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6 **Integrated Tick Management in the Upper Midwest: Impact of Invasive Vegetation**  
7 **Removal and Host-targeted Acaricides on *Ixodes scapularis* Infestation and *Borrelia***  
8 ***burgdorferi* Prevalence of Small Mammals**

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19 **ABSTRACT**

20           Integration of tick management strategies has been suggested to overcome ecological  
21 variation in tick, host, pathogen, and habitat, yet empirical evidence assessing combined  
22 treatment effect on blacklegged ticks, *Ixodes scapularis* Say, is limited. In this 5-year study  
23 (2014-2018) we tested whether combining two methods targeting tick/mammal interactions  
24 could reduce juvenile *I. scapularis* parasitism of two small mammal species, *Peromyscus*  
25 *leucopus* Rafinesque and *Tamias striatus* Linnaeus. Infection of small mammals with *Borrelia*  
26 *burgdorferi* was used to evaluate host exposure to feeding ticks. Using a factorial design,  
27 removal of invasive vegetation (Amur honeysuckle, *Lonicera maackii* Ruprecht and common  
28 buckthorn, *Rhamnus cathartica* Linnaeus) was coupled with deployments of permethrin-treated  
29 cotton nesting materials (tick tubes) and evaluated against control sites. Removal of invasive  
30 vegetation resulted in lower captures of *T. striatus* suggesting that treatment impacted reservoir  
31 activity in the plots. Deployments of permethrin-treated cotton were effective at reducing the  
32 frequency of juvenile *I. scapularis* parasitism of *P. leucopus* by 91% across the study compared  
33 to controls. However, tick tubes did not offer consistent protection against mouse exposure to *B.*  
34 *burgdorferi* exposure. An additive negative effect was detected for juvenile tick intensity on *P.*  
35 *leucopus* when tick tubes were combined with invasive vegetation removal. We conclude that  
36 integration of these two methods provides very limited benefit and that permethrin treatment  
37 alone offers the best option for reducing *I. scapularis* infestation on *P. leucopus*.

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## 41 ***1. INTRODUCTION***

42           Lyme disease (LD) is the most common vector-borne disease in North America and  
43 Europe (Bacon et al. 2008). The incidence of LD has steadily increased over the past two  
44 decades and the geographic range has expanded dramatically (Kilpatrick et al. 2017). In the  
45 northeastern and upper midwestern United States, LD is caused by the spirochete bacteria  
46 *Borrelia burgdorferi* sensu stricto and *B. mayonii* (Burgdorfer et al. 1982, Pritt et al. 2016),  
47 which are transmitted through the bite of an infected *Ixodes scapularis* Say (blacklegged tick)  
48 (Kilpatrick et al. 2017). Blacklegged ticks are also vectors of several other tick-borne pathogens  
49 in humans including *B. miyamotoi*, *Anaplasma phagocytophilum*, *Babesia microti*, Powassan  
50 virus, and *Ehrlichia muris* (Barbour 2017). Continued emergence of tick-borne pathogens  
51 necessitates more effective tick control strategies. The complex ecological relationships between  
52 habitat, ticks, wild hosts, and tick-borne pathogen often limit broad effectiveness of single  
53 interventions (Williams et al. 2018). Integration of more than one of the currently available  
54 strategies has been suggested as one way to overcome ecological variation, but empirical  
55 evidence for the effectiveness of integrated tick management strategies (ITM) is limited  
56 (Bloemer et al. 1990, Williams et al. 2018, Eisen and Stafford 2020).

57           Juvenile *I. scapularis* ticks will blood feed on a wide variety of vertebrate hosts but the  
58 largest proportion of ticks are fed by small mammals (Giardina et al. 2011, Kilpatrick et al.  
59 2017) Vertebrate hosts have varying degrees of reservoir competency, thereby limiting pathogen  
60 transmission (Donahue et al. 1987, Telford et al. 1988, Mather et al. 1989). White-footed mice  
61 (*Peromyscus leucopus* Rafinesque) are particularly effective at infecting local tick populations  
62 with *B. burgdorferi* due to their abundance and extended infectivity (Linske, Williams, Stafford,  
63 et al. 2018) and juvenile ticks parasitizing these hosts are highly likely to successfully feed to

64 completion. These mice are also adept at colonizing a variety of habitats, including fragmented  
65 tracts in residential neighborhoods, and establishing high population densities when conditions  
66 permit (Barko et al. 2003, Nupp and Swihart 2011). When they are in close proximity to humans  
67 and domestic animals, *P. leucopus* sustain and infect ticks thereby increasing the risk of local  
68 exposure to tick-borne disease. In addition to mice, other hosts such as shrews (*Blarina*  
69 *brevicauda* Say) and chipmunks (*Tamias striatus* Linnaeus) are known to be highly  
70 spirochetemic reservoirs perpetuating the *B. burgdorferi* enzootic cycle (Schmidt and Ostfeld  
71 2001, LoGiudice et al. 2003, Brisson et al. 2008)

72         One method of tick control that targets the host is the commercially available “tick tube”.  
73 Tick tubes offer a permethrin-treated nesting material which white-footed mice gather and use  
74 towards the construction of their nests. Superficial application of the acaricide to mice kills  
75 parasitizing ticks and prevents pathogen transmission (Spielman 1988). Studies have revealed  
76 reduced infestation of larval ticks on mice as well as a decrease in the abundance of host-seeking  
77 nymphs in the year following tick tube deployment (Mather et al. 1987, 1988, Deblinger and  
78 Rimmer 1991). However, small-scale residential studies in New York and Connecticut found  
79 that use of tick tubes caused a significant reduction in mouse infestation by ticks but did not  
80 reduce questing nymphs following treatment (Daniels et al. 1991, Stafford 1991, 1992, Ginsberg  
81 1992). Variability of mouse treatment has been documented and remains a potential area of  
82 improvement; however, has never been extensively evaluated.

83         Habitat modification has also been used for tick control, especially in relation to invasive  
84 plant species. Invasives are characterized by rapid infiltration of the local ecosystem and  
85 establishment as the dominant species thereby threatening native biological diversity (Mattos and  
86 Orrock 2010). While biological invasions have a direct effect on competing species, the indirect

87 consequences on wildlife communities have been shown to influence ticks and pathogen  
88 transmission (Allan et al. 2010). These exotic plants may facilitate interaction between ticks and  
89 competent hosts in two ways; 1) modification of the density and distribution of reservoirs by  
90 offering cover from predators and/or providing an ample food source for generalist feeders  
91 (Berryman and Hawkins 2006, Allan et al. 2010, Mattos and Orrock 2010, Linske, Williams,  
92 Ward, et al. 2018), or 2) generation of favorable microclimates that modify tick desiccation-  
93 induced tick mortality and allow sustained periods of unhindered questing that increase the  
94 probability of tick encounter with hosts (Stafford 1994, Williams et al. 2009). Dense-growing  
95 invasive species such as Japanese barberry (*Berberis thunbergii* de Candolle), common  
96 buckthorn (*Rhamnus cathartica* Linnaeus) and Amur honeysuckle (*Lonicera maackii* Ruprecht)  
97 have been positively associated with questing tick abundance (Lubelczyk et al. 2004, Elias et al.  
98 2006). Removal of invasive plants can reduce the abundance of *B. burgdorferi* infected *I.*  
99 *scapularis* adults that are seeking hosts as well as juvenile ticks that are parasitizing *P. leucopus*;  
100 this is thought to occur because of modification of abiotic conditions in altered stands (Williams  
101 et al. 2009, 2017, Williams and Ward 2010, Linske, Williams, Ward, et al. 2018). However, the  
102 generalizability of these results to other invasive plant species requires further study.

103 We reported that implementation of invasive vegetation removal or permethrin-treated  
104 cotton reduced the density of host-seeking *B. burgdorferi*-infected *I. scapularis* nymphs across a  
105 multi-year study (Mandli et al. 2021). However, when these interventions were combined as a  
106 part of an integrated approach no additive effect was observed. To further elucidate the  
107 underlying mechanisms of these interventions, we examined points of hosts and vector contact.  
108 These included treatment effect on tick infestation prevalence, intensity of infestation, and *B.*  
109 *burgdorferi* prevalence in two small mammal species, *P. leucopus* and *T. striatus*. Evaluation of

110 treatment influence on small mammal abundance and activity was completed through animal  
111 captures.

