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Original article

Integrated control of juvenile *Ixodes scapularis* parasitizing *Peromyscus leucopus* in residential settings in Connecticut, United States

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ABSTRACT

Lyme disease continues to be the most common vector-borne disease in the United States with an estimated 330,000 human cases annually. In the eastern United States, the blacklegged tick, *Ixodes scapularis*, is the primary vector of the Lyme disease spirochete, *Borrelia burgdorferi*, and the white-footed mouse, *Peromyscus leucopus*, is a primary reservoir host. In four residential neighborhoods in Connecticut over three years, we tested the effectiveness of different low-toxicity integrated tick management approaches to control larval and nymphal *I. scapularis* parasitizing *P. leucopus*. Combinations of white-tailed deer, *Odocoileus virginianus*, reduction, broadcast application of the entomopathogenic fungus *Metarhizium anisopliae*, and distribution of fipronil-based rodent-targeted bait boxes were evaluated against an experimental control. Deer reduction with no other intervention likely forced juvenile *I. scapularis* to obtain blood meals from available reservoir hosts, resulting in increased exposure of *P. leucopus* to *B. burgdorferi* compared to control sites. The *M. anisopliae*/bait box and the deer reduction/*M. anisopliae*/bait box treatment combinations resulted in 94% and 85% reductions in larvae parasitizing *P. leucopus* that tested positive for *B. burgdorferi*, respectively, compared to control. Deer reduction alone resulted in only a 3% reduction, likely because parasitizing juvenile *I. scapularis* were not targeted by bait box-delivered fipronil. Unless there is community support to reduce and maintain deer at very low densities (< 5 deer/km^2), it is clear that a combination of *M. anisopliae*/fipronil-based bait boxes offers an effective, localized, low-toxicity option for reducing *I. scapularis* parasitizing *P. leucopus* without complications from host switching.

1. Introduction

Lyme disease is the most common vector-borne disease in the United States with 275,589 cases reported to Centers for Disease Control and Prevention between 2008 and 2015 (Schwartz et al., 2017). *Borrelia burgdorferi*, the major etiological agent of Lyme disease in the Northeast, is transmitted to humans through the bite of infected blacklegged ticks, *Ixodes scapularis* (Burgdorfer et al., 1982; Barbour and Fish, 1993; Eisen and Eisen, 2016). Other *I. scapularis*-borne pathogens such as *Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia miyamotoi*, and Powassan virus are known to cause anaplasmosis, babesiosis, hard tick-borne relapsing fever, and Powassan encephalitis, respectively. The ongoing emergence of these diseases underscores the urgent need for innovative and more effective tick management interventions.

Although *I. scapularis* are able to feed on numerous hosts of varying size (Piesman and Spielman, 1979), those most often associated with larval and nymphal stages are small mammals, most notably white-footed mice, *Peromyscus leucopus* (Spielman et al., 1984). As adults, their preferred hosts are white-tailed deer, *Odocoileus virginianus* (Wilson et al., 1988). White-footed mice are reservoir hosts for *Borrelia burgdorferi* sensu stricto, *A. phagocytophilum*, and *B. microti* (Stafford et al., 1999; Levin et al., 2002; Bunikis et al., 2004). In contrast, white-tailed deer are reservoir incompetent for these pathogens, but are of vital importance as a reproductive host for adult *I. scapularis*.

Risk of acquiring a tick-borne pathogen may be greatest on residential properties (i.e. peridomestic; Falco and Fish, 1988; Stafford et al., 2017; Mead et al., 2018) and therefore, managing risk in such settings is of vital importance. Personal protection and tick checks are only effective if conducted daily but vigilance may wane over time (Gould et al., 2008). As alternatives to personal protection, homeowners may use a variety of management approaches with the objective of reducing their contact with infected ticks (Stafford, 2007; Stafford et al., 2017). These approaches can be further categorized into

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landscape management and direct targeting of host-seeking ticks, reservoir hosts (i.e. rodents), and reproductive hosts (i.e. deer).

Acaricides have been a major tool in tick management and their broadcast application is effective for short-term control in localized areas (Ginsberg and Stafford, 2005). While barrier acaricide application can be effective in reducing *I. scapularis* abundances, it does not necessarily translate into reduction in human-tick encounters or tick-borne disease incidence (Hinckley et al., 2016). In residential backyard settings it is unlikely that the entire area would be treated and residents may acquire ticks in untreated areas (e.g., vegetable gardens, ornamental shrub borders, woodlots; Hinckley et al., 2016). Acaricide alternatives such as host-targeted approaches and use of broadcast applications of biological agents are attractive because they minimize the amount of chemical needed to control ticks while at the same time potentially impacting a larger area compared to a barrier spray.

