

B. Project description

1. Project Category: IPM Issues.

2. Project Summary

Lyme disease is a bacterial infection transmitted in the northeastern United States by the deer tick *Ixodes scapularis*. Over 20,000 cases are reported to US Centers for Disease Control annually. In northern New England, three deer tick-borne diseases now infect people, dogs and farm animals. The use of synthetic pesticides to control these medically important arthropods, while effective, remains controversial due to non-target impact, health risk, and persistence. Recently, certain minimal risk, botanically-derived compounds have gained interest for inclusion in IPM strategies to control deer ticks. Based on positive results from a preliminary project, we propose to test the efficacy of a rosemary oil-based insecticide, Eco-Exempt IC2 (IC2), to control all stages of the deer tick in southern Maine. Working with licensed applicators, we will record the abundance of nymphal and adult *I. scapularis* ticks before and following applications of IC2, bifenthrin (a widely used synthetic pyrethroid), and water during the peaks of their seasonal abundance. Multiple study grids will be established within triplicate 70m X 70m grids in forested, infested habitat. In addition, we will examine the compound's effect on all of the tick's life stages in their environment by exposing them to treated leaf litter within enclosures. The effects of IC2 on non-target organisms, including pollinators will also be examined by plot count surveys and pitfall traps. Ancillary studies will examine the persistence of IC2 when sprayed at the beginning of the deer tick nymphal season, and its effectiveness against *Dermacentor variabilis*, the American dog tick.

Background and Justification.

This proposal extends and amplifies a preliminary study, funded by an IPM Minigrant, to document the effectiveness of IC2, a minimal risk (25B), botanical compound to control deer ticks (*Ixodes scapularis*), the vectors of the agent of Lyme disease and at least two other diseases (anaplasmosis, babesiosis) of man and domestic animals.

The Problem. Over the last twenty years, these ticks have invaded northern New England. In Maine, where deer ticks are now found even in the northernmost counties (1), reported cases have doubled in the last two years (2). In 2008, New Hampshire has reported over 1100 human cases through October, and Vermont over 300 (3). Canine Lyme disease is widespread in the region. Repeated canine serosurveys (4, 5) have demonstrated both the statewide advance of transmission as well as very high levels of local positivity for both Lyme disease and canine anaplasmosis (6). In addition, horses suffer from Lyme disease and from anaplasmosis, with fever, lameness, anorexia, edema and ataxia (7). Their treatment can be expensive and is not always successful. In a recent serosurvey we found up to 42% of 89 horses from four southern Maine counties positive for Lyme antibodies, and 17% positive for antibodies to *Anaplasma phagocytophilum* (unpublished data). Yet another deer tick-transmitted disease, babesiosis, has now been reported in more than a dozen humans in southern Maine. While there is an effective anti-Lyme vaccine for dogs, none is available for humans or horses, and no vaccines exist for the other two deer tick-transmitted diseases. Prevention of tick-borne disease rests, therefore, on the public's awareness of the risk of tick bite and the practice of integrated pest management, including habitat modification and the judicious use of acaricides. Several synthetic pesticides have been shown to be effective in controlling deer ticks (8), but have significant adverse effects on non-target organisms (including pollinating bees and marine wildlife, both important to Maine's economy), risks to applicators, the environment, and a long re-entry time required in pastures where animals would be exposed not only externally, but from contaminated feed.

Increasing attention has been given recently to the acaricidal properties of essential oils derived from plants (9-11). Many essential oils have long been used in the food and fragrance industries, and have been generally recognized as safe (GRAS) by the U.S. Environmental Protection Agency. EcoEXEMPT®IC2 (EcoSMART Technologies, Inc., Franklin, TN) (IC2) is a botanical product including rosemary (10%), peppermint oil (2%), oil of wintergreen, and vanillin. It is a food-grade pesticide exempted for registration by the EPA and is stated by its manufacturer for use indoors, outdoors, in environmentally sensitive areas (including freshwater wetlands and shorelines), directly on farm animals and pets, and around children. Its label states that it is effective against ticks, but no specific information about its acaricidal properties is given. The manufacturer has provided a supplemental label for the product's use in pastures and other grazing areas.