## 112 **2. MATERIALS AND METHODS**

### 113 **2.1 Study location:**

114 Field work took place at the University of Wisconsin (UW) Arboretum located in  
115 Madison, WI (43.047764, -89.422732) between May 2014 and August 2018. All sites were  
116 established in a temperate forest dominated by red oak (*Quercus rubra* Linnaeus) with associated  
117 shagbark hickory (*Carya ovata* Miller), and white pine (*Pinus strobus* Linnaeus). Dense invasive  
118 vegetation encompassed a majority of the forest understory. This location was confirmed as  
119 having an established *I. scapularis* population in 2012.

120 In 2014, sixteen non-adjacent 50 x 50 m experimental sites were established, representing  
121 the average size of residential lots based on United States home sale census data from 2013 (U.S.  
122 Census Bureau 2017). Site sizes (0.25 ha) approximate the lower end of the estimated home  
123 range of the white-footed mouse (0.2-0.6 ha). Distance between sites ranged from 35-610 m  
124 indicating some potential for mouse cross plot activity (Wolff 1985). Each site was verified for  
125 the growth of common buckthorn (*Rhamnus cathartica*) and Amur honeysuckle (*Lonicera*  
126 *maackii*), while hosting comparable concentrations of native plant species. Prior to the 2015 field  
127 season, an additional control site was created and one of the original sites was relocated due to  
128 flooding concerns.

### 129 **2.2 Experimental design:**

130 Sites were randomly assigned to one of four treatment combinations consisting of  
131 vegetation removal and permethrin-treated cotton in a two-by-two factorial design. Treatments  
132 consisted of the following: *control* sites underwent no invasive vegetation removal and received

133 tick tubes containing untreated cotton nest material (CTRL), *vegetation removal-only* sites  
134 underwent invasive vegetation removal and received tick tubes containing untreated cotton nest  
135 material (VR), *permethrin-treated cotton-only* sites underwent no invasive vegetation removal  
136 and received tick tubes containing permethrin-treated cotton nest materials (PTC), and *combined*  
137 sites received both invasive vegetation removal and tick tubes containing permethrin-treated  
138 cotton nesting material (PTC + VR).

### 139 **2.3 Invasive vegetation removal:**

140 During plot establishment in May 2014 and 2015 (relocation of site and establishment of  
141 new site), common buckthorn and Amur honeysuckle were cleared using loppers, handsaws, and  
142 brush cutters from a 20 x 20 m central plot embedded in the full 50 x 50 m experimental site.  
143 Non-target plant species and fallen trees were left undisturbed. In years following plot  
144 establishment, new growth of the targeted invasive species was removed prior to each field  
145 season in early May. An impact of vegetation removal on infesting *I. scapularis* immature ticks  
146 and the prevalence of *B. burgdorferi* infection in small mammals is expected within the same  
147 year of treatment. Assuming VR treatment influences small mammal activity in plots, captures  
148 are expected to decrease immediately.

### 149 **2.4 Tick tube deployment:**

150 Twenty-five tubes were deployed across each site approximately 10 m apart, in line with  
151 recommended densities of commercial tick tubes and with an emphasis on placement at preferred  
152 rodent habitat at the base of trees or along downed logs. Permethrin-treated cotton was prepared  
153 from the Tengard SFR One Shot commercial permethrin product during 2014-2016 (United  
154 Phosphorus Inc., King of Prussia PA, USA) or the ProZap Insectrin X 10% permethrin  
155 concentrate in 2017 and 2018 (CHEM-TECH, LTD., Lexington KY, USA) (providers were

156 changed due to availability). Stock solutions were diluted with water to produce a 10%  
157 experimental formulation that was applied to cotton using a 7.6 liter pump sprayer (Roundup®,  
158 Marysville, OH). Untreated cotton was prepared with water in a similar fashion, but in a separate  
159 location using dedicated equipment. Cotton tube reservoirs were prepared by cutting 6.1 cm (2.4  
160 in) x 3.05 m (10 ft) PVC tubing into lengths of approximately 15.2 cm (6.0 in). Cotton was  
161 stuffed into each tube until a 2.54 cm (1 in) lip without cotton was generated at both ends (17  
162 cotton balls/tube). Tick tube deployment occurred once prior to the peak of nymphal and larval  
163 activity during 2014-2015 (mid-May) and twice in 2016-2017 (mid-May and early to mid-July)  
164 with the second deployment designed to target ticks later in the season. Tubes were recovered  
165 from the field in October or November prior to snowfall, cleaned, and reused each year. An  
166 impact of permethrin-treated cotton on infesting *I. scapularis* ticks and the prevalence of *B.*  
167 *burgdorferi* infection in white-footed mice should be detectable within the same year of  
168 treatment. PTC is expected to have no impact on small mammal captures.

169 Cotton nesting material uptake was monitored in 2014 through 2018 at the time of second  
170 tick tube deployment or tube collection. A lack of observed uptake in early 2017 and 2018  
171 prompted the quantification of material following the first deployment (May-July: ~ 55 days  
172 later) and again following the second deployment (July-October: ~ 70 days later) in both years.

### 173 **2.5 Small mammal capture:**

174 Animal trapping and sampling procedures were reviewed and approved by the  
175 Institutional Animal Care and Use Committee of the University of Wisconsin-Madison  
176 (#A005400). Small mammals were trapped using Sherman live traps (H.B. Sherman Traps Inc.,  
177 Tallahassee, FL, USA) once a month from June to August for three consecutive days. A pre-bait  
178 night was included to acclimate mice to the presence of traps and increase trapping success.

179 Traps were initially baited with steel-cut oats and an apple chunk as a source of water, however,  
180 low animal captures in June 2014 prompted the addition of peanut butter to the bait formulation  
181 thereafter. Traps were checked three times a day at 8:00, 12:00, and 16:00 to capture a broad  
182 spectrum of nocturnal and diurnal species; white-footed mice, eastern chipmunk, eastern grey  
183 squirrel (*Sciurus carolinensis* Gmelin), southern flying squirrel (*Glaucomys volans* Linnaeus),  
184 and northern short-tailed shrew. Each site contained five pairs of Sherman traps deployed in a  
185 cross-like orientation; a single pair was located at the center of the site and four pairs were  
186 placed 10 m away in each cardinal direction (Figure 1). Traps were placed in areas of increased  
187 small mammal activity and away from tick tubes.

188 Animal processing occurred onsite. Captured small mammals were identified by species,  
189 aged, sexed, and weighed. Small mammals were temporarily sedated with isoflurane, fitted with  
190 a uniquely numbered ear tag, and a 2.0 mm ear biopsy was collected and stored in 70% ethanol.  
191 Ticks removed from animals were placed in 70% ethanol until identification. All ticks were  
192 identified to species and life stage using established taxonomic keys (Clifford et al. 1961, Durden  
193 and Keirans 1996). If animals were recaptured within the same month, they were checked for  
194 ticks and freed without further processing. Once recovered from the effects of isoflurane,  
195 animals were released at the origin of capture.

#### 196 **2.6 B. burgdorferi s.s. screening of ear biopsies:**

197 Genomic DNA was extracted from bisected ear biopsies using the Bioline Isolate II  
198 Genomic DNA Extraction Kit (Bioline USA Inc., Taunton MA, USA) or the DNeasy Blood and  
199 Tissue Kit (Qiagen, Valencia, CA) in accordance with the manufacturer's animal tissue protocol.  
200 Samples were tested for *B. burgdorferi* s.s DNA by nested polymerase chain reaction (PCR)

201 targeting the outer surface protein B operon as previously described (Caporale and Kocher 1994,  
202 Lee et al. 2014).

### 203 **2.7 Statistical Analysis:**

204 *Nesting material uptake:* For 2017 and 2018, the number of remaining cotton balls were  
205 summarized as mean residual cotton balls per tick tube by treatment by site during each  
206 deployment. Differences between PTC and untreated cotton (UTC) removal were compared  
207 using Welch's two sample t-tests.