Due to the complex ecology of ticks and tick-borne diseases, single intervention strategies are limited in their time to effectiveness, duration, or efficacy. Integrating treatments that target different life stages and aspects of tick-borne disease ecology (e.g., vector, reservoir hosts, and reproductive hosts) may overcome the shortcomings of single interventions. Integrative treatments that are effective with low toxicity typically have the most public acceptance. *Metarhizium anisopliae* (Met52; Novozymes Biologicals, Inc., Salem, VA) is a naturally occurring, entomopathogenic fungus pathogenic to *I. scapularis* that offers an environmentally acceptable alternative to synthetic acaricides. In field settings, Met52 was effective at reducing nymphal tick abundance, specifically when applied during peak seasonal activity (Bharadwaj and Stafford, 2010; Stafford and Allan, 2010).

Fipronil-based rodent bait boxes (Select TCS™, Tick Box Technology Corporation, Norwalk, CT) target juvenile *I. scapularis* parasitizing small rodents, particularly *P. leucopus*. The first field trial of a fipronil-based bait box prototype resulted in a reduction in the numbers of host-seeking nymphal *I. scapularis* by > 50% and the number of questing nymphs infected with *B. burgdorferi* by 67% (Dolan et al., 2004). Deployment of the now commercially-available bait box, fitted with a protective metal shroud to alleviate eastern grey squirrel, *Sciurus carolinensis*, damage, resulted in signifi cant declines of *I. scapularis* parasitizing *P. leucopus* by 88% in the first year and 97% in the second year (Schulze et al., 2017).

Reducing deer to 5.1 deer/km^2 dramatically reduced *I. scapularis* abundances and Lyme disease cases on a Connecticut peninsula (Kilpatrick et al., 2014). However, deer reduction has not yet been shown to be effective for *I. scapularis* control in settings of a non-insular nature (Kugeler et al., 2016). It also is important to note that deer-targeted treatments have lagged effects because of long generation times and assessment of their efficacy must take place over multiple years (Brei et al., 2009).

We previously reported that ITM approaches incorporating differing combinations of deer reduction, fipronil-based rodent bait boxes, and barrier applications of Met52 led to significant reductions in questing nymphal *I. scapularis* and *B. burgdorferi*-infected questing nymphal *I. scapularis* over three years (Williams et al., 2018). The present study documents the impacts of the same ITM approaches on juvenile *I. scapularis* parasitizing *P. leucopus* and *P. leucopus* serologically positive for

2. Materials and methods

2.1. Study area

This study was conducted from January 2013 through September 2015, in the town of Redding, Connecticut, USA (41.3044°N, 73.3928°W). Redding encompasses 83 km^2 in Fairfield County in southwestern CT where Lyme disease has become endemic. From 2009 through 2015, 8–18 human cases of Lyme disease (87–197 cases/100,000 population) were reported annually in Redding (Connecticut Department of Health, 2018).

Cooperating residential properties were distributed within four targeted 2.6 km^2 study neighborhoods. The four neighborhoods were selected based on uniformity in size and landscape characteristics. Residential properties within target neighborhoods were selected based on the presence of woodland-lawn edge of at least 100 m, as well as accessibility and homeowner participation. Average property size was approximately 2.0 ha with similar vegetative characteristics at each residence; ornamental shrubs around the house with a lawn area transitioning to open deciduous woodlands with little understory present. Selected properties were included in the study based on homeowners' permission to access their land. While a few properties had shared boundaries, the majority were not contiguous to one another. Cooperating properties received differing combinations of a broadcast spray of Met52 (*M. anisopliae*), white-tailed deer removal, or distribution of fipronil-based rodent bait boxes.

2.2. Treatment assignments

The four neighborhoods were randomly assigned treatment designations. In 2013, there were 21 cooperating residential properties: 6 within the control group, 6 within the rodent fipronil bait box and Met52 treatment combination, 5 within the deer removal treatment, and 4 within the deer removal, rodent fipronil bait box, and Met52 treatment combination. In 2014 and 2015, 17 properties were added to the control and treatment groups to increase sample sizes. For this interval, there were a total of 12 within the control treatment, 13 within the bait box/Met52 treatment, 8 within the deer removal treatment, and 5 within the deer removal/bait box/Met52 treatment (n = 38).

