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ORIGINAL ARTICLE

Topical Treatment of White-Tailed Deer with an Acaricide for the Control of *Ixodes scapularis* (Acari: Ixodidae) in a Connecticut Lyme Borreliosis Hyperendemic Community

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Abstract

The 4-Poster device for the topical treatment of white-tailed deer, *Odocoileus virginianus* (Zimmermann), against ticks using the acaricide amitraz, was evaluated in a Lyme borreliosis endemic community in Connecticut. As part of a 5-year project from 1997 to 2002, 21–24 of the 4-Posters were distributed at residential sites in Old Lyme, CT, in a core treatment area of $\approx 5.2\,km^2$ in fall 1997. The 4-Posters were active October to mid-December and March into May, corresponding to the peak periods of activity for adult *Ixodes scapularis* in this particular area. Corn consumption ranged from 361 to 4789 kg/month for October and November and 696–3130 kg/month during April. Usage of 4-Posters by deer generally was high (>90%), except during acorn masts in fall 1998 and 2001. Amitraz was applied by rollers at the estimated rate of 1.3 g active ingredient/ha/year. The abundance of host-seeking *I. scapularis* nymphs declined significantly ($p < 0.001$) in the core treatment area, as compared to a control community in Old Saybrook, CT, through 2004, over the project period from 1998 to 2003, from $9.3/100m^2$ to $0.97/100m^2$, rising to $1.90/100m^2$ in 2004. From 1999 through 2003, there were 46.1%, 49.6%, 63.4%, 64.6%, and 70.2% reductions, respectively, in the nymphal tick population in comparison with the untreated community and initial tick abundance in 1998. Control of *I. scapularis* adults declined to only 19.1% in 2004; 2 years after the treatment of deer was discontinued. Differences in nymphal tick abundance between the control and core treatment area were significant in 1999 ($p = 0.042$) and highly significant in 2001 ($p < 0.001$) and 2002 ($p = 0.002$). The passive topical application to deer of the acaricide amitraz resulted in a significant decrease in the population of free-living *I. scapularis* nymphs in the treated core in Connecticut.

Key Words: 4-Poster—Acaricide—Host-targeted—*Ixodes scapularis*—Lyme disease—*Odocoileus virginianus*—Tick-borne disease—Tick control—White-tailed deer.

Introduction

White-tailed deer, *Odocoileus virginianus* Zimmermann, are the principal host for the adult stage of the blacklegged tick, *Ixodes scapularis* (Main et al. 1981), and tick populations are closely correlated with the abundance of this host and, indirectly, the transmission of *Borrelia burgdorferi* and the incidence of Lyme borreliosis (Wilson et al. 1985, 1990, Stafford et al. 1998). The role of the blacklegged tick as a vector of human pathogens, the incidence of these diseases in the United States, and the central role of deer in maintaining populations of these ticks are detailed elsewhere (Fish and Childs 2009), and we present only specific information with regard to Connecticut.

In Connecticut, deer abundance increased from an estimated 49,472 in 1996 to 76,344 in a 1999/2000 aerial survey (Gregonis 2000). The treatment and control sites in Old Lyme and Old Saybrook, respectively, are endemic for Lyme borreliosis with incidences before the start of the project in 1996 of 765 and 272 cases per 100,000 population, respectively (Connecticut Department Public Health, www.dph.state.ct.us). The purpose of this study was to evaluate the 4-poster device for the topical treatment of white-tailed deer against *I. scapularis* as part of a northeastern regional tick management

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project with other 4-posters sites in Rhode Island, New York, New Jersey, and Maryland (Pound et al. 2009a).

Materials and Methods

Study sites

The treatment site was a neighborhood in the southern area of the town of Old Lyme, CT, of approximately 576 ha (2.4 by 2.4 km) ($\approx$2.2 mi^2) and bordered by I-95 on the north, Mile Creek Road on the west, Route 156 on the south, and Four Mile River Road on the east (centered $\approx$ latitude 41°18.389′, longitude 72°16.664′). A peripheral zone without 4-Posters ranging 2.4 km from the core was used to provide a measure of a gradient of control outward from the core treatment area. The control area (no 4-Posters) was a neighborhood along Schoolhouse Road north of I-95 in the town of Old Saybrook covering an area of $\approx$331 ha (1.3 by 2.5 km) ($\approx$1.28 mi^2) (centered $\approx$ latitude 41°18.498′, longitude 72°24.972′). This town is separated from Old Lyme by the Connecticut River. All the devices were established on private residential property (with landowner permission).

Placement of 4-Posters

Twenty-three 4-Posters were placed at 23 residential sites in September 1997, set in woodlands behind or adjacent to the landscaped residence, and filled with whole kernel corn. Corn and apples were also placed on the ground to serve as attractant during an initial acclimation period of 2–3 weeks before the addition of acaricide. At the request of several homeowners, we painted all the devices black to make them less conspicuous. An additional device was added September 1998. Observations on the rate of corn consumption, direct observations of deer and other animals at the devices, observations on acaricide use from the rollers, and other information were used to optimize or adjust placement and service of the 4-Posters and provide an indication of usage. Each cohort of adult *I. scapularis* is active in the fall and following spring season (Daniels et al. 1989), the 4-Posters were active during five fall-spring seasons of adult tick activity.

