EFFECTS OF ECTOPARASITE REMOVAL ON *PEROMYSCUS LEUCOPUS* AND

OCHROTOMYS NUTTALLI WITHIN A FOREST ECOSYSTEM

by

JAMES OLIN MOREE

(Under Direction of Gary W. Barrett)

ABSTRACT

There are a growing number of case studies that describe the role of parasites affecting host population dynamics through effects on survival and reproduction. There exists, however, a need for experimental removal studies, especially regarding ectoparasites. We examined changes in reproductive success, survival, and abundance of wild rodents following ectoparasite removal. *Frontline Plus®* was applied to 2 species of small mammals, *Peromyscus leucopus* and *Ochrotomys nuttalli*, in an attempt to control ectoparasites. We established 4 treatment and 4 control transects in forest-edge habitat. Tick parasitism in *P. leucopus* was significantly reduced 28 days following treatment, compared with control values. Body mass appeared to impact treatment efficacy; increases in body mass resulted in higher rates of tick parasitism following application in *P. leucopus*. Botflies were effectively excluded in both small mammal species for 28 days following application. We found no evidence that treatment application increased reproductive success, survival, or abundance of *P. leucopus* or *O. nuttalli.*

Key words: American dog tick, botfly, *Cuterebra fontinella*, *Dermacentor variabilis*, forest-edge habitat, *Frontline Plus®*, golden mouse, *Ochrotomys nuttalli*, *Peromyscus leucopus*, white-footed mouse

EFFECTS OF ECTOPARASITE REMOVAL ON *PEROMYSCUS LEUCOPUS* AND

OCHROTOMYS NUTTALLI WITHIN A FOREST ECOSYSTEM

by

JAMES OLIN MOREE

B.S., Ecology, University of Georgia, 2010

B.S., Biology, University of Georgia, 2010

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment

of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GA

2012

EFFECTS OF ECTOPARASITE REMOVAL ON *PEROMYSCUS LEUCOPUS* AND

OCHROTOMYS NUTTALLI WITHIN A FOREST ECOSYSTEM

by

JAMES OLIN MOREE

Major Professor: Gary W. Barrett

Committee: Sonia M. Altizer
Terry L. Barrett
Nicole L. Gottdenker

Electronic Version: Approved

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2012

Acknowledgments

In closing, I thank my family and friends who encouraged me throughout my graduate education. My parents, Chip and Jennifer Moree, and my grandfather, Lamer Moree, have been incredible sources of support, as well as my two brothers, Lamar and William. This is a reflection of your confidence in me over the years.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Parasites play an important role in small mammal ecology. They can manifest significant impact on the health of small mammal populations, as demonstrated by delayed summer breeding in white-footed mouse (*Peromyscus leucopus*) populations (Anderson and May 1978; Hudson et al. 1992; Vandegrift et al. 2008). Parasites also can have consequential impact on individuals through reduced ability to forage, defend territory, avoid or defend against predation, affect immunological response, or slow growth or development. These factors lower individual animal fitness by reducing reproduction and/or fecundity (Jaenike 1996; Møller 1997; Stearns 1992). For example, treatment of *P. leucopus* for gastrointestinal helminths resulted in increased body condition, growth rate, survival, and reproduction (Vandegrift et al. 2008)

Among the many examples of host-parasite interactions, studies on small mammals and their ectoparasites have been surprisingly sparse despite the role they play in the spread of terrestrial disease (Devevey and Christe 2009). For example, a recent paper on Lyme disease highlights how a misunderstanding of the parasite-host relationship led to the development of misguided public health strategies for curbing the transmission of this disease (Brisson et al. 2008). As emerging and expanding zoonosis continue to threaten human health and well-being, it is important to gain a better understanding of the relationship between parasites, vectors, and their hosts (Pongsiri et al. 2009).

A loss of fecundity has been reported in the common vole (*Microtus arvalis*) and Columbian ground squirrel (*Spermophilus columbianus*) due to ectoparasitism, but decreased individual

survivorship due to such parasitism has garnered much less attention (Deter et al. 2007; Neuhaus 2003). A controlled study of flea (*Nosopsyllus fasciatus*) parasitism on *M. arvalis* found that mean life span of deparasitized animals was 36 percent longer than parasitized animals while maintaining larger body sizes (Devevey and Christe 2009). Further work with fleas has shown that the effects of ectoparasitism may be more subtle. Differences in gerbil (*Gerbillus andersoni*) age (juveniles versus adults) as well as intensity of flea infestation affected body mass changes (Hawlena et al. 2007). Additionally, flea infestation changed *G. andersoni's* foraging strategy by reducing foraging effectiveness and altering management of predation risk (Raveh et al. 2011). We hypothesize that removing ectoparasites will increase longevity and reproductive success in 2 species of small mammals, the white-footed mouse (*P. leucopus)* and golden mouse (*Ochrotomys nuttalli*), living in their natural forest habitat.

    P. leucopus are considered a ubiquitous habitat generalist found from Mexico to southern Canada; in the United States they range from the eastern seaboard to the Midwest (Lackey et al. 1985; Pratt and Barrett 2012). They inhabit deciduous forests, as well as highly fragmented, human-impacted areas. *P. leucopus* have numerous endo- and ectoparasites serving as a vector and/or reservoir for various pathogens and diseases, some of which are zoonotic (Durden 2006). This fact, along with their broad geographic distribution, makes *P. leucopus* a target species to study in terms of disease impacts on the host, as well as vector transmission and infection. The effects of endoparasites on *P. leucopus* have been widely documented (Munger and Karasov 1989; Pedersen and Greives 2008; Schwanz et al. 2011; Vandegrift et al. 2008). The impact of *P. leucopus* as a reservoir and vector also have been duly examined, especially in the case of Lyme disease (Brunner and Ostfeld 2008; LoGiudice et al. 2003; Richer et al. 2011; Schwanz et al.

2011; Tsao et al. 2004). However, with the exception of botflies (*Cuterebra fontinella*), effects of ectoparasites on small mammal populations have not been widely investigated. It is worth noting, that studies of *C. fontinella* have struggled to document conclusive evidence of negative, neutral, or positive effects of the ectoparasite on *P. leucopus* (Burns et al. 2005; Catts 1982; Cramer and Cameron 2006, 2010; Jaffe et al. 2005; Jennison et al. 2006; Klein et al. 2010).

O. nuttalli inhabits a much smaller geographic range than *P. leucopus* and are typically less abundant (Pratt and Barrett 2012). *O. nuttalli* are found from central Florida to northwestern Virginia, and from the Atlantic coast west to parts of Texas, Oklahoma, Missouri, Illinois, and Kentucky (Feldhamer and Linzey 2008; Linzey and Packard 1977). Though similar to *P. leucopus* in body size, life history, reproduction cycles, and food and habitat preferences, *O. nuttalli* are considered to be more of a habitat specialist and quite docile in behavior (Feldhamer and Linzey 2008; Linzey and Packard 1977). Likely due to sparse distribution and lower abundance, less is known about the effects of ectoparasitism in *O. nuttalli*. Some known parasites of *O. nuttalli* have been reported, but little is known regarding how these various parasites affect individual animals or population dynamics (Durden et al. 2004; Forrester 1992; Jennison et al. 2006; Linzey and Packard 1977).

Few studies have investigated how ectoparasites affect small mammal population dynamics in a natural habitat. We hypothesis that ectoparasite exclusion by *Frontline Plus®* application would increase longevity and reproductive success in *P. leucopus* and *O. nuttalli*. Work with African Cape ground squirrels (*Xerus inauris*), using *Frontline Plus®,* found increases in reproductive success, lower rates of allogrooming, and a higher resting metabolic rate when compared with control individuals (Hillegass et al. 2010; Scantlebury et al. 2007). However,

researchers combined endoparasite and ectoparasite treatments, making it impossible to distinguish benefits conferred independently from either treatment. Additionally, Barile et al. (2005), using fipronil treatments, were able to reduce tick populations 60 to 80 percent in the first year, and 90 to 100 percent in the second year by targeting small mammals, including white-footed mice. Though they were able to reduce total tick populations, they did not individually monitor the small mammals, thus, no population-level effects were observed. Furthermore, they did not control dosage or number of total treatments per individual. Thus, we designed a yearlong mark/recapture study targeting white-footed and golden mice for controlled application of *Frontline Plus®* to exclude ectoparasites to gain a better understanding of the role that ectoparasites play in small mammal population dynamics within a natural forest habitat.

LITERATURE CITED

ANDERSON, R. M. AND R. M. MAY. 1978. Regulation and stabilization of host-parasite population

   interactions. Journal of Animal Ecology 47:219-267.

