2016

Evaluating the Effect of Minimal Risk Natural Products for Control of the Tick, *Ixodes scapularis*

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EVALUATING THE EFFECT OF MINIMAL RISK NATURAL PRODUCTS FOR CONTROL OF THE TICK, *IXODES SCAPULARIS*

BY

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

MASTER IN SCIENCE

IN

ECOLOGY AND ECOSYSTEMS SCIENCE

UNIVERSITY OF RHODE ISLAND

2016

MASTER OF SCIENCE THESIS

OF

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2016

ABSTRACT

I evaluated the knock-down and residual activity of eleven minimal risk natural products (MRNP) against host-seeking nymphal stage blacklegged ticks (*Ixodes scapularis* Say) using a novel micro-plot product screening system in a landscape setting similar to a wooded residential property. The micro-plot system reduced variability between testing sites typically seen in larger field trials and provided the opportunity to compare results of studies conducted under the same environmental conditions, saving both time and money by confining product application and tick sampling to a 0.3 m diameter arena. By seeding the arenas with a known number of laboratory-raised blacklegged tick nymphs, I was able to further reduce the variability and improve product screening reproducibility across years. The products evaluated included CedarCide PCO Choice, EcoPCO® EC-X, Met52® EC, EcoEXEMPT® IC^2, EcoSMART® Organic® Insecticide, Essentria™ IC^3, nootkatone, Progaea, Tick Guard, Tick Killz and Tick Stop. Five of the eleven products tested (EcoPCO® EC-X, Met52® EC, EcoEXEMPT® IC^2, Essentria™ IC^3 and nootkatone) were found to have a statistically significant ($P < 0.05$) "knockdown" effect (meaning the product was applied while ticks were in the arenas), and only two of them, EcoPCO® EC-X and nootkatone, displayed significant "residual" tick-killing activity after weathering for 2 weeks. I found relatively inconsistent results with botanical oil-based products tested multiple times, indicating batch-to-batch variability, as well as variability between formulations. The results of my study suggest a need for better quality control and/or efficacy testing of botanical oil and other minimal risk natural products. Such MRNP screening can provide consumers with an improved ability to make more informed decisions about the level of tick encounter protection they might expect from

products they may be purchasing because they believe them to be environmentally safer.

KEYWORDS: blacklegged ticks, *Ixodes scapularis*, natural products, botanical oils, biopesticides, bifenthrin

ACKNOWLEDGEMENTS

I want to thank my major professor, Dr. Thomas Mather, for his continuous support and encouragement over the years. It's been a long journey, and it would not have been possible without him.

I would also like to thank my committee members: Dr. Howard Ginsberg, Dr. Steven Alm, Dr. Gavino Puggioni and Dr. Sandra Ketrow for overseeing this work and helping me along the way.

I would also like to thank the members of the Center for Vector-borne Disease and Dr. Alm's lab, past and present, for their technical support and assistance. I would like to give a special thank you to Wendy M.C. Shattuck, Matthew Requintina and Karen Frost for their help and support over the years, they helped make the work go easily and I couldn't have done this without them.

I would like to thank the following people for donating their products for our trials: Patrick Ryan (Envincio) for the EcoPCO® EC-X and Essentria™ IC^3 in 2012; Chris Fletcher (Bartlett Tree Experts) for the Talstar® Professional (2012 ,2013 ,2015) and UP-Star Gold (2014); Jerrod Leland (Novozyme) for the Met52® EC (2012, 2013); Trevor Nelson (Pure Pest Solutions) for the EcoEXEMPT® IC^2 and EcoADJUVANT® (2013), Essentria™ IC^3 (2013, 2014), and Progaea (2013); Marc Dolan (CDC) for the nootkatone (2014); Wildflower Farms for the Tick Stop (2014); and Brian Kelly (East End Tick Control) for the Tick Guard (2015). This research was supported by the Centers for Disease Control and Prevention Award 1U01CK000186.

DEDICATION

I would like to dedicate this thesis to my family. My parents, Alan and Carolyn, have taught me to have a loving appreciation for the outdoors, and as long as I believe in myself, I can do anything I set my mind to. My siblings, Kristen, Shawn and Adam, and their amazing families have always been supportive and encouraged me, in our traditionally sarcastic "Dyer" way. I love them all.

PREFACE

This thesis is a culmination of four years of evaluating minimal risk natural products using a novel micro-plot study.

This thesis is in partial fulfillment for the degree of Master of Science in Biological and Environmental Sciences with a specialization in Ecology and Ecosystems Science and is presented in manuscript format, according to the rules of the Journal of Medical Entomology.