Specific Needs. Over the last 20 years, our research has focused on the environmental factors that influence the spread and establishment of the deer tick in Maine (see www.mmcni.org/lyme/research.html), with the principal goal of developing new control strategies. In the fall of 2007 we conducted a preliminary evaluation of IC2 on two *I.*

scapularis-infested horse farms in southern Maine when the owner of two Belgian draft horses, both Lyme-infected and requiring expensive antibiotic treatment, requested an environmentally safe treatment that would allow rapid re-entry of his horses to pasture. Prior to application in late October at the peak of the adult tick season, from 10 to 69 *I. scapularis* were collected per person-hour on the pastures, whereas none was collected one week after treatment, nor in early May, 2008. Those data supported a successful application for an IPM Minigrant to develop more data on the effectiveness of IC2 on non-pasture tick-infested sites during the 2008 nymphal and adult tick seasons (see below). They also brought a request for presentation to the Maine Large Animal Veterinarians Association, where considerable concern regarding Lyme disease in horses was evident.

Stakeholder Priorities. For both FY 2007 and 2006, one of the PMAP priorities was the development of “IPM tactics for critical or emerging pests of regional or national magnitude”, and among the General IPM priorities for the Northeast in 2006 was “tick management for communities and residences”. Listed among New York State’s 2006 Livestock/Field Crop IPM priorities is IPM of significant pests affecting pastures. An effective, environmentally safe IPM against vector ticks will, of course, benefit all in the northeastern and north central United States who work or recreate where they are present. It is particularly needed in sensitive environments, including farms, recreational trails, parks, wetlands, and, here in Maine, areas adjacent to blueberry fields and lobster fishing grounds.

Table 1. Ticks flagged per hour in preliminary IC2 study, 2008.

Experiment	Period	Stage of Interest	Treatment	Ticks/hour		
Peak Nymph Spray	Pre-spray 5-, 2-wk	Nymph	IC2	12		
			Post-spray 1-, 2-wk	Nymph	Bifenthrin	0
					IC2	0
		Reference		79		
	Post-spray 5-wk	Larva	Bifenthrin	540		
			IC2	3520		
			Reference	9530		
	Post-spray 14-wk	Adult	Bifenthrin	0		
			IC2	2		
			Reference	32		
	Peak Adult Spray	Pre-spray 1-, .5-wk	Adult	Bifenthrin	63	
				IC2	55	
Reference				47		
Post-spray 1.5-wk		Adult	Bifenthrin	0		
			IC2	0		
			Reference	46		

Ongoing Work. In June, 2008, we were awarded an IPM Minigrant to study IC2’s impact on both the nymphal and adult stage of the deer tick. In an oak-maple habitat in Cape Elizabeth Maine, where *I. scapularis* is plentiful, we established three 1ha (100m x 100m) spray grids. On July 16th, at the height of the *I. scapularis* nymphal season, we sprayed the grids with either IC2 (4 oz./gal., 4 gal./1000 sq. ft.), SPECKoZ®, 0.06% Bifenthrin (1.0 oz./1000 sq. ft.), or water (the IC2 vehicle). On October 24th, at the height of the adult tick season, we sprayed two new, nearby, 1ha grids (re-using the initial control site) with IC2, Bifenthrin or water. For the nymphal (summer) spray we flagged the IC2 grid at 5 and 2 weeks

pre-spray, and all grids at 1, 2, 5, and 14 weeks post-spray. For the adult (autumn) spray we flagged all grids 1 and .5 week pre-spray and 1.5 week post-spray. The results are summarized in Table 1. While preliminary, they are consistent with a total knock-down

of both stages of the tick which, despite the volatility of the botanical oils, appears, with respect to nymphs, to be maintained up to 5 weeks and reflected in few molting to adults in the fall. The presence of surviving larvae 5 wks post-spray suggest that future research should also include egg and larval development. If, as these findings suggest, IC2 is an effective alternative to more environmentally hazardous synthetic pesticides, its effect on each stage of the tick should be ascertained to guide the development of an effective seasonal application schedule. Although not a known vector of human diseases in northern New England, *D. variabilis*, the dog tick, is a significant pest, infesting people, dogs, and other animals in grassy habitats during the late spring and early summer. The susceptibility of this tick to IC2 also merits investigation.

Regional application. Although our original study examined IC2's specific benefits for use on pastures, the compounds application is universal, and therefore the results of the study we have designed, which tests it in high-risk habitat at seasonal peaks, will apply throughout the northeastern and north central regions of the United States where the vast majority of the nation's Lyme disease cases are reported.

Objectives and Anticipated Impacts.