BARILE, J., ET AL. 2005. A novel tick management system and its role in reducing the incidence

   of Lyme disease. Pp. 371-374 in Proceedings of the Fifth International Conference on Urban

   Pests (C. L. Lee and W. H. Robinson, eds.). P&Y Design Network, Malaysia.

BRISSON, D., D. E. DYKHUIZEN, AND R. S. OSTFELD. 2008. Conspicuous impacts of

   inconspicuous hosts on the Lyme disease epidemic. Proceedings of the Royal Society of

   London, British Biological Sciences 275:313-319.

BRUNNER, J. L., AND R. S. OSTFELD. 2008. Multiple causes of variable tick burdens on small-

   mammal hosts. Ecology 89:2259-2272.

BURNS, C. E., B. J. GOODWIN, AND R. S. OSTFELD. 2005. A prescription for a longer life? Botfly

   parasitism in the white-footed mouse. Ecology 86:753-761.

CATTS, E. P. 1982. Biology of New World botflies: Cuterebridae. Annual Review of Entomology

   27:313-338.

CRAMER, M. J., AND G. N. CAMERON. 2006. Effects of botfly (*Cuterebra fontinella*) parasitism

   on a population of white-footed mice (*Peromyscus leucopus*). Journal of Mammalogy

   87:1103-1111.

CRAMER, M. J., AND G. N. CAMERON. 2010 Effects of botfly parasitism on movements of

   Peromyscus leucopus. American Midland Naturalist 163:455-462.

DETER, J., J. F. COSSON, Y. CHAVAL, N. CHARBONNEL, AND S. MORAND. 2007. The intestinal nematode *Trichuris arvicolae* affects the fecundity of its host, the common vole *Microtus arvalis*. Parasitological Research 101:1161-1164.

DEVEVEY, G. AND P. CHRISTE. 2009. Flea infestation reduces the lifespan of the common vole. Parasitology 136:1351-1355.

DURDEN, L. A. 2006. Taxonomy, host associations, life cycles, and vectorial importance of ticks parasitizing small mammals. Pp. 91-102 in Micromammals and Macroparasites (S. Morand, B. R. Krasnov, and R. Poulin, eds.). Springer-Verlag, Tokyo, Japan.

DURDEN, L. A., R. N. POLUR, T. NIMS, C. W. BANKS, AND J. H. OLIVER, JR. 2004. Ecotparasites and other epifaunistic arthropods of sympatric cotton mice and golden mice: Comparisons and implications for vector-borne zoonotic diseases. Journal of Parasitology 90:1293-1297.

FELDHAMER, G. A. AND D. W. LINZEY. 2008. The golden mouse: taxonomy and natural history. Pp. 21-35 in The Golden Mouse: Ecology and Conservation (G. W. Barrett and G. A. Feldhamer, eds.). Springer, New York.

FORRESTER, D. J. 1992. Parasites and diseases of wild mammals in Florida. University Press, Gainsville, Florida.

HAWLENA, H., I. S. KHOKHLOVA, Z. ABRAMSKY, AND B. R. KRASNOV. 2006. Age, intensity of infestation by flea parasites and body mass loss in a rodent host. Parasitology 133: 187-193.

HILLEGASS, M. A., J. M. WATERMAN, AND J. D. ROTH. 2010. Parasite removal increases reproductive success in a social African ground squirrel. Behavioral Ecology 21:696-700.

HUDSON, P. J., A. P. DOBSON, AND D. NEWBORN. 1992. Regulation and stability of a free-living host-parasite system - *Trichostrongylus tenuis* in red grouse. I. Monitoring and parasite reduction experiments. Journal of Animal Ecology 61:477-486.

JAENIKE, J. 1996. Population-level consequences of parasite aggregation. Oikos 76:155-160.

JAFFE, G., D. A. ZEGERS, M. A. STEELE, AND J. F. MERRIT. 2005. Long-term patterns of botfly parasitism in *Peromyscus maniculatus, P. leucopus,* and *Tamias striatus*. Journal of Mammalogy 86:39-45.

JENNISON, C.A., L. R. RODAS, AND G. W. BARRETT. 2006. *Cuterebra fontinella* parasitism on *Peromyscus leucopus* and *Ochrotomys nuttalli*. Southeastern Naturalist 5:157-164.

KLEIN, G. P., C. C. CHRISTOPHER, G. N. CAMERON, AND G. W. BARRETT. 2010. Effect of botfly parasitism on vertical habitat use by *Peromyscus leucopus*. Northeastern Naturalist 17:223-228.

LACKEY, J. A., D. G. HUCKABY, AND B. G. ORMISTON. 1985. *Peromyscus leucopus*. Mammalian Species 247:1-10.

LINZEY, D. AND R. PACKARD. 1977. *Ochrotomys nuttalli.* Mammalian Species 75:1-6.

LOGIUDICE, K., R. S. OSTFELD, K. A. SCHMIDT, AND F. KEESING. 2003. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. Proceedings of the National Academy of Science, USA 100:567-571.

MØLLER, A. P. 1997. Parasitism and the evolution of host life history. Pp. 105-127 in Host-parasite evolution, general principles, and avian models (D. H. Clayton and J. Moore, eds.). Oxford University Press, New York, USA.

MUNGER, J. C., AND W. H. KARASOV. 1989. Sublethal parasites and host energy budgets:

tapeworm infection in white-footed mice. Ecology 70:904-921.

NEUHAUS, P. 2003. Parasite removal and its impact on litter size and body condition in Columbian ground squirrels (*Spermophilus columbianus*). Proceedings of the Royal Society of London, B. Biological Sciences 270:213-215.

PRATT, N. L. AND G. W. BARRETT. 2012. Timing of breeding in *Ochrotomys nuttalli* and *Peromyscus leucopus* is related to a latitudinal isotherm. Landscape Ecology 27:599-610.

PEDERSEN, A. B., AND T. J. GREIVES. 2008. The interaction of parasites and resources cause crashes in a wild mouse population. Journal of Animal Ecology 77:370-377.

PONGSIRI, M. J., ET AL. 2009. Biodiversity loss affects global disease ecology. BioScience 59:945-954.

RAVEH, A., B. P. KOTLER, Z. ABRAMSKY, AND B. R. KRASNOV. 2011. Driven to distraction: detecting the hidden costs of flea parasitism through foraging behavior in gerbils. Ecology Letters 14:47-51.

RICHER, L. M., M. AROSO, T. CONTENTE-CUOMO, L. IVANOVA, AND M. GOMES-SOLECKI. 2011. Reservoir targeted vaccine for Lyme borreliosis induces a yearlong neutralizing antibody response to OspA in white-footed mice. Clinical and Vaccine Immunology 18:1809-1816.

SCANTLEBURY, M., J. M. WATERMAN, M. HILLEGASS, J. R. SPEAKMAN, AND N. C. BENNETT. 2007. Energetic cost of parasitism in the Cape ground squirrel *Xerus inauris*. Proceedings of the Royal Society of London, B. Biological Sciences 274:2169-2177.

SCHWANZ, L. E., M. J. VOORDOUW, D. BRISSON, AND R. S. OSTFELD. 2011. *Borrelia burgdorferi* has minimal impact on the Lyme disease reservoir host *Peromyscus leucopus*. Vector-born and Zoonotic Diseases 11:117-124.

STEARNS, S.C. 1992. The evolution of life histories. Oxford University Press, Oxford, United
Kingdom.

TSAO, J., J. BUNKIS, C. J. LUKE, M. G. LUNA, D. FISH, AND A. G. BARBOUR. 2004. *Borrelia burgdorferi* infection in a natural population of *Peromyscus leucopus* mice: a longitudinal study in an area where Lyme borreliosis is highly endemic. Journal of Infectious Diseases 189:139-147.

VANDEGRIFT, K. J., T. R. RAFFEL, AND P. T. HUDSON. 2008. Parasites prevent summer breeding in white-footed mice, *Peromyscus leucopus*. Ecology 89:2251-2258.

CHAPTER 2: EFFECTS OF ECTOPARASITE REMOVAL ON *PEROMYSCUS LEUCOPUS*

AND *OCHROTOMYS NUTTALLI* WITHIN A FOREST ECOSYSTEM

INTRODUCTION

Small mammals play a crucial role in experimental field studies (Barrett and Peles 1999), and previous studies have used small mammals as an ecological model species (Wolff 1999). Many life history attributes of small mammals make them ideal models because detailed information on numerous species of small mammals in various habitats have been published. Because small mammals perform well in mark/recapture field studies, researchers can evaluate survivorship, reproductive success, home range size, and trophic-level dynamics at the population, community, ecosystem, and landscape levels (Barrett and Peles 1999). In addition, small mammals have short lives, typically disperse at adulthood, and frequently exhibit behavioral responses to seasonal change. This affords insight into processes of colonization, extinction, dispersal, and persistence, providing vital information among levels of organization.