208 The effect of the treatments, PTC, VR and PTC+VR, on animal captures, juvenile *I.*  
209 *scapularis* infestation prevalence, intensity of infestation, and host infection prevalence for two  
210 species (*P. leucopus* and *T. striatus*) was assessed through multivariate analyses using  
211 generalized linear models (GLMs) for individual years and generalized linear mixed effect  
212 models (GLMMs) when accounting for random effects in the cumulative study. Data from  
213 recaptured animals in a given trapping session (year + month) were condensed to a single data  
214 point (distinct animal) per plot for data analysis. The cumulative 5-year model fixed effects  
215 included *Year, Month, Treatment*. *Site* was included as a random effect in GLMMs to control for  
216 natural variation among plots and autocorrelation (Richer et al. 2014, Cayol et al. 2018). Annual  
217 GLMs included treatments and month as fixed effects. Model selection was carried out based on  
218 corrected Akaike Information Criterion for small sample sizes (AICc) (Burnham and Anderson  
219 2002). The simplest model containing treatment effects within 2 AICc of the model with the  
220 lowest AICc value was selected. Post-hoc tests assessing relative excess risk due to interaction  
221 (RERI-delta method) and multiplicative interaction were completed (VanderWeele and Knol  
222 2014). Analysis was carried out in R, version 1.4.1717 (R Core Team 2021). GLMMs and GLMs

223 were developed in the lme4 package (Bates et al. 2015). Analyses utilized CTRL as reference  
224 unless otherwise stated.

225 *Small mammal captures (SMC)*: All capture events regardless of recapture status,  
226 summarized as the number of *P. leucopus* or *T. striatus* captures per trap day by plot x trapping  
227 session. Captures were modeled using negative binomial regression (with log-link function) and  
228 relative risk was expressed as incidence rate ratios (IRRs) with a 95% confidence interval.

229 *Tick infestation prevalence (INF)*: Reported as a positive bivariate outcome (i.e. the  
230 presence or absence of at least one immature tick on a host). Direct comparison of the prevalence  
231 of infestation by tick life stage between *P. leucopus* and *T. striatus* was completed using a chi-  
232 square analysis with Yates' continuity correction. IP was modeled using logistic regression (with  
233 logit-link distribution) and relative risk was expressed as odds ratios (OR) with a 95%  
234 confidence interval.

235 *Tick intensity of infestation (INT)*: Density of immature ticks (larvae and nymphs)  
236 parasitizing all infested individual hosts. BI was modeled using negative binomial regression  
237 (with log-link function) and relative risk was expressed as IRRs with a 95% confidence interval.

238 *B. burgdorferi infection in hosts (BbP)*: Reported as a positive bivariate outcome (ie:  
239 infected vs uninfected host). Direct comparison of infection prevalence between *P. leucopus* and  
240 *T. striatus* was completed using a chi-square analysis with Yates' continuity correction. BbP was  
241 modeled using logistic regression (with logit-link distribution) and relative risk was expressed as  
242 ORs with a 95% confidence interval.

### 243 **3. RESULTS**

#### 244 **3.1 Treatment implementation:**

245 Initial removal of invasive vegetation was completed in both 2014 and 2015, but  
246 continued maintenance was required in order to keep Amur honeysuckle and common buckthorn  
247 from reinvading sites. Cotton was actively removed from tick tubes during the first three years  
248 (2014 - 2016) with less than 5% of cotton nesting material remaining in tubes or on the ground  
249 nearby. In 2017, 48% (3456/7225) of the cotton balls remained at the conclusion of the first  
250 deployment (May-July). Permethrin-treated cotton was removed less than UTC with a mean 11.2  
251 (95% CI: 9.53 – 12.8) and 5.44 (3.59 – 7.38) residual balls per tube respectively ( $t = 4.18$ ,  $p$ -  
252 value  $< 0.001$ ). During the second deployment in 2017 (July-October), only 3% (227/7225) of  
253 the cotton nesting material was left uncollected. At the second deployment, no difference in  
254 removal of treated or untreated nest material was detected with an average 0.695 (95% CI: 0.390  
255 – 1.04) (PTC) and 0.391 (95% CI: 0.187– 0.618) (UTC) residual balls per tube ( $t = 1.399$ ,  $p$ -  
256 value = 0.19). In 2018, twenty-two tick tubes could not be found following the first deployment.  
257 Of those recovered, 27% (1865/6851) of the cotton balls remained. Similar to 2017, PTC was  
258 removed less frequently than UTC with a mean 8.13 (6.29 – 9.80) and 1.46 (0.64 – 2.57) balls  
259 remaining per tube respectively ( $t = -6.09$ ,  $p$ -value  $< 0.001$ ). Values following the second  
260 deployment were poorly catalogued, limiting our ability to quantitatively evaluate cotton uptake.  
261 Still, anecdotal observations indicate near complete cotton removal of both cotton treatments by  
262 the end of the 2018 season, matching what was reported in previous years.

### 263 ***3.2 Small rodent capture***

264 Over the course of this study, we sampled small mammals on 7,560 trap days; 16 sites  
265 were trapped on 3 occasions (1440 trap days/year) in 2014 and 17 sites were trapped on 3  
266 occasions (1530 trap days/year) from 2015-2018. Ten traps were consistently placed at each site  
267 while trapping. During our study only two small rodent species were reliably captured and

268 maintained tick burdens, *P. leucopus* (0.181 captures/trap day) and *T. striatus* (0.060  
269 captures/trap day). We encountered other mammal species including *G. volans* (0.002  
270 captures/trap day), *B. brevicauda* (0.001 captures/trap day), and *S. carolinensis* (0.001  
271 captures/trap day). Overall, 901 *P. leucopus* were captured 1,371 times and 266 *T. striatus* were  
272 captured 456 times (Suppl. Table 1 and 2). Rates of recapture varied between mammal species;  
273 white-footed mice were captured one to six times (601, 180, 81, 30, 7, 2 mice respectively) and  
274 chipmunks were captured one to seven times (161, 59, 28, 8, 5, 3, 1 chipmunk respectively) with  
275 a single outlier that was captured 11 times. Because some plots were relatively close together,  
276 mice and chipmunks were sometimes recaptured on another plot. Of the 901 unique mice that  
277 were trapped, 2.2% (20) were also captured on another treatment site at some time. Of the 266  
278 chipmunks that were captured, 4.5% (12) were recaptured at another treatment site. This suggests  
279 that movement between plots was low.

280         The likelihood of *P. leucopus* capture was not significantly different between treatments  
281 at the annual and cumulative 5-year level with the exception of PTC in 2015 (Tables 1A & 2).  
282 Captures exhibited clear temporal variation in which the likelihood of *P. leucopus* capture  
283 increased in July and August compared to June (Table 1A). Using 2014 as reference, the  
284 probability of white-footed mouse capture significantly declined during 2015 and 2017 but  
285 recovered to similar levels in 2016 and 2018 thereby suggesting a fluctuating two-year trend in  
286 the population. The likelihood of *T. striatus* capture was not associated with treatments across  
287 the entire study period; however, captures were negatively associated with VR in 2014, 2015,  
288 and 2016 (Tables 1B & 3). Similar to mice, chipmunk captures exhibited temporal variability.  
289 Across the study, the probability of capturing a chipmunk was significantly lower in August

290 compared to June. Using 2014 as reference, the likelihood of *T. striatus* capture significantly  
291 increased in 2017 and 2018 by 80% and 126% respectively.

### 292 **3.3 Juvenile Ixodes scapularis parasitizing small rodents**

293 A total of 530 juvenile ticks (478 larvae, 52 nymphs) were collected from all small  
294 rodents; 414 juvenile ticks (403 larvae / 11 nymphs: CTRL = 6, VR = 2, PTC = 0, PTC+VR = 3)  
295 were obtained from *P. leucopus* and 116 juvenile ticks (75 larvae / 41 nymphs) from *T. striatus*.  
296 Larvae were more likely to infest mice (OR = 1.70,  $\chi^2 = 6.29$ , df 1, p-value = 0.012) while  
297 nymphs were more likely to infest chipmunks (OR = 5.82,  $\chi^2 = 16.9$ , df 1, p-value < 0.001). No  
298 other captured mammal species presented any observed *I. scapularis*. Across the study, 17% (n =  
299 1101) of all distinct white-footed mice were infested with at least one juvenile tick, whereas 16%  
300 (n = 318) of all distinct chipmunks were infested. Mean intensity of infestation was 2.27  
301 (median: 2.0, range: 1-13) ticks per distinct *P. leucopus* and 2.27 (median: 1.0, range: 1-26) ticks  
302 per distinct *T. striatus*.