Specific Aims:

- To assess the ability of an environmentally safe, rosemary oil-based pesticide, EcoEXEMPT IC2 (IC2), to control *Ixodes scapularis*, the vector tick of Lyme disease.
- To assess, in the field and in tick enclosures, the toxicity of IC2 on the individual life stages of the tick.
- To assess the impact of IC2 on non-target arthropods
- To assess, in tick enclosures and in the field, the acaricidal efficacy of IC2 to *D. variabilis*, the dog tick.

Anticipated Impacts (re: National Road Map for Integrated Pest Management):

- Demonstration of an effective, botanical acaricide for inclusion in an integrated management program to reduce the risk of tick-borne diseases, including Lyme disease.
- Demonstration of an acaricide with potential use in residential settings, on farms with grazing livestock, in recreational areas, and other sensitive environments.
- Demonstration of the effectiveness of this compound on each stage of the deer tick's life cycle, and its impact on non-target arthropods.
- Knowledge of its ability to control the dog tick, *D. variabilis*.

Evaluation: As described in the Approach and Procedures section, below, this project, which compares tick counts before and several times after the application of IC2 and both positive and negative controls on triplicate spray sites for each, has been designed to provide data with robust statistical power.

Approach and Procedures

Study Area: Field studies will be conducted on private property in the southern coastal Maine town of Cape Elizabeth (43°34'N, 70°13'W) in Cumberland County, during 2009-2010. Cape Elizabeth typifies the dominant forest community of post-agricultural woodlands in southern Maine--a mosaic of second-growth deciduous and mixed forest, successional and managed fields, and fresh- and saltwater wetlands. The woodlands are characterized by red oak (*Quercus rubra*), red maple (*Acer rubrum*), black cherry (*Prunus serotina*), apple (*Malus* spp.), spruce (*Picea* spp.) and eastern white pine (*Pinus strobus*). Understory is generally open, containing invasive species such as barberry (*Berberis* spp.), Eurasian honeysuckles (*Lonicera* spp.), and native species such as high-bush blueberry (*Vaccinium corymbosum*), meadowsweet (*Spiraea latifolia*), speckled alder (*Alnus rugosa*), and winterberry holly (*Ilex verticillata*), (12, 13). All understory species, with the exception of alder, are highly attractive to native pollinators.

I. Spray Efficacy Experiments: In year one, two experiments will test the efficacy of spraying during the *I. scapularis* 1) nymphal peak, and 2) adult peak. The experimental design is a multiple before-after-control-impact (MBACI) sampling program. To spatially assign treatments to spray grids we will employ a randomized complete block design with three replicates of each of the three treatments (IC2, Bifenthrin, and Reference [water, the vehicle used for IC2]). The impact of treatments over time will be assessed with repeated measures on sample plots. The experimental unit is the spray grid and the sampling unit is the plot at time t_i .

Spatial and Temporal Layout: In spring of year one, we will establish fifteen 70 x 70m spray grids in uniform stands of forest. Each grid will fit entirely within forested habitat. We will randomly assign spray treatments to

grids. Three grids each will receive water (Reference), IC2 (experiment 1), Bifenthrin (experiment 1), IC2 (experiment 2), and Bifenthrin (experiment 2). We will divide each spray grid into forty-nine 10 x 10m plots and consider the 24 plots along the border of the spray grids buffer plots. Among the twenty-five interior plots, we will randomly select seven, resulting in a total of 21 plots per treatment per experiment. Repeated measurements over time will incorporate tick seasonality into the study design. The seasonal prevalence of questing adult dog ticks and adult and nymphal deer ticks is shown in Figure 1. **Experiment 1:** The peak *I. scapularis* nymphal spray will be timed near the middle of peak nymph season, ~June 20th. We will flag each plot for nymphs twice pre-application and at 1-, 2- 4-, 10-wk post-application for larvae, and 16-wk post-application for adults. We will flag these plots again in the 2nd-year in mid-June.