Numerous studies have addressed the effects of perturbations on small mammal population dynamics, such as pesticides (Barrett 1988), radiation (Green 1968), nutrient enrichment (Hall et al. 1991), heavy metals (Levine et al. 1989), and fire (Crowner and Barrett 1979). Few studies, however, have focused on the use of chemical compounds to improve the survivorship of a population by reducing parasitism in order to increase reproductive success of small mammals in their natural environment. We selected 2 small mammal species, the white-footed mouse (*Peromyscus leucopus*) and the golden mouse (*Ochrotomys nuttalli*) for experimental field

analysis. We identified 2 ectoparasites known to effect metabolism and reproductive success of these 2 small mammal species, the American dog tick (*Dermacentor variabilis*) and botfly (*Cuterebra fontinella*) (Jennison et al. 2006; Vandegrift et al. 2008), to quantify reproductive success between treatment and control populations. *Frontline Plus®* (active ingredient fipronil; 9.8%) was employed under natural habitat conditions in an attempt to increase reproductive success, survivorship, and abundance of small mammals within a deciduous forest habitat.

Frontline Plus® is advertised as a safe and effective method for killing fleas and ticks that commonly parasitize domestic animals, namely, dogs and cats (Kidd and James 1991; Penaliggon 1997). Various laboratory studies have shown it to be fast acting (12 hours for fleas), long lasting (30 days), and waterproof (Dryden et al. 2000; Everett et al. 2011; Jacobson et al. 2004). *Frontline Plus®* accomplishes this efficiency by binding to lipids in hair follicles and sebaceous glands from which it is slowly released to provide long-term effectiveness (Cochet et al. 1997).

Fipronil ($C_{12}H_4Cl_2F_6N_4OS$) is an insecticide of the phenylpyrazole chemical family that disrupts nerves in the brain and spinal cord of insects by reducing the ability of nerve cells to transmit nerve impulses (Jackson et al. 2009; Tingle et al. 2003). First marketed in the United States in 1996, fipronil is used in fire ant baits, cockroach poisons, flea products, termite control, and turf, golf course, and agriculture products (Cox 2005). The United States Environmental Protection Agency (US EPA) classifies fipronil as a carcinogen because experiments with laboratory animals resulted in benign and malignant tumors, aggressive behavior, kidney damage, altered thyroid function, smaller offspring, and slowed sexual development (Diaz 2005; Ohi et al. 2004; US EPA 1994, 1998). Fipronil can persist in soils and contaminate water, and is

11

considered toxic to birds, lizards, fish, crawfish, shrimp, bees, and copepods (Cox 2005). Insects

can develop resistance to fipronil (Liu et al. 2004). Fipronil resistance developed in Taiwanese

diamondback moths (*Plutella xylostella*) in just 2 years; and after 5 years, the dose required to

kill a moth had increased hundredfold (Kao and Cheng 2001). Though fipronil is distributed by

a variety of companies under various brand names, our investigation focused on *Frontline Plus®*

(fipronil concentration: 100g/l) produced by Merial Limited, Deluth, Georgia.

Most anti-parasitic drugs typically are tested on laboratory-bred animals, and their use

restricted to laboratory conditions. While such tests are useful, we argue that the effects of such

compounds would be more fully understood if studies were designed to investigate such effects

when applied under natural habitat conditions (Odum et al. 1968; Barrett 1968; Barrett et al.

1976). Performance of a chemical under laboratory conditions may not reflect performance as

compared with a natural environment. For example, climate, habitat quality, and dietary

diversity are controlled factors in a laboratory setting that vary in a natural habitat. These factors

can influence the efficacy of a chemical application or treatment.

There exists an abundance of controlled experiments testing the efficacy of *Frontline Plus®*

on laboratory animals (Jackson et al. 2009). These studies have focused mainly on flea and tick

control for dogs and cats at various time intervals (Dryden et al. 2000; Jacobson et al. 2005;

Tanner et al. 1997). However, there exists a paucity of information regarding *Frontline Plus®*

research conducted as natural field trials. *Frontline Plus®* effects on African Cape ground

squirrels (*Xerus inauris*) represent one of the few small mammals for which field experiments

have been performed. Ground squirrels treated for ectoparasites using *Frontline Plus®* increased

body mass and time feeding, decreased allogrooming rates, and had a fourfold increase in

reproductive success (Hillegasse et al. 2010; Scantlebury et al. 2007). Unfortunately, the

Frontline Plus® treatment in these studies was paired with an endoparasite treatment, making it

difficult to determine what portion of the results can be attributed to *Frontline Plus®* treatment

for ectoparasite removal. A 3-year study using *Frontline Plus®* on small mammals, including

the Eastern chipmunk **(***Tamias striatus***)** and white-footed mouse (*P. leucopus*), reduced tick

populations by 60 percent or more (Barile at al. 2005; Dolan et al. 2004). However, amounts of

Frontline Plus® application were not standardized per animal, and animals could have received

multiple treatments during a single foraging session.

We designed a replicated field investigation in a deciduous forest habitat to quantify the

effects of *Frontline Plus®* on the white-footed and golden mouse. This investigation was

conducted during all seasons to quantify changes in population dynamics between control and

treated small mammals.

MATERIALS AND METHODS

Experimental site.—The study was conducted at the HorseShoe Bend (HSB) Ecology

Experimental Research Site located in Clarke County, in proximity to Athens, Georgia (33°57'N,

83°23'W). HSB is a 14.2-hectare (35-ac) peninsula created by a meander of the North Oconee

River. The site is composed of upland and lowland deciduous forests. White oak (*Quercus alba*)

and American beech (*Fagus grandifolia*) dominate the upland, whereas river birch (*Betula nigra*)

and sweetgum (*Liquidambar styraciflua*) were the most abundant in the lowland. Greenbriar

(*Smilax*), Amur and Japanese honeysuckle (*Lonicera maackii* and *L. japonica*, respectively),

water oak (*Q. nigra*), and Chinese privet (*Ligustrum sinense*) were common in both habitat

types.

Trapping regime.—The study was conducted from 23 November 2010 to 19 November 2011. Eight experimental transects (each 0.14-ha) were positioned along forest-edge habitat to capture white-footed mice (*P. leucopus*) and golden mice (*O. nuttalli*) (Pratt and Barrett 2012). Four were randomly selected as treatment transects, whereas the remaining 4 served as control transects (Figure 1). Transects were evenly divided between the upland and lowland habitats; each transect contained 8 capture stations positioned in a linear pattern approximately 10 ± 2 meters apart. Transects were located along an old field or a gravel road approximately 3 meters into forest-edge habitat to maximize capture because both small mammal species prefer edge habitat (Barrett 2008; Feldhamer and Morzillo 2008; Wolf and Batzli 2002; Yahner 1992).

Each capture station consisted of a live trap (H. B. Sherman Traps, Incorporated, Tallahassee, Florida) and a nest box (Lewellen and Vessey 1999). During the peak winter breeding months, nest boxes contained non-absorbent cotton. Both live traps (n = 64) and nest boxes (n = 64) were placed 1.5 meters aboveground on trunks of trees. Live traps baited with sunflower seeds, were placed on L-shaped platforms positioned on tree trunks (Christopher and Barrett 2006). Nest boxes were attached to tree trunks by bungee cords located in thick stands of *L. sinense, L. maackii,* and *L. japonica*. Water oak acorns (n =12) were placed in the nest boxes as supplemental food on 23 November 2010, 14 January and 19 February 2011. Nonabsorbent cotton was removed from nest boxes on 7 May 2011, then each replaced with a live trap situated at ground-level for a total of 128 live traps for the duration of the study.

Live traps and nest boxes were checked twice weekly in alternating fashion throughout the peak breeding season. Live traps were set 2 hours before nightfall, and checked the following morning at 8:00 a.m. Captured individuals were identified to species, sexed, weighed to the

14

nearest gram, and examined for reproductive condition (open or closed vaginal orifice; pregnant or lactating; abdominal or scrotal testes). Each captive animal received an ear tag for identification (Neway Products, Murray, Utah), and then was released at the site of capture. Captured animals weighing less than 10 grams were considered juveniles. Black rat snakes (*Elaphe obsoleta*) are proficient predators of both species of small mammals (Blouin-Demers and Weatherhead 2001; Blouin-Demers et al. 2000). *E. obsoleta* discovered in and around nest boxes were removed and translocated to a distant forest habitat.