303 The prevalence of infestation exhibited substantial temporal variation that coincided with  
304 the tick phenology discussed in Mandli et al. 2021 (Mandli et al. 2021); *P. leucopus* infestation  
305 corresponded to larval questing patterns and *T. striatus* infestation matched peak nymphal  
306 patterns (Table 1A&B).

307 As for treatments, PTC significantly reduced the likelihood of infestation on white-footed  
308 mice by 91% across the study but had no significant impact on INT (Table 1A). PTC was  
309 negatively associated with IP in 2014 and 2015 and exhibited complete inhibition of infestation  
310 on mice in 2016 and 2018 (Table 2). In 2017, CTRL exhibited a large decline in infestation  
311 prevalence which impacted comparative assessments of PTC. Even so, mice captured on PTC  
312 sites exhibited nearly complete absence of tick infestation. At the cumulative level, VR exhibited

313 no significant impact on the probability of infestation. However, during the first year of  
314 treatment *P. leucopus* INF was negatively associated with VR, with a 62% decrease. The  
315 combination of PTC and VR was negatively associated with INT at the cumulative level. As  
316 such, additive interaction was detected between treatments for *P. leucopus* (Table 1A). The  
317 likelihood of tick infestation on *T. striatus* was not associated with treatments at the annual or  
318 cumulative 5-year level (Table 1B & 3). Moreover, none of the explored effects were associated  
319 with INT in chipmunks.

### 320 **3.4 Small rodent *B. burgdorferi* infection**

321 A strong dichotomy existed between chipmunk and mouse *B. burgdorferi* infection (OR  
322 = 5.15,  $\chi^2 = 115.8$ , df 1, p-value < 0.001). In total 14% (142/1013) of *P. leucopus* and 46%  
323 (105/230) of *T. striatus* ear biopsies from unique animals were infected with *B. burgdorferi*.  
324 Thirteen mice became infected with *B. burgdorferi* over the course of a given season; three were  
325 captured in CTRL plots, four from VR plots, four from PTC plots, and two from PTC+VR plots.  
326 Of note, one of these mice moved from a CTRL to a PTC+VR plot and another moved from  
327 CTRL to PTC so they may have become infected outside PTC-treated plots. Annual *B.*  
328 *burgdorferi* prevalence in *P. leucopus* and *T. striatus* was variable (Table 1A&B). The likelihood  
329 of *B. burgdorferi* infection in *P. leucopus* was negatively associated with PTC (= lower  
330 infection) at the cumulative level but remained variable in each individual year (Table 1A).  
331 Infection with *B. burgdorferi* in chipmunks was not associated with any explored effects.

## 332 **4. DISCUSSION**

333 Results from this study demonstrate that PTC is a reliable deterrent of juvenile  
334 blacklegged tick parasitism on *P. leucopus*. This affirms the causal mechanism of PTC reduction  
335 of DON and DIN as effective inhibition of tick and host contact. PTC resulted in immediate

336 reductions to INF on white-footed mice that persisted throughout much of the study; however,  
337 intensity of infestation on infested mice did not differ from CTRLs suggesting an all or nothing  
338 response. Reductions to tick infestation imposed by VR were short-lived. Significant reductions  
339 to INF on mice were constrained to the first year of study; however, intensity of infestation did  
340 not differ from CTRL. Despite reductions in INF for both treatments in 2014, no interaction was  
341 detected. Neither treatment exhibited an impact on *T. striatus* tick infestation or intensity of  
342 infestation. This was not unexpected; chipmunks exhibit little interest in PTC and have a larger  
343 home range which takes them well outside the boundaries of the VR plots.

344         Permethrin-treated nesting materials are expected to inhibit all tick feeding, including  
345 infected nymphs, in an attempt to disrupt spirochete transmission. As such, we expected that *P.*  
346 *leucopus* captured in sites with deployed tick tubes would have lower *B. burgdorferi* infection  
347 compared to CTRL sites. While PTC exhibited a cumulative reduction to prevalence of *B.*  
348 *burgdorferi* in mice, the impact was inconsistent from year to year, probably because the  
349 prevalence of infection and the number of mice tested per treatment each year were low.  
350 Invasive vegetation removal had no influence on *B. burgdorferi* prevalence in mice. Given a lack  
351 of effect on tick parasitism, it was no surprise that treatments had no impact on chipmunk  
352 infection with *B. burgdorferi*.

353         Dense vegetation provides refuge for small mammals, especially white-footed mice,  
354 thereby facilitating interaction between ticks and hosts (Allan et al. 2010, Mattos and Orrock  
355 2010, Guiden and Orrock 2017). As such, we initially hypothesized that removal of invasive  
356 vegetation would decrease mouse relative abundance. However, this study showed that the  
357 likelihood of capturing a *P. leucopus* was indistinguishable between treatments annually and  
358 across the cumulative 5-year study. Linske et al (2018b) reported a similar lack of difference in

359 *P. leucopus* captures after the removal of Japanese barberry from 30 x 30 m plots. Surprisingly,  
360 *T. striatus* exhibited lower rates of capture among VR plots in 2014-2016, perhaps suggesting  
361 that removal of invasive plants may act as a deterrent to activity of this diurnal species. PTC does  
362 not offer any advantage in small mammal survivability (Mather et al. 1987); therefore, it was no  
363 surprise that chipmunk and mouse captures in the PTC plots were not different from CTRLs.  
364 While not the main focus of this study, our data shows significant variation in *T. striatus* and *P.*  
365 *leucopus* captures between years as well as among months. This is likely a result of fluctuating  
366 environmental and ecological factors including weather (Madison et al. 1984), predator  
367 abundance (Mattos and Orrock 2010), and food availability (Ostfeld, Jones, et al. 1996) and  
368 population dynamics (Wang et al. 2008), and seasonal population growth (Wolff 1986, Mather et  
369 al. 1987).

370 Results from this study suggest that permethrin-treated cotton may offer complete protection  
371 against *I. scapularis* infestation in an area of low tick abundance. Across the study, only 6% of  
372 all distinct mice captured (n = 490) from PTC or PTC+VR sites were infested with at least one  
373 tick. The body burdens on these infested mice were similar compared to CTRLs suggesting that  
374 these may be mice that originated from untreated locations and indeed, we documented  
375 movement of mice from control to treated plots. Other PTC trials have similarly documented all  
376 or nothing responses (Mather et al. 1987, Deblinger and Rimmer 1991). Furthermore, Ginsberg  
377 et al. (1992) demonstrated that mice with detectable levels of permethrin on hair were  
378 completely free of parasitizing ticks. Assuming PTC treatment of mice offers complete  
379 protection against infestation, animals with parasitizing ticks in areas of permethrin treatment are  
380 likely untreated. Exposing mouse movement patterns under different conditions as well as  
381 nesting behaviors related to acaricide application are key to refining this strategy for broader use.

382           We saw an overall reduction in the prevalence of *B. burgdorferi* in mice captured in PTC  
383 plots, but sampling was underpowered and low prevalence and small sample sizes precluded the  
384 ability to detect annual patterns for most years. Reducing host infection is key to minimizing  
385 human risk because individual mice can infect hundreds of ticks. Given that the action of  
386 permethrin-treated cotton includes the inhibition of infected nymph feeding on mice, we  
387 expected that *P. leucopus* captured in PTC and PTC+VR sites would have lower *B. burgdorferi*  
388 infection compared to CTRL sites. Our data revealed a complete lack of parasitizing nymphs at  
389 PTC sites and three instances of nymph parasitism at PTC+VR sites. While frequency of  
390 parasitizing nymphs was low, a few mice captured in sites with permethrin-treated cotton  
391 became infected over the season. However, several of these mice originated in untreated areas.  
392 Studies describing the impact of PTC on the prevalence of *B. burgdorferi* in mice are lacking,  
393 but other host-targeted systems, like fipronil-bait boxes, have described an inability to reduce  
394 prevalence of infection of *P. leucopus* at small scales (< 1.0 ha) (Dolan et al. 2004, Schulze et al.  
395 2017). Both host-targeted approaches are constrained by an area of effect. Assuming mice closer  
396 to a tick tube have a higher probability of becoming treated, a larger area of treatment is more  
397 likely to generate protection at its center. Mouse home ranges are estimated to be approximately  
398 0.2-0.6 hectares (0.5-1.5 acres), but this can vary depending on population density and season  
399 (Wolff 1985). Landscape features (man-made structures), other nesting substrates, and mice with  
400 unusually expansive home ranges could further complicate estimates of area of effect. Further  
401 investigation is needed to understand spatial constraints of PTC and other host-targeted  
402 approaches in order to refine deployment strategies.

