THE SEASONAL OCCURRENCE AND CONTROL OF

ECTOPARASITES ON THE FOX SQUIRREL,

SCIURUS NIGER (RODENTIA:

SCIURIDAE)

By

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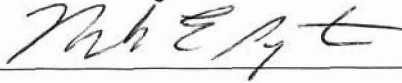the requirements for
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THE SEASONAL OCCURRENCE AND CONTROL OF

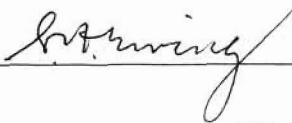# ECTOPARASITES ON THE FOX SQUIRREL,
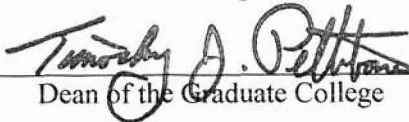
SCIURUS NIGER (RODENTIA:

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Acknowledgments

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Introduction

Project Overview. The fox squirrel (*Sciurus niger* L.) has a range extending along the eastern coast of the United States, continuing westward into the central part of the country and north into Canada. With well-developed musculature and long digits and claws, the fox squirrel is well adapted for climbing trees. Fox squirrels can be found in rural and urban settings and can move readily between the two. A propensity for inhabiting attics and backyard trees puts the fox squirrel, and any ectoparasites thereof, in continual association with human beings and companion animals. Consequently, the fox squirrel is likely to transfer ectoparasites, and thus, disease vectors, from relatively sparsely populated rural settings to urban areas. Relatively little is known about the seasonal ectoparasite burden of the fox squirrel in Oklahoma, but several studies have found the fox squirrel to host a wide variety of ectoparasites. These studies gave no insight into the ectoparasite burden of fox squirrels in an urban setting compared with squirrels in a rural setting. Additionally, none of these studies indicated that attempts had been made to control the ectoparasites on fox squirrels. If the fox squirrel is, indeed, the host of a wide variety of potential ectoparasite disease vectors, then it is worthwhile to investigate strategies to control these ectoparasites.

Surveying Ectoparasites of the Fox Squirrel. In order to evaluate the fox squirrels' role in the transfer of ectoparasites, animals were surveyed to determine seasonal ectoparasite load from May of 2000 to December of 2001. The data collected were analyzed so that a comparison could be made between squirrels from rural and urban habitats.

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The Treatments. Two commercially available insecticides, fipronil (Merial Animal Health) and moxidectin (Cydectin®, Fort Dodge Animal Health) provided for easy topical application and were ideal for treatment of fox squirrels. Fipronil, marketed as Frontline® and Top Spot®, is a member of the phenylpyrazole class of insecticides and is registered for use on companion animals for the control of fleas and ticks. It is an extremely active molecule and disrupts the central nervous system in arthropods through the gamma aminobutyric acid (GABA) channel. It does not, however, bind to GABA receptors in vertebrates and is, thus, quite selective with a wide safety margin. Fipronil is also registered for use on crops, and for the prevention termite damage, but resistance has not developed as is the case with many other pesticides. For instance, while the brown dog tick, *Rhipicephalus sanguineus* (Latreille) (Acari:Ixodidae), has shown significant resistance to permethrin, and coumaphos and moderate resistance to amitraz, there was no resistance to fipronil (Miller et al. 2001). Fipronil has also been shown to be effective against the cat flea, *Ctenocephalides felis* (Bouché), without evidence of resistance having developed (Ritzhaupt et al. 2000).

Moxidectin is an endectocide in the milbemycin chemical class and shows the distinctive mode of action typical of macrolytic lactones. Moxidectin binds selectively with the glutamate-gated chloride ion channels that are important in invertebrate nervous systems, thus interfering with neurotransmission. Moxidectin is marketed for the control of both internal and external parasites of livestock and is a ready-to-use, pour-on formulation. A fermentation product of *Streptomyces cyaneogriseus*, moxidectin is related to the avermectins, such as ivermectin, but has distinct methoxine and dimethylbutenyl side chains and thus, a distinct effect. Resistance to moxidectin has not

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been reported, and it has proved effective against a variety of internal parasites as well as flies, lice (Webb et al, 1991), psoroptic mange mites (Parker et al. 1999), and the cattle tick, *Boophilus microplus* (Canestrini) (Acari:Ixodidae), (Guglielmone et al. 2000), attesting to its value as an acaricide. Minimal side effects of topical moxidectin treatment have been reported, although ingestion has caused respiratory failure in dogs (Beal et al. 1999). It has been shown that vegetative management, such as mowing and thinning tree cover, and acaricidal premise treatments, though cost effective (Meyer et al. 1982), provide only temporary control because ticks are continually reintroduced into treated areas by wild hosts that move freely in and out of the treated areas (Zimmerman et al. 1988).Treating animals, such as squirrels, that come and go from an area with an effective pesticide could prevent some of this reintroduction.

Objectives. The objectives of this study were threefold. The first objective was to determine what types of ectoparasites occur on the fox squirrel during each month of the year in central Oklahoma and to decide if control methods were warranted. A second objective was to compare ectoparasite burden of squirrels collected in an urban habitat and those collected in a rural habitat. The third objective was to evaluate topical treatment with fipronil and moxidectin as potential methods of controlling ectoparasites, particularly ticks, on fox squirrels; thus, inhibiting the fox squirrel from taking part in the life cycle of these ectoparasites. A comparison of these two pesticides was made as a beginning step in designing self-treating devices for the control of ectoparasites on fox squirrels.

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Review of Published Literature

General Biology. The fox squirrel, *Sciurus niger* Linnaeus, is an abundant animal with a wide distribution throughout the eastern and central United States and into southern Canada (Koprowski 1994). Introductions have occurred in California, Oregon, Colorado, Idaho, New Mexico, North Dakota, Texas, and Washington. A late emigrant from Europe to Florida, the fox squirrel has changed little morphologically since its introduction. This is evidenced by the fact that fossils from the Miocene era found in both Europe and North America are identical to animals found today (Koprowski 1994). Fox squirrels are in the family Sciuridae in the order Rodentia, in which 1,650 species are classified, including 365 species of squirrels in seven families.

Sciurus niger is a tree squirrel of medium size, with a body mass ranging from 507-1361g, total body length from 454-698mm (with the tail ranging from 200-300mm) and no sexual dimorphism in size or color. The scientific name is derived from ancient Greek and gives insight into the fox squirrels' coloration. *Sciurus* is derived from *skia*, meaning shadow or shade, and *oura* meaning tail. *Niger* refers to the dark coloration of many specimens. Dorsal coloration of the fox squirrel varies from buff to orange, and the ventral surface is white to cinnamon in color (Koprowski 1994).

Fox squirrels have survived in captivity for as long as thirteen years, but the life expectancy in the wild rarely exceeds 7.5 years (Koprowski et al. 1988). Age is a dominance factor, and an aged squirrel may significantly influence the genetic and phenotypic composition of a population. Males reach sexual maturity at eleven months of age and will mate with many females throughout life. The female may bear young at eight months of age, but most do not reproduce until older than one year. Females select

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the strongest male to mate with, but do not typically mate with the same male twice. Squirrels may mate at any time, but breeding typically occurs from November to February with a December peak, and from April to July with a June peak. Mating is conducted in a specific order, beginning with a series of tail motions that proceed to an identification kiss in which the squirrels touch noses, groom, and then to copulate. (McClosky and Shaw 1977). Litter size ranges from 1.97 to 3.35 on average. In addition to playing a role in the mating ritual, the tail of the fox squirrel is thought to play an important role in thermoregulation (Muchlinski and Shump 1979). Unlike most rodents, the tail of the fox squirrel is quite bushy; it serves to hold body heat better than a nude tail.

Found in a variety of forest settings, populations of *S. niger* are most dense where trees that produce winter-storable foods, such as acorns, pecans, and other nuts, are plentiful. Fox squirrels cache food; this allows animals to mass large amounts of food when food is plentiful and the cost is low, and eat when food is scarce and the cost is high (Kotler et al. 1999). The species of trees commonly serving as a habitat for fox squirrels are oaks (*Quercus*), hickories (*Carya*), walnuts (*Juglans*), and pines (*Pinus*) (Koprowski 1994). Well adjusted to living in a forest setting, the fox squirrel is adapted for climbing owing to sharp claws, elongated digits, and well developed musculature.

Vehicular trauma and predation are the most common causes of mortality. *Sciurus niger* has numerous natural predators, including mammalian, reptilian, and avian species. Timber rattlesnakes (*Crotalus horridus*), black rat snakes (*Elaphe obsoleta*), red-tailed hawks (*Buteo jamaicensis*), great horned owls (*Bubo virginianus*), opossums (*Didelphis virginiana*), weasels (*Mustela frenata*), raccoons (*Procyon lotor*), red foxes (*Vulpes*

vulpes), gray foxes (*Urocyan cinereoargenteus*), bobcats (*Felis rufus*), wolves (*Canis lupus*), coyotes (*C. latrans*), and domestic dogs and cats all take their toll on squirrel populations (Koprowski 1994).

Sciurus niger is active throughout the year with daily activity peaking at two hours after sunrise and two to four hours before sunset during the spring and fall, with an additional peak between 1000-1200 hours during the summer. The winter season shows a peak in activity at midday with high activity in the early morning, associated with breeding behavior. Weather seems to have little effect on activity with the exception of high wind (>14.5 km/h), which decreases activity (Koprowski 1994). Squirrels are nest builders and are adaptable in their nesting sites, often using as many as nine nests through the course of a year (Svihla 1931). Nests are constructed of a platform of twigs placed on a tree limb, a shell of twigs and leaves, and an inner lining of a woven material. Summer nests are not as sturdy and may consist of simply a platform and a thin shell. (Koprowski 1994). Squirrels are known to abandon nests that become too heavily infested with parasites (Svihla 1931).

Sciurus niger has a variable range, covering between 0.85 to 17.2 ha for females and 1.54 to 42.8 ha for males. The large number of squirrels in any given area and the extensive range cause overlap of home ranges, but territoriality has not been seen (Koprowski 1994).

Trapping and Handling. Squirrels, including *S. niger*, can be successfully live-trapped using a variety of baits and trap settings. Traps are often baited with peanut butter, corn, sunflower seeds, nuts, or fruits and placed in or around trees. Trees with easily accessible horizontal branches are the usual trap sites. Traps can also be placed on

the ground, making them easier to set and to move, but non-target captures and incidences of bait theft are increased. Traps can also be attached vertically to a tree trunk, but success is decreased with this technique (Huggins and Gee 1995). A prebaiting period is often utilized, allowing animals to become accustomed to the presence of the trap. Factors that often affect trapping success include trap type, set, bait, prebaiting period, season, and trapper experience (Huggins and Gee 1995). Squirrel density and number of traps will also have an impact on trapping success. Trapping success for fox squirrels has been reported at 28.9 captures/100 trap days (Huggins and Gee 1995). Because squirrels often have two activity peaks, traps should be checked twice daily to prevent mortality among captured animals (Huggins and Gee 1995). Fur dyes and ear tags are useful means of marking captured animals.

Means for handling captured squirrels include manual restraint using heavy gloves and a canvas bag, or chemical restraint depending on specific needs (Arnez 1997). If minimal contact with the squirrels is required, then canvas bags are adequate. Close contact and manipulation of the animal will require chemical restraint due to the squirrels' disagreeable nature and tendency to bite. Ketamine-hydrochloride is a widely used anesthetic administered through intramuscular injection at a dosage of 36.0 mg/kg (Arnez 1997). This sedative has been shown to be effective within two to four minutes and provides twelve to twenty minutes of sedation. Squirrels typically recover within two to three hours with minimal side effects. This drug does cause mild convulsions in some animals, and this risk may be reduced through the use of alternative drugs such as diazepam, acepromazine, or xylazine (Arnez 1997). Squirrels recover more quickly if left in covered cages that reduce external stimuli such as noise, movement, or intense light.

Ectoparasites of Squirrels. The fox squirrel is the reported host of numerous types of ectoparasites, though limited studies have been conducted to determine the squirrels' role in the biology of many of these ectoparasites.

A survey of 100 squirrels in southern Kansas was conducted by Graham and Uhrich (1943). Though the collection method was not given, a variety of ectoparasites were collected. Sixty-three percent of the squirrels examined had either *Hoplopsyllus affinis* Baker (Siphonaptera:Pulicidae) or *Orchopeas howardii* Baker (Siphonaptera:Ceratophyllidae), 46% were infested with *Neohaematopinus sciurinus* Mjöberg (Anoplura:Polyplacidae), while 7% were infested with *Dermacentor variabilis* (Say) (Acari:Ixodidae). Furthermore, 15% of the squirrels were infested with mites of the family Trombiculidae, and 1% with mites of the family Sarcoptidae. Seven percent of the squirrels examined were free of ectoparasites.

An ectoparasite survey of 26 squirrels from LeFlore County, Oklahoma was conducted during 1978-1979 (Koch and Dunn 1980). Animals were collected using live traps, and were anesthetized for examination. Of the 26 squirrels, 16 were infested with larvae and nymphs of *Amblyomma americanum* (L.) (Acari:Ixodidae), five were infested with larvae and adults of *D. variabilis*, and two were infested with nymphal *Ixodes scapularis* Say (Acari:Ixodidae).

During the same period, a survey of 88 squirrels was conducted in Tennessee that focused on fleas and lice (Durden 1980). Squirrels were live trapped, anesthetized with chloroform, and brushed to collect ectoparasites. Three species of lice were discovered: *Hoplopleura sciuricola* Ferris (Anoplura: Hoplopleuridae), *Neohaematopinus sciuri* Janké (Anoplura:Polyplacidae), and *Enderleinellus longiceps* Kellogg and Ferris

(Anoplura:Enderleinellidae). Only one species of flea, *O. howardii*, was collected. The data indicated that louse populations peaked in mid-winter and mid-summer, whereas the flea population had a slight peak in the spring but stayed fairly constant throughout the year.

A study that focused on ticks was conducted in Kansas between 1989 and 1992 (Brillhart et al. 1994). Animals were live trapped using a Sherman folding trap, baited with peanut butter and oatmeal, and anesthetized with ether prior to examination. Of the 28 fox squirrels examined, two were infested with nymphal *A. americanum* and three with all mobile life stages of *D. variabilis*.

The most in-depth survey was conducted in Florida between 1988 and 1993 and included 119 squirrels (Coyner et al. 1996). Animals were collected by shotgun or as roadkill and were examined for both endo and ectoparasites. Ectoparasites were collected by brushing the fur and removing attached specimens with forceps. Of the squirrels examined, 75% were free of internal parasites, but most were infested with some variety of ectoparasite. In total, nine species of arthropods were found. The only flea recovered was *O. howardii*, and this species occurred on 70% of the squirrels collected, though there was no seasonal pattern observed. *Neohaematopinus sciurinus, H. sciuricola*, and *E. longiceps* were collected from 20%, 18%, and 2% of the squirrels, respectively. *Amblyomma americanum* was the only tick recovered, and it was present on only 7% of the animals collected. In addition, one larval specimen of *Cuterebra* sp. (Diptera:Cuterebridae) was collected. The authors concluded that the low prevalence of many of these ectoparasites indicated that they were likely accidental parasites of the fox squirrel.

Although limited studies have been conducted involving *S. niger*, many more have involved the gray squirrel, *Sciurus carolinensis* Ord. These squirrels are closely related and often share the same habitat, so it is likely that they share ectoparasites. One such study, conducted in Virginia (Parker 1968), included 15 gray squirrels, but the collection method was not mentioned. The method of ectoparasite collection, however, was unusual. Corpses were placed in tubs of 5% sodium hydroxide to dissolve the hair, and ectoparasites were collected from the tubs. Six squirrels were infested with *O. howardii*, eight were infested with *N. sciuri*, seven with *H. sciuricola*, six with *E. longiceps*, and four with larval *Cuterebra* sp. One squirrel was completely free of ectoparasites; one was infested with 1,358 ectoparasites.

A study conducted in North Carolina (Parker and Holliman 1971) involved nine gray squirrels, collected either as roadkill or after drowning. Nine were infested with *O. howardii* and *N. sciuri*, and five had been invaded by larval *Cuterebra* sp. No ticks were collected. The potential ectoparasite loss due to drowning was not discussed.

In a study conducted in Jacksonville, Florida in 1974 (Nixon et al. 1991), fifteen gray squirrels were collected each month of the year. Animals were live trapped using peanut butter and pecans as bait. Once collected, the animals were sedated and brushed to collect ectoparasites. Of the 180 squirrels examined, 80 were infested with *O. howardii*, 96 with *N. sciuri*, 58 with *E. longiceps*, 51 with *H. sciuricola*, and 2 with *D. variabilis*. Fleas were most prevalent between January and March, while lice where present in large numbers throughout the year.

A study involving the gray squirrel was conducted in Missouri between June 1993 and July 1996 that focused on the incidence of *A. americanum* (Kollars Jr. et al. 2000).

Animals were live trapped using peanut butter and oats as bait and were anesthetized using ketamine hydrochloride. Ten gray squirrels were examined; 91% were infested with larval *A. americanum*, collected between April and October, and 21% with nymphal *A. americanum*, collected between March and November.

Other reported ectoparasites of the fox squirrel include: Acarina – *Amblyomma maculatum* Koch, *A. tuberculatum* Marx, *Haemaphysalis leporispalustris* (Packard), *Ixodes cooki, I. hearlei*; Siphonaptera – *Ctenocephalides felis* (Bouchè), *Echidnophaga gallinacea* (Westwood), *Opisodasys robustus* Jordan; Diptera – *Cuterebra emasculator* Fitch (Koprowski 1994).

Diseases of Fox Squirrels. Little work has been done to determine the fox squirrels' role in the transmission of diseases, though many of the ectoparasites found on the fox squirrel are incriminated as vectors of disease. Most studies done to determine the role of squirrels in disease transmission have included the squirrel in general mammalian surveys. Several of these studies have included the gray squirrel rather than the fox squirrel, but the same comparison can be made between the squirrels and that done with the ectoparasite load of the two species.

In Oklahoma, Rocky Mountain spotted fever (RMSF) is a disease of great concern. Caused by the bacterial organism *Rickettsia rickettsii*, this disease is prevalent in foci throughout the state and is transmitted by *D. variabilis*. While no studies have been conducted on squirrels in the state of Oklahoma to determine if they harbor the disease agent in the wild, several studies have been conducted in other states. Five gray squirrels were captured in Mississippi in 1974 and 1975 during a survey of small mammals (Norment et al. 1985). Sera was drawn from the animals and tested for reactivity to

RMSF. One of the five squirrels was serologically positive for the disease agent. Two other studies were conducted in Connecticut in 1979 and 1984. The first study involved two gray squirrels collected in a small mammal survey (Magnarelli et al. 1979). One of the two tested positive for RMSF. The second study involved five gray squirrels (Magnarelli et al. 1985). No ticks were found on any of the squirrels, and none of them tested positive for RMSF. Collection details were not provided.