2.3. Met52 application

The active ingredient in Met52, *M. anisopliae*, is a naturally-occurring soil-borne fungus that causes green muscardine disease in insects and has been shown to be pathogenic to *I. scapularis* nymphs and adults in the laboratory (Zhioua et al., 1997; Benjamin et al., 2002; Kirkland et al., 2004; Bharadwaj and Stafford, 2012) and in the field (Bharadwaj and Stafford, 2010). The deer removal/bait box/Met52 and bait box/Met52 properties received broadcast applications of Met52, containing 11% w/w of *M. anisopliae* or 5.5×10^9 colony forming units (CFU)/gram of product. The Met52 was applied at a rate of 0.63-0.96 ml/m^2, twice per season in early June and July of 2013, 2014, and 2015 or as

pularis parasitizing *P. leucopus* and *P. leucopus* serologically positive for antibodies to *B. burgdorferi*. Our aim with the treatment combinations was to target *I. scapularis* at multiple life stages, impact *I. scapularis* both on and off hosts, and disrupt *B. burgdorferi* transmission using least-toxic treatment measures in a residential setting. We predicted the greatest reductions in both parasitizing juvenile *I. scapularis* and *P. leucopus* serologically positive for *B. burgdorferi* antibodies would occur in residences receiving all three ITM treatments in combination.

twice per season in early June and July of 2013, 2014, and 2015 or as dry weather conditions dictated. Each property was sprayed by a licensed applicator (Connecticut Tick Control, Norwalk, CT) within an area encompassing 3 m on either side of the woodland-lawn transect. Unfortunately, due to nymphal peak activity occurring in mid-May when night temperatures are too cold to humanely capture and process *P. leucopus* in Connecticut, we have no pre-Met52 application data to document effectiveness on parasitizing nymphal *I. scapularis* ticks after Met52 spraying. However, use of Met52 in an ITM approach was effective on questing nymphs at the same treatment locations within each year (Williams et al., 2018). Our expectation was that Met52 treatment would result in a reduction in nymphal attachment on *P. leucopus* within weeks of application.

2.4. Deer removal

Deer were removed by professional sharpshooters (White Buffalo, Inc., Moodus, CT, USA) throughout the deer removal and the deer removal/bait box/Met52 treatments as regulated hunting as a management tool in non-insular suburban settings has yet to achieve area-wide densities much below 17 deer/km^2 (Williams et al., 2013). Deer were concentrated with bait on cooperating homeowners' properties and euthanized with a single 0.223 caliber bullet to the center of the brain, consistent with American Veterinary Medical Association and Sikes and Gannon (2011) standards for humane euthanasia and in compliance with The Connecticut Agricultural Experiment Station's Institutional Animal Care and Use Committee (#P18-13) and Connecticut Department of Energy and Environmental Protection's Volunteer Authorization (#1315006b). All venison was donated through local charities (WhiteTail Solutions, LLC, Town of Redding Food Pantry, Bridgeport Rescue Mission). Target deer densities sought for *I. scapularis* reduction were below 4.0 deer/km^2 on each of the two research areas. Deer were removed from January-March of each year, largely after the end of the regulated hunting season. Deer densities were determined in each year by aerial counts over snow (see Williams et al., 2018). Our expectation was that deer removal would result in a reduction in juvenile *I. scapularis* attachment on *P. leucopus* over the course of the following year (for larvae) or two years (for nymphs).

2.5. Rodent-targeted fipronil bait boxes

The fipronil-based rodent bait boxes (Select TCS™) targeted juvenile *I. scapularis* parasitizing small rodents. Bait boxes were distributed on plots approximately every 10 m along property perimeters, several meters in from where the lawn ended and wooded habitat began, where the majority of *I. scapularis* are found in residential areas (Stafford and Magnarelli, 1993). In 2013 at the bait box/Met52 treatment properties, 65 bait boxes were placed at the 6 properties and 47 were distributed at the 4 deer removal/bait box/Met52 properties in May. All bait boxes were replaced with new boxes at the end of June/beginning of July at each location. Boxes were removed completely and inspected for usage and bait consumption in early fall 2013. In both 2014 and 2015, 152 bait boxes were placed at the 13 bait box/Met52 properties and 61 at the 5 deer removal/bait box/Met52 properties initially in May of each year. Boxes also were replaced midseason and were retrieved in early fall. All rodent bait boxes showed signs of heavy usage for all years of the study. Our expectation was that the bait box treatment would result in a reduction in juvenile *I. scapularis* attachment on *P. leucopus* within a few days post-deployment.

date. *Peromyscus leucopus* abundance was calculated as the total number of captures divided by the number of trap nights for each treatment for each year in order to make comparisons between treatments and years with varying sampling effort.

Sedated *P. leucopus* were returned to Sherman traps and allowed to recover from the effects of isoflurane. Once alert, they were released to the property and trap location from which they were originally captured. *Peromyscus leucopus* capture and handling protocols were approved by the Wildlife Division of the Connecticut Department of Energy and Environmental Protection (#1315006b) and The Connecticut Agricultural Experiment Station's Institutional Animal Care and Use Committee (P18-13) in accordance with the American Society of Mammologist's guidelines for the use of wild animals in research (Sikes and Gannon, 2011).