403           Spirochete infection in mice may also occur prior to PTC treatment. Tick tubes were  
404 deployed at the same time each year, however increasingly warmer temperatures earlier in the

405 season may have advanced tick phenologies and increased mouse and tick interactions prior to  
406 treatment (Levi et al. 2015). Another possible explanation is the reported presence of immature  
407 ticks in mouse nests. Larson et al. 2019 found unfed blacklegged tick nymphs among nests  
408 collected in May. Moreover, ticks were also observed in nests collected in August, suggesting  
409 the possibility of overwintering in these locations. Given our lack of understanding of the  
410 longevity of permethrin treatment on mice and the stability of the acaracide on cotton substrates  
411 in the field, we do not know if PTC in nests remains effective by spring. Therefore, it is possible  
412 that mouse exposure begins in the nest. Further research assessing the longevity and  
413 transferability of permethrin in the field is needed to determine when deployments are most  
414 efficacious. If nymphal tick overwintering in nests or early questing does occur, it may be  
415 effective to provide PTC late in the fall prior to mouse torpor.

416 Low cotton collection in 2017 was likely a function of a mouse abundance. Mouse captures  
417 were significantly lower than any other year of study. As a result, *P. leucopus* home ranges may  
418 have increased and mice may have moved more outside the bounds of our study sites (Wolff  
419 1985, Ostfeld, Miller, et al. 1996). Mice exhibited lower levels of INF in CTRL sites that  
420 resembled PTC sites suggesting cross treatment. All three instances of cross plot migration that  
421 we observed in 2017 were a result of mice moving between areas of permethrin-treated cotton to  
422 those without. Under these conditions, PTC appeared less effective as compared to CTRL likely  
423 as a result of some treated mice entering these areas. The use of multi-year studies (> 2) is  
424 necessary for interpretation of these low-density events.

425 Invasion of exotic vegetation can have a dramatic impact on local ecology thereby modifying  
426 host and tick interactions. However, across this study, VR had little impact on capture rates, tick  
427 infestation frequency and intensity, and *B. burgdorferi* prevalence of *P. leucopus*, yet significant

428 reductions in DIN (density of infected questing nymphs) were documented in Mandli et al 2021.  
429 These results suggest that some alternative factor besides larval tick survivorship on or infection  
430 by white-footed mice in VR plots altered DIN the following year (T+1). The presence of  
431 alternative hosts could explain the reduction in densities of infected nymphs. *Tamias striatus* and  
432 *B. brevicauda* were both captured on our sites and are known to be competent *B. burgdorferi*  
433 reservoirs (Schmidt and Ostfeld 2001, LoGiudice et al. 2003, Brisson et al. 2008). The absence  
434 of chipmunks in VR sites coupled with their high rates of *B. burgdorferi* infection could account  
435 for declines in DIN (T+1). While chipmunks are typically associated with higher rates of  
436 nymphal tick attachment (Schmidt et al. 1999), chipmunks do feed larvae and some of these  
437 must contribute to the population of infected nymphs. Future work could benefit from nymph  
438 bloodmeal analysis to better understand the sources of tick infection (Cadenas et al. 2007).  
439 Interventions that discourage competent hosts or encourage incompetent hosts presence have the  
440 potential to dilute pathogen presence (Linske et al. 2018). Strategic application of VR as an  
441 intervention will require further investigation of how it influences alternative species in order to  
442 justify its application.

443 Here, we evaluated the impact of integrated environmental control strategies on *B.*  
444 *burgdorferi* prevalence and *I. scapularis* parasitism of two small mammal species in south  
445 central Wisconsin. Our results demonstrate that PTC reliably reduced mouse infestation by  
446 juvenile ticks, however it does not completely eliminate the role of mice in maintaining  
447 spirochete transmission. VR removal had no detectable impact on white-footed mouse  
448 infestation, infection, or abundance suggesting an effect on tick feeding and/or spirochete  
449 transmission in alternative reservoir hosts. Continued examination of treatment impact on host  
450 and tick interactions remains a key part of understanding variation in treatment effectiveness.

451 Additional work is needed to refine these strategies and determine which combinations offer the  
452 cost-effective option for the general public.

453

454

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- 643
- 644

645 **FIGURES**

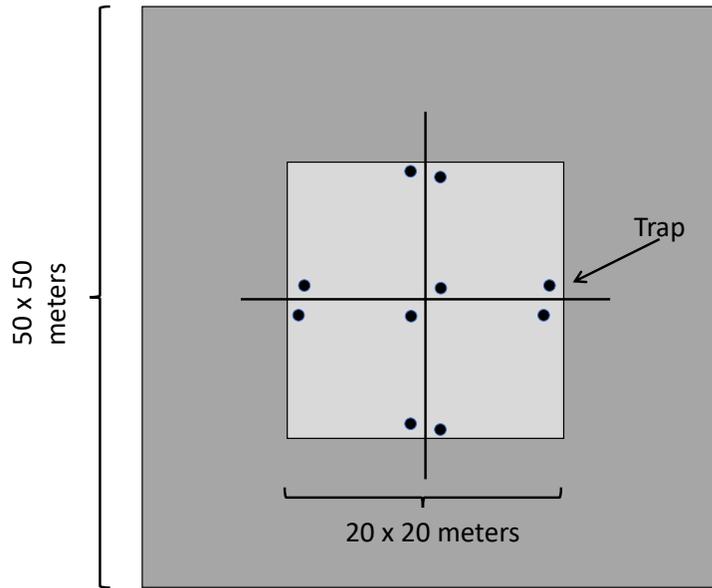
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**Figure 1:** Trapping schematic and dimensions for individual plot. Traps are represented by black circles.



650

651 **TABLES**

652 **Table 1.** Results from the impact of year, month, permethrin-treated cotton (PTC), vegetation removal (VR), and the combination of PTC and VR on small  
 653 mammal captures (SMC), juvenile tick infestation prevalence (INF), tick intensity of infestation (INT), *B. burgdorferi* infection prevalence (BbP) across the study  
 654 for two small mammal species: A) *Peromyscus leucopus* and B) *Tamias striatus* (Data from GLMM). Baseline effects are year: 2014, month: June, Treatment:  
 655 CTRL. Additive (RERI) and multiplicative interaction terms reported between treatments Differences between minimal model and best model given as  $\Delta$  AICc.

**A**

	Captures				Infestation Prevalence			Intensity of Infestation			Infection Prevalence		
	Fixed Effects	IRR	95% CI	p-value	OR	95% CI	p-value	IRR	95% CI	p-value	OR	95% CI	p-value
<i>P. leucopus</i>	2015	0.63	0.5 - 0.8	< 0.001	2.12	1.2 - 3.8	0.009	1.28	0.9 - 1.8	0.130	4.86	2.6 - 9.3	< 0.001
	2016	1.05	0.8 - 1.4	0.707	0.50	0.3 - 0.9	0.010	1.03	0.7 - 1.4	0.874	2.71	1.5 - 5.0	0.001
	2017	0.43	0.3 - 0.6	< 0.001	0.37	0.2 - 0.8	0.016	0.90	0.5 - 1.6	0.721	4.49	2.2 - 9.1	< 0.001
	2018	1.01	0.8 - 1.3	0.932	0.65	0.4 - 1.1	0.111	1.06	0.8 - 1.5	0.708	2.03	1.1 - 3.8	0.031
	July	2.51	2.0 - 3.1	< 0.001	0.24	0.1 - 0.4	< 0.001	0.66	0.4 - 1.0	0.042	1.62	1.0 - 2.7	0.074
	August	1.64	1.3 - 2.1	< 0.001	2.08	1.3 - 3.5	0.002	1.34	1.0 - 1.8	0.056	1.58	0.9 - 2.8	0.117
	PTC	0.79	0.6 - 1.1	0.131	0.09	0.04 - 0.2	< 0.001	0.93	0.6 - 1.5	0.774	0.50	0.3 - 0.9	0.027
	VR	0.81	0.6 - 1.1	0.172	1.02	0.6 - 1.9	0.942	1.19	0.9 - 1.5	0.143	0.73	0.4 - 1.3	0.291
	PTC+VR	0.83	0.6 - 1.1	0.236	0.18	0.09 - 0.4	< 0.001	0.62	0.4 - 1.0	0.034	0.57	0.3 - 1.1	0.075
	RERI	-0.07	-0.2 - 0.08		-0.10	-0.7 - 0.5		-0.43	-0.7 - -0.1		-0.02	-0.4 - 0.4	
Multiplicative	1.31	0.8 - 2.1		1.90	0.6 - 5.6		0.56	0.3 - 1.1		1.58	0.6 - 4.0		

