-free cattle dropping and its relationship to increased dung fouling of rangeland pastures. *J. Econ. Ecol.* **77**, 133–141 (1984).

24. MacLusky, D. S. Some estimates of the areas of pasture fouled by the excreta of dairy cows. *Grass Forage Sci.* **15**, 181–188 (1960).

25. Junco, M. *et al.* Effect of macrocyclic lactones on nontarget coprophilic organisms: a review. *Parasitol. Res.* **120**, 773–783 (2021).

26. Floate, K. D. Endectocide use in cattle and fecal residues: Environmental effects in Canada. *Can. J. Vet. Res.* **70**, 1–10 (2006).

27. Jacobs, C. T. & Scholtz, C. H. A review on the effect of macrocyclic lactones on dung-dwelling insects: Toxicity of macrocyclic lactones to dung beetles. *Onderstepoort J. Vet. Res.* **82**, 1–8 (2015).

28. Floate, K. D. & Fox, A. S. Indirect effects of ivermectin residues across trophic levels: *Musca domestica* (Diptera: Muscidae) and *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae). *Bull. Entomol. Res.* **89**, 225–229 (1999).

29. Verdú, J. R. *et al.* Low doses of ivermectin cause sensory and locomotor disorders in dung beetles. *Sci. Rep.* **5**, 1–10 (2015).

30. Floate, K. D., Wardhaugh, K. G., Boxall, A. B. A. & Sherratt, T. N. Fecal residues of veterinary parasiticides: Nontarget effects in the pasture environment. *Annu. Rev. Entomol.* **50**, 153–179 (2005).

31. Floate, K. D. Endectocide residues affect insect attraction to dung from treated cattle: Implications for toxicity tests. *Med. Vet. Entomol.* **21**, 312–322 (2007).

32. Conforti, S. *et al.* Comparative effects of the parasiticide ivermectin on survival and reproduction of adult sepsid flies. *Ecotoxicol. Environ. Saf.* **163**, 215–222 (2018).

33. Herd, R. P., Sams, R. A. & Ashcraft, S. M. Persistence of ivermectin in plasma and faeces following treatment of cows with ivermectin sustained-release, pour-on or injectable formulations. *Int. J. Parasitol.* **26**, 1087–1093 (1996).

34. Alvinerie, M. *et al.* Persistence of ivermectin in plasma and faeces following aministration of a sustained-release bolus to cattle. *Res. Vet. Sci.* **66**, 57–61 (1999).

35. Errouissi, F., Alvinerie, M., Galtier, P., Kerbœuf, D. & Lumaret, J. P. The negative effects of the residues of ivermectin in cattle dung using a sustained-release bolus on *Aphodius constans* (Duft.) (Coleoptera: Aphodiidae). *Vet. Res.* **32**, 421–427 (2001).

36. Soll, M. D. *et al.* An eprinomectin extended-release injection formulation providing nematode control in cattle for up to 150 days. *Vet. Parasitol.* **192**, 313–320 (2013).

37. CVMP. *Final CVMP Assessment Report for LongRange*. (2018).

38. Forbes, A. B. LongRange™ (eprinomectin 5%) extended-release injection parasiticide and the utility of extended-activity antiparasitics in cattle. *Vet. Parasitol.* **192**, 308–312 (2013).

39. Nieman, C. C. *et al.* Eprinomectin from a sustained release formulation adversely affected dung breeding insects. *PLoS One* **13**, 1–11 (2018).

40. Sommer, C. & Steffansen, B. Changes with time after treatment in the concentrations of ivermectin in fresh cow dung and in cow pats aged in the field. *Vet. Parasitol.* **48**, 67–73 (1993).

41. Henegouwen, A. V. B. & Foster, G. N. A key to the British species of *Sphaeridium*. *Coleopt.* **28**, 1–12 (2019).

42. Gordon, R. D. & Skelley, P. E. *A monograph of the Aphodiini Inhabitating the UnitedSstates and Canada (Coleoptera: Scarabaeidae: Aphodiini).* (The American Entomological Institute, 2007).

43. Brunke, A., Newton, A., Klimaszewski, J., Majka, C. & Marhsall, S. Staphylinidae of eastern Canada and adjacent United States. Key to subfamilies; Staphylininae: Tribes and subtribes, and species of Staphylinina. *Can. J. Arthropod Identif.* **12**, (2011).

44. McAlpine, J. F. *et al. Manual of Nearctic Diptera Volume 1*. (Research Branch, Agriculture Canada, 1981).

45. McAlpine, J. F. *et al. Manual of Nearctic Diptera Volume 2*. (Research Branch, Agriculture Canada, 1987).

46. Floate, K. D. Off-target effects of ivermectin on insects and on dung degradation in southern Alberta, Canada. *Bull. Entomol. Res.* **88**, 25–35 (1998).

47. Bolker, B. M. *et al.* Generalized linear mixed models: A practical guide for ecology and evolution. *Trends Ecol. Evol.* **24**, 127–135 (2009).

48. SAS® Institute. SAS Studio Release 3.81. (2020).

49. R Core Development Team. R: A Language and Environment for Statistical Computing. (2021).

50. Oksanen, J. F., Blanchet, G., Friendly, M., Kindt, R. & Legendre, P. Vegan Package: Community Ecology. (2020).

51. Signorell, A. *et al.* DescTools: Tools for Descriptive Statistics. (2021).

52. Floate, K. D. *et al.* Validation of a standard field test method in four countries to assess the toxicity of residues in dung of cattle treated with veterinary medical products. *Environ. Toxicol. Chem.* **35**, 1934–1946 (2016).

53. Finch, D., Schofield, H., Floate, K. D., Kubasiewicz, L. M. & Mathews, F. Implications of endectocide residues on the survival of aphodiine dung beetles: A meta-analysis. *Environ. Toxicol. Chem.* **39**, 863–872 (2020).

54. Floate, K. D., Colwell, D. D. & Fox, A. S. Reductions of non-pest insects in dung of cattle treated with endectocides: a comparison of four products. *Bull. Entomol. Res.* **92**, 471–481 (2002).

55. Mohr, C. O. Cattle droppings as ecological units. *Ecol. Monogr.* **13**, 275–298 (1943).

56. Jochmann, R., Lipkow, E. & Blanckenhorn, W. U. A field test of the effect of spiked ivermectin concentrations on the biodiversity of coprophagous dung insects in Switzerland. *Environ. Toxicol. Chem.* **35**, 1947–1952 (2016).

57. Jochmann, R. & Blanckenhorn, W. U. Non-target effects of ivermectin on trophic groups of the cow dung insect community replicated across an agricultural landscape. *Basic Appl. Ecol.* **17**, 291–299 (2016).

58. Fincher, G. T. Injectable ivermectin for cattle: Effects on some dung-inhabiting insects. *Environ. Entomol.* **21**, 871–876 (1992).

59. Madsen, M. *et al.* Treating cattle with ivermectin: Effects on the fauna and decompsition of dung pats. *J. Appl. Ecol.* **27**, 1–15 (1990).

60. Wolf, B. U., Puniamoorthy, N., Schäfer, M. A., Scheffczyk, A. & Römbke, J. Sepsid flies confirm their suitability as bioassays of pharmaceutical residues (ivermectin) in cattle dung. *Ecotoxicol. Environ. Saf.* **89**, 21–28 (2013).

61. Iwasa, M., Nakamura, T., Fukaki, K. & Yamashita, N. Nontarget effects of ivermectin on coprophagous insects in Japan. *Environ. Entomol.* **34**, 1485–1492 (2005).

62. Floate, K. D., Spooner, R. W. & Colwell, D. D. Larvicidal activity of endectocides against pest flies in the dung of treated cattle. *Med. Vet. Entomol.* **15**, 117–120 (2001).

63. Wardhaugh, K. G., Holter, P., Whitby, W. A. & Shelley, K. Effects of drug residues in the faeces of cattle treated with injectable formulations of ivermectin and moxidectin on larvae of the bush fly, *Musca vetustissima* and the house fly, *Musca domestica*. *Aust. Vet. J.* **74**, 370–374 (1996).

64. Ambrožová, L. *et al.* Lasting decrease in functionality and richness: Effects of ivermectin use on dung beetle communities. *Agric. Ecosyst. Environ.* **321**, 1–9 (2021).

2.9 Figures & Tables

Figure 2.1. A molded pat (500 g cow dung) on a Styrofoam™ plate and a layer of damp sand. A chicken wire exclosure prevents disturbance by birds and small mammals. This set up was used to test the toxicity of LongRange® eprinomectin residues on dung colonizing insects.

Figure 2.2. Molded dung pats on Styrofoam™ plates, covered by chicken wire domes (Fig. 2.1), in the field in a randomized grid pattern for insect colonization. Each orange flag marks an individual dung pat location.

Figure 2.3. Emergence cages (11 L pails) in a controlled environment room. Each cage contains a dung pat on a Styrofoam™ plate and layer of sand that had been exposed in the field to insect colonization for 7 to 10 days (Fig. 2.1, Fig. 2.2).

Figure 2.4. Dung seeded with 25 newly-hatched house fly larvae was added to bioassay cups containing damp sand to assess the insecticidal activity of LongRange® eprinomectin residues. This photo shows the number of flies surviving to emerge as adults after 3 weeks. Lids placed on the cups caused high humidity that occasionally promoted the growth of mold on the dung.

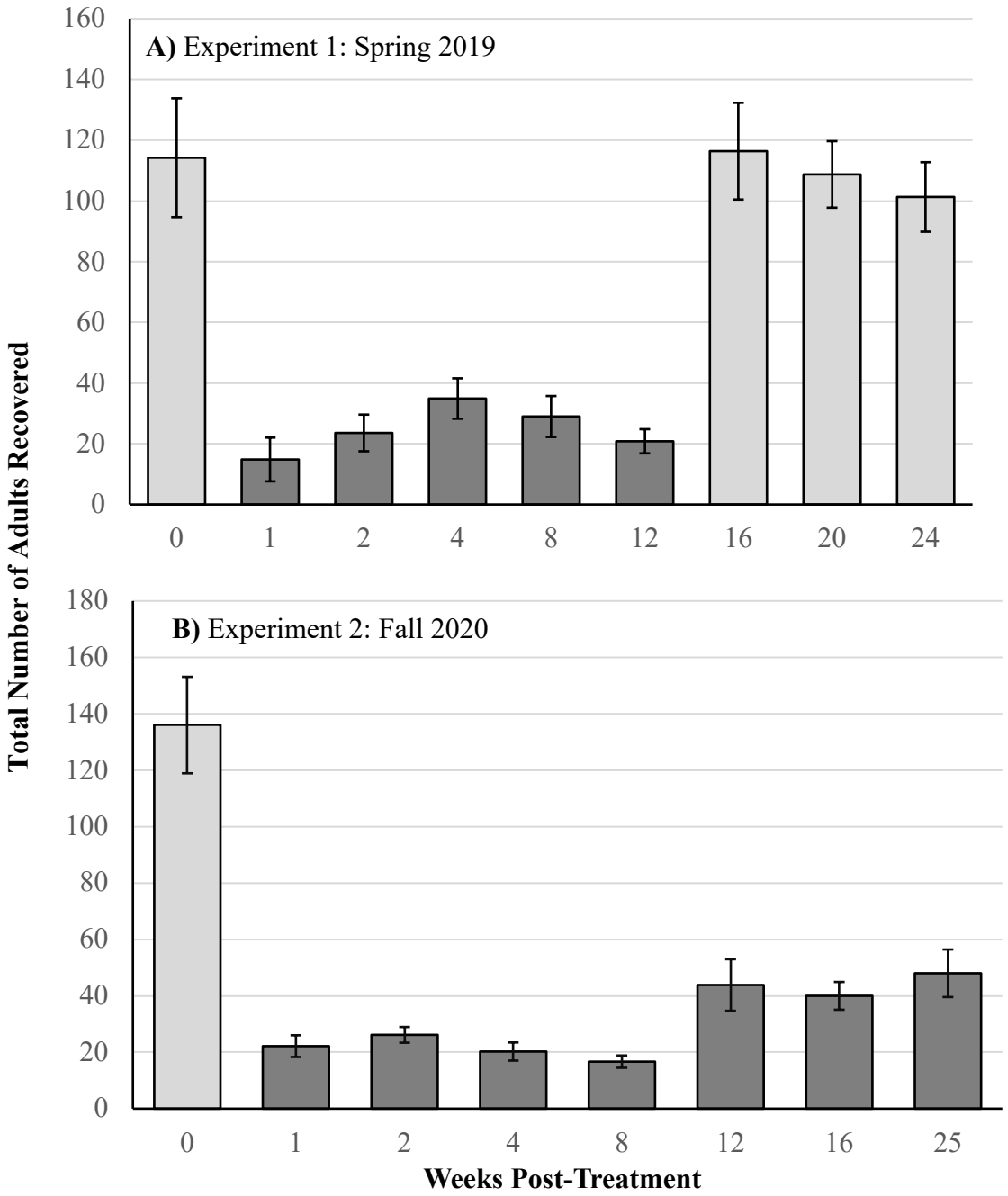

Figure 2.5. Mean (± SE) abundance of adult insects recovered from dung collected prior to treatment (0 weeks) and 1, 2, 4, 8, 12, 16, 20, and 24/25 weeks post-treatment of cattle with LongRange® eprinomectin. Replicate pats (n = 12) were placed outdoors in June 2019 (**A**) and September 2020 (**B**) for 7-10 days. Dark grey bars represent statistically significant reductions from the control.

