

STUDIES OF DUNG-DWELLING INSECTS IN CATTLE-GRAZING REGIONS OF THE  
COLUMBIA BASIN, WASHINGTON STATE

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of  
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COLUMBIA BASIN, WASHINGTON STATE

Abstract

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Dung is an important resource for microbes, nematodes, insects and annelids. The study presented here focused on the dung-dwelling insects of pasture ecosystems. The pattern of succession and manipulation of dung results in exploitation and resource utilization by many organisms. The activities of dung insects cause the degradation of dung and the cycling of nutrients back into the environment, which is crucial for soil fertility and forage growth in a pasture environment. However, the presence of flies, beetles and wasps have been reported to be affected negatively by the presence of veterinary parasiticides (VPs), thus disrupting succession and dung degradation. VPs are commonly used in the Pacific Northwest (PNW) region of the U.S. and worldwide to control parasites of livestock.

Studies were conducted over a two-year period in the Columbia Basin region of Washington, U.S. to examine the succession of insects in cattle dung and the effects of pour-on formulations of ivermectin and doramectin on those insects. The succession study examined the dung-dwelling insects emerging over three-day intervals from

artificial pats placed in three microhabitats (natural grassland, non-irrigated pasture and irrigated pasture) in which cattle graze. The results for Experiment 1 (2007) revealed no differences in insect emergence through time, while Experiment 2 (2008) showed that significantly more Diptera emerged from dung on day 2 than 3 or 4. Numbers of flies emerging was greatest in natural grassland for both experiments, while numbers of beetles emerging was lowest in the natural grassland.

VP experiments were examined the insects emerging from artificial pats from cattle treated with ivermectin, doramectin or no VPs. The results of these experiments varied between years with reduced emergence from dung treated with VPs for the Diptera (Experiment 3: 2008), lower emergence rates from control dung for Hymenoptera (Experiment 2: 2007), and no reductions in Coleoptera emergence in either experiment. This study suggests that VPs negatively affected dung-dwelling insects but varied by organism as well as from year to year in Washington State in the summer. These studies highlight the importance of characterizing the ecology of the dung insect community in the PNW region of the U.S.

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## Introduction

The dung of herbivores is often the most important resource for dung communities (Dormont et al. 2007). Microbes, fungi, nematodes, insects and annelids all use dung for food and habitat (Mohr 1943, Laurence 1954, Holter 1979, Lussenhop et al. 1980, Floate et al. 2005). The activities of these organisms are important in the recycling of nutrients in pasture ecosystems. These activities include the mixing of dung with the soil, leading to improvement in soil characteristics and to increases in forage quality (Bang et al. 2005). The members of the dung community that are focused on in these studies are the dung-dwelling insects. These insects include primarily species within the orders Diptera, Coleoptera and Hymenoptera. Insects in these taxa are suited for exploiting this ephemeral resource.

Herbivore dung is a patchy, ephemeral resource that is utilized as food and habitat by arthropods often leading to competition (Price 2004). The short-lived nature of the dung pat as a nutritive resource is primarily due directly to environmental factors.

Temperature and humidity have a direct impact on the conditions of the pat and this in turn affects colonization of the manure (Mohr 1943, Laurence 1954, Holter 1979, Dickinson et al. 1981, Floate et al. 2005). Herbivore dung desiccates in the inland Pacific Northwest shrub-steppe environment from the time it is deposited until it has been fully utilized by the dung community. This process of desiccation is seasonally dependent upon solar radiation, temperature, soil type, soil pH, and rainfall (Fincher 1970, Price 2004). After deposition, a dung pat begins to form a crust on the outer surface as it loses water. This crust causes the desiccation of the interior of the pat to

slow dramatically, which maintains a moist environment. The formation of the crust also forms a barrier for access to the interior of the pat for certain arthropods. The drying of the interior of pats is not homogeneous and will depend primarily upon the activity of the dung animal community on and within the pat. Desiccation continues over time from the outer edges toward the interior until the dung pat is broken apart by animal activity or has dried completely. This process is primarily driven by the environmental factors mentioned above, but the presence of dung-dwelling organisms also affects how rapidly dung desiccates.

The species diversity, distribution, and composition of animal fauna that colonize dung are influenced by the type of dung, surrounding flora, colonizing and inhabiting fauna, as well as the environmental conditions of solar radiation, temperature, soil type, soil pH, and rainfall (Fincher 1970, Price 2004). The dung-dwelling community has also been shown to be affected by the quality of herbivore dung, which is a direct result of seasonal variation in forage (Greenham 1972, Omaliko 1981, Gittings and Giller 1998, Finn and Giller 2002). The location of a dung pat within a pasture can influence the colonization and subsequent development of dung-dwelling organisms (Mohr 1943). For example, succession will differ between dung deposited in shade beneath a tree and in an open pasture. These studies together show an impact of a multitude of factors on the colonization and succession of dung pats by the dung-dwelling community.

An important concept in the field of ecology, succession has been defined as community change that is due to climatic, topographic, edaphic and biotic causes

(Philips 1934). Succession in herbivore dung has been little studied (Mohr 1943, Laurence 1954, Gittings and Giller 1998, Finn and Giller, 2000, Floate et al. 2005, Lee and Wall 2006) although it can be characterized as degradative succession.

Degradative succession is characterized by serial replacement of species that occur on a short time-scale, in which different species invade a resource and then disappear after having used resources while making other resources available (Doube 1987).

Degradative succession ends because the resource has been fully exploited and utilized. The colonization and succession of a dung pat is very much a degradative successional type and involves many organisms, which all take advantage of the dung pat in the brief time it is available.

As mention above the dung community that is involved in succession is composed primarily of bacteria, fungi, nematodes, annelids, and arthropods. The first colonizers of dung make the habitat more suitable for later succession organisms (Holter 1979).

Again the focus of this study and thus the focus of this review is on the dung insect community. The first insect colonizers of dung are adult Diptera. Flies arrive to feed and lay eggs within the dung immediately after it has been voided from the bovine. As the pat begins to form a crust the activity of the adult flies begins to diminish. Adult Coleoptera are typically the next groups of insects to arrive. The peak colonization of dung feeding beetles is from the first to the fifth day after dung deposition and rapidly diminishes from day 15 to 25 once the dung pat is fragmented by insect activity (Floate et al. 2005). Arrival of parasitic hymenoptera coincides with the colonization of flies and beetles. Parasitic wasps oviposit on developing insect larvae within the dung pat. Some

of the last organisms to exploit the dung pat and aid in incorporating organic material into the surrounding soil are earthworms (Holter 1977, 1979, Svendsen et al. 2005). The activities of these animals lend to the cycling of nutrients and the eventual degradation and incorporation of the manure into the soil.

The dung community is instrumental in the degradation of dung pats and the recycling of nutrients back into the pasture environment promoting healthy pasture land and thus healthy and robust livestock. The removal of fecal material from the ground surface is the most obvious impact of dung-dwelling fauna. Dung removal is a critical process whereby the dung community prevents the loss of nutrients in a pasture ecosystem. Coleoptera, have been documented to reduce the loss of nitrogen through ammonia volatilization and increase the uptake of nitrogen by plants thus increasing soil fertility (Nichols et al. 2008). The return of nutrients to the soil by the dung community, especially Coleoptera, has been documented to contribute to higher yields in forage on which livestock graze (Bang et al. 2005). In fact, nutrient cycling as well as plant composition and soil fertility are affected by fecal degradation (Wall and Strong 1987, Herd 1995). Without dung degradation there would be less forage available for grazing livestock. In addition to less forage, Omaliko (1981) demonstrated that grazing cattle reject the herbage around a pat for up to 12 weeks after deposition of the dung pat. The breakdown of dung reduces the amount of fouled grassland, which is beneficial to animal husbandry practices (Wall and Strong 1987).

Nematodes and other parasitic arthropods that cause economic injury to livestock have been well documented to be controlled by veterinary parasiticides (VPs; Schmidt 1983, Madsen et al. 1990, Williams et al. 1999, Floate et al. 2005, Floate 2007). Forms of VPs known as endectocides are used throughout the world to treat livestock for internal parasites. These VPs include such chemical compounds as avermectins (eprinomectin, ivermectin, and doramectin) and milbemycins (moxidectin) (Herd 1995, Floate et al. 2005, Floate 2007). VPs are administered orally, subcutaneously, or topically and are metabolized by the animal. The compounds are then excreted in the animal's feces or urine into the environment (Williams et al. 1999, Floate et al. 2005, Wardhaugh 2005). In the pasture ecosystem these compounds come into contact with a diversity of organisms, particularly those that utilize the feces of livestock for growth, development, and reproduction. VPs may negatively impact dung-dwelling insects and cause a reduction in pat degradation, resource cycling and thus pasture ecosystem health.

The negative impact of VPs on dung beetles, flies and wasps has been documented by a number of studies (Schmidt 1983, 1987, Floate 1998, Madsen et al. 1990, Fichner 1992, Dadour et al. 2000, Suarez et al. 2003, Iwasa et al. 2003, 2005, Floate 2006, Errouissi et al. 2007, Iwasa et al. 2008). While these studies conclude that VPs negatively impact dung fauna, the severity of these impacts is not uniform across all studies, environment, and groups of organisms. Arthropod taxon, chemical formulation of the VPs, and the environmental factors of the region at which the study was conducted may all determine the extent to which dung-dwelling organism are affected (Wall and Strong 1987, Floate 1998). Some studies have also concluded that specific

VPs have no negative impacts on the insect community (Kryger et al. 2005, 2006, 2007). It is clear from these contradictory studies that more research must be conducted on the impacts of VPs on insect communities in a diversity of habitats and ecosystems.

My research was to examine the dung-dwelling insect community and degradative succession in the Pacific Northwest region of the U.S. (PNW) and to characterize the effects of several commonly applied VPs of cattle on populations of beetles, flies, and parasitoids in the PNW. I conducted two experiments over two years in order to characterize succession of dung-dwelling insects in the PNW. I also conducted a series of experiments over two years to determine the effects of pour-on formulations of ivermectin and doramectin, two commonly used VPs in the PNW rangeland cattle system, on dung-dwelling beetles, flies, and parasitoids. All of these experiments were designed to use the most standardized attributes from previous studies. Until the present study no investigations had been done in the PNW to examine the impacts of VPs on the dung insects. Taken together, these experiments will not only provide new information on the dung insect community found in the pasture ecosystem in the state of Washington but will also provide important new data for researchers, extension specialists and cattlemen on best management practices for maintaining healthy pasture ecosystems.

# THE SUCCESSION OF DUNG-DWELLING BEETLES, FLIES, AND PARASITOIDS WITHIN DIFFERENT GRAZING ENVIRONMENTS

## **Abstract**

Ecological succession is a predictable change in the structure of an ecological community. This concept is important in community dynamics because the impacts of a particular organism on the environment could allow another organism to exploit resources not previously available. This phenomenon is extremely important in dung, which is considered an ephemeral resource that is suitable as food and habitat for a short period of time due to desiccation and competition between arthropods. The purpose of this study was to examine succession of the dung insect community over three day intervals. A two-year study was conducted in the Columbia Basin region of Washington, USA. The succession of dung-dwelling insect fauna was examined by collecting dung from cattle, forming artificial pats and placing the pats in two managed and one natural microhabitats (natural grassland, non-irrigated pasture and irrigated pasture). Cattle were actively grazing in the managed pastures but were not present in the natural grassland. Placing the pats in the three locations allowed insects to colonize the pats in three areas varying in abiotic conditions. The results for Experiment 1:2007 revealed no differences in insect assemblage by day over a three week interval. In Experiment 2: 2008 significantly more Diptera emerged from pats on day 2 than on subsequent days. Mean number of fly emergence was greatest in pats in natural grassland for both experiments, while mean number of beetle presence was lowest in the natural grassland. This study suggested that insect emergence differed only modestly between 2, 3 and 4 days post excretion of dung. However, the presence of



dung-dwelling insects may differ depending on location. Future studies on dung insect communities should examine insect communities in a variety of local microhabitats in an effort to understand to what extent various abiotic and biotic conditions have on dung-dwelling insects both in the Pacific Northwest and worldwide.

## Introduction

Succession is central to the field of ecology and explains the predictable change in the structure of an ecological community. Succession has been defined as community change that is due to climatic, topographic, edaphic and biotic causes (Philips 1934). A classic study of succession presented in Cowles (1899) examined the succession of vegetation on the Indiana Dunes on the shore of Lake Michigan. In the study Cowles documented the abiotic and biotic conditions that affected plant presence on the sand dunes. Succession events may differ depending on the resources that organisms exploit. Degradative succession has been described as serial replacements that occur on a short time-scale, typically months to at the most several years, where different species invade and disappear using some resources while making others available (Doube 1987). Degradative succession ends when the resource has been fully utilized. Succession of a resource is important because the activity of one organism may indirectly impact the degradation of a dung pat by making it more suitable for other organisms (Holter 1979). Understanding succession is pivotal to understanding community dynamics associated with dung pats.

Dung is considered an ephemeral resource that can only provide suitable food and habitat for a short period of time due to desiccation and competition between arthropods (Price 2004). Dung of domestic livestock can vary in attractiveness to colonizing arthropods depending on the animal that produced the dung. For example, Dormont et al. (2007) demonstrated that specific species of dung beetles exhibit preferences among cow, sheep, horse, or deer dung and will colonize resources based on these

preferences. The dung of these species was studied because they can be the most important resources for dung insect communities (Dormont et al. 2007). The dung of one species of livestock may vary in quality with season due to the quality or type of forage ingested by the grazing animal (Greenham 1972, Omaliko 1981, Gittings and Giller 1998, Finn and Giller 2002). The location of a dung pat within a pasture also influences colonization by dung-dwelling fauna (Mohr 1943, Greenham 1972). This study will focus on the colonization and succession of cattle dung by insects.

Animal waste products in the form of manure, or dung, not only contain many nutrients but are also important habitats for many arthropod species (Mohr 1943). Immediately after deposition dung is colonized by arthropods but is only a suitable habitat for a short time. The short-lived nature of the dung pat is often due directly to abiotic factors.

Temperature and humidity have a direct impact on the conditions of the dung and whether colonizing fauna will exploit the resource (Mohr 1943, Laurence 1954, Holter 1979, Floate et al. 2005). Herbivore dung typically goes through a process of desiccation from the time it is deposited until it has been completely utilized by the dung organisms that have colonized the pat (Mohr 1943, Laurence 1954, Floate et al. 2005).

The process of desiccation is dependent primarily on abiotic conditions (Mohr 1943, Laurence 1954, Greenham 1972). As a dung pat begins to dry, it forms a hard crust on the outer surface. Once this crust has formed, desiccation of the pat interior slows dramatically. The formation of the crust has been shown to limit access of some organisms to the pat (Mohr 1943). The drying of the interior of the pat is not homogeneous, but varies with the activity of the organisms feeding and dwelling on the

pat. The pat continues to dry over time from the outer edges toward the interior until it is degraded by the dung-dwelling organisms and/or completely desiccates.