Devices with relatively low deer usage (i.e., infrequent visits <1–2 weeks) were relocated to high deer use (i.e., 6–7 visits/week) areas in December 1998 for a total of 16 sites of which 8 had two feeders. Beginning fall 1999, there were 16 active sites with 7 having two feeders located at each site for a total of 23 feeders. At the end of fall 1999, one 4-Poster was shut down based on a record of low corn consumption and on telemetry observations of deer movement and response to the devices within the area (Williams 2000). Only 22 feeders at 15 sites were in use in spring and fall 2000 and 2001, and spring 2002. Consequently, 7 of the 15 sites had two feeders at each site for over half of the study period.

Application of amitraz

An experimental use permit (No. 11312-EUP-101) was obtained from the U.S. Environmental Protection Agency for the application of Point-Guard® (Hoechst Roussel Vet, Somerville, NJ) (2% amitraz) to white-tailed deer. Deer were treated topically with amitraz during the principal activity periods of adult *I. scapularis* in the fall and spring ($\approx$151 days), which in Connecticut is October to December, when temperatures usually become too cold for tick activity, and March into May (Table 1). Initially, 25 mL of amitraz was applied per roller per week or 100 mL per 4-Poster (Table 1). Point-Guard was not added to the rollers until late October the second year due to poor usage during a major acorn mast. Only 2.3 L of Point-Guard was applied to the rollers that fall to six active 4-Posters. During the third year of the project, as described in detail in Pound et al. (2009a), 25 mL of the acaricide was placed on each of the larger rollers weekly during October and increased to 40 mL/roller in November and December. The rate of 40 mL of acaricide per roller per week was maintained through fall 2000. Fifty milliliters of the acaricide was placed on each roller during March 2001 and increased to

Table 1. Period of Amitraz Application, Amount of Amitraz Applied, and Deer Usage of the 4-Posters in Old Lyme, CT, 1997–2002

Year	Season	Treatment period[a]	No. of 4-Posters	Amount applied: Per roller (mL)	Amount applied: Total (L)	No. (%) of deer marked[b]
Year 1	Fall 1997	October 9–December 15	23	25	25.0	20 (62.5)
	Spring 1998	March 11–May 26	23	25	12.3	9 (90)
Year 2	Fall 1998	October 20–December 8	24	25	2.3[c]	—
	Spring 1999	March 31–April 27	23	40	3.3	9 (69)
Year 3	Fall 1999	October 7–December 16	23	25–40	28.3	11 (92)
	Spring 2000	April 13–May 1	22	40	10.7	17 (94)
Year 4	Fall 2000	October 11–December 11	21	40	21.1	25 (93)
	Spring 2001	March 28–May 8	22	50–60	30.2	—
Year 5	Fall 2001	October 8–November 24	22	50	22.0	10 (43)
	Spring 2002	March 17–April 26	22	50	25.0	—

[a]Period when amitraz was applied to the roller, does not include period of acclimation or other days when 4-Posters were baited with corn without acaricide on the rollers.
[b]Number of marked deer observed by spotlight after placement of marker rollers on the 4-Posters; specific observation dates are found in the text.
[c]Applied to only six devices.

60 mL per roller in April and May. In fall 2001 and spring 2002, 50 mL weekly/roller was applied to the rollers.

Impact on deer

Deer were captured in both treatment and control areas by remote immobilization with a dart gun, as described in detail in Pound et al. (2009a), from a tree over bait during the first 2 years of the project. The Connecticut Agricultural Experiment Station's Institutional Animal Care and Use Committee and the Wildlife Division of the Connecticut Department of Environmental Protection approved all Connecticut work with deer. Because no deer could be obtained from Old Saybrook control area in 1997, deer sampling was transferred to the New York site [see Daniels et al. (2009), this issue] to increase sample size and measure the amount of amitraz on the hair coat of treated animals. Amitraz residues were sampled using filter paper wipes from the head, foreleg, and groin area of six deer in the core treatment area (Bedford, NY) in October 1998. Another sample was taken from 18 deer in Bedford in October 1999 and delivered to the Connecticut Agricultural Experiment Station. Samples were taken by rubbing 5.5-cm circular filter paper (Whatman No. 5) over approximately 58 cm^2 (9 in^2) of hair surface and frozen until analyzed for the presence of amitraz. Sample wipes were folded into 40 mL screw cap vials with 5 mL hexane overnight. Samples were analyzed with two separate gas chromatographs (GC); Hewlett-Packard GC 5890 with a mass selective detector and SPB-5 column (No. 2-4035; Supelco, Bellafonte, PA) or (Varian GC, Palo Alto, CA) with ion trap detector and DB-608 column (J&W 123-1730, Santa Clara, CA). An amitraz standard in hexane was prepared from stock (0.01 g/10 mL) for each run, diluted to 1.00, 0.20, and 0.02 mL/10 mL. Results in parts per million were multiplied by the 5 mL hexane in sample extraction and reported in μg/sample.