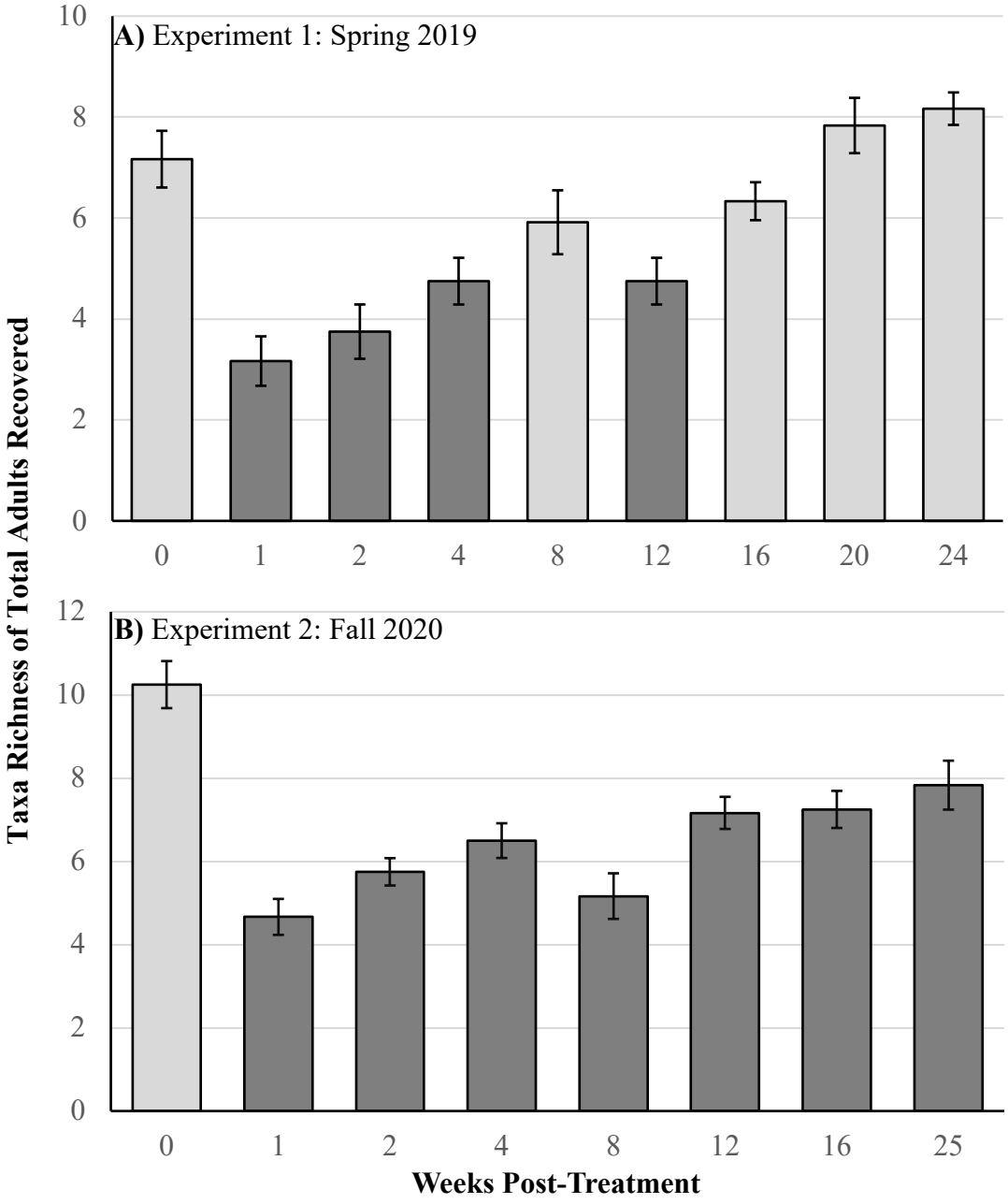

Figure 2.6. Mean (± SE) taxa richness (number of taxa) of adult insects recovered from dung collected prior to treatment (0 weeks) and 1, 2, 4, 8, 12, 16, 20, and 24/25 weeks post-treatment of cattle with LongRange® eprinomectin. Replicate pats (n = 12) were placed outdoors in June 2019 (**A**) and September 2020 (**B**) for 7-10 days. Dark grey bars represent statistically significant reductions from the control.

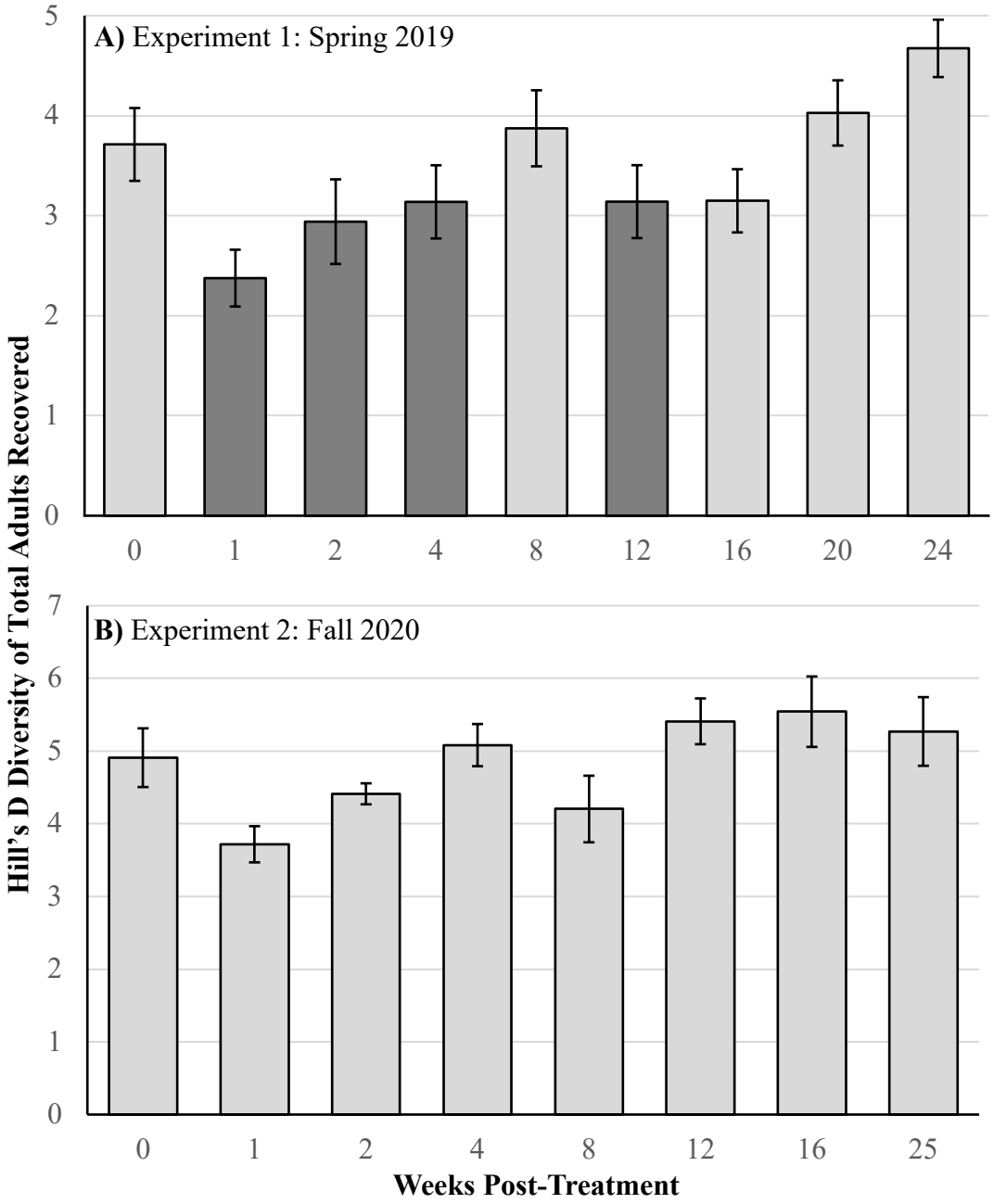

Figure 2.7. Mean (± SE) Hill's D diversity index (= exp[Shannon Index H']) of adult insects recovered from dung collected prior to treatment (0 weeks) and 1, 2, 4, 8, 12, 16, 20, and 24/25 weeks post-treatment of cattle with LongRange® eprinomectin. Replicate pats (n = 12) were placed outdoors in 2019 (**A**) and 2020 (**B**) for 7-10 days. Dark grey bars represent statistically significant reductions from the control.

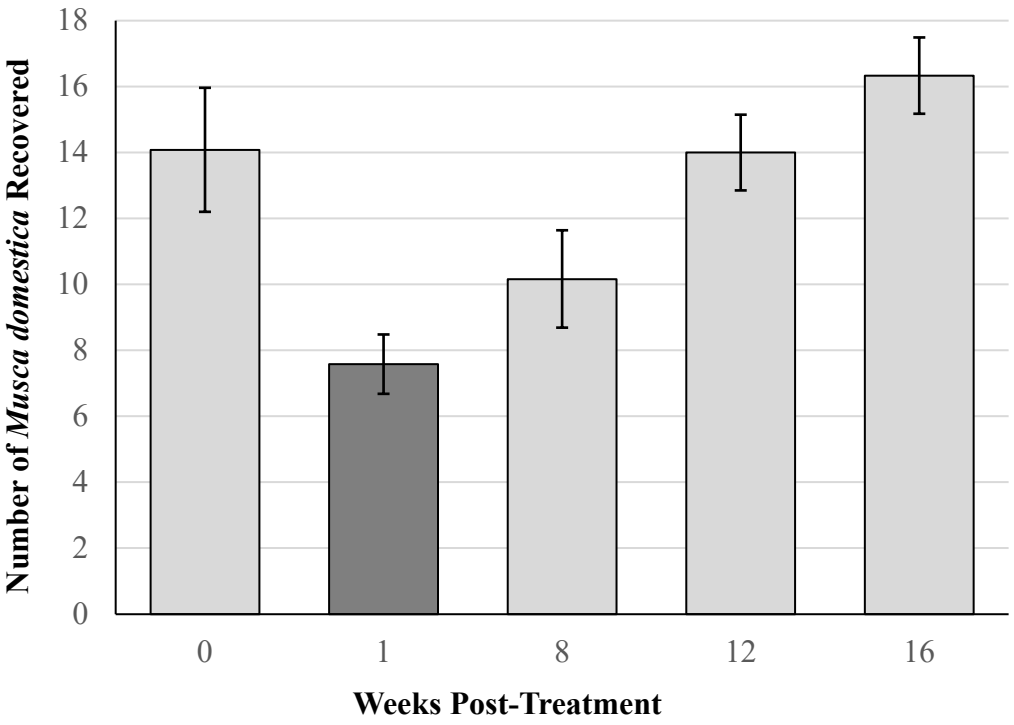

Figure 2.8. Mean (± SE) number of *Musca domestica* (house fly) recovered from dung collected prior to treatment (0 weeks) or 1, 8, 12, or 16 weeks post-treatment of cattle with LongRange® eprinomectin in 2019. Dark grey bars represent statistically significant reductions from the control.

Table 2.1. Mean (± SE) number of adults of individual taxa recovered from eprinomectin-treated cattle dung pats. Dung was collected from cattle at 0 (control) and 1, 2, 4, 8, 12, 16, 20 and 24 weeks post-treatment. Bold font represents treatments that were significantly different from controls ($p < 0.05$). Dung was collected from cattle treated in 2018 and placed outdoors for colonization June 2019. Test statistics are based on Type III Tests of Fixed Effects (df = 8, 88).

Taxonomic group	Control	1 Week	2 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks	20 Weeks	24 Weeks	Duration of effect	F Stat	p-value
COLEOPTERA												
Ptiliidae	47.2 ± 16.3	**3.3 ± 1.6**	**10.7 ± 4.0**	**5.2 ± 2.1**	**3.0 ± 0.8**	**9.2 ± 3.9**	54.2 ± 17.7	19.9 ± 7.9	24.0 ± 7.9	**12 - 16 weeks**	8.11	< **0.0001**
Scarabaeidae												
Otophorus haemorrhoidalis	8.8 ± 3.5	**0.3 ± 0.2**	**0.8 ± 0.4**	**0.5 ± 0.4**	**0.8 ± 0.3**	**0.7 ± 0.3**	7.7 ± 3.7	38.8 ± 11.2	11.0 ± 4.2	**12 - 16 weeks**	14.27	< **0.0001**
Planolinus vittatus	5.3 ± 2.1	0.0 ± 0.0	2.7 ± 0.9	3.3 ± 1.4	4.6 ± 2.9	1.8 ± 0.9	10.2 ± 3.3	8.5 ± 2.7	7.3 ± 3.0	No effect	2.29	**0.0283**
Staphylinidae												
Aleocharinae	22.2 ± 3.2	**1.3 ± 0.4**	**6.9 ± 1.2**	**12.3 ± 3.8**	**9.1 ± 1.6**	**5.4 ± 1.4**	30.2 ± 7.9	19.2 ± 3.4	27.6 ± 3.3	**12 - 16 weeks**	16.40	< **0.0001**

Table 2.1. (*Continued*)

Taxonomic group	Control	1 Week	2 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks	20 Weeks	24 Weeks	Duration of effect	F Stat	p-value
DIPTERA												
Cecidomyiidae	1.0 ± 0.8	3.0 ± 2.1	0.5 ± 0.4	0.3 ± 0.1	0.08 ± 0.08	0.0 ± 0.0	0.2 ± 0.1	0.6 ± 0.4	0.0 ± 0.0	No effect	1.43	0.197
Ceratopogonidae	12.3 ± 6.7	**0.0 ± 0.0**	**0.0 ± 0.0**	7.0 ± 5.8	**0.08 ± 0.08**	**0.3 ± 0.3**	**0.3 ± 0.2**	**0.8 ± 0.5**	**0.2 ± 0.1**	**24 weeks +** [A]	2.20	**0.035**
Chironomidae	3.3 ± 3.3	0.0 ± 0.0	0.0 ± 0.0	1.8 ± 1.4	0.0 ± 0.0	0.0 ± 0.0	0.08 ± 0.08	0.0 ± 0.0	0.0 ± 0.0	No effect	0.99	0.449
Sphaeroceridae	8.4 ± 4.9	**0.2 ± 0.2**	**0.3 ± 0.3**	**1.4 ± 1.2**	**1.3 ± 0.5**	**0.8 ± 0.4**	**1.2 ± 2.1**	9.9 ± 4.3	17.3 ± 3.7	**16 - 20 weeks**	14.27	**< 0.0001**
HYMENOPTERA	1.2 ± 0.6	0.2 ± 0.1	**0.0 ± 0.0**	0.08 ± 0.08	**0.0 ± 0.0**	**0.0 ± 0.0**	0.3 ± 0.3	0.9 ± 0.6	2.1 ± 0.5	**16 - 20 weeks**[B]	4.53	**0.0001**

[A] Based on results of Year 2 (Table 2.2) and previous studies, the value for 4 weeks is considered here to be a biological meaningful (albeit statistically insignificant) reduction in insect number. See Discussion for details.

[B] Based on results of Year 2 (Table 2.2) and previous studies, values for 1, 4 and 12 weeks are considered here to be a biological meaningful (albeit statistically insignificant) reduction in insect number. See Discussion for details.

Table 2.2. Mean (± SE) number of adults of individual taxa recovered from eprinomectin-treated cattle dung pats. Dung was collected from cattle at 0 (control) and 1, 2, 4, 8, 12, 16 and 25 weeks post-treatment. Bold font represents treatments that were significantly different from controls ($p < 0.05$). Dung was collected from cattle treated in 2019 and placed outdoors for colonization September 2020. Test statistics are based on Type III Tests of Fixed Effects (df = 7, 77).