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**B**

	Captures				Infestation Prevalence			Intensity of Infestation			Infection Prevalence		
	Fixed Effects	IRR	95% CI	p-value	OR	95% CI	p-value	IRR	95% CI	p-value	OR	95% CI	p-value
<i>T. striatus</i>	2015	0.69	0.3 - 1.4	0.319	2.45	0.6 - 9.0	0.202	0.96	0.4 - 2.2	0.915	0.40	0.1 - 1.2	0.112
	2016	0.83	0.4 - 1.8	0.630	0.09	0.2 - 0.5	0.007	0.52	0.09 - 2.9	0.452	0.57	0.2 - 1.8	0.274
	2017	1.80	0.9 - 3.6	0.098	0.21	0.6 - 0.8	0.019	1.11	0.5 - 2.7	0.812	1.00	0.3 - 2.9	0.971
	2018	2.26	1.1 - 4.5	0.021	0.29	0.08 - 1.0	0.047	0.74	0.3 - 1.9	0.520	0.96	0.4 - 2.5	0.875
	July	1.35	0.8 - 2.2	0.229	0.04	0.01 - 0.1	< 0.001	0.76	0.3 - 2.0	0.581	1.18	0.6 - 2.2	0.711
	August	0.43	0.2 - 0.8	0.003	0.43	0.2 - 1.2	0.111	0.60	0.3 - 1.4	0.222	0.93	0.4 - 2.3	0.840
	PTC	2.47	0.7 - 9.0	0.169	0.67	0.3 - 1.6	0.365	0.45	0.2 - 1.6	0.203	0.95	0.5 - 1.8	0.966
VR	0.53	0.1 - 2.0	0.353	1.09	0.4 - 3.1	0.868	0.68	0.2 - 2.1	0.516	0.88	0.4 - 1.9	0.865	

	<b>PTC+VR</b>	0.61	0.2 - 2.3	0.466	2.38	0.8 - 6.7	0.099	0.41	0.1 - 1.3	0.125	0.58	0.2 - 1.4	0.234
	<b>RERI</b>	-1.20	-3.1 - 0.7		0.98	-2.5 - 4.4		-0.01	-0.8 - 0.8		-0.34	-0.9 - 0.2	
	<b>Multiplicative</b>	0.47	0.07 - 3.1		3.24	0.7 - 14.2		1.34	0.3 - 6.2		0.70	0.2 - 2.3	

**Table 2.** Results from the impact of permethrin-treated cotton (PTC), vegetation removal (VR), and the combination of PTC and VR on small mammal captures (SMC), juvenile tick infestation prevalence (INF) and intensity of infestation (INT), *B. burgdorferi* infection prevalence (BbP) by each year for *P. leucopus* (Data from GLM). Baseline effect is Treatment: CTRL. Differences between minimal model and best model given as  $\Delta AICc$ .

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	Capture				$\Delta AICc = -7$	Infestation Prevalence			$\Delta AICc = 54$	Intensity of Infestation			$\Delta AICc = -3$	Infection Prevalence			$\Delta AICc = 6$
	Fixed Effects	IRR	95% CI	p-value		OR	95% CI	p-value		IRR	95% CI	p-value		OR	95% CI	p-value	
2014	PTC	1.16	0.7 – 1.9	0.525	0.10	0.03 - 0.3	< 0.001	0.82	0.4 - 1.5	0.544	0.15	0.02 - 0.6	0.016				
	VR	1.04	0.7 – 1.6	0.872	0.38	0.1 – 0.9	0.036	1.19	0.8 - 1.8	0.431	0.29	0.06 - 1.0	0.066				
	PTC+VR	0.93	0.04 – 2.4	0.875	0.12	0.04 – 0.3	< 0.001	0.65	0.3 - 1.2	0.193	0.09	0.005 – 0.5	0.026				
2015	PTC	0.94	0.6 – 1.6	0.912	0.10	0.03 - 0.3	< 0.001	1.03	0.5 - 2.1	0.935	0.55	0.2 - 1.6	0.286				
	VR	0.71	0.4 - 1.2	0.211	0.53	0.2 – 1.4	0.207	0.72	0.4 - 1.2	0.278	1.29	0.4 - 3.8	0.636				
	PTC+VR	0.12	0.7 – 1.8	0.622	0.11	0.03 – 0.3	< 0.001	0.38	0.1 - 1.0	0.075	0.69	0.2 – 2.0	0.488				
2016	PTC	0.78	0.4 – 1.3	0.332	9.0E <sup>-9</sup>	1.2E <sup>-215</sup> – 8.2E <sup>25</sup>	0.992	0.34	0.05 – 1.4	0.184	0.77	0.3 - 1.9	0.572				
	VR	1.05	0.6 – 1.6	0.862	2.54	1.1 – 6.0	0.030	1.58	0.9 – 2.8	0.110	0.62	0.2 - 1.5	0.308				
	PTC+VR	0.73	0.4 – 1.2	0.237	0.15	0.02– 0.6	0.015	0.54	0.08 – 2.4	0.451	0.80	0.3 – 2.0	0.637				
2017	PTC	0.48	0.2 - 0.96	0.040	1.30	0.06 – 14.7	0.833	0.50	0.03 – 3.4	0.535	1.08	0.3 - 3.9	0.912				
	VR	0.65	0.3 – 1.3	0.201	4.36	0.8 – 34.1	0.110	0.88	0.3 – 3.3	0.831	1.24	0.4 - 3.9	0.722				
	PTC+VR	0.56	0.3 – 1.1	0.099	3.40	0.5 – 27.8	0.207	0.67	0.2 – 2.8	0.566	0.75	0.2 – 2.9	0.694				
2018	PTC	0.74	0.5 – 1.2	0.235	1.2E <sup>-8</sup>	1.9E <sup>-309</sup> – 4.9E <sup>31</sup>	0.993	0.53	0.2 – 1.2	0.177	0.44	0.1 - 1.3	0.172				
	VR	0.78	0.5 – 1.3	0.333	0.86	0.4 – 2.0	0.727	1.29	0.8 – 2.0	0.256	0.75	0.2 - 2.0	0.584				
	PTC+VR	0.80	0.5 - 13	0.360	0.19	0.05 – 0.6	0.006	0.69	0.3 – 1.6	0.419	0.69	0.2 – 1.9	0.477				

**Table 3.** Results from the impact of permethrin-treated cotton (PTC), vegetation removal (VR), and the combination of PTC and VR on small mammal captures (SMC), juvenile tick infestation prevalence (INF) and intensity of infestation (INT), *B. burgdorferi* infection prevalence (BbP) by each year for *T. striatus* (Data from GLM). Baseline effect is Treatment: CTRL. Differences between minimal model and best model given as  $\Delta$  AICc.