Observations of deer usage and population estimates

The amount of corn consumed was recorded weekly to determine the relative usage of the feeders by deer. The percentage of deer using the feeders was estimated by comparing the number of deer marked versus unmarked during spotlighting observations. Extra rollers were coated with a combination of petroleum jelly and microglass beads, and the consistency of the mixture was adjusted with mineral oil at lower temperatures. Marking agent rollers were placed on the feeders 2 days before making observations. Deer were observed from a vehicle at night, traces of the reflective marking agent being readily visible with a spotlight and binoculars. In fall 1997, there were three observation periods: 3 (50%), 5 (36%), and 12 (100%) deer were marked on October 8, November 6, and December 20, respectively. Other specific observation dates were 12 April 1998, 27 March and 7 November 1999, and 21 March and 30 November 2000. Two observations were made fall 2001, 4 November and 8 December 2001, with 7 (47%) and 3 (38%) marked, respectively (Table 1).

Deer census

The distribution and abundance of deer in the treatment and control region was estimated by aerial survey counts during the winter months when there was >10 cm of snow cover for visual contrast. The method followed was similar to Beasom et al. (1981). Counts were conducted on 26 February 1999 and on 22 January 2004 by helicopter flying 200-m-wide north–south transects, respectively, at an elevation of 60 m and an air speed of approximately 43 km/h. Deer were counted and locations recorded on a topographic map. In 1999, the area surveyed was 12.0 km^2 focusing primarily on 7.38 km^2 around the core in Old Lyme and 5.82 km^2 in Old Saybrook. In 2004, an area of 12.0 km^2, again focusing on the core 7.38 km^2 area, and 7.92 km^2 was surveyed for Old Lyme and Old Saybrook, respectively.

The difference between the number of animals seen and true population size is the visibility bias (Dunn et al. 2002). The standard correction factor of 0.80 used by wildlife agencies was used for the 1999 population estimates. To calculate a correction factor in 2004, the helicopter was flown over a control site of known deer population size in Bridgeport, CT (Stafford et al. 2003), which contained 41 animals at the time of the 2004 survey. This resulted in correction factor of 0.73 (30 of 41 or 73% of deer were observed).

Tick surveillance

Relative tick abundance of larval and nymphal *I. scapularis* was monitored from summer 1998 through summer 2004, by dragging the leaf litter and vegetation with a 1×1.2 m white flannel-like cloth attached along one side to a wooden dowel to form a tick drag (Falco et al. 1992). Sample plots (each 100 m^2) were established in the woodlands at properties through the core treatment area, outside the treatment area, and within the control community. Each plot was sampled by one pass over the entire area and checking the drag at each 10-m segment. There were 16 sample plots within the core treatment area, 4 in the peripheral zone outside the core treatment area, and 12 sample sites in the control area, which was increased to 14 plots in 2002. Only six sample sites outside the core area were established at the beginning of the study because of dense vegetation at most of the residential properties adjacent to the core treatment area. No suitable sites were available to the north or south of the treatment core. One property became unavailable beyond 1998, and an additional plot was established at Rocky Neck State Park to the east of the core treatment area to compensate. However, very few ticks were recovered over the entire project period at the state park plots (14 nymphs and 133 larvae), and these sites were dropped from the final analysis. Of the remaining four outer core sites, three were on the west side of the core treatment area, where many deer visiting the western 4-Posters reside as indicated from telemetry and aerial survey data. The area to the west of the core is relatively undeveloped with extensive woodlands, a river, and associated wetlands. Ticks were sampled biweekly from May through August for the nymphal and larval stages of *I. scapularis* and two to three times during the fall and spring peak activity periods for adult *I. scapularis*. Ticks were removed from the tick drag, counted, placed in vials, and returned to the laboratory.

Infection rates in ticks

A subsample of the nymphal ticks ($n = 2328$) was tested for *B. burgdorferi* by indirect fluorescent antibody staining of tick mid-gut tissues with murine monoclonal antibody H5332, directed to outer surface protein A (OspA), and fluorescein-conjugated antibodies as previously described (Magnarelli et al. 1987).

Statistical analysis

Relative larval and nymphal tick abundance per 100 m^2 was compared between the treatment and control areas and between years by the nonparametric Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks using SigmaStat (SPSS, Chicago, IL) (Fox et al. 1995). Dunn's test was used for an all pairwise comparison of differences between experimental groups. Transformation of the counts was insufficient to normalize the data for a parametric analysis of variance. The season cutoffs were mid-May through July for the nymphs, except for 1998, 2000, and 2001, which included counts in the first week of August, and mid-July through August for the larvae, although mid-July counts were not used if no ticks were collected, and larval tick activity was not evident. The proportion of infected ticks between the treatment and control sites was compared with the z-statistic using SigmaStat.

Percent reduction was calculated using the average number of ticks collected per site visit using the following modification of Abbott's formula (Henderson and Tilton 1955):

$$\text{Reduction} = 100\ (1 - X_c Y_t / X_t Y_c)$$

where X_c and X_t are the pretreatment averages in the control and experimental properties, respectively, and Y_c and Y_t are the posttreatment averages in the control and experimental properties, respectively. Reductions are based on mean number of ticks per 100 m^2 during the peak seasonal activity of the nymphs (mid-May through July) and larvae (mid-July through August or just August, depending upon seasonal emergence).