Taxonomic group	Control	1 Week	2 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks	25 Weeks	Duration of effect	F Stat	p-value
COLEOPTERA											
Hydrophilidae											
Cercyon sp.	3.3 ± 0.9	**0.2 ± 0.2**	**0.2 ± 0.1**	**0.4 ± 0.2**	**0.2 ± 0.1**	**0.3 ± 0.3**	**0.3 ± 0.2**	**0.8 ± 0.3**	**25 weeks +**	5.34	**< 0.0001**
Cryptopleurum sp.	12.7 ± 2.5	6.3 ± 2.4	7.9 ± 1.9	**4.1 ± 1.6**	**4.0 ± 1.6**	9.0 ± 3.6	8.2 ± 2.0	13.1 ± 3.6	**8-12 weeks[A]**	2.08	**0.0552**
Ptiliidae	10.8 ± 4.5	**0.9 ± 0.6**	**1.8 ± 0.99**	3.9 ± 1.2	**2.3 ± 0.84**	7.3 ± 3.8	8.3 ± 3.6	11.2 ± 3.3	**8-12 weeks[B]**	4.71	**0.0002**
Scarabaeidae											
Aphodius pedellus	5.7 ± 0.95	5.7 ± 1.1	5.4 ± 0.99	3.6 ± 0.70	**1.7 ± 0.58**	4.8 ± 0.74	6.0 ± 1.2	4.7 ± 0.7	No effect[C]	3.22	**0.0047**
Staphylinidae											
Aleocharinae	3.3 ± 0.6	4.3 ± 1	4.5 ± 0.71	3.2 ± 0.69	2.9 ± 0.93	8.4 ± 1.2	7.0 ± 0.8	3.5 ± 0.9	No effect	4.28	**0.0005**
Staphylininae	5.3 ± 1.0	3.0 ± 0.9	3.8 ± 0.62	3.0 ± 0.67	**2.3 ± 0.5**	6.8 ± 1.8	5.6 ± 0.89	3.6 ± 0.7	No effect[C]	2.86	**0.0104**

Table 2.2. (*Continued*)

Taxonomic group	Control	1 Week	2 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks	25 Weeks	Duration of effect	F Stat	p-value
DIPTERA											
Ceratopogonidae	3.3 ± 1.3	0.0 ± 0.0	0.08 ± 0.08	0.2 ± 0.1	0.3 ± 0.2	0.7 ± 0.4	0.7 ± 0.4	5.7 ± 3.3	No effect	2.59	**0.0186**
Chironomidae	1.2 ± 0.8	0.0 ± 0.0	0.08 ± 0.08	0.08 ± 0.08	0.0 ± 0.0	1.3 ± 0.8	0.4 ± 0.3	1.5 ± 1.4	No effect	1.02	0.4214
Psychodidae	13.4 ± 7.3	**0.0 ± 0.0**	**0.08 ± 0.08**	**0.0 ± 0.0**	**0.08 ± 0.08**	**0.2 ± 0.1**	**0.5 ± 0.3**	**0.9 ± 0.7**	**25 weeks +**	3.28	**0.0042**
Scatopsidae	2.2 ± 0.6	1.4 ± 0.6	1.8 ± 1.1	1.3 ± 0.4	0.83 ± 0.4	3.0 ± 0.8	1.8 ± 0.73	1.1 ± 0.2	No effect	1.17	0.3294
Sepsidae											
Sepsis sp.	68.4 ± 15.8	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.08 ± 0.08**	**0.0 ± 0.0**	**0.08 ± 0.08**	**0.2 ± 0.2**	**25 weeks +**	3.09	**0.0063**
Sphaeroceridae	5.3 ± 1.2	**0.3 ± 0.1**	**0.4 ± 0.3**	**0.3 ± 0.1**	**0.2 ± 0.2**	**1.1 ± 0.4**	**0.6 ± 0.3**	**1.1 ± 0.5**	**25 weeks +**	8.78	**< 0.0001**
HYMENOPTERA	1.2 ± 0.4	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.3 ± 0.1**	**0.08 ± 0.08**	**0.08 ± 0.08**	**0.08 ± 0.08**	**0.0 ± 0.0**	**25 weeks +**	6.01	**< 0.0001**

[A] Based on previous studies, the values for 1 and 2 weeks is considered here to be a biological meaningful (albeit statistically insignificant) reduction in insect number. See Discussion for details.

[B] Based on results of Year 1 (Table 2.2) and previous studies, values for 1, 4 and 12 weeks are considered here to be a biological meaningful (albeit statistically insignificant) reduction in insect number. See Discussion for details.

[C] The statistically significant values in 4 and 8 weeks is considered here to be artifact and not biologically meaningful in terms of residue toxicity. See Discussion for details.

2.10 Appendix

Appendix 2.1. Generalized linear mixed model information for all individual taxa, total abundance, and taxa richness statistically analyzed from data collected in June 2019.

Taxonomic group	Distribution	Link Function	Replicate Covariance
COLEOPTERA			
Ptiliidae	Negative Binomial	Log	No
Otophorus haemorrhoidalis	Negative Binomial	Log	No
Planolinus vittatus	Shifted-*t*	Identity	No
Aleocharinae	Negative Binomial	Log	No
DIPTERA			
Cecidomyiidae	Shifted-*t*	Identity	No
Ceratopogonidae	Shifted-*t*	Identity	No
Chironomidae	Shifted-*t*	Identity	No
Sphaeroceridae	Geometric	Log	No
HYMENOPTERA	Shifted-*t*	Identity	No
Taxa richness	Poisson	Log	**Yes**
Abundance	Geometric	Log	**Yes**

Appendix 2.2. Generalized linear mixed model information for all individual taxa, total abundance, and taxa richness statistically analyzed from data collected in September 2020.

Taxonomic group	Distribution	Link Function	Replicate Covariance
COLEOPTERA			
Cercyon sp.	Geometric	Log	No
Cryptopleurum sp.	Geometric	Log	No
Ptiliidae	Negative Binomial	Log	No
Aphodius pedellus	Negative Binomial	Log	No
Aleocharinae	Negative Binomial	Log	No
Staphylininae	Negative Binomial	Log	No
DIPTERA			
Ceratopogonidae	Shifted-*t*	Identity	No
Chironomidae	Shifted-*t*	Identity	No
Psychodidae	Shifted-*t*	Identity	No
Scatopsidae	Geometric	Log	No
Sepsis sp.	Shifted-*t*	Identity	**Yes**
Sphaeroceridae	Negative Binomial	Log	No
HYMENOPTERA	Shifted-*t*	Identity	No
Taxa richness	Poisson	Log	**Yes**
Abundance	Negative Binomial	Log	No

Chapter 3: Insect attraction to dung defecated by cattle treated with extended-release LongRange® eprinomectin

3.1 Abstract

Coprophilous insects that colonize fresh dung pats provide important ecological services on cattle pastures. Attraction by insects to these pats is a response to the release of odors, which can be altered by the presence of residues in dung of cattle treated with veterinary medicines. Here I examined the effect of residues in dung of cattle treated with the parasiticide LongRange® eprinomectin on insect attraction. In 2019 and 2020, I compared catches of coprophilous insects on native grassland from pitfall traps baited with dung from untreated cattle (week 0, control) or with dung from treated cattle (1, 8, 12, or 16 weeks post-treatment). Treatment did not appear to affect total insect abundance, taxa richness or community diversity. The only exception was a significant reduction in the recovery of total beetles in dung collected 12 weeks-post treatment in 2019, mainly associated with the dung beetle *Chilothorax distinctus* (Coleoptera: Scarabaeidae). Recovery of individual taxa was examined for 20 taxa between both years. In comparisons between the control and the dung collected 1 week post-treatment (i.e., dung with the highest level of residue), residues increased recovery of the dung beetles *Aphodius pedellus* and *Onthophagus nuchicornis* (Coleoptera: Scarabaeidae), of featherwinged beetles (Coleoptera: Ptiliidae) and of blow flies (Diptera: Calliphoridae). Comparisons between the control and other treatments (dung collected 8, 12 and 16 weeks post-treatment) identified five cases for which residues either enhanced (2 cases) or reduced (3 cases) insect recovery. A high level of variation in insect numbers across

samples due to changes in seasonal activity likely confounded the detection of additional treatment effects. These collective results confirm previous reports that: 1) parasiticide residues in cattle dung can affect colonization by coprophilous insects, and 2) the nature of effect appears to be largely unpredictable, likely being influenced by the type of chemical, its concentration in the dung, the insect taxon, and other factors.

3.2 Introduction

Coprophilous (dung-loving) insects provide important ecosystem services on cattle pastures. These insects include a broad range of orders but largely consist of beetles (Coleoptera), flies (Diptera), and parasitoid wasps (Hymenoptera)[1–4]. Through their feeding and tunneling activities, they scatter, aerate, and bury dung pats to return nutrients to soils and prevent the fouling of pastures with undegraded dung[5–9]. Rapid dung degradation also reduces dung-breeding populations of pests (e.g., horn fly [*Haematobia irritans*], stable fly [*Stomoxys calcitrans*], and face fly [*Musca autumnalis*]) and parasites that affect cattle[4,10]. These pest populations are further reduced through non-pest insects via competition, predation, and parasitism[10–14]. Thus, limiting these ecosystem services can potentially reduce overall pasture efficacy and cattle health[10–12,14–16].

Insects are attracted to dung in response to an odor plume of volatile organic compounds (VOCs) released from the fresh deposit[17,18]. VOCs are produced by the decomposition of organic compounds within the pat, driven by a host of microbiota, fungi, and other insects, and are dependent on the diet of the animal voiding the dung[18]. Some VOCs may be common to dung from a large number of animal species, whereas

other VOCs may be associated with only a small number of animals[19–21]. The presence of specific VOCs and their relative concentration in the odor plume allows insects to distinguish between source species of animal and the age of the deposit to locate their preferred type of dung[18,21]. Some insects preferentially colonize dung from animals with similar types of diets (i.e., herbivore, omnivore, carnivore), whereas other insects distinguish between animal species that have been maintained on similar diets (e.g., cattle versus horses)[20,22]. Information for dung insect orders other than coleopterans is limited, although processes involving VOCs are likely driving similar behaviours associated with attraction and preference[23].

Veterinary medicines applied to livestock may alter the VOCs of dung voided by treated animals and thus alter insect preference for dung. Altered attraction has been best documented for dung of cattle treated with the well-known macrocyclic lactone (ML) drugs (e.g., ivermectin, eprinomectin, doramectin, moxidectin). They confer protection against both internal (endo) and external (ecto) parasites and for this reason are commonly called endectocides[24,25]. Following treatment with endectocides, animals excrete residues in their dung comprised mostly of unmetabolized active ingredient[26–28]. The results of numerous studies have shown that these residues can alter rates of colonization of dung from treated animals, although the pattern is unpredictable[29–33]. In a series of three experiments, Floate *et al*. compared captures of coprophilous beetle species in pitfall traps baited with dung from untreated cattle or dung from cattle treated 1 and 4 weeks previously with a topical (pour-on) formulation of ivermectin[29]. Their results demonstrated high inter-specific variability in colonization rates of treated dung, but even for the same species, results varied between sites, years, season, and with residue

concentration[29]. Other factors may include the active ingredient (ML), the formulation of the product (e.g., topical, injectable), the reproductive status of the insect (e.g., gravid, or non-gravid), and the diet of the treated animal[29]. It seems unlikely that insects are responding to VOCs emitted by the residues directly, given that endectocides are reported to bind tightly to particulate matter in dung[34]. Rather, endectocides likely alter the gut microbiome of the treated animal and subsequently the VOCs emitted from their dung. This hypothesis is supported in part by a study by Bernal *et al*., where it was shown that the amino acids present in dung were altered in dung of cattle treated with ivermectin compared to untreated dung[35].

LongRange® eprinomectin (LR) is a relatively new endectocide on the market, boasting an extended-release formulation with two periods of active ingredient release following subcutaneous injection. Whereas previous topical or injectable formulations of endectocides provide protection against nematode parasites for 14-42 days post-treatment, LR can confer protection for up to 150 days post-injection[36,37]. Animals treated with this product excrete a peak concentration of residue approximately one week post-treatment in their feces and a second, smaller peak about 12 weeks post-treatment and a decrease in concentration in between peaks at 8 weeks[38,39]. The bimodal peak of excretion following treatment with LR has been documented for cattle in both blood plasma and in dung[26,39].

To date, only Nieman *et al*. have investigated the non-target effects of LR residues in dung of treated cattle (see Chapter 2), but they only examined its insecticidal activity[39]. No studies have examined the effects of LR residues in dung on attraction and only one study has previously investigated the effects of eprinomectin residues on

attraction[30]. Results of that study indicated that residues increased attraction for 8 taxa and reduced attraction for 12 taxa[30]. However, this study was limited to presence or absence of residues and did not investigate variations in concentration[30].

In the current study, I report results from a two-year pitfall trapping study that examines the attraction/repellency to coprophilic insects of different LR concentrations of residues in dung of treated cattle. This study was completed in the fall of 2019 and repeated in the fall of 2020 on a native grassland pasture in southern Alberta. Whereas most studies on endectocide residues were generally limited to dung beetles, this study also considered dipterans and carrion beetles in one of the two years. If residues repel coprophilous insects from dung of treated cattle, this could mitigate against the insecticidal effects of residues. However, this may also reduce the beneficial services provided by these insects. In contrast, if residues enhance attraction, the risk of coprophilous species being exposed to insecticidal residues would be magnified.

3.3 Materials & Methods

3.3.1 Site Description

Experiments were completed at the Purple Springs Grazing Reserve (PSGR). The PSGR comprises ca. 6 800 ha near the village of Purple Springs in southern Alberta, Canada, which is approximately 70 km east of the city of Lethbridge[40]. Approximately 30% of the overall reserve is native grassland pasture that can support up to 250 cattle each year from May to October[40]. In 2019, traps were placed in an exclosure that prevented access from grazing cattle (30 X 50 m; lat: 49.8264°, long: -111.8938°). In 2020, traps were placed approximately 4km NE of the exclosure (lat: 49.8435°, long: -

111.8454°) in response to greater insect recovery at this site in 2019 in a different study. Research on the PSGR was conducted in accordance with Temporary Field Authorizations issued by Alberta Environment and Parks (TFA181933, TFA193826).

3.3.2 Cattle Treatment & Dung Collection

Dung used in my 2019 experiments (Year 1) was collected from animals treated one time with LR in 2018. An initial collection of fresh dung occurred immediately prior to treatment to act as a control (week 0). Prior to the LR treatment, the animals had not previously been treated with parasiticides. Subsequent collections of fresh dung occurred at 1, 2, 4, 8, 12, 16, 20, and 24 weeks post-treatment of cattle. Dung was collected from multiple pats, bulked per collection date, and mixed by hand. Due to the necessity of assessing all treatments concurrently, dung was frozen (-20C°) until use. Because of the stability of residues in dung, freezing was not expected to alter their composition appreciably[41]. This is supported by Nieman *et al*., who chemically analyzed eprinomectin residues in dung following a freezing period of approximately 2 years[39].