	Fixed Effects	Capture			$\Delta$ AICc = -1	Infestation Prevalence			$\Delta$ AICc = -2	Intensity of Infestation			$\Delta$ AICc = -11	Infection Prevalence			$\Delta$ AICc = -8
		OR	95% CI	p-value		OR	95% CI	p-value		IRR	95% CI	p-value		OR	95% CI	p-value	
2014	PTC	0.62	0.2 – 2.5	0.498		5.36	0.9 – 4.5	0.078		1.20	0.3 – 8.2	0.823		0.33	0.03 – 2.6	0.309	
	VR	0.16	0.03 – 0.8	0.024		5.6E <sup>-8</sup>	N/A – 2.8E <sup>241</sup>	0.996		1.67	0.4 – 5.8	0.429		0.66	0.06 – 7.5	0.733	
	PTC+VR	0.29	0.07 – 1.2	0.086		3.00	0.2 – 45.5	0.395		2.00	0.4 – 14.4	0.423		0.66	0.08 – 5.3	0.697	
2015	PTC	0.56	0.1 – 2.5	0.411	$\Delta$ AICc > 5	0.42	0.1 – 1.5	0.187	$\Delta$ AICc = -3	0.68	0.3 – 1.5	0.343	$\Delta$ AICc = -5	0.71	0.2 – 3.0	0.633	$\Delta$ AICc = -6
	VR	0.13	0.02 – 0.6	0.005		0.56	0.07 – 3.6	0.549		1.23	0.4 – 3.1	0.682		1.22	0.1 – 9.6	0.848	
	PTC+VR	N/A	N/A	N/A		N/A	N/A	N/A		N/A	N/A	N/A		N/A	N/A	N/A	
2016	PTC	1.65	0.5 – 6.0	0.411	$\Delta$ AICc > 2.0	N/A	N/A	N/A	$\Delta$ AICc = N/A	N/A	N/A	N/A	$\Delta$ AICc = N/A	0.67	0.1 – 3.8	0.646	$\Delta$ AICc = -7
	VR	0.07	0.003 – 0.5	0.020		N/A	N/A	N/A		N/A	N/A	N/A		6.4E <sup>7</sup>	0.0 – N/A	0.996	
	PTC+VR	0.59	0.1 – 2.4	0.453		N/A	N/A	N/A		N/A	N/A	N/A		1.00	0.1 – 9.1	0.999	
2017	PTC	2.68	0.8 – 9.7	0.118	$\Delta$ AICc = -1	0.07	0.004 – 0.5	0.023	$\Delta$ AICc = 1	0.15	0.006 – 3.2	0.187	$\Delta$ AICc = -15	2.33	0.6 – 9.4	0.220	$\Delta$ AICc = -4
	VR	1.47	0.4 – 5.4	0.546		0.26	0.03 – 1.4	0.139		0.45	0.08 – 3.1	0.375		0.70	0.2 – 3.1	0.638	
	PTC+VR	0.45	0.1 – 1.8	0.245		1.92	0.1 – 50.8	0.643		0.15	0.01 – 1.28	0.075		0.58	0.08 – 7.7	0.689	
2018	PTC	1.87	0.6 – 6.5	0.312	$\Delta$ AICc = -6	2.25	0.3 – 47.0	0.494	$\Delta$ AICc = -3	1.33	0.3 – 9.6	0.740	$\Delta$ AICc = -11	0.83	0.3 – 2.7	0.763	$\Delta$ AICc = -5
	VR	1.21	0.4 – 4.3	0.766		6.32	0.9 – 126.5	0.105		2.40	0.7 – 15.4	0.252		1.19	0.3 – 4.4	0.789	
	PTC+VR	1.24	0.4 – 4.4	0.727		2.08	0.3 – 20.0	0.478		1.33	0.3 – 9.6	0.740		0.45	0.1 – 1.6	0.235	

659 **SUPPLEMENTARY**

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**Supplementary Table 1:** Mean (median, range) captures per plot trap day, infestation prevalence and mean (median, range) intensity infestation of juvenile *I. scapularis* on *P. leucopus*, and *B. burgdorferi* infection prevalence by treatment and year for *P. leucopus*.

		<i>P. leucopus</i>			
	Treatment	Captures	Infestation Prevalence	Intensity of Infestation	% Infected
2014	CTRL	2.3 (1.8, 0-7)	35% (25/71)	2.2 (2.0, 1-5)	16% (11/70)
	VR	2.1 (1.7, 0-5)	20% (12/61)	2.7 (2.0, 1-8)	5% (3/59)
	PTC	2.4 (2.0, 0-6)	8% (6/77)	1.8 (1.5, 1-3)	3% (2/74)
	PTC + VR	2.1 (2.2, 0-5)	11% (7/65)	1.4 (1.0, 1-3)	2% (1/58)
	<b>Annual Total</b>	<b>2.2 (1.8, 0-7)</b>	<b>18% (50/274)</b>	<b>2.2 (2.0, 1-8)</b>	<b>7% (17/261)</b>
2015	CTRL	1.4 (1.0, 0-3)	49% (24/49)	2.7 (2.0, 1-9)	27% (12/45)
	VR	1.0 (1.0, 0-2)	37% (11/30)	1.9 (1.0, 1-5)	32% (8/25)
	PTC	1.4 (1.3, 0-3)	11% (4/37)	2.8 (2.0, 1-6)	17% (6/36)
	PTC + VR	1.6 (1.3, 0-4)	11% (4/38)	1.0 (1.0, 1-1)	20% (7/35)
	<b>Annual Total</b>	<b>1.3 (1.3, 0-4)</b>	<b>28% (43/154)</b>	<b>2.3 (1.0, 1-9)</b>	<b>23% (33/141)</b>
2016	CTRL	2.6 (2.7, 0-5)	15% (14/96)	1.9 (1.0, 1-5)	18% (16/88)
	VR	2.7 (2.3, 0-7)	29% (21/73)	3.0 (2.0, 1-13)	12% (8/66)
	PTC	2.0 (2.3, 0-3)	0% (0/63)	0.0 (0.0, NA)	15% (8/55)
	PTC + VR	1.9 (2.0, 1-4)	4% (2/55)	1.0 (1.0, 1-1)	15% (8/53)
	<b>Annual Total</b>	<b>2.3 (2.0, 0-7)</b>	<b>13% (37/287)</b>	<b>2.5 (1.0, 1-13)</b>	<b>15% (40/262)</b>
2017	CTRL	1.3 (1.3, 0-4)	4% (2/46)	2.0 (2.0, 1-3)	22% (10/45)
	VR	0.9 (0.7, 0-2)	17% (4/24)	1.8 (1.5, 1-3)	26% (6/23)
	PTC	0.6 (0.3, 0-2)	5% (1/19)	1.0 (1.0, 1-1)	24% (4/17)
	PTC + VR	0.8 (0.7, 0-1)	15% (3/20)	1.3 (1.0, 1-2)	18% (3/17)
	<b>Annual Total</b>	<b>0.9 (0.7, 0-4)</b>	<b>9% (10/109)</b>	<b>1.6 (1.0, 1-3)</b>	<b>23% (23/102)</b>
2018	CTRL	2.6 (2.3, 0-5)	24% (24/101)	2.2 (1.0, 1-8)	15% (13/86)
	VR	2.0 (2.0, 0-4)	23% (14/60)	2.9 (2.0, 1-8)	12% (6/51)
	PTC	1.9 (1.7, 0-4)	0% (0/59)	0.0 (0.0, NA)	7% (4/55)
	PTC + VR	2.1 (1.7, 0-6)	7% (4/57)	1.5 (1.5, 1-2)	11% (6/55)
	<b>Annual Total</b>	<b>2.2 (2.0, 0-6)</b>	<b>15% (42/277)</b>	<b>2.3 (2.0, 1-8)</b>	<b>12% (29/247)</b>
Cumulative	CTRL	2.0 (1.7, 0-7)	25% (89/363)	2.3 (2.0, 1-9)	19% (62/334)
	VR	1.7 (1.3, 0-7)	25% (62/248)	2.6 (2.0, 1-13)	14% (31/224)
	PTC	1.7 (1.3, 0-6)	4% (11/255)	2.1 (2.0, 1-6)	10% (24/237)
	PTC + VR	1.7 (1.3, 0-6)	9% (20/235)	1.3 (1.0, 1-3)	11% (25/218)
	<b>Project Total</b>	<b>1.8 (1.3, 0-7)</b>	<b>17% (182/1101)</b>	<b>2.3 (2.0, 1-13)</b>	<b>14% (142/1013)</b>

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**Supplementary Table 2:** Mean (median, range) captures per plot trap day, infestation prevalence and mean (median, range) intensity of infestation of juvenile *I. scapularis* on *T. striatus*, and *B. burgdorferi* infection prevalence by treatment and year for *T. striatus*