It has been reported that *D. variabilis* harbors the Lyme spirochete (*Borrelia burgdorferi*) in the wild as well as *Rickettsia rickettsii*. Similarly, *A. americanum* has been reported to harbor the causative agent of Lyme disease (Piesman and Sinsky 1988) . Studies have shown, however, that neither of these ticks is capable of successfully transmitting the spirochete. *Ixodes scapularis*, on the other hand, is capable of both harboring and transmitting this disease agent (Piesman and Sinsky 1988). Animals on which *I. scapularis* feeds are likely to be infected with *B. burgdorferi* (Cooney and Burdorfer 1974), and this includes the fox squirrel.

Hepatozoonosis is a disease that has long been known to occur in wildlife, first being reported from an animal indigenous to the neartic region in 1829 from the California ground squirrel (Clark 1958). A different species of the disease agent, *Hepatozoon americanum*, has recently been reported from several domestic dogs, bringing hepatozoonisis to the forefront of many research programs (Panciera et al. 1999) While it is a different species of *Hepatozoon* that affects fox squirrels, the disease agent is prevalent nonetheless. A study conducted in Maryland revealed that 22 of 24 fox squirrels examined were infected with *Hepatozoon* sp. (Clark 1958). The investigators were unable to determine the particular species present, but some work was done to

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determine a vector. Several common ectoparasites of squirrels were considered, including the flea *O. howardii*, and a mite, *Echinolaelaps echidinus*. It was determined that fleas are unable to transmit the disease, but *E. echidinus* was able to transmit *Hepatozoon* sp. under laboratory conditions.

A study conducted in Tennessee between 1969 and 1972 focused on the zoonotic potential of small mammal ectoparasites (Cooney and Burgdorfer 1974). Six fox squirrels were captured, sedated, examined, and released. Of the six squirrels, one was infested with *A. americanum* larvae and nymphs and one with *D. variabilis* adults. According to the author, *A. americanum* has been incriminated as a vector of tularemia (*Francisella tularensis*), and *D. variabilis* has been incriminated as the vector of numerous diseases, including RMSF. It is suggested in their study that fox squirrels play a role in the life cycles of these disease-causing agents.

Other disease agents present in fox squirrels include plague (*Yersinia pestis*), California encephalitis, Western equine encephalitis, and leptospirosis (*Leptospira grippotyphosa*) (Koprowski 1994). Rabies is known but rare in fox squirrels.

Ectoparasite Control. Although no studies have been conducted with the intention of controlling ectoparasites on fox squirrels, such studies have been carried out with other rodents. Many studies have been conducted using rodenticidal baits to control rodent populations, and the concept of attracting an animal to a bait station to deliver some sort of chemical is not new, nor is the concept of applying pesticides directly to an animal to prevent infestations. Each of these methods has proved effective.

One study focused on controlling fleas on the Mexican wood rat (*Neotoma mexicana* Baird). Polyvinylchloride tubes were lined with carpeting treated with liquid

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permethrin (Cage et al. 1997). These tubes were baited with peanut butter and corn and placed in areas known to be populated with rats. The rats were surveyed prior to placement of the tubes to determine ectoparasite load. The treated tubes proved to be effective, as a significant reduction in flea and tick numbers were seen in just one week. No determination was made as to how long the treatment was effective.

A somewhat similar study focused on the control of *D. variabilis* using polyvinylchloride tubes lined with felt (Sonenshine and Haines 1985). The felt was treated with 1% diazinon diluted in vegetable oil, and each tube was coated with peanut butter to attract animals. This treatment was also effective, resulting in the recovery of 10.4 ticks/animal on treated animals and 37.3 ticks/animal on untreated animals.

One of the most successful and widely known ectoparasite control projects focused on white-tailed deer, *Odocoileus virginianus* (Zimmermann). In this study, white-tailed deer were fed whole kernel corn treated with 10mg of ivermectin per 0.45kg of corn at a rate of 0.45kg/deer per day in a confined treatment pasture (Pound et al. 1996). An adjacent pasture contained an equal number of deer that were fed untreated corn. The study was conducted between February and September of 1992 and 1993. Following treatment, there were 83.4% fewer adult *A. americanum*, 92.4% fewer nymphal *A. americanum*, and 100% fewer larval masses of *A. americanum* in the treated pasture. No change in tick population was observed in the control pasture. This study demonstrated that freely consumed acaricidal bait can significantly reduce the abundance of all stages of *A. americanum*.

Direct application of pesticides is also effective. A study conducted in 1980 involved the direct application of acaricides to beef cattle at 10-day intervals (Barnard et

al. 1983). This treatment resulted in an 86.7% reduction in the population of *A.*

americanum larvae, a 72.7% reduction in nymphs, and a 46.2 % reduction in the number

of adults. This study utilized several acaricides including dioxithion, toxaphene, and

malathion. A comparison was not drawn between the effectiveness of each, but the

overall control provided was significant. All of these studies indicate that attracting

animals to some variety of bait station for the delivery of a pesticide treatment is effective

in controlling ectoparasites, though none assessed longevity of the treatments.

Materials and Methods

Survey Overview. The ectoparasite survey was conducted in rural and urban settings in Payne County, Oklahoma, and a comparison of seasonal ectoparasite occurrence in these areas was made. Payne County is located in northern Oklahoma with a latitude of 36.08 and a longitude of −96.97. Payne County has a land area of 180,522 hect., an annual precipitation of 119.63cm, a January average temperature of 5.56°C and a July average temperature of 27.11°C. Fox squirrels involved in the survey were harvested by firearm, collected as roadkill if in good condition and recently killed, or live-trapped using a Havahart® small-mammal trap, and either marked and released or taken to the laboratory to become part of the live colony. Those squirrels that were tagged and released were not examined again if recaptured. Thirteen of the original squirrels captured were subjected to histopathological examination at the Oklahoma Animal Disease Diagnostic Laboratory (OADDL). Survey data were evaluated as to whether the squirrel was collected in a rural or an urban habitat. A focused urban survey was conducted between May 2001 and September 2001 in which trapping was confined to an area within the Stillwater city limits. The survey included 200 squirrels, total.

Trapping. Fox squirrels were live-trapped using Havahart® small mammal traps (Model 1078). These traps were larger than the squirrel and allowed movement within the trap once the squirrel had been captured. Traps were placed in trees that had nearly horizontal branches protruding at accessible heights and of a sufficient diameter to maintain the weight of the trap and a trapped animal. Higher branches were reached using a six-meter extension ladder.

Traps were secured to the branches using durable but malleable wire cut to sufficient length to encircle the limb. One wire was passed through the mesh of the trap at the rear and one near the front. It was crucial that the wire not interfere with the trigger mechanism of the trap and prevent the trap from springing. Additionally, the traps had to be tightly secured or squirrels would not enter them.

The traps were baited with a mixture of sunflower seeds and creamy peanut butter. The bait was placed on a 15.25cm X 15.25cm square of mesh cloth (tulle). The cloth was then gathered about the bait and twisted tightly forming a ball. The stem of the twist was stapled and excess cloth was trimmed off. In order to prevent bait theft by birds, opossums, raccoons, squirrels, or other animals, the bait was placed in a closed wire-mesh cylinder (15.24cm X 5.08cm), which was secured inside the trap with wire. This allowed the scent and appearance of the bait to attract animals but did not allow the animals access to the bait.

The traps were checked twice daily following the normal activity time of squirrels (0800-1000h and 1400-1600h). Bait remained in the trap for a period of ten days and was then replaced with fresh bait. No prebaiting period was utilized, as it did not seem to increase trapping success in preliminary trapping attempts. Bait was also replaced in the event of heavy rain or theft, if any destruction of the package occurred, if it lost its scent, became too dry, or was otherwise damaged.

Harvest by Firearm and Collection of Roadkill. In areas where it was difficult to trap squirrels or to check the traps with reasonable ease, harvesting the squirrels by firearm became the best option. Shooting of squirrels involved first knowing where squirrels could be found. This was done through observation of the area or by

recommendation of the landowner. Arriving at the location shortly before the known activity time of the squirrels was important. Squirrels were harvested with a .22 caliber rifle or 4-10 gauge shotgun and were ideally shot in the cranial region to prevent loss of parasites through damage to the carcass and for humane reasons. These types of firearm were chosen because they caused minimal damage to the carcass.

Squirrels that were killed by automobiles were only collected and examined if the extent of the trauma was minimal and if the animal was killed very recently, as judged by onset of rigor mortis or appreciable decline in body temperature. These squirrels were handled in the same manner as those harvested by firearm as described in the following section.

Handling of Killed Squirrels. Squirrels that were killed in the field were placed immediately into plastic storage bags (ZipLoc®, 1 gallon size) and taken to the medical entomology laboratory at Oklahoma State University (OSU) and examined thoroughly over a white surface and under bright lights. Examination involved rubbing the fur of the animals vigorously to remove loose ectoparasites and examination of the body by parting the fur and collecting ectoparasites with forceps. Each animal was examined, and data were recorded as to type of ectoparasite, date of collection, and squirrel number. Squirrels harvested in this manner were numbered in succession with those collected by live traps.

Handling of Live Squirrels. Squirrels collected in live traps were taken to the medical entomology laboratory for examination. Animals were coaxed from the trap into a net bag, sedated with 0.2cc of xylazine (Rompun®, Bayer Animal Health) through intramuscular injection and placed in a container with a lid to reduce the light intensity

until the drug took effect. Once the squirrel was incapacitated, it was checked thoroughly for ectoparasites by rubbing the fur vigorously and combing through the hair over a white surface under bright lights. Ectoparasites were placed in vials labeled with the squirrel number and the date and taken to the campus laboratory for identification. The squirrels were ear tagged and the number of the tag recorded. The squirrels were returned to their traps and held until they recovered from the drug, then released. If there was a lack of animals in the squirrel colony, the animals were not released but were added to the laboratory squirrel colony.

Histopathological Examination. Following examination for ectoparasites, the first 13 squirrels collected alive were transported to the Oklahoma Animal Disease Diagnostic Laboratory and euthanized via asphyxiation with carbon dioxide. A ventral midline incision was made, followed by an additional incision to open the chest cavity. Internal parasites were collected by removing digesta from the stomach and intestinal tract and examining it for the presence of endoparasites. Following examination for abnormalities of the digestive tract and internal organs, samples of several organs, including the kidney, spleen, liver, heart, and lungs were taken. In addition, samples of smooth muscle from a front and rear limb were taken. These samples were trimmed to pieces no larger than $2.54cm^2$. The samples were fixed in formalin and placed in a sealed container for 24 hours. Following tissue fixation, the tissue was cut into very thin slices and placed in tissue analysis trays to be analyzed by a pathologist. Additionally, squirrels that bit laboratory personnel severely enough to break the skin were tested for the rabies virus by the Oklahoma Department of Health in Oklahoma City.

Handling of Ectoparasite Specimens. All ectoparasites collected were stored in 70% isopropyl alcohol, with the exception of engorged immature ticks, which were placed in plastic cups and allowed to molt into the next life stage for ease of identification. A damp cloth was included in the cup to provide a moisture source for the prevention of desiccation and to increase humidity to aid in molting. Ectoparasite specimens were handled and identified differently depending on type, though all identification was carried out through the use of standard taxonomic keys and identification guides. Fleas and larval and nymphal ticks were placed on cavity slides and coated with lactic acid. The underside of the slide was heated with an open flame to clear the specimen and a binocular microscope was used for identification. Adult ticks were identified by sight. Lice were cleared as described previously, and identification was accomplished with the aid of a phase-contrast microscope. Following identification, specimens were returned to vials containing alcohol. Identification of lice was verified by Mr. Don Arnold, the curator of the K.C. Emerson Museum, located in the Department of Entomology and Plant Pathology at Oklahoma State University. Voucher specimens were also deposited in this museum.

Handling in the Laboratory (Keeping Squirrels in a Colony). Squirrels were placed in a large cage measuring 1.95m X 4.80m X 1.20m constructed of 30cm steel pipe and chicken wire with a mesh size of $15cm^2$. Doors were constructed of a more substantial wire cloth with a mesh size of $1.27cm^2$. This mesh was also used to reinforce the chicken wire in areas around the feeders and in places where squirrels tended to chew on the wire. The cage was divided into four individual cells, each with an area of 1.95m X 1.20m X 1.20m. Each cell housed a maximum of four squirrels. The cage was elevated

off the floor using paving stones and placed over an existing drain channel in the laboratory. Waste was washed down the channel each day and collected in a wire basket to prevent drain blockage. Animals were fed black oil sunflower seeds and water *ad libitum*. A wooden sleeping box measuring 30cm X 20cm X 20cm was originally provided for each animal for the dual purpose of animal welfare and ease in removing squirrels from the cage once they were inside. Each box was attached to the cage in such a fashion that it could easily be removed. Once it was seen that the squirrels were destroying the wooden boxes, they were replaced with steel mailboxes hung from the top of the cage. The metal construction of the mailboxes prevented destruction by the squirrels. The mailboxes were removed from the cage and cleaned every two weeks. The squirrels were provided with wood pieces to chew to prevent overgrowth of their teeth.

Comparison of Squirrels from Rural and Urban Habitats. Squirrels were sorted according to trap site into two groups: squirrels collected in a rural habitat and squirrels collected in an urban habitat. All squirrels collected inside the city limits of Stillwater were considered to be urban squirrels, while all those collected outside the city limits were considered to be rural. Squirrels collected in an urban habitat were further divided into the following subdivisions:

Urban Resembling Rural: Areas with dense trees and large separations between houses

Semi-urban: Areas with scattered trees and less space between houses

Urban: Areas with sparse trees and houses close together

Preliminary Infestations. Squirrels were removed from the colony cage by opening the back access door of the cage and holding a net bag in front of the sleeping

boxes. The squirrels were coaxed into the bag and removed from the cage in this way. The squirrels were removed one at a time to prevent escape and so that each squirrel could be ear tagged, if not already tagged. Heavy gloves were worn at all times while handling animals. Each squirrel was sedated with 0.2cc of xylazine through intramuscular injection and placed in a container with a lid until the drug took effect. Once the squirrel was incapacitated, it was removed from the net bag and fitted with a nylon sleeve about its midsection. Twenty-five nymphal ticks from the Oklahoma State University Tick Rearing Facility were placed under the sleeve, and the squirrel was then placed in a small cage (40.64cm X 17.80cm) with 2.54cm X 1.27cm mesh over a water reservoir. To minimize the stress of confinement, the laboratory room containing the squirrels was kept dark and quiet except during examination of the water and feeding. If squirrels handled confinement particularly badly, their cages of were covered with towels so that movements in the laboratory would not startle them. One trial was carried out using *A. maculatum* nymphs and one with *R. sanguineus* nymphs for the dual purpose of testing the squirrels' competence as a host for these tick species and simply for practice in handling the squirrels during an infestation.

A cotton rat (*Sigmodon hispidus* Say and Ord) was used as a control in each trial because data had been collected for the rats in previous studies and it was known that these tick species fed readily on the rats. The rat was removed from its cage and also fitted with a nylon sleeve about its midsection. It was then placed in an isolation cage that restricted its movement and prevented it from grooming the sleeve off. The ticks were placed on the rat under the sleeve and the rat remained in the isolation cage for 24 hours. Following this period, the rat was placed back into its cage and placed over a water

reservoir. All animals were provided with water *ad libitum* and fed once daily. The rat was fed a laboratory rodent diet and the squirrels were fed bait balls, described previously in the trapping section.

The water beneath the animals was checked daily for flat and engorged ticks by placing the reservoir on an illuminated table. This procedure allowed one to distinguish ticks from other debris. Ticks collected from the water were blotted on a paper towel to remove excess surface moisture, counted, and placed in small plastic cups. The animal identification and the date were recorded on the cup lid. Each tick was weighed within an hour of removal from the water to prevent weight loss due to meal concentration by the tick. Damp paper was placed in the cup with engorged ticks to provide humidity and the ticks were allowed to molt to the adult stage. Following examination, the water in the reservoir was discarded and replaced with fresh water. Percent of ticks that fed to engorgement as well as average weight after feeding were calculated.

Medication Study Overview. A comparison between two topical insecticides, fipronil (Merial Animal Health) and moxidectin (Cydectin®, Fort Dodge Animal Health), was made. The infestation trials involved eight squirrels for each trial. Four were treated with an insecticide and four were untreated controls that were sham-treated with peanut oil or water. A solution of 9.7% fipronil (9.7mg/kg) and peanut oil was mixed according to the average body weight of the squirrels to give a total volume of 3ml while moxidectin was ready to use from the container (1ml for each 8.21kg of body weight). An average squirrel weight of 600g was used to calculate the appropriate dosage of each medication. Ticks used in the trials were laboratory reared and had not been in contact

with any medications or acaricides. Squirrels used in the fipronil trials were not used in the moxidectin trials.

Infestation and Treatment of Squirrels with Fipronil. Squirrels were removed individually from the colony cage in the same fashion discussed previously. Each squirrel was sedated with 0.2cc of xylazine administered through intramuscular injection and was placed in a closed plastic container to reduce light until the drug took effect. Once a squirrel was incapacitated, it was fitted with a nylon sleeve about its midsection and was infested with 50 *D. variabilis* nymphs. The four squirrels that were to be treated with fipronil were marked with an ear punch. One hole was placed in the left ear of each principal. The squirrels were then placed in small cages over a water reservoir and allowed to recover completely from the sedative. The water beneath all squirrels was checked twice daily for ticks and the number of ticks recovered was recorded. After being thoroughly examined, the water in the reservoirs was discarded and replaced with fresh water.

On the third day of the trial, all ticks that were likely to attach had done so. At this time, the four control animals were each treated topically between the ears with 3ml of peanut oil and the four treatment animals were each treated with fipronil diluted in peanut oil to give a total volume of 3ml. Application of the solution was accomplished with a separate pipette for each squirrel. The water beneath all squirrels was checked each day, and the number of ticks, both flat and engorged, was recorded. Ticks that were engorged or partially engorged were weighed within an hour of collection and percentage of ticks that fed to engorgement and engorgement weight were recorded for the treatments and controls. At the end of the ten-day trial, the squirrels were returned to the colony cage.

The duration of the trial was determined by the recovery of ticks from the water reservoir. The trial was concluded when no ticks had detached for two consecutive days.

Longevity of Fipronil. In order to determine how long the medication was effective, squirrels that had been treated were reinfested 15 or 30 days after the original treatment. It was not possible to use the same squirrels for both the 15-day and 30-day trials because the stresses of confinement proved fatal to squirrels after repeated infestations and two of the treated squirrels died upon return to the colony cage. Thus, one group of squirrels was used to test the effectiveness of fipronil 15 days post-treatment and eight entirely different squirrels were used to evaluate the effectiveness of fipronil 30 days post-treatment. The same eight squirrels used in the above infestation were reinfested after 15 days and handled in the same fashion described above. Eight separate squirrels were utilized in the same manner described above. These squirrels were infested with ticks, treated with fipronil, and reinfested 30 days post-treatment.

Infestation and Treatment of Squirrels with Moxidectin. Squirrels were removed individually from the colony cage, sedated, and infested with 50 *D. variabilis* nymphs in the same fashion described previously (p.21). Squirrels that were to be treated with moxidectin were marked with an ear punch, and one hole was made in the left ear of each principal. The squirrels were then placed in small cages over a water reservoir and allowed to recover from the sedation. The water beneath the squirrels was checked twice daily for ticks and the number of ticks recovered was recorded.