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Evaluating the effect of minimal risk natural products for control of the tick, *Ixodes scapularis* (Acari: Ixodidae)

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This thesis is prepared in manuscript format for submission to the
Journal of Medical Entomology.

BACKGROUND

Ticks are obligate ectoparasites, relying on the blood meal from a host to complete each life stage. They are native to a variety of unique habitats, which affords them the opportunity to encounter and feed on a wide variety of hosts, in turn dictating the specific pathogens which they can later transmit. Worldwide, ticks are known to transmit over 20 different emerging or Category A-C pathogens of medical and veterinary importance including viruses, bacteria and parasites (Balashov 1972).

In southern New England, there are three common species of ticks which are known to bite humans and transmit disease-causing pathogens: American Dog ticks (*Dermacentor variabilis*), Lone Star ticks (*Amblyomma americanum*) and blacklegged ticks (*Ixodes scapularis*) which are more commonly known as deer ticks. All are found predominantly within their own distinct habitats and are known to transmit their own assortment of pathogens. Blacklegged ticks are vectors of the bacterial agent causing Lyme disease, the most commonly occurring tick-borne disease in North America with an estimated 300,000 human cases acquired annually in the United States (Kuehn 2013); less commonly, these same ticks also are capable of transmitting the pathogens that cause anaplasmosis, babesiosis, a tick-borne relapsing fever, and Powassan virus encephalitis.

Blacklegged ticks are predominantly found in landscapes containing deciduous forests, but due to demographic and wildlife population trends, human tick encounters have steadily increased and the spatial distribution of these ticks have expanded, even into residential backyards. This increase in tick encounter rates along with advances in medical diagnostics, patient screening, and disease reporting have also contributed to the steady increase of confirmed Lyme disease cases over the past few decades (Berardi et al. 1988, Pearson 2014, CDC 2016a, 2016b)

To combat rising levels of tick-borne disease incidence among humans and pets, there is an increasing need to take measures that protect against tick bites. From a personal protection viewpoint, (1) identifying and avoiding tick habitat, (2) using repellents, especially long-lasting clothing-only repellents with the active ingredient permethrin, and (3) performing daily tick checks all can help reduce the risk of potential tick bites (Faulde and Uedelhoven 2006, Faulde et al. 2006, Miller et al. 2011, Vaughn and Meshnick 2011, Eisen and Dolan 2016). Environmental measures also can be taken, including (1) landscape management to reduce tick habitat (e.g., cutting back low hanging branches to increase the amount of sunlight and reduce humid environments, clearing leaves and controlled burning to reduce tick habitat, stacking wood piles to reduce rodent (tick hosts) habitat), (2) use of host targeted strategies to kill ticks before they can feed (e.g., Max Force bait boxes and Damminix tick tubes), and (3) using a broadcast acaricide application often called a "perimeter spray", referring to targeted spraying of the habitat most frequented by these ticks in the residential landscape (Mount 1981, Mather et al. 1987, Deblinger and Rimmer 1991, Schulze et al. 1995, Hubálek et al. 2006, Piesman 2006, Stafford 2007, Ginsberg 2014). Some homeowners have become somewhat suspicious of possible side effects of spraying synthetic chemical pesticides on their property which creates a potential barrier to effective tick bite protection. However, a growing trend favors a more natural tick treatment like botanical oils or biopesticides.

In 1996, the Environmental Protection Agency (EPA) amended the Federal Insecticide, Fungicide & Rodenticide Act to exclude from regulation a class of pesticides they termed "Minimum Risk Pesticides". These products are deemed to "pose little to no risk to human health or the environment" but must meet 6 conditions in order to qualify, one being that their active ingredients being listed as a qualifying ingredient on EPA's minimal risk products list (40 CFR 152.25(f)(1)) (EPA 2016). Once approved, makers of these products are exempted from registering with the EPA under clause 25(b), and 25(b) exempt products generally fall solely under the regulation of individual States where they are distributed. Accordingly, these products do not undergo the same rigorous testing and analysis required of most pesticides. Using a novel microplot design in a field trial, this study was conducted to help amend this possible oversight by screening the tick-killing efficacy of commercially available and experimental minimal risk natural products (MRNPs) claiming to reduce tick abundance.

INTRODUCTION

Annual cases of Lyme disease, the most commonly reported tick-borne disease in the United States, have been increasing consistently over the past 20 years, especially in the northeastern United States (CDC 2016a). Since 2005, in Rhode Island alone, the Lyme disease incidence rate has increased from 3.6 to 54 cases per 100,000 residents compared to the national rate of 7.9 cases per 100,000 (CDC 2016b). Furthermore, in Rhode Island, it is estimated that more than 300 additional cases go unreported every year (CDC 2016b).