		<i>T. striatus</i>				
		Treatment	Captures	Infestation Prevalence	Intensity of infestation	% Infected
2014	CTRL		0.8 (0.2, 0-3)	12% (2/17)	1.0 (1.0, 1-1)	60% (6/10)
	VR		0.1 (0.0, 0-1)	0% (0/4)	0.0 (0.0, NA)	50% (2/4)
	PTC		0.5 (0.0, 0-3)	42% (5/12)	1.2 (1.0, 1-2)	33% (2/6)
	PTC + VR		0.2 (0.0, 0-1)	25% (2/8)	2.0 (2.0, 1-3)	50% (3/6)
	Annual Total		0.4 (0.0, 0-3)	22% (9/41)	1.3 (1.0, 1-3)	50% (13/26)
2015	CTRL		0.6 (0.0, 0-4)	47% (9/19)	2.4 (1.0, 1-7)	35% (6/17)
	VR		0.3 (0.0, 0-1)	33% (2/6)	3.0 (3.0, 1-5)	40% (2/5)
	PTC		1.0 (0.3, 0-5)	27% (6/22)	1.7 (2.0, 1-2)	28% (5/18)
	PTC + VR		0.0 (0.0, 0-0)	0% (0/0)	0.0 (0.0, NA)	N/A
	Annual Total		0.5 (0.0, 0-5)	36% (17/47)	2.2 (2.0, 1-7)	33% (13/40)
2016	CTRL		0.4 (0.0, 0-2)	0% (0/13)	0.0 (0.0, NA)	33% (3/9)
	VR		0.03 (0.0, 0-0)	0% (0/1)	0.0 (0.0, NA)	100% (1/1)
	PTC		0.7 (0.3, 0-3)	0% (0/18)	0.0 (0.0, NA)	31% (4/13)
	PTC + VR		0.3 (0.0, 0-2)	38% (3/8)	1.0 (1.0, 1-1)	40% (2/5)
	Annual Total		0.4 (0.0, 0-3)	8% (3/40)	1.0 (1.0, 1-1)	36% (10/28)
2017	CTRL		0.6 (0.0, 0-6)	28% (5/18)	6.6 (2.0, 1-26)	46% (6/13)
	VR		0.9 (0.0, 0-4)	9% (2/22)	3.0 (3.0, 1-5)	38% (6/16)
	PTC		1.7 (0.8, 0-7)	3% (1/36)	1.0 (1.0, 1-1)	67% (18/27)
	PTC + VR		0.3 (0.3, 0-1)	22% (2/9)	1.0 (1.0, 1-1)	33% (1/3)
	Annual Total		0.9 (0.3, 0-7)	12% (10/85)	4.2 (1.5, 1-26)	53% (31/59)
2018	CTRL		0.7 (0.2, 0-4)	4% (1/25)	1.0 (1.0, 1-1)	52% (11/21)
	VR		0.9 (0.2, 0-5)	21% (5/24)	2.4 (2.0, 1-4)	59% (10/17)
	PTC		1.3 (1.2, 0-3)	9% (3/35)	1.3 (1.0, 1-2)	50% (11/22)
	PTC + VR		0.9 (0.0, 0-4)	14% (3/21)	1.3 (1.0, 1-3)	35% (6/17)
	Annual Total		0.9 (0.3, 0-5)	11% (12/105)	1.8 (1.5, 1-4)	49% (38/77)
Cumulative	CTRL		0.6 (0.0, 0-6)	18% (17/92)	3.4 (1.0, 1-26)	46% (32/70)
	VR		0.4 (0.0, 0-5)	16% (9/57)	2.7 (2.0, 1-5)	49% (21/43)
	PTC		1.1 (0.7, 0-7)	12% (15/123)	1.4 (1.0, 1-2)	47% (40/86)
	PTC + VR		0.3 (0.0, 0-4)	22% (10/46)	1.3 (1.0, 1-3)	39% (12/31)
	Project Total		0.6 (0.0, 0-7)	16% (51/318)	2.3 (1.0, 1-26)	46% (105/230)

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**Supplementary Table 3:** Mean (median, range) captures per plot trap day, infestation prevalence and mean (median, range) intensity of infestation of juvenile *I. scapularis* on *P. leucopus*, and *B. burgdorferi* infection prevalence by treatment and month for *P. leucopus*.

		<i>P. leucopus</i>			
	Treatment	Captures	Infestation Prevalence	Intensity of infestation	% Infected
June	CTRL	1.3 (1.2, 0-4)	20% (15/76)	2.5 (1.0, 1-9)	13% (9/69)
	VR	0.8 (0.7, 0-2)	33% (13/40)	1.7 (2.0, 1-4)	12% (4/34)
	PTC	0.8 (0.3, 0-3)	11% (4/37)	2.8 (2.0, 1-6)	6% (2/33)
	PTC + VR	1.1 (1.0, 0-3)	10% (5/48)	1.2 (1.0, 1-2)	16% (7/44)
	Monthly Total	1.0 (0.7, 0-4)	18% (37/201)	2.1 (1.0, 1-9)	12% (22/180)
July	CTRL	2.9 (2.7, 0-7)	9% (16/171)	1.3 (1.0, 1-3)	22% (35/158)
	VR	2.7 (2.2, 0-6)	9% (11/126)	1.5 (1.0, 1-5)	15% (17/117)
	PTC	2.5 (2.5, 0-6)	1% (1/130)	1.0 (1.0, 1-1)	10% (12/123)
	PTC + VR	2.2 (1.8, 0-6)	4% (4/96)	1.3 (1.0, 1-2)	10% (9/88)
	Monthly Total	2.6 (2.7, 0-7)	6% (32/523)	1.3 (1.0, 1-5)	15% (73/486)
August	CTRL	1.8 (1.5, 0-5)	50% (58/116)	2.5 (2.0, 1-8)	17% (18/107)
	VR	1.7 (1.0, 0-7)	46% (38/82)	3.3 (2.0, 1-13)	14% (10/73)
	PTC	1.8 (2.0, 0-5)	7% (6/88)	1.8 (1.5, 1-3)	12% (10/81)
	PTC + VR	1.7 (1.5, 0-5)	12% (11/91)	1.4 (1.1, 1-3)	10% (9/86)
	Monthly Total	1.8 (1.3, 0-7)	30% (113/377)	2.6 (2.0, 1-13)	14% (47/347)

**Supplementary Table 4:** Mean (median, range) captures per plot trap day, infestation prevalence and mean (median, range) intensity of infestation of juvenile *I. scapularis* on *T. striatus*, and *B. burgdorferi* infection prevalence by treatment and month for *T. striatus*.

		<i>T. striatus</i>			
	Treatment	Captures	Infestation Prevalence	Intensity of infestation	% Infected
June	CTRL	0.8 (0.2, 0-6)	28% (11/40)	4.7 (2.0, 1-26)	45% (14/31)
	VR	0.6 (0.0, 0-4)	40% (9/23)	2.7 (2.0, 1-5)	50% (8/16)
	PTC	1.1 (0.3, 0-7)	21% (8/39)	1.4 (1.0, 1-2)	26% (16/28)
	PTC + VR	0.3 (0.0, 0-2)	40% (6/15)	1.2 (1.0, 1-2)	25% (2/8)
	Monthly Total	0.7 (0.0, 0-7)	29 (34/117)	2.8 (2.0, 1-26)	45% (37/83)
July	CTRL	0.8 (0.3, 0-4)	8% (3/36)	1.0 (1.0, 1-1)	45% (13/29)
	VR	0.7 (0.0, 0-5)	0% (0/30)	0.0 (0.0, NA)	46% (11/24)
	PTC	1.5 (0.8, 0-5)	2% (1/56)	2.0 (2.0, 2-2)	51% (21/41)
	PTC + VR	0.5 (0.0, 0-4)	0% (0/19)	0.0 (0.0, NA)	40% (6/15)
	Monthly Total	0.9 (0.3, 0-5)	3% (4/141)	1.25 (1.0, 1-2)	47% (51/109)
August	CTRL	0.3 (0.0, 0-1)	19% (3/16)	1.0 (1.0, 1-1)	50% (5/10)
	VR	0.1 (0.0, 0-1)	0% (0/4)	0.0 (0.0, NA)	67% (2/3)
	PTC	0.6 (0.3, 0-3)	21% (6/28)	1.3 (1.0, 1-2)	35% (6/17)
	PTC + VR	0.2 (0.0, 0-1)	33% (4/12)	1.5 (1.0, 1-3)	50% (4/8)
	Monthly Total	0.3 (0.0, 0-3)	22% (13/60)	1.3 (1.0, 1-3)	45% (17/38)