Experimental treatment methods.—To reduce ectoparasitism on *P. leucopus* and *O. nuttalli*, *Frontline Plus®* (active ingredient fipronil) was applied to captured small mammals within treatment transects, using a controlled amount applied by micropipette (Merial Limited, Duluth, Georgia). *Frontline Plus®* is traditionally used on larger domestic mammals, such as dogs and cats (Kidd and James 1991; Penaliggon 1997), and has a fipronil concentration of 9.8 percent. A topical dosage (7.5mg/kg) of fipronil was calculated for individuals in our treatment transects (Carpenter 2004). We calculated a dosage of 0.8 micro-liters for animals greater than 10 grams; animals smaller than 10 grams were not treated to avoid toxic exposure. We chose a conservative dosage of fipronil for our investigation because of the absence of field studies involving wild populations of small mammals. A conservative dose was chosen to caution residual effects on the local habitat, as well as to reduce negative or toxic effects of *Frontline Plus®* application in our small mammal species. Controlled trials testing fipronil exposure in white-footed and golden mice should be useful for calculating higher dosage levels if field studies are continued.

Initially, all visible parasites were to be recorded. However, field observations of lice and mites, including species and abundance, proved difficult. Host animals would require intensive handling, and in some cases anesthesia, to fully evaluate parasite burdens, thus, field evaluation was impractical. Prior to initiating the study, ectoparasites were collected from small mammals for a period of one month and delivered to Lance Durden, Georgia Southern University, for species identification. No fleas were observed or collected. Of the collected ticks, all specimens were identified as *D. variabilis*. Bot fly specimens from our site were identified as *C. fontinella*. Thus, we established *D. variabilis* and *C. fontinella* as target ectoparasites for long-term field monitoring.

Each captured small mammal was examined methodically for ectoparasites in the field. While a researcher scruffed the animal, another individual used a headlamp (Black Diamond Equipment, Salt Lake City, Utah) and tweezers to carefully examine the ears, head, abdomen, and rump for attached ticks and botflies. Observed parasites were recorded. Regardless of whether the animal was captured in a treatment or control transect, it went through an examination process. Treatments were applied every 21 days to maintain effectiveness. An individual from a treatment transect had its recapture history examined to determine if it had been treated during the previous 21 days. If not, the above amount of *Frontline Plus®* was applied by micropipette to the center dorsal area between the ears of the mouse; then the mouse was released on site. We followed guidelines approved by the *American Society of Mammalogists* for the Use of Wild Animals in Research (Sikes et al. 2011) and The University of Georgia Animal Care and Use Committee (AUP #A2010 7-116).

Control animals remained untreated for the duration of the experiment. Treatment animals were categorized into 5 phases used to quantify application effectiveness. Phase classification was determined by the number of days since the last treatment. The Initial phase refers to animals captured and treated the first time. The Adjustment phase (days 1-14 following treatment) represents the time it takes for the *Frontline Plus®* application to become effective. The Effective phase (days 14-28) is the period of maximum treatment effectiveness. The Decline phase (days 28-42) refers to the projected declining concentration of *Frontline Plus®* in experimental animals. The Not Effective phase is the fifth classification and refers to 42 or more days following treatment. In this phase, the application is thought to be ineffective.

Despite the location of separate transects, a few individuals traversed control and treatment transects; these mice were considered dispersers. Dispersers were permanently categorized as treatment animals after the application of a single treatment, even if they returned to a control transect.

Reproductive Success.—We measured reproductive success by assessing the number of new individuals entering the population. All unmarked animals weighing less than or equal to 15 grams were classified as juveniles approaching adulthood that had yet to disperse from their natal site (Krohne et al. 1984; Stickel 1968). This cutoff body mass of 15 grams was determined based on growth and development studies of white-footed mice. Rogers and Beauchamp (1973) found that male and female white-footed mice approach maturity at approximately 38 days. Goundie and Vessey (1986) also found that young individuals left the natal site on average of 5.6 weeks, or approximately 38 days. Having established length of time to maturity, we researched a mean cutoff body mass at maturity. Derrickson (1988) found that mice averaged 14.6 grams at

42 days. Guetzow and Judd (1981) reported an identical body mass for a population of white-footed mice in Texas; their mice also averaged 14.6 grams for 42 day-old individuals. Furthermore, Lackey (1973) found that white-footed mice in Michigan weighed, on average, 15.7 grams at 42 days old. Thus we established 15 grams as the transition body mass for juveniles entering into the adult population.

Statistical Analyses.—The Minimum Number Know Alive (MNKA) was used to estimate small mammal species abundance in both treatment and control transects (Krebs 1999). Using MNKA, survivorship and longevity were calculated. Rates of parasitism were compared between treatment and control transects. Reproductive success among females between the treatment and control transects also was calculated using an ANOVA. A chi-square analysis was used to determine differences in sex ratios based on a 1:1 (M:F) ratio. The criterion for statistical significance was *P ≤ 0.05*.

The relationship of *Frontline Plus®* treatment to presence of parasites was analyzed using a mixed-effects logistic regression model, where the response was binomial presence-versus-absence. Two basic models were used. The first model compared treated mice to control mice, each treatment phase at a time; the second model compared the treatment phase of treated mice with one another, and not to the control mice. This approach recognizes control mice as a different cohort than treated mice, but that treatment phase occur within the same individual. Pairwise comparisons of the 5 phases were calculated using Tukey's adjustments for multiple comparisons.

A mixed-effects ANOVA model was used to determine if there was a significant body mass difference between sexes, and between control and treatment mice. A Fisher Exact Test was used

to determine if there was a relationship between period of capture and presence of ticks or botflies. A logistic regression was used to predict the presence or absence of ticks based on whether or not a parasite was found on a mouse on the previous date of capture. This logistic regression function predicted the probability that a parasite will be present when a parasite was present on a mouse on the previous date of capture.

A Poisson regression was used to compare the relationship between the treatment phase to the number of ticks and botflies present. This regression was used to predict the number of parasites expected to be present on any particular capture occasion. This prediction was based on the specific phase of treatment. Because repeated measures were recorded on individual mice, it was necessary to account for the identity of each mouse similarly to the logistic regression. Therefore, the identities of the mice were considered a "random" effect in each model. These models were "mixed-effects models" due to the inclusion of the "fixed" effect of treatment and the random effect of an individual mouse treated. Similarly to the logistic regression, the same 2 basic approaches of comparison were used (control versus treated mice, and phases of treatment compared with one another among treated mice). This analysis was performed for both ticks and botflies.

The relationship between sex, body mass, and treatment with presence of parasites also was analyzed using mixed-effects logistic regression models. The presence of ticks or botflies was predicted based on sex, body mass, and treatment phase. Pairwise comparisons of the 5 phases were calculated using a Tukey's Test.

The Poisson regression also was utilized to examine the relationship of sex, body mass, and treatment phase to number of parasites, instead of just presence or absence; here the number of

parasites was predicted based on sex and body mass of each mouse at each phase of *Frontline Plus®* treatment. Each mouse was considered a random effect; these models again were mixed-effects models. The same 2 basic approaches from the previous analyses were repeated, the first model compared treated to control mice, each treatment phase at a time in separate models, while accounting for sex and body mass; the second model compared the treatment phases of the treated mice with one another and not to the control, while accounting for sex and body mass. All analyses were completed using SAS 9.3. The criterion for statistical significance was $P \leq 0.05$.

RESULTS

White-footed mice.—A total of 143 white-footed mice (78 M; 65 F) was investigated during this study; 50 individuals were control animals, and 93 individuals were treatment animals (Table 1). No significant difference was found between control and treatment mice based on sex (Fisher exact test $P = 0.67$). 19 individuals traversed control and treatment transects. Although the mean body mass of these dispersers was more than control or treatment individuals, differences were not significant ($F = 1.75$, $P = 0.18$), nor where body mass differences based on sex ($F = 2.04$, $P = 0.16$).

Tick Parasitism.—Period of capture, as a predictor of the presence of ticks, was not significant ($P = 0.31$). Logistic regression analyses indicated no relationship between presence or absence of ticks based on whether a tick was present during the previous capture ($X^2 = 0.09$; *d. f.* $= 1$; $P = 0.76$), as well as how long that capture was from the previous capture ($X^2 = 0.06$; *d.f.* $= 1$; $P = 0.81$). Table 2 shows the probability of tick presence during each of the 5 phases of *Frontline Plus®* effectiveness. Individual models comparing treatment to control mice regarding

tick presence or absence by each phase were not significant (i.e., none of the phases showed a significant reduction in presence of ticks when compared to control values).