Dung used in my 2020 experiments (Year 2) was collected from animals treated one time with LR in 2019 and followed the same procedure as Year 1 with the exception that week 20 was not collected and a collection occurred at week 25, instead of week 24, due to unavoidable consequences of federal government COVID-19 restrictions in place at the time. All cattle used for dung collections were cared for in accordance with the guidelines of the Canadian Council for Animal Care and with the approval of the Lethbridge Research and Development Centre Animal Care Committee (Protocols 1826, 1916 and 1929).

Subsamples of dung from each collection date in both years were set aside to chemically measure residues. However, due to unforeseen circumstances, these analyses were not completed. Thus, concentrations of residues in dung were presumed to closely follow analyses completed by Nieman *et al*. with peaks at 1 and 12 weeks post-treatment, a low point in between peaks at 8 weeks post-treatment, and a steady decrease following the second peak[39].

In both years, only dung from week 0, and 1, 8, 12, and 16 weeks post-treatment were used for the pitfall traps due to the logistics and time constraints of set up and insect processing. Other collections were used in Chapter 2 for toxicity studies. The collection intervals chosen for the current study were expected to provide a range of residue concentrations based on the reported excretion profile by Nieman *et al*.; i.e., control (0 weeks) high (1 week post-treatment), intermediate (8 and 12 weeks post-treatment), and low (16 weeks post-treatment)[39].

3.3.3 Trap Set Up

The dung-baited pitfall traps used for the current research were as previously described by Kadiri et al and Bezanson *et al*.[1,42]. In brief, traps consisted of two yellow, nested, 2 L plastic buckets where the top was flush with the ground (Fig 3.1). Each trap was filled with approximately 5 cm of a 1:1 diluted, non-toxic antifreeze (propylene glycol) preservative solution. A few drops of dish soap were also added to break the surface tension of the liquid.

Baits were comprised of the dung (250 g/bait) that was initially frozen after collection. Prior to making the baits, dung from the initial collections was thawed for 3

days and mixed by hand. Dung was then measured using a scoop and wrapped in 3-ply cheesecloth before being tied off with a twist tie to prevent insects from entering the dung. Baits were then refrozen (-20 °C) until the day of use. The twist tie also served to suspend the dung bait over the opening of the trap from a sturdy 2.5 cm metal mesh. The mesh was pegged into the ground to prevent small vertebrates from entering, while still allowing access for a range of insects (Fig. 3.1). To facilitate the dispersal of odors emitted by the baits, traps were placed in areas with less foliage, or the foliage was trimmed down surrounding the traps. Bezanson *et al*. previously showed that baits retain their attractiveness for about 3 days and the general attractiveness is not affected by the freezing and storage process[42].

Four circles (3 m diameter) were laid out within the 30 x 50 m study site, each of which was separated by a distance of at least 15 m. Along the circumference of each circle, five traps were placed equidistant so that each trap was approximately 5 m apart (Fig. 3.2). In Year 2, a similar design was used except the circle diameter was 10 m with traps approximately 6 m apart and circles were placed approximately 30 m apart. Traps were rebaited every three or four days, with traps within a circle randomly assigned one of the five types of bait (0, 1, 8, 12, or 16 weeks post-treatment). Insects recovered in traps during the previous trap period were removed at this time. Collected insects were rinsed in water to remove antifreeze residues and then stored in 70% EtOH until they could be processed.

In Year 1, traps were maintained from August 30 to September 27, 2019 (8 trap collections x 4 samples/treatment = 32 samples/treatment). In Year 2, traps were

maintained from August 4 to September 18, 2020 (14 trap collections x 4 samples/treatment = 56 samples/treatment).

3.3.3 Insect processing

In Year 1, processing and identification of insects was limited to coprophilous beetles (Coleoptera: Scarabaeidae, Histeridae, Hydrophilidae) and larger coprophilous flies (Diptera: Anthomyiidae, Calliphoridae, Muscidae, Sarcophagidae, Scathophagidae, Sepsidae). Other taxa were not considered due to time constraints. In Year 2, Silphidae (carrion beetles) and Ptiliidae were recovered in large numbers and were added to identifications. Dipterans, however, were dropped from consideration, given the large amount of time required to process them in Year 1. Insects were identified at least to family but ideally to genus and species. Coleopterans were identified using pinned reference material (LeRDC, Floate lab) and taxonomic keys[43–45]. Dipterans were also identified using taxonomic keys[46,47].

3.4 Statistical Analyses

To assess the effect of residues on the recovery of insects in the pitfall traps, the data analyses were performed on the following response variables: abundance (total individuals), richness (number of identified taxa), and diversity (Hill's D diversity = exp[Shannon Index H']). The mean abundances of individual taxa identified were also analysed. However, to avoid the potential of type II errors (false negatives), individual taxa with fewer than 50 individuals summed across all trap collections were excluded from individual abundance analyses. Because dipterans were not processed from pitfall

traps in Year 2, separate sets of analyses were done for beetles (Year 1, Year 2) and flies (Year 1). Analyses of beetles in individual years were kept separated based on three factors. First, the pitfall traps were operated in different locations between years. Second, the pitfall traps were operated at slightly different periods of time between years. Third, the dung was collected from different groups of cattle between years.

The data were analysed as previously described in Chapter 2, as follows. Because most of the data were non-normally distributed (Shapiro-Wilks test, $p < 0.05$) and variance showed significant heterogeneity (Levene's test, $p < 0.05$) for measures of abundance and richness, data were statistically analyzed using generalized linear mixed models (GLMMs). This was done to avoid the use of rank transformations, which can decrease statistical power[48]. The GLMMs assessed the fixed effects of treatment (weeks post-treatment) and the random effects of replicate. The replicate was designated as a random effect because each collection period per trap was used as an individual sample rather than bulked per replicate. Modelled analyses were completed using the GLIMMIX procedure in SAS® Studio (Statistical Analytic Software)[49]. The distributions for the models (Poisson, negative binomial, or geometric) were chosen based on best fit as determined by the Bayesian information criterion (BIC) after 1000 iterations. If none of these distributions converged after the set number of iterations, a shifted-*t* distribution was used. Finally, the replicate number was used as part of the covariance structure following the same criteria as above (i.e., if the model converged after 1000 iterations and the BIC was lower than the initial model).

Initial tests for a significant difference were based on Type III Tests of Fixed Effects (Wald *F*). In the event of a statistically significant outcome ($p < 0.05$), a post-hoc

Dunnett's test was then used to determine if any of the weeks post-treatment were significantly different (higher or lower) compared to the control treatment (0 weeks). Additional information for the models used in Year 1 and Year 2 are outlined in Appendices 3.1 and 3.2, respectively.

Hill's D diversity data were normally distributed (Shapiro-Wilks test, $p > 0.05$) and variance showed no significant heterogeneity (Levene's test, $p > 0.05$). Therefore, diversity data were assessed using a one-way ANOVA followed by a post-hoc Dunnett's test in the event of a statistically significant result ($p < 0.05$). Hill's D diversity was calculated in R Studio using the Vegan package[50,51]. Statistical analyses were completed in R Studio using base R and the DescTools package[50,52].

3.5 Results

In Year 1, a total of 25,440 insects were processed from the pitfall traps, representing 14 taxa of coleopterans and at least 19 taxa of dipterans. Following identification and comparison with the literature (i.e., checked for dung association), 24,624 individuals (representing 14 taxa of coleopterans and 9 taxa of dipterans; 97 % of the total number counted) remained for statistical analyses. Overall, *Chilothorax distinctus* was the most abundant insect in the pitfall traps and accounted for 74.9% of the total number of all individuals counted. The most abundant dipteran family was Anthomyiidae, which accounted for 4.4% of total individuals.

In Year 1, an effect of treatment was detected on total coleopteran abundance ($F_{4, 152} = 5.05$, $p = 0.0008$). A subsequent post-hoc Dunnett's test revealed that fewer coleopterans were recovered in traps baited with dung collected 12 weeks post-treatment

of cattle with (Fig. 3.3A). However, given the dominance of *C. distinctus*, I considered the possibility that they might be masking treatment effects. Thus, I repeated the abundance analyses without *C. distinctus*. Although Type III Tests of Fixed Effects indicated a statistically significant difference between treatments ($F_{4, 152} = 2.80$, $p = 0.028$), a subsequent post-hoc Dunnett's test revealed no effect ($p > 0.05$) of treatment compared to the control treatment on abundance. There were no detected differences in treatments for coleopteran taxa richness ($F_{4, 152} = 1.09$, $p = 0.365$, Fig. 3.4A) or diversity ($F_{4, 155} = 0.15$, $p = 0.962$, Fig. 3.5A). Similarly, there were no detected differences in treatments for dipteran abundance ($F_{4, 152} = 2.10$, $p = 0.083$, Fig. 3.6), taxa richness ($F_{4, 152} = 1.07$, $p = 0.373$, Fig 3.7), or diversity ($F_{4, 155} = 1.13$, $p = 0.344$, Fig 3.8). Out of the taxonomic groups retained for analyses, individual tests of abundance were run on 12 (i.e., those represented by at least 50 individuals summed across all traps). The results of individual taxa abundance comparisons showed 3 cases of attraction towards treated dung, 2 cases of repulsion against treated dung, and 7 cases that showed no significant effect (Table 3.1).

In Year 2, a total of 10,002 insects were processed from the pitfall traps, representing over 40 taxa of coleopterans. Following identification and comparison with the literature (i.e., checked for dung association), 9,744 individuals (representing 23 taxa of coleopterans and 97% of the total number counted) remained for statistical analyses. The two most abundant taxa, *Onthophagus nuchicornis and C. distinctus,* accounted for 47.3% and 39.4% of total individuals, respectively. Measures of abundance ($F_{4, 272} = 0.20$, $p = 0.9388$, Fig 3.3B), taxa richness ($F_{4, 272} = 0.51$, $p = 0.7257$, Fig 3.4B), and diversity ($F_{4, 275} = 0.93$, $p = 0.450$, Fig 3.5B) showed no significant difference from

control levels. Out of the taxonomic groups retained for analyses, individual tests of abundance were run on 8 (i.e., those represented by at least 50 individuals summed across all traps). Only 2 taxa showed a significant increase in traps baited with dung collected 1 week post-treatment of cattle with LR compared to control (0 weeks) baited traps, while there was no effect of treatment on the abundance of 6 taxa (Table 3.2).

3.6 Discussion

Although the results of community-level analyses between years were consistent, similar measures reported in the literature are infrequent and mostly inconsistent with the current study. The literature is largely focused on insect abundance, often only of individual taxa, with no known reports on taxa richness or diversity. In contrast with the current study, Floate reported more than a two-fold increase in abundance from traps baited with dung voided by cattle treated with ivermectin in one experiment and, in following experiments, a 1.4- and 1.8-fold decrease with the same formulation[29]. Variation between experiments was attributed to the variation in the diet of the cattle, although clear effects were noted in both cases[29]. Similarly, Errouissi & Lumaret reported coleopterans broadly preferred dung voided by cattle treated with ivermectin[53]. Other studies also show cases of overall coleopteran attraction or repellency, although the effect is inconsistent[32,33,54]. However, Strong & Wall and Strong *et al.* showed no significant effect on the frequency of colonization by Aphodiine beetles in dung voided by cattle treated with ivermectin and, in the latter study, moxidectin[55,56]. Finally, Beynon *et al.* also noted no significant difference in biomass of coleopterans or dipterans attracted to dung voided by cattle treated with ivermectin[57]. However, a lack of statistically significant

results in this case may be attributed to large variation in biomass within and between samples or variation in methodology. Thus, the literature broadly supports that effects on attraction can impact colonization of dung insects, although this generalization is somewhat contrasting with the current study and largely only representative of coleopterans.

In both years of the current study, results were consistent in that there were no significant effects on abundance, taxa richness, or diversity analyses. Only total coleopteran abundance in Year 1 showed a significant repellency effect. However, when *C. distinctus* were removed and the data were reanalysed, there was no longer a significant change in any treatment group. Thus, coleopteran abundance trends in Year 1 were dictated by a single, highly abundant species and community abundance as a whole was unchanged. A lack of significant changes in community analyses are speculated to be caused by a combination of factors. First, variation within each treatment group was large, likely caused by the seasonality of some insects being highly abundant in certain collection periods but not others. Seasonality of insects and the impact on variation in trapping has been discussed previously in Floate[30]. Second, individual taxa with opposing preferences may have masked any broader trends. Finally, for measures of richness and diversity, processed insects were mostly limited to abundant groups, increasing the probability they would be present in all traps.

Reports of individual coleopteran taxa are contrasting between years and only somewhat consistent with the literature. For example, in the current study, *Aphodius pedellus* showed a repellency effect in Year 1, but an attractive effect in Year 2. In contrast, in the only attraction study for a formulation of eprinomectin, Floate showed

that there was no effect on *A. pedellus* (as *A. fimetarius*)[30]. However, an earlier study by Floate using ivermectin showed *A. pedellus* (as *A. fimetarius*) had no preference in the spring, but preferred untreated dung in the fall[29]. Thus, presence of this species appears to be highly variable, and previous reports are both contrasting and consistent with the current study. Similar comparisons can be made for *C. distinctus* and *O. nuchicornus*, both of which were also inconsistent between years. In the case of *C. distinctus,* Floate also reported a repellency effect of distinctus to eprinomectin residues, which is consistent with the results in Year 1, albeit inconsistent with results in Year 2 of which there was no effect[30]. However, Floate also previously reported inconsistent results for *C. distinctus* in two different studies, often with variations occurring in sequential years[29,30]. Finally, the abundance of *C. distinctus* was highly variable in both years of the current study due to high catches in some late collections compared to the few recovered in early collections. In one case nearly 2,700 individuals were recovered from a single trap over 3 days in late September. Due to this variation in seasonal abundance, effects of residue on attraction may have been masked[30]. Thus, although the effects are ambiguous, reports in the literature of variation between years are consistent with results in the current study.

Although the cause for variation in the current study is unknown, it is possible to rule out many of the known confounding factors such as formulation, season, and cattle diet as they were kept consistent between both years. Similarly, the same dung collection periods were used to bait the traps, ruling out large differences in residue concentration. I speculate that a combination of three factors could be responsible for variation between years. First, the weather may be a confounding factor, as temperature and precipitation between years were inconsistent. Second, the site at which studies were conducted was

changed between years. Although both were conducted at PSGR within 5 km of each other, the site in Year 2 had considerably more overgrown foliage in addition to being an active grazing site of cattle compared to the exclosure used in Year 1. Finally, dung was collected from different groups of cattle between years, which could introduce variation in dung quality due to animal variability.