Results

Corn consumption patterns and deer usage of the 4-Posters

Corn consumption patterns and observations of marked deer indicated that the local deer readily acclimated to the 4-Posters and usage was relatively high through most of the project. Average monthly corn consumption was highest in the first (1997–1998) and third (1999–2000) years, and lowest during the second year (1998–1999) of the project. The greatest use of corn was during October, November, and April, and the total amount of corn used during each month of 4-Poster activity is provided in Table 2. Corn consumption steadily increased from September through November 1997 and picked up the following spring for a total usage of 14,063 kg for the first treatment year. At the beginning of the project in fall 1997, usage rates initially were moderate, then declined, and subsequently peaked at 100% (50%, 36%, and 100% of observed deer had marking agent on them on 8 October, 6 November, and 20 December, respectively) (Table 1). The reduction in November was likely the result of cold temperatures preventing marking agent from being properly applied as corn consumption increased in November.

During the second year of the project, evidence of 4-Poster usage by deer was not detected until late October, and fall consumption was down by 82% from the first year. The abundance of acorns in fall 1998 may explain the substantial decline in corn consumption, and full utilization of the 4-Posters did not resume until the third year. No spotlight observations were taken because deer usage was very low based on low corn consumption and only six of the devices were active. Deer utilization largely rebounded the following spring with 69% of the deer observed ($n = 13$) marked in March and corn consumption increased. Usage of the 4-Posters by deer was not detected until late October in 1999, but a high percentage of the deer (92% of 12), as measured by marking agent, were utilizing the feeders by November, which recorded the greatest corn consumption (4789 kg) for the entire study. Corn usage varied considerably through the study area and, except for the second year, ranged from 4.5 to 336 kg per month at each site. Only five feeder sites, each with two 4-Posters, along the western or central parts of the core had consistent relatively high deer usage (greater than 680 kg/month) over the entire course of the project. These sites generally accounted for at least 42% of the total consumption each year. Deer usage continued to be above 90% in years 3 and 4 (Table 1). A moderate oak acorn mast was experienced for the last 2 years of the project, and the fall corn consumption declined accordingly during the peak months, although total corn consumption during the fourth year was only slightly less than the year before. Usage by deer was detected within days of filling the 4-Posters in fall 2000. This indicated that deer had become accustomed to the availability of food from the devices. Fewer (43% of 23) marked deer were observed in fall 2001, probably due to the moderate acorn mast.

TABLE 2. TOTAL MONTHLY CORN CONSUMPTION (KG) BETWEEN YEARS DURING KEY MONTHS OF OCTOBER–DECEMBER AND APRIL, AND FOR EACH ADULT TICK SEASON IN OLD LYME, CT, FROM 1997 TO 2002

	Year				
Month	*1997–1998*	*1998–1999*	*1999–2000*	*2000–2001*	*2001–2002*
September	1209	0	828	0	0
October	1879	361	3695	1533	1615
November	2723	381	4789	2028	1433
December	2601	742	1027	1538	1751
March	687	742	0	653	807
April	2533	696	1077	2699	3130
May	2431	0	755	635	485
Total September–December	8412	1484	10,339	5099	4799
Total March–May	5651	1438	1832	3987	4422

Over the course of the entire 5-year project, corn was available for 31 months; 47,463 kg was distributed at a cost of ≈$7.00/22.7 kg for a total expense of ≈$14,636.

Deer census

During the 1999 aerial survey, a total of 76 deer were counted, 70 within the 7.38 km^2 around the core treatment site, and 45 deer were counted in the control site in Old Saybrook. Of the 70 deer observed in Old Lyme, 48 (68.6%) were located in the western half of the core treatment site, corresponding with corn consumption at the 4-Posters. Few deer were observed in the forested wetlands along the western side of the study area. The density estimates for Old Lyme and Old Saybrook in 1999 were 11.58 deer/km^2 (30.7/mi^2) and 9.65 deer/km^2 (25/mi^2), respectively. In the 2004 survey, a total of 145 deer were counted, 120 within the core 7.38 km^2 in Old Lyme and 99 deer were observed in Old Saybrook. This provided an estimate of 198.17 (±28.2) and 135.3 (±23.2) deer in the treatment and control areas, respectively. Therefore, the deer density in Old Lyme in 2004 was estimated at 22.27 deer/km^2 (57.7/mi^2) and in Old Saybrook, 17.11 deer/km^2 (44.3/mi^2). Over the course of the project, the estimated deer population increased in Old Lyme by a factor of 1.88 and in Old Saybrook by a factor of 1.77.

