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Control of Immature *Ixodes scapularis* (Acari: Ixodidae) on Rodent Reservoirs of *Borrelia burgdorferi* in a Residential Community of Southeastern Connecticut

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ABSTRACT A 3-yr community-based study was conducted on residential properties on Mason's Island, Mystic, CT, to determine the efficacy of a rodent-targeted acaricide (fipronil) to control immature *Ixodes scapularis* (Say) on *Peromyscus leucopus*. Results indicated that modified commercial bait boxes were effective as an acaricide delivery method for reducing nymphal and larval tick infestations on white-footed mice by 68 and 84%, respectively. Passive application of fipronil significantly reduced the infection rate of *Borrelia burgdorferi* among white-footed mice by 53%. Moreover, the abundance of questing *I. scapularis* adults on treated properties was reduced by 77% and fewer were infected with spirochetes (31%) compared with untreated sites (47%) after 3 yr of treatment. Likewise, the abundance of host-seeking nymphs was significantly reduced on treated properties by >50%. Finally, infection rates in flagged nymphal ticks for both *B. burgdorferi* and *Anaplasma phagocytophilum* were reduced by 67 and 64%, respectively, after only 2 yr of treatment. Results from this 3-yr trial indicate that the use of fipronil passively applied to reservoir animals by bait boxes is an environmentally acceptable means to control ticks, interrupt the natural disease transmission cycle, and reduce the risk of Lyme disease for residents of treated properties.

KEY WORDS *Ixodes scapularis*, *Borrelia burgdorferi*, host-targeted, fipronil

LYME DISEASE HAS BECOME the most common vector-borne disease in the United States (Dennis 1995). The number of reported Lyme disease cases reached an all-time high in 2002 with 23,763 cases (Centers for Disease Control 2004). The blacklegged tick, *Ixodes scapularis* (Say), serves as the principal vector in the northeastern United States. Due to their epidemiological importance in Lyme disease transmission (Piesman et al. 1987), nymphal *I. scapularis* are the primary targets for control (Schulze et al. 2001). Moreover, the discovery that *I. scapularis* transmits several diseases to humans in addition to Lyme disease, including babesiosis, and human granulocytic ehrlichiosis (Lane et al. 1991, Goodman et al. 1996, Stafford et al. 1999), developing strategies that are effective and environmentally acceptable for the control of *I. scapularis* has become an important public health issue in many residential and recreational areas of the United States (Lane et al. 1998).

Applications of insecticides and acaricides are frequently proposed as the primary means of reducing exposure to tick-borne diseases. Several studies have demonstrated the effectiveness of areawide applications of acaricides for controlling host-seeking *I. scapularis* nymphs (Schulze et al. 1991, 1994; Stafford et al. 1991a, b; Solberg et al. 1992; Curran et al. 1993), although these efforts were seasonal and short term. Schulze et al. (1987, 1988) sought to control adult populations of *I. scapularis* by using ground spraying equipment to apply diazinon and carbaryl to natural habitat. Additional studies incorporated granular formulations of carbaryl, chlorpyrifos, and diazinon to effectively reduce immature *I. scapularis* (Schulze et al. 1991, Stafford et al. 1991a). The application of areawide pesticides, however, is not widely accepted due to growing public concerns about adverse environmental effects, toxicity, and impact on nontarget organisms (Schmidtmann 1994, Fish 1995, Gage et al. 1997, Lane et al. 1998, Schulze et al. 2001). Alternative methods to control Lyme disease vector ticks include vegetation management and landscape modifications, reduction of host species populations,

G.O.M. and M.C.D. are coinventors on three patents pending: 1) Control of Arthropod Vectors of Parasitic Diseases U.S. Patent Ser. No.09/595,034, 2) Control of Arthropods on Rodents U.S. Patent Ser. No. 09/595,177, and 3) Methods to Control Arthropods. A licensing agreement has been signed between the Centers for Disease Control and Prevention and Bayer Environmental Science.

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and biological control (Wilson et al. 1988, Deblinger et al. 1993, Piesman and Gray 1994).

Novel approaches to controlling tick populations include host-targeted methods. These methods are desirable due to the reduced risk of acaricide exposure to nontarget species and minimal environmental contamination. In general, these host-targeted methods require a great deal less pesticide to be effective compared with areawide application techniques (Mather et al. 1987, 1988; Gage et al. 1997; Lane et al. 1998). However, for host-targeted techniques to be effective, an efficient means of applying or delivering acaricides to host species is required. For example, several studies were carried out using permethrin-treated cotton nesting material to control ticks and fleas on rodent hosts. This method has been used to take advantage of nest-building behaviors of white-footed mice, *Peromyscus leucopus* (Mather et al. 1987, 1988; Deblinger et al. 1991; Stafford 1991b), woodrats, and/or other species of *Peromyscus* (Sonenshine and Haines 1985, Beard et al. 1992, Leprince and Lane 1996). Gage et al. (1997) developed a rodent-targeted bait tube approach for controlling ectoparasitic disease vectors by passive application of liquid permethrin. This method resulted in a 99% reduction of *Ixodes spinipalpis* (Nuttall) and *Dermacentor andersoni* (Stiles) and 11 species of fleas on Mexican woodrats (Baird) throughout 12 mo of study in Colorado. Lane et al. (1998) reported comparable results by using a similar permethrin-treated bait tube technology on woodrat reservoirs and Lyme disease vectors in California.

This article describes a 3-yr study using a host-targeted method for controlling immature *I. scapularis* parasitizing white-footed mice on Mason's Island, Mystic, CT. The efficacy of a rodent-targeted acaricide (fipronil, 0.75% active ingredient [AI] topical formulation) was evaluated. In addition, the utility of modified commercially available Protecta Jr. bait boxes as an acaricide delivery method was determined as was the ability of fipronil to impact the natural transmission cycle of tick-borne pathogens, primarily *Borrelia burgdorferi* and *Anaplasma phagocytophilum*. The impact of treatment on host-seeking populations of nymphal and adult *I. scapularis* was evaluated. The principal goal of the current study was to test the hypothesis that passively applied fipronil in a rodent bait box effectively reduced the number of host-seeking *I. scapularis.*

Materials and Methods

Laboratory Bioassay of Fipronil. Laboratory bioassays were conducted to determine the lowest concentration of 20 μl of fipronil formulation required to kill ≥90% of *I. scapularis* nymphs on treated outbred mice through 42 d posttreatment. Four test groups ranging in concentration from 0.43 to 1.0% and one control group consisting of five mice each (25 mice in total) were treated with 20 μl of fipronil applied with a pipette between the scapulae. Mice were challenged with 10 nymphal *I. scapularis* at 14, 28, and 42 d after treatment.