On the third day of the trial, all ticks that were likely to attach had done so. At this time, each of the four control animals was treated topically between the ears with 62.5µl of water and each of the four principals was treated with 62.5µl of moxidectin.

Application of the solution was accomplished with a pipette, and separate pipette tips were used for each squirrel. The water beneath each squirrel was checked each day and the number of ticks, both flat and engorged, was recorded. Ticks that were engorged or partially engorged were weighed and percent of ticks that fed to engorgement and weight after feeding were calculated for the treatments and controls. The trial lasted 14 days and the squirrels were then returned to the colony cage. The trial was considered complete when no ticks had been recovered for two consecutive days.

Longevity of Moxidectin. In order to determine how long the medication was effective, squirrels that had been treated were reinfested 15 days after the original treatment. This reinfestation was conducted in the same fashion as those described previously. In this instance, the treatment did not appear to be effective and the planned 30-day post-treatment trial was abandoned.

Statistical Analysis. Data collected during the survey, and in both the fipronil and moxidectin trials, were evaluated using PC SAS Version 8.1 (SAS Institute Incorporated, Cary, NC, 2000). Means and standard deviations were calculated for the survey using PROC MEANS. Percentage data for the presence of ectoparasites on fox squirrels were analyzed in a contingency table with a Chi Square test using PROC FREQ. In the acaricide trials, PROC TTEST was used to perform t-tests to determine differences in the means. Whenever unequal variances were detected with an F-test, a pseudo t-test was used along with a Satterthwaite approximation for degrees of freedom. Percentages of engorgement of ticks were transformed using an arcsin square root transformation.

Results

Survey as a whole. In the ectoparasite survey, the first squirrel was collected on May 21, 2000, and the last squirrel included in the survey was collected on December 17, 2001. In that time, 200 fox squirrels were collected and examined, and 147 (73.5%) were infested with at least one species of ectoparasite. Of the 200 squirrels, 108 (54%) were live trapped, 81 (40.5%) were harvested by firearm, and 11 (5.5%) were collected as roadkill. A total of 69 (34.5%) squirrels were collected from a rural habitat and 131 (65.5%) were collected from an urban habitat.

Fox squirrels were infested with a wide variety of ectoparasites, including ticks and other mites, fleas, and lice. Two flea (Siphonaptera) species were recovered from the fox squirrel, *Orchopeas howardii* Baker, and *Nosopsyllus fasciatus* (Bosc). Two species of lice (Anoplura) were also found on the fox squirrel, *Neohaematopinus sciurinus* Mjöberg and *Hoplopleura sciuricola* Ferris. Four tick species were collected, and included all life stages of *Dermacentor variabilis* (Say), larvae and nymphs of *Amblyomma americanum* (L.), nymphs of *Ixodes scapularis* Say, and larvae of *Haemaphysalis leporispalustris* (Packard). The total number of specimens of each ectoparasite species collected is presented in Table 1. The average number of each type of ectoparasite collected each month is presented in Table 2.

Seasonal Incidence of Ectoparasites. Most types of ectoparasites showed a seasonal activity pattern, but this was not uniformly true. There was not a strong seasonal pattern in flea populations on the fox squirrel, but population peaks were seen in

Table 1. Total number of ectoparasite specimens collected, by species.

Order	Species	Total # Collected
Anoplura	*Neohaematopinus sciurinus*	1,463
Anoplura	*Hoplopleura sciuricola*	168
Siphonaptera	*Orchopeas howardii*	464
Siphonaptera	*Nosopsyllus fasciatus*	6
Acari	*Amblyomma americanum*	1,525[a]
Acari	*Dermacentor variabilis*	68[a]
Acari	*Ixodes scapularis*	4[a]
Acari	*Haemaphysalis leporispalustris*	1[a]

[a] This total includes all mobile life stages. Total numbers of each life stage collected are presented in Table 3.

Table 2. Average number of ectoparasites recovered from the fox squirrel, by month and year.

Month	# Squirrels Col.	Avg. # Fleas/Sq.	Avg. # Lice/Sq.[a]	Avg. # Ticks/Sq.
May 2000	4	1.5 ± 0.58	7.25 ± 14.50	2.0 ± 2.70
June 2000	9	2.44 ± 2.40	20.1 ± 44.02	2.22 ± 2.53
July 2000	2	12.0 ± 0.0	0.0 ± 0.0	0.50 ± 0.707
August 2000	7	0.57 ± 1.13	5.57 ± 14.30	64.29 ± 118.03
September 2000	6	0.83 ± 0.75	0.17 ± 0.42	5.66 ± 8.24
October 2000	14	0.35 ± 1.08	2.57 ± 6.02	0.57 ± 0.94
November 2000	7	1.86 ± 2.91	21.4 ± 28.27	0.0 ± 0.0
December 2000	8	1.63 ± 2.72	43.25 ± 69.23	0.0 ± 0.0
January 2001	13	4.78 ± 10.54	1.31 ± 2.28	0.0 ± 0.0
February 2001	2	1.0 ± 0.0	2.0 ± 2.82	0.50 ± 0.707
March 2001	8	8.38 ± 11.84	0.38 ± 0.74	0.0 ± 0.0
April 2001	6	2.33 ± 2.33	0.0 ± 0.0	4.50 ± 5.72
May 2001	8	3.75 ± 3.99	2.88 ± 5.38	0.75 ± 1.21
June 2001	32	1.0 ± 2.25	0.53 ± 1.16	0.97 ± 1.82
July 2001	21	0.71 ± 1.48	0.90 ± 1.92	0.38 ± 0.80
August 2001	13	0.0 ± 0.0	0.15 ± 0.38	0.31 ± 0.85
September 2001[b]	7	7.14 ± 16.29	81.7 ± 215.76	142.0 ± 325.74
October 2001	19	4.26 ± 7.92	4.57 ± 11.89	1.26 ± 2.08
November 2001	12	2.0 ± 1.35	4.42 ± 6.20	0.33 ± 0.49
December 2001	2	1.5 ± 2.12	7.5 ± 7.77	0.0 ± 0.0

Standard deviations are listed beside each mean.

[a] Only adult lice that could be positively identified were included in the calculation of the mean number of lice occurring each month.

[b] One squirrel collected during September, 2001 had been injured by a predator prior to collection. This animal could not climb trees and, thus, was spending large amounts of time on the ground. This squirrel had a much greater number of ectoparasites than other squirrels and the large means and standard deviations for the month are a product of this.

March of 2001 (8.38 fleas/sq.), and in September of 2001 (7.14 fleas/sq.). Flea populations were lowest in August (0.57 fleas/squirrel), September (0.83 fleas/squirrel), and October (0.35 fleas/squirrel) of 2000, and in June (1.0 fleas/squirrel), July (0.71 fleas/squirrel), and August (0.0 fleas/squirrel) of 2001. Populations were also low in November (1.86 fleas/squirrel) and December (1.63 fleas/squirrel) of 2000. Low populations were also observed in November and December of 2001 (2.0 fleas/squirrel and 1.5 fleas/squirrel respectively) (Table 2). *Orchopeas howardii* was the predominant flea species collected, and specimens of *N. fasciatus* were collected only in the warm months of May, June, and July of 2000 (Figure 1) and only from five squirrels.

The seasonal activity pattern of lice showed peaks during the cooler months of the year, though this was species dependent (Figure 2). *Hoplopleura sciuricola* was the only louse species collected during December of 2001. In August and October of 2000, *H. sciuricola* accounted for 78% and 79% of the louse specimens collected, respectively. *Neohaematopinus sciurinus* was a far more prevalent anopluran species on the fox squirrel, accounting for 91% of the specimens collected, and was recovered in all but four months of the study. Populations of *N. sciurinus* peaked in the cooler months, including December of 2000 (43.25 lice/squirrel). Additional peaks were seen in June of 2000 (20.1 lice/squirrel) and September of 2001 (87.1 lice/squirrel), but this was due to a large numbers of lice being collected from a single animal in each of those months.

Mites, though less common than other types of ectoparasites, were present on fox squirrels. All mites, other than ticks, collected from squirrels were identified only to the suborder Gamasida. No definite seasonal trend of mite populations was observed; population peaked in November 2000 (0.71 mites/squirrel) and again in October 2001

(2.26 mites/squirrel). Mites were not found during the hotter part of the year including the months of June, July, and August in 2000 or 2001. Figure 3 presents the average numbers of mites collected through each month of the survey.

Far more species of ticks were found on fox squirrels than any other type of ectoparasite. While ticks were not present during every month of the year, it was clear that different species and life stages of ticks occurred at different times of the year. Table 3 lists the total number of each tick species collected during each month of the survey as well as the number of squirrels collected that month.

Ticks were the least prevalent in December 2000 and 2001, and in January and February of 2001. Additionally, fox squirrels collected in March of 2001 were devoid of ticks. The most prevalent tick collected from the fox squirrel was *A. americanum*. Larval stages of this tick were found in great number in August of 2000 and September of 2001, but were rarely found in other months and were only collected from a small number of squirrels. These few squirrels were heavily infested with larvae, and 859 ticks were recovered from one animal. This was an injured animal, but large numbers of larvae were present on healthy squirrels as well (Table 3). *Amblyomma americanum* nymphs were collected from fox squirrels between May and October of 2000 and between April and September of 2001. Squirrels were not heavily parasitized by nymphs, and adults were not collected from fox squirrels at all. Another tick collected in great number from fox squirrels was *D. variabilis*, which was present in every parasitic life stage. Larvae were collected between August and October of 2000 and 2001, and one larval tick was collected in February of 2001. Nymphs were collected between July and September of 2000 and between July and November of 2001. Neither the nymphs nor the
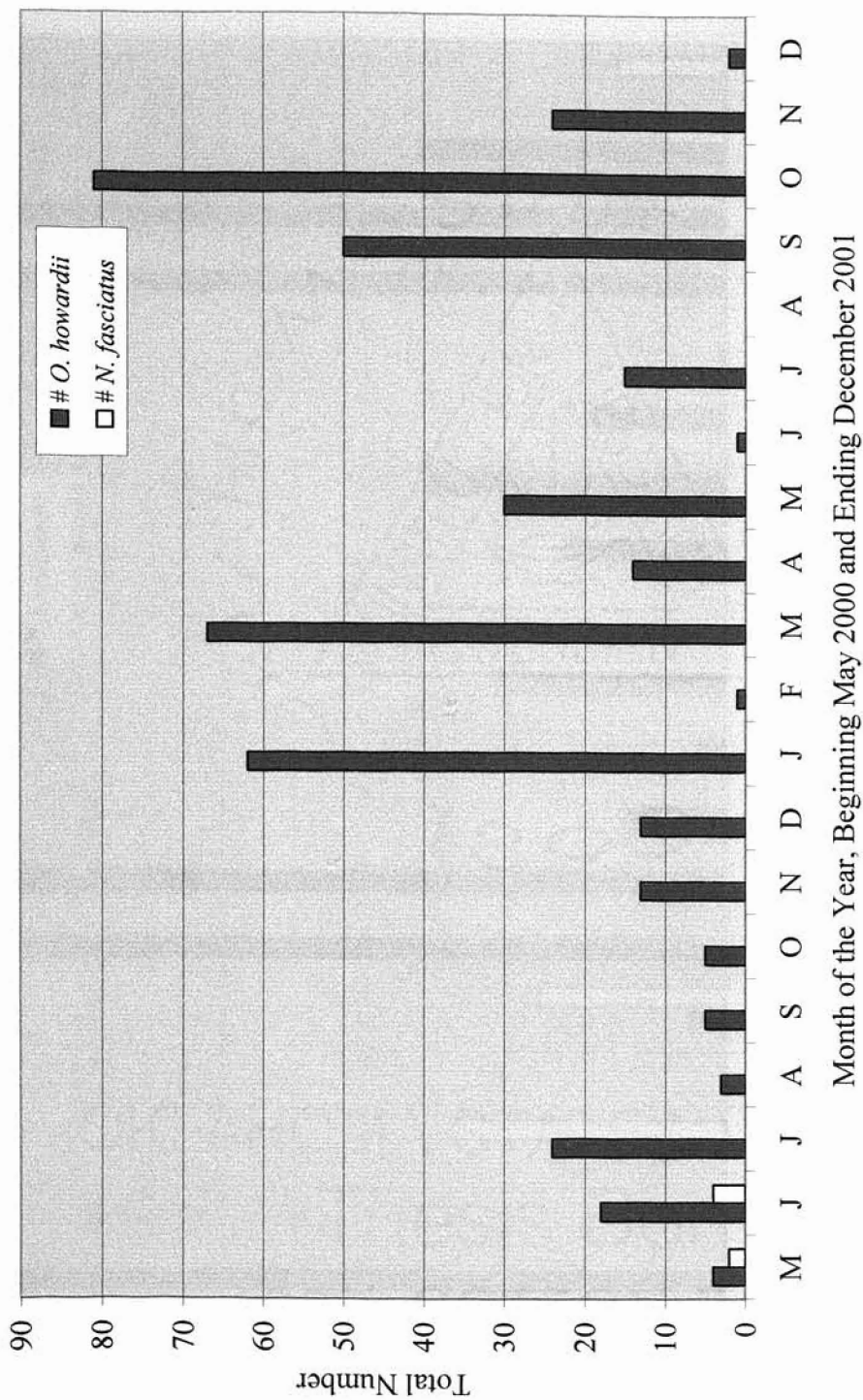
31

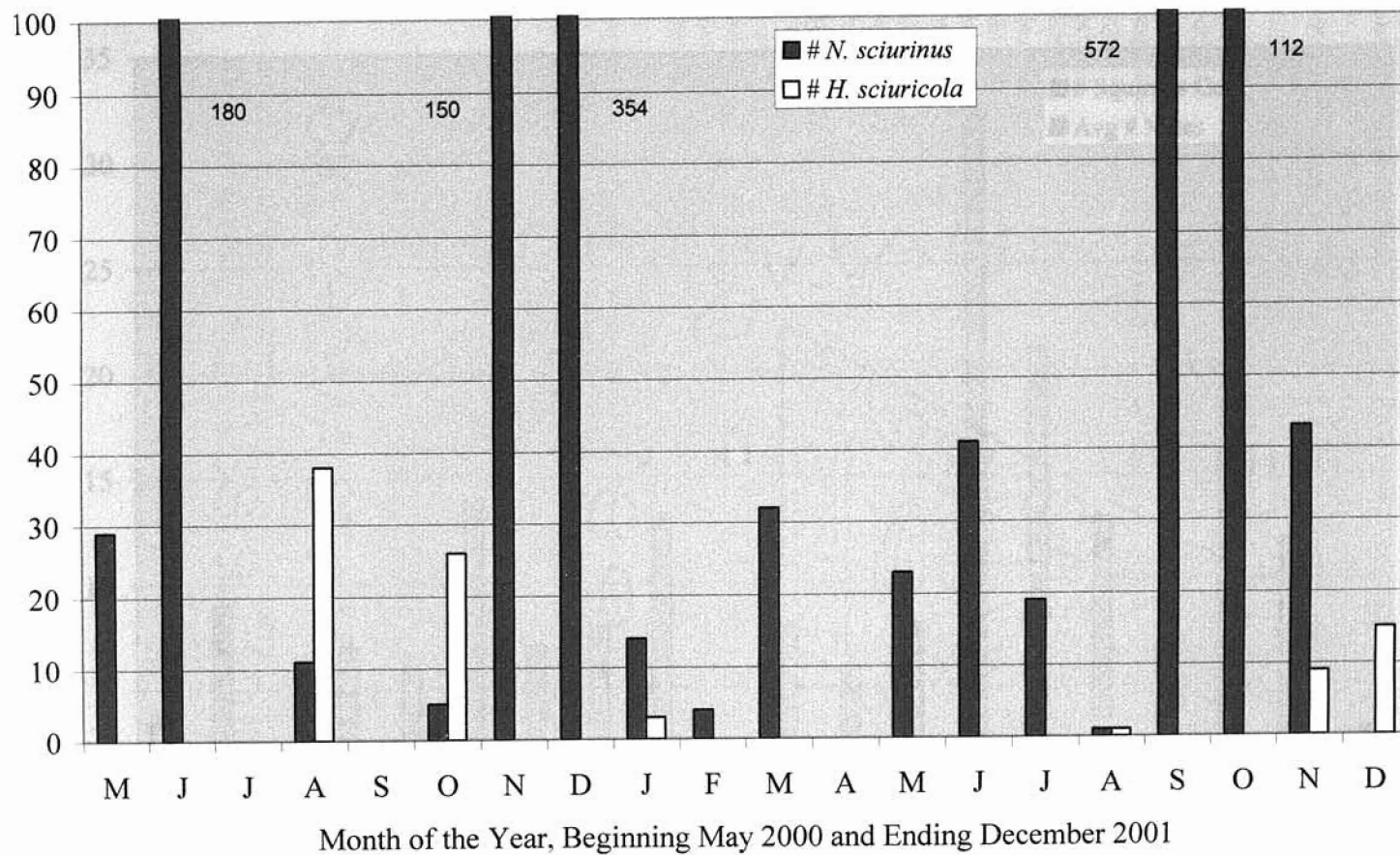**Figure 1.** Total number of fleas collected each month, by species.

Figure 2. Total number of lice collected each month, by species.

Figure 3. Average number of mites other than ticks collected from fox squirrels each month.

Table 3. Total number of specimens of each tick species collected from the fox squirrel, by month.

Month	# Squir Col.	#I. scap. Ny.	#A. amer. Lv.	#A. amer. Ny.	#D. var. Lv.	#D. var. Ny.	#D. var. Ad.	#H. leporis. Lv.
May '00	4	2	0	6	0	0	0	0
June '00	9	0	0	9	0	0	11	0
July '00	2	0	0	0	0	1	0	0
Aug. '00	7	0	411	14	3	1	2	0
Sept. '00	6	1	0	28	1	2	0	0
Oct. '00	14	0	3	2	3	0	0	0
Nov. '00	7	0	0	0	0	0	0	0
Dec. '00	8	0	0	0	0	0	0	0
Jan. '01	13	0	0	0	0	0	0	0
Feb. '01	2	0	0	0	1	0	0	0
Mar. '01	8	0	0	0	0	0	0	0
April '01	6	0	0	24	0	0	3	0
May '01	8	1	0	3	0	0	2	0
June '01	32	0	0	22	0	0	8	0
July '01	21	0	0	2	0	1	5	0
Aug. '01	13	0	0	2	0	0	2	0
Sept. '01	7	0	977	14	2	2	0	0
Oct. '01	19	0	8	0	14	2	0	0
Nov. '01	9	0	0	0	0	3	0	1
Dec. '01	2	0	0	0	0	0	0	0

Lv=larva, Ny=nymph, Ad=adult, *I. scap.=Ixodes scapularis*, *A. amer.=Amblyomma americanum*, *D. var.=Dermacentor variabilis*, *H. leporis.=Haemaphysalis leporispalustris*

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larvae were present in large numbers on any animal. Adult *D. variabilis* were found in June and August of 2000 and between April and August of 2001. Similar to the immatures, the adults were not found in large numbers on any animal. Two other species of ticks were less common. Only four specimens of *I. scapularis* were collected and only in the nymphal stage during the months of May 2000 and 2001 and in September of 2000. Only one specimen of *H. leporispalustris*, a larva, was collected in November of 2001.