2.7. Peromyscus leucopus exposure to Borrelia burgdorferi sensu stricto

Antibody response to *B. burgdorferi* sensu stricto in *P. leucopus* sera was determined using an enzyme-linked immunosorbent assay as previously described (Magnarelli et al., 1991, 1997, 2006). Positive cutoff values were derived from the mean plus three standard deviations of net absorbance values of sera from 13 *B. burgdorferi*-naïve laboratory-reared *P. leucopus*. Readings for *P. leucopus* sera were deemed positive if net optical densities exceeded 0.18, 0.15, and 0.11 for the respective serum dilutions 1:160, 1:320, and > 1:640. *Borrelia burgdorferi* infection in *P. leucopus* was calculated as the number of *B. burgdorferi*-positive *P. leucopus* captures ($\geq$1:160) divided by the number of trap nights for each treatment for each year to standardize values.

2.8. Parasitizing juvenile Ixodes scapularis and Borrelia burgdorferi infection

The number of juvenile *I. scapularis* parasitizing captured *P. leucopus* was recorded but life stages not differentiated. Engorged or partially engorged juvenile *I. scapularis* were removed from *P. leucopus*, transported to the laboratory, life stage determined, and larvae were tested for pathogen uptake using polymerase chain reaction as described previously (Williams et al., 2018). Engorged and partially engorged larvae were tested for *B. burgdorferi* from 2013 to 2015 and those from 2014 and 2015 were also tested for *Anaplasma phagocytophilum* and *Babesia microti*. Additionally, the mean number of larvae that tested positive for *B. burgdorferi* was determined for *P. leucopus* captured in August of all three years, when larval activity is at its peak in Connecticut (Stafford, 2007).

2.6. Peromyscus leucopus capture

Peromyscus leucopus were trapped on all participating properties on multiple occasions using Sherman live traps (LFAHD folding trap, H. B. Sherman Traps, Inc., Tallahassee, FL, USA) from June to August in all years. From 7–10 Sherman traps were placed at approximately 15-m spacing in the vicinity of the fipronil boxes and Met52 treated areas where applicable, baited with peanut butter in the afternoon, and checked the following morning. Each round of trapping for all treatment locations was conducted within the same week.

Captured *P. leucopus* were temporarily sedated using the inhalant anesthetic isoflurane (Piramal Critical Care, Inc., Bethlehem, PA, USA). They were then fitted with a uniquely-numbered ear tag (National Band and Tag Co., Newport, KY, USA) and blood-sampled via cardiac puncture (100–150 µl). Several weeks elapsed between each trapping round, permitting us to document ear tag number and then sample recaptured mice in the same manner. Blood samples were transported from field sites to the laboratory with freezer packs in an insulated container. Later the same day, blood samples were centrifuged to separate serum from whole blood. Sera were stored at −80° C for analysis at a later

2.9. Statistical analyses

To determine differences in the number of *P. leucopus* captures and the number of *P. leucopus* captures that were positive for *B. burgdorferi* antibodies between treatments, we determined the total number of each from each property for each year. To standardize those data and compare among treatments with differing trapping effort, we divided those totals by the number of trap nights and multiplied by 100 (for data presentation purposes only) at each location for each year. Total number of *P. leucopus* and *B. burgdorferi*-infected *P. leucopus* captures/trap night/property/year were then subjected to two-way analysis of variance (ANOVA) with year and treatment as factors. Tukey's Honest Significant Difference (HSD) was used to check for differences in multiple comparisons between means. While we realize repeated measures ANOVA would have been more appropriate, the fact that there were an unequal number of properties included in Year 1 as compared to Years 2–3 would have omitted data from 17 properties from the 2014 and 2015 datasets which would have substantially reduced robustness.

Due to an overabundance of zero values, data from the number of parasitizing juvenile *I. scapularis*/captured *P. leucopus* could not be successfully normalized using standard transformations. As a result, the

average number of parasitizing *I. scapularis*/captured *P. leucopus* was determined for each treatment over each of the three years. These average data were then subjected to one-way repeated measures ANOVA with year as the subject and treatment as the factor. Tukey HSD was used to determine differences in multiple comparisons between means.

Due to small sample size of larvae taken from *P. leucopus* in the bait box/Met52 treatment area, likely due to treatment effectiveness, we were forced to sum all *B. burgdorferi*-infected and uninfected parasitizing engorged larvae data for each treatment area and for mean infected engorged larvae/captured *P. leucopus* in August for all three years. We then used Pearson's chi-square test with Yate's correction for continuity for small sample sizes to determine multiple pairwise differences in treatment combinations for *B. burgdorferi*-infection in engorged larvae from *P. leucopus* by treatment and mean number of infected larvae/*P. leucopus* from August 2013, 2014, and 2015 combined. Resulting data were deemed significantly different at $P < 0.05$. All statistical analyses were done in SigmaPlot (Version 13.0, Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Treatments

Bait box distribution, Met52 application, and deer removals were properly executed in all three years. In all, 87 deer were removed; 31, 11, and 6 from the deer removal only treatment and 20, 14, and 5 from the deer removal/bait box/Met52 treatment area in 2013, 2014, and 2015 respectively. Due to hunter interference and resulting safety concerns, we were forced to suspend deer removal efforts prematurely and did not achieve target densities. More detailed information on deer removals, density estimation, and impacts from hunter interference can be found in Williams et al. (2018).