The dung community is composed of microbes, fungi, nematodes, insects and annelids (Mohr 1943, Laurence 1954, Holter 1979, Lussenhop et al. 1980, Floate et al. 2005).

Although the microbes, nematodes, and fungi are important members of the dung community, the focus of this study is on the insect community. The first insect colonizers of dung are adult Diptera, which arrive immediately after dung deposition to feed and oviposit within the dung. Once the pat begins to dry and a crust begins to form colonization rates by adult is reduced. The next groups of insects to colonize dung pats are dung-feeding and carnivorous Coleoptera. Peak colonization by these beetles is from the first to the fifth day after dung deposition and rapidly diminishes from day 15 to 25 once the dung pat has been fragmented by insect activity (Floate et al. 2005).

Coinciding with the colonization of flies and beetles is the arrival of parasitic hymenoptera that attack developing insect larvae within the dung pat. Carnivorous beetles, such as the larvae of *Sphaeridium* (Hydrophilidae, Coleoptera) attack fly and beetle larvae found in and around the dung (Mohr 1943, Sowig 1997). Earthworms are late colonizers that exploit the resources of the dung pat and aid in incorporating organic material from the degraded pat into the surrounding soil (Holter 1977, 1979, Svendsen et al. 2005). The foraging activities of these animals together cause the cycling of nutrients and the fragmentation of the dung pat leading to its eventual degradation and incorporation into the soil.

Dung-dwelling organisms are essential for the degradation of dung and the recycling of nutrients in the environment. In managed pastures these organisms contribute to the maintenance of healthy pastures. The most obvious impact of dung organisms is the removal of fecal material from the pasture surface. By removing fecal material, dung fauna prevent the loss of nutrients. Some species of Coleoptera have been documented to reduce the amount of nitrogen that is lost through ammonia volatilization, thus increasing available nitrogen for plant uptake (Nichols et al. 2008). The removal of dung not only affects nutrient cycling, but soil fertility as well as plant composition (Wall and Strong 1987, Herd 1995). By returning nutrients to the soil, beetle-activity contributes to higher yields in forage on which livestock graze (Bang et al. 2005). Degradation of dung can lead to less forage avoided by grazing livestock. Omaliko (1981) demonstrated that grazing cattle reject the herbage around a pat up to 12 weeks after deposition of the dung pat. The breakdown of dung reduces the amount of fouled grassland, and so is beneficial to animal husbandry (Wall and Strong 1987).

Dung communities have been extensively studied in Europe (Holter 1979, Wall and Lee, 1987, Gittings and Giller 1998, Finn and Giller 2000, 2002, Lee and Wall 2006, Dormont et al. 2007) Australia (Dadour et al. 2000, Wardhaugh et al. 2001), Asia (Iwasa et al. 2005, 2008, Bang et al. 2007), and South America (Suarez et al. 2003, Iglesias et al. 2006). Most of the above mentioned studies have focused on how veterinary parasiticides affect survival of dung-feeding fauna. There are relatively few ecological studies that report on succession of the insect dung community through time (Mohr 1943, Laurence 1954, Gittings and Giller 1998, Finn and Giller, 2000, Floate et al. 2005,

Lee and Wall 2006). This study contributes new information on succession and community dynamics of dung-dwelling insects in North America. The previous studies have focused on the Mid-Atlantic (Price 2004), Midwest (Mohr 1943), Southeast (Bertone et al. 2005), and Southern regions (Fincher et al. 1970, Schmidt 1983, Fincher 1992) of the US, and Alberta in Canada (Floate 1998, 2006, Floate et al. 2001, 2002), with no data available as yet for the Pacific Northwest of North America. The current study examines the colonization and succession of insects in cattle dung over a three day period following the deposition of artificial pats in three microhabitats within the Columbia Basin of Washington State, USA. I hypothesized that composition of the dung insect assemblage would differ over the four day period, would vary seasonally, and would differ between microhabitats. The three microhabitats were chosen because they represent the three major habitats in which cattle graze in the Columbia Basin region of Washington State. It is important to study areas that differ in abiotic conditions because of the well documented effects that environmental conditions have on the longevity and exploitation of dung. The approach we used was to follow the experimental design outlined by Floate (1998, 2006) and Floate et al. (2002). Briefly, I collected dung from cattle, homogenized it, and set out artificial dung pats. This particular method was used to standardize the size of artificial dung pats across experiments; pat size may often vary among studies, and this variation has led to difficulties in interpretation of results (Finn and Giller 2000, 2002).

## **Materials and Methods**

### *Experiment 1: Succession of Beetles, Flies, & Parasitoids in August (2007)*

Experiments were conducted for three successive weeks beginning 7 August 2007 near Prosser, Benton Co., WA to determine the pattern of succession of adult dung-dwelling insect fauna by surveying the emergence of first generation offspring. Three different locations were chosen to represent the different microhabitats in the area: a natural grassland (NG) (46° 21' 28" N, 119° 43' 25" W) environment, a non-irrigated pasture (NI) (46° 15' 34" N, 119° 38' 53"W), and an irrigated pasture (IP) (46° 14' 7" N, 119° 43' 45" W). The natural grassland (NG) environment was not managed in any way and was completely surrounded by similar type of habitat. The non-irrigated pasture (NI) received no controlled irrigation. The only constant supply of water was from a creek, and adjacent to this creek was one of the few places in which green forage was consistently present. The irrigated pasture (IP) was kept green by flooding at brief intervals. This pasture was surrounded by other irrigated pasture that also maintained grazing livestock. These areas differed from one another in the type of foliage and amount of moisture present.

To assess the pattern of succession of dung-dwelling insects in these three habitats standardized dung pats were constructed from dung collected from a herd of mixed Hereford and Black Baldy cattle near Washington State University's Irrigated Agricultural Research and Extension Center Prosser, Benton Co., WA. The cattle had not been treated with any veterinary parasiticides for a minimum of 12 weeks. Thus the dung was considered to be free of veterinary parasticide residues (VPs). This was verified by Floate (1998), which reported reductions in insect fauna up to 12 weeks. Other studies have reported negligible concentrations of the VP ivermectin in the feces

of cattle after 4 weeks (Laffont et al. 2001, Iwasa et al. 2005). Cattle were monitored, and freshly deposited dung was collected within five minutes after deposition over a five day interval, mixed, and stored in Ziploc® storage bags (SC Johnson, Racine, WI, USA) at -40°C in the week prior to the start of the succession experiments. Twenty-four hours prior to each experiment, dung was thawed, divided into 0.5 liter dung pats, and set on a 2 cm deep bed of sand covering the top of a Styrofoam plate (23 cm diameter). Three holes were put in the bottom of the plates to allow rainfall to drain. Artificial dung pats were then placed 3 m apart along a transect at each of the three locations.

Six dung pats were randomly removed from each site at each of 3 different time points (48, 72, and 96 hours post-placement in the field) and placed in emergence buckets. Emergence buckets were 11 liter white plastic buckets (United States Plastic Corporation, Lima, OH, USA) that had been fitted with fine mesh sleeves to contain emerging adult insects. The buckets were placed in a fully enclosed cattle barn at WSU IAREC, Prosser, WA away from direct sunlight. All buckets were in the main part of the barn and were exposed to the same temperature, humidity, and lighting. Distilled water (50 mL) was added to each pat every other week after arthropods had begun to emerge, to prevent desiccation of developing insects. The dung pats were monitored daily for a period of seven weeks for adult insect emergence. Once adult insects began to emerge from the pats they were aspirated and stored in 70% ethanol to be sorted, counted, and identified to family level.

*Experiment 2: Succession of Beetles, Flies & Parasitoids in Spring through Fall (2008)*



Experiments were conducted monthly from May to September near Prosser, Benton Co., WA to determine the pattern of succession of adult dung-dwelling insect fauna. As in the previous year's experiment, the same three locations were used (a natural grassland environment, a non irrigated pasture, and an irrigated pasture).

To assess the pattern of succession of dung-dwelling insects, dung pats were constructed from fresh dung (<5 minutes old) collected from grazing cattle. The dung was collected in the morning hours (8:00-11:00) the day pats were to be placed in the field. Cattle were monitored, and once a pat was excreted it was immediately collected to insure that the dung was extremely fresh. Fresh dung was used in an effort to replicate field conditions. Collected dung was mixed, divided into 0.5 liter dung pats and stored in Ziploc® bags until deployed in the field. At each of the three different locations, the 0.5 liter dung pats were placed on a 2 cm bed of sand on top of a 30 x 30 cm piece of wax paper. Wax paper was used instead of Styrofoam plates to facilitate access to dung pats by walking insects. At each microhabitat site, artificial dung pats were spaced 3 m apart along a transect.

The collection of dung pats, monitoring of buckets for the emergence and collection of adult insects collected was conducted in the same manner as detailed above in Experiment 1: 2007.

### **Statistical Analysis**

The effects of time (2007: 3 weeks, 3 days within each week; 2008: 4 months, 3 days within each month) on counts of arthropods emerging from dung pats were assessed using a repeated measures analysis of variance; analyses were done using PROC MIXED (SAS Institute 2002). The count data were transformed as necessary to meet normality assumptions by taking the square root of each value. The six pats for each collection and site were first averaged before transformation, thus providing a single observation per collection date and site. The ar(1) covariance structure was used to account for correlations among the repeated measures. I specified the Kenward-Rogers procedure in PROC MIXED to adjust degrees of freedom, as this adjustment is recommended for PROC MIXED when repeated measures analyses are done (Littell et al. 2006). The 3 habitat types were not compared statistically due to lack of replication of this factor; I instead present the raw emergence numbers for each site (=habitat type).

## **Results**

### *Experiment 1: Succession of Beetles, Flies, & Parasitoids in August (2007)*

#### Diptera

The families of flies that colonized the artificial dung pats were Ceratopogonidae, Stratiomyidae, Phoridae, Sepsidae, Sphaeroceridae, Anthomyiidae, Muscidae, Calliphoridae and Sarcophagidae. The two families that comprised the majority of the Diptera that emerged in this experiment were the Sphaeroceridae and Sarcophagide comprising 46 and 40 percent of total Diptera emergence. Most of the Diptera emerging from pats were obtained from the NG and NI sites, with considerably lower rates of

emergence from pats at the IP site (Fig. 1). Numbers of Diptera emerging from pats were not affected either by week of the study (mean emergence = 3.55, 2.97, and 4.84 [SE = 1.03] specimens per pat for weeks 1-3, respectively (Fig. 2:  $F_{2,4.4} = 4.0$ ,  $P = 0.10$ ) or days within week (means of 3.65, 3.44, and 4.27 [SE = 1.03] specimens per pat for days 2-4, respectively: Fig. 3:  $F_{2,8} = 1.0$ ,  $P = 0.42$ ). The lack of a significant day effect implies that there was no measureable difference in emergence of flies from pats whether they had been left in the field for 2, 3, or 4 days, which is consistent with the hypothesis advanced in the Introduction. The lack of a significant week effects suggests that there was no difference in the emergence of flies from pats by week, which does not support my central hypothesis.

### Coleoptera

The Coleoptera that were present in this experiment were composed of Carabidae, Hydrophilidae, Staphylinidae, Scarabaeidae, Histeridae and Curculionidae. The majority of the Coleoptera consisted of Scarabaeidae in the genus *Aphodius*, which comprised 93 percent of total beetle presence. The presence of Coleoptera was mostly obtained from the NP and NI sites with considerably fewer beetles present at the NG site (Fig. 4). Beetle presence was not affected by week of the study (mean presence = 4.32, 4.89 and 3.98 [SE = 1.85] specimens per pat for weeks 1-3, respectively: Fig. 5:  $F_{2,4.6} = 1.10$ ,  $P = 0.41$ ) or days within week (means of 4.66, 4.49 and 4.05 [SE = 1.81] per pat for days 2-4, respectively: Fig. 6:  $F_{2,7.4} = 1.94$ ,  $P = 0.21$ ). The lack of significance by day indicates there was no difference in the presence of Coleoptera in pats left in a field for 2, 3, or 4 days. This result did not agree with the hypothesis set forth in the introduction.

As with the flies, there was no significant effect of week, which also does not support my hypothesis.

### Hymenoptera

Hymenoptera were present but emergence rates were so low that statistical analysis could not be conducted.

### *Experiment 2: Succession of Beetles, Flies & Parasitoids in Spring through Fall (2008)*

### Diptera

As with the 2007 trial, the of flies that colonized the artificial dung pats consisted of Ceratopogonidae, Stratiomyidae, Phoridae, Sepsidae, Sphaeroceridae, Anthomyiidae, Muscidae, Calliphoridae and Sarcophagidae. However in this experiment the Sarcophagidae were the dominant family comprising 66 percent of total fly emergence. As observed in Experiment 1:2007, few Diptera emerged from pats at the IP site (Fig. 7). Daily emergence rates per pat were not affected by month of the study (means between 1.32 and 3.32 [SE = 1.00: Fig. 8:  $F_{3,7.1} = 0.8$ ,  $P = 0.52$ ). However, there was a significant day effect, with numbers of Diptera emerging declining between days 2 and 4 (Figure 1:Fig. 9:  $F_{2,14.5} = 6.2$ ,  $P = 0.011$ ). This result appears to contradict my hypothesis in the Introduction that fly emergence would be independent of the number of days that a pat was left in the field. It is unclear why emergence rates were highest for pats left in the field for the shortest amount of time.

### Coleoptera

Like Experiment 1:2007 the Carabidae, Hydrophilidae, Staphylinidae, Scarabaeidae, Histeridae and Curculionidae comprised the Coleoptera for this experiment. The majority of the Coleoptera consisted of Scarabaeidae in the genus *Aphodius*, which comprised 91 percent of total beetle presence. Coleoptera numbers were highest in the NI samples with considerably fewer beetles emerging from the NP and NG samples (Fig. 10). Beetle presence was not affected by month of the study (mean between 1.18 and 3.16 [SE = 0.86]: Fig. 11:  $F_{3, 8.4} = 1.00$ ,  $P = 0.42$ ) or days within month (means of 2.68, 2.35 and 1.78 [SE= 0.49] per pat for days 2-4, respectively: Fig. 12:  $F_{2, 16} = 1.73$ ,  $P = 0.21$ ). The lack of significant month or day effects suggests that dung beetle emergence did not vary by day, or (on a larger time scale) by month. These results are inconclusive.

### Hymenoptera

As in Experiment 2: 2007 the numbers of parasitic wasps that emerged were low. The emergence data were also highly clumped, in that many wasps (10 or more) emerged from specific pats while no wasps emerged for a number of pats. The low and clumped populations of wasps precluded no statistical analysis.