Study Site. This field trial was conducted from April through September on Mason's Island, New London County. Mason's Island is a coastal island in southeastern Connecticut ≈4.5 by 3.4 km and predominated by oak hardwoods. Individual properties varied from 1/10 to 2.23 hectares. Figure 1 shows the locations of fipronil-treated properties and untreated control areas, and Table 2 describes the bait box design, fipronil formulation, and number of treated properties evaluated from 1999 to 2001 on Mason's Island., Mystic, CT. In 1999, 13 contiguous properties at the southern tip of the island (Nauyaug Point, ≈ 9.9 ha) received fipronil-treated bait boxes. In May 2000, six properties located on the northern-most part of the island and an additional 25 properties near the center of the island received fipronil-treated bait boxes (designated New Areas). This increased the number of fipronil-treated properties to a total of 44 developed land parcels (≈40 ha). In May 2001, the number of fipronil-treated properties was expanded to a total of 154 properties (≈150 ha; additional 110 properties designated new areas 2001). Five sites located in the undeveloped natural area near the center of the island were used as untreated controls during all 3 yr (Fig. 1).

Box Design and Fipronil Formulation. Modified Protecta Jr. (Bell Laboratories, Madison, WI) mouse bait boxes (fipronil-treated bait boxes) were used in this study (Fig. 2A). A cotton yarn wick was stapled to the underside of the lid so that when the lid was closed and locked, the wick was suspended just above the floor and immediately inside the entry to the feeding chamber. Two nontoxic Detex monitoring bait blocks (Bell Laboratories, Madison, WI) were placed in the feeding chamber of each box. The cotton wick was treated with ≈2–3 ml of fipronil topical formulation (Bayer Environmental Science, Montvale, NJ), and the lid was closed and locked with a setscrew before placement. A 0.43% (AI) topical oil formulation of fipronil was used in May and June 1999. This was replaced by a 0.75% (proprietary issue) (AI) formulation of fipronil in July 1999 and used through September 2001 (Table 2).

During year 3 of the study, a bait box produced by Bayer Environmental Science (Prototype 2; Fig. 2B) was used from 15 May to 25 July 2001. This particular Prototype contained either three Detex monitoring bait blocks or a scented cotton ball treated with a 5-ml solution of an orange/almond extract. The fipronil-containing wick consisted of a piece of dental cotton secured with a plastic clip suspended above the floor of the box just preceding the entrance to the feeding chamber. The 154 treated properties on the island were divided with approximately one-half receiving Prototype 2 fipronil-treated boxes containing Detex monitoring bait blocks and one half receiving Prototype 2 fipronil-treated boxes containing the scented cotton ball. This prototype exhibited severe overall limitations and we were unable to determine the effectiveness of boxes containing a scented versus a food bait and the prototype 2 boxes were replaced by 30 July 2001 with the modified Protecta Jr. mouse bait box used in 1999 and 2000 (Table 2).

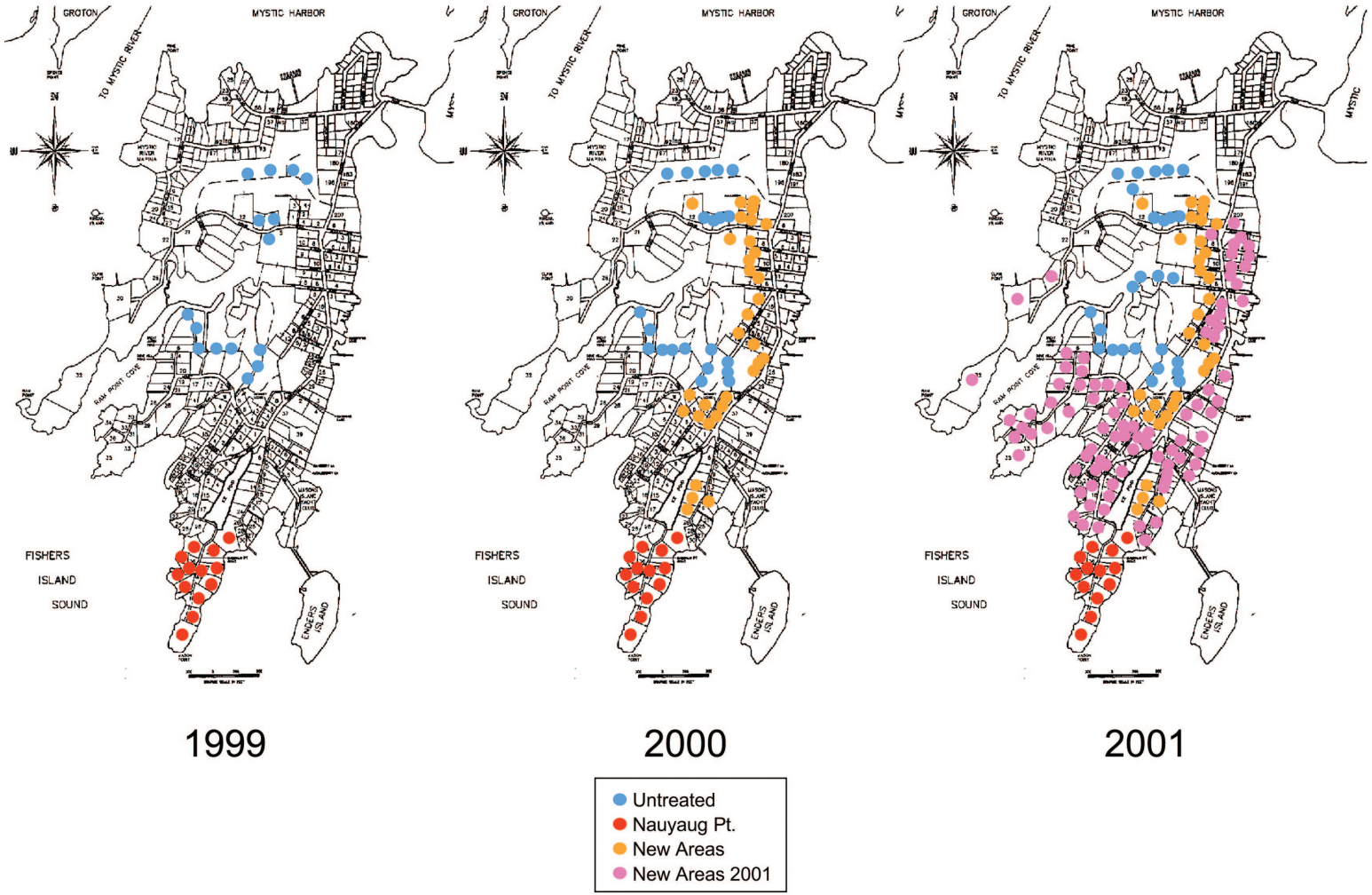

Fig. 1. Map of Mason's Island showing individual land plots and properties receiving fipronil-treated bait boxes,1999–2001. In 1999, 13 properties (red dots) received Protecta Jr. fipronil-treated bait boxes on the southern tip of the island (Nauyaug Pt.). In 2000, 31 additional properties (orange dots) received Protecta Jr. fipronil-treated bait boxes (new areas, 44 total treated properties). In 2001, 110 additional properties (pink dots) were added (154 total treated properties, new areas 2001). Five sites located in the undeveloped center of the island served as untreated controls during all 3 yr (blue dots).