3.2. Peromyscus leucopus capture

There were a total of 4592 trap nights for the three years; the 21

($F_{3,85} = 10.04$, $P < 0.001$) there were no differences between years ($F_{2,85} = 0.67$, $P = 0.51$) or the treatment by year interaction ($F_{6,85} = 1.02$, $P = 0.42$). No differences existed among treatments in 2013, but in both 2014 and 2015, there were significantly more *B. burgdorferi*-infected *P. leucopus*/trap night in the deer removal only treatment compared to the other three among which no differences were detected (Fig. 2). There was also a higher overall percent infection in *P. leucopus* in the deer removal only treatment (150 positive of 211 sera, 71%) compared to control (104 of 244, 43%), bait box/Met52 (96 of 202, 48%), and deer removal/bait box/Met52 treatment (55 of 106, 52%) over the three years (Fig. 2).

3.4. Juvenile Ixodes scapularis parasitizing Peromyscus leucopus

A total of 1250 juvenile *I. scapularis* were documented parasitizing *P. leucopus* across all treatments. More than half (55%) of *P. leucopus* captures (n = 428) had no parasitizing *I. scapularis*, 26% had either one (n = 121) or two (n = 81). Very few *P. leucopus* had high burdens, 29 (3.7%) had ≥ 10 *I. scapularis* recorded. Significant differences in juvenile *I. scapularis*/captured *P. leucopus* occurred between treatments over the three years ($F_{3,6} = 11.65$, $P < 0.01$). *Peromyscus leucopus* captured from the deer removal/bait box/Met52 treatment area had significantly fewer parasitizing juvenile *I. scapularis* than did *P. leucopus* from the control area (Fig. 3). Additionally, *P. leucopus* captured from the bait box/Met52 treatment area had significantly fewer parasitizing juvenile *I. scapularis* than did *P. leucopus* captured from both the control and deer removal only areas (Fig. 3).

While the majority of engorged or partially engorged juvenile *I. scapularis* were larvae (74%), we confirmed 85 engorged or partially engorged nymphs over the three years. Of these, 74% were sampled from *P. leucopus* within control (n = 21) and deer removal (n = 42) areas. A total of 13 engorged nymphs were sampled from *P. leucopus* in the bait box/Met52 treatment area and 9 from the deer removal/bait box/Met52 treatment area.

3.5. Borrelia burgdorferi infection in engorged larvae parasitizing white-footed mice

properties were each trapped on eight occasions in 2013 and the 38 properties on four occasions in both 2014 and 2015. There were an average of 78.0 trap nights/property (SD = 6.1) in 2013 and 38.8/property (SD = 3.1) in both 2014 and 2015. There were 774 mouse captures across all treatments of which 555 were unique mice. Of all unique mice, 400 were captured once only (72%), 115 were captured twice (21%), 25 thrice (5%), 10 four times (2%), two five times (0.4%), two six times (0.4%), and one was captured on seven occasions (0.2%). Captures were overwhelmingly white-footed mice (84.1%), but we also captured and released 91 eastern chipmunks, *Tamias striatus*, 27 meadow voles, *Microtus pennsylvanicus*, 23 northern short-tailed shrews, *Blarina brevicauda*, two red squirrels, *Tamiasciurus hudsonicus*, a wood frog, *Lithobates sylvaticus*, a gray squirrel, and a Carolina wren, *Thryothorus ludovicianus*.

While our treatment variables were not intended to alter mouse abundances, we did detect significant differences in mouse captures/trap night between treatments ($F_{3,85} = 4.55$, $P < 0.01$) and between years ($F_{2,85} = 14.38$, $P < 0.001$) and the treatment by year interaction ($F_{6,85} = 2.57$, $P < 0.03$). Mean mouse captures/trap night were significantly higher in 2014 ($\overline{X} = 22.9 \pm 2.4$) compared to 2013 ($\overline{X} = 12.1 \pm 2.5$; $P < 0.001$) and 2015 ($\overline{X} = 16.1 \pm 2.3$; $P = 0.001$), while 2013 and 2015 did not differ ($P = 0.15$; Fig. 1).