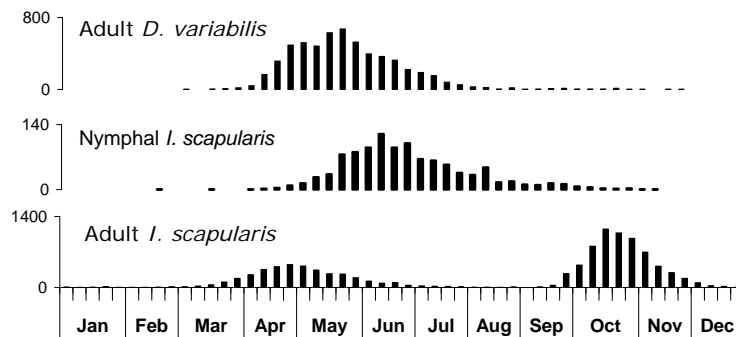


Figure 1. Seasonality of ticks submitted to MMCR, 1991-2007.

Experiment 2: The peak *I. scapularis* adult spray will be timed near the middle of adult season, ~October 20th. We will flag each plot twice for adults pre-application, at 1-, 2-wk post-application (flagging is usually limited in November), and in the 2nd-year in May.

Protocols: Spraying will be conducted by the licensed applicators of Atlantic Pest Solutions, Inc. of Kennebunkport, Maine, supervised by the company's owner, Mr. Ted St. Amand. Just prior to each spray, the depth and make-up of the leaf litter will be measured within each of the seven 10 x 10 m flagging plots within each spray grid. IC2 (4oz./gal), will be applied at 4 gals./1000 sq. ft. and Bifenthrin (0.06% as SPECKoZ®) at 1 oz./1000 sq. ft. We will collect ticks using 1m² flags of light-colored corduroy attached to a ~150cm pole, dragging the flags over the dry leaf litter and understory vegetation at temperatures >10°C. We will flag each plot for a total of 10 minutes, dragging for 1min, inspecting for ticks, and repeating this protocol until the entire plot has been flagged. We will place ticks in plastic vials containing a dampened plaster of Paris base for transportation to the laboratory, where we will enumerate and identify ticks to species and stage.

Analysis: We will enter data into an existing MS-Access database, and use SAS® for data manipulation and statistical analysis. Tick flagging rates will be described as ticks/hr. The main hypothesis for each experiment is that there will be fewer ticks/hr on plots treated with either organic compound relative to reference plots, both before and after the spray. The second hypothesis is that there will be no near-term (within 5wk) post-spray differences in ticks/hr for IC2 versus Bifenthrin. The analytical approach will be analysis of variance (ANOVA) in a generalized linear mixed model (14, 15).

II. Enclosure Experiments: We will employ tick enclosures, as described in Rand, et al. (16) to examine the impact of the three treatments, as they are applied in the field, on the survival of all stages of *I. scapularis* and adult *D. variabilis*. The enclosures consist of polyethylene pails modified by holes providing drainage and air exchange, buried with their lids at the level of the leaf litter and filled with soil to within 10 cm of the rim. The method involves monitoring ticks or eggs, sealed in a sleeve (per Yuval and Spielman, 1990 [17]) made of 95 micron nylon mesh screening. We will examine (1) nymphal and adult *I. scapularis* and adult *D. variabilis* survival, (2) acaricide persistence, and (3) egg and larval survival.

Protocols: **Experiment 1:** At the time of the June spray, we will place one sleeve each of freshly flagged *I. scapularis* nymphs, previously flagged adults, and previously flagged adult dog ticks (10-15 ticks each) within an enclosure in each spray grid, then cover them with treated leaves. For one week we will examine each sleeve daily for tick motility, and where motility in the acaricide-treated enclosures persists, weekly until one month post-spray. **Experiment 2:** We will collect egg masses from the first week of oviposition (late May) of spring-flagged, gravid female *I. scapularis* fed on a New Zealand White rabbit in the animal facility of the Maine Medical Center Research Institute (MMCRI/ IACUC Approval pending). We will estimate egg numbers on the basis of the weight of counted aliquots of single eggs. We will place ~100 eggs in two

sleeves in each of the nine enclosures. We will place one sleeve in the field immediately to allow development to start. We will hold the other sleeve at 4°C until the June spray date. Then, we will cover all sleeves with 10cm of leaves that had been freshly sprayed with IC2, bifenthrin or water. We will examine them at 1-wk intervals, and then at shorter intervals after the appearance of larvae in any enclosure, counting the numbers of mobile and immobile larvae with a hand lens. (Over a 4-year span in Cape Elizabeth, oviposition to eclosion times averaged 45 days [16]). **Experiment 3:** To determine how long sprayed leaf litter remains toxic to deer ticks, we will add one sleeve containing 5 - 10 freshly flagged nymphs to each of the three enclosures in the IC2, bifenthrin, and control areas at 24 and 48 hours, 1- and 2-wk following treatment, or until the sprayed leaves become ineffective. We will examine the sleeves and count the number of live ticks at day 1, day 2, and 1-wk following placement.