## **Discussion**

The results of our experiment do not support my central hypothesis that the composition of the dung insect assemblage would differ over the four day period, would vary seasonally, and would differ between microhabitats. In Experiment 1: 2007 fly emergence did not differ by week or day (Fig. 2, 3). In Experiment 2: 2008 emergence

rates of flies did not differ by month (Fig. 8). However, in Experiment 2: 2008 there were more flies emerging on day 2 than day 3 and 4 (Fig. 9). The reason for lower emergence rates on days 3 and 4 than day 2 is most likely due to an increase in mortality of developing fly larvae due to longer exposure to field conditions. There was no significant difference in beetle emergence rates with week or day (Fig. 5, 6) in Experiment 1: 2007, or with month or day in Experiment 2: 2008 (Fig. 11, 12). The lack of time effects did not meet my original predictions that beetle emergence would differ over the three day period.

I did see some interesting trends in arthropod emergence among locations. There was a stark contrast of mean insect emergence of flies and beetles between the three different locations: natural grassland (NG), non-irrigated (NI), and irrigated (IP). For Experiment 1: 2007, Diptera emergence was greatest at the NG site and least at the IP site (Fig. 1). In contrast Coleoptera presence was highest at the IP site and lowest at the NG site (Fig. 4). In Experiment 2: 2008 the greatest number of flies again emerged from pats from the NG site, followed by the NI site then the IP site (Fig. 7). However, beetle presence was highest in the NI site, then the IP site with the NG site having the lowest abundance (Fig. 10). There is similarity between the emergence of dung-dwelling flies in Experiment 1: 2007 and Experiment 2: 2008. In both years greater fly emergence was from pats in NG with IP having the least amount of fly emergence (Fig. 1, 7). Floate (1998) reported that summing data across taxa can conceal the sensitivity of individual dung-dwelling species to perturbations. In the current study this was unavoidable due to low representation of individuals at the species, genus, and in some cases, family level.

The choice of the three different locations (natural grassland, non-irrigated pasture, and irrigated pasture) represent a range of conditions experienced by dung-dwelling organisms. A study conducted by Hutton and Giller (2003) examined how different farm management practices affected populations of dung insects. They reported that organic farms (with no veterinary parasiticide usage and no input of synthetic fertilizers and pesticides) had greater abundance, biomass, and diversity of dung beetles compared to farms that had varying inputs of VPs, fertilizers and pesticides. That study, coupled with the current study, demonstrates that different types of pasture management may affect dung-dwelling insects.

The results from this research demonstrated that dung-dwelling insects did not differ in rates of emergence over three day intervals or by season. Insect emergence did seem to differ by location and by taxonomic order. This finding is important because abiotic conditions vary within the Pacific Northwest Region of USA. It is important to study dung community dynamics in different environmental and geographic conditions. Future studies should be conducted over multiple seasons in order to assess seasonal fluctuations of insect populations as well as to determine how different grazing schemes or VP use by cattle producers affect dung-dwelling insects. The results of this study reinforce the need for additional research of the dung inhabiting organisms in the Pacific Northwest.

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## Figure Captions

### **Fig. 1**

Experiment 1: 2007- Fly Emergence by Location. Mean values of emergence of Diptera from dung pats in natural grassland (NG), non-irrigated pasture (NI) and irrigated pasture (IP). Data are untransformed.

### **Fig. 2**

Experiment 1:2007- Fly Emergence by Week. Mean values  $\pm$  SE of Diptera emerging from dung pats by week. Data are square root transformed.

### **Fig. 3**

Experiment 1:2007- Fly Emergence by Day. Mean values  $\pm$  SE of emerging Diptera from dung pats by day. Data are square root transformed.

### **Fig. 4**

Experiment 1:2007- Beetle Presence by Location. Mean values of Coleoptera present from dung pats in natural grassland (NG), non-irrigated pasture (NI) and irrigated pasture (IP). Data are untransformed.

### **Fig. 5**

Experiment 1:2007- Beetle Presence by Week. Mean values  $\pm$  SE of number of beetles present in dung pats by week. Data are square root transformed.

### **Fig. 6**

Experiment 1:2007- Beetle Presence by Day. Mean values  $\pm$  SE of number of beetles present in dung pats by day. Data are square root transformed.

**Fig. 7**

Experiment 2: 2008- Fly Emergence by Location. Mean values of emergence of Diptera from dung pats in natural grassland (NG), non-irrigated pasture (NI) and irrigated pasture (IP). Data are untransformed.

**Fig. 8**

Experiment 2:2008- Fly Emergence by Month. Mean values  $\pm$  SE of Diptera emerging from dung pats by Month. Data are square root transformed.

**Fig. 9**

Experiment 2:2008- Fly Emergence by Day. Mean values  $\pm$  SE of emerging Diptera from dung pats by day. Data are square root transformed.

**Fig. 10**

Experiment 2:2008- Beetle Presence by Location. Mean values of Coleoptera present from dung pats in natural grassland (NG), non-irrigated pasture (NI) and irrigated pasture (IP). Data are untransformed.

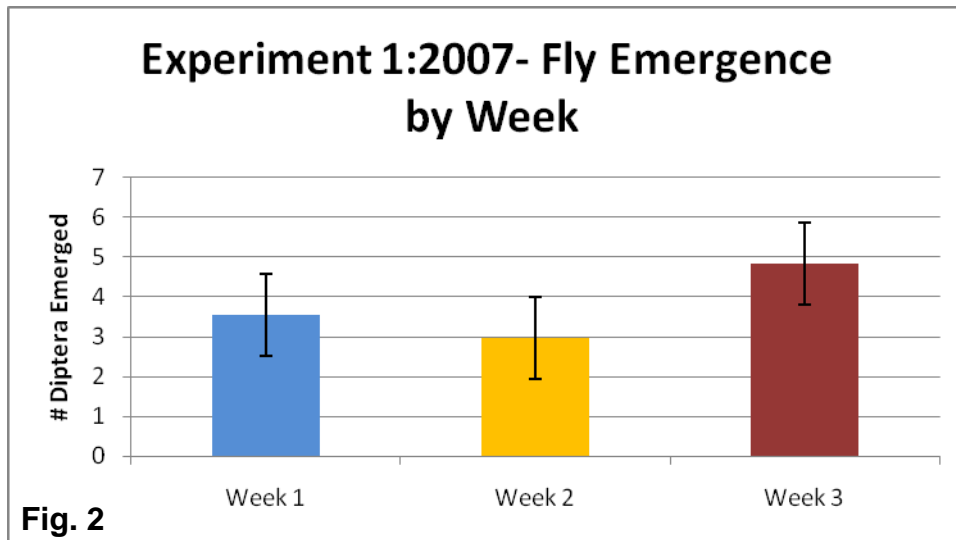
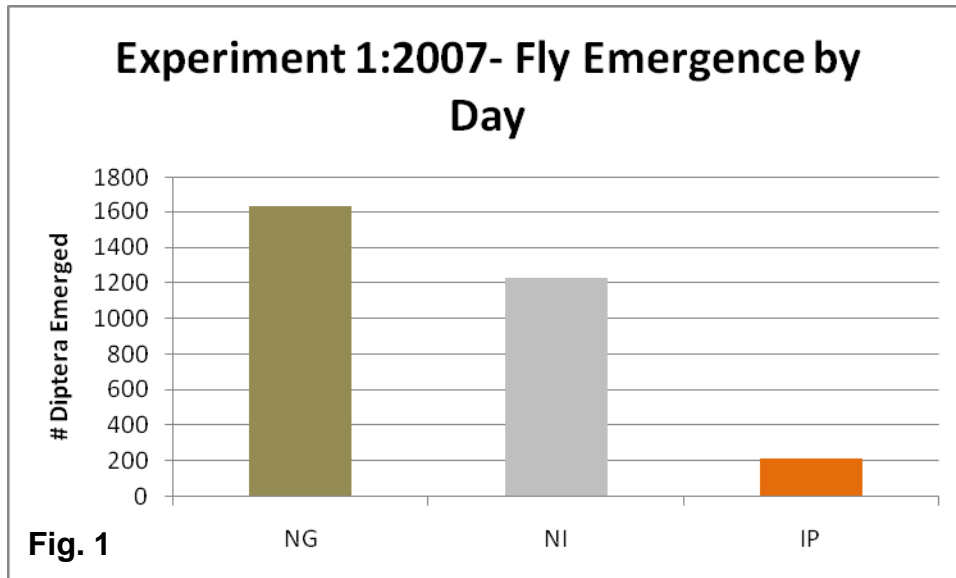
**Fig. 11**

Experiment 2:2008- Beetle Presence by Month. Mean values  $\pm$  SE of number of beetles present in dung pats by Month. Data are square root transformed.

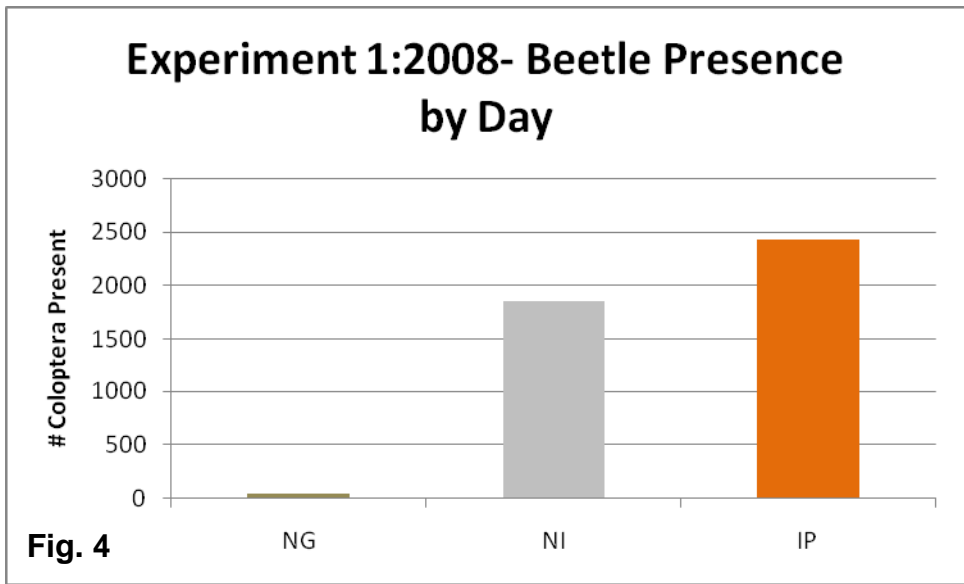
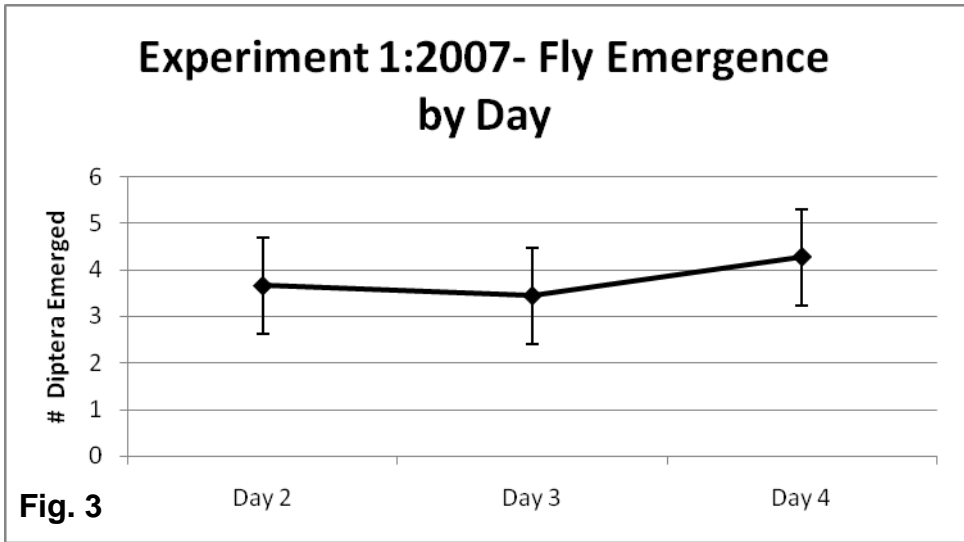
**Fig. 12**

Experiment 2:2008- Beetle Presence by Day. Mean values  $\pm$  SE of number of beetles present in dung pats by day. Data are square root transformed.