Certain results from Year 1 suggested that residue concentration influenced coleopteran preference for untreated or treated dung, as has been reported previously in the literature. Relative to the control, fewer *A. pedellus* were recovered with baits containing intermediate concentrations of residue, whereas no effect of either high or low concentrations of residue was detected. In contrast, *O. nuchicornus* preferred high and low concentrations, but not intermediate concentrations. Other studies have reported a similar phenomenon. For example, Römbke *et al.* showed that using a formulation of ivermectin that *Volinus distinctus* preferred untreated dung, but only compared to dung voided 3 and 4 days post treatment[32]. In contrast, there was no effect for dung voided 2 or 7 days post-treatment, despite similarly containing residues[32]. In another example, Floate showed that *Sphaeridium scarabaeoides*, a species of Hydrophilidae, was repelled by dung voided from cattle treated with a formulation of ivermectin 1 week post-treatment, but not 4 weeks post-treatment[29]. Other reports for altered attraction with varying concentrations of residues are somewhat frequent, although these studies are mostly focus on ivermectin with only one study reporting on doramectin[30,53,54]. The results from the current study in combination with previous literature show that altered attraction likely does not occur as a dichotomy, nor on a scale. In other words, altered attraction is not solely dependent on presence vs. absence of residue, nor does increasing concentrations

of residue beget a stronger effect, and vice versa. However, the reason for this is unknown and speculation on the topic is limited.

Reports in the literature for taxa of dipterans are similarly variable, although more consistent than the coleopterans, albeit comparisons are limited to only a few studies. The most extensive study of dipterans was completed by Floate *et al.*, who investigated attraction to dung containing either ivermectin, doramectin, eprinomectin, or moxidectin residues and is the only known study to investigate eprinomectin in any formulation[30]. Results for Calliphoridae in the current study are contrasting with Floate *et al.* who reported repellency or no effect to dung voided by treated cattle, compared to the case of attraction in the current study[30]. Similarly, reports of *Adia* sp. (Family: Anthomyiidae) in the same study showed 2 cases of repellency to residues of eprinomectin, compared to only 1 case of no effect[30]. In contrast, when all cases of *Adia* sp. are considered for all formulations, *Adia* sp. only showed a preference for untreated dung in 6 cases, whereas there was no effect in 5 cases[30]. Thus, reports of no effect were almost equally as frequent and consistent with the current study. Finally, results of *Ravinia* sp. and *Scathophaga stercoraria* in the current study are also somewhat consistent with reports in Floate *et al*.[30]. They showed 6 and 11 cases of no effect compared to only 2 and 4 cases of repellency to residues, respectively[30]. Reports of dipterans in other studies are also consistent with the current results. The lack of an effect on *S. stercoraria* in the current study is consistent with reports by Webb *et al.* who found that they were equally abundant in pastures of cattle treated with either ivermectin or doramectin[58]. Finally, Beynon *et al*. found no overall effect on dipteran attraction, which is broadly consistent with the current findings for most families of dipterans[57]. I speculate contrasting results

could be caused by differences in the species composing the families between studies, which were unknown or unreported, especially in the cases of Anthomyiidae and Calliphoridae. Additionally, differences in formulation and the season in which studies were conducted could also be responsible, as shown in previous studies[29,30]. Thus, although broad, results are not inconsistent with the literature.

Finally, members of the genus *Nicrophorus* showed no effects on attraction. However, this is not an unexpected result. Members of this genus belong to the family Silphidae, more commonly known as the carrion beetles. Similar to dung insects, carrion beetles also respond and are attracted to VOCs emitted by their food source, hence their inclusion in this study[59]. I speculate that if they are attracted to the VOCs of dung, they do not discriminate between quality or type. This theory is supported by Stavert *et al*., who showed that the VOCs of herbivore dung and carrion vary considerably, with only minor overlap[21]. Alternatively, the carrion beetles may not have been attracted to the dung at all, but rather the presence of decaying beetles within the pitfall trap itself. Thus, the effect of residues in dung on carrion beetle attraction likely does not warrant further study.

3.7 Conclusion

Overall, residues can have an effect on attraction, but reports in the literature are largely inconsistent across endectocides, formulations, concentrations, and species of insect investigated. As shown in the current study, LR has its own unique effect on attraction, even when compared to a pour-on formulation of eprinomectin. Furthermore, it is not just the presence or absence of residues in dung, but rather there is evidence that

their relative concentrations influence individual insect choice. Finally, it is clear that differences in season can influence attraction to dung treated with residues, thus future studies of LR could be conducted to assess the impacts on attraction in the spring, as both years of the current study were conducted in the fall.

There are many knowledge gaps that remain to be addressed. First and foremost, future studies should be done to identify the mechanisms behind altered attraction caused by endectocide residues in relation to VOCs. With this, we may better understand the factors involved that are leading to such contrasting results in the literature. Furthermore, studies should continue to analyze the insect community as a whole where feasible instead of investigating individual orders (i.e., coleopterans) or individual species of insects to better capture a realistic picture of altered attraction. Finally, studies should investigate effects on community composition, not just abundance, richness, or diversity metrics. Correspondingly, insects should be identified to the lowest possible taxonomic resolution to better facilitate comparisons in the literature.

Two criticisms of the current study are raised, although they were largely unavoidable. First, not all random factors were standardized between years, including the potential effect of weather in addition to a minor change in trapping site. Thus, future studies in attraction should aim to eliminate any potential source of variation between repetition and isolated lab studies may be of use to better study attraction with fewer variables. Second, it is unclear how freezing the dung prior to use may impact attraction or alter VOCs. Although freezing has previously been shown to retain the insecticidal activity of MLs, and does not impact general attractiveness, it is unknown if it could alter

specific VOCs such that attraction is altered. Thus, future studies are required to investigate this effect.

3.8 Literature Cited

1. Kadiri, N., Lumaret, J. P. & Floate, K. D. Functional diversity and seasonal activity of dung beetles (Coleoptera: Scarabaeoidea) on native grasslands in southern Alberta, Canada. *Can. Entomol.* **146**, 291–305 (2014).

2. Floate, K. D. & Gill, B. D. Seasonal activity of dung beetles (Coleoptera: Scarabaeidae) associated with cattle dung in southern Alberta and their geographical distribution in Canada. *Can. Entomol.* **130**, 131–151 (1998).

3. Bertone, M. *et al.* Seasonal activity and species composition of dung beetles (Coleoptera: Scarabaeidae and Geotrupidae) inhabiting cattle pastures in North Carolina. *Ann. Entomol. Soc. Am.* **98**, 309–321 (2006).

4. Floate, K. D. Arthropods in Cattle Dung on Canada's Grasslands. in *Arthropods of Canadian Grasslands (Volume 2): Inhabitants of a Changing Landscape* 71–88 (Ottawa: Biological Survey of Canada, 2011).

5. Nichols, E. *et al.* Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. *Biol. Conserv.* **141**, 1461–1474 (2008).

6. Manning, P., Slade, E. M., Beynon, S. A. & Lewis, O. T. Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. *Agric. Ecosyst. Environ.* **218**, 87–94 (2016).

7. Pecenka, J. R. & Lundgren, J. G. The importance of dung beetles and arthropod communities on degradation of cattle dung pats in eastern South Dakota. *PeerJ* **6**, e5220 (2018).

8. Lee, C. M. & Wall, R. Cow-dung colonization and decomposition following insect exclusion. *Bull. Entomol. Res.* **96**, 315–322 (2006).

9. Doube, B. M. Ecosystem services provided by dung beetles in Australia. *Basic Appl. Ecol.* **26**, 35–49 (2018).

10. Fincher, G. T. Effects of dung beetle activity on the number of nematode parasites acquired by grazing cattle. *J. Parasitol.* **61**, 759–762 (1975).

11. Fincher, G. T. *Biological Control of Dung-Breeding Flies Affecting Pastured Cattle*. *Beef Cattle Handbook* (1914).

12. Fincher, G. T. Dung beetles as biological control agents for gastrointestinal parasites of livestock. *J. Parasitol.* **59**, 396–399 (1973).

13. Walsh, G. C. & Cordo, H. A. Coprophilous arthropod community from argentina with species of potential use as biocontrol agents against pest flies. *Environ. Entomol.* **26**, 191–200 (1997).

14. O'Hea, N. M., Kirwan, L. & Finn, J. A. Experimental mixtures of dung fauna affect dung decomposition through complex effects of species interactions. *Oikos* **119**, 1081–1088 (2010).

15. Anderson, J. R., Merritt, R. W. & Loomis, E. C. The insect-free cattle dropping and its relationship to increased dung fouling of rangeland pastures. *J. Econ. Ecol.* **77**, 133–141 (1984).

16. MacLusky, D. S. Some estimates of the areas of pasture fouled by the excreta of dairy cows. *Grass Forage Sci.* **15**, 181–188 (1960).

17. de Jongh, E. Navigation and orientation in Coleoptera: A review of strategies and mechanisms. *Anim. Cogn.* **24**, 1153–1164 (2021).

18. de Bruyne, M. & Baker, T. C. Odor detection in insects: Volatile codes. *J. Chem. Ecol.* **34**, 882–897 (2008).

19. Dormont, L., Jay-Robert, P., Bessière, J. M., Rapior, S. & Lumaret, J. P. Innate olfactory preferences in dung beetles. *J. Exp. Biol.* **213**, 3177–3186 (2010).

20. Dormont, L., Rapior, S., McKey, D. B. & Lumaret, J. P. Influence of dung volatiles on the process of resource selection by coprophagous beetles. *Chemoecology* **17**, 23–30 (2007).

21. Stavert, J., Drayton, B., Beggs, J. & Gaskett, A. The volatile organic compounds of introduced and native dung and carrion and their role in dung beetle foraging behaviour. *Ecol. Entomol.* **39**, 556–565 (2014).

22. Dormont, L., Epinat, G. & Lumaret, J. P. Trophic preferences mediated by olfactory cues in dung beetles colonizing cattle and horse dung. *Environ. Entomol.* **33**, 370–377 (2004).

23. Sladecek, F. X. J., Segar, S. T. & Konvicka, M. Early successional colonizers both facilitate and inhibit the late successional colonizers in communities of dung-inhabiting insects. *Eur. J. Entomol.* **118**, 240–249 (2021).

24. Burg, R. W. *et al.* Avermectins, new family of potent anthelmintic agents: Isolation and chromatographic properties. *Antimicrob. Agents Chemother.* **15**, 368–371 (1979).

25. Hotson, I. K. The avermectins: A new family of antiparasitic agents. *J. South African Vet. Assoc.* **53**, 87–90 (1982).

26. Aksit, D., Korkut, O., Aksoz, E. & Gokbulut, C. Plasma disposition and faecal excretion of eprinomectin following topical and subcutaneous administration in non-lactating dairy cattle. *N. Z. Vet. J.* **64**, 207–211 (2016).

27. González Canga, A. *et al.* The pharmacokinetics and metabolism of ivermectin in domestic animal species. *Vet. J.* **179**, 25–37 (2009).

28. Steel, J. W. Pharmacokinetics and metabolism of avermectins in livestock. *Vet. Parasitol.* **48**, 45–57 (1993).

29. Floate, K. D. Does a repellent effect contribute to reduced levels of insect activity in dung from cattle treated with ivermectin? *Bull. Entomol. Res.* **88**, 291–297 (1998).

30. Floate, K. D. Endectocide residues affect insect attraction to dung from treated cattle: Implications for toxicity tests. *Med. Vet. Entomol.* **21**, 312–322 (2007).

31. Webb, L., Beaumont, D. J., Nager, R. G. & McCracken, D. I. Field-scale dispersal of *Aphodius* dung beetles (Coleoptera: Scarabaeidae) in response to avermectin treatments on pastured cattle. *Bull. Entomol. Res.* **100**, 175–183 (2010).

32. Römbke, J. *et al.* Effects of the parasiticide ivermectin on the structure and function of dung and soil invertebrate communities in the field (Madrid, Spain). *Appl. Soil Ecol.* **45**, 284–292 (2010).

33. Holter, P., Sommer, C., Grønvold, J. & Madsen, M. Effects of ivermectin treatment on the attraction of dung beetles (Coleoptera: Scarabaeidae and Hydrophilidae) to cow pats. *Bull. Entomol. Res.* **83**, 53–58 (1993).

34. Halley, B. A., Nessel, R. J. & Lu, A. Y. H. Environmental aspects of ivermectin usage in livestock: General considerations. in *Ivermectin and Abamectin* 162–172 (Springer, 1989).

35. Bernal, J. L., Del Nozal, M. J., Salas, M., Galante, E. & Lumaret, J. P. HPLC determination of residual ivermectin in cattle dung following subcutaneous injection. *J. Liq. Chromatogr.* **17**, 2429–2444 (1994).

36. Soll, M. D. *et al.* An eprinomectin extended-release injection formulation providing nematode control in cattle for up to 150 days. *Vet. Parasitol.* **192**, 313–320 (2013).

37. CVMP. *Final CVMP Assessment Report for LongRange*. (2018).

38. Forbes, A. B. LongRange™ (eprinomectin 5%) extended-release injection parasiticide and the utility of extended-activity antiparasitics in cattle. *Vet. Parasitol.* **192**, 308–312 (2013).

39. Nieman, C. C. *et al.* Eprinomectin from a sustained release formulation adversely affected dung breeding insects. *PLoS One* **13**, 1–11 (2018).

40. Government of Alberta. *Purple Springs provincial Grazing Reserve; Southeast Region, South Saskatchewan Operational Region*. (2017).

41. Sommer, C. & Steffansen, B. Changes with time after treatment in the concentrations of ivermectin in fresh cow dung and in cow pats aged in the field. *Vet. Parasitol.* **48**, 67–73 (1993).

42. Bezanson, G. A., Dovell, C. D. & Floate, K. D. Changes in the recovery of insects in pitfall traps associated with the age of cow dung bait fresh or frozen at the time of placement. *Bull. Entomol. Res.* **111**, 340–347 (2021).

43. Monk, E., Hinson, K., Szewczy, T., Oench, H. D. & Mccain, C. M. Key to the Carrion Beetles (Silphidae) of Colorado & Neighboring States. 1–14 (2016).

44. Henegouwen, A. V. B. & Foster, G. N. A key to the British species of *Sphaeridium*. *Coleopt.* **28**, 1–12 (2019).

45. Gordon, R. D. & Skelley, P. E. *A Monograph of the Aphodiini Inhabitating the United States and Canada (Coleoptera: Scarabaeidae: Aphodiini).* (The American Entomological Institute, 2007).

46. McAlpine, J. F. *et al. Manual of Nearctic Diptera Volume 1*. (Research Branch, Agriculture Canada, 1981).

47. McAlpine, J. F. *et al. Manual of Nearctic Diptera Volume 2*. (Research Branch, Agriculture Canada, 1987).

48. Bolker, B. M. *et al.* Generalized linear mixed models: A practical guide for ecology and evolution. *Trends Ecol. Evol.* **24**, 127–135 (2009).