3.3. *Peromyscus leucopus* exposure to *Borrelia burgdorferi*

The majority of captured *P. leucopus* sera samples (99.0%; n = 763) were tested for antibody presence to *B. burgdorferi* and 53% were positive (n = 405). While we detected significant differences in *B. burgdorferi*-infected *P. leucopus*/trap night between treatments

A total of 243 engorged larvae were removed from *P. leucopus* and retained for analysis. Of these, 76 (33%) were positive for *B. burgdorferi*. Additionally, of the 191 *I. scapularis* tested from 2014 to 2015, 31 were positive for *A. phagocytophilum* (16%) and 10 positive for *Babesia microti* (5%). The majority of engorged larvae were sampled from the control (n = 131) and deer removal (n = 88) areas (90%). Of these, 28% from the control area were positive for *B. burgdorferi* while 36% in the deer removal treatment were positive (Table 1). Very few larvae were sampled from mice in the bait box/Met52 treatment (n = 8) and the deer removal/bait box/Met52 treatment (n = 16), likely due to the presence of bait boxes directly targeting parasitizing juvenile *I. scapularis*. Only 2 of 16 larvae collected in the deer removal/bait box/Met52 treatment were positive for *B. burgdorferi* (13%). Only 8 larvae from 7 different *P. leucopus* were removed and tested from within the bait box/Met52 treatment area, but 6 (75%) were positive for *B. burgdorferi* (Table 1).

There was significantly higher infection in engorged larvae in the bait box/Met52 treatment as compared to control ($\chi^2 = 8.07$, $P < 0.01$), deer removal only ($\chi^2 = 4.58$, $P < 0.02$), and the deer removal/bait box/Met52 treatment ($\chi^2 = 9.38$, $P < 0.001$). Additionally, deer removal only treatment had significantly higher infection than did the deer removal/bait box/Met52 treatment ($\chi^2 = 3.50$, $P < 0.041$).

3.6. Larvae positive for *Borrelia burgdorferi* parasitizing mice in August

The bait box/Met52 treatment combination resulted in 17-fold fewer *B. burgdorferi*-infected larvae/*P. leucopus* ($\overline{X} = 0.02$) than in the

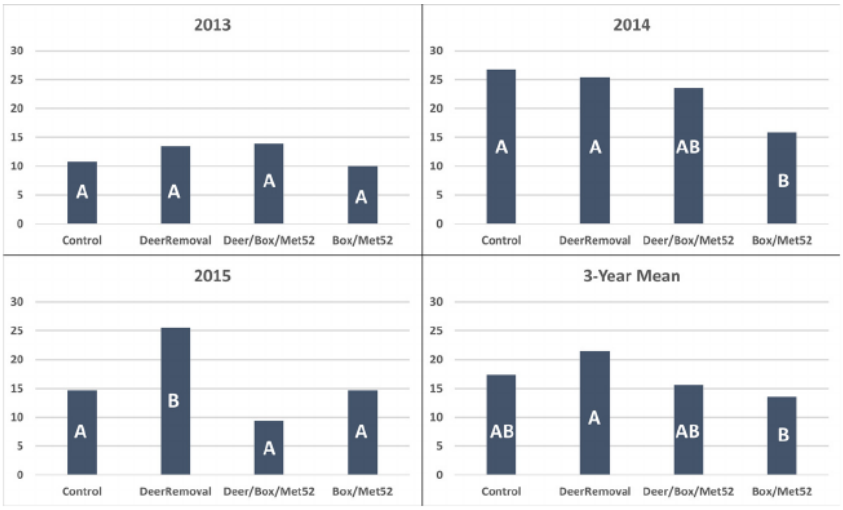
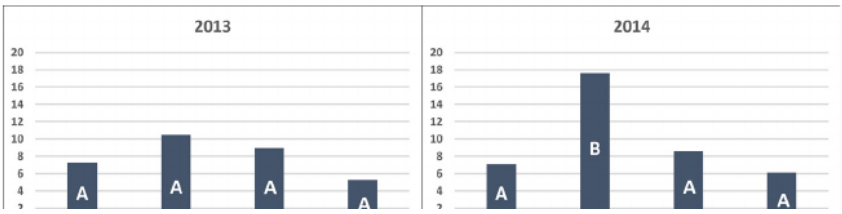


Fig. 1. Mean number *Peromyscus leucopus* captures/trap night/treatment/year. Means with the same letter are not significantly different from each other within each sub chart.



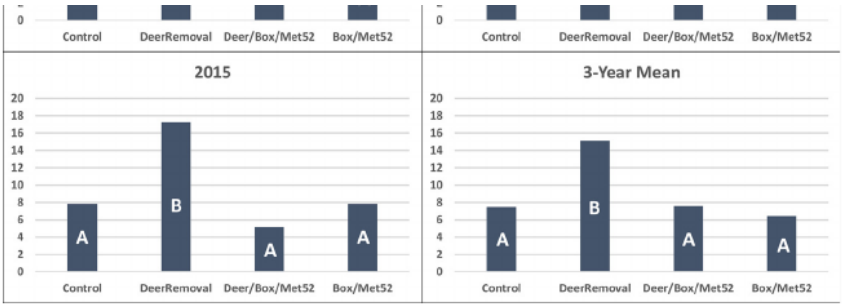


Fig. 2. Mean number of *Borrelia burgdorferi*-infected *Peromyscus leucopus* captures/trap night/treatment/year. Means with the same letter are not significantly different from each other within each year.