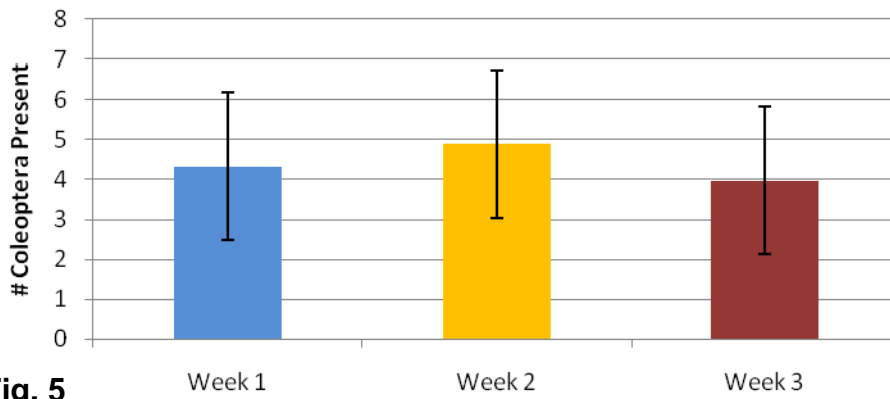
## Figures





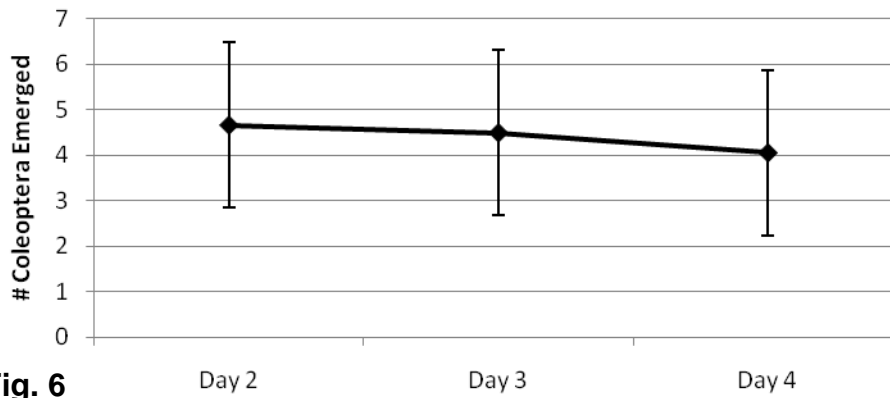


### Experiment 1:2007- Beetle Presence by Week

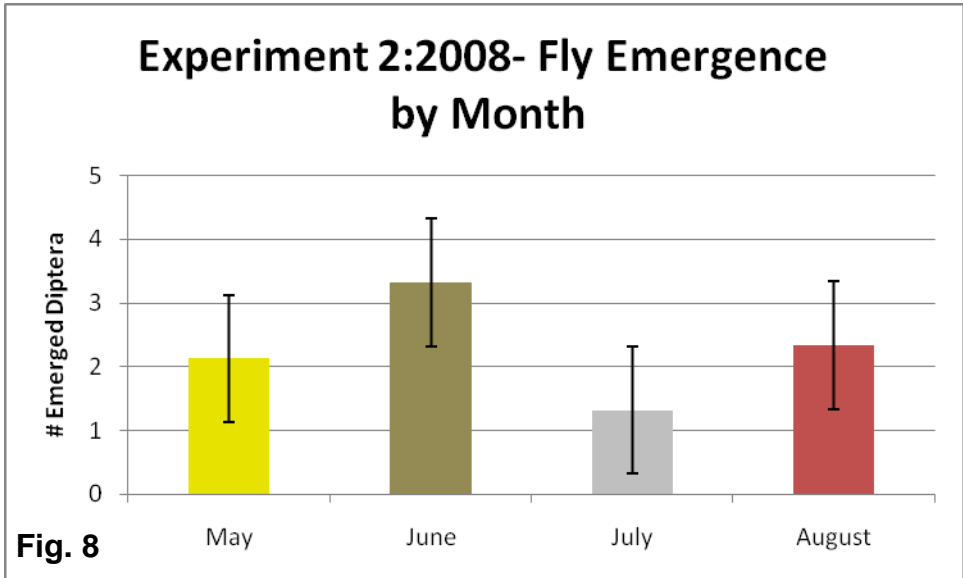
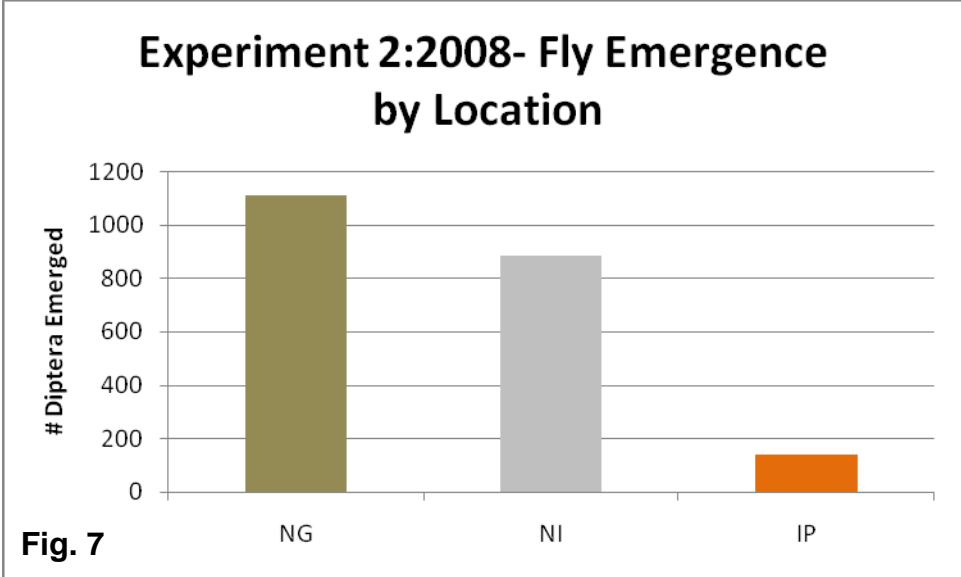


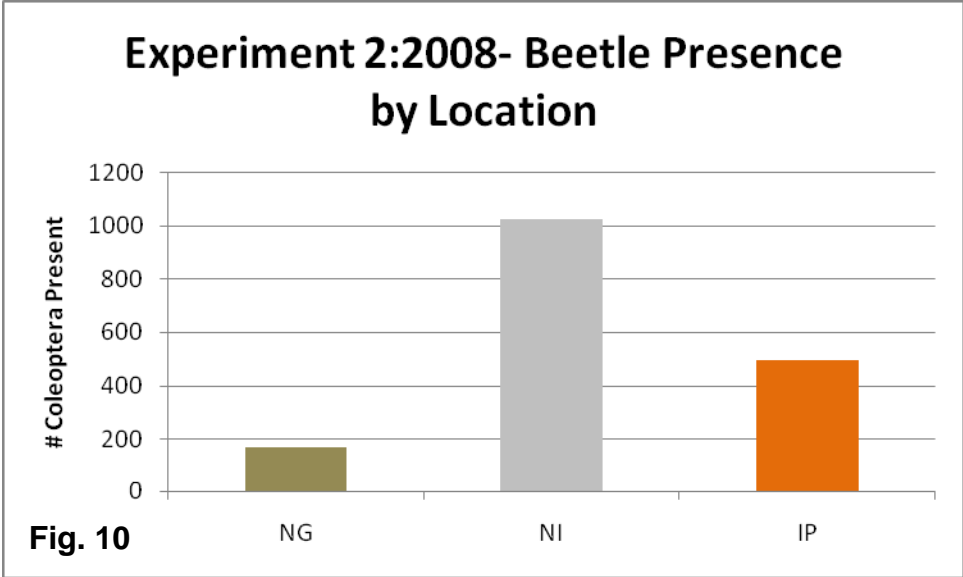
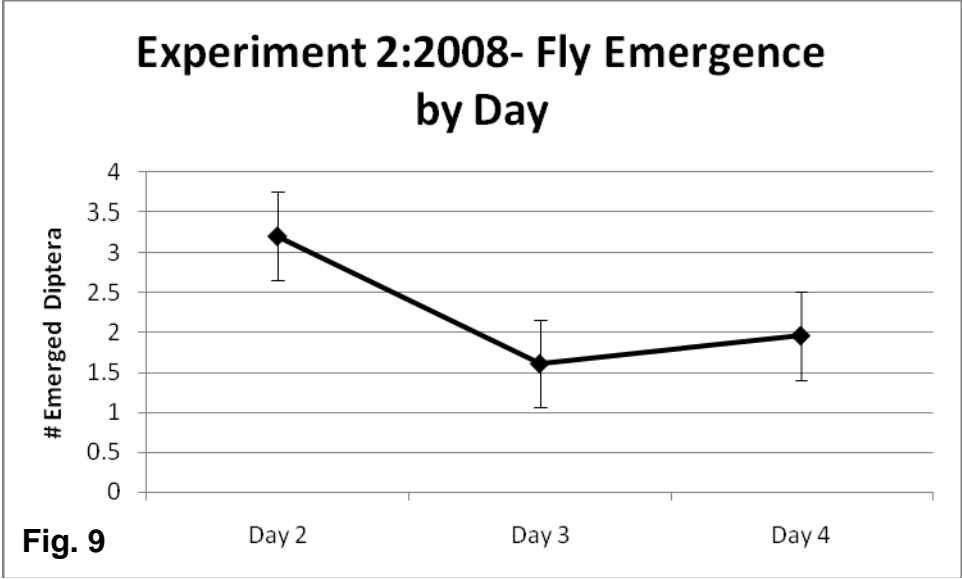
**Fig. 5**

### Experiment 1:2007 Beetle Presence by Day

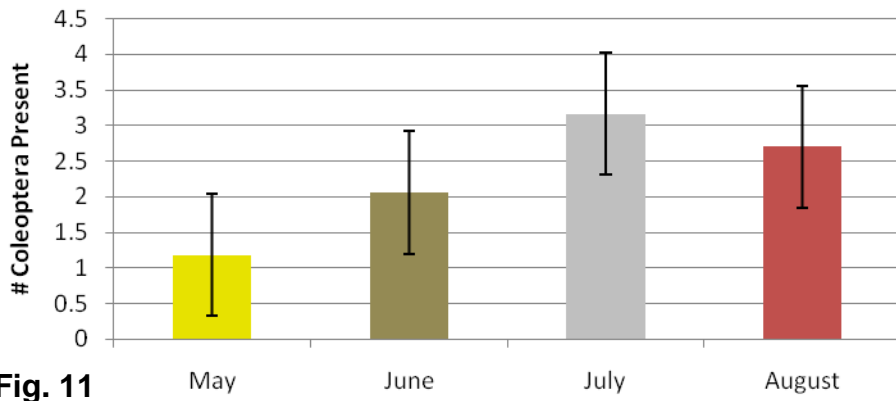


**Fig. 6**



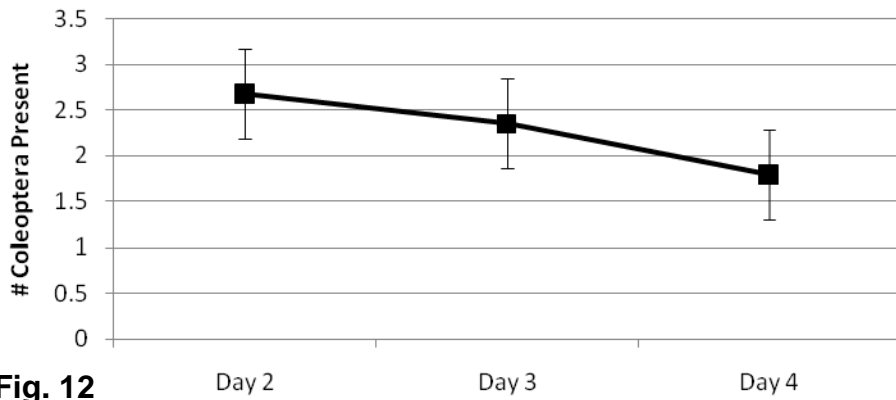


### Experiment 2:2008- Beetle Presence by Month



**Fig. 11**

### Experiment 2:2008- Beetle Presence by Day



**Fig. 12**

THE EFFECTS OF VETERINARY PARASITICIDES ON EMERGENCE OF DUNG  
INHABITING BEETLES, FLIES, AND PARASITOIDS FROM DUNG  
WITHIN A PASTURE ECOSYSTEM

**Abstract**

Veterinary parasiticides (VPs) are used frequently to control internal parasites of livestock. These powerful medicines are metabolized, degraded by the host animal, and excreted in the urine or feces. In this form non-target organisms can be exposed to the parasiticides. The purpose of this study was to determine what effects VP residues in cattle dung might have on dung-dwelling insects. Experiments were conducted over a 2-year period to examine the effects of VPs on colonizing dung fauna in the Columbia Basin region of Washington, USA. In 2006, 2007, and 2008 cattle were left untreated or treated with commercial formulations of either ivermectin or doramectin. Ivermectin in various formulations is the predominant parasiticide used in Washington State and has been used commercially on livestock for over 20 years (Ferguson et al. 2006) Doramectin is used less frequently but it is readily available.

After treatment cattle were monitored, and manure was collected at regular intervals. Once collected, artificial dung pats were formed and placed in the field where they were colonized by insects. Pats were removed from the field and monitored for insect emergence. The results of the three experiments varied, with reduced emergence from dung treated with VPs for the Diptera in one experiment, but not in the other experiment. Emergence rates for Hymenoptera were significantly lower in control pats compared to pats from treated cattle in one experiment. There were no reductions in

Coleoptera emergence from treated dung in any experiment. This study suggests that VPs negatively affected some dung-dwelling insects under summer conditions at the central Washington State study site, but with variable impact across insect taxa or years. Additional studies should be conducted during different seasons to determine how VPs coupled with seasonal variation of environmental factors may affect the dung insect community. This study highlights the importance for continual study of the impacts of VPs on the dung community in the Pacific Northwest Region of USA as well as for geographical regions worldwide.

## Introduction

Organisms that use animal dung as a resource have an integral role in the degradation of dung and the recycling of nutrients in most terrestrial ecosystems. The community that colonizes livestock dung in a managed pasture environment is composed of a diverse community of bacteria, fungi, nematodes, annelids, and arthropods. The insects that are associated with dung include beetles, flies, and parasitoid wasps that feed on bacteria, fungi, plant material, and other insects found in dung (Floate 1998). The first colonizing insects to arrive at a freshly deposited dung pat are adult Diptera. These insects arrive immediately after dung deposition to feed and oviposit within the dung. Once a pat has begun to age and dry it forms a hard layer on the outer surface that limits access to moist dung, while at the same time slowing the release of odors known to attract flies (Floate et al. 2005). The next groups of insects to arrive are the dung feeding Coleoptera, with peak colonization from the first to the fifth day after dung deposition and ending 15 to 25 days after deposition due to fragmentation of the pat (Floate et al. 2005). Beetle colonization diminishes rapidly from day 15 to 25 once the dung pat has become fragmented. The arrival of parasitic Hymenoptera coincides with the colonization of flies and beetles. Parasitic Hymenoptera oviposit on fly and beetle larvae developing within the dung. The foraging activities of the dung insect assemblage cause the cycling of nutrients and the fragmentation of the dung pat leading to its eventual degradation and incorporation into the soil.

Many factors affect the species diversity, distribution, and composition of the animal dung community, including the species and population density of herbivores depositing



dung, surrounding flora, forage quality, the colonizing fauna, solar radiation, temperature, soil type, soil pH, and rainfall (Fincher 1970, Price 2004). In addition to these factors, seasonal variation in the forage of livestock has been documented to impact fungi and insects of the dung community (Greenham 1972, Omaliko 1981, Gittings and Giller 1998, Finn and Giller 2002). Another factor influencing the dung-dwelling community is the location of a dung pat in the environment. Dung that is deposited in a pasture versus a wooded area may be colonized by different organisms (Mohr 1943). These studies highlight the complexity of the dung ecosystem, a short-lived ephemeral habitat and resource.

Veterinary parasiticides control nematodes and other parasitic arthropods in livestock and are widely used especially in the Pacific Northwest of the U.S. (Schmidt 1983, Madsen et al. 1990, Williams et al. 1999, Floate et al. 2005, Ferguson et al. 2006, Floate 2007). A form of veterinary parasiticides (VPs) known as endectocides are commonly used to treat livestock for internal parasites and include such veterinary medicines as avermectins (eprinomectin, ivermectin, and doramectin) and milbemycins (moxidectin) (Herd 1995, Floate et al. 2005, Floate 2007). VPs are administered orally, subcutaneously, or topically and are metabolized by the animal. Once metabolized the compounds are released into the environment via feces and urine (Williams et al. 1999, Floate et al. 2005, Wardhaugh 2005). In the pasture ecosystem these compounds come into contact with a diversity of organisms, particularly those that utilize the feces of livestock for growth, development, and reproduction.

A large number of studies have concluded that VPs negatively affect beetles, flies and parasitoids (Schmidt 1983, Madsen et al. 1990, Fincher 1992, Floate 1998, Dadour et al. 2000, Suarez et al. 2003, Iwasa et al. 2003, 2005, Floate 2006, Errouissi et al. 2007, Iwasa et al. 2008). Although these studies conclude that VPs negatively affect dung insects, the severity of these impacts are not uniform across all studies and for all organisms. Variation in the effects of VPs is dependent upon the species, chemical formulation of the VP, and the abiotic factors of the geographic region where the study was conducted (Wall and Strong 1987, Floate 1998). Studies have determined that some VPs have no negative impacts on the insect community (Kryger et al. 2005, 2006, 2007). For example, Kryger et al. (2005) concluded that there were no negative effects of fluazuron, an acaricide, as well as ivermectin on dung-dwelling insects and deduced that this was due to high rainfall during the experiment possibly diluting any VP residues in the dung. It is clear from these studies that both abiotic and biotic factors are important to consider when determining the impacts of VPs on insect communities and that these experiments should be conducted across diverse habitats and ecosystems.