49. SAS® Institute. SAS Studio Release 3.81. (2020).

50. R Core Development Team. R: A Language and Environment for Statistical Computing. (2021).

51. Oksanen, J. F., Blanchet, G., Friendly, M., Kindt, R. & Legendre, P. Vegan Package: Community ecology. (2020).

52. Signorell, A. *et al.* DescTools: Tools for Descriptive Statistics. (2021).

53. Errouissi, F. & Lumaret, J. P. Field effects of faecal residues from ivermectin slow-release boluses on the attractiveness of cattle dung to dung beetles. *Med. Vet. Entomol.* **24**, 433–440 (2010).

54. Lumaret, J. P. *et al.* Field effects of ivermectin residues on dung beetles. *Appl. Ecol.* **30**, 428–436 (1993).

55. Strong, L. & Wall, R. Effects of ivermectin and moxidectin on the insects of cattle dung. *Bull. Entomol. Res.* **84**, 403–409 (1994).

56. Strong, L., Wall, R., Woolford, A. & Djeddour, D. The effect of faecally excreted ivermectin and fenbendazole on the insect colonisation of cattle dung following the oral administration of sustained-release boluses. *Vet. Parasitol.* **62**, 253–266 (1996).

57. Beynon, S. A., Peck, M., Mann, D. J. & Lewis, O. T. Consequences of alternative and conventional endoparasite control in cattle for dung-associated invertebrates and ecosystem functioning. *Agric. Ecosyst. Environ.* **162**, 36–44 (2012).

58. Webb, L., Beaumont, D. J., Nager, R. G. & McCracken, D. I. Effects of avermectin residues in cattle dung on yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae) populations in grazed pastures. *Bull. Entomol. Res.* **97**, 129–138 (2007).

59. Kalinová, B., Podskalská, H., Růžička, J. & Hoskovec, M. Irresistible bouquet of death -how are burying beetles (Coleoptera: Silphidae: Nicrophorus) attracted by carcasses. *Naturwissenschaften* **96**, 889–899 (2009).

3.9 Figures & Tables

Figure 3.1. Example of dung-baited pitfall trap set-up used to assess insect attraction to dung treated with LongRange® eprinomectin. The metal grid prevents small vertebrates from falling in while also allowing insects access.

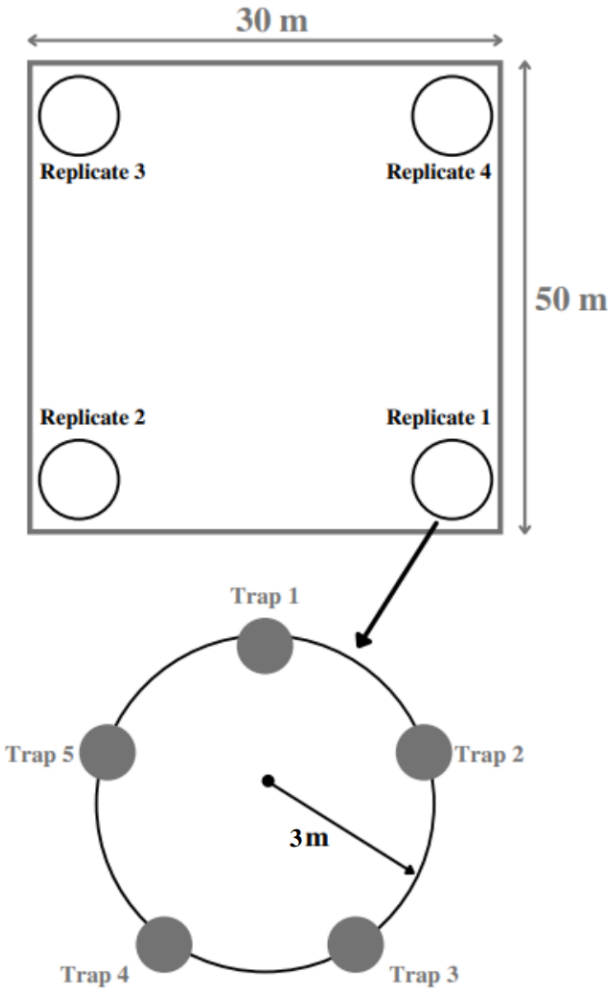

Figure 3.2. Arrangement of dung-baited pitfall traps within an exclosure at Purple Spring Grazing Reserve. Open circles represent replicates of identical trap arrangements. Solid circles represent individuals traps (Fig. 3.1). Traps were used to test for altered attraction of LongRange® eprinomectin residues on coprophilous insects. Image is not to scale.

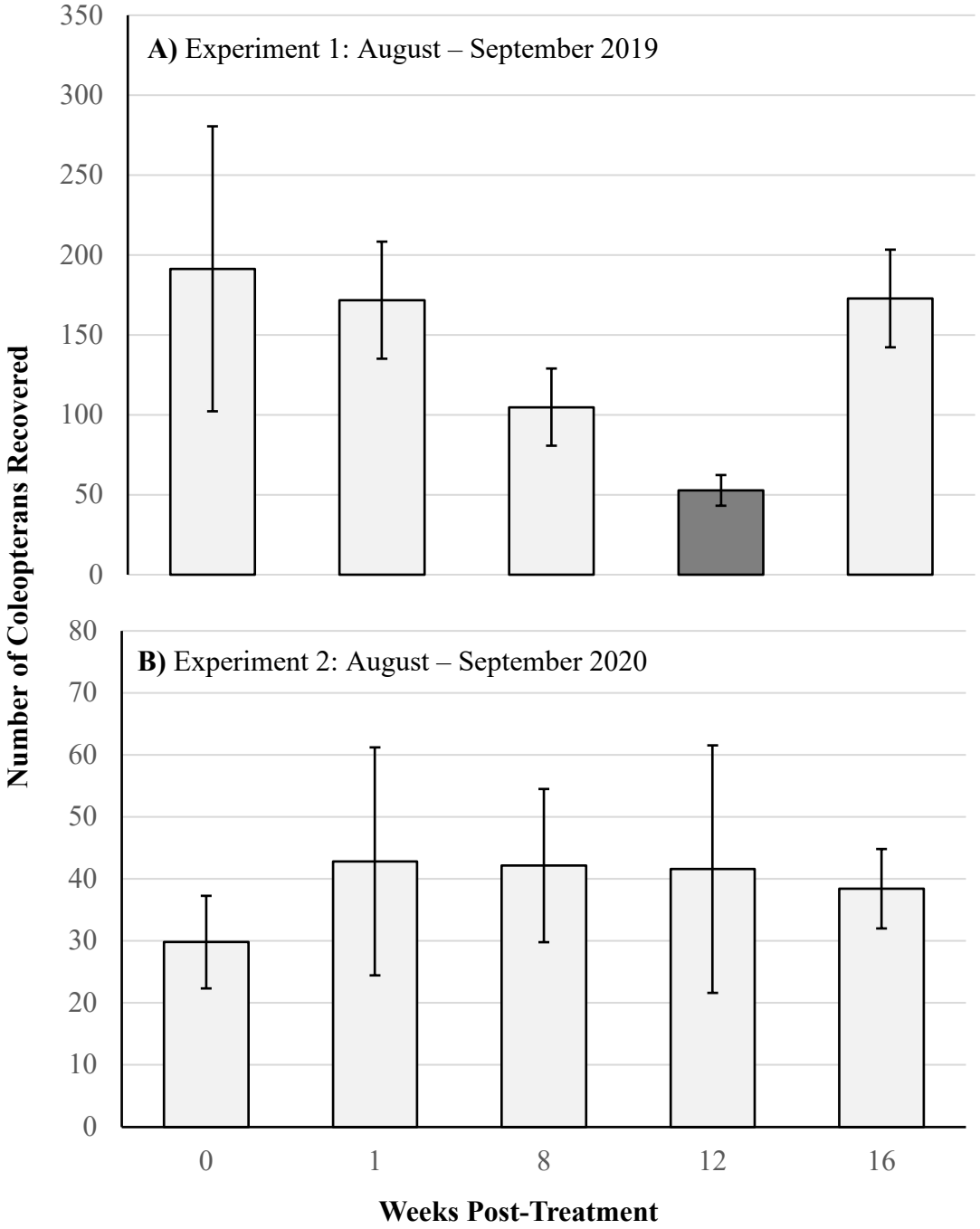

Figure 3.3. Mean (± SE) abundance of coleopterans recovered per pitfall trap per 3-4 day collection period at Purple Springs Grazing Reserve in 2019 (**A**) and 2020 (**B**). Traps were baited with dung collected from cattle prior to treatment at 0 weeks (control) or dung collected at 1, 8, 12, or 16 weeks post-treatment with LongRange® eprinomectin. Dark gray bars represent statistically significant differences from the control.

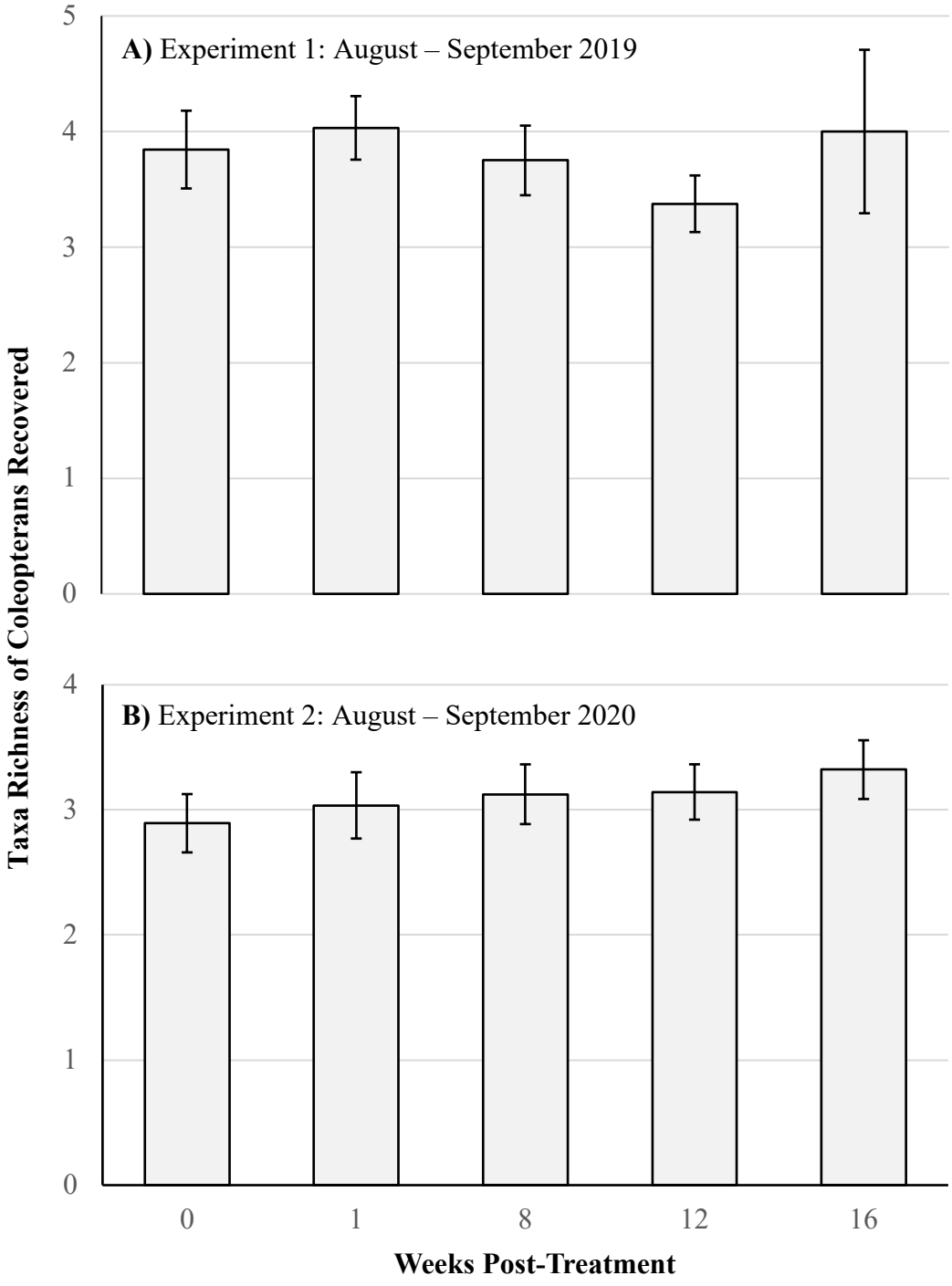

Figure 3.4. Mean (± SE) taxa richness of coleopterans recovered per pitfall trap per 3-4 day collection period at Purple Springs Grazing Reserve in 2019 (**A**) and 2020 (**B**). Traps were baited with dung collected from cattle prior to treatment at 0 weeks (control) or dung collected at 1, 8, 12, or 16 weeks post-treatment with LongRange® eprinomectin. No statistically significant differences were found.

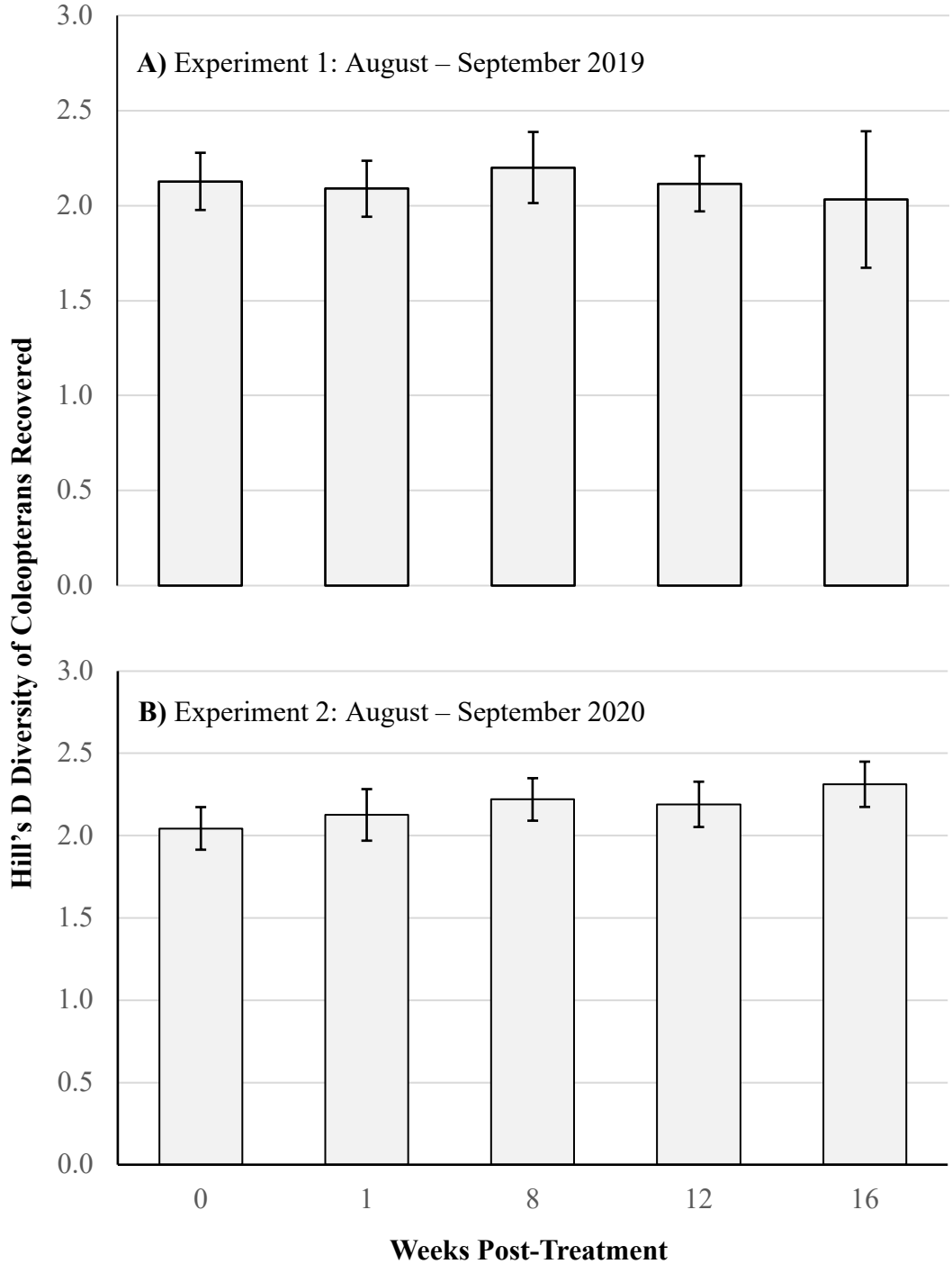

Figure 3.5. Mean (± SE) Hill's D diversity (= exp[Shannon Index H']) of coleopterans recovered per pitfall trap per 3-4 day collection period at Purple Springs Grazing Reserve in 2019 (**A**) and 2020 (**B**). Traps were baited with dung collected from cattle prior to treatment at 0 weeks (control) or dung collected at 1, 8, 12, or 16 weeks post-treatment with LongRange® eprinomectin. No statistically significant differences were found.