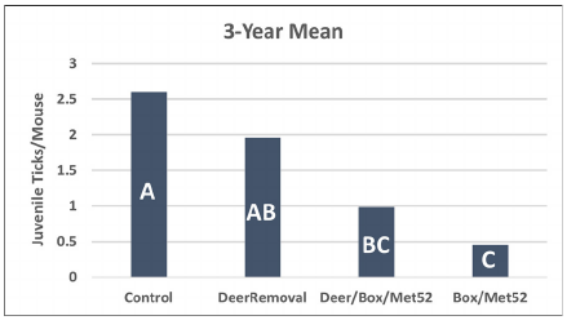


Fig. 3. Three-year (2013–2015) mean of juvenile *Ixodes scapularis* parasitizing captured *Peromyscus leucopus*/treatment.

control area ($\overline{X}$ = 0.34/*P. leucopus*) and 16.5-fold fewer than in the deer removal treatment area ($\overline{X}$ = 0.33/*P. leucopus*). The deer removal/bait box/Met52 treatment combination was not as effective ($\overline{X}$ = 0.05 infected larvae/*P. leucopus*), but still resulted in near 7-fold fewer infected larvae/*P. leucopus* than for the control and deer removal areas. Infected larvae/*P. leucopus* did not differ between the control and deer removal areas (χ^2 = 0.02, *P* = 2.66) nor did it differ between the deer removal/bait box/Met52 and bait box/Met52 areas (χ^2 = 1.3, *P* = 0.18; Table 2).

Table 1
Borrelia burgdorferi infection in engorged and partially engorged larval *Ixodes scapularis*. Rows with the same letter assignments were not significantly different.

Treatment	*Bb*-negative	*Bb*-positive	% Infected
Control	95	36	27% (AB)
Deer Removal.	56	32	36% (B)
Deer/Box/Met52	14	2	13% (A)
Box/Met52	2	6	75% (C)

4. Discussion

Results of this study indicate that significant reduction in juvenile stages of *I. scapularis* parasitizing its primary reservoir host for many disease-causing pathogens can be achieved using an ITM approach. As with questing nymphs, we found the most effective treatment

Table 2
Pearson's chi-square test values with Yate's correction for multiple comparisons between treatment means for *B. burgdorferi*-infected larvae/captured mouse in August 2013–2015.

Treatment	X^2	*P* Value
Deer vs Control	0.02	2.66
Deer vs Box/Met 52.	32.6	< 0.0001
Deer vs Deer/Box/Met52	25.0	< 0.0001
Control vs Box/Met52	34.0	< 0.0001
Control vs Deer/Box/Met52	26.3	< 0.0001
Deer/Box/Met52 vs Box/Met52	1.3	0.18

combination for reducing both juvenile *I. scapularis* and larvae parasitizing *P. leucopus* that tested positive for *B. burgdorferi* was the combination of a spray application of the entomopathogenic fungus *M. anisopliae* and distribution of fipronil-based rodent bait-boxes (Williams et al., 2018). This ran counter to our original expectations that more intervention, also including deer removal, would result in better control of *I. scapularis*. However, given protests from hunters and resulting limited deer removals, this result was not surprising.

None of our interventions were intended to impact *P. leucopus* 1980; Magnarelli et al., 1995; Garnett et al., 2011; Williams et al., 2018).

The same response did not occur in the deer removal/bait box/Met52 treatment area. We do not think that it was the result of fewer deer being removed, but rather the presence of Met52 impacting questing nymphs as well as fipronil bait boxes targeting juvenile *I. scapularis* on small rodents. Mean number of juvenile *I. scapularis* parasitizing *P. leucopus* in the bait box/Met52 treatment was not significantly different from the deer removal/bait box/Met52 treatment combination. Surprisingly, mean parasitizing juvenile *I. scapularis*/*P. leucopus* in the deer removal/bait box/Met52 treatment combination did not differ from the deer removal only treatment either. We suspect that as the result of host switching by juvenile *I. scapularis* due to deer removals, burdens increased on *P. leucopus* in the deer removal/bait box/Met52 treatment combination. The bait box/Met52 treatment area did not include deer removals and therefore, *P. leucopus* found within did not sustain the same increased tick load.

Similar to Dolan et al. (2004) and Schulze et al. (2017) who used fipronil-based bait boxes only, we were successful in significantly reducing parasitizing juvenile *I. scapularis* burdens on *P. leucopus* using an ITM approach, but did not reduce *P. leucopus* exposure to *B. burgdorferi*.