Comparison of Ectoparasite Species on Squirrels from Rural and Urban Areas. As was mentioned earlier, 65% of the squirrels collected came from an urban setting and 35% from a rural setting, and the tick burden carried by squirrels from each habitat was different. For the purpose of this study, rural squirrels were those collected outside the Stillwater city limits, and urban squirrels were those collected from within the city limits. Of the squirrels collected from an urban area, 58 (44.3%) were infested with fleas, while 37 (53.6%) of the squirrels collected from a rural setting were infested with fleas, indicating that there was not a significant difference in flea burden between the habitats ($P = 0.2082$). In an urban habitat, 48 squirrels (36.6%) were infested with lice, while 23 (33.3%) of the squirrels collected in a rural setting were infested with lice. There was not a significant difference in louse burden between the two habitat types ($P = 0.6421$). Of the squirrels collected in an urban habitat, 28 (24.1%) were infested with ticks, while 40 (58%) of the squirrels collected from a rural habitat were infested with ticks. There was a significant difference in tick burden between the two habitat types ($P < 0.0001$)

When the urban habitat was further divided into subcategories, the average number of ectoparasites collected from fox squirrels decreased, and the diversity of tick species also decreased as habitat became more urbanized (Table 4). In the habitat type designated "urban resembling rural", the 15 squirrels collected were infested with a total of 887 ticks (59.1 ticks/squirrel), 77 fleas (5.13 fleas/squirrel), and 573 lice (38.2 lice/squirrel). In the habitat designated "semi-urban", the 79 squirrels collected were infested with a total of 27 ticks (0.34 ticks/squirrel), 229 fleas (2.89 fleas/squirrel), and 655 lice (8.29 lice/squirrel). In the habitat designated "urban", the 37 squirrels collected were infested with a total of 3 ticks (0.08 ticks/squirrel), 24 fleas (0.65 fleas/squirrel), and 111 lice (3.00 lice/squirrel) (Table 5).

A number of the squirrels collected were not infested with ectoparasites, and the average number of ectoparasites collected from infested squirrels when uninfested squirrels were excluded was different. In the habitat type designated "urban resembling rural", the 10 infested squirrels collected carried 88.7 ticks/squirrel, 7.70 fleas/squirrel, and 57.3 lice/squirrel. In the habitat type designated "semi-urban", the 60 infested squirrels collected carried 0.45 ticks/squirrel, 3.82 fleas/squirrel, and 10.92 lice/squirrel. In the habitat type designated "urban", the 18 infested squirrels collected carried 0.17 ticks/squirrel, 1.33 fleas/squirrel, and 6.17 lice/squirrel.

Because ticks are a primary concern when considering the transmission of diseases from wildlife to humans and companion animals, a comparison was made between the tick burden of squirrels collected in rural and urban habitats. As previously mentioned, a greater percentage of rural squirrels were infested with ticks than urban squirrels, but the average number of ticks collected per squirrel from rural squirrels was

Table 4. Total number of specimens of each tick species collected from the fox squirrel, by habitat designation.

Species	Life stage	Rural	Urban Res. Rural	Semi-Urban	Urban
A. americanum	Lv.	537	859	1	0
A. americanum	Ny.	103	16	3	0
D. variabilis	Lv.	12	2	6	0
D. variabilis	Ny.	7	3	4	0
D. variabilis	Ad.	25	4	6	3
I. scapularis	Ny.	4	0	0	0
H. leporispalustris	Lv.	1	0	0	0

Table 5. Average number of each type of ectoparasite collected from fox squirrels in each habitat designation.

Habitat Type	# Squirrels Collected	Avg. # Fleas	Avg. # Lice	Avg. # Ticks
Rural	69	2.02	5.13	9.91
Urban Res. Rural	15	5.13	38.2	59.1
Semi-Urban	79	2.89	8.29	0.34
Urban	37	0.65	3.00	0.08

not significantly greater (P = 0.7209). A total of 684 ticks were collected from the 69 squirrels (9.99 ticks/squirrel) trapped from rural habitats, whereas 904 ticks were collected from urban squirrels (6.98 ticks/squirrel). When squirrels that were not infested with ticks were excluded from the calculation, the average number of ticks on squirrels collected from a rural area was 11.59 ticks/squirrel, while the average number of ticks collected from urban squirrels was 10.27 ticks/squirrel. There was not a significant difference between these two means (P = 0.6361). A larger number of tick species (four) was collected from squirrels trapped in rural habitats than from those collected in an urban habitats (three species). The rabbit tick, *H. leporispalustris* was only collected from a squirrel trapped in a rural habitat.

Results of Histopathological Examination. None of the 13 squirrels that underwent histopathological examination was found to have any variety of abnormality or illness. All were examined thoroughly for the presence of *Hepatozoon* sp., but the parasite was not detected in muscle biopsies. In addition, only one of the examined squirrels was found to be infected with an internal parasite; the parasite was identified as a nematode, but identification was not taken further than this. Because of the cost of these examinations and the lack of positive results, the examinations were halted after thirteen squirrels. It did not seem productive to sacrifice squirrels without positive results. The one squirrel tested for rabies was found to be free of the virus.

Preliminary Infestations. Nymphal *Amblyomma maculatum* Koch fed readily on the fox squirrel under laboratory conditions, as did nymphal *Rhipicephalus sanguineus* (Latreille). For most squirrels, a higher percent of *A. maculatum* nymphs fed to

engorgement than fed to engorgement on the cotton rat (Figure 4). In addition, the average weight post-feeding of *A. maculatum* nymphs was higher after feeding on fox squirrels than on the cotton rat (Figure 5). Both percent engorgement and weight post-feeding were similar for nymphal *R. sanguineus* feeding on fox squirrels and the cotton rat (Figures 6 and 7, respectively). While neither of these tick species was collected during the ectoparasite survey, these infestations indicate that the fox squirrel is a susceptible host, and that it is likely a behavioral trait or differences in habitat rather than a physiological trait that keeps these species of tick from infesting fox squirrels.

Results of Treatment with Fipronil. Fipronil provided very effective control that lasted at least 30 days. Separate squirrels were used for the 15-day and 30-day trials, and two sets of results are representative of this. During the initial infestation, ticks that fed on treated squirrels failed to engorge 100% of the time, whereas a significantly higher percentage (20% to 44%) of ticks that fed on untreated squirrels fed to engorgement ($P = 0.0019$) (Figure 8). Additionally, the difference in engorged weights between ticks that partially fed on treated squirrels and those that fed on untreated squirrels was highly significant ($P = 0.0036$) (Figure 9). A subsequent infestation showed similar results. Ticks that fed on treated squirrels 15 days post-treatment failed to engorge 100% of the time, whereas a significantly higher percentage (12% to 82%) of ticks feeding on untreated squirrels fed to engorgement ($P = 0.0282$) (Figure 10). The difference in engorged weights between ticks that partially fed on treated squirrels and those that fed on untreated squirrels was highly significant ($P = 0.0028$) (Figure 11).

The second trial, conducted to test the effects of fipronil on feeding ticks 30 days post-treatment, had similar results. In the initial infestation, ticks that fed on treated
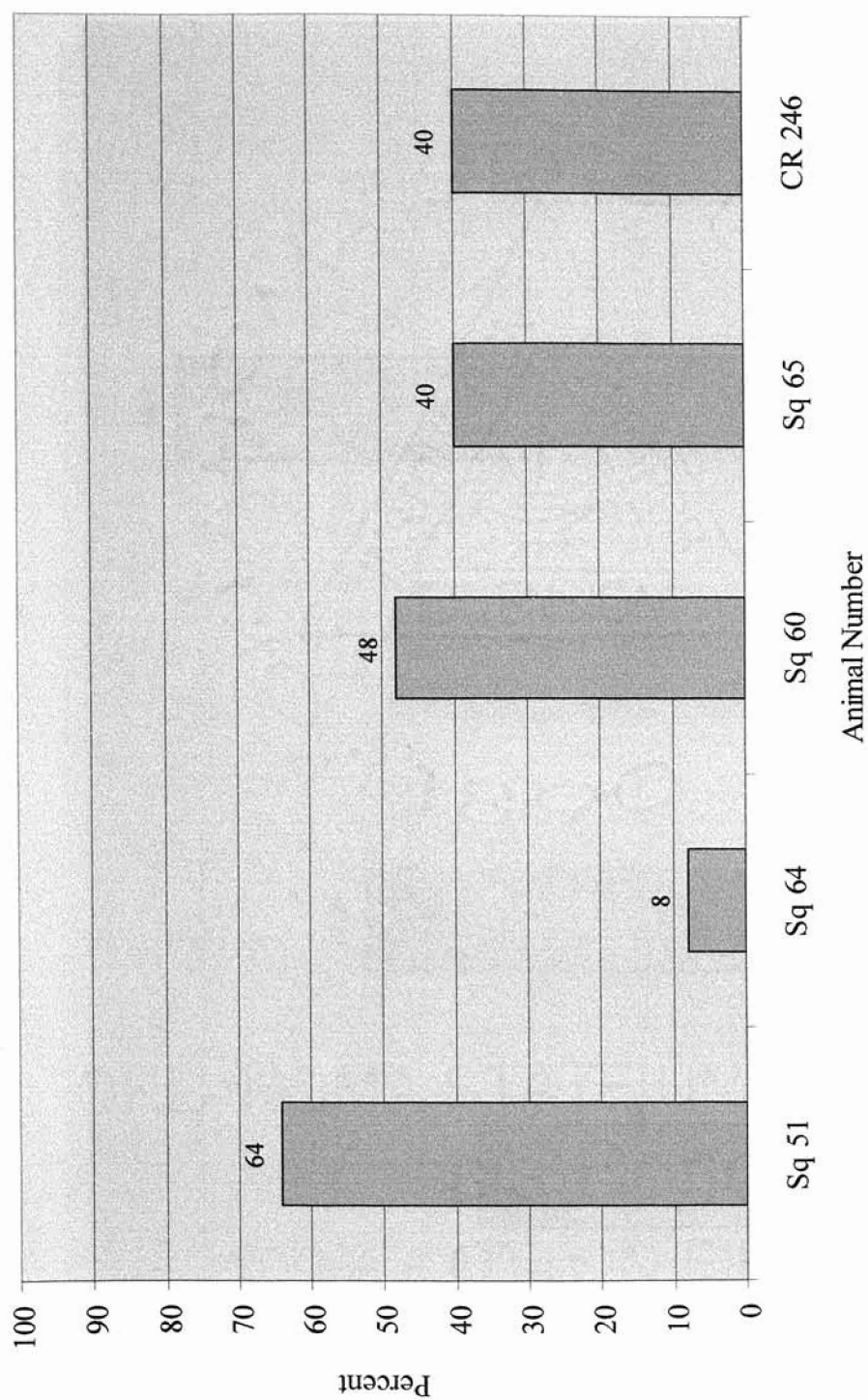
40

Figure 4. Percent of *Amblyomma maculatum* nymphs that fed to engorgement on the fox squirrel, *Sciurus niger,* and the cotton rat, *Sigmodon hispidus.*
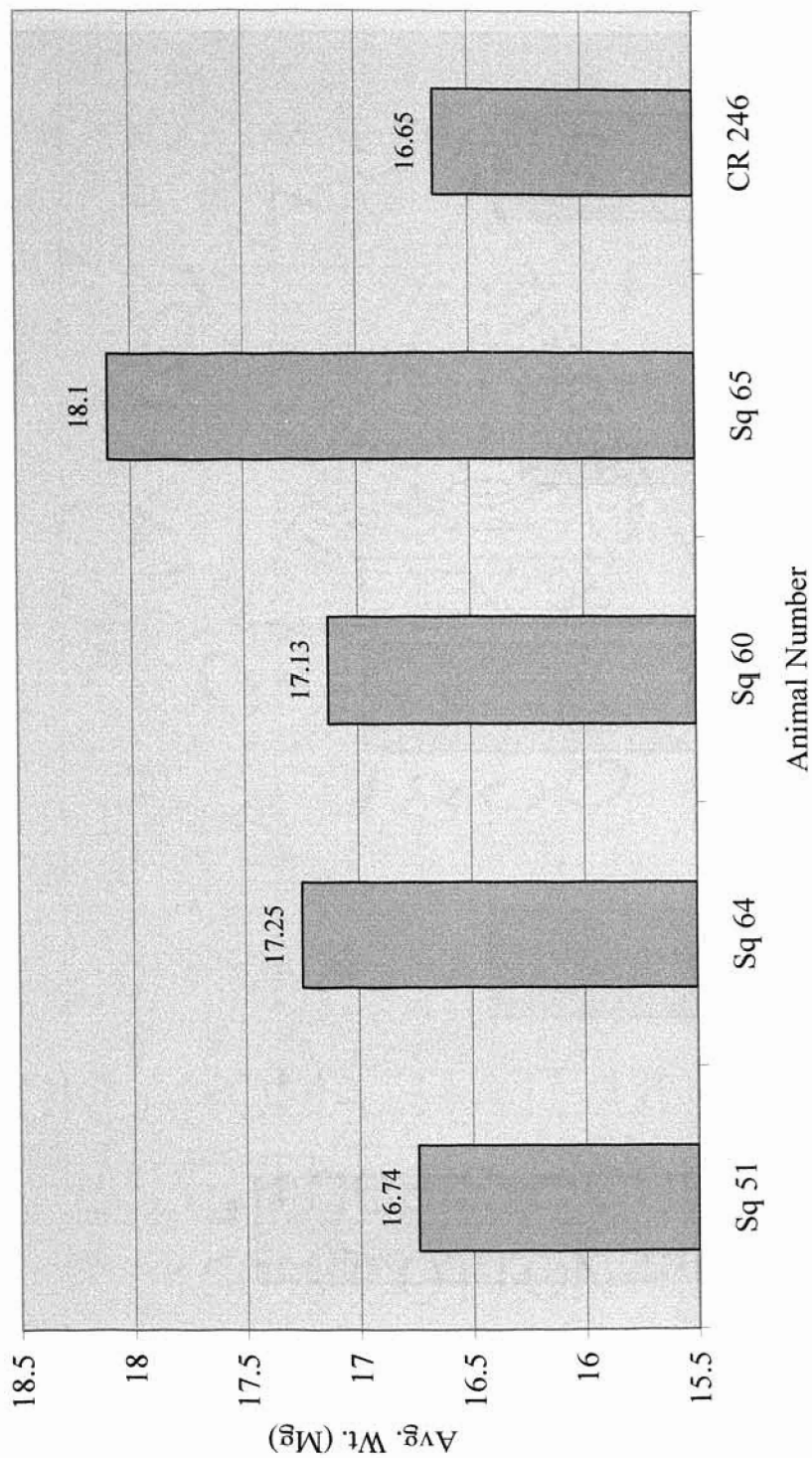
Figure 5. *Average weight of Amblyomma maculatum nymphs that fed on the fox squirrel, Sciurus niger and the cotton rat, Sigmodon hispidus.*
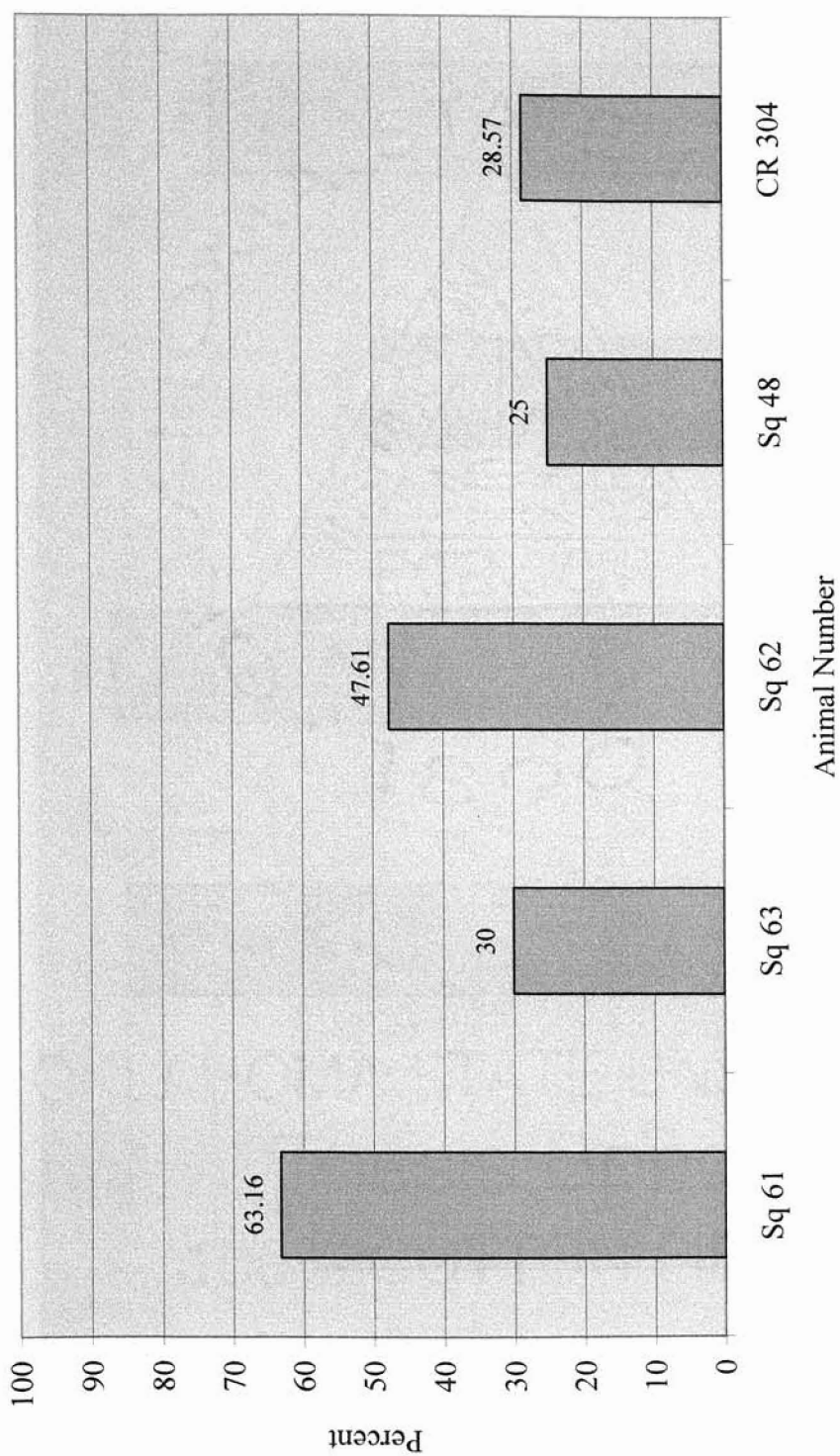
42