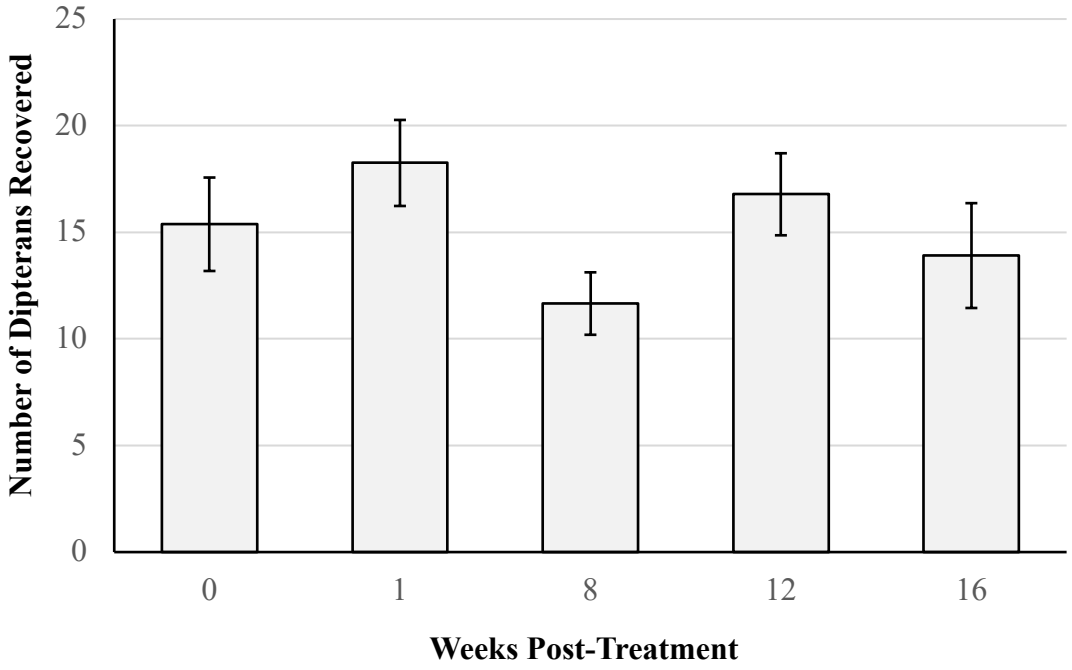

Figure 3.6. Mean (± SE) abundance of dipterans recovered per pitfall trap per 3-4 day collection period at Purple Springs Grazing Reserve in 2019. Traps were baited with dung collected from cattle prior to treatment at 0 weeks (control) or dung collected at 1, 8, 12, or 16 weeks post-treatment with LongRange® eprinomectin. Pitfall traps were run from August – September 2020. No statistically significant differences were found.

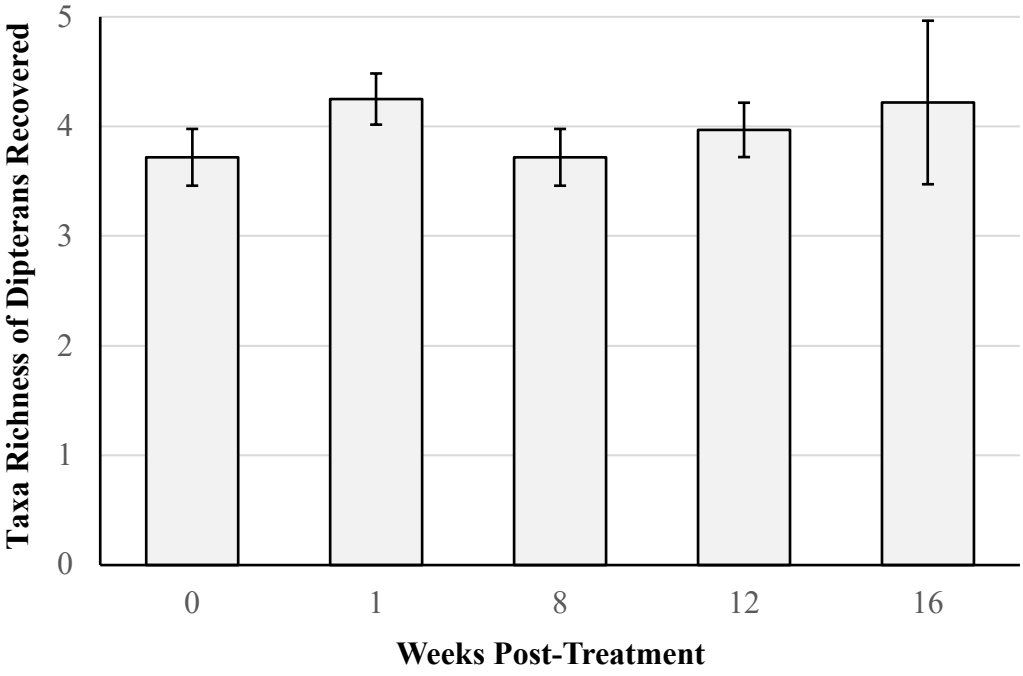

Figure 3.7. Mean (± SE) taxa richness of dipterans recovered per pitfall trap per 3-4 day collection period at Purple Springs Grazing Reserve in 2019. Traps were baited with dung collected from cattle prior to treatment at 0 weeks (control) or dung collected at 1, 8, 12, or 16 weeks post-treatment with LongRange® eprinomectin. No statistically significant differences were found.

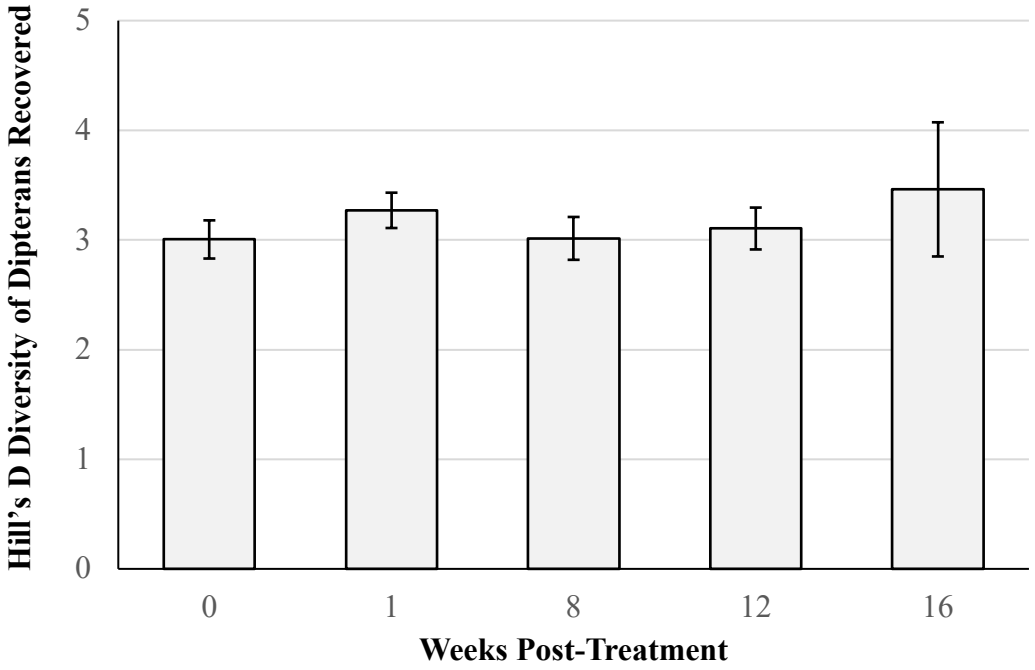

Figure 3.8. Mean (± SE) Hill's D diversity (= exp[Shannon Index H']) of dipterans recovered per pitfall trap per 3-4 day collection period at Purple Springs Grazing Reserve in 2019. Traps were baited with dung collected from cattle prior to treatment at 0 weeks (control) or dung collected at 1, 8, 12, or 16 weeks post-treatment with LongRange® eprinomectin. No statistically significant differences were found.

Table 3.1. Mean (± SE) number of adults of individual taxa recovered per dung-baited pitfall trap per collection date. Dung was collected from cattle at 0 (control) and 1, 8, 12 and 16 weeks post-treatment with LongRange® eprinomectin. Bold font represents treatments that were significantly different from controls. Dung was collected from cattle treated in 2018 and traps were run from August 30 to September 27, 2019. Test statistics are based on a Type III Test of Fixed Effects (df = 4, 152).

Taxonomic Group	Control	1 Week	8 Weeks	12 Weeks	16 Weeks	F Stat	P-Value
COLEOPTERA							
Histeridae	0.44 ± 0.15	0.72 ± 0.21	0.88 ± 0.28	0.44 ± 0.12	**1.3 ± 0.37**	2.39	**0.0535**
Hydrophilidae							
Sphaeridium lunatum	0.44 ± 0.26	0.38 ± 0.26	0.31 ± 0.18	0.41 ± 0.16	0.62 ± 0.32	0.37	0.828
Sphaeridium scarabaeoides	0.41 ± 0.14	0.28 ± 0.14	0.31 ± 0.10	0.31 ± 0.15	0.38 ± 0.15	0.13	0.970
Scarabaeidae							
Aphodius pedellus	2.0 ± 0.69	1.6 ± 0.57	**0.50 ± 0.16**	**0.50 ± 0.15**	0.72 ± 0.21	3.90	**0.0048**
Canthon praticola	2.2 ± 0.71	4.3 ± 1.1	3.2 ± 0.95	1.7 ± 0.63	4.5 ± 1.3	2.30	0.0614
Chilothorax distinctus	173.6 ± 89.1	142.6 ± 35.5	79.8 ± 22.4	**37.2 ± 8.5**	143.1 ± 25.4	8.38	**< 0.0001**
Onthophagus nuchicornus	11.8 ± 2.6	**21.4 ± 4.0**	19.5 ± 4.7	12.0 ± 3.2	**21.8 ± 4.1**	2.95	**0.0219**
DIPTERA							
Anthomyiidae	6.6 ± 1.2	8.5 ± 1.1	5.3 ± 0.83	7.3 ± 1.1	6.4 ± 0.84	1.46	0.218
Calliphoridae	0.41 ± 0.14	**1.4 ± 0.35**	0.94 ± 0.33	0.94 ± 0.29	1 ± 0.30	1.89	0.116
Muscidae							
Neomyia cornicina	5.1 ± 1.1	4.5 ± 0.81	2.6 ± 0.53	4.9 ± 0.94	3.6 ± 0.59	1.84	0.123
Sarcophagidae							
Ravinia sp.	2.5 ± 0.34	2.6 ± 0.40	2.5 ± 0.36	2.7 ± 0.39	2.8 ± 0.43	0.06	0.992
Scathophagidae							
Scathophaga stercoraria	2.8 ± 0.66	3.3 ± 0.66	2.5 ± 0.47	3.3 ± 0.76	2.4 ± 0.42	0.58	0.678

Table 3.2. Mean (± SE) number of adults of individual taxa recovered per dung-baited pitfall trap per collection date. Dung was collected from cattle at 0 (control) and 1, 8, 12 and 16 weeks post-treatment with LongRange® eprinomectin. Bold font represents treatments that were significantly different from controls. Dung was collected from cattle treated in 2018 and traps were run from August 30 to September 27, 2020. Test statistics are based on a Type III Test of Fixed Effects (df = 4, 272).

Taxonomic Group	0 Weeks (Control)	1 Week	8 Weeks	12 Weeks	16 Weeks	F Stat	P-Value
COLEOPTERA							
Histeridae	0.32 ± 0.18	0.41 ± 0.17	0.18 ± 0.06	0.14 ± 0.06	0.39 ± 0.12	1.10	0.3577
Ptiliidae	0.07 ± 0.03	**0.59 ± 0.20**	0.13 ± 0.06	0.13 ± 0.06	0.23 ± 0.08	3.94	**0.0040**
Scarabaeidae							
Aphodius pedellus	0.23 ± 0.09	**0.79 ± 0.27**	0.21 ± 0.07	0.34 ± 0.11	0.43 ± 0.14	2.40	**0.0501**
Chilothorax distinctus	4.6 ± 3.6	19.3 ± 17.9	17.7 ± 12.2	20.4 ± 19.8	6.7 ± 5.3	0.97	0.6152
Onthophagus nuchicornus	20.9 ± 5.9	13.1 ± 3.2	16.8 ± 2.7	17.3 ± 3.0	14.5 ± 2.9	0.67	0.6104
Silphidae							
Nicrophorus hybridus	1.21 ± 0.49	1.0 ± 0.50	0.79 ± 0.21	1.4 ± 0.50	1.0 ± 0.34	0.50	0.7327
Nicrophorus marginatus	0.30 ± 0.17	0.48 ± 0.30	0.32 ± 0.15	0.38 ± 0.14	0.60 ± 0.29	0.68	0.6033
Nicrophorus obscurus	0.96 ± 0.35	1.3 ± 0.86	0.98 ± 0.30	1.2 ± 0.38	1.1 ± 0.36	0.13	0.9713

3.10. Appendix

Appendix 3.1. Generalized linear mixed model information for all individual taxa statistically analyzed in addition to the total abundance and taxa richness of coleopterans and dipterans. Data was collected from dung-baited pitfall traps August to September 2019.