Amitraz residues and use

In Old Lyme, five deer were tranquilized at 4-Poster sites from 23 October through 5 November 1997, examined for ticks, and released. An average of eight engorged female ticks (range 2–17) were observed, principally in the leg and groin area (75.8% of 41 ticks), suggesting some control in the head region. Unfortunately, no deer could be obtained from the control area for comparison. Consequently, a decision was made to obtain project pesticide samples for analysis by the Connecticut Agricultural Experiment Station from deer captured in New York. An average (SEM) of only 10.2 (3.1), 1.6 (0.3), and 0.97 (0.3) μg was detected from the head/neck, foreleg, and hindleg/groin wipes, respectively, of six New York deer in October 1998, despite the lack of a competing food source (i.e., acorns) and heavy usage of the feeders by the deer. By contrast, an average of 1573.25 (360.9) μg (range 3.1–3800 μg) was detected on the head region filter paper wipes from 18 deer in New York in October 1999. Of the 17 deer sampled (only a head sample was available for one animal), 7 had detectable residues on the groin wipe, and 10 had detectable residues on the foreleg wipe. An average of 68.3 (43.9) and 10.5 (5.0) μg amitraz was detected on the foreleg and groin wipes, respectively.

The change to a larger capacity roller and more frequent application of amitraz increased delivery of amitraz to the deer, especially to the head and neck region where the majority of *I. scapularis* attach on deer (Schmidtmann et al. 1998). In Old Lyme, ≈180 l of Point-Guard was dispensed over the course of the project (Table 1). With 0.07 kg of amitraz active ingredient (AI) per 3.78 L of product, an average of 0.66 kg AI was dispensed annually over 518 ha, or 1.29 g of amitraz AI/ha/year.

Impact on tick abundance

Although there was a statistically significant difference in larvae between years in the core treatment area ($H = 23.004$, df = 6, $p < 0.001$), the only difference that was significant in the all pairwise multiple comparison procedure (Dunn's method) was between 1998 and 2002. There was no significant difference in larvae between years for the outer core ($H = 4.198$, df = 6, $p = 0.650$). While there was a significant difference between years in the control ($H = 13.497$, df = 6, $p = 0.036$), differences in median values among years could not be distinguished by the Dunn's multiple comparison procedure at $p = 0.05$. The greatest difference in ranks was between 2003 and 2004, when the mean number of larvae collected increased to 108.8/100m^2, one of the highest annual mean densities recorded (Table 3). Larval distributions are extremely focal and a collection at or near an actual egg mass can skew the numbers (>1000) dramatically. From a different perspective, the proportion of plots in which larvae were recovered ranged from 43.8% to 53.6% in the core, except in 2002 and 2003, when larvae were detected in only 10.0% and 26.7% of the plots, respectively. In 2002, there was a significant difference between the core and control areas in the proportion of plots from which larvae were recovered ($z = 2.298$, $p = 0.022$). By 2004, a year in which the tick population was expected to rebound, larvae were recovered from 53.6% of the sampled plots in the core, the highest proportion for any year of the project. In the control, 38.9–52.4% of the plots produced larvae, except in 2000 and 2004 when larvae were recovered from 70.8% and 68.2% of the plots, respectively. There was no difference in the proportion of infested plots between the core and control in 2004 ($z = 0.755$, $p = 0.435$). In a few cases, the majority of all larvae collected were collected at one site or on one occasion. In 2000, one plot in the core accounted for 64.4% of 2763 larvae (with 1290 collected on one date), while the most that one plot contributed in the control was 37.2% of the 2372 larvae collected in 2000 and 46.4% of 2355 larvae collected in 2003. Control of larvae may have been higher than immediately apparent from the total numbers. Usage of the 4-Posters was particularly high in fall 1999 with larger capacity rollers being used, which should result in a notable impact on nymphs in 2001. Nymphal abundance was reduced in the core in 2001 (Table 3).

There was a significant difference between years for the nymphs in the core and outer core ($H = 129.856$, df = 6, $p < 0.001$; $H = 52.776$, df = 6, $p < 0.001$, respectively). The highest mean (and median) nymphal tick abundance in the core was observed in 1998 and 2000 with the lowest tick density observed in 2003 (Table 3). Pairwise comparisons (Dunn's method) for the core revealed that, with the exception of 2000, the years 1999–2004 were significantly different ($p < 0.05$) from 1998 and that there were no annual significant differences in tick density after 2000. Nymphal abundance in 1999 was not significantly different from that from 2001 to 2004, while 2000 was significantly different from 2001 through 2004. In the outer core area, median tick abundance in 1998 and 1999 was significantly greater than tick abundance in 2003 and 2004, and 2000 was significantly different from all subsequent years.

In the control, the highest tick densities were observed in 1998 and 2000 with 6.8 and 6.4 nymphs/100m^2, respectively (Table 3). By contrast, tick densities were the lowest observed thorough the study in 2003 and 2004, reflecting a general decline in tick abundance in portions of southeastern Connecticut in recent years (Stafford, unpublished data). The Kruskal–Wallis ANOVA on ranks indicated a statistical

TABLE 3. MEAN NUMBER OF *IXODES SCAPULARIS* NYMPHS AND LARVAE COLLECTED FROM CORE, OUTER CORE, AND CONTROL SITES, MAY THROUGH AUGUST 1998–2004, AND SAMPLE SIZE DURING THE PEAK TICK SEASON FOR NYMPHS AND LARVAE, RESPECTIVELY