No significant differences were found when comparing treatment to control mice by phase regarding total number of ticks present. However, pairwise comparisons of the various phases of the treated mice with one another revealed that the Effective phase had significantly fewer ticks per mouse per occasion than the Not Effective phase (Tukey-adjusted P-value = 0.02).

Regression models shown in Table 3 incorporated the effect of mouse body mass and indicate that treatment mice in the Effective phase have significantly lower probabilities of tick presence than control mice ($P \leq 0.05$). In addition, heavier control mice were less likely to have ticks than lighter control mice, whereas heavier treatment mice were more likely to have ticks than lighter treated individuals ($P \leq 0.05$). Within treatment phases of white-footed mice, females were more likely to have lesser numbers of ticks during the Effective phase, when compared to males in the Effective phase ($P \leq 0.05$). The more a treatment mouse weighed during the Effective phase, the less effective was the treatment when compared with a lighter individual ($P \leq 0.05$).

Botfly Parasitism.—Period of capture, as a predictor of the presence of botflies, was not significant ($P = 0.15$). A Fisher exact test showed no significant relationship regarding the probability of having a botfly compared with the presence of a botfly on the previous capture ($P = 1.00$). Table 4 shows the probability of botfly presence during each of the 5 phases of *Frontline Plus*® effectiveness. Analysis of botflies between treatment and control mice indicates that during the Effective phase, botflies were significantly less likely to be present on a treatment mouse compared to a control mouse ($P \leq 0.05$).

Significant differences were found in the number of botflies present in the Effective phase between treatment and control groups (Table 5). Additionally, pairwise comparisons between the Effective and Not Effective phases and between the Effective and Initial phases resulted in Tukey-adjusted *P*-values of 0.06 and 0.08, respectively, depicting values slightly greater than our criterion significance value of *P ≤ 0.05*.

Body mass and sex did not have a significant influence on *Frontline Plus®* effectiveness regarding whether mice had botflies present (Table 6). Additionally, neither body mass nor sex influence the relationship between treatment phase and the number of botflies found per mouse, per capture date (Table 7).

Golden Mice Parasitism.—A total of 33 golden mice (20 M; 13 F) were monitored in this study, yielding 8 control and 25 treatment individuals. No significant difference in body mass was found between transect type or by sex (Table 8). Sixteen individuals traversed control and treatment transects.

A logistic regression analysis was used to determine if golden mice exhibited tick parasitism at a lower rate than white-footed mice. After comparing the Not Effective phase (*P = 0.84*), combined treatment phases (*P = 0.55*), and the control groups (*P = 0.32*), there is no evidence indicating golden mice are less likely to present with a tick, compared with the same cohort of white-footed mice. Table 9 shows percentages of captures in which ticks were observed by species. No botflies were observed in the control or treatment golden mice thus, no statistical analyses were performed.

Longevity and Reproduction.—Differences in longevity between white-footed and golden mice proved inconclusive. Additionally, there were no significant effects on reproduction within

22

treatment transects for white-footed ($P = 0.39$) or golden ($P = 0.64$) mice when compared with control transects for each species, respectively.

DISCUSSION

Fipronil research has been dominated by controlled, laboratory studies utilizing laboratory-bred research animals (Cox 2005). This information is useful for manufacturers because it helps define the upper limits of the chemical effectiveness of fipronil, while avoiding the complexities of animals functioning in a natural environment. However, laboratory studies alone do not provide a full understanding of fipronil and its properties. We recommend that laboratory investigations be coupled with natural field studies using wild populations of small mammals to ascertain how natural conditions might alter efficacy. Though difficult to design and execute, field investigations include variables, such as weather condition, diet, social and reproductive behavior, natural predation, and parasitism that typically are controlled or absent in a laboratory setting. These variables could have a significant impact an individual mammal, altering expected results of fipronil applications.

Previous studies involving fipronil treatment of wild populations of small mammals represent 2 basic approaches, but also raise important questions. One study targeted white-footed mice (*P. leucopus*) and Eastern chipmunks (*T. striatus*) for fipronil treatment in an effort to develop a tick-management system (Barile et al. 2005). Chemical treatments were applied indirectly using bait to attract small mammals through a corridor with a fipronil treated wick. This method proved effective, reducing tick populations 60 to 80 percent in the first year, and 90 to 100 percent in the second year. Unfortunately, this approach does not provide information about small mammal population dynamics. It is unclear if and how small mammals benefited from the treatment, or

were harmed by it. Furthermore, the total number of individuals treated, and how many treatments each individual received, could not be evaluated. Thus, we chose direct applications on marked animals in the field, controlling the amount of chemical application per individual, number of treatments, and time between treatments. Though more labor intensive, this method allowed us to test the length of effectiveness and collect specific ectoparasite data, while monitoring small mammal population dynamics within their natural habitat.

In two other studies, *Frontline Plus®* treatment on African Cape ground squirrels (*X. inauris*) was designed to measure treatment effects with regard to parasitism. Direct applications were sprayed on marked individuals once a month. Reproductive success in treated females was 4 times greater than control females; treated individuals also had lower rates of allogrooming (Hillegasse et al. 2010). Scantlebury et al. (2007) found treatment animals gained more body mass and had a higher resting metabolic rate than control animals. However, both of these studies combined ectoparasite treatments with endoparasite treatments, thus rendering it impossible to distinguish positive or negative impacts of each treatment independent of one another, though when combined, they did confer benefits to individual animals. Moreover, these studies were performed only on female ground squirrels during their breeding season. For our investigation, we concentrated our attention on ectoparasites on an annual basis. Internal parasites obviously play an important role in population dynamics (Vandegrift et al. 2008), but our approach allowed us to independently focus on treatment of ectoparasites. Our yearlong experiment monitored *Frontline Plus®* application response in 2 species of small mammals, namely the white-footed mouse and the golden mouse, including individuals of both sexes.

White-footed Mice and Ticks.—Results show varying levels of *Frontline Plus®* effectiveness. In white-footed mice, no significant reduction of ticks was found in treatment transects compared to control values. It would appear that *Frontline Plus®* was ineffective, but upon closer investigation by treatment phase, this overall value may be misleading. Only 12 percent of the control animal observations resulted in an incidence of tick parasitism, whereas the 5 treatment phase values ranged from 7 percent in the Effective phase to 17 percent in the Decline phase. Results from analysis of the 5 treatment phases indicate that *Frontline Plus®* did significantly reduce ticks on mice during the Effective phase, compared to the Not Effective phase. We found that the effectiveness wears off 42 or more days following application, at which time tick parasitism increases.

An analysis of treatment mice in the Effective phase compared to control mice, when accounting for body mass, also reflects a significantly lower probability of tick presence. Accounting for body mass was crucial in our investigation. Though we used a standard rate of *Frontline Plus®* application for all treated individuals greater than 10 grams, evidence suggests that slight differences in body mass can have significant impact on the presence or absence of ticks. Heavier control mice were less likely to have ticks than lighter control mice, whereas heavier treated mice were more likely to have ticks than lighter treated mice. We suggest that treatments could be more effective if dosage was tailored for a more specific range of body mass. We propose that instead of the same, conservative dosage applied to all individuals greater than 10 grams, that separate dosages be calculated for small mammals from 10 to 15 grams, 16 to 20 grams, and greater than 21 grams.

Female white-footed mice were more likely to have lower counts of ticks during the

Effective phase compared to male white-footed mice in the same phase. One explanation might

be differences in body mass between male and female treatment individuals. For example,

treated males weighed on average 17.9 grams, whereas females averaged 16.9 grams. As

indicated earlier, heavier treated mice were more likely to have ticks than lighter treated mice.

Another explanation might involve the life history of the white-footed mouse. Males typically

have larger home range sizes that overlap multiple female home ranges (Lackey et al. 1985).

Covering a larger home range area could increase tick exposure.

White-footed Mice and Botflies.—Frontline Plus® applications appear to be very effective

against botflies. Though not significant, there exists the possibility that significantly fewer

botflies would be present during the Effective phase than in these other phases should additional

studies be repeated. Only 0.28 percent of the white-footed mice in the Effective phase had botfly

parasitism, contrasted with 5.25 percent of the control observations. It should be noted that body

mass and sex of white-footed mice did not influence the effectiveness of application on whether

or not botflies were present. This is an interesting botfly response compared to the percentage of

tick parasitism in which body mass and sex played a significant role.

Because there is a paucity of information linking *Frontline Plus®* application to botfly

response, we can only speculate as to why treatments were more effective on botflies than ticks.