Taxonomic group	Distribution	Link Function	Replicate Covariance
COLEOPTERA			
Histeridae	Negative Binomial	Log	No
Sphaeridium lunatum	Negative Binomial	Log	No
Sphaeridium scarabaeoides	Negative Binomial	Log	No
Aphodius pedellus	Geometric	Log	
Canthon praticola	Negative Binomial	Log	No
Chilothorax distinctus	Negative Binomial	Log	**Yes**
Onthophagus nuchicornus	Geometric	Log	No
Taxa richness	Negative Binomial	Log	**Yes**
Total abundance	Negative Binomial	Log	No
DIPTERA			
Anthomyiidae	Negative Binomial	Log	No
Calliphoridae	Negative Binomial	Log	No
Neomyia cornicina	Geometric	Log	No
Ravinia sp.	Geometric	Log	No
Scathophaga stercoraria	Geometric	Log	No
Taxa richness	Negative Binomial	Log	**Yes**
Total abundance	Negative Binomial	Log	No

Appendix 3.2. Generalized linear mixed model information for all individual taxa statistically analyzed in addition to the total abundance and taxa richness. Data was collected from dung-baited pitfall traps August to September 2020.

Taxonomic group	Distribution	Link Function	Replicate Covariance
COLEOPTERA			
Histeridae	Negative Binomial	Log	No
Ptiliidae	Negative Binomial	Log	No
Aphodius pedellus	Negative Binomial	Log	No
Chilothorax distinctus	Negative Binomial	Log	No
Onthophagus nuchicornus	Negative Binomial	Log	No
Nicrophorus hybridus	Negative Binomial	Log	No
Nicrophorus marginatus	Negative Binomial	Log	No
Nicrophorus obscurus	Negative Binomial	Log	No
Taxa richness	Poisson	Log	No
Total abundance	Negative Binomial	Log	No

Chapter 4: Concluding Remarks & Future Directions

4.1 Summary & General Remarks

The goal of my thesis was to assess the non-target effects associated with use of the novel parasiticide LongRange® eprinomectin (LR) on rates of colonization, diversity, and abundance of coprophilous (dung-loving) insects. The community of insects that inhabit dung is complex but ultimately provides important services to pasture ecosystems such as rapid dung burial, nutrient cycling, and the natural biocontrol of pest insects[1–5]. Cattle treated with parasiticides may excrete residues that maintain their insecticidal activity and are toxic to coprophilous insects or affect their colonization of a dung pat. Residues present in the dung of cattle are of particular concern for LR, for which treated animals excrete a pulse of residue in the first week post-treatment, followed by a second pulse approximately 12 weeks post-treatment[6,7]. Furthermore, it has been shown that LR residues continue to be excreted for at least 20 weeks post-treatment[6]. Only one other study has investigated the non-target effects of LR[6]. The results of that study confirmed concerns regarding the toxicity of residues, but was limited in terms of the taxa examined, the duration of tested effects, and it did not assess the effect of residues on insect attraction[6]. In two data chapters (Chapters 2 & 3), the current thesis aimed to address concerns that may be raised about LR residues on the dung insect community and contributes to the limited literature on this novel formulation.

In Chapter 2, I conducted a field study to investigate the toxicity of residues on insects breeding in dung of cattle treated with LR to build and expand upon the work pioneered by Nieman *et al.*[6]. The field study, replicated in each of two years, revealed

that certain taxa (e.g., Ptiliidae, *Otophorus haemorrhoidalis, Cercyon* sp., Ceratopogonidae, Psychodidae, *Sepsis* sp., Sphaeroceridae, Hymenoptera) were highly susceptible to residues such that effects on the insect community (abundance, richness, diversity) were detectable for dung from cattle treated up to and including 25 weeks post-treatment. This is the first time residues of any parasiticide have been shown to suppress insect emergence for this length of time, likely due to the unique formulation of LR. Although it is unknown if residue toxicity persists beyond this range, results suggest that, if cattle are treated in the spring, they will defecate residues at high enough concentrations to impact some taxa of dung breeding insects for the entirety of the grazing season.

Also included in Chapter 2, was a companion study completed in the lab to test the toxicity of LR residues in dung of treated cattle on the larvae of house flies (*Musca domestica*). In a comparison of dung from untreated cattle or cattle treated 1, 8, 12, and 16 weeks previously, an effect of treatment was only detected for 1 week post-treatment. Thus, results from the bioassay showed that house flies are only susceptible to the highest concentrations of residues but are otherwise highly resistant. The insecticidal activity of residues in dung of treated cattle may be either beneficial or undesirable, depending on the taxa affected. House flies, although mostly only a nuisance pest of cattle, are closely-related to face fly (*Musca autumnalis*) which are vectors of the bacteria *Moraxella bovis* and can cause pink eye in cattle[8,9]. In contrast, if residues reduce the numbers of dung beetles, this could result in the loss of essential ecosystem services[10]. Thus, it may initially seem that the toxicity of residues is beneficial to some extent but this trade-off with the loss of beneficial insects is short-lived and unequivocal.

The consequences to dung-breeding insects of sustained LR use on pastured cattle will be influenced by their ability to develop resistance to faecal residues, which presumably will depend upon the number of generations a given insect taxon has per year. This is concerning for species that have only one or two new generations per year, specifically species of coleopterans. For example, *Otophorus haemorrhoidalis* populations were suppressed for 12 to 16 weeks post-treatment in the current study, but it has been shown they likely only have one or two generations in a year[11,12]. If these intermittent events align with the highest toxicity weeks, such as weeks 1 or 12 post-treatment of cattle with LR, resilience to lower concentrations of residues may not be as mitigating as it may initially seem. In contrast, most pests of livestock have multiple generations per year. For example, the horn fly (*Haematobia irritans*) experiences relatively short generation times with only a couple of weeks between each new generation[13,14]. Thus, certain pest insects could acquire resistance much faster compared to those insects that have fewer generations in a year. Consideration for these insects that have contrasting life-history strategies in dung could usefully address this issue.

Chapter 3 investigated the attraction response of coprophilous insects to dung of cattle treated with LR. Dung-baited pitfall traps were employed in each of two subsequent years to better understand how residues may influence dung colonization. Analyses at the community level (abundance, richness, diversity) revealed there was no preference for untreated or treated dung overall. Results for individual taxa showed minor cases of attraction or repellency, but these patterns were inconsistent between years (i.e., for Histeridae, *Aphodius pedellus, Chilothorax distinctus, Onthophagus nuchicornus*).

This phenomenon of contrasting results with year is not uncommon in the literature, although no consensus has been reached for why this occurs. Although factors such as formulation, active ingredient, cattle diet, and season were standardized between years in the current study, other important factors such as temperature and moisture conditions were not. Thus, I speculate that a change of site and differences in weather between years may in part be responsible for the observed differences.

The consequences of insects being more attracted to, or repelled by, dung containing residues has further implications on the pasture ecosystem services they provide. An overall repellency effect may benefit dung insects as they are less likely to colonize highly toxic dung. However, this may also reduce insect activity and thus slow the rate of dung degradation and nutrient cycling. In contrast, if residues increase insect activity in dung, pat degradation may be accelerated but the effects of toxicity could be amplified within the dung insect community. Thus, neither situation is entirely beneficial nor detrimental.

4.2 Future Considerations

Future investigations into the toxicity of residues in dung, for both LR and other formulations of parasiticides, should continue to include lab components where feasible. Although field studies more accurately represent the "natural" situation on pasture while involving the entire community of coprophilous insects, they are often limited by the stochasticity of natural systems. For example, the random colonization of pats cannot be controlled and often leads to large variation that could mask trends caused by toxicity. Similarly, mortality factors such as predation, parasitism, and competition may influence

the results. Thus, lab bioassays or tightly controlled field experiments on dung-breeding insects retain value in the literature and should continue to be conducted on a broad range of members from the dung-breeding community.

Although studies concerning the effects of parasiticides on attraction are common, there are still considerable knowledge gaps, some of which may explain the contrasting results of such studies. Primarily, the critical information on how residues alter dung to cause this change in attraction is unknown. I speculate that residues indirectly alter the volatile organic compounds (VOCs) emitted from dung, but this has not been tested explicitly. Thus, chemical-based studies comparing the difference in VOCs in treated vs. untreated dung may clarify the relationship between residues and attraction.

To date, the continuous use of parasiticides across subsequent years and their effect on dung insect communities at the level of the pasture remains a subject of debate. In other words, it is unclear whether non-target effects are sufficient to cause additive changes within the insect community, or to the broader pasture food web through bioaccumulation, with applications of parasiticides across multiple years or at what point communities of dung insects can no longer recover. Thus, future research on community recovery, long-term usage of parasiticides, and bioaccumulation is warranted. Studies could include multiple-year pitfall-trapping experiments on pastures where cattle are being continuously treated across multiple years, similar to smaller-scale studies by Krüger and Scholtz[15,16] or Verdú *et al.*[17]. Similarly, to better understand recovery of the insect community, studies could expand on the experiments by Ambrožová *et al.*, who showed that changes in the insect community were persistent for at least 8 weeks after parasiticides were no longer detectable in dung[18].

Finally, although there are many studies characterizing the non-target effects of residues on the dung insect community, the literature is largely focused on formulations of macrocyclic lactones (MLs) with little regard to other chemical groups in formulations available. Even within the MLs, most studies report on formulations of ivermectin with fewer focusing on its sister chemicals (i.e., eprinomectin, doramectin, moxidectin). This is of no surprise, as formulations of MLs, especially ivermectin, are more prevalent in the market compared to others[19,20]. However, future studies may aim to investigate other formulations and chemical classes in equally as much depth as ivermectin.

4.3 Conclusion

To better understand the impact of parasiticide residues in dung, studies on both lethal and non-lethal effects associated with current and continuous usage across years are critical. Furthermore, it is important to consider the coprophilous insect community as a whole because many taxa play diverse roles within the dung pat and often respond in different ways to residues. Finally, special consideration should be given to those formulations and chemical classes that are currently understudied. Although this thesis begins to address some of the concerns of the potential non-target effects of LR to dung-breeding insects, there are likely many more effects that will require further investigation.

4.4 Literature Cited

1. Kadiri, N., Lumaret, J. P. & Floate, K. D. Functional diversity and seasonal activity of dung beetles (Coleoptera: Scarabaeoidea) on native grasslands in southern Alberta, Canada. *Can. Entomol.* **146**, 291–305 (2014).

2. Manning, P., Slade, E. M., Beynon, S. A. & Lewis, O. T. Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. *Agric. Ecosyst. Environ.* **218**, 87–94 (2016).

3. Nichols, E. *et al.* Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. *Biol. Conserv.* **141**, 1461–1474 (2008).

4. Pecenka, J. R. & Lundgren, J. G. The importance of dung beetles and arthropod communities on degradation of cattle dung pats in eastern South Dakota. *PeerJ* **6**, e5220 (2018).

5. Floate, K. D. Arthropods in Cattle Dung on Canada's Grasslands. in *Arthropods of Canadian Grasslands (Volume 2): Inhabitants of a Changing Landscape* vol. 2 71–88 (Ottawa: Biological Survey of Canada, 2011).

6. Nieman, C. C. *et al.* Eprinomectin from a sustained release formulation adversely affected dung breeding insects. *PLoS One* **13**, 1–11 (2018).

7. Forbes, A. B. LongRange™ (eprinomectin 5%) extended-release injection parasiticide and the utility of extended-activity antiparasitics in cattle. *Vet. Parasitol.* **192**, 308–312 (2013).

8. Gerhardt, R. R., Allen, J. W., Greene, W. H. & Smith, P. C. The tole of face flies in an episode of infectious bovine keratoconjunctivitis. *J. Am. Vet. Med. Assoc.* **180**, 156–159 (1982).

9. Arends, J. J. & Wright, R. E. Transmission of *Moraxella bovis* in the laboratory by the face fly (Diptera: Muscidae). *J. Econ. Entomol.* **75**, 816–818 (1982).

10. Anderson, J. R., Merritt, R. W. & Loomis, E. C. The insect-free cattle dropping and its relationship to increased dung fouling of rangeland pastures. *J. Econ. Ecol.* **77**, 133–141 (1984).

11. Holter, P. Resource utilization and local coexistence in a guild of scarabaeid dung beetles (*Aphodius* spp.). *Oikos* **39**, 213–227 (1982).

12. Mohr, C. O. Cattle droppings as ecological units. *Ecol. Monogr.* **13**, 275–298 (1943).

13. Melo, L. R. B. *et al.* Development and number of generations of *Haematobia irritans* (Diptera: Muscidae) in bovine fecal masses in the semiarid region of Brazil. *Vet. Parasitol. Reg. Stud. Reports* **20**, 100411 (2020).

14. Floate, K. D., Lysyk, T. J. & Gibson, G. A. P. *Haematobia irritans* L., Horn Fly, *Musca domestica* L., House Fly, and *Stomoxys calcitrans* (L.), Stable Fly (Diptera: Muscidae). in *Biological Control Programmes in Canada, 2001-2012* (eds. Mason, P. G. & Gillepsie, D.) 182–191 (CABI Publishing, 2013).

15. Krüger, K. & Scholtz, C. H. Changes in the structure of dung insect communities after ivermectin usage in a grassland ecosystem. I. Impact of ivermectin under drought conditions. *Acta Oecologica* **19**, 425–438 (1998).

16. Krüger, K. & Scholtz, C. H. Changes in the structure of dung insect communities after ivermectin usage in a grassland ecosystem. II. Impact of ivermectin under high-rainfall conditions. *Acta Oecologica* **19**, 439–451 (1998).

17. Verdú, J. R. *et al.* Ivermectin residues disrupt dung beetle diversity, soil properties and ecosystem functioning: An interdisciplinary field study. *Sci. Total Environ.* **618**, 219–228 (2018).

18. Ambrožová, L. *et al.* Lasting decrease in functionality and richness: Effects of ivermectin use on dung beetle communities. *Agric. Ecosyst. Environ.* **321**, 1–9 (2021).

19. Ulrich-Schad, J. D. *et al.* What and why: South Dakota rangeland livestock producers' usage of parasiticides. *Rangel. Ecol. Manag.* **79**, 190–200 (2021).

20. Barton, C. H. J., Dale, E. F., Dixon, C. & Coles, G. C. Survey of parasite control on beef farms in south-west England. *Vet. Rec.* **159**, 682–684 (2006).