Stage	*Year*	*No. sites*	n[a]	*Total*[b] *nymphs*	*Mean (SEM) nymphs/100 m*^{2}	n[a]	*Total*[b] *larvae*	*Mean (SEM) larvae/100 m*^{2}
Core	1998	16	80	746	9.32 (0.83)	32	2149	67.16 (13.47)
	1999	16	96	257	2.68 (0.35)	49	1736	35.43 (8.43)
	2000	16	75	333	4.44 (0.57)	30	2457	80.60 (43.84)
	2001	15	80	158	1.97 (0.40)	48	2199	45.81 (12.70)
	2002	15	75	73	1.67 (0.31)	30	430	14.67 (9.53)
	2003	15	75	75	0.97 (1.24)	27	887	29.57 (18.73)
	2004	15	73	139	1.90 (0.29)	28	1771	63.25 (22.59)
O. Core	1998	6	28	402	14.38 (3.48)	18	395	21.94 (12.23)
	1999	6	36	367	10.19 (3.74)	18	751	41.72 (27.58)
	2000	6	30	226	7.53 (1.50)	18	3463	288.58 (195.32)
	2001	6	30	73	2.43 (0.86)	12	130	7.22 (4.75)
	2002	6	29	138	4.76 (1.22)	18	589	47.00 (26.71)
	2003	6	30	39	1.30 (0.42)	12	614	51.17 (24.07)
	2004	6	30	112	3.73 (1.90)	12	616	51.33 (20.63)
Control	1998	9	30	205	6.83 (0.98)	18	483	26.83 (11.65)
	1999	12	72	246	3.42 (0.42)	36	3925	109.03 (33.04)
	2000	12	60	383	6.38 (0.69)	24	2359	58.29 (36.75)
	2001	14	70	264	3.88 (0.59)	42	1520	36.19 (7.83)
	2002	14	70	233	3.33 (0.42)	28	1446	51.64 (16.37)
	2003	14	66	151	2.29 (0.44)	27	2355	87.22 (52.05)
	2004	14	55	94	1.66 (0.28)	22	2794	108.82 (30.82)

[a]n = number of site visits, number sample plots × number of biweekly visits included in the calculation.
[b]Total number collected during peak season tick activity, not entire summer. Nymphs are based on mid-May through July counts, and larvae are based on mid-July through August counts. Core and outer core statistics do not include one site with larval count > 1000.

difference between years ($H = 48.252$, df = 6, $p < 0.001$), largely due to the lower number of ticks recovered in 2003 and 2004. Dunn's multiple comparison test detected differences between 2003 and 2004 at $p = 0.05$ with 1998, 2000, and 2001. In 1999, there was a significant difference between the core and control ($T = 6059.5$; $n = 72$, 80; $p = 0.042$). In 2001 and 2002, there was a highly significant difference in nymphal abundance between the core and control ($T = 6371.5$; $n = 70$, 79; $p < 0.001$ and $T = 5875.5$; $n = 70$, 75; $p = 0.002$, respectively). This reflects the years following the greatest treatment of deer with amitraz. The failure to detect a difference in nymphal abundance between the control and core treatment area in 2000 may be due to the lack of treatment of deer in fall 1998.

The overall reductions in larval numbers calculated between 1998 and 1999–2004 in the treatment core relative to the control was 87.0%, 75.0%, 49.4%, 88.6%, 86.5%, and 76.9%, respectively. The lowest mean larval numbers in the control and outer core were recorded in the first summer of sampling in 1998. By contrast, the lowest mean number of larvae from the core was in 2002. Except for 1999, no reductions in larval numbers were noted in the outer core in comparison with the control.

In the treatment core, nymphal tick abundance dropped substantially from 1998 to 1999, rose in 2000, and dropped to the lowest levels by 2002 and 2003. The reduction in the total number of *I. scapularis* nymphs from 1998 to 1999–2004 in the treatment core relative to the control was 46.1%, 49.6%, 63.4%, 64.6%, 70.2%, and 19.1%, respectively. Despite the regional decline in tick abundance, the lowest calculated reduction in nymphal abundance was during the final year of the project, 2 years after deer treatments were discontinued. Some reductions in the nymphs were noted in the outer core relative to the control in 2001 and 2003 of 51.7% and 60.5%, respectively.

Infection rate

Over the course of the study, the prevalence of *B. burgdorferi* in the nymphal ticks as detected by indirect fluorescent antibody did not differ between the treatment area (9.7% of 1554 tested) and the control area (8.3% of 774 tested) ($z = 1.023$, $p = 0.306$). Trends in the proportion of infected ticks for both sites were similar and were significantly different from each other only during 2000 with fewer ticks in Old Saybrook (5.1% of 254) containing spirochetes than in Old Lyme (13.3% of 346) ($z = 3.195$, $p = 0.001$). The highest rates were obtained in 1999 and 2002 with spirochetes observed in 17.6% of 216 and 14.5% of 234 nymphs tested, respectively.