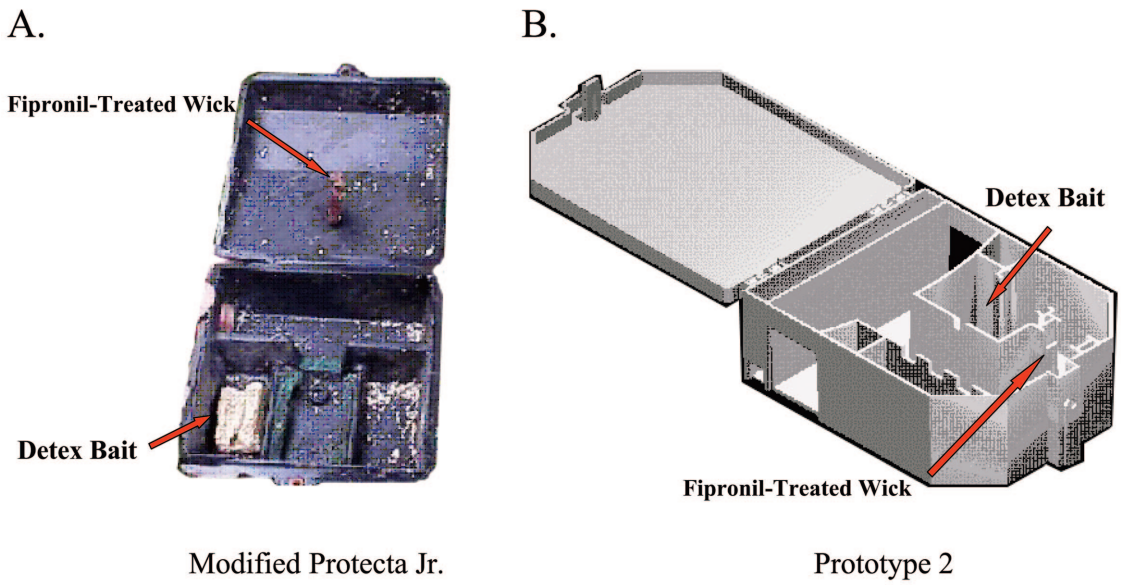

Fig. 2. (A) Picture of the modified Protecta Jr. bait box used during 1999, 2000, and August and September 2001. The wick consisted of a fipronil-treated cotton string attached to the lid of the box. (B) Prototype 2 bait box used from May through July 2001. The wick was constructed from cotton dental packing and became turgid when treated with fipronil oil formulation making access to the bait chamber very difficult. The location of the wick in relation to the bait chamber and the narrowed access run between the wick and bait (arrows) adversely affected acceptance and use by targeted rodents, resulting in sublevel treatment of animals.

Fipronil Treatment and Bait Box Maintenance. Based upon pretreatment mouse distribution and population data, bait boxes were set out on properties as follows: In May 1999, 13 properties (Nauyaug Pt.) received 125 fipronil-treated bait boxes. In 2000, 44 properties in total were treated with 315 fipronil-treated bait boxes. By 2001, 154 properties received 1,700 fipronil-treated bait boxes (Table 2). Boxes were spaced ≈10 m apart along the interface of maintained landscaping (predominantly lawn) with woodlot, stonewall, or scrub brush consisting of both native plants and feral cultivars to specifically target mouse/tick habitat (Maupin et al. 1991, Frank et al. 1998). In addition to targeting specific ecotone, bait boxes also were placed near woodpiles and outbuildings. The number of boxes per property ranged from 5 to 20 (average of 10 boxes per property), depending on property size and presence of suitable mouse habitat. In 1999, initial treatment with fipronil began on 17 May, and at 4-wk intervals each box was rebaited with one to two Detex bait blocks as needed, and the wick was replenished with 2–3 ml of fipronil formulation as needed throughout the trial. A record of relative use by white-footed mice was kept for each box during the trial. The amount of bait consumed, feces within the bait box, presence of dirt and debris on treated wicks, and depletion of fipronil from wicks determined relative use.

In 2000, initial treatment with fipronil began on 19 May (245 boxes on 34 properties) and at 2-wk intervals, each box was checked to add bait and fipronil as necessary. The remaining 70 boxes were set out on an additional 10 properties during the week of 23 June. A record of relative use by white-footed mice was kept for each box during the trial. In 2001, initial treatment with the Aventis Prototype 2 (Fig. 2B) fipronil-treated bait box began on 15 May, and boxes were left in place until they were replaced with the modified Protecta Jr. mouse bait box (Fig. 2A) by 30 July. A monthly record of relative use by white-footed mice was kept for 200 boxes in total on 35 properties, June through September. Treatment was terminated the final week of September for each year. A tree and lawn care company specializing in tick control (SavaTree, Old Saybrook, CT) was contracted to disperse and maintain bait boxes during 2000 and 2001.

Collection of Rodent and Tick Specimens. White-footed mice, *P. leucopus*, were live-trapped using 7.6 by 7.6 by 25-cm. Sherman mouse traps (Sherman Trap Co., Tallahassee, FL). In 1999, pretreatment trapping was conducted in April and May and in May only during 2000 and 2001. Posttreatment trapping was conducted at monthly intervals from June through September all 3 yr to determine 1) distribution and density of mice on properties, 2) tick burdens on treated and untreated animals, and 3) prevalence of infection with *B. burgdorferi* in mice in treated and untreated sites. Live traps were baited with a peanut butter/oatmeal mixture and set within a 5-m radius of bait stations in a representative sample of treated sites (13/13 treated properties in 1999, 34/44 treated properties in 2000, and 35/154 treated properties in 2001) during the posttreatment period. Additionally, 140 live

traps were set in a total of five untreated control areas near the undeveloped center portion of the island during the same time intervals.