Figure 6. Percent of *Rhipicephalus sanguineus* nymphs that fed to engorgement on the fox squirrel, *Sciurus niger*, and the cotton rat, *Sigmodon hispidus*.
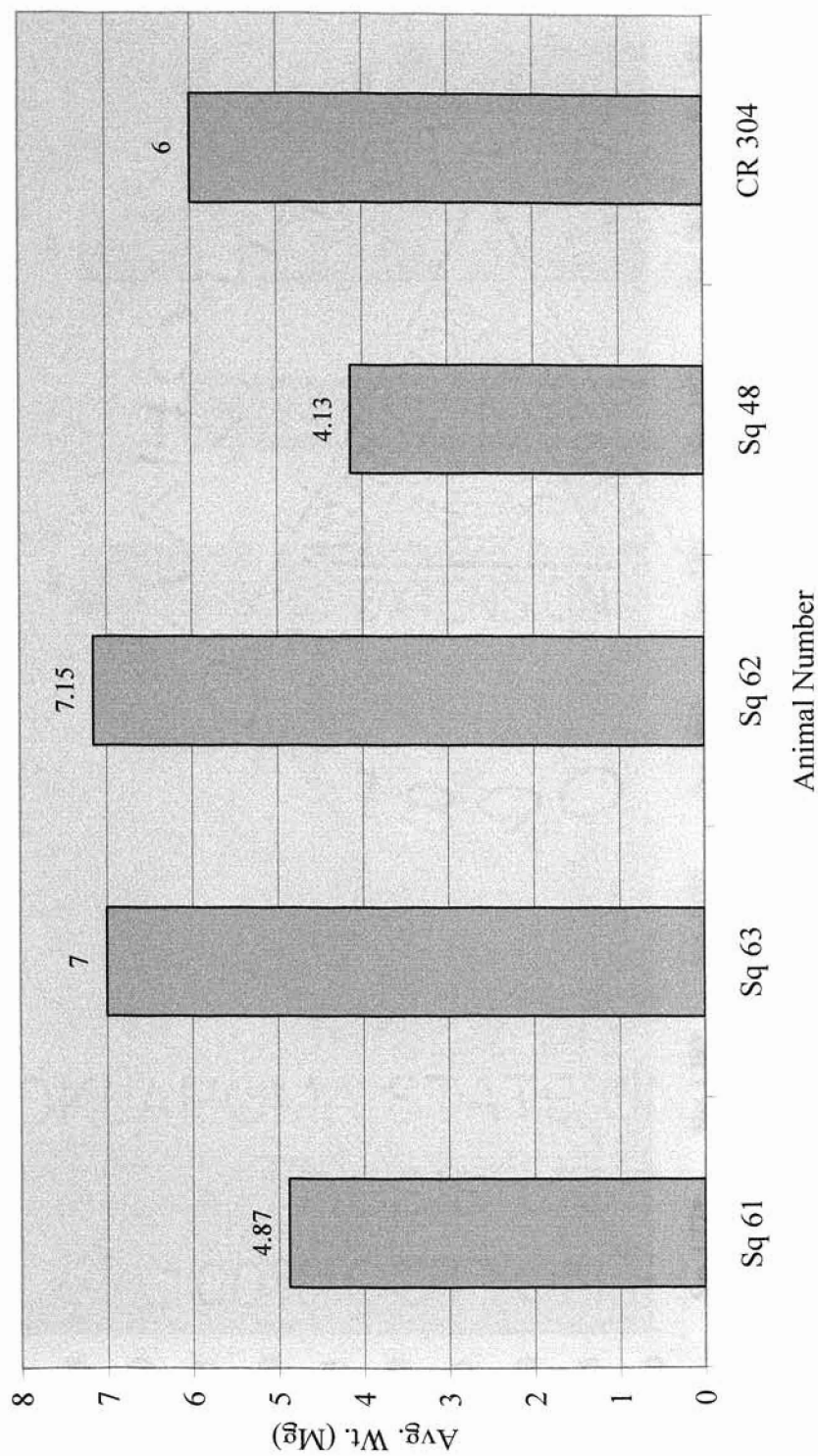
Figure 7. Average weight of *Rhipicephalus sanguineus* nymphs that fed to engorgement on the fox squirrel, *Sciurus niger*, and the cotton rat, *Sigmodon hispidus*.
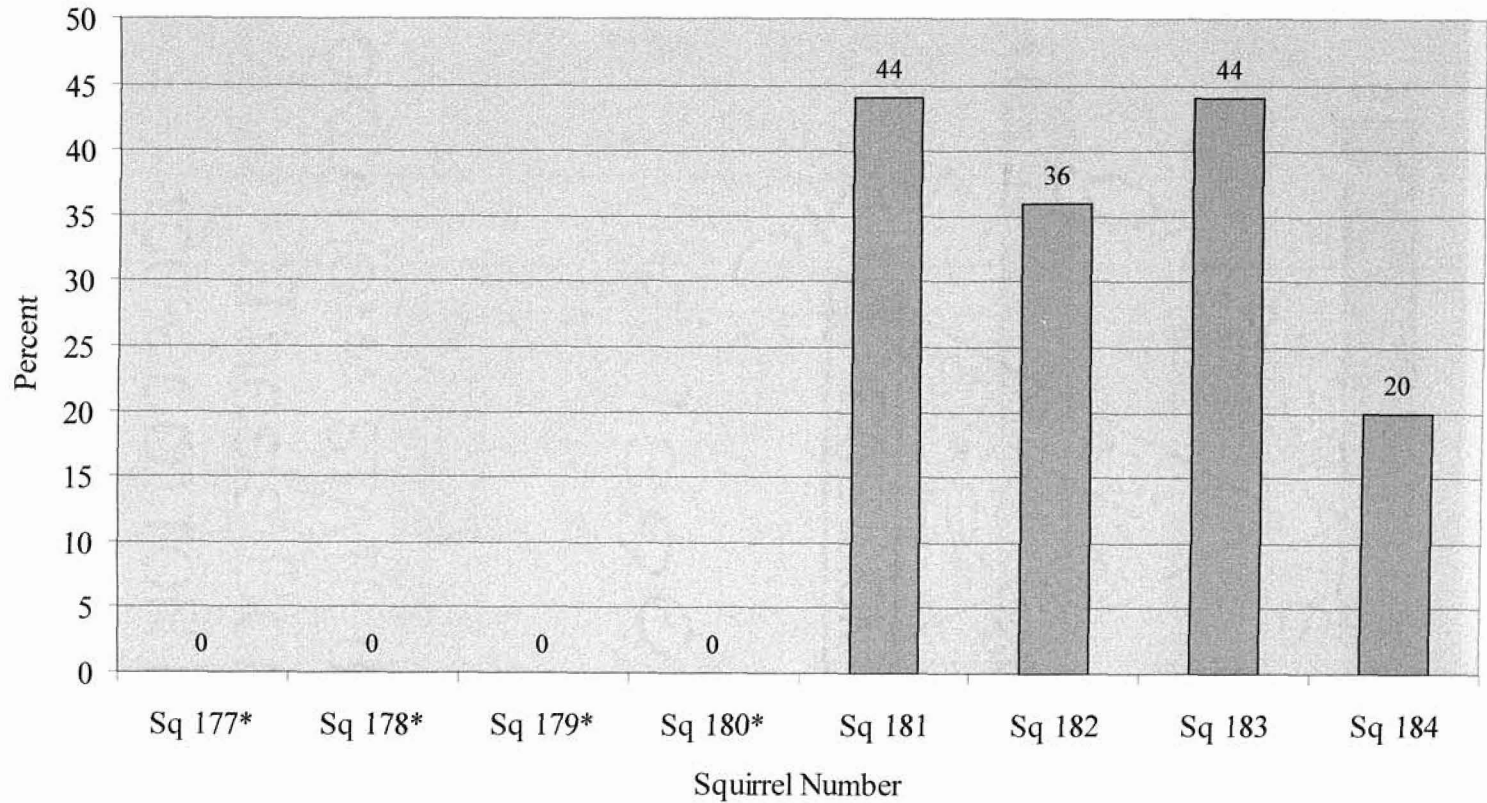
Figure 8. Percent of *Dermacentor variabilis* nymphs that fed to engorgement on fox squirrels after being treated with fipronil three days post-infestation. Treated squirrels are indicated with an asterisk. The percentage of ticks that fed to engorgement was significantly lower for ticks that fed on treated squirrels (P = 0.0019).
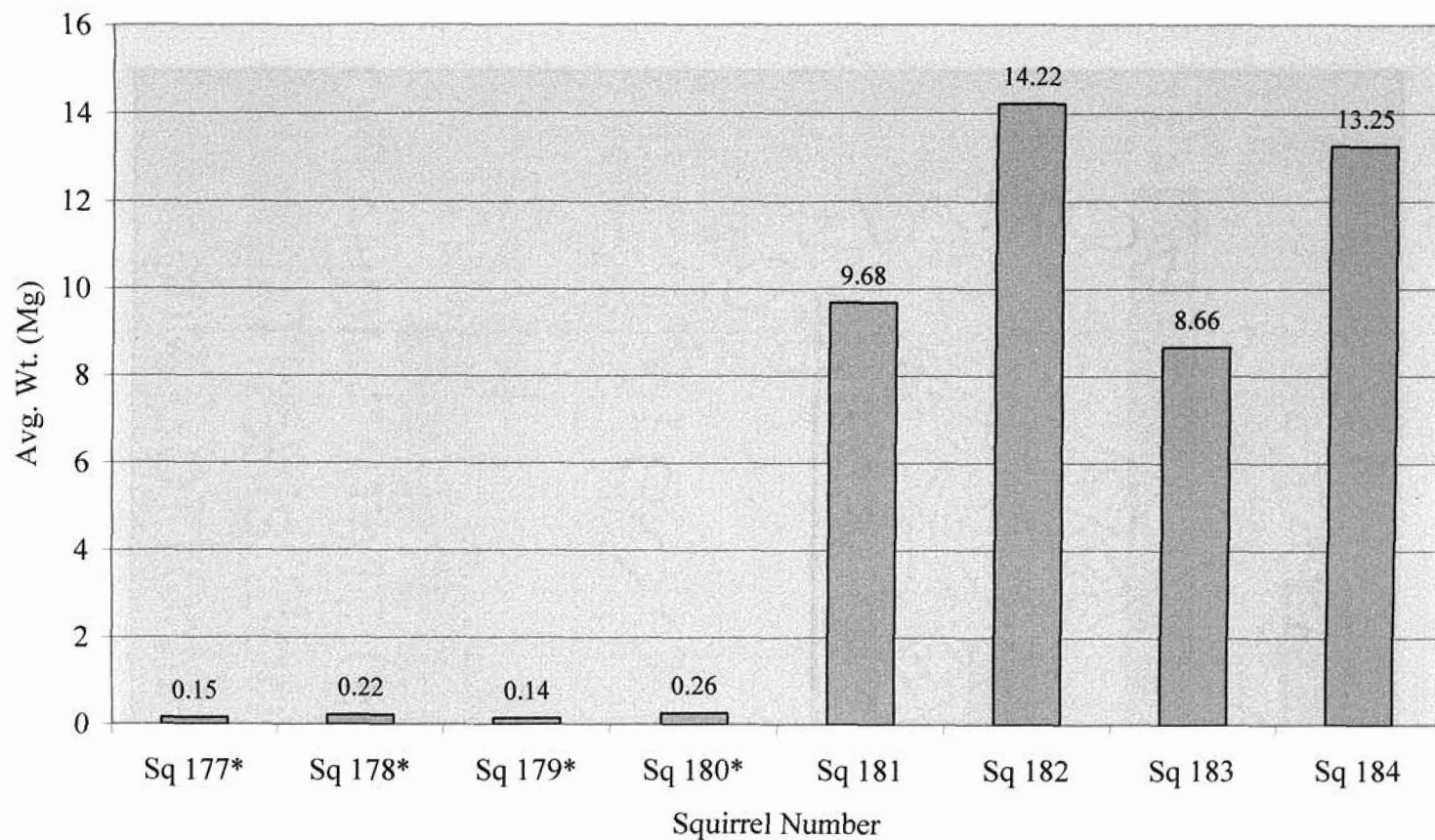
Figure 9. Average weight of engorged *Dermacentor variabilis* nymphs after being treated with fipronil three days post-infestation. The difference in weights between ticks that fed on treated squirrels and those that fed on untreated squirrels was highly significant ($P = 0.0036$). Treated squirrels are indicated with an asterisk.
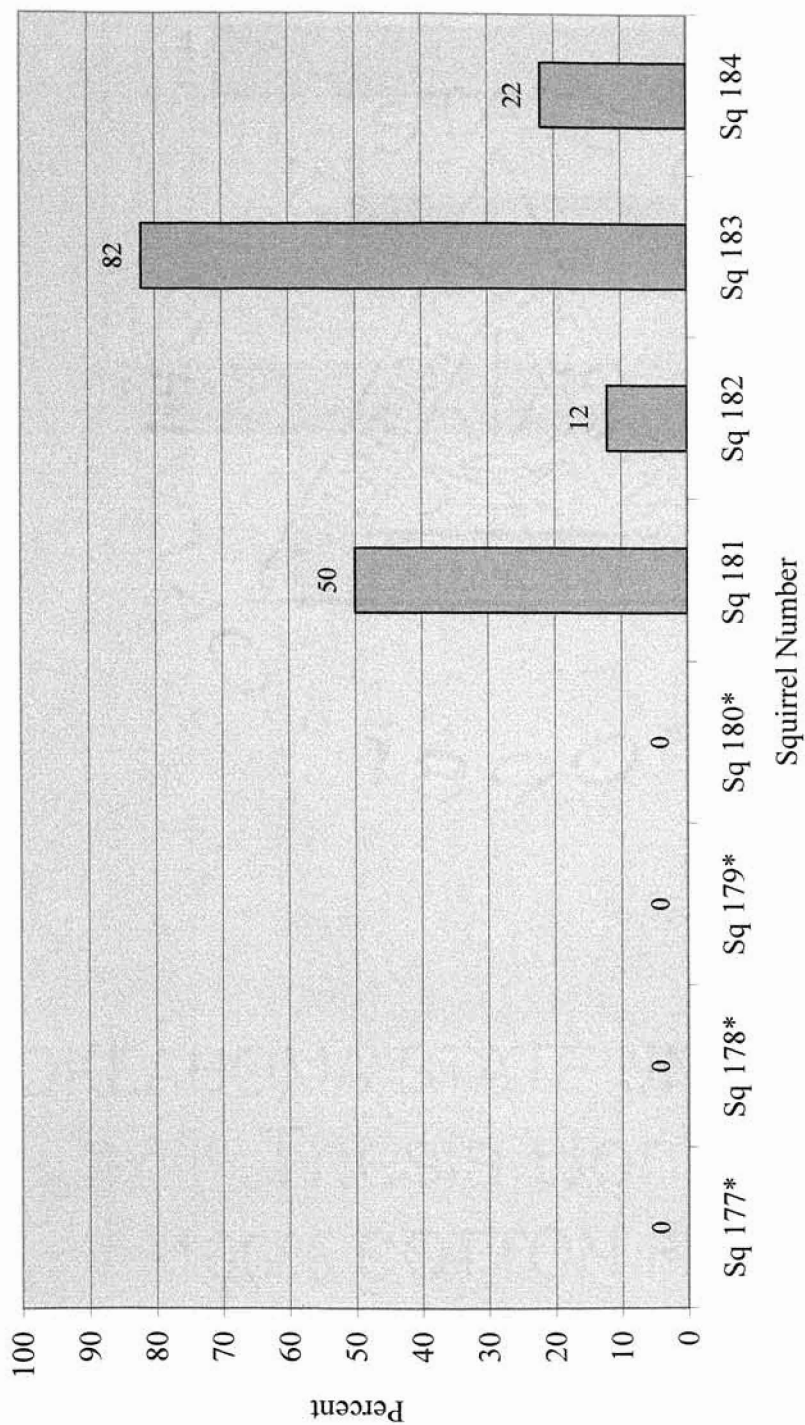
Figure 10. Percent of *Dermacentor variabilis* nymphs that fed to engorgement on fox squirrels 15 days post-treatment with fipronil. Treated squirrels are indicated with an asterisk. The percentage of ticks that fed to engorgement was significantly lower for ticks that fed on treated squirrels (P = 0.0282).
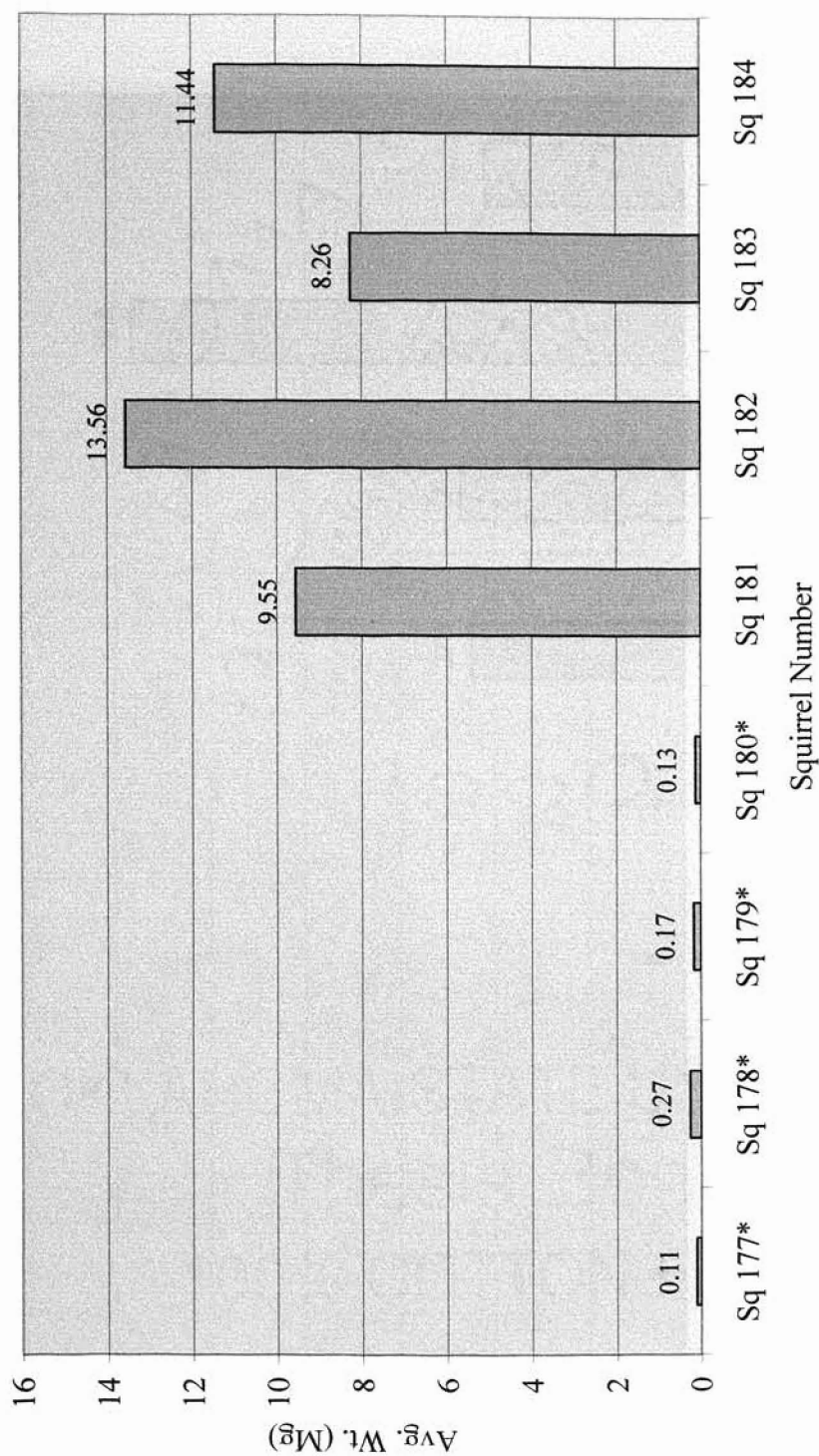
Figure 11. Average weight of engorged *Dermacentor variabilis* nymphs 15 days post-treatment with fipronil. The difference in weight between ticks that fed on treated squirrels and those that fed on untreated squirrels was highly significant (P = 0.0028). Treated squirrels are indicated by an asterisk.