A closer examination regarding the biology of botflies following application may provide the

answer. Botflies develop in the subcutaneous layer of their host, from which they open a warble

pore, facilitating the exchange of gas and waste (Catts 1982*). Frontline Plus®* is applied directly

to the skin of experimental individuals, where it attaches to hair follicles and sebaceous glands to

create lasting effectiveness (Cochet et al. 1997). Thus, botflies are constantly exposed to the chemical application as they grow and develop. *Frontline Plus®* is very effective against developing parasites, often disrupting, slowing, or halting growth by altering nervous system function (Bloomquist 1993, 1996). Additionally, botflies have less flexibility when parasitizing a host, including no evidence indicating their ability to switch hosts. Ticks, in contrast, can easily drop from a host to seek another individual or in this case, small mammal species. If a tick senses a toxic chemical, the parasite has a greater ability to escape or avoid danger, whereas botflies are restricted to a single host chosen by timing and proximity (Catts 1982). Resistance to fipronil has developed in some insect species (Liu et al. 2004), including ticks of the family Ixodidae (Castro-Janer 2010). Ticks, a commonly targeted species in domestic and commercial management strategies, have a higher probability of having been previously exposed to fipronil. Therefore, ticks have a higher likelihood of developed resistance compared with a non-target species like botflies. A lack of previous fipronil exposure for botflies results in maximum chemical effectiveness following application.

Golden Mouse Parasitism.—Conclusive evidence regarding effects of *Frontline Plus®* application on golden mice was difficult to quantify. As seen in Table 9, the percent of tick parasitism was lower in golden mice when compared with white-footed mice for every cohort we analyzed, though not significantly different. This could be because the sample size of golden mice was too small to adequately make a valid comparison with white-footed mice.

An absence of botfly parasitism within our experimental population of golden mice warrants closer examination. For example, botflies are known parasites of golden mice (Catts 1982; Jennison et al. 2006). Reports of botfly parasitism in golden mice exist for Florida (Pearson

1954), Georgia (Durden et al. 2004), Mississippi (Clark and Durden 2002), and Tennessee

(Linzey 1968). Furthermore, there exists published research from our site showing measurable

levels of botfly parasitism in white-footed and golden mice (Jennison et al. 2006), There are

several possible explanations regarding the absence of botflies in our experimental golden mouse

population. Firstly, the *Frontline Plus®* application was extremely effective in eliminating

botflies, as observed in the white-footed mouse results previously discussed. However, this does

not explain the absence of botflies in control transects. Secondly, a smaller sample size could be

partly responsible. Thirdly, a closer look at golden mouse life history may provide further clarity.

Golden mice differ from white-footed mice in that golden mice prefer a two-dimensional, semi-

arboreal, habitat space instead of the larger, three-dimensional arboreal space inhabited by white-

footed mice (Christopher and Barrett 2006; Klein et al. 2010). This difference in use of habitat

space may lower golden mouse exposure to botfly larvae, thus reducing parasitism when

compared with white-footed mice in the same area (Jennison et al. 2006). Fourthly, higher levels

of sociability found in golden mice could provide another explanation for lower levels of botfly

parasitism. Golden mice are typically found in communal nests (Luhring and Barrett 2008;

Stueck et al. 1977). It is possible that treatment of some individuals in a social group will reduce

ectoparasitic burden for all members because they huddle and exhibit higher rates of

allogrooming (Hillegasse 2010). This behavior allows freshly applied fipronil to pass among

individuals, and ectoparasites from untreated individuals could jump to treated individuals and

die. This can be significant in golden mice, where up to 8 individuals have been recorded

sharing the same nest (Barbour 1942). Furthermore, Madden and Clutton-Brock (2009) found

no differences in parasite burdens between treated and untreated individuals of the same social group.

Reproduction Analysis.—Though our reproduction and longevity results were inconclusive, additional confidence in the juvenile body mass value was verified when analyzing the body mass distribution of white-footed and golden mice in our study. Of the 143 white-footed mice in our study, only 19 mice (10 M, 9 F) were dispersers, or mice that moved between treatment and control transects. These dispersers weighed on average 18.8 grams for males and 17.3 grams for females. Of the 33 experimental golden mice, 16 individuals (10 M, 6 F) dispersed. Golden mouse dispersers weighed, on average, 16.8 grams for males and 17.1 grams for females. Therefore, an untagged mouse weighing 15 grams or less is more likely to be a juvenile, rather than a mature adult dispersing into new habitat.

Other studies indicate that fipronil can disrupt reproduction and development in rats, delaying sexual maturity (Ohi 2004). Though this only occurred at the highest dosage levels tested, it could have an impact on mouse development, increasing age, and body mass of early adulthood (US EPA 1998). Additionally, wild populations generally develop slower, meaning a laboratory individual may reach a target body mass more rapidly compared to wild individuals (Goundie and Vessey 1986).

Conclusive evidence on the effects of *Frontline Plus*® application in terms of reproduction could not be deduced. For example, we found no significant difference regarding when individual white-footed mice entered the control population compared with new individuals in the treatment transects, based on age and body mass criteria. It is possible that several individuals were born in tree cavities or ground hibernacula located within control or treatment

29

transects that never entered either population. This highlights difficulties researchers face when designing and conducting studies on populations in their natural forest habitat.

Future Studies.—We recommend implementing more clearly defined dosages for specific body mass ranges (10-15 g, 16-20 g, 20+ g) for white-footed and golden mice. Both species are of similar body mass and periods of activity (Christopher and Barrett 2006). Increasing the dosage amount in treatment transects may increase effectiveness or refine the level in which *Frontline Plus®* becomes toxic to wild populations. Additionally, establishing transects at greater distances apart would discourage mice from dispersing between experimental transects. Greater emphasis also needs to be placed on reproductive data to help monitor population and community-level effects of *Frontline Plus®* application. Increased focus should be placed on recording pregnant and lactating females, litter size at birth, and when individuals reach adulthood to fully comprehend the effect of *Frontline Plus®* application on small mammal population dynamics.

Because the winter breeding latitudinal isotherm for *P. leucopus* and *O. nuttalli* is south of the 35°N latitude (Pratt and Barrett 2012), we recommend that a similar ectoparasite removal study be performed north of this isotherm (i.e., a site located during the summer breeding season). Previous studies of tick and botfly life history indicate high incidence and abundance of parasites during summer months (Goodwin et al. 2001; Jennison et al. 2006; Ostfeld et al. 2006; Reye et al. 2010). Performing an investigation when reproduction and peak parasitism overlap should provide greater insight into the relationship between ectoparasite removal and small mammal reproduction.

Though not generally a target species of *Frontline Plus®* treatments, botflies were clearly affected by ectoparasite treatment. Eliminating botflies in treatment groups also could provide a greater understanding of how botfly parasitism affects small mammal growth and metabolism. Reports pertaining to botfly parasitism highlight the difficulties researchers have faced in quantifying the energetic cost of botfly parasitism (Cramer and Cameron 2006, 2010; Timm and Cook 1979). This investigation focused on improving the reproductive quality of 2 small mammal populations subjected to ectoparasitism—a management strategy worthy of future study.

LITERATURE CITED

BARBOUR, R. W. 1942. Nests and habitat of the golden mouse in eastern Kentucky. Journal of

Mammalogy 23:90-91.

BARILE, J., ET AL. 2005. A novel tick management system and its role in reducing the incidence

of Lyme disease. Pp. 371-374 in Proceedings of the Fifth International Conference on Urban

Pests (C. L. Lee and W. H. Robinson, eds.). P&Y Design Network, Malaysia.

BARRETT, G. W. 1968. The effects of an acute stress on a semi-enclosed grassland ecosystem.

Ecology 49:1019-1035.

BARRETT, G. W. 1988. Effects of sevin on small mammal populations in agricultural and old-

field ecosystems. Journal of Mammalogy 69:731-739.

BARRETT, G. W. 2008. The golden mouse: a levels-of-organization perspective. Pp. 3-19 in The

Golden Mouse: Ecology and Conservation (G. W. Barrett and G. A. Feldhamer, eds.).

Springer, New York.

BARRETT, G. W. AND J. D. PELES. 1999. Small mammal ecology: a landscape perspective. Pp. 1-

10 in Landscape Ecology of Small Mammals (G. W. Barrett and J. D. Peles, eds.). Springer-

Verlag, New York.

BARRETT, G. W., G. M. VAN DYNE, AND E. P. ODUM. 1976. Stress ecology. BioScience 26:192-

194.