Upon capture, individual mice were anesthetized with methoxyflurane and ectoparasites removed with fine forceps and placed in 70% ethanol for species identification. Ear biopsies were taken, surface sterilized, and cultured in BSK-H media for isolation of *B. burgdorferi* (Sinsky and Piesman 1989). Cultures were read by dark-field microscopy every 7 d for 4 wk (Piesman et al. 1986) before cultures were deemed negative. In addition, ≈300 μl of whole blood was taken by cardiac puncture, and each mouse was ear-tagged, weighed, overall body measurements recorded (total body length, tail length, right ear length, and right hind foot length), and subsequently released at the point of capture. Because fipronil is undetectable to ticks before they attach to a host, determination of whether ticks were alive or dead at the time of collection was made by microscopic examination. Ticks that were motile and contained fresh blood in midguts were considered alive. However, ticks that did not demonstrate evidence of blood in the midgut were nonmotile and in obvious stages of physical degradation (emaciated, missing setae and/or legs that broke apart easily) were considered moribund at the time of collection. These ticks were not used when determining infestation rates or ticks per mouse. Ticks matching this description were recovered from treated areas only.

Animals were handled according to approved protocols on file with the Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases Animal Care and Use Committee.

Surveys for Host-Seeking Larval, Nymphal, and Adult *I. scapularis*. To determine the impact of fipronil-treatment on tick densities of treated properties compared with untreated controls, flagging for ticks with a 1-m^2 white cloth drag was conducted at monthly intervals on those treated and untreated control areas that mice were sampled. A standard method of flagging treated properties and untreated areas was followed; flagging was conducted where ticks were most likely to be present for each property, and properties were sampled for the same duration (Maupin et al. 1991, Stafford and Magnarelli 1993). Due to varying vegetation type and density of flagged properties, number of tick(s) per time of effort was used as a standard measurement to determine tick densities. Surveys for host-seeking stages of deer ticks were conducted as follows: April and May for adults, June and July for nymphs, and July, August, and September for larvae (Piesman et al. 1987, Ginsberg and Ewing 1989, Maupin et al. 1991, Stafford and Magnarelli 1993). A representative sample (≥50) of nymphs and adults was tested for infection with *B. burgdorferi* by nested polymerase chain reaction (PCR) as described previously by Zeidner et al. (2001) and for *A. phagocytophilum* by standard PCR as described by Zeidner et al. (2000), during 2000 and 2001.

Statistical Analysis. To determine the specific activity of laboratory bioassays to control application in a field setting, the modified Abbott's formula (Mount 1981) was used for primary comparisons between least (active ingredient) of fipronil in laboratory bioassays, percentage of reductions of ticks on mice in treated areas compared with untreated areas, as well as monthly percentage of reductions of ticks on mice and number of nymphs flagged per minute in the new areas. To test the hypothesis that fipronil-treated bait boxes could significantly reduce the number of ticks on mice, questing ticks, and infection rates of mice and ticks, χ^2 tests were used. χ^2 was used to determine significance (at $P \leq 0.05$) of tick infestations rates on mice, ticks per mouse, numbers of infected mice, and percentages of young of the year infected comparing treated areas to untreated areas. χ^2 also was used to determine significance for percentage of reduction of adult and nymphal ticks infected with *B. burgdorferi* and *A. phagocytophilum* in Tables 3 and 4 (SAS release 8.01 software).

Table 1. Bioassay of fipronil in Adonis formulation required to kill *I. scapularis* nymphs

Test group[a]	14 d[b] (%)	28 d (%)	42 d (%)
Control[c]	72[e]	76	74
1.00%[d] (200 μg/mouse)	0	0	2
0.75% (150 μg/mouse)	0	2	6
0.50% (100 μg/mouse)	0	8	24
0.43% (85 μg/mouse)	0	16	30

[a] Five mice were challenged with 10 nymphal *I. scapularis* per group; a total of 25 mice and 750 ticks were tested as described under *Materials and Methods.*

[b] Mice were challenged at 14, 28, and 42 d after treatment.

[c] Control mice received rapeseed oil formulation with no fipronil.

[d] Mice received a single dose, 20 μl, of 1, 0.75, 0.5, or 0.43% (AI) fipronil applied by pipette between the scapulae.

[e] Number of replete nymphs collected from each group are listed as a percentage.

Results

Bioassay of Fipronil. Laboratory bioassays conducted on a total of four concentrations of fipronil resulted in complete protection through 14 d after treatment (Table 1). Mice treated with 1% fipronil remained completely protected at 28 d, whereas the first signs of successful tick feeding were observed at 28 d in the remaining three groups receiving single doses of 0.43, 0.5, and 0.75% fipronil. However, the percentage of replete nymphs (16%) at even the lowest concentration of fipronil (0.43%) was dramatically lower than the control group (76% of nymphs fed to repletion). At 42 d after treatment, only 6% of nymphal ticks fed to repletion in the group treated with 0.75% fipronil compared with 74% on untreated animals (Table 1). Percentage of control for 0.75% fipronil at 42 d remained adequately high at 92% (modified Abbott's formula). Results of this trial were the basis for using 0.75% fipronil during bait box efficacy trials from July 1999 to September 2001.

Relative Use of Bait Boxes by Mice. Relative acceptance and use of these devices by *P. leucopus* in 1999

Table 2. Bait box design, fipronil formulation, and no. of treated properties evaluated from 1999 to 2001 on Mason's Is., Mystic, CT

Yr	Mo	No. properties	Area[a]	Box design	Bait	Wick	% Fipronil
1999	May–June	13	NP	Protecta Jr.	Detex	Cotton String	0.43
1999	July–Sept.	13	NP	Protecta Jr.	Detex	Cotton String	0.75
2000	May–Sept.	44	NP, NA	Protecta Jr.	Detex	Cotton String	0.75
2001	May–July	74	NA 2001	Prototype 2	Scented cotton[b]	Dental Wick	0.75
2001	May–July	80	NP, NA	Prototype 2	Detex	Dental Wick	0.75
2001	Aug.–Sept	154	NP, NA	Protecta Jr.	Detex	Cotton String	0.75

[a] NA, New Areas; NA 2001, new areas added in 2001; NP, Nauyaug Pt.
[b] Scented cotton balls were treated with a 5-ml solution of an orange/almond extract.

on fipronil-treated properties was ≥85% in June and increased to >95% from July through September. During year 2 of the study, 365 Protecta Jr. fipronil-treated bait boxes were placed on 44 properties. Maintenance records provided by pest management professionals from SavaTree (Old Saybrook, CT) indicated that in early June, 77% of the stations were being used, and by the end of June, bait box use increased to 90%. From July through late September, bait boxes were consistently >99% used.