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Figure 12. Percent of *Dermacentor variabilis* nymphs that fed to engorgement on fox squirrels after being treated with fipronil three days post-infestation. Treated squirrels are indicated with an asterisk. The percentage of ticks that fed to engorgement was significantly lower for ticks that fed on treated squirrels (P = 0.0084).

Figure 13. Average weight of engorged *Dermacentor variabilis* nymphs after being treated with fipronil three days post-infestation. The difference in weight between ticks that fed on treated squirrels and those that fed on untreated squirrels was highly significant (P = 0.0047). Treated squirrels are indicated with an asterisk.
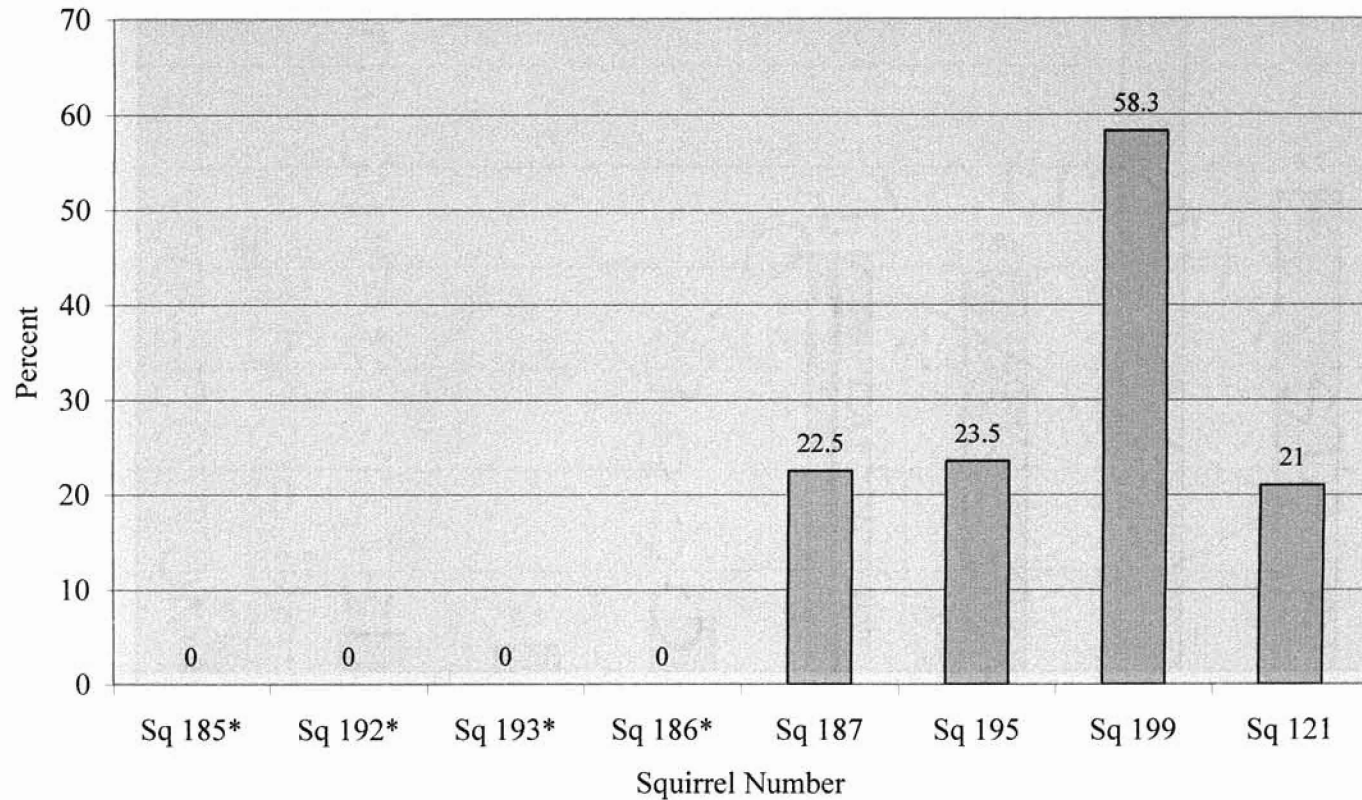
Figure 14. Percent of *Dermacentor variabilis* nymphs that fed to engorgement on fox squirrels 30 days post-treatment with fipronil. Treated squirrels are indicated with an asterisk. The percentage of ticks that fed to engorgement was significantly lower for ticks that fed on treated squirrels (P = 0.0119).
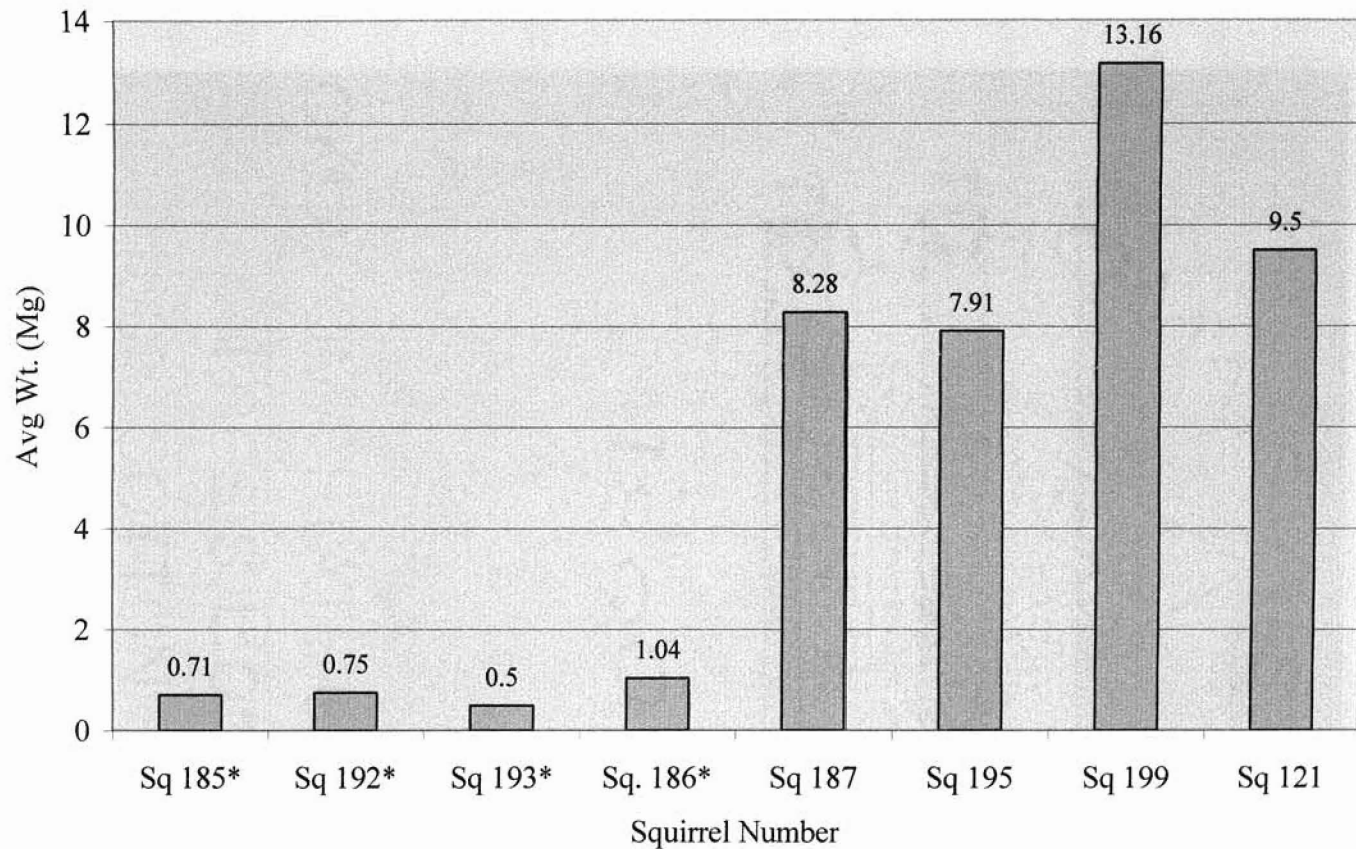
Figure 15. Average weight of engorged *Dermacentor variabilis* nymphs 30 days post-treatment with fipronil. The difference in weight between ticks that fed on treated squirrels and those that fed on untreated squirrels was highly significant (P = 0.0016). Treated squirrels are indicated with an asterisk.
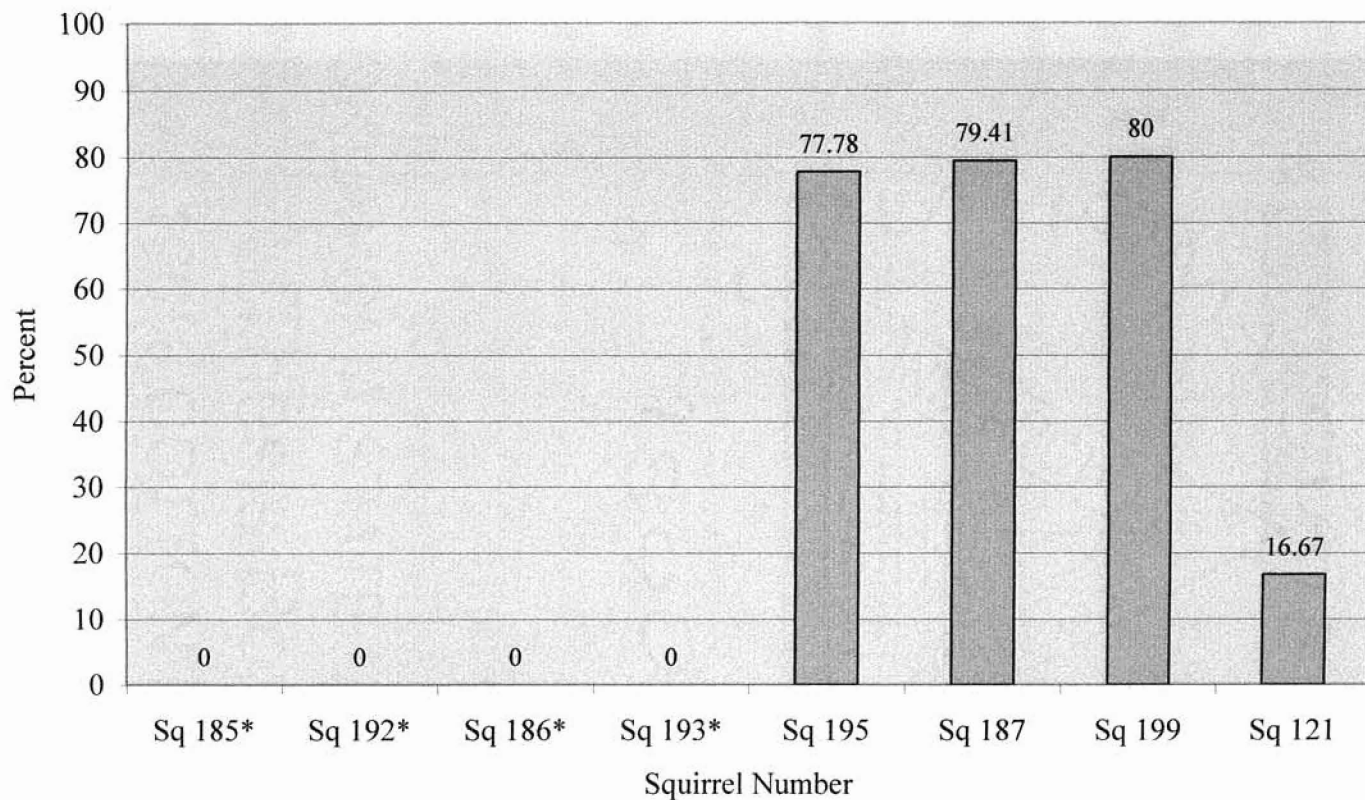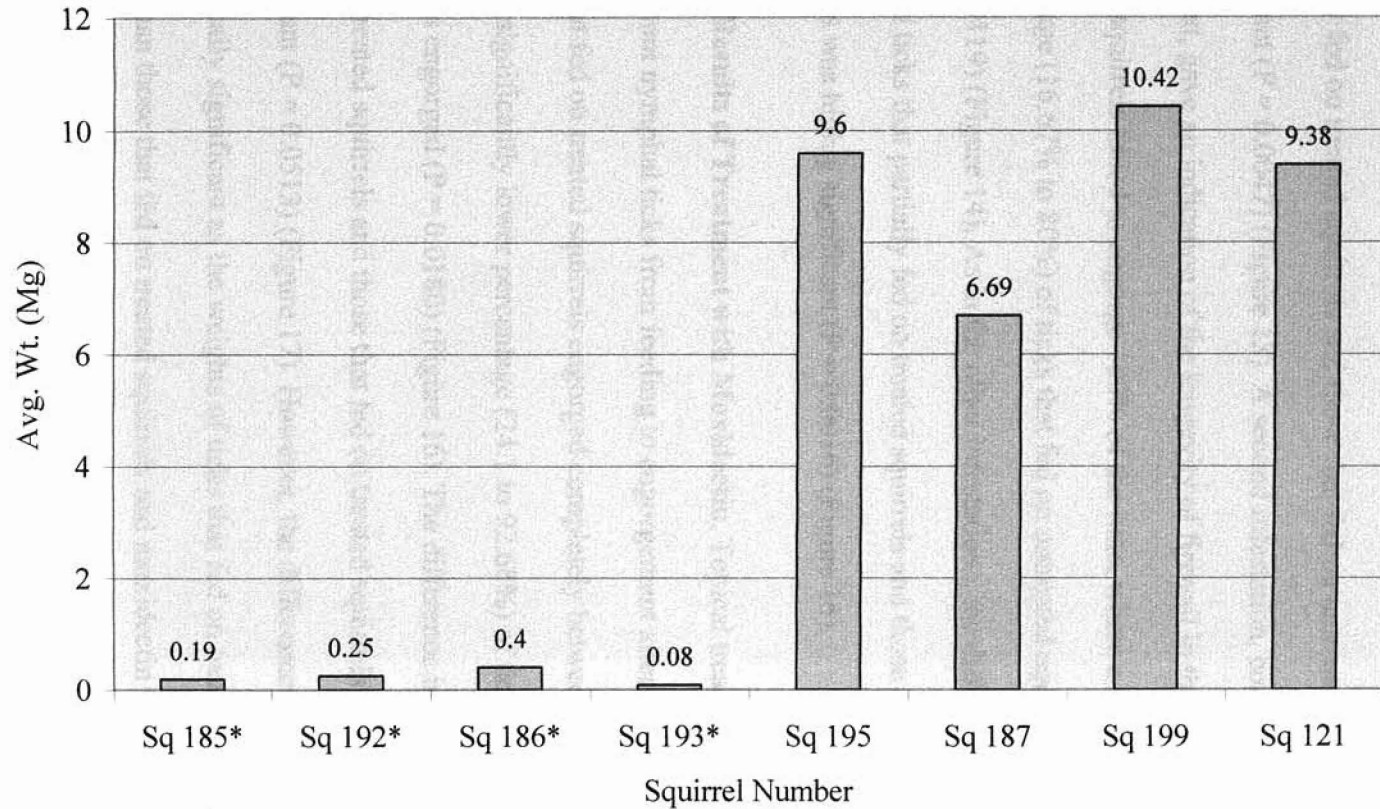
squirrels failed to engorge 100% of the time, while a significantly higher percentage

(21% to 58.3%) of ticks that fed on untreated squirrels fed to engorgement (P = 0.0084)

(Figure 12). Again, in this trial, the difference in engorged weights between ticks that

partially fed on treated squirrels and those that fed on untreated squirrels was highly

significant (P = 0.0047) (Figure 13). A second infestation, conducted 30 days post-

treatment, gave an indication of the longevity of fipronil in the blood. Ticks that fed on

treated squirrels failed to engorge 100% of the time, where a significantly higher

percentage (16.67% to 80%) of ticks that fed on untreated squirrels fed to engorgement

(P = 0.0119) (Figure 14). As in the other infestations, the difference in engorged weights

between ticks that partially fed on treated squirrels and those that fed on untreated

squirrels was highly significant (P = 0.0016) (Figure 15).

Results of Treatment with Moxidectin. Topical treatment with moxidectin did

not prevent nymphal ticks from feeding to engorgement after 15 days. In the initial trial,

ticks that fed on treated squirrels engorged completely between 0 to 22.7 % of the time,

while a significantly lower percentage (24.1 to 92.68%) of ticks that fed on untreated

squirrels engorged (P = 0.0180) (Figure 16). The difference in weights between ticks that

fed on treated squirrels and those that fed on treated squirrels was not statistically

significant (P = 0.0513) (Figure 17). However, the differences in weight were

biologically significant as the weights of ticks that fed on treated squirrels were typically

lower than those that fed on treated squirrels and moxidectin was having some effect. It is

likely that a larger sample size would result in statistical significance being shown.