To determine the effects of VPs on the insect community in dung from rangeland cattle in south-central Washington State, I conducted a study to examine the effects of two different pour-on formulations of VPs on the emergence of first generation dung-colonizing insects in an irrigated pasture habitat located in the Columbia Basin region of Washington State, USA. My hypothesis is that survival of insects would be lower in dung from VP-treated cattle than dung from untreated cattle, and that this effect would decrease over time as VP residues decreased over time. I predicted that there would be

a decrease in the total numbers of insects emerging from dung deposited by cattle treated with pour-on formulations of ivermectin and doramectin compared to dung from untreated cattle. I also predicted that as the VPs were metabolized by the cattle post-treatment, the numbers of insects to completing development in the dung would increase over time as has been shown in other studies (Floate 1998). This study is important because there is very little information on how VPs affect the colonization and survival of dung-dwelling arthropods in the Pacific Northwest region of the United States, despite the diversity of similar studies done elsewhere in North America. This study was done to add to the current knowledge on the effects of VPs on dung-dwelling insects in North America (Schmidt 1983, Fincher 1992, Floate 1998, 2006, Floate et al. 2001, 2002). This study was done not only to add to the current knowledge in North American but also to increase the knowledge of the effects of VPs in well studied dung communities in Asia (Iwasa et al. 2005, 2008), Australia (Dadour et al. 2000, Wardhaugh et al. 2001), Europe (Errouissi et al. 2001, Hempel et al. 2006, Webb et al. 2007), South America (Suarez et al. 2003, Iglesias et al. 2006) and South Africa (Kryger et al. 2005, 2006, 2007). The studies conducted on the effects of VPs consist of both laboratory bioassays (Schmidt 1983, Madsen et al. 1990, Dadour et al. 2000, Floate et al. 2001, Iwasa et al. 2005, 2008, Kryger 2006, 2007) and studies conducted under field conditions (Schmidt 1983, Fincher 1992, Floate 1998, 2006, Wardhaugh et al. 2001, Floate et al. 2002, Suarez et al. 2003, Iwasa et al. 2005, 2008 Kryger et al. 2005 Webb et al. 2007).

## **Materials and Methods**

*Experiment 1: The effects of Ivomec and Ivomec Plus on Beetles, Flies & Parasitoids (2006)*

To determine the effects of veterinary parasiticides (VPs) applied to cattle on dung-dwelling insect fauna in the Pacific Northwest, I conducted an experiment on cattle in an irrigated pasture setting in Prosser, Benton Co., WA. Cattle were a mix of Hereford and Black Baldy cows for a total of 33 cows sampled. The herd was divided into three treatment groups and was treated by individual cow as follows: ivermectin at the label rate (500 µg/kg body weight of IVOMEC<sup>®</sup> Pour-On For Cattle treatment; Merial: Duluth, GA); ivermectin + clorsulon at the label rate (500 µg/kg body weight of IVOMEC<sup>®</sup> Plus Injection for Cattle; Merial: Duluth, GA), and the control received no treatment. Each cow possessed an ear tag with a unique number, which was used to identify individual cows after they had been moved back into their pastures.

Dung was collected 3, 7, 14 and 21 days post-treatment with the specific VPs as described above. Cattle were monitored, and dung was collected within five minutes of deposition. Dung from each collection day was homogenized among individual cows within treatments, divided into 0.5 L pats, and held at -40°C in Ziploc<sup>®</sup> storage bags (SC Johnson, Racine, WI, USA). Dung pats were thawed twenty four hours prior to being used in the experiment.

On 11 July 2007, following the protocol outlined in Floate (1998), 0.5 liter dung pats were placed in a pasture. Each pat was set on a 2 cm deep bed of sand covering a 23 cm Styrofoam plate that had three holes cut in the bottom to allow rainwater to drain.

The Styrofoam plates were placed in a pasture adjacent to a second pasture containing grazing cattle, to ensure that dung-dwelling insects were in the study area. The dung pats were placed in a 6 x 2 randomized grid design with 1 m between each dung pat. One replicate of day and treatment were present within the grid.

Five days after putting out the artificial pats, they were removed from the pasture and placed into emergence cages. The protocol highlighted in Floate (1998) was used with some modification. Emergence cages were 11 liter white plastic buckets (United States Plastic Corporation, Lima, OH, USA) fitted with fine mesh sleeves to contain emerging adult insects. All of the buckets were placed in the same covered barn (at Washington State University Irrigated Agriculture Research and Extension Center, Prosser, WA), to ensure that all emergence buckets were exposed to similar conditions of temperature and humidity. The cover also prevented desiccation of pats due to direct sunlight. Distilled water (50 mL) was added to each pat every other week after emergence of arthropods was first noted, to prevent desiccation of developing insects. The dung pats were monitored daily over a seven week interval for adult insect emergence. Once adult insects began to emerge from the pats they were aspirated and stored in 70% ethanol and collected insects were sorted, counted, and identified to family level.

*Experiment 2: The effects of Dectomax and Ivomec on Beetles, Flies & Parasitoids (2007)*

This experiment was conducted in Prosser, Benton Co., WA to determine the effects of two formulations of VPs applied to cattle on dung beetles, flies, and parasitoids. Two

herds (comprising a mix of Hereford and Black Baldy cows) were each divided into three treatment groups and treated as follows: doramectin at the label rate (500 µg/kg body weight of DECTOMAX® Pour-on; Pfizer: Exton, Pa); Ivermectin (500 µg/kg body weight of IVOMEC® Pour-On For Cattle; Merial: Duluth, GA); and the untreated Control group. Once treated, the two herds were grazed on their respective pastures located four miles apart in Prosser, Benton Co. WA.

Dung was collected immediately before VP treatment and then weekly beginning with the first week of treatment and ending following the eighth week post-treatment. Cows were monitored, and fresh dung that was less than five minutes old was collected from individual cows of each treatment group and stored separately at -40°C in Ziploc® storage bags. Individual cows had ear tags that were used to determine their treatment. Twenty four hours prior to the start of the experiment dung was allowed to thaw.

On 25 July 2007, following the protocol outlined in Floate (1998), 0.5 L artificial dung pats were formed and placed in a pasture on a 2 cm deep bed of sand covering a 23 cm Styrofoam plate as described above. Styrofoam plates were then placed in a pasture adjacent to grazing cattle. The dung pats were placed in a 50 x 6 randomized grid design with 1 m between each dung pat. One replicate of each cow, day, and treatment were present within the grid.

Due to the lack of adult insect emergence from Experiment 1 (2006), the artificial pats were left in the field for two days rather than five. Over the five day period the average

air temperature was 25.7 °C with average relative humidity at 51.5 %, and these conditions caused the pats to dry completely and become very hard despite adding water to the pats at intervals after they had been placed in the emergence cages (Agweathernet 2008, Washington State University). Two days is sufficiently long to attract beetles and flies according to previous research (Mohr 1943, Floate et al. 2005) and my preliminary studies. After the two days, the dung pats were removed (on 27 July 2007) from the pasture and placed into emergence cages. Following the protocol highlighted in Floate (1998) and described above, insects were collected from dung pats in emergence buckets, stores in 70% ethanol and identified to family level. Due to low emergence numbers in some families it was not possible to conduct statistical analysis on the families independently. In order to analyze differences it was necessary to sum the families of the Diptera and Coleoptera and conduct the analysis by order. Floate (1998) reported that summing across all flies can conceal the sensitivity of individual species to the residues of VPs. This was unavoidable in the current study due to low representation of individuals at the species, genus, and in some cases, family level.

*Experiment 3: The effects of Dectomax and Ivomec on Beetles, Flies & Parasitoids (2008)*

Experiment 3 was conducted from June to August in Prosser, Benton Co., WA to repeat Experiment 2: 2007, with some modification. Three herds of cattle were treated with topical pour-on formulations of two different VPs. Two herds of cows were all Hereford cows. The third herd contained all heifers, which were a mix of Hereford and Black Baldy. As described above, the three herds were each divided into three treatment

groups and treated as follows: the doramectin treatment received the label rate (500 µg/kg body weight of DECTOMAX® Pour-on; Pfizer: Exton, Pa), ivermectin at label rate (500 µg/kg body weight of IVOMEC® Pour-On For Cattle; Merial: Duluth, GA), and the control group received no treatment.

Dung was collected 1, 4, 7 and 21 days post-treatment of VPs; dung from control cows was collected on these same dates. Dung from each collection day was homogenized within treatment by herd, divided into 0.5 L pats, and held at -40°C in Ziploc® storage bags. As mentioned above, each cow had an ear tag used to identify treatment group. Twenty four hours prior to being used in the experiment the dung pats were allowed to thaw.

Each experiment was placed outdoors in a pasture adjacent to where cattle were grazing in order to maximize insect colonization as previously described. The artificial pats were placed on a 2 cm bed of sand on top of a 30 x 30 cm piece of wax paper. Wax paper was used instead of Styrofoam plates to maximize colonization of insects that reach the pat by walking. The pats were arranged according in a randomized complete block design and blocked by herd, with one replicate of each day and treatment present. Within each block replicates were arranged in a 4 x 3 m grid design with 1 m between each replicate. Adjacent blocks were separated by a distance of 2 m.

After two days hours the pats were removed from the field and placed in emergence buckets as previously described. Adult insects were aspirated as they emerged,



collected into 70% ethanol and counted. As in Experiment 2: 2007 it was not possible to conduct statistical analysis on the families independently due to low emergence numbers in some families. In order to analyze differences it was necessary to sum the families of the Diptera and Coleoptera and conduct the analysis by order.

### **Statistical Analysis**

Statistical analysis of the data for Experiment 1: 2006 was not conducted because no insects emerged. This was most likely due to desiccation of all the dung pats from the experiment; the pats were hard and dry and no organisms completed development. For the 2007 data, effects of treatment (Control, Ivomec, Dectomax), time (weeks 1-8 post-treatment), and herd on counts of arthropods emerging from dung pats were assessed using a repeated measures analysis of variance, as the data consisted of repeated observations through time for each cow. Emergence counts were often clumped among artificial dung pats; no emergence occurred from some artificial pats while others produced large numbers of insects. These emergence patterns led to difficulties in meeting the assumptions of ANOVA therefore the count data were normalized by square root transformation. The analyses were done using PROC MIXED in SAS (SAS Institute 2002). Normality assumptions were assessed by calculating the Shapiro-Wilk statistic using PROC UNIVARIATE (SAS Institute 2002). When transformation of the data failed to meet assumptions of normality, the untransformed counts were instead modeled using the negative binomial distribution.

Analyses were done using PROC GLIMMIX in SAS (SAS Institute 2002). A small constant (0.05) was added to each count for the Hymenoptera, as the analysis failed to converge for this taxon using the original data regardless of transformation. The ar(1) covariance structure was used to account for correlations among the repeated measures across days. The ilink option was used to back transform least squares means and standard errors to the original count response variable. Degrees of freedom were adjusted using the Kenward-Rogers adjustment (Littell et al. 2006). In the event of a significant interaction between treatment and the other factors, tests on simple effects were done using the slice command.

For the 2008 data, repeated measures analysis of variance was used to assess effects of treatment (control, Ivomec, Dectomax), month (June, July, August), and days (1, 4, 7, or 21 days post-treatment) on counts of arthropods emerging from dung pats. Methods of analysis and data transformations were similar to those described above for the 2007 data.

## **Results**

*Experiment 2: The effects of Dectomax and Ivomec on Beetles, Flies & Parasitoids (2007)*

### Diptera

The Dipteran species that colonized the artificial dung pats were composed of individuals of Ceratopogonidae, Stratiomyidae, Phoridae, Sepsidae, Sphaeroceridae, Anthomyiidae, Muscidae, Calliphoridae and Sarcophagidae. The Sarcophagidae were

the most numerous of the families present comprising 75 percent of total fly emergence. Treatment with VP's had no effects on fly emergence, as shown by absence of significant treatment effects in the ANOVA (mean emergence per pat (SE): Control = 2.33 (0.11); Doramectin = 2.29 (0.11); Ivermectin = 2.26 (0.11); Fig. 1;  $F_{2, 29} = 0.09$ ,  $P = 0.91$ ); interactions of treatment with herd, week, or week x herd were also non-significant ( $P > 0.10$  in all cases). Emergence did fluctuate significantly by day (Fig. 2;  $F_{7, 203} = 5.8$ ,  $P < 0.0001$ ), with peak counts occurring at 14 and 21 days. Emergence showed somewhat different patterns through time between the two herds (Fig. 3;  $F_{7, 203} = 10.9$ ,  $P < 0.0001$ ); rates of emergence were very low in week 1 for one of the two herds.

### Coleoptera

The Coleoptera families that colonized the artificial pats included the Carabidae, Hydrophilidae, Staphylinidae, Scarabaeidae, Histeridae and Curculionidae. The majority of the Coleoptera consisted of Scarabaeidae in the genus *Aphodius*, which are primarily dung feeding beetles and are ubiquitous in North America in herbivore dung. The genus *Aphodius* comprised 77 percent of the total Coleoptera present. The presence of beetles did not differ by treatment of VPs (mean emergence per pat (SE): Control = 5.7 (0.4), Doramectin = 5.4 (0.4), Ivermectin = 4.5 (0.4); Fig. 4;  $F_{2, 21} = 2.10$ ,  $P = 0.14$ ). There was a significant treatment by day interaction (Fig. 5;  $F_{14, 147} = 2.03$ ,  $P = 0.02$ ) with lower beetle presence on day 28 in the Ivermectin treatment. Differences between herds through time in beetle emergence led to a significant herd by day interaction (Fig. 6;  $F_{7, 147} = 2.97$ ,  $P = 0.006$ ).

## Hymenoptera

The emergence of Hymenoptera was affected by VP treatment (mean emergence per pat (SE); Control= 0.80 (0.25), Doramectin= 1.37 (0.17), Ivermectin= 1.76 (0.17): Fig. 7;  $F_{2, 29} = 5.3$ ,  $P = 0.01$ ). Dung from cattle that had been left untreated produced the highest rates of emergence occurring in dung from Ivermectin treated cattle. None of the interaction terms involving treatment were significant. Emergence did fluctuate significantly by day (Fig.8;  $F_{7, 203} = 5.9$ ,  $P < 0.0001$ ), with peak counts occurring at 21 days post treatment. Emergence rates also differed through time between the two herds leading to a significant herd by time interaction (Fig. 9;  $F_{7, 203} = 3.8$ ,  $P = 0.0007$ ). Averaged over days, emergence rate depended upon the source of the dung (Fig. 10;  $F_{1, 29} = 22.25$ ,  $P < 0.0001$ ).