A total of 0.5 liters of 0.43% (AI) fipronil and 0.65 liters of 0.75% (AI) fipronil were used to treat 125 bait boxes during 1999. This volume translates to a total of 8.65 g (0.31 oz) of fipronil used on 13 properties. During the second year, a total of 4 liters (30 g) of 0.75% (AI) fipronil was used to treat 315 bait boxes bimonthly. Less than 0.1 g (AI) was applied to each bait box on 44 properties situated on ≈40 ha during the 4.5 mo of treatment.

During year 3, a total of 1,700 Prototype 2 bait boxes were placed on 154 properties by 15 May 2001. All 1,700 bait boxes contained a wick treated with 0.75% (AI) fipronil. Of these boxes, 850 contained three Detex nontoxic monitoring bait blocks (total of 78 properties), and 850 contained a cotton ball scented with 5 ml of an orange/almond extract (total of 76 properties) (Table 2). Relative use of bait boxes was determined as in the previous 2 yr, by recording use data on a total of 200 boxes located on 35 properties. From 15 May through 12 June, ≈20% of the bait boxes with the scented cotton ball and 30% of the bait boxes with the Detex bait blocks were being used. The bait box prototype used from May through July (Prototype 2) had severe overall design flaws. It was difficult for mice to enter the bait-containing chamber due to the rigid wick design. These bait boxes were replaced with a modified Protecta Jr. bait box by 30 July. All 1,700 bait boxes contained wicks treated with 0.75% (AI) fipronil and two Detex monitoring bait blocks. Relative use for these boxes was 88 and 93% for August and September, respectively.

Abundance of Ticks and Mice. A total of 6,248 trap nights resulted in the capture of 1,821 *P. leucopus* (29.2% trap success rate), the principal reservoir for *B. burgdorferi* in the northeastern United States. A total of 6,025 ticks was collected from these mice. Eighty-four percent of the ticks were larval and nymphal *I. scapularis* and the remainder were immature *Dermacentor variabilis* (Say). The total number of ticks collected during all 3 yr resulted in 4,647 ticks collected from 666 *P. leucopus* (6.98 ticks per mouse) from the untreated control areas, 919 ticks were collected from a total of 299 *P. leucopus* (3.07 ticks per mouse) from the New Areas, and 78 ticks were collected from a total of 452 *P. leucopus* (0.17 ticks per mouse) from Nauyaug Pt.

Reduction of Ticks on Treated Mice. The immature stages of *I. scapularis* that parasitize white-footed mice are nymphs that are most active from April to July, whereas larvae feed mainly from July to September. Ticks on the 13 properties of Nauyaug Pt. during all 3 yr were significantly controlled. Figures 3A and 4A show that a total of two pretreatment collections made in April and May 1999 resulted in 16.3 and 29.3% of mice infested with ticks (Fig. 3A) and the mean ticks per mouse were 0.31 and 0.37, respectively (Fig. 4). The mean number of *I. scapularis* nymphs recovered from white-footed mice was much lower on Nauyaug Pt. compared with the untreated control areas (Fig. 4A). Weather conditions during the weeks before and during pretreatment collections made in April and May 1999 were extremely rainy, windy, and cold and may have been responsible for inhibiting normal host-seeking activity patterns for *I. scapularis* nymphs on the highly exposed and windswept Nauyaug Pt. area compared with the more protected untreated control sites. Nevertheless, live nymphs were not recovered on mice after fipronil treatment, whereas nymphs were present on mice in the untreated control areas through August 1999 (Fig. 4A). It was observed during June and July 1999 (Fig. 4A) that several mice in the fipronil-treated area had nymphs attached to the head area, but these ticks had died in situ before feeding. No dead ticks attached to mice were ever observed in the untreated control areas.

Pretreatment collections made in May 2000 and 2001 on Nauyaug Pt. resulted in a 25 (Fig. 3B) and 10% (Fig. 3C) infestation rate with 0.36 (Fig. 4B) and 0.25 (Fig. 4C) mean ticks per mouse. Posttreatment collections for Nauyaug Pt. were performed June through September all 3 yr. No nymphs and only two engorged larvae (one mouse in August and one mouse in September) were recovered from mice in the fipronil-treated area in 1999 (Fig. 4A). Posttreatment collections made in 2000 through September resulted in only one unmarked (no ear tag) *P. leucopus* infested with 15 larval *I. scapularis* (Fig. 4B). No nymphal deer ticks

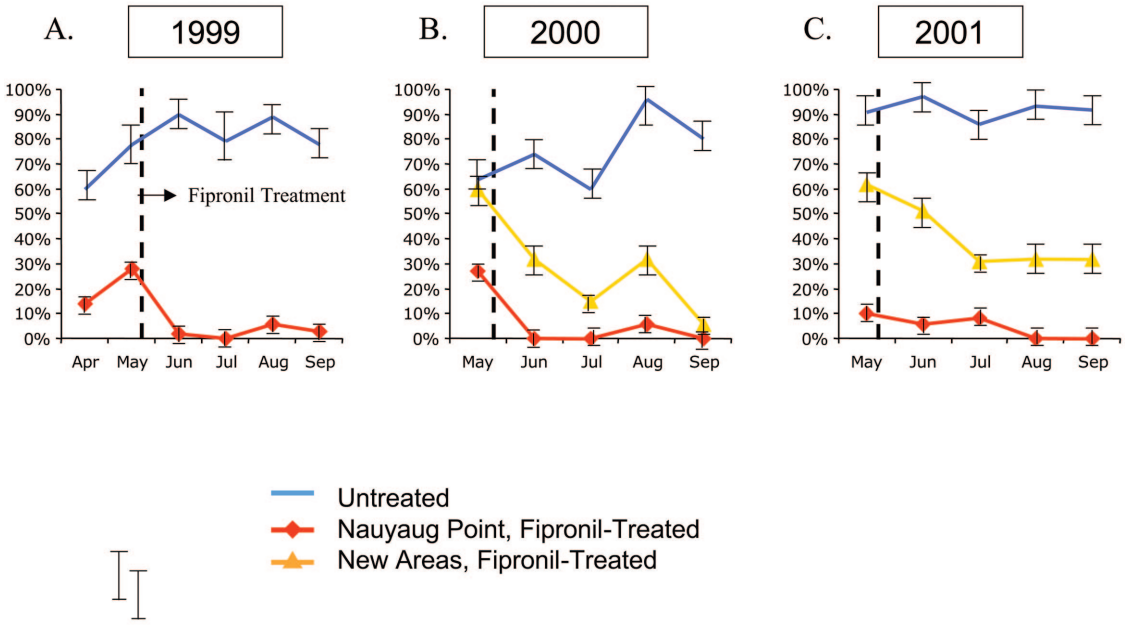

Fig. 3. (A) Infestation rates of *P. leucopus* on Nauyaug Pt. compared with untreated areas. Pretreatment collections were performed in April and May, posttreatment in June to September. Fipronil-treated bait boxes were in place by 15 May all 3 yr. (B) Infestation rates of mice including New Areas. (C) Infestation rates during 2001. 15 May–July, properties received Prototype 2 bait boxes; modified Protecta Jr. boxes were used August and September.