In the second trial, conducted 15 days post-treatment with moxidectin, ticks that

fed on treated squirrels engorged 7.5 to 50 % of the time while ticks that fed on untreated
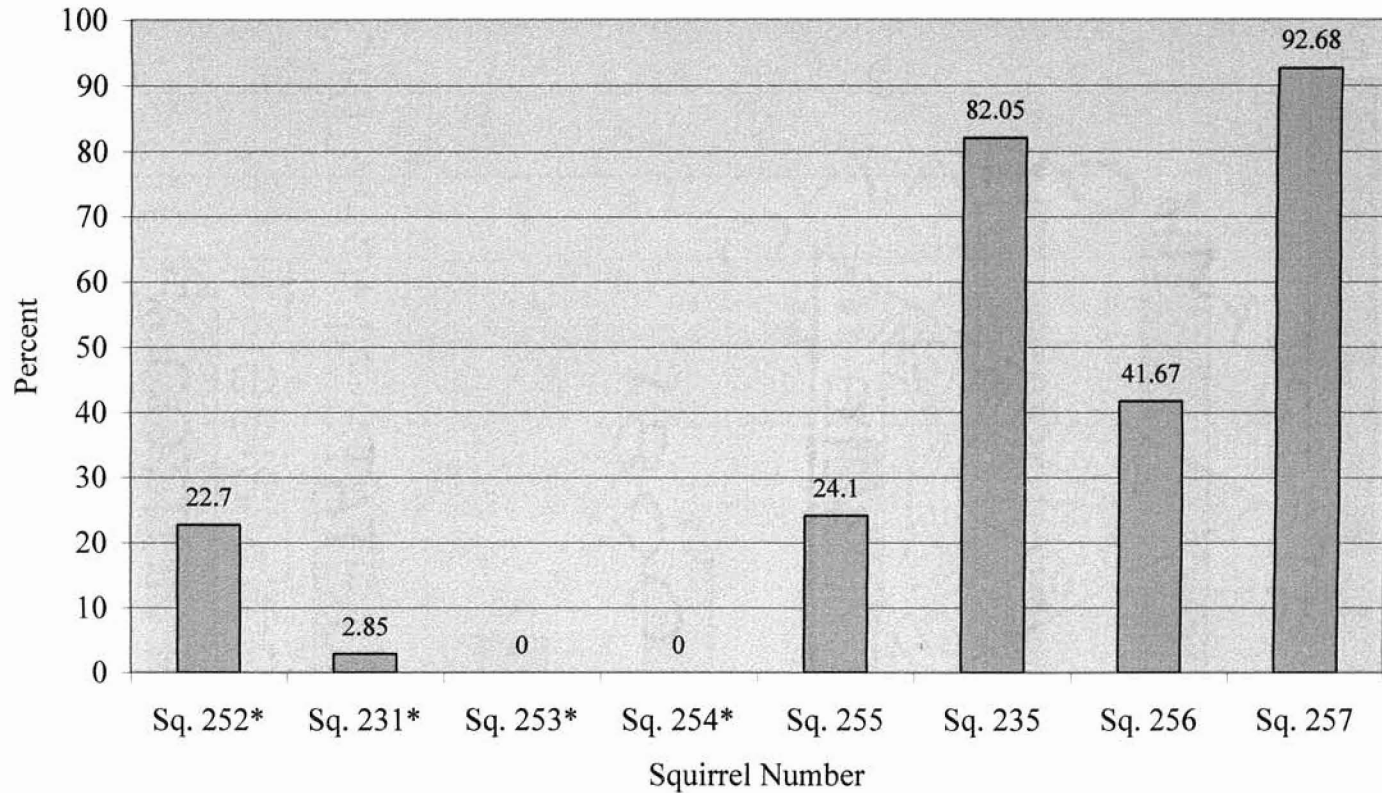
Figure 16. Percent of *Dermacentor variabilis* nymphs feeding to engorgement on fox squirrels after treatment with moxidectin three days post-infestation. Treated squirrels are indicated with an asterisk. The percentage of ticks that fed to engorgement was significantly lower for ticks that fed on untreated squirrels (P = 0.0180).
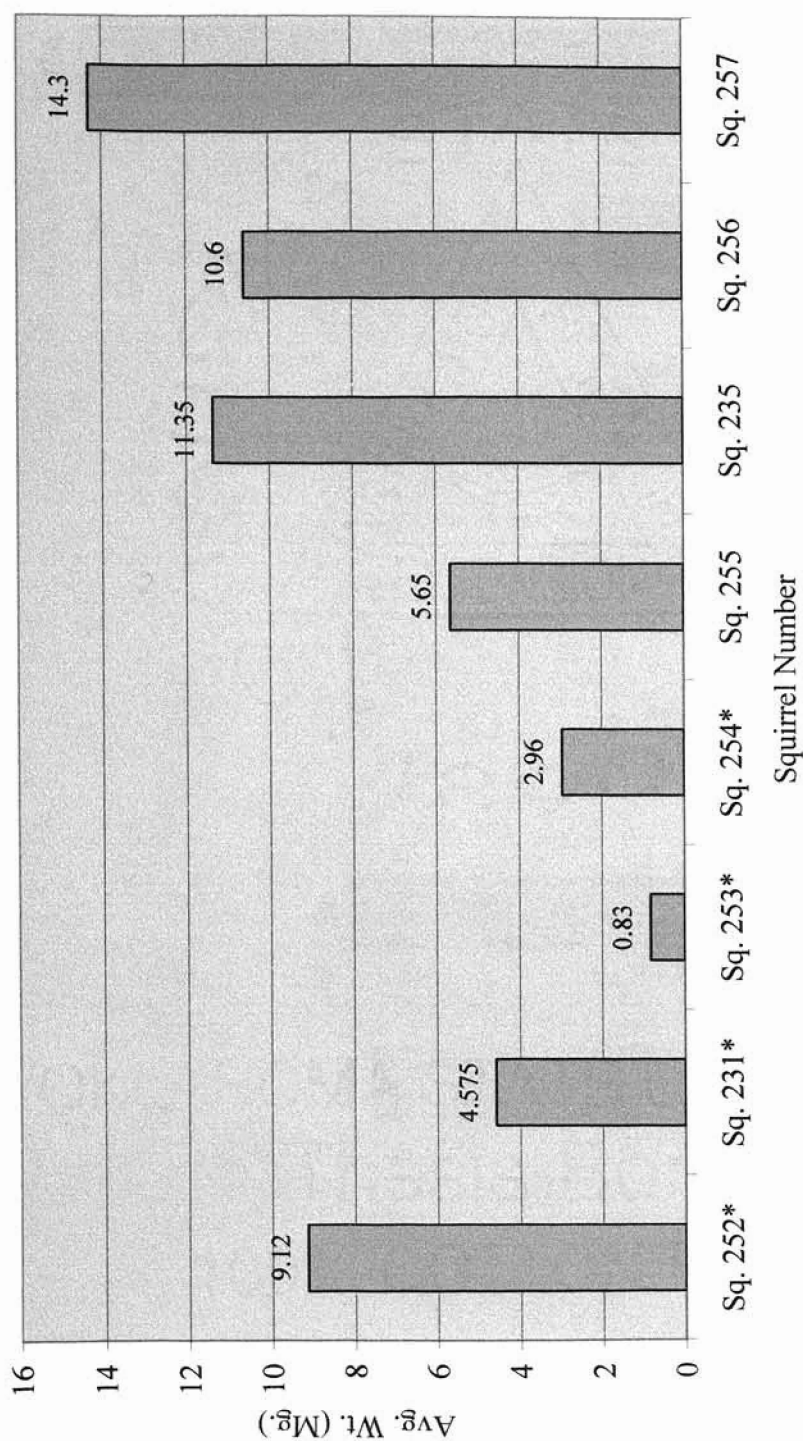
Figure 17. Average weight of engorged *Dermacentor variabilis* nymphs after treatment with moxidectin three days post-infestation. There was not a significant difference between the weights of ticks that fed on treated squirrels and those that fed on untreated squirrels (P = 0.0513), though moxidectin is having some effect. Treated squirrels are indicated with an asterisk.
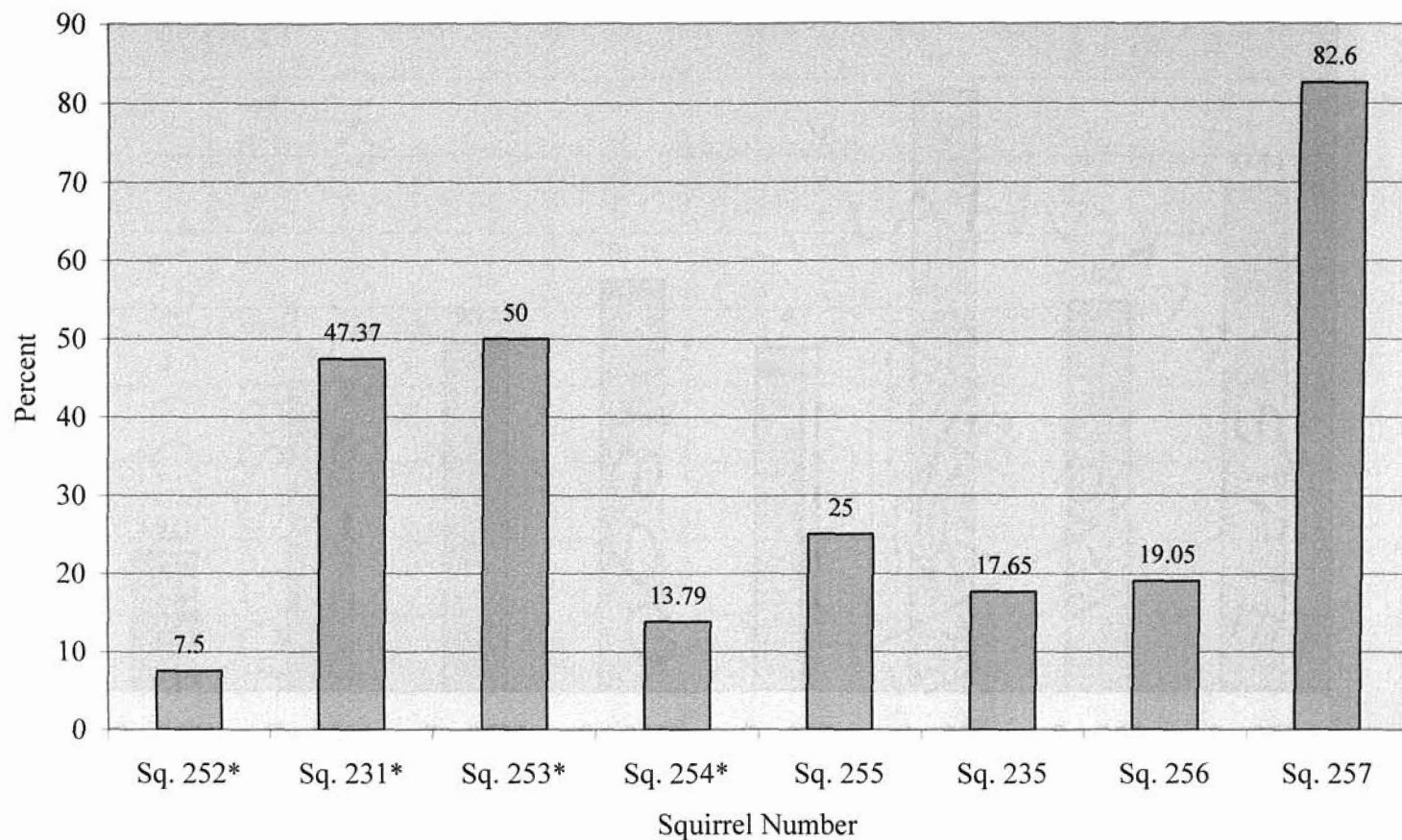
Figure 18. Percent of *Dermacentor variabilis* nymphs that fed to engorgement on fox squirrels 15 days post-treatment with moxidectin. Treated squirrels are indicated with an asterisk. There was not a significant difference in the percentage of ticks that fed to engorgement on treated and untreated squirrels (P = 0.6988).
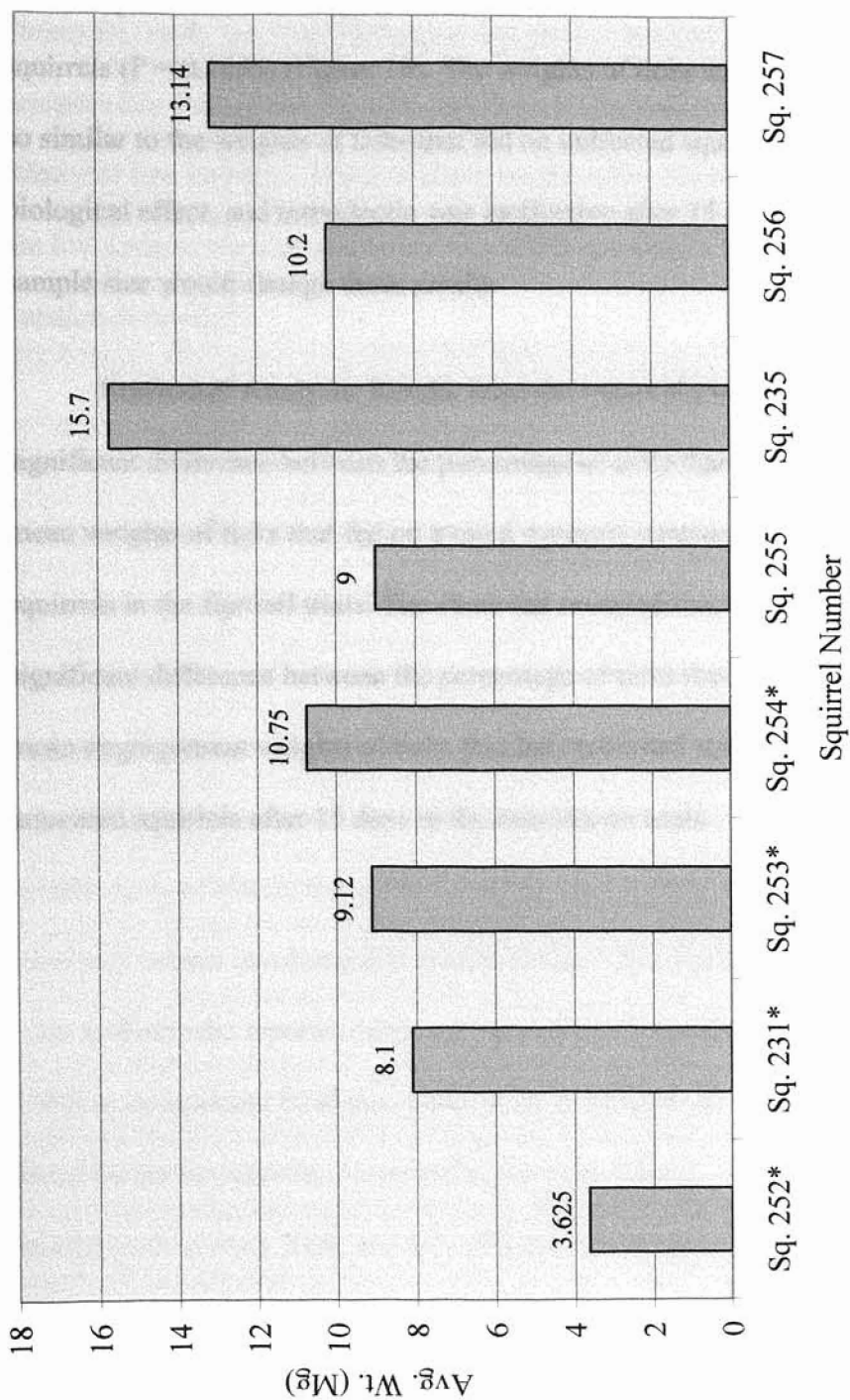
Figure 19. Average weight of engorged *Dermacentor variabilis* nymphs 15 days post-treatment with moxidectin. There was not a significant difference between the weights of ticks that fed on treated squirrels and those that fed on untreated squirrels (P = 0.1036). Treated squirrels are indicated with an asterisk.

squirrels engorged 17.65 to 82.6 % of the time, and these results were not significantly different (P = 0.6988) (Figure 18). The average engorgement weight of ticks that fed on treated squirrels was not significantly lower than that of ticks that fed on untreated squirrels (P = 0.1036) (Figure 19). The weights of ticks that fed on treated squirrels was so similar to the weights of ticks that fed on untreated squirrels that there was no biological effect, and moxidectin was ineffective after 15 days. It is unlikely that a larger sample size would change these results.

Statistical Analysis. Results from the t-tests show that there was a statistically significant difference between the percentage of ticks that fed to engorgement and the mean weights of ticks that fed on treated squirrels compared to ticks that fed on untreated squirrels in the fipronil trials. The same test revealed that there was not a statistically significant difference between the percentage of ticks that fed to engorgement and the mean engorgement weights of ticks that fed on treated squirrels and those that fed on untreated squirrels after 15 days in the moxidectin trials.

Discussion

Overall Survey Results and Seasonality. The variety of ectoparasites recovered during this study is a clear indication that the fox squirrel is a host to numerous types of ectoparasites throughout the year, but these ectoparasites were collected, generally, in relatively low numbers. Many of these ectoparasites are incriminated disease vectors, and the fox squirrel plays an important role in transporting these ectoparasites from one location to another.

Ectoparasite burdens changed with seasons of the year, but not necessarily in the way that was expected. The occurrence patterns of fleas were a good example of this. It is widely accepted that flea populations rise in the winter in nesting hosts, due mainly to the longer hair coat of the host. Squirrels do have a longer coat in the winter than in the summer, and this would lead to the assumption that flea populations would indeed be higher in the winter. This, however, was not the case. *Orchopeas howardii* Baker population peaks were seen in warm months, such as May and September, and in cooler months such as March, but not in winter months (Figure 1). There was no seasonal incidence pattern involving this species of flea. This finding was consistent with that of other authors who reported slight spring peaks in populations of *O. howardii* (Durden 1980) or no seasonal incidence peaks at all (Coyner et al. 1996). The other species of flea found on the fox squirrel, *Nosopsyllus fasciatus* (Bosc), was found only during the warm months such as May, June, and July (Figure 1) and only six specimens were collected from five squirrels. Because no references to the occurrence of this flea on fox squirrels were found in the literature, no generalization could be made about the expected seasonal populations of this species. Additionally, few specimens of this species were collected,

and one cannot speculate on the seasonal incidence of this flea based upon data collected in this study. Many of the squirrels examined in this study were not infested with any fleas.

Just as flea populations are typically at their highest point during the winter months of December, January, and February, lice populations often increase due to thicker hair coats of squirrels. Population peaks of both species of lice collected from the fox squirrel occurred in the colder months of the year (Nov., Dec., Jan.), and this observation was consistent with the literature. Both species of lice collected are known ectoparasites of the fox squirrel (Durden 1980, Coyner et al. 1996). A peak in louse population occurred in June and this observation may be a distortion owing to large numbers of lice being found on a single animal. This animal did not appear healthy, and this likely accounted for the unusually severe infestation. The winter peaks and the presence of lice throughout the year were consistent with the literature (Durden 1980, Nixon et al. 1991). Both species of lice collected exhibited different seasonal incidence patterns. Populations of *Neohaematopinus sciurinus* Mjöberg tended to be constant throughout the year with slight winter peaks, while *Hoplopleura sciuricola* Ferris were found only during the months of August, October, and January (Figure 2). The available literature did not provide insight into the seasonal patterns of the different species on squirrels, but it is conjectured that the longer winter hair coat of the squirrel may play a role in the development of *H. sciuricola* whereas *N. sciurinus* does not require a specific length of the hair coat, or that one species may be more sensitive to hormonal changes in the host occurring as a response to seasonal changes.

The occurrence of parasitic mites on fox squirrels exhibited seasonal activity. Gamasid mites were only collected during the colder part of the year (Figure 3), and it is likely that these mites benefit from the longer hair coat of the squirrel that makes grooming more difficult. Additionally, the largest number of mites collected on a single squirrel occurred on an unhealthy animal that had ceased normal grooming behavior and was spending large amounts of time on the ground. The available literature did not refer to the seasonal incidence of Gamasid mites on fox squirrels, so it is not known if the seasonal pattern revealed in this study is typical.