Analysis: In experiments one and two we will implement the among-treatment multiple comparison of proportions of ticks surviving with a chi-square test procedure. In experiment three we will model the proportion of ticks surviving as a function of both treatment and number of weeks post-spray (GLMM).

III. Ancillary Experiments:

I. scapularis pre-nymphal season and *D. variabilis*: In year 2, **Experiment 1** we will compare the acaricides' ability to prevent the appearance of nymphs and will monitor any continuing effect. The *I. scapularis* pre-nymphal spray will be timed as nymphs are first appearing, ~ May 15th. We will flag each plot for nymphs twice pre-application and at 1-, 2-, 4-, 8-, for larvae at 14-wk post-application and for adults at 20-wk post application. **Experiment 2** will evaluate IC2's effect on *D. variabilis*, which is a larger and hardier tick than *I. scapularis*. The adult *D. variabilis* spray will be timed for the adult season, approximately May 30th. We will flag each plot for adults twice pre-application and at 1- and 2-wk post-application. Design, protocols, and analysis mirror that of the spray efficacy experiment of year 1.

Non-target arthropods: Many beneficial insects are soil-dwelling and thus it is imperative to assess any lethal and sublethal effects on non-target arthropods. Given current concern over pollinator declines (18, 19) we are including native bees in our assessment of IC2. In collaboration with Dr. Joseph Staples (Department of Environmental Science, University of Southern Maine) and Dr. Constance Stubbs (School of Biology and Ecology, University of Maine), we will assess the impact of the two acaricides on both soil arthropods and soil nesting pollinators (primarily native bees such as *Andrena* and many species of *Bombus*). For all arthropods, we will deploy an array of 16 pitfall traps (4 x 4, 14m apart with a 14m side buffer) within each spray grid, filled with 30 ml 70% ethanol or propylene glycol and collected after 24-30 hours, after Schulz et al. (20). Pre- and post-spray sampling on the grids will follow the schedule for tick flagging. In addition, for the soil nesting bees, we will monitor pollinator abundance and behavior (possible sublethal effects- disorientation, erratic flight) pre- and post-application on any flowering vegetation within 24 randomly selected, 2 x 2m plots (21, 22). Soil Arthropods

will be identified to family. Native bees will be identified to genus. We will compare total abundance and diversity for specific taxa among spray treatments (ANOVA).

Evaluation Plans

The results of this project will be submitted for publication in journals related to tick-borne diseases, their prevention and control, as have our previous many studies over the last 20 years (www.mmcri.org/lyme/research.html). Over that period we have developed strong ties to state agencies concerned with vector-borne diseases which have broad public outreach (Maine's Center for Disease Control, Department of Agriculture, Department of Inland Fisheries and Wildlife; New Hampshire Division of Public Health Services; Vermont Department of Health). Through our tick identification program, repeated canine serosurveys, and now an equine serosurvey, we are in close touch with both small and large animal veterinarians. In addition, as one of four groups surveying for the mosquito vectors of arborviruses, we closely associate with the pesticide industry in southern Maine. In 2007, our staff provided 20 individual talks to concerned groups and is frequently interviewed by the media. Thus, we have a particular ability to communicate whatever role we may show IC2 to have as an element of IPM for tick control.

Cooperation, Institutional Units, and Key Personnel Involved.

This project will be conducted by the staff of the Vector-borne Disease Laboratory (VBDL), a unit of the Maine Medical Center Research Institute. The Parent Organization is the Maine Medical Center. Robert P Smith, MD, MPH and Peter W. Rand, MD, Co-Directors of the laboratory, will oversee the project. Field Biologist Charles Lubelczyk will establish the study grids, coordinate with applicators, and oversee tick flagging. Susan Elias, a small mammal ecologist and statistician, will oversee data management and analysis. Both she and Research Associates Eleanor Lacombe and Bruce Cahill will assist in flagging, identifying ticks to species and stage, and monitoring the enclosures. Consultants will include Theodore St. Amand, owner of Atlantic Pest Solutions, who will oversee pesticide applications, and Professors Joseph Staples (University of Southern Maine) and Constance Stubbs (University of Maine), who will advise regarding arthropod collections.

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