BLOOMQUIST, J. R. 1996. Ion channels as targets for insecticides. Annual Review of Entomology

41:164-190.

BLOOMQUIST, J. R. 1993. Toxicology, mode of action and target site-mediated resistance to insecticides acting on chloride channels. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 106:301-314.

BLOUIN-DEMERS, G. AND P. J. WEATHERHEAD. 2001. Habitat use by black rat snakes (*Elaphe obsoleta obsoleta*) in fragmented forests. Ecology 82:1882-1896.

BLOUIN-DEMERS, G., K. A. PRIOR, AND P. J. WEATHERHEAD. 2000. Patterns of variation in spring emergence of black rat snakes (*Elaphe obsoleta obsoleta*). Herpetologica 56:175-188.

CARPENTER, J. 2004. Exotic Animal Formulary. 3rd ed. Turtleback Press, St. Louis, Missouri.

CASTRO-JANER, E., L. RIFRAN, P. GONZALEZ, C. NIELL, J. PIAGGIO, A. GIL, AND T. T. S. SCHUMAKER. 2010. *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) resistance to fipronil in Uruguay evaluated by in vitro bioassays. Veterinary Parasitology 178:172-177.

CATTS, E. P. 1982. Biology of New World botflies: Cuterebridae. Annual Review of Entomology 27:313-338.

CHRISTOPHER, C. C., AND G. W. BARRETT. 2006. Coexistence of white-footed mice (*Peromyscus leucopus*) and golden mice (*Ochrotomys nuttalli*) in a southeastern forest. Journal of Mammalogy 87:102-107.

CLARK, K. L., AND L. A. DURDEN. 2002. Parasitic arthropods of small mammals in Mississippi. Journal of Mammalogy 83:1039-1048.

COCHET, P., P. BIRCHEL, M. BROMET-PETIT, N BROMET, AND A. WEIL. 1997. Skin distribution of fipronil by microautoradiography following topical administration to the beagle dog. European Journal of Drug Metabolism and Pharmacokinetics 22:211-216.

COX, C. 2005. Fipronil, insecticide factsheet. Journal of Pesticide Reform 25:1:10-15.

CRAMER, M. J., AND G. N. CAMERON. 2006. Effects of bot fly (*Cuterebra fontinella*) parasitism on a population of white-footed mice (*Peromyscus leucopus*). Journal of Mammalogy 87:1103-1111.

CRAMER, M. J., AND G. N. CAMERON. 2010 Effects of bot fly parasitism on movements of *Peromyscus leucopus*. American Midland Naturalist 163:455-462.

CROWNER, A. W. AND G. W. BARRETT. 1979. Effects of fire on the small mammal component of an experimental grassland community. Journal of Mammalogy 60:803-813.

DERRICKSON, E. M. 1988. Patterns of postnatal growth in a laboratory colony of *Peromyscus leucopus*. Journal of Mammalogy 69:57-66.

DIAZ, S. L. 2005. Efficacy of fipronil in the treatment of pediculosis in laboratory rats. Laboratory Animals 39:331-335.

DOLAN, M., ET AL. 2004. Control of immature *Ixodes scapularis* (Acari: Ixodes) on rodent reservoirs of *Borrelia burgdorferi* in a residential community of southeastern Connecticut. Journal of Medical Entomology 41:1043-1054.

DRYDEN, M. W., M. DENEBERG, AND S. BUNCH. 2000. Control of fleas on naturally infected dogs and cats and in private resident with topical spot applications of fipronil or imidacloprid. Veterinary Parasitology 93:69-75.

DURDEN, L. A., R. N. POLUR, T. NIMS, C. W. BANKS, AND J. H. OLIVER, JR. 2004. Ectoparasites and other epifaunistic arthropods of sympatric cotton mice and golden mice: Comparisons and implications for vector-borne zoonotic diseases. Journal of Parasitology 90:1293-1297.

EVERETT, W. R., S. J. GROSS, P. T. TANNER, AND D. S. CARITHERS. 2011. Immediate and residual speed of kill of Frontline Plus® (fipronil+(S)-methoprene) against Rdl-homozygous fleas on

dogs assessed at twelve, eighteen, and twenty-four hours post-treatment and following

subsequent weekly infestations. International Journal of Applied Research in Veterinary

Medicine 9:120-123.

FELDHAMER, G. A. AND A. T. MORZILLO. 2008. Relative abundance and conservation: Is the

golden mouse a rare species? Pp. 117-133 in The Golden Mouse: Ecology and Conservation

(G. W. Barrett and G. A. Feldhamer, eds.). Springer, New York.

GOODWIN, B. J., R. S. OSTFELD, AND E. M. SCHAUBER. 2001. Spatiotemporal variation in Lyme

disease host and and vector: black-legged ticks on white-footed mice. Vector Borne and

Zoonotic Diseases 1:129-138.

GOUNDIE, T. R. AND S. H. VESSEY. 1986. Survival and dispersal of young white-footed mice born

in nest boxes. Journal of Mammalogy 67:53-60.

GREEN, E. 1968. Genetic effects of radiation on mammalian populations. Annual Review of

Genetics 2:87-120.

GUETZOW, D. D., AND F. W. JUDD. 1981. Postnatal growth and development in a subtropical

population of *Peromyscus leucopus texanus*. Southwestern Naturalist 26:183-191.

HALL, A. T., P. E. WOODS, AND G. W. BARRETT. 1991. Populations dynamics of the meadow vole

(*Microtus pennsylvanicus*) in nutrient-enriched old-field communities. Journal of

Mammalogy 72:332-342.

HILLEGASS, M. A., J. M. WATERMAN, AND J. D. ROTH. 2010. Parasite removal increases

reproductive success in a social African ground squirrel. Behavioral Ecology 21:696-700.

JACKSON, D., C. B. CORNELL, B. LUUKINEN, K. BUHL, AND D. STONE. 2009. Fipronil technical

fact sheet. National Pesticide Information Center, Oregon State University Extension Services.

JACOBSON, R., ET AL. 2004. The ability of fipronil to prevent the transmission of *Borrelia burgdorferi*, the causative agent of Lyme disease to dogs. Journal of Applied Research in Veterinary Medicine 2:39-45.

JENNISON, C.A., L. R. RODAS, AND G. W. BARRETT. 2006. *Cuterebra fontinella* parasitism on *Peromyscus leucopus* and *Ochrotomys nuttalli*. Southeastern Naturalist 5:157-164.

KAO, C. AND E. Y. CHENG. 2001. Insecticide resistance in *Plutella xylostella*. L. XI. Resistance to newly introduced insecticides in Taiwan (1990-2001). Journal of Agriculture Research in China 50:80-89.

KIDD, H., AND D. R. JAMES. 1991. The agrochemicals handbook. Royal Society of Chemistry Information Services, Cambridge, United, Kingdom.

KLEIN, G. P., C. C. CHRISTOPHER, G. N. CAMERON, AND G. W. BARRETT. 2010. Effect of bot fly parasitism on vertical habitat use by *Peromyscus leucopus*. Northeastern Naturalist 17:223-228.

KREBS, C. J. 1999. Ecological methodology. 2nd ed. Benjamin Cummings, Menlo Park, California.

KROHNE, D. T., B. A. DUBBS, AND R. BACCUS. 1984. An analysis of dispersal in an unmanipulated population of *Peromyscus leucopus*. American Midland Naturalist 112:146-156.

LACKEY, J. A. 1973. Reproduction, growth and development in high-latitude and low-latitude populations of *Peromyscus leucopus* (Rodentia). Ph.D. dissertation, University of Michigan,

Ann Arbor, Michigan.

LACKEY, J. A., D. G. HUCKABY, AND B. G. ORMISTON. 1985. *Peromyscus leucopus*. Mammalian Species 247:1-10.

LEVINE, M. B., A. T. HALL, G. W. BARRETT, AND D. H. TAYLOR. 1989. Heavy metal concentrations during ten years of sludge treatment to an old-field community. Journal of Environmental Quality 18:411-418.

LEWELLEN, R. H. AND S. H. VESSEY. 1999. Estimating *Peromyscus leucopus* densities using live- trap and nest-box censuses. Journal of Mammalogy 80:400-409.

LINZEY, D. W. 1968. An ecological study of the golden mice, *Ochrotomys nuttalli*, in the Great Smokey Mountains National Park. American Midland Naturalist 79:320-345.

LINZEY, D. W., AND R. PACKARD. 1977. *Ochrotomys nuttalli.* Mammalian Species 75:1-6.

LIU, H., E. W. CUPP, K. M. MICHER, A. GUO, AND N. LIU. 2004. Insecticide resistance and cross-resistance in Alabama and Florida strains of *Culex quinquefasciatus*. Journal of Medical Entomology 41:408-413.