Discussion

The passive treatment of deer with amitraz using the 4-Poster in Connecticut resulted in a decrease of about 64–70% in the population of *I. scapularis* nymphs, and a significant difference in the tick population between the treatment and control areas was observed in 3 years of the project. Overall, a similar reduction was documented for the five sites of the entire project [see Brei et al. (2009) and Pound et al. (2009b), this issue]. The impact appears to have flattened out at, as the level of control in 2002 was only 1.2% greater than 2001, but increased by 5.6% in 2003. Deer usage of the 4-Posters was initially moderate as the animals acclimated to the devices and then increased to over 90%. Based on the analysis of wipes of the hair on the deer at the New York study site using similar amitraz rates and treatment protocols as Connecticut, the amount of amitraz delivered to the animals was initially lower than expected. This was due in part to heavier than expected usage in New York and consequently, at times, insufficient material on the rollers combined with the density of the winter hair coat. This raised concern about the actual amount of amitraz deer were receiving at all project sites, including Connecticut. While control of the ticks on the deer was undoubtedly less than might have been achieved (ticks were recovered from the sample of treated deer in Old Lyme), fewer ticks were recovered in the head region, where most adult *I. scapularis* attach on deer, than in the groin. By late fall, most, if not all, of the deer were being treated based on observations of marked animals. Subsequently, there was a reduction in the number of nymphal ticks in the core treatment area 2 years following the 1997–1998 treatment season. This is concordant with an impact following through the 2-year tick life cycle. Clearly, the topical treatment of deer with an acaricide can reduce the abundance of host-seeking ticks.

The application issues were addressed by the second year with larger capacity rollers and, in Connecticut, reallocation of the feeders to maximize exposure based on corn consumption patterns, observed deer distributions from the aerial survey, and responses to bait based on radio telemetry data (Williams 2000). Two 4-Posters were placed at the most heavily frequented locations to help distribute usage among visiting animals. The residues obtained from the hair of deer at the New York site in 1999 with the new treatment protocols and rollers showed substantial increases from 1998 levels. However, in Connecticut, little treatment of deer was obtained in the second year due to competing food sources (i.e., acorns), which was probably reflected in higher nymphal tick numbers 2 years later in summer 2000. Nymphal tick numbers were high in the control area in 2000 as well. Larval numbers in the core treatment area had declined in 1999, while increasing at other sites. By fall 1999, deer usage of the 4-Posters had resumed high levels (>90%) and continued through spring 2001, based on visual observations of marked deer and corn consumption. A smaller acorn mast in fall 2002 reduced usage of the 4-Posters, but not as drastically as in fall 1998. Periodic acorn masts can severely impact the use and effectiveness of treating deer in northeastern states when corn is used as the attractant.

Oaks have periodic mast crops (years in which flower and acorn production is exceptionally high) every 2–11 years depending upon the species (red oak 3–5 years, black oak 2–3 years, and white oak 4–10 years) and growing conditions (Schopmeyer 1974). Red oak is the most common oak in Connecticut, and it takes 2 years to produce acorns. A lack of synchrony in mast between species means that the availability of acorns will vary geographically and temporally in areas where the 4-Posters are used. Indeed, that is what happened during the regional tick control project with some sites having no, a few, or several years of oak mast production. The Connecticut Department of Environmental Protection has been surveying hunter perceptions of the fall acorn crop since 1993. In 1998, 94% of hunters responding to the survey ranked the acorn crop as moderate to abundant, particularly in the region where our study was conducted (Kilpatrick et al. 1999). Availability of alternative food resources will have to be considered in use of the 4-Poster to treat deer.

Other major issues with the project concern the volume of corn consumed and its impact on the local deer population and the pesticide that will be used as this technology moves forward. There may be concern by wildlife officials or agricultural groups about the amount of corn consumed and its impact on the deer population. However, supplemental feeding of free-ranging deer with corn has been found to have only a slight positive impact on survival and little effect on migration (Lewis and Rongstad 1998). Survival increased with autumn or winter supplemental feed only during the severest winters. Deer abundance increased in both Old Lyme and Old Saybrook study sites over the course of the study, but by similar rates (1.9 and 1.8, respectively), although other factors such as any differences in mortality rates could influence the apparent population growth. Similarly, the impact of feeders on home range is minimal (Lewis and Rongstad 1998, Williams 2000, Kilpatrick 2002). In a study in Groton, CT, Kilpatrick and Spohr (2002) found that feeders had no effect on home range or core-area size, but that feeders could shift core areas of activity within deer home ranges. In Old Lyme, 11 deer within the treatment area were fitted with radio transmitters, and telemetry data were collected over a 1-year period (January–December 1999) during 4-Poster dormancy and activity (Williams 2000). The mean annual home range was found to be 57.7 ha, ranging from 34.7 to 100.1 ha, with a mean core area size of 8.4 ha (range 4.4–13.0 ha). With multiple active 4-Posters available in most home ranges, every deer showed some spatial home range movement in response to a 4-Poster within their home range, including core area shifts, addition of another core area closer to a second active 4-Poster, and a reduction of home range and core area around an active 4-Poster. Williams and DeNicola (2000) concluded that if bait site overlap is not desired, the 4-Posters should be distributed every 50–60 ha and placed in wooded areas to shift core areas away from residences. No deer in the Groton or Old Lyme study used bait stations or 4-Posters outside their annual home range (Williams 2000, Kilpatrick 2002).