*Experiment 3: The effects of Dectomax and Ivomec on Beetles, Flies & Parasitoids (2008)*

## Diptera

As in Experiment 2: 2007 the species of Diptera that colonized the dung pats were composed of Ceratopogonidae, Stratiomyidae, Phoridae, Sepsidae, Sphaeroceridae, Anthomyiidae, Muscidae, Calliphoridae and Sarcophagidae. Sarcophagidae were again the most numerous of the families present comprising 92 percent of total fly emergence. In contrast to results obtained in 2007, emergence of Diptera was affected by VP treatment (Fig. 11; treatment:  $F_{2,28.6} = 5.2$ ,  $P = 0.01$ ). Treatment of cattle with either Doramectin or Ivermectin led to lowered emergence rates from dung than observed

emerging from dung collected from untreated cattle (Figure 11,  $P < 0.05$  by LSD test). None of the interaction terms involving treatment were significant. Emergence appeared to peak at day 4 (Fig. 12; day:  $F_{3,53} = 5.29$ ,  $P = 0.0029$ ). Finally, emergence rates decreased between the June and August samples (Fig. 13; month:  $F_{2,28.6} = 12.0$ ,  $P = 0.0002$ ).

### Coleoptera

The Coleoptera families that colonized the artificial pats were the same as in Experiment 2: 2007, which included the Carabidae, Hydrophilidae, Staphylinidae, Scarabaeidae, Histeridae and Curculionidae. The majority of the Coleoptera consisted of Scarabaeidae in the genus *Aphodius*, which are primarily dung feeding beetles and are ubiquitous in North America in ruminant herbivore dung. The genus *Aphodius* comprised 96 percent of the total Coleoptera present. As was observed in Experiment 2:2007 emergence of beetles was not affected by treatment ( (Fig. 14;  $F_{2,30.1} = 0.45$ ,  $P = 0.64$ ) in the rates of emergence. None of the interaction terms involving treatment were significant. Beetle presence did significantly differ by day with the most beetles present on Day 1 post-treatment (Fig. 15;  $F_{3, 50} = 13.26$ ,  $P < .0001$ ). Significantly more beetles were present in pats from August than July, with June having the lowest numbers of beetles of the three months (Fig 16;  $F_{2, 30.1} = 22.14$ ,  $P < 0.0001$ ).

### Hymenoptera

Due to the low number of wasps emerging from cages, no statistical analysis was conducted.

## Discussion

These experiments were conducted to test the hypothesis that survival of dung-dwelling insects would be negatively affected by the residues of veterinary parasiticides in cattle dung, and that over time this effect would decrease. I predicted that the total numbers of insects emerging from dung deposited by cows that had been treated with pour-on formulations of ivermectin and doramectin would be lower than emergence from dung deposited by untreated cattle. This result was observed for Diptera in Experiment 3: 2008. In Experiment 2:2007 emergence rates were significantly higher in the ivermectin and doramectin treatments compared to control. However there were no differences in emergence by treatment for the Coleoptera in either experiment. I also predicted that as the VPs were metabolized by the cattle post-treatment the numbers of insects able to survive on the dung would increase over time, as has been shown in other studies (Floate 1998). There was no difference in emergence from dung containing VP residues for flies, beetles or wasps through time.

In Experiment 2: 2007 Diptera emergence was lower at day 7 in herd 2 for unknown reasons (Fig. 3). There was also a significant difference in emergence by day with peak emergence on day 21 (Fig. 2). Although rates of emergence fluctuate there is no support for a consistent trend of emergence through time. In Experiment 3: 2008 fly emergence was significantly higher on day 4 than days 1, 7, and day 21 (Fig. 8). As with Experiment 2: 2007 the difference does not seem to support a trend of emergence. Also, in Experiment 3: 2008 there were significantly more flies emerging in June than July and August (Fig. 12). This difference is most likely due to biology of Diptera. For

example, Sarcophagidae, which comprised the majority of the flies that emerged from our dung pats, overwinter in a pupal diapause. The emerging of diapausing flies could explain why more flies were collected in June than July and August.

The effects of veterinary parasiticides on flies differed among the experiments. In Experiment 2: 2007, there were no differences observed between treatments for Diptera (Fig. 1). However in Experiment 3: 2008 dung collected from cattle treated with both ivermectin and doramectin caused reduced emergence in the number of Diptera (Fig.11). All flies that emerged from the artificial pats were presumed to be offspring of adult flies that oviposited and then went in search of new oviposition sites.

The results of Experiment 3: 2008 are consistent with many other studies of flies that have been conducted measuring the effects of VPs on the dung community throughout the world (Schmidt 1983, Floate 1998, Madsen et al. 1990, Fichner 1992, Dadour et al. 2000 Suarez et al. 2003, Iwasa et al. 2003, 2005, Floate 2006, Errouissi et al. 2007, Iwasa et al. 2008). It is possible that no effects on Diptera emergence were observed in Experiment 2: 2007 because it was conducted for a short period during the month of July. Performing the experiment in this manner only allows for a snapshot of the colonizing insects to be sampled. An experiment that is conducted many times over the better part of a field season, such as Experiment 3: 2008, should allow for a more complete sampling of community dynamics and presence. Conducting an experiment to survey dung insects in this manner could provide a more complete perspective of the

community dynamics of dung-dwelling insects in the pasture and offers the opportunity to determine the impacts of VPs on specific insect assemblages that colonize dung.

Although dung from cattle treated with VPs did lead to lower emergence rates by Diptera, these effects were not observed for the Coleoptera. In Experiment 3: 2008 the presence of more beetles on days 1 and 4 than days 7 and 21 may be attributed again to the attraction of beetles to VP volatiles in the cattle dung but this cannot be confirmed in this study (Floate 2007) (Fig. 12) Greater abundance of beetles were observed in Experiment 3: 2008 from June through August (Fig 16). No effects were found in the presence of Coleoptera in emergence cages containing dung from cattle treated with VPs.

The fact that beetles were not affected by VP residues in dung is consistent with the findings of Suarez et al. (2003); there were no differences in the numbers of Scarabaeidae recovered during the experiment from pats containing residues of ivermectin or doramectin and untreated pats. Suarez et al. (2003) also reported no repellent effect on beetle colonization in pats from animals treated with ivermectin. As stated previously Floate (2007) demonstrated that ivermectin had a strong attractive effect on a number of species of dung flies, beetles and wasps. The experimental design of this study did not make it possible to assess whether or not ivermectin had a repellent or attractive effect in artificial pats from animals. In both Experiment 2: 2007 and Experiment 3: 2008 dung beetles were collected as they were found in the emergence buckets. This method did not allow me to distinguish the colonizing parental



generation from the F1 generation. That is, the beetles that were found in the buckets likely consisted of colonizers and their offspring. Since the two groups were not differentiated, the possible attraction of dung beetles to the pats could not be determined. Although the level of attraction could not be determined, there was no impact of VPs on the presence of beetles in the buckets. The lack of any effects of VPs on beetles suggests the need for additional and more in depth study of the dung community in this region to be confident in this result across beetle families and for different environmental conditions.

In Experiment 2: 2007 the third order of insects that emerged from dung was parasitic Hymenoptera. This order was composed solely of the family Braconidae. In the 2007 experiment, I observed significantly lower rates of emergence of wasps from control dung compared to dung from the Doramectin and Ivermectin treatments (Fig. 7). A possible explanation for this observation is that ovipositing hymenoptera were more attracted to the dung containing residues of VPs. Floate( 2007) determined that families of Diptera, Coleoptera and Hymenoptera were attracted to dung that contained residues of VPs. Another possible explanation is that the ability of the host to encapsulate and kill parasitoid eggs or larvae was decreased due to the presence of VP residues. Floate and Fox (1999) reported higher rates of parasitoid emergence from dung containing ivermectin at 0.01 ppm than from control dung containing no VP residues. However, when they exposed fly larvae to higher concentrations of ivermectin there was significantly more mortality of larvae and less parasitoid emergence. From these results it was concluded that at low concentrations ivermectin can prevent developing host

larvae from killing parasitoid eggs thus increasing the rates of parasitoid emergence.

With no effects of VP residues detected in Experiment 2: 2007 on host larvae it is likely that hampering of host defenses is a possible explanation for the significant increase in emergence rates of parasitoids from treated dung in my experiment.

The results of this study have implications for dung community dynamics. The reduction of Diptera emergence in Experiment 3: 2008 can lead to reduced fly larvae presence in dung pats and thus lead to a reduction in rates of dung degradation. This concept has been reported in many studies (Wall and Strong, 1987, Madsen et al. 1990, Floate 1998, Floate et al. 2002). This is important because a reduction in insect activity is the mechanism that is important rather than the VP itself (Floate 2002). The reduction of insect activity caused by VPs may not be the only reason that dung is not degraded. Lee and Wall (2006) suggest that the VP chemicals may affect the breakdown of dung directly by altering the consistency of the dung. This may ultimately affect both weathering of dung, and the rates at which dung is colonized by arthropods. It is difficult to support the possible reduction in the degradation of animal dung by insect reduction with the findings of these two studies because in both Experiment 2: 2007 and Experiment 3: 2008 colonizing and reproducing beetles were not affected by VP residue presence.

Previous studies found that adult (Fincher 1992, Suarez et al. 2003) and larval (Iwasa et al. 2005, 2008) stages of beetles were not affected by VP residues in dung, which coincides with the findings of this study. Beetles were collected for up to seven weeks

after the dung was removed from the field. This follows the protocol in Floate (1998) where the progeny of the colonizing beetles were collected beginning six weeks after the pats had been removed from the field. The lack of VP effect is important because of the role that dung beetles have within a pasture ecosystem. Dung beetles are important in the reduction of the loss of nitrogen, through ammonia volatilization, and the increase in soil fertility because they increase the amount of nitrogen in the soil that plants can uptake by burying dung in the soil (Nichols et al. 2008). Along with returning nutrients to the soil, coprophagous beetles contribute to higher yields in forage on which livestock graze (Bang et al. 2005). This is important because if dung is not degraded then nutrients remain in the pat and are not incorporated into the soil and the forage that is in the vicinity of dung may be avoided by grazing livestock (Nichols et al. 2008). Omaliko (1981) observed that cattle reject grazing around a pat up to 12 weeks after deposition of the dung pat. The degradation of dung impacts nutrient cycling within a pasture, plant composition, soil fertility (Wall and Strong 1987, Herd 1995), as well as reduces the amount of fouled grassland avoided by cattle. Dung degradation ultimately leads to better and sustainable animal husbandry practices (Wall and Strong 1987).

The experimental design that was used in this study was modified from year to year which introduced several potential pitfalls to this work. One such potential problem was that since the cattle were in herds and then divided, treated with VPs, and then allowed to graze together the potential for allogrooming must be taken into account.

Allogrooming is a documented phenomenon where one cow will lick another cow. This was observed in my study once the herds were treated and allowed to graze in their

respective pastures. Allogrooming happened most often between cows and their calves, although the calves were not included in the study. Laffont et al. (2001) observed that cattle that were allowed to lick themselves after being treated with a pour-on formulation of ivermectin eliminated the VP at a rate 33 times higher than cattle that were not allowed to lick themselves. It was also reported that cattle allowed to allogroom had 70 percent of the drug unchanged in their dung (Laffont et al. 2001). It was concluded that a large amount of the pour-on formulation was ingested by the cattle and moved directly through the digestive tract into the feces (Laffont et al. 2001). In this experiment allogrooming presents the problem of cross contamination between treated cattle and between treated and non-treated cattle. This situation was unavoidable because we were working with cattle owned by volunteer rancher-cooperators. These cattle producers use rotational grazing, often in small pastures, and it was not logistically possible to divide the herds by treatment groups for long periods of time. Although allogrooming presents a potential confounding factor the possible variability in forage quality between treatment groups that might be observed if treatment groups were grazed in separate pastures was eliminated.

The second potential problem that could have confounded our results was the time of year when the experiments were conducted. This was evident with the failure of insects to develop in the dung of Experiment 1: 2006. In Washington State a majority of producers who treat their cattle once a year with VPs do so in the fall, while those who treat twice a year do so in the fall and spring corresponding with other herd management practices and not specifically for treatment with VPs (Ferguson et al.

2006). These experiments were conducted during the summer months where VP residues might not be present in the field and environmental conditions are poor for insect colonization. VP experiments were introduced at a time of the year when they are not typically found in cattle dung in this area. It is important to conduct these studies in the fall and spring when most producers are actually treating their cattle. During these times the insects are at different physiological states. In the spring insects are emerging from diapause and in the fall they preparing to enter diapause. Dung-dwelling insect fauna could respond differently to VP residues in dung depending on their physiological state.

The results from this research show that dung from cattle treated with pour-on formulations of ivermectin and doramectin do not have any effects on beetle species, associated with dung in the Columbia Basin of central Washington State during the summer months. However, flies did respond negatively to fecal residues of VPs as evidenced by a decrease in emergence compared to non-treated control dung. Parasitic Hymenoptera had lower emergence rates in dung containing no residues compared to doramectin and ivermectin. These results underline the need for continual study of the impacts of endectocide compounds in different environmental and geographic conditions.

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## Figure Captions

### Fig 1.

Experiment 2: 2007- Fly Emergence by Treatment. Mean values  $\pm$  SE of individual Diptera emerging from Control, Doramectin and Ivermectin dung pats. Data are square root transformed.

### Fig. 2

Experiment 2:2007- Fly Emergence by Day. Mean values  $\pm$  SE of Diptera emerging from dung pats by day post-treatment with VPs. Data are square root transformed.

### Fig. 3

Experiment 2: 2007- Fly Emergence by Herd by Day. Mean values  $\pm$  SE of individual Diptera emerging from dung pats in the two herds by day post-treatment with VPs. Data are square root transformed.

### Fig. 4

Experiment 2: 2007- Beetle Presence by Treatment. Mean values  $\pm$  SE presence of individual Coleoptera from Control, Doramectin and Ivermectin dung pats. Data are square root transformed.

### Fig. 5

Experiment 2: 2007- Beetle Presence by Treatment by Day. Mean values  $\pm$  SE presence of individual Coleoptera from Control, Doramectin and Ivermectin dung pats by day post-treatment with VPs. The data are square root transformed.

### Fig. 6

Experiment 2: 2007- Beetle Presence by Herd by Day. Mean values  $\pm$  SE of individual Coleoptera present from dung pats in the two herds by day post-treatment with VPs.

Data are square root transformed.

**Fig. 7**

Experiment 2: 2007- Wasp Emergence by Treatment. Mean values  $\pm$  SE of individual Hymenoptera emerging from Control, Doramectin and Ivermectin dung pats. Data are

square root transformed.

**Fig. 8**

Experiment 2:2007- Wasp Emergence by Day. Mean values  $\pm$  SE of Hymenoptera emerging from dung pats by day post-treatment with VPs. Data are square root

transformed.