were collected off white-footed mice during the second season of fipronil-treatment on the 13 properties on Nauyaug Pt. (Fig. 4B). Posttreatment data recorded for June 2001 (Fig. 3C) resulted in a slight increase in the number of infested mice (12%), and the number of ticks/mouse (2.8) (Fig. 4C). This is most likely a direct result of the problems experienced with the bait box prototype that was used on the island from May through the end of July. Upon replacement of these boxes (30 July 2001) with the original proto-

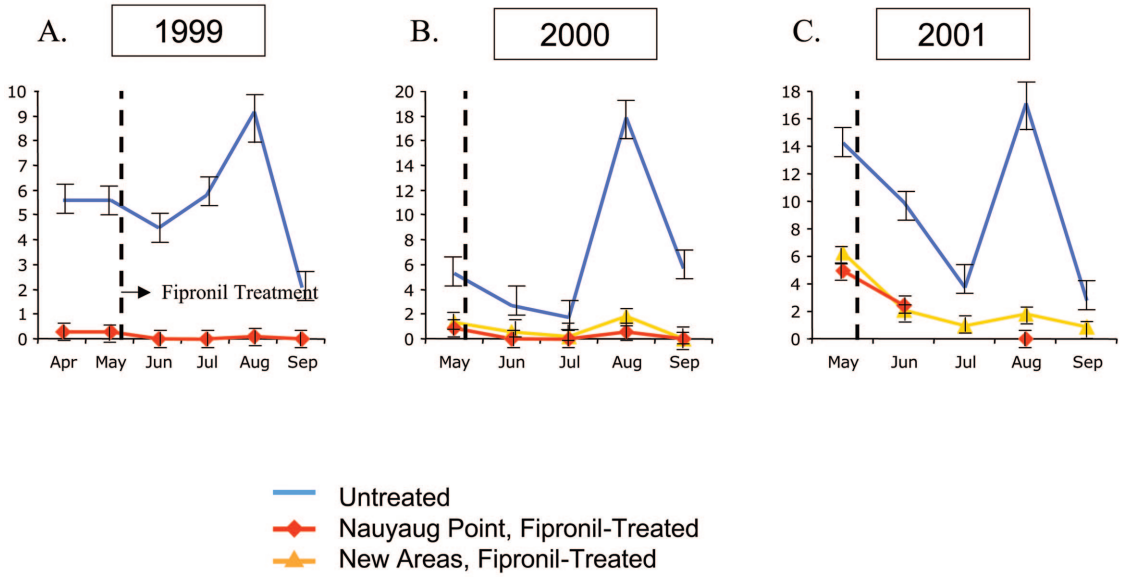

Fig. 4. (A) Average ticks per mouse in 1999 for Nauyaug Pt. (fipronil-treated) compared with untreated control areas. Pretreatment trapping conducted during April and May 1999 and May only of the following years. (B) Average ticks per mouse in 2000 and 2001, including the New Areas. Note that nymphal peak activity occurs during June and the larval peak during August.

Table 3. Comparison of adult *I. scapularis* populations after fipronil treatment in 1999 and 2000, Mason's Island Mystic, CT

Study areas	No. adults collected	Adults collected/min flagging	% adults infected with *B. burgdorferi*	% adults infected with *A. phagocytophila*
Untreated areas 2000	298	1.86[a]	48	22.1
Untreated areas 2001	295	1.5	45	20
New areas 2000	ND	ND	ND	ND
New areas 2001	95	0.33[b]	31.1[c]	11.1[c]
Nauyaug Pt. 2000	58	0.36[d]	34[e]	9.7[e]
Nauyaug Pt. 2001	4	0.013	Insufficient sample[f]	Insufficient sample

ND, not done, treatment was initiated in 2000.
[a] Average number of ticks per minute for five untreated control areas.
[b] Average number of ticks per minute for 31 treated properties, New Areas.
[c] $P < 0.001$ (χ^2).
[d] Average number of ticks per minute for 13 treated properties, Nauyaug Pt.
[e] Sample size (four ticks) was too small for testing; ≥10 ticks required as determined by χ^2.

type, a modified Protecta Jr. bait box, no ticks were collected from 54 *P. leucopus* trapped during August and September 2001 (Figs. 3C and 4C).

Twenty-one of the 31 properties in the New Areas were sampled for mice and ticks. Figures 3B and 4B show that in May, tick infestation rates on mice in these New Areas were similar to those in untreated controls. Posttreatment results on these 21 properties reflect a dramatic reduction in the percentage of infested mice and the number of ticks per mouse within 3 wk of the initial application of fipronil and tick numbers were significantly curtailed through the end of September. Using the modified Abbott's formula monthly percent reductions of ticks on mice in this new area during year 2 were calculated as follows: June, 86%; July, 45%; August, 87%; and September, 96% reduction. A total of 22 properties in the New Areas were sampled for mice and ticks during 2001. Pretreatment collections for the New Areas resulted in a 62.2% infestation rate (Fig. 3C) with 6.3 ticks per mouse (Fig. 4C) compared with a 91% infestation rate (Fig. 3C) and 13.9 ticks per mouse in the untreated areas (Fig. 4C). As noted in the previous year, 2000, posttreatment results in 2001 on these 22 properties reflect a significant reduction of ticks on mice ($P < 0.001$) throughout the treatment period (Figs. 3C and 4C). The mean number of ticks per mouse declined steadily from 2.1 in June to 0.9 in September (Fig. 4C). The only increase occurred during the larval peak in August (1.8 ticks per mouse) (Fig. 4C). However, this number represents an 89% reduction compared with the number of ticks per mouse (17.1) in the untreated areas (Fig. 4C) ($P < 0.001$).