Point-Guard is no longer available commercially. Amitraz was selected for application to deer in the regional tick project because this acaricide can be used on beef and dairy cattle and swine with no withholding period for milk or posttreatment slaughter. Preliminary laboratory trials with topical application of 2% amitraz on mice found that protection against *I. scapularis* nymphs dropped from 100% on day 1 to 68%, 50%, and 8% by 7, 14, and 21 days posttreatment, respectively (G.O. Maupin, unpublished data). A 64–70% reduction was obtained with the dispersal of $\leq$1.3 g amitraz AI/ha/year and confined to deer, not broadcast through the environment. This does not include product remaining on the rollers and discarded or material delivered to nontarget animals such as raccoons. By contrast, calculated application rates for broadcast sprays with the synthetic pyrethroids cyfluthrin, cyhalothrin, deltamethrin, and permethrin are $\approx$51.6, 38.6, 90.0, and 476.0 g AI/ha/year, respectively (assuming 1 application/year for control of *I. scapularis* nymphs at lowest label rate). A longer acting or more effective compound could either reduce the frequency of application and the volume of feed needed to attract and keep deer visiting the device or improve control. Solberg et al. (2003) found that the application of 10% permethrin (Brute®; Y-Tex, Cody, WY) to deer provided 100% control of ticks on deer, 91–100% control of questing adult, nymphal, and larval *I. scapularis* and 70–95% reduction on white-footed mice.

It was initially unclear if the treatment of deer had impacted the incidence of Lyme borreliosis in the core treatment area. The overall incidence of Lyme borreliosis had generally declined in both Old Lyme and Old Saybrook over the period of the project. The incidence of Lyme disease in Old Lyme and Old Saybrook in 1996 was 765 and 272 cases per 100,000 population declining in 2002 to 392 and 145 per 100,000 population, respectively. However, it was possible to determine the incidence of Lyme borreliosis in the treatment, control, and other untreated areas from active Lyme borreliosis surveillance data, and a significant difference was found in the erythema migrans cases of Lyme borreliosis before and after the 4-posters were used in the treated community and between the treated community and elsewhere in Old Lyme (Garnett, Connally, Stafford, and Cartter, unpublished data). The computer simulation model LYMESIM (Agricultural Research Service, U.S. Department of Agriculture, Gainesville, FL) was developed to assess the population dynamics of *I. scapularis* and transmission of *B. burgdorferi* (Mount et al. 1997a). Simulations of various management strategies with the model have indicated that the acaricidal treatment of deer to be the most cost-effective, long-term strategy of choice for large areas that could prevent the most cases of Lyme borreliosis (Mount et al. 1997b, Hayes et al. 1999). A 10-year simulation run assuming 95% tick mortality on treated deer indicated that 70% to over 90% of the deer population in a targeted area must be treated to produce a large reduction in the density of infected nymphs (Mount et al. 1997b). Treatment of 90% of the deer reduced nymphal density by 95% in year 5, while nymphs were reduced by only 84% after 10 years when only 70% of the animals are treated. In a simulation of eight interventions in a hypothetical community of 10,000 people over 4 years with an annual Lyme borreliosis incidence of 0.01 cases per person per year (Hayes et al. 1999), the treatment of deer prevented most cases of Lyme borreliosis under all scenarios except the best use of a human Lyme borreliosis vaccine, which is no longer available. The reduction of 64–70% achieved by years 5 and 6 under field conditions in Connecticut may reflect lower tick mortality on the deer due to efficacy in the distribution and residual presence of amitraz on treated or partly treated animals, as usage was generally high except for those seasons with an acorn mast. Reduction in risk of Lyme borreliosis would be associated with the decline in the tick population, as the prevalence of *B. burgdorferi* between the treated and untreated sites was, except for 1 year, statistically the same. There was no indication of a short-term rise in tick infection rates with the treatment of deer, which has resulted from actual and computer-simulated herd reductions (Gray et al. 1992, Mount et al. 1997b, Stafford et al. 2003, Rand et al. 2004).

In Connecticut, the deer population has risen from an estimated 49,472 animals in 1993 to approximately 76,000 in 1999–2000 and 2003 (Gregonis 2000, 2003), with some of the highest densities in southwestern portion of the state where deer management options are limited. The rising deer population has led to increased vehicle collisions and excessive damage to forests, agricultural crops, nursery stock, and landscape plantings (Warren 1997), but an increasing impetus for managing urban and suburban populations of deer appears to be the rising incidence of Lyme borreliosis. The exclusion or reduction of deer has been shown to reduce tick abundance (reviewed in Stafford and Kitron 2002). Exclusion by fencing is expensive and may be limited by local statutes. The reduction or virtual elimination of these animals is controversial, and it may be difficult to reduce deer sufficiently to affect tick populations or the dynamics of disease transmission except on islands or other geographically isolated tracts. It is probable that the high incidence of Lyme borreliosis will continue or rise in the absence of an acceptable vaccine or vector management program. In summer 2003, the U.S. Environmental Protection Agency registered a 10% topical permethrin formulation for application to deer to control ticks via the 4-Poster device (4-Poster™ Tickicide; Y-Tex), pesticide registrations have been obtained in at least 33 states, and the 4-Poster is commercially available (David Weld, American Lyme Disease Foundation, personal communication).

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Disclosure Statement

No competing financial interests exist.

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