**Fig. 9**

Experiment 2: 2007- Wasp Emergence by Herd by Day. Mean values  $\pm$  SE of individual Hymenoptera emerging from dung pats in the two herds by day post-treatment with

VPs. Data are square root transformed.

**Fig. 10**

Experiment 2: 2007- Wasp Emergence by Herd. Mean values  $\pm$  SE of individual Hymenoptera emerging from dung pats in the two herds. Data are square root

transformed

**Fig. 11**

Experiment 3: 2008- Fly Emergence by Treatment. Mean values  $\pm$  SE of individual Diptera emerging from Control, Doramectin and Ivermectin dung pats. Data are square root transformed.

**Fig. 12**

Experiment 3:2008- Fly Emergence by Day. Mean values  $\pm$  SE of Diptera emerging from dung pats by day post-treatment with VPs. Data are square root transformed.

**Fig. 13**

Experiment 3:2008- Fly Emergence by Month. Mean values  $\pm$  SE of Diptera emerging from dung pats by month. Data are square root transformed.

**Fig. 14**

Experiment 3:2008- Beetle Presence by Treatment. Mean values  $\pm$  SE presence of individual Coleoptera from Control, Doramectin and Ivermectin dung pats. Data are square root transformed.

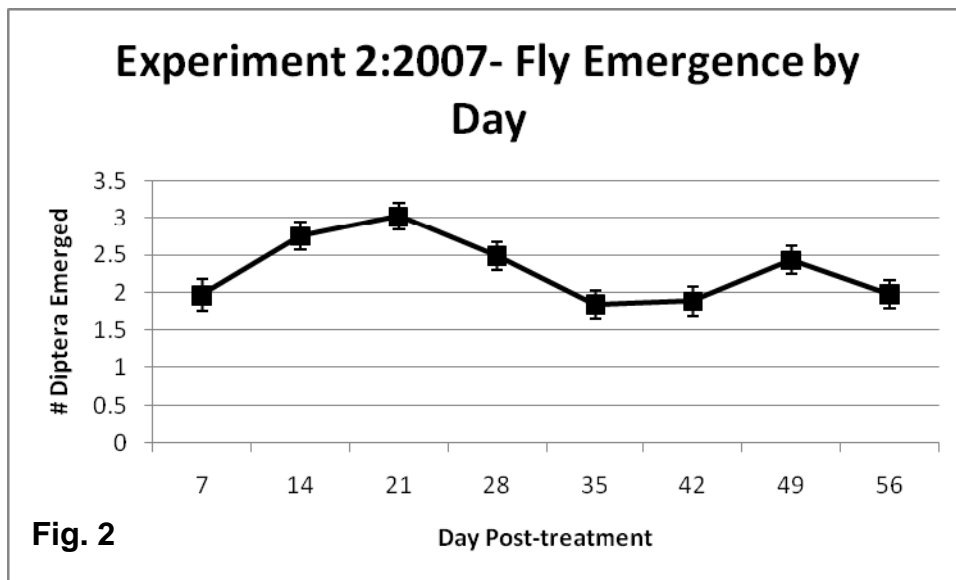
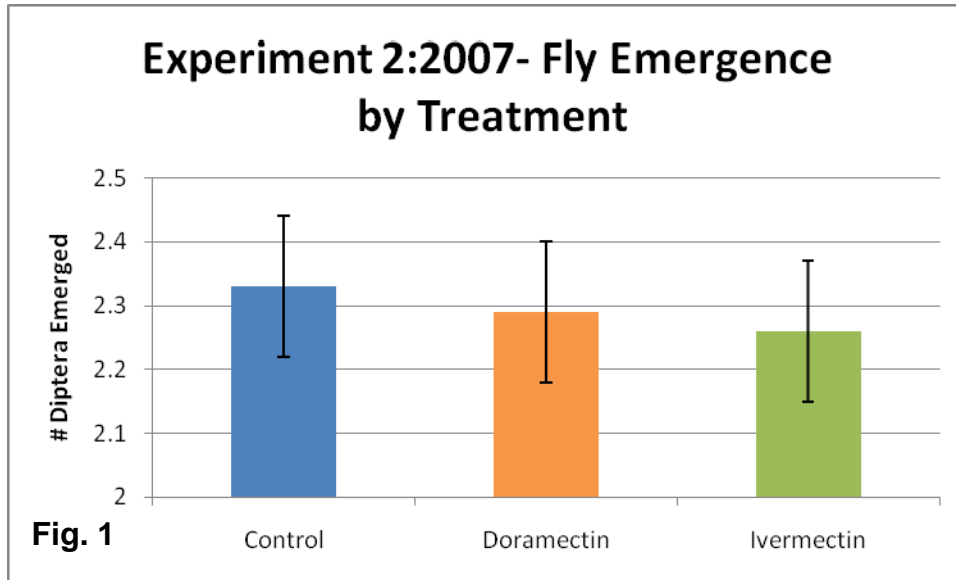
**Fig. 15**

Experiment 3:2008- Beetle Presence by Day. Mean values  $\pm$  SE of individual Coleoptera present from dung pats by day post-treatment with VPs. Data are square root transformed.

**Fig. 16**

Experiment 3:2008- Beetle Presence by Month. Mean values  $\pm$  SE of individual Coleoptera present from dung pats by month. Data are square root transformed.

## Figures





### Experiment 2:2007- Fly Emergence by Herd by Day

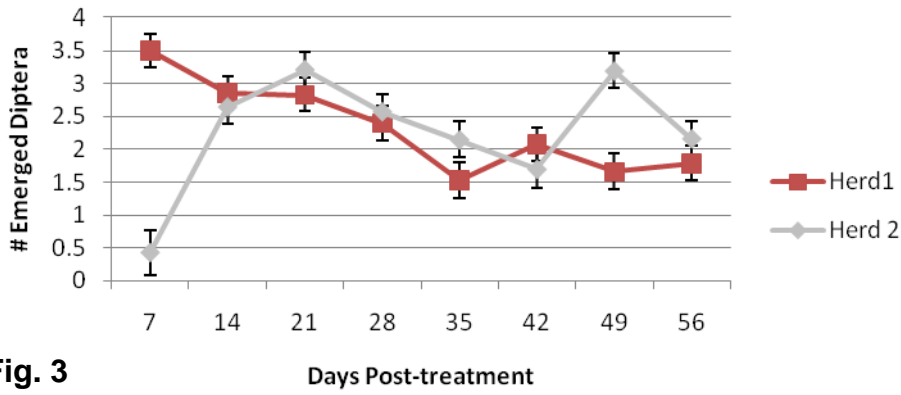


Fig. 3

### Experiment 2:2007- Beetle Presence by Treatment

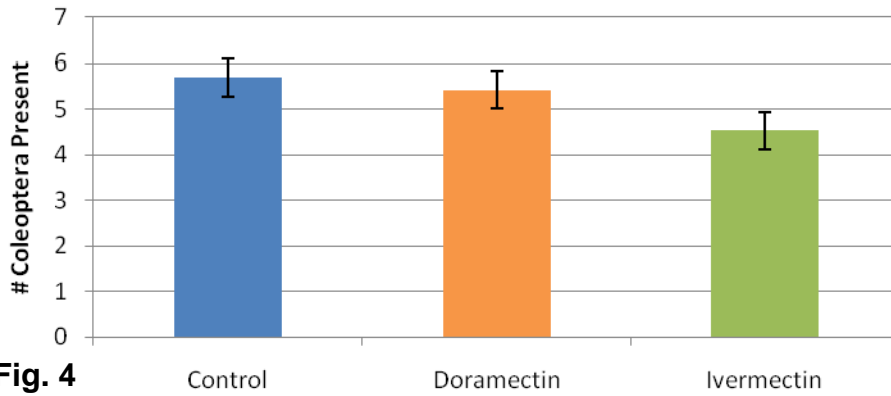


Fig. 4

### Experiment 2:2007- Beetle Presence by Treatment by Day

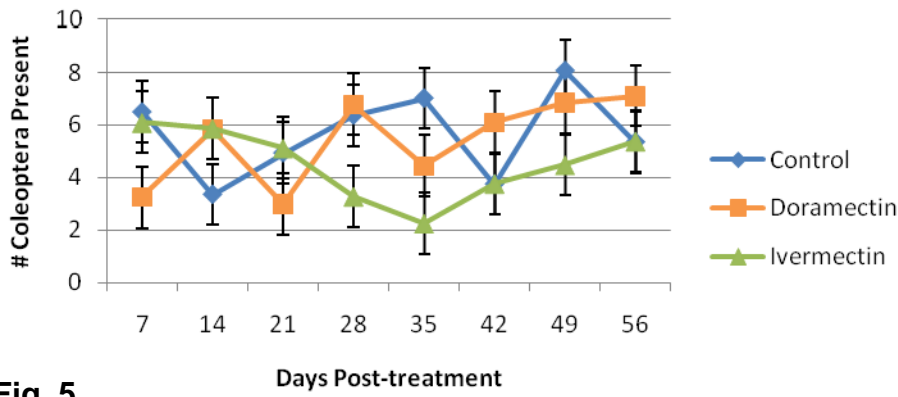


Fig. 5

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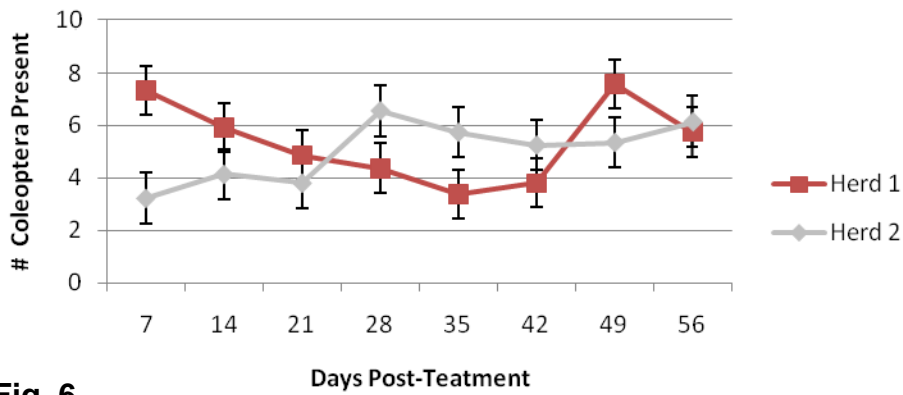


Fig. 6

### Experiment 2:2007- Wasp Emergence by Treatment

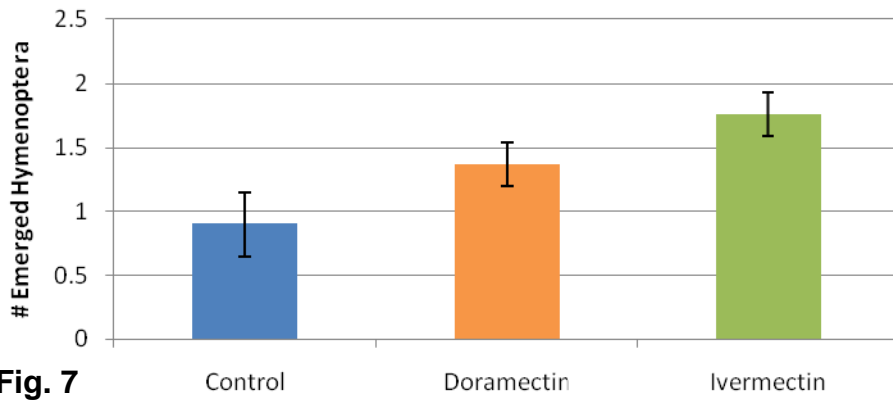


Fig. 7

### Experiment 2:2007-Wasp Emergence by Day

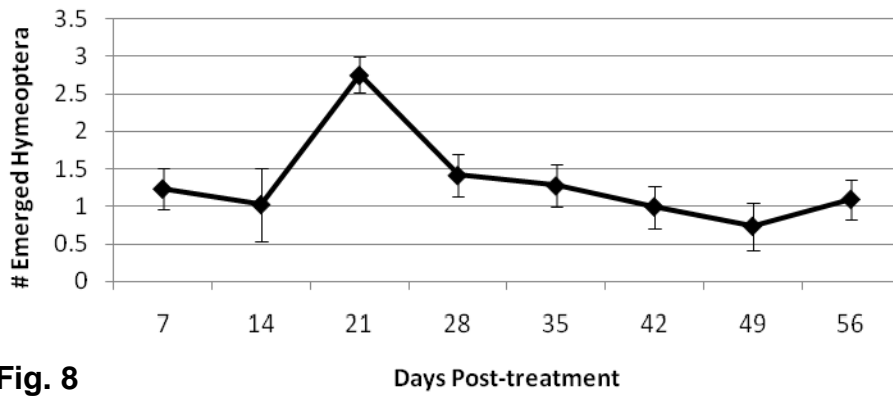


Fig. 8

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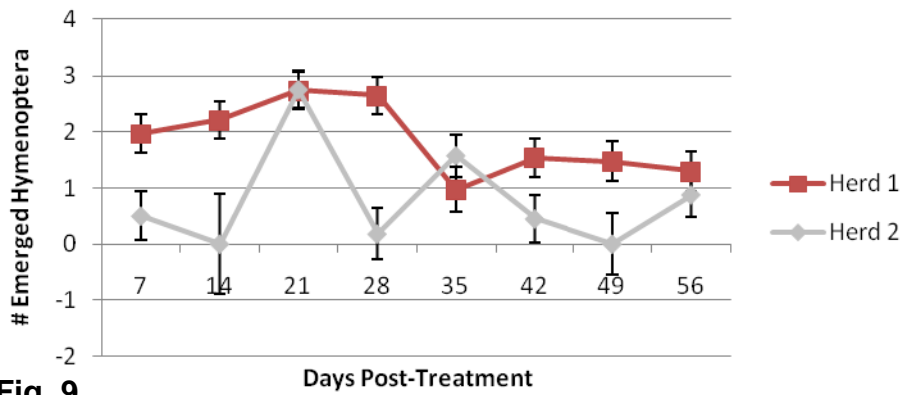