None of our interventions were intended to impact *P. leucopus* abundances; *P. leucopus* and small rodent abundances in general were consistent between treatments within each year, with a few exceptions. While beyond the scope of this project, our finding that *P. leucopus* abundances differed significantly between years corroborated the results of numerous other studies, likely due to fluctuating environmental variables such as hard mast abundance (Wolff, 1996; McCracken et al., 1999; Elias et al., 2004), overwintering survival (Vogt and Lynch, 1982), precipitation (Madison et al., 1984), and mesopredator abundances (Eagan et al., 2011) among others.

Additionally, we did not detect any differences in the abundance of *P. leucopus* with sera positive for antibodies to *B. burgdorferi* between control, bait box/Met52, and deer removal/bait box/Met52 treatments in 2013, 2014, 2015, or the 3-year mean. We expected *P. leucopus* captured on properties receiving bait boxes to have lower *B. burgdorferi* exposure compared to control properties, as the intent of the bait box use included targeting infected nymphs on reservoir hosts in an attempt to interrupt the pathogen transmission cycle. However, we did document the presence of engorged nymphal *I. scapularis* parasitizing *P. leucopus* in both treatment areas that received bait boxes. In the deer removal only treatment, we did have an impact on prevalence of exposure of *P. leucopus* to *B. burgdorferi*, but counter to the intended direction. While no differences were detected between treatments in 2013, there were significantly more *P. leucopus* exposed to *B. burgdorferi* in the deer removal only treatment in both 2014 and 2015 as well as the 3-year mean.

The increase in exposure to *B. burgdorferi* was not our intent, but again, was not unexpected due to our inability to achieve targeted deer reductions over the course of the project. Nevertheless, while deer densities reported to negatively impact *I. scapularis* abundances were not achieved, 31 deer were euthanized from the deer removal only treatment area in 2013 alone. This would not have impacted *I. scapularis* abundances immediately, but the absence of 31 large-bodied, incompetent hosts from a relatively small area may have created a void for juvenile host-seeking *I. scapularis* which were forced to prioritize blood meals on other hosts, particularly competent hosts like *P. leucopus* (see Williams et al., 2018). Consequently, *P. leucopus* exposure to *B. burgdorferi* increased both in 2014 and 2015. This result re-emphasizes the importance of white-tailed deer as both pathogen dilution and reproductive hosts for juvenile stages of *I. scapularis*. Adult *I. scapularis* are most often associated with large hosts like white-tailed deer as 95% of females require them for their final blood meal (Wilson et al., 1990), but juvenile stages do not have the same host size requirements as adults and can therefore, feed on nearly any vertebrate host they come in contact with (Watson and Anderson, 1976; Anderson and Magnarelli,

Neither Dolan et al. (2004) nor Schulze et al. (2017) detected any nymphal *I. scapularis* parasitizing *P. leucopus* post-bait box deployment, yet we documented engorged nymphs from all treatment areas, including those that utilized bait boxes. Initially, we thought this was due to high *P. leucopus* abundances and resulting over-use of bait boxes leading to insufficient fipronil treatment as we had 4.5 times the capture rate than did Schulze et al. (2017); 0.121 first-captured *P. leucopus*/trap night vs. 0.027, respectively. But Dolan et al. (2004) reported *P. leucopus* capture success of over 29%, which was far greater than our success rate of 17%. We then suspected that there may have been differences in the number of bait boxes deployed at each property, but our nearly 12 boxes/property exceeded that of both Dolan et al. (2004) (10 boxes/property) and Schulze et al. (2017) (8 boxes/property).

We conclude that our inability to affect *B. burgdorferi* exposure was the result of a combination of both property configuration and *P. leucopus* abundance. On smaller individual properties with lower *P. leucopus* abundances as in Schulze et al. (2017), bait boxes were successful in eliminating parasitizing nymphal *I. scapularis* and presumably interruption of the pathogen transmission cycle. On larger properties with high *P. leucopus* abundances, bait boxes were successful in eliminating parasitizing nymphal *I. scapularis* and reducing *B. burgdorferi* infection in *P. leucopus* by 53% when virtually all properties ($n = 157$) in a contiguous array had bait boxes ($n \approx 1700$) deployed (Dolan et al., 2004). On large, individual properties with moderate *P. leucopus* abundances as in our study, a single ring of bait boxes with differing combinations of Met52 application and deer removal significantly reduced juvenile *I. scapularis* burdens on *P. leucopus*, but not to levels sufficient to interrupt the *B. burgdorferi* pathogen transmission cycle. A second ring of bait boxes would likely have broken the transmission cycle, but at $40/installed box, this strategy would likely be cost prohibitive to most homeowners ($\approx$ $1,000/season). However, for low-toxicity, localized relief of both parasitizing juvenile and *B. burgdorferi*-infected larval *I. scapularis*, it seems clear that a combined treatment of fipronil-based rodent bait boxes and a broadcast application of Met52 can be effective.

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