Ticks were the most diverse group of ectoparasites found on the fox squirrel and also the most seasonally distinct (Table 3). All of the tick species collected have been identified previously on the fox squirrel. The life cycle and seasonal incidence patterns of many species of ticks are well documented. In this study, tick seasonal activity on fox squirrels is consistent with the literature. The present study revealed that *Amblyomma americanum* (L.) larvae fed on fox squirrels between August and October of 2000 and 2001, though few squirrels were infested with the larvae. The larvae of *A. americanum* are known to feed on small mammals, birds, and reptiles, and are active between July and September depending upon habitat type (Semtner and Hair 1973). A longer activity period was observed by Hair and Howell (1970) who reported larval activity between June and November. Larval masses often number thousands of ticks, and it was thus not surprising that large numbers of larvae were collected from individual fox squirrels in August and September. Because high numbers of larvae were present late in the ticks' activity period, it is likely that the fox squirrel contributes to the number of overwintering nymphs, thus playing a role in the number of ticks present the next year. It is unlikely,

however, that the fox squirrel plays a major role overall in the life cycle of larval *A. americanum*, as the squirrels that were infested with the larvae had encountered larval masses, and these squirrels were few in number. Nymphs of *A. americanum* are also known to parasitize small mammals between June and September (Semtner and Hair 1973). This study found that nymphs were active on fox squirrels between April and October, which is similar to the activity pattern reported by Hair and Howell (1970) who observed *A. americanum* nymphs to be active between March and October. The seasonal prevalence of *A. americanum* seen in this study also agrees with a study conducted between June 1993 and July 1996 (Kollars et al. 2000). Their study showed that *A. americanum* nymphs were found on squirrels between March and November, where larvae were present between April and October. The seasonal activity of *A. americanum* was expressed again in a study conducted in Tennessee in 1985 and 1986. *Amblyomma americanum* larvae were collected from gray squirrels between July and September while nymphs were collected between April and June (Zimmerman et al. 1988). Lone star ticks are known to occur in higher number in wooded areas than in open fields, and this makes the fox squirrel an ideal host (Fleetwood et al. 1984, Meyer et al. 1982). Adult *A. americanum* prefer to feed on large mammals such as cattle and deer, and it was thus not surprising that no adult specimens were collected from the fox squirrel.

Another species of tick common on fox squirrels was *Dermacentor variabilis* (Say). This tick is far more particular in host choice as an immature than *A. americanum* in that it feeds almost exclusively on small rodents (Harwood and James 1979). The larvae are active between June and October, and it is uncommon for larvae to survive the winter without finding a host (Samuel et al. 2001). The occurrence of larvae on fox

squirrels in our study was similar to the seasonal pattern presented by Samuel et al. (2001). The majority of larvae were collected in August, September, and October with one specimen collected in February that is inconsistent with the normal activity time. In this study, nymphs were collected between August and November. According to the literature, nymphal *D. variabilis* are active between June and October, and this is consistent with the occurrence seen in this study. Adult *D. variabilis* were collected from fox squirrels between June and August. Zimmerman et al. (1988) demonstrated that adults were active and feeding on gray squirrels between the months of April and June, and this is not consistent with the activity seen in this study, but the slight variation in seasonal activity may be due to habitat. Adults are known to prefer dogs as a host, but will feed on most mammals (Harwood and James 1979). This explains the presence of adults in the ears of fox squirrels between April and August, the peak season of activity for the adults.

A less common, but still important, tick collected from the fox squirrel was *Ixodes scapularis* Say. Only four specimens of this tick were collected, and it was thus difficult to speculate as to the seasonal activity of this tick on fox squirrels. *Ixodes scapularis* was present only as a nymph and only between May and September, but this was only slightly surprising. Adults prefer to feed on larger mammals and are active during the fall, winter, and spring (Kollars et al. 1999). This preference for large mammals excludes the fox squirrel from being a normal host. Immatures parasitize birds, reptiles, and small mammals during the spring and summer with larval population peaks in July and nymphal population peaks in June (Kollars et al. 1999). Thus, it was not surprising to find nymphs feeding on the fox squirrel in May and June, though the absence of larvae was

surprising. For example, cotton rats (*Sigmodon hispidus* Say and Ord) collected from the same habitats during the same time of year were infested with the larvae of *I. scapularis*. Both animals would spend time on the ground in wooded areas, and one would expect a similar parasite load to be present. This was not the only incidence in which the cotton rat and the fox squirrel differed in tick burden. Throughout the survey, *Amblyomma maculatum* Koch was not collected from the fox squirrel in any life stage. Cotton rats collected at the same times of the year and from identical trap sites were infested heavily with larvae and nymphs of this species. A tentative explanation is that *A. maculatum* is most common in open areas and prefers hosts that forage extensively on the ground (Samuel et al. 2001). Fox squirrels do not frequent this habitat type or display this foraging behavior during June and July, when immature *A. maculatum* are most active (Samuel et al. 2001). Cotton rats, on the other hand, move from a wooded area to open areas in search of food and forage exclusively on the ground, thus acquiring immature *A. maculatum,* and then return to the wooded areas for security. The amount of time spent on the ground resulted in consistently higher numbers of ectoparasites being recovered from cotton rats than from fox squirrels. Without exception, squirrels that matched cotton rats in ectoparasite burden were unhealthy animals, often obviously injured and unable to climb trees. These squirrels were forced to spend increased time on the ground and thus acquired similar burdens to the cotton rats. Wounded or unhealthy animals cease normal grooming behavior and typically possess higher numbers of ectoparasites than healthy animals. Future studies directly comparing the ectoparasite burden of cotton rats to that of fox squirrels and evaluating the effects of injury and illness on ectoparasite burden would be interesting and worthwhile.

Only one specimen of *Haemaphysalis leporispalustris* (Packard), a larva, was collected from the fox squirrel, and speculation about seasonal activity is made difficult by the low number of specimens collected. This tick prefers rabbits as hosts, but will attack other small mammals and domestic dogs and cats (Harwood and James 1979). In climates where this tick is prevalent, all life stages can be found throughout the year, and this explains the occurrence of this species on a fox squirrel in November, a month in which larvae of other species are not typically active.

Ectoparasite Burdens in Rural vs. Urban Habitats. Ectoparasite burdens were not significantly higher on squirrels collected in rural areas than on those collected in urban areas, though the ectoparasites burden decreased as the squirrels' habitat became more urbanized. This was not surprising, as many of the factors that contribute to parasitism are more prevalent in rural settings. This includes a greater number of animals to serve as potential hosts, more dense vegetation to prevent the light-induced desiccation of ectoparasites questing for a host, and the lack of efforts to control parasite populations. Ticks, for instance, were far more prevalent on rural squirrels, and this can be directly attributed to the environment in which the squirrels were collected. Rural squirrels have a far greater opportunity to acquire tick infestations than those in urban areas. Many other animals, such as deer, coyotes, opossums, cotton rats, and raccoons share a habitat with squirrels in a rural setting, and each of these do their part to perpetuate the tick population. A squirrel leaving a tree to forage may acquire ticks that have previously fed on any of these animals. An urban squirrel may be the only animal present in some urban neighborhoods, aside from domestic animals that are likely to have been treated with pesticides to keep tick populations down. Squirrels crossing an urban yard may only

acquire ticks if that particular squirrel or another squirrel has deposited ticks there previously. Because of decreased vegetation and nesting sites in many urban areas, the squirrel population may be less when compared with rural populations. For this reason, squirrels in urban areas have less contact with other squirrels and have less opportunity to pass along ectoparasites that rely on physical contact for perpetuation, such as fleas and lice.

These data do not suggest that urban squirrels were free of ectoparasites, as the percentage of squirrels infested with fleas and lice in urban habitats was not significantly different from the percentage of squirrels infested in a rural habitat. Of the squirrels collected from an urban environment, 21.4% were infested with ticks and each was carrying an average of 5.76 ticks. However, when the urban habitat was subdivided, it was clear that squirrels inhabiting less urban areas were infested with a greater number of ectoparasites (Table 5). In areas within the city limits where trees were plentiful and houses were spaced farther apart, squirrels had similar, even greater numbers of ectoparasites than squirrels in rural areas. There was a linear decline in ectoparasite numbers as the habitat became more urban. Very few ticks were seen in the most urbanized areas, and this was likely due to the lack of hosts for the ectoparasites. Squirrel numbers were decreased in these areas due to limited nesting space and minimal sources of winter storable food.

The mean number of ticks recovered from urban squirrels was not significantly different from that of rural squirrels, and when squirrels that were not infested with ectoparasites were excluded, the average number of ticks collected from fox squirrels in rural areas was similar to that of squirrels collected in urban areas. With few other

animals to serve as hosts, fox squirrels were the most likely source of transfer of ticks from one yard to another in urban areas. This was one reason that implementing control methods for the ectoparasites of fox squirrels was a useful endeavor.

Zoonotic Potential of Fox Squirrel Ectoparasites. A second and perhaps more convincing reason to control ectoparasites of fox squirrels was that many have been incriminated as vectors of arthropod-borne diseases. Of the three types of ectoparasites collected (ticks, fleas, and lice), both ticks and fleas can pose a serious risk to both humans and companion animals. Lice are considered to be extremely host specific and thus squirrel lice do not pose a threat.

Fleas are also host specific, but not to the extreme that lice are. While fleas prefer to feed on a specific type of animal, they will feed on any available mammal if it is necessary for survival. And while squirrel fleas may not often leave squirrels except to infest other squirrels, flea eggs deposited in attic nests will produce fleas, and the only available hosts may be humans or companion animals once the squirrels move on. Studies have indicated that the majority of fleas infesting squirrels are female (Amin and Sewell 1977). This increases the possibility that squirrel fleas will become a problem by increasing the reproductive potential. In addition, fleas may remain inactive as pupae for extended periods, and removal of squirrels from an attic may not immediately remove a flea infestation but will remove the primary host of the fleas, forcing them to seek secondary hosts. The two species of fleas collected in this survey, *O. howardii* and *N. fasciatus*, have both been incriminated as vectors of rural plague and allergic reactions, such as flea bite dermatitis, which are common in instances where flea populations are high (Harwood and James 1979).

The risk of acquiring a disease from ticks is much greater. All of the species of ticks collected from fox squirrels have been incriminated as disease vectors, and nearly all of these species were collected in urban settings where transmission to humans and companion animals is more likely. The species of tick responsible for many arthropod-borne diseases, *D. variabilis*, was collected from fox squirrels in every habitat type from the most rural to the most urban. This tick is the incriminated vector of Rocky Mountain spotted fever (*Rickettsia rickettsii*) in Oklahoma as well as tularemia (*Francisella tularensis*), tick paralysis, and cytauxzoon (*Cytauxzoon felis*) in cats (Eldridge and Edman 2000). Each of these diseases is potentially detrimental to humans or companion animals, and each of these diseases could be brought into yards and homes through ticks feeding on fox squirrels.

Another common disease vector, *A. americanum*, was prevalent on fox squirrels in urban areas. While this tick was not present in the most urbanized settings, it was present in both semi-urban areas and urban areas resembling rural habitats. This tick is the incriminated vector of several diseases, including Q fever, tularemia, and ehrlichiosis (*Ehrlichia ewingii* and *E. chaffiensis*) (Eldridge and Edman 2000). These diseases of man and companion animals could easily be brought into urban areas through ticks feeding on fox squirrels.

The two other species of ticks collected from fox squirrels, while fewer in number, have disease transmission potential as well. As the vector of the Lyme spirochete (*Borrellia burgdorferi*) in the eastern and central United States, *I. scapularis* has become the focus of numerous control efforts. This species has also been shown to transmit tularemia under laboratory conditions. While Lyme disease is not a considerable

problem in Oklahoma, preventing the transfer of this tick from rural to urban areas by way of the fox squirrel is important, as Lyme disease is spreading throughout the country, and may become significant in Oklahoma in the future. The least common tick collected from the fox squirrel, *H. leporispalustris*, feeds mainly on rabbits, but can transmit tularemia to man or companion animals (Harwood and James 1979). As each of these tick species are capable of transmitting diseases, developing a method of controlling ticks that feed on fox squirrels is shown to be even more important. If the fox squirrel can be removed from the life cycles of these tick species, the risk of contracting tick-borne disease can be reduced. In addition, the lesser risk of contracting flea-borne disease can be minimized if flea populations can be managed on fox squirrels.

The Effectiveness of Fipronil Treatment. Fipronil was clearly an effective product for the short-term control of ticks on fox squirrels. Not only did topical treatment cause failure of ticks to feed to engorgement, but feeding ticks ceased feeding and detached, consuming a significantly decreased blood meal than ticks feeding on untreated squirrels. Fipronil was very long lasting as a systemic pesticide, giving complete control for at least 30 days. While no other studies have been conducted in which fipronil was applied to fox squirrels, these results were consistent with other studies that have proven fipronil to be effective in controlling ticks on other animals (Miller et al. 2001). No side effects to the fipronil treatment were seen, though the quantity of solution applied to the squirrels seemed to cause annoyance to the squirrels, which shook their heads repeatedly in an effort to dry themselves. The oily texture of the solution also caused the fur to matte, and the animals groomed the treated area more aggressively than other areas.

A disadvantage of fipronil was that a large quantity of fipronil diluted in peanut oil was needed, but the dose could be decreased by increasing the concentration of the fipronil solution. Fipronil was impressive as far as the speed in which it acted. Squirrels that were treated began dropping ticks within twelve hours, and treated squirrels had ceased dropping ticks within four days of treatment. Non-treated squirrels began dropping engorged ticks on day five and continued to do so until day eight. This indicates that ticks feeding on treated squirrels had ample opportunity to feed to engorgement but were unable to do so. In addition, flat ticks that detached from the squirrels before treatment were alive and actively questing. Partially fed ticks detaching following treatment were either dead or not questing. This pesticide deserves additional research and could be suitable for application in a self-treating device if the necessary dosage could be decreased. The longevity of the chemical in the blood of the animal would allow treatments to be spaced apart, both minimizing the amount of fipronil in the environment and insuring that animals were not being over-treated. This study only explored longevity up to 30 days, and a follow up study to evaluate the effectiveness after a longer period would be worthwhile.

The Effectiveness of Moxidectin Treatment. Moxidectin was ineffective as a control method for ectoparasites on fox squirrels. While a lesser percentage of ticks fed to engorgement after feeding on treated squirrels in the initial trial, indicating biological significance, average weights after feeding were not significantly different ($p>.05$), and the treatment was entirely ineffective after 15 days. These results are in contrast to other studies that have shown the effectiveness of moxidectin as an acaricide. Used in an injectable formulation, moxidectin was effective in controlling psoroptic mange (Parker

et al. 1999). While their study was not intended to evaluate the effectiveness of moxidectin against ticks, the acaricidal value of this product was certainly evaluated. A study that can be directly compared involved the topical treatment of cattle with moxidectin for the control of the cattle tick, *Boophilus microplus* (Canestrini). Significantly (p<.05) fewer ticks were collected from treated animals than those that had not been treated. Additionally, this study reported that the engorgement weight of ticks collected from treated animals was lower than that of ticks collected from untreated animals, though significance of the weight data was not provided (Guglielmone et al. 2000). Their results were in stark contrast to the findings of this study and indicated that moxidectin may prevent ticks from attaching to an animal after treatment but may be less effective against ticks that are already feeding. The number of squirrels treated with moxidectin was relatively small, and it is likely that a larger sample size would result in a statistical difference in tick weights after the initial treatment, but it is unlikely that moxidectin would be proven effective after 15 days, even with a larger sample size.

Summary and Conclusions

The results of this study show that the fox squirrel (*Sciurus niger* L.) is the host of numerous types of ectoparasites and that many of these ectoparasites are incriminated disease vectors in other studies. The results also show that, on average, squirrels are infested with relatively low numbers of ectoparasites. In instances where ectoparasite burdens were especially high, the squirrels either appeared to be unhealthy or had recently acquired large numbers of larval ticks though contact with a larval mass. Many of the ectoparasites that were collected, however, did display a seasonal incidence pattern.

In concurrence with published literature, *Orchopeas howardii* Baker was the flea most commonly collected from fox squirrels, and its occurrence reflected no seasonal pattern. In addition, the flea *Nosopsyllus fasciatus* (Bosc) was collected in the summer of 2000, and records of this flea occurring on fox squirrels were not found in the literature reviewed for this study. Two species of lice were collected, *Neohaematopinus sciurinus* Mjöberg and *Hoplopleura sciuricola* Ferris, each displaying individual seasonal patterns not unlike those seen in prior studies. Ticks were the most diverse group of ectoparasites collected from fox squirrels, with four different species being observed. All mobile life stages of *Dermacentor variabilis* (Say) as well as larvae and nymphs of *Amblyomma americanum* (L.) were the most prevalent ticks collected as well as nymphs of *Ixodes scapularis* Say and a larva of *Haemaphysalis leporispalustris* (Packard). Each of these ticks were present in seasonal patterns consistent with the published literature, though it was difficult to speculate on the seasonal incidence of ticks that were collected in low number, such as *H. leporispalustris* and *I. scapularis*.

In addition to showing the diverse seasonal ectoparasite burden of the fox squirrel, this study revealed that a squirrel's habitat directly impacted this burden. Squirrels collected in rural areas and in urban areas that closely resembled rural areas were more heavily burdened with ectoparasites than those from semi urban and urban habitats. Squirrels in urban settings apparently had less opportunity to become infested with ectoparasites, and thus carried a lesser burden. Though no census was carried out during the course of this study, squirrels in urban areas were more likely to be the source of ectoparasites spread from yard to yard because fewer animals appeared to be present to act as hosts. It was interesting to note that when squirrels that were not infested with any species of ectoparasite were excluded from the calculation of average ectoparasite burden, the average number of ectoparasites collected from rural and urban habitats was similar, though the average number still declined as the habitat became more urban. Because of the relatively low numbers of ectoparasites collected and the high proportion of squirrels that carried no ectoparasite burden, it is unlikely that the squirrel is as important a host for ectoparasites as other rodents. This may be because of less time spent on the ground. Many of the ectoparasites collected, however, have been incriminated as disease vectors in other studies, and the fox squirrel did play a role in the transport of these ectoparasites, particularly in urban areas. Therefore, it was worthwhile to investigate means for controlling these ectoparasites.

The second portion of this study was an evaluation of two topical acaricides for use on fox squirrels. Fipronil and moxidectin were evaluated for effectiveness and longevity. Fipronil proved to be a very effective product, controlling 100% of feeding ticks for at least thirty days after treatment. Ticks feeding on fipronil-treated squirrels

took significantly smaller blood meals than those feeding on untreated squirrels, and ticks that fed on treated squirrels failed to feed to engorgement 100% of the time even 30 days post-treatment. Moxidectin was not as effective as a method of control. A lower percentage of ticks that fed on untreated squirrels fed to engorgement than those that fed on untreated squirrels after the initial treatment, implying a biological difference, but there was not a statistically significant difference in engorged weight between ticks from treated and untreated squirrels. Additionally, the percentage of ticks feeding to engorgement was nearly identical for ticks that fed on treated squirrels 15 days post-treatment and those that fed on untreated squirrels. Again, there was no significant difference in engorgement weight between ticks from treated and untreated squirrels.

These data show that fipronil provides effective control of ticks on fox squirrels, and future research applying this substance to squirrels outside a laboratory setting would be worthwhile. Future studies should also evaluate more closely the role of the fox squirrel in transporting ectoparasites from one location to another, compare the ectoparasites burden of fox squirrels with that of cotton rats in the same area, and evaluate the role of sickness in determining the ectoparasites burden of fox squirrels. Additionally, a study conducted to determine the impact of ectoparasites on the general health of fox squirrels would be worthwhile.

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VITA

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