Fig. 9

### Experiment 2:2007- Wasp Emergence by Herd

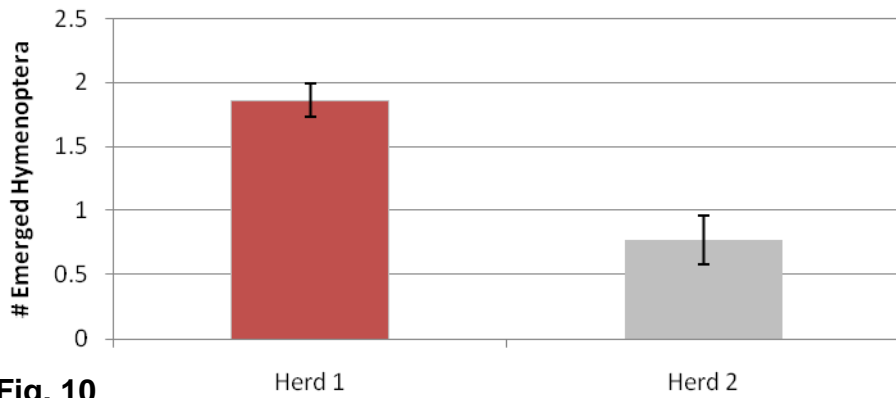


Fig. 10

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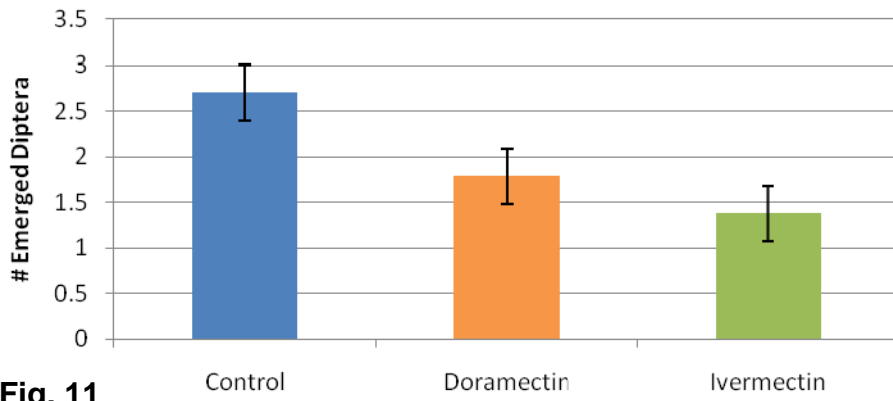


Fig. 11

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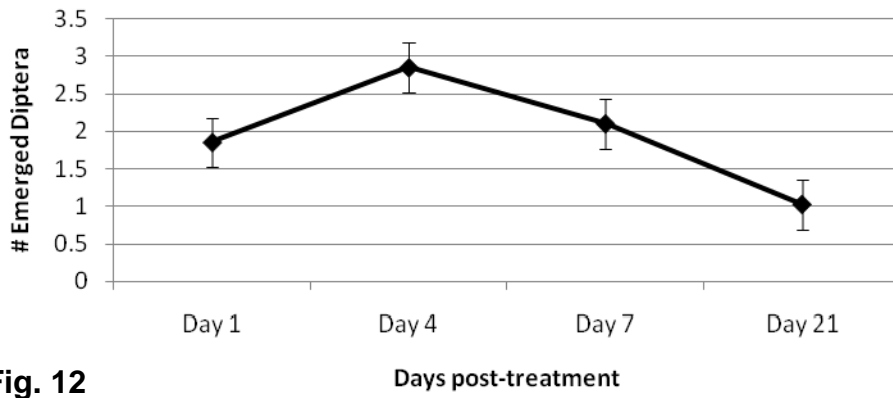


Fig. 12

### Experiment 3:2008- Fly Emergence by Month

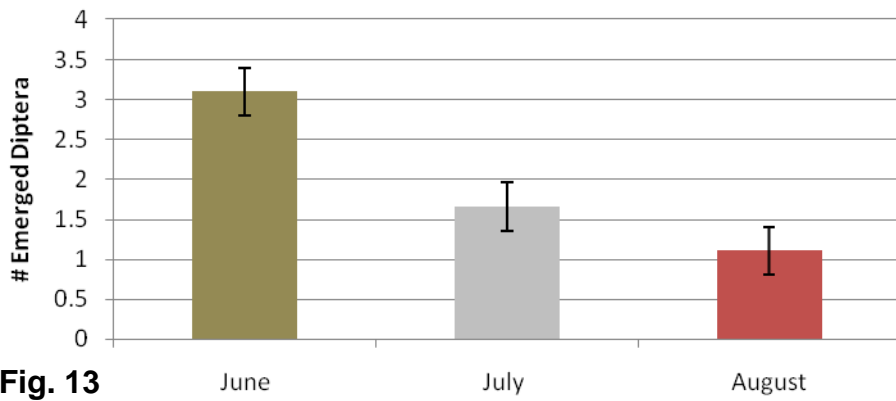


Fig. 13

### Experiment 3:2008- Beetle Presence by Treatment

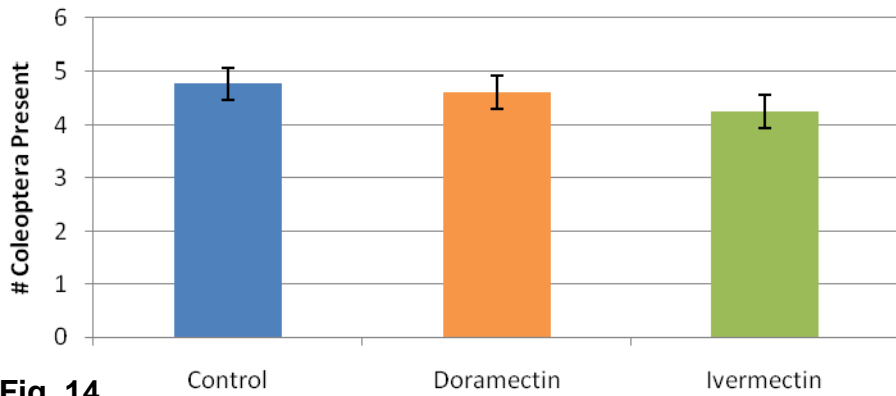


Fig. 14

### Experiment 3:2008- Beetle Presence by Day

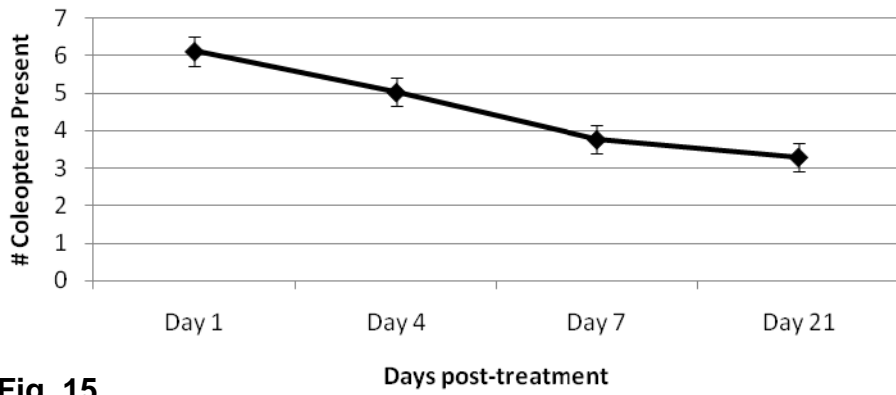


Fig. 15

### Experiment 3:2008- Beetle Presence by Month

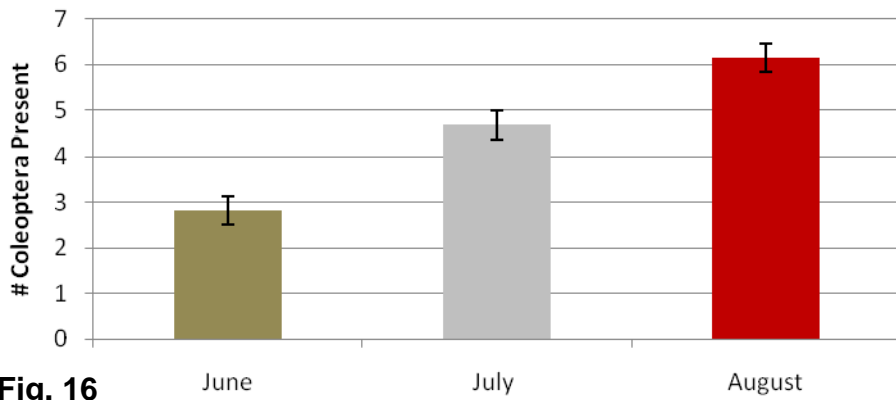


Fig. 16

## Discussion

The goal of my research was to examine the dung-dwelling insect community and degradative succession in the Pacific Northwest region of the US (PNW), and to characterize the effects of veterinary parasiticides (VPs) used to treat grazing cattle on the beetles, flies, and parasitoids in the PNW. I conducted two experiments over two years in order to characterize succession of dung-dwelling insects in the PNW. I also conducted a series of experiments over two years to determine the effects of two commonly used VPs in the PNW rangeland cattle system, ivermectin and doramectin, on dung-dwelling beetles, flies, and parasitoids. From these studies a number of insects of the orders Diptera, Coleoptera and Hymenoptera emerged from dung pats.

Diptera included the families Ceratopogonidae, Stratiomyidae, Phoridae, Sepsidae, Sphaeroceridae, Anthomyiidae, Muscidae, Calliphoridae and Sarcophagidae. In all studies, specimens of Sarcophagidae were the most common flies to emerge from the dung pats. Beetles consisted of the families Carabidae, Hydrophilidae, Staphylinidae, Scarabaeidae, Histeridae and Curculionidae. The Scarabaeidae were most abundant, consisting entirely of beetles in the genus *Aphodius*. Of the wasps the only family to emerge from the dung pats in both experiments was Braconidae.

My first study was conducted to test the hypothesis that emergence of insects from dung would differ by day and by season. I predicted that the fly emergence would be present equally in all days but that beetle presence would be lower in day 2 increasing in numbers through days 3 and 4. This was predicted because fly colonization is



reported to peak within a few hours of dung excretion where colonization by beetles peaks between day 1 and 5 (Floate et al. 2005). The results of my experiment did not support the hypothesis in either of the two succession experiments.

In the succession study there were no differences in the emergence of colonizing insect's offspring by day or by month except in Experiment 2: 2008 where I observed more flies emerging on day 2 than day 3 and 4. A probable explanation for this result is that mortality of developing larvae was reduced because dung pats were removed from the harsh environmental field conditions and placed into more moderate conditions of the bull sale barn. Another interesting result was the difference in mean insect emergence of flies and beetles between the three different locations; natural grassland (NG), non-irrigated (NI), and irrigated (IP). In Experiment 1: 2007 Diptera emergence was greatest in the NG site and least in the IP site. The opposite was true in Coleoptera, with presence highest in the IP site and lowest in the NG site. In Experiment 2: 2008 the greatest number of flies emerged from pats from the NG site followed by the NI site then the IP site. However, beetle presence was highest in the NI site, then IP site with the NG site having the lowest abundance. In both Experiment 1: 2007 and Experiment 2: 2008 fly emergence was greatest from pats in the NG site, with the IP site having the least amount of fly emergence. Although these differences were observed the means from the sites could not be compared statistically because they were not replicated. It was not possible to compare mean species emergence because of low representation at the species, genus, and in some cases, family level. It has been documented that

that summing across all insects of an order can conceal the sensitivity of individual species to treatments (Floate 1998).

My second study was conducted to test the hypothesis that the survival of dung-dwelling insects would be negatively affected by the presence of veterinary parasiticides in dung and that over time this effect would decrease. I predicted that the total numbers of insects emerging from cattle dung treated with pour-on formulations of ivermectin and doramectin would be lower compared to untreated dung. A reduction in insect emergence from dung deposited by cattle that had been treated with VPs was observed in Experiment 3: 2008 for the Diptera. While reduced rates of Hymenoptera emergence occurred in the control pats. However there were no differences in emergence by treatment for the Coleoptera in either experiment. I also predicted that as the VPs were metabolized by the cattle post-treatment the numbers of insects able to survive on the dung would increase over time, as has been shown in other studies (Floate 1998). No difference in emergence of insects from dung containing VP residues for flies, beetles or wasps was observed through time.

The reduction of emergence of Diptera in Experiment 3: 2008 is consistent with many other studies of flies that have been conducted measuring the effects of VPs on the dung community throughout the world (Schmidt 1983, Floate 1998, Madsen et al. 1990, Fichner 1992, Dadour et al. 2000 Suarez et al. 2003, Iwasa et al. 2003, 2005, Floate 2006, Errouissi et al. 2007, Iwasa et al. 2008). It is possible that no negative effects were observed in Diptera emergence in Experiment 2: 2007 because the experiment

was only conducted once in July. Perhaps an experiment conducted repeatedly over a field season, such as Experiment 3: 2008, allows for a more complete sampling of community dynamics and presence. Conducting an experiment in this way provides a more complete perspective of the community dynamics of dung-dwelling insects in the pasture and allows the impacts of VPs on insect assemblages that colonize dung to be examined.

Unlike the results of the Diptera, dung from cattle treated with VPs did not have an effect on the presence of Coleoptera. This result is not surprising given that Suarez et al. (2003) reported there were no differences in the number of Scarabaeidae recovered from pats containing residues of ivermectin or doramectin and untreated pats. Floate (2007) reported that attraction of dung beetles as well as flies and wasps may be altered in dung containing residues of VPs. Suarez et al. (2003) reported that no repellent effect on beetle colonization in pats from animals treated with ivermectin was observed. In my study dung beetles were collected as they were found in the emergence buckets in both Experiment 2: 2007 and Experiment 3: 2008. This collection method did not allow me to distinguish the colonizing parental generation from the F1 generation. Thus the beetles that were in the buckets represented both the colonizers and their offspring. Since the generations could not be differentiated the possible attraction of dung beetles to the pats could not be determined. Although the level of attraction could not be determined, there was no impact of VPs on the presence of beetles in the buckets.

Parasitic Hymenoptera were also affected by the presence of VP residues in the dung with a reduction of parasitoid emergence in the Control treatment (Experiment 2: 2007). An observation has been reported by Floate and Fox (1999). The mechanism as to why there is a reduction in wasp emergence is still not known. In their study Floate and Fox (1999) postulated that reductions in parasitoid emergence are due to the inability of host larvae to encapsulate and kill parasitoids eggs due to the effects of VP residues in the dung. Since there were no differences in host emergence in Experiment 2: 2007 the hampering of host defenses in the presence of VP residues is the most likely explanation for increased rates of emergence from the Ivermectin and Doramectin treatments. More in-depth research on lethal and sub-lethal effects of VP residues on Hymenoptera and their host needs to be conducted in this system to untangle these effects.

The results from the succession study indicate that dung-dwelling insects did not differ in their assemblage over a three day period or by week or month. Although, mean insect emergence did seem to differ by location and by order. The VP study revealed that dung from cattle treated with pour-on formulations of ivermectin and doramectin do impact dung-dwelling flies in the Columbia Basin of central Washington State during the summer months. Flies are negatively impacted by fecal residues of VPs as evidenced by a decrease in emergence compared to non-treated control dung. Parasitic Hymenoptera are also affected by the presence of VPs with higher emergence rates in control dung than doramectin and ivermectin. The results of these experiments underline the complexity of the dung-dwelling insect community as well as the variation

that can exist due to varying environmental conditions. These studies highlight the importance for the continual study of the dung-dwelling community not only in the PNW region of the U.S. but in other regions varying in both environment and geographical conditions worldwide.

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