Impact of Fipronil Treatment on the Transmission of *B. burgdorferi*. Each captured mouse was tested for infection with *B. burgdorferi.* Spirochete-infected *P. leucopus* (67.9% of 666 mice) were present at all five untreated collection sites. In comparison, fewer mice (29.1% of 452 mice) ($P < 0.05$) were infected in the Nauyaug Pt. area, even though the distribution of spirochete-infected mice remained relatively high (84.5% of properties) after 3 yr of fipronil tick control. Likewise, the proportion of infected mice in the New Areas (41.3% of 299 mice) was lower than the untreated areas (data not shown). To further evaluate the impact of fipronil on the incidence of *B. burgdorferi* transmission, we examined rates of infection among naïve young of the year (≤17 g) (Schug et al. 1991). During this 3-yr study, 36.4% of young mice in the untreated areas were spirochete-infected during the pretreatment period (May), and 32.1% became infected on recapture from June through September. Similarly, during 3 yr of fipronil application on the Nauyaug Pt. properties, 30% of young mice became infected during pretreatment periods, whereas only 3.4% developed infection during the treatment period (87.1% control) ($P = 0.0213$). Protection against *B. burgdorferi* transmission was afforded to young mice in the New Areas with results similar to those observed on Nauyaug Pt.; only 10.3% became infected during the treatment period compared with 42.9% during the pretreatment period ($P = 0.0187$).

Impact of Fipronil Treatment on Questing *I. scapularis* Populations. Table 3 shows results of flagging data for adult *I. scapularis* in April for 2000 and 2001. The number of questing adults flagged on Nauyaug Pt. were >73.7-fold less abundant and fewer were infected with *B. burgdorferi* (*Bb*) and *A. phagocytophila* (*Ap*) (34 and 9.7%, respectively) than adults on untreated sites (46.5 and 21.1%, 2-yr average) ($P < 0.001$ for both *Bb* and *Ap*) compared with untreated areas. Adults flagged in New Areas were >3.3-fold less abundant and also exhibited overall lower infection rates for *Bb* and *Ap* (31.1 and 11.1%, respectively) ($P < 0.001$ for both *Bb* and *Ap*). Table 4 shows densities of host-seeking nymphs in June 2000 and 2001. Tick densities and spirochete infection rates were essentially identical in untreated areas (189 nymphs, 1.03 nymphs per minute and 26% infected) and the New Areas (141 nymphs, 0.94 nymphs per minute and 24% infected) during 2000. The number of nymphs per minute for the New Areas during 2001 was reduced by 68% (95 nymphs, 0.35 nymphs per minute, 8% infected with *Bb*) ($P < 0.001$). During June 2000, infection rates in questing nymphs from the untreated areas and new areas was 13 and 11% for *Ap*, respectively. Infection rates in nymphs from the New Areas in June 2001 resulted in a significant decrease to 4% for *Ap* ($P < 0.001$). A total of 11 host-seeking nymphs (four nymphs in 2000 and seven in 2001) were collected on

Table 4. Comparison of nymphal *I. scapularis* populations after fipronil treatment in 1999 and 2000, Mason's Island Mystic, CT

Study areas	No. nymphs collected	Nymphs collected/min flagging	% nymphs infected w/ *B. burgdorferi*	% nymphs infected w/*A. phagocytophila*
Untreated areas 2000	189	1.03[a]	26	13
Untreated areas 2001	214	0.93	20	12
New areas 2000	141	0.94[b]	24	11
New areas 2001	95	0.35	8[c]	4[c]
Nauyaug Pt. 2000	4	0.03[d]	Insufficient sample[e]	Insufficient sample
Nauyaug Pt. 2001	7	0.039	Insufficient sample	Insufficient sample

[a] Average number of ticks per minute for five untreated control areas.
[b] Average number of ticks per minute for 31 treated properties, New Areas.
[c] $P < 0.001$ (χ^2).
[d] Average number of ticks per minute for 13 treated properties, Nauyaug Pt.
[e] Sample size (four nymphs in 2000, seven nymphs in 2001) was too small for testing; ≥10 ticks required as determined by χ^2.

the 13 properties on Nauyaug Pt. during 2000 and 2001 (0.0345 nymphs per minute, 2-yr average). These 11 nymphs were not tested for the presence of *Bb* or *Ap* due to the small sample size.

Questing larvae were not quantified nor tested for infection with tick-borne pathogens as with nymphs and adults. Rather, host-seeking larvae were flagged on treated and untreated sites to determine relative distribution. August is the month of peak larval abundance, and the number of treated properties infested with larvae was compared with untreated sites for larval infestations for that month. During August from 1999 to 2001, five-fifths untreated sites had questing larvae. During this same period, 13 treated properties on Nauyaug Pt. were infested at rates of 54, 77, and 38%, respectively. Sixty (2000) and 100% (2001) of 34 treated properties in New Areas were infested with larvae in August. Although not all treated properties had questing larvae, the low infestation rates and mean tick burdens (Figs. 3 and 4) among captured mice indicate a high level of host-targeted control. Also, the moderate-to-high levels of larval reinfestation of treated properties suggest that control may be required annually to maintain low risk levels of tick-transmitted diseases.

Discussion

The results of this 3-yr study demonstrated that the community-wide use of a novel host-targeted approach to tick control is highly effective in a Lyme disease endemic community. Fipronil-treated bait boxes effectively controlled immature ticks on rodents and provides a new and highly effective community-wide strategy to prevent Lyme disease and other tick-borne illnesses in humans. Tick infestations on Nauyaug Pt. declined within the first month after placement of the bait boxes on 13 properties in May 1999, and remained extremely low through September 2000. Posttreatment collections made over the 3-yr period when modified Protecta Jr. bait boxes were in place resulted in a total of two *I. scapularis* larvae collected from two mice during 1999 and only one mouse with 15 ticks in 2000. This adult male *P. leucopus* was untagged, and most likely entered the study plot from a neighboring area.

From 1999 to 2000 the number of treated properties was expanded to include the center of the island that parallels an undeveloped natural area. This undeveloped area on the center of the island contained four-fifths untreated areas and allowed us to further evaluate bait box efficacy. Tick infestation rates on mice in these New Areas were nearly identical to untreated controls. However, posttreatment results reflected a significant reduction of ticks on mice throughout the treatment period. Overall results on fipronil-treated properties demonstrated a nearly 80% reduction of *I. scapularis* nymphal tick populations after the first year of treatment and a 96% reduction over 2 yr.

Applications of these devices not only dramatically reduce the total number of *I. scapularis* ticks but also, as we have demonstrated here, decrease the abundance of ticks infected with *Bb* and *Ap* in these endemic areas. By effectively controlling ticks on mice, questing adults on Nauyaug Pt. and the New Areas were 73.7- and 3.3-fold less abundant, and the number of nymphs per minute in the New Areas was reduced by 68% compared with untreated areas. Control of immature questing ticks on Nauyaug Pt. afforded minimal risk due to tick bite by nymphal deer ticks for residents of treated properties. In addition, risk of infective tick bites decreases further in successive years of application.