LUHRING, T. M., AND G. W. BARRETT. 2008. Nesting ecology of the golden mouse: An *oikos* engineer. Pp. 151-165 in The Golden Mouse: Ecology and Conservation (G. W. Barrett and G. A. Feldhamer, eds.). Springer, New York.

MADDEN, J. R., AND T. H. CLUTTON-BROCK. 2009. Manipulating grooming by decreasing ectoparasite load causes unpredicted changes in antagonism. Proceedings of the Royal Society of London, B. Biological Sciences 276:1263-1268.

ODUM, E. P., G. W. BARRETT, AND H. R. PULLIAM. 1968. Testing pesticides at the ecosystem level. Association of Southeastern Biologists Bulletin 15:48.

OHI, M., P. R. DALSENTER, A. J. M. ANDRADE, AND A. J. NASCIMENTO. 2004. Reproductive

adverse effects of fipronil in Wistar Rats. Toxicology Letters 146:121-127.

OSTFELD, R. S., F. KEESING, A. PRICE, V. L. HORNBOSTEL, AND M. A. BENJAMIN. 2006.

Controlling ticks and tick-borne zoonoses with biological and chemical agents. BioScience

56:383-394.

PEARSON, P. G. 1954. Mammals of Gulf Hammock, Levy County, Florida. American Midland

Naturalist 51:468-480.

PENALIGGON, J. 1997. Getting to grips with fleas on pet dogs and cats. Pesticide Outlook 8:19-

23.

PRATT, N. L. AND G. W. BARRETT. 2012. Timing of breeding in *Ochrotomys nuttalli* and

Peromyscus leucopus is related to a latitudinal isotherm. Landscape Ecology 27:599-610.

ROGERS, J. G. AND G. K. BEAUCHAMP. 1973. Relationships among three criteria of puberty in

Peromyscus leucopus noveboracensis. Journal of Mammalogy 55:461-462.

REYE, A. L., J. M. HUBSCHEN, A. SAUSY, AND C. P. MULLER. 2010. Prevalence and seasonality of

tick-borne pathodens in questing *Ixodes ricinus* ticks frin Luxembourg. Applied and

Environmental Microbiology 76:2923-2931.

SCANTLEBURY, M., J. M. WATERMAN, M. HILLEGASS, J. R. SPEAKMAN, AND N. C. BENNETT.

2007. Energetic cost of parasitism in the Cape ground squirrel *Xerus inauris*. Proceedings of

the Royal Society of London, British Biological Sciences 274:2169-2177.

SIKES, R. S., W. L. GANNON, AND THE ANIMAL CARE AND USE COMMITTEE OF THE AMERICAN

SOCIETY OF MAMMALOGISTS. 2011. Guidelines of the American Society of Mammalogists

for the use of wild animals in research. Journal of Mammalogy 92: 235-253.

STICKEL, L. F. 1968. Home range and travels. Pp. 373-411 in Biology of *Peromyscus* (Rodentia) (J. A. King, ed.). American Society of Mammalogist.

STUECK, K. L., M. P. FARRELL, AND G. W. BARRETT. 1977. Ecological energetics of the golden mouse based on three laboratory diets. Acta Theriologica 22:309-315.

TANNER, P. A., AND M. N. ROMANO. 1997. Comparative studies to evaluate the flea control efficacy of Frontline Top Spot against Advantage flea adulticide in the dog and cat. Proceedings from the American Association of Veterinary Parasitologists; July 19-22, Reno, Nevada; Abstract 19.

TIMM, R. M., AND E. F. COOK. 1979. The effect of botfly larvae on reproduction in white-footed mice, *Peromyscus leucopus*. American Midland Naturalist 101:211-217.

TINGLE, C. C., J. A. ROTHER, C. F. DEWHURST, S. LAUER, AND W. J. KING. 2003. Fipronil: environmental fate, ecotoxicology, and human health concerns. Reviews of Environmental Contamination and Toxicology 176:2-3.

U.S. EPA. 1994. Data evaluation report: combined chronic toxicity/carcinogenicity/rats. MRID No. 429186-48: pp. 11, 19, 20, 27, 28.

U.S. EPA. 1998. Fipronil for use on rice (Regent®, Icon®) and pets (Frontline®). Health Effects Division risk assessment: pp. 16, 19-22, 41.

VANDEGRIFT, K. J., T. R. RAFFEL, AND P. T. HUDSON. 2008. Parasites prevent summer breeding in white-footed mice, *Peromyscus leucopus*. Ecology 89:2251-2258.

WOLF, M. AND G. O. BATZLI. 2002. Effects of forest edge on populations of white-footed mice, *Peromyscus leucopus*. Ecography. 2:193-199.

WOLFF, J. O. 1999. Behavioral Model Systems. Pp. 11-40 in Landscape Ecology of Small

Mammals (G. W. Barrett and J. D. Peles, eds.). Springer-Verlag, New York.

YAHNER, R. H. 1992. Dynamics of a small mammal community in a fragmented forest. American Midland Naturalist 127:381-391.

Table 1. White-footed mice based on treatment classification and body mass distribution in grams.

Type	Sex	n	Mean body mass (g)	S. D.
Control	M	25	16.8	3.40
	F	25	16.1	4.92
Treatment	M	43	17.9	3.20
	F	31	16.9	3.03
Disperser (Treatment)	M	10	18.8	2.52
	F	9	17.3	2.87

Table 2. Logistic regression models (5) comparing presence of ticks in treatment phases to

control values.

Treatment phase	d. f.	F - value	P - value
Initial	1, 141	0.26	0.61
Adjustment	1, 96	0.43	0.51
Effective	1, 102	3.08	0.08
Decline	1, 69	0.57	0.45
Not Effective	1, 73	0.33	0.57

Table 3. Logistic regression models comparing treatment Effective phase presence of ticks to

control presence of ticks, including body mass*.

Effective phase	d. f.	F - value	P - value
Effective	1, 101	5.50	0.02
Body mass	1, 547	0.07	0.79
Effective/Body mass	1, 547	4.42	0.04

*Control value comparison is implied.

Table 4. Logistic regression models (5) comparing presence of botflies in treatment phases to

control values.

Treatment phase	d. f.	F - value	P - value
Initial	1, 141	0.32	0.57
Adjustment	1, 96	2.20	0.14
Effective	1, 102	8.27	0.01
Decline*			
Not Effective	1, 73	0.01	0.94

*No test result is shown for the Decline phase because no mice were found to have botflies

during this phase. However, due to a small number of observations during this period, a

simplified Fisher exact test indicates that treated mice observations during the Decline phase

were not significantly different from control mice ($P = 0.36$).

Table 5. Poisson regression models comparing average treatment phase counts of botflies with

control counts of botflies.

Treatment phase	*d. f.*	*F* - value	*P* - value
Initial	1, 141	0.44	0.51
Adjustment	1, 96	2.28	0.13
Effective	1, 102	8.37	0.01
Decline*			
Not Effective	1, 73	0.03	0.87

*No test is shown for the Decline phase because no mice were found to have botflies during this

phase.

Table 6. Single logistic regression model comparing presence of botflies across all treatment

phases.

Effect	d. f.	F - value	P - value
Phase	3, 500	0.66	0.58
Body mass	1, 500	2.42	0.12
Body mass/Phase	3, 500	0.36	0.78

Table 7. Single Poisson regression model comparing all treatment phase counts of botflies.

Effect	d. f.	F - value	P - value
Phase	3, 500	0.67	0.57
Body mass	1, 500	2.42	0.12
Body mass/Phase	3, 500	0.37	0.77

Table 8. Golden mice based on treatment classification and body mass distribution in grams.

Type	Sex	n	Mean body mass (g)	S. D.
Control	M	3	15.5	4.44
	F	5	17.0	2.84
Treatment	M	7	17.4	2.81
	F	2	16.4	2.05
Disperser (Treatment)	M	10	16.8	4.88
	F	6	17.1	5.43

Table 9. Comparing rates of tick parasitism between golden mice and white-footed mice as a

percent of total captures.

| Phase | Golden mice | | White-footed mice | |
	Total captures	Tick parasitism (% of captures)	Total captures	Tick parasitism (% of captures)
Not effective	8	12.5%	60	15.0%
All treatment	63	4.8%	439	7.7%
Control	40	5.0%	357	10.9%

Fig. 1.—Aerial photograph of HorseShoe Bend Ecology Experimental Site depicting the research design and arrangement of the 8 transects within the meander of the North Oconee River. White dots indicate capture stations.