Of particular interest is the impact that fipronil-treatment had on the natural enzootic transmission cycle of *B. burgdorferi* among *I. scapularis* and *P. leucopus.* Ear-biopsy culture results performed on *P. leucopus* demonstrated that the transmission cycle of Lyme disease on Mason's Island is very intense. The average infection rate of *P. leucopus* in the untreated areas during this 3-yr study was 67.9%. Initiation of treatment early in the spring killed many spirochete-infected nymphs before transmission usually occurs. Percentage of reduction of infected mice on Nauyaug Pt. and New Areas after treatment were 87.2 and 72.8%, respectively. In addition, infection rates among naïve young of the year (<17 g) (Schug et al. 1991) were significantly reduced. In untreated areas 32.1% (27/84) of young mice became infected, whereas in treated areas, only 3.4 (2/59) and 10.3% (7/68) of young mice became infected on Nauyaug Pt. and the New Areas, respectively. As a result, larval ticks that

successfully feed on these uninfected mice will not be infected with *Borrelia* once they molt to nymphs. Moreover, given the effectiveness, use of only a small amount (72.65 g) of fipronil for ≈60.7 hectares of risk area ensures minimal environmental impact to reduce risk of Lyme borreliosis in this community. Thus, fipronil and this unique delivery system should be considered a strong candidate for use as a safe, effective tool for controlling Lyme disease and other tick-borne diseases in other areas of the United States.

A total of 6,428 trap nights resulted in 1,750 captures representing 1,225 individual *P. leucopus.* One might be concerned that using a food lure in bait boxes might potentially increase rodent populations. However, during 3 yr of treatment, the number of mice captured actually decreased by ≥2% each year, and there was no significant difference in capture success between treated and untreated areas.

In both the treated and untreated areas during 1999 and 2000, >70% of the mice were captured only once, and the proportion of individual captures in the treated and untreated areas during 2001 were 67 and 65%, respectively, indicating constant migration in and out of the study area (data not shown). These observations underscore the need for an acaricide with considerable residual activity and adequate potency to protect mice from tick infestation. Fipronil, a recently developed phenylpyrazole insecticide/acaricide, is an ideal compound to afford the required protection for this type of host-targeted delivery system. These studies demonstrated this compound to be effective at low concentrations with a long half-life (≥42 d) of acaricidal activity. Fipronil is highly regarded for its effective and safe control of fleas and ticks and is widely used as a spot-on treatment for companion animals (Hutchison et al. 1998) with long-lasting effects (Metzger and Rust 2002). Unlike permethrin, a widely used acaricide, ticks are unable to detect fipronil on topically treated animals. In addition, fipronil has the characteristic of displacing mechanically to areas of the skin far from the site of treatment along with low percutaneous passage (Cochet et al. 1997).

Developing community-wide strategies that are effective yet environmentally sound for the control of *I. scapularis* has become paramount in many Lyme disease endemic areas of the northeastern and upper midwestern United States. Since the discovery that *I. scapularis* transmits several disease agents to humans, including Lyme borreliosis, babesiosis, and ehrlichiosis, many studies have been conducted to evaluate tick control techniques. To date, single, well-timed areawide applications of acaricides provide the most effective reduction of ticks (Stafford 1991a, b; Schulze et al. 2001). However, many environmental factors, including, temperature and precipitation, can affect the onset and duration of questing nymphal tick periods making a single well-timed application difficult. Host-targeted bait boxes can be set out in early May and ensure effectiveness throughout seasonal, peak nymphal activity.

Likewise, areawide applications tend to be seasonal and short term, and are often criticized for their impact on the environment. Commercial pesticides, such as permethrin, cyfluthrin, carbaryl, and diazinon, are generally applied at rates ranging from 0.5 to 2% (AI), 4.5 kg/ha (Schulze et al. 2001), and are formulated with a synergist such as piperonyl butoxide, for areawide treatments (Panella et al. 1997). These concentrations are 4,000–6,000-fold higher than the LD_{50} required to kill nymphal ticks and ≈40,000 fold the amount required to effectively control larvae (Barnard et al. 1981, Maupin and Piesman 1994, Panella et al. 1997). The fact that commercial pesticides preparations delivered at these concentrations are highly effective is not surprising. In light of this, we effectively controlled immature ticks on mice using host-targeted control methods in which very small quantities of active ingredient (0.75%) were required for effective control with minimal impact on the environment. For example, a total of 8.65 g of fipronil was used to treat 9.9 ha in 1999.

Although areawide acaricides and vegetation management can be effective in reducing tick populations, it is highly desirable to provide the public with additional options for controlling medically important arthropods. Of primary interest are those methods that focus on targeting the hosts of *I. scapularis.* One such method has been designed to take advantage of nest-building behaviors of white-footed mice (Mather et al. 1987, Deblinger and Rimmer 1991) and woodrats to treat these rodents with permethrin-treated cotton nest material. However, some studies using this methodology failed to reduce the number of host-seeking nymphs (Daniels et al. 1991, Wilson and Deblinger 1993). A second method developed by Gage et al. (1997) and Lane et al. (1998) demonstrated host-targeted liquid permethrin delivered in bait tubes as an extremely effective method for controlling ticks and fleas on wood rats in Colorado and California. A third methodology used a "four-poster" device that attracts deer, the principal host for adult *I. scapularis* (Barbour and Fish 1993), to a food source (Pound et al. 2000). As the deer feed, they are passively treated with an acaricide for controlling ticks. Although effective, these devices overall require more labor and maintenance to achieve quality levels of control compared with our host-targeted techniques (Barnes and Kartman 1960, Barnes et al. 1974, Gage et al. 1997). During these studies, one individual pest management professional (SavaTree) successfully deployed a total of 1,700 bait boxes on 154 properties in 5 d on Mason's Island in 2001. Moreover, using these devices in combination as part of an integrated pest management approach would facilitate the targeting of all three stages of deer ticks for complete control during a single season and warrants further investigation.

Results of subsequent research at several mainland sites are in accordance with levels of control seen on our island setting (G.O.M., unpublished data). Continuing collaborative developmental efforts between CDC and Bayer Environmental Science (previously Aventis Environmental Science) are underway to produce a bait box that meets EPA safety and registration criteria, will last several weeks without maintenance,

and will accommodate use by eastern chipmunks, a secondary but important host for immature *I. scapularis* and *B. burgdorferi* (Anderson et al. 1985, Slajchert et al. 1997).

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