The public generally understands that blacklegged ticks, *Ixodes scapularis* Say carry the Lyme disease-causing bacterium and transmits it to people and pets during blood feeding (Childs et al. 1998, Herrington Jr 2004). They also are familiar with the bull's eye rash that is characteristic of Lyme disease. Although public awareness regarding tick bite-associated health risks is increasing, a large gap in tick-bite prevention knowledge and action still exists. Despite being well versed in the consequences of tick exposure, the public is largely uneducated, inexperienced, and prone to foregoing the most effective tick bite prevention behaviors and activities (Herrington Jr 2004, Gould et al. 2008, Connally et al. 2009). Many factors likely contribute to this, including: 1) lack or improper use of protective measures such as repellents and wearing repellent-treated clothes, 2) difficulty in finding attached and feeding ticks and 3) failure to recognize and avoid tick habitat.

Along with host-targeted strategies and landscape manipulations, suppressing the tick population with an area-wide treatment using chemical pesticides is considered one of the most effective methods for reducing tick encounter risk on residential properties. For control of the blacklegged tick, a broadcast application method often called a "perimeter spray" is used, referring to targeted spraying of the habitat most frequented by these ticks in the residential landscape (Piesman 2006, Stafford 2007). If applied correctly using effective products, perimeter sprays can significantly reduce tick encounter risks for family members, including pets, within their own yard (Stafford 2007). However, due to concerns about potential human toxicity/carcinogenicity, environmental contamination (including groundwater), and toxicity toward non-target organisms and pets (Childs et al. 1998), recent consumer trends suggest that homeowners are embracing newer, "greener" natural alternatives over industry standard synthetic chemical pesticides which have historically been proven effective. Though possibly less damaging to the environment, the natural pesticides, which may include various botanical oils, biopesticides, and abrasives, or a combination of these, have not been thoroughly tested. Also, due to their "natural" active ingredients, they do not fall under the same Environmental Protection Agency (EPA) regulations as do the industry standard chemicals. Such minimal risk natural products (MRNPs) are usually considered exempt from federal regulation under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), and need not be registered with the EPA, but may need to be listed within the states they are distributed (EPA 2016).

Traditionally, field plots used for evaluating efficacy of acaricides to control blacklegged ticks using the "area-wide" method typically range from 100 m^2 to hectares in size and must be replicated extensively to support enough tick collection numbers for statistical analyses. Such studies are labor intensive and expensive, presenting a significant impediment to evaluating tick control products. Moreover, when conducted across residential sites, ecological variability often results in variances much larger than means. This study simultaneously evaluates an array of MRNPs in a novel micro-plot system that simulates ecological conditions found in typical residential sites in the northeast U.S. where blacklegged ticks are highly endemic. Using field-derived but laboratory-reared nymphal blacklegged ticks, I compared the tick-killing knockdown and residual activity of some of these products to highly effective formulations of bifenthrin, the current industry standard which has been proven effective against ticks (Stafford 2007, Elias et al. 2013).

MATERIALS & METHODS

Field Plot Set-up. This study was conducted over four nymphal blacklegged tick seasons, June-August (2012-2015), in a plot of woods located at the University of Rhode Island's East Farm. Professional contractors surveyed and prepared the site by cutting down some of the smaller trees to open up the dense tree canopy in Fall 2011, creating a 0.5 ha study area similar to that of a wooded residential property. The site was left undisturbed until the following Spring (2012) to allow restoration of the natural leaf litter. The area was covered by a predominantly oak canopy and the substrate consisted of natural leaf litter and un-mown grasses. Six plots were laid out within the newly established study area, and each of them was further divided into five or six micro-plots, each containing two 0.3 m diameter PVC rings (arenas) spaced 1.5 m apart and tampered into the ground (Fig 1). Each micro-plot was assigned a different Minimal Risk Natural Product (MRNP) treatment based on a randomized block design, resulting in a total of six replicates per treatment. The two arenas within each micro-plot represented a knockdown (KD) treatment and a residual (RESID) treatment. A total of 60 first generation, lab-reared nymphal blacklegged ticks were placed into each knockdown arena to provide a 24 hour acclimation prior to applying MRNPs to the arenas to allow tick dispersal into the leaf litter. The residual arenas were sprayed at the same time as the knockdown arenas, but were allowed to weather for 2 weeks before ticks were added to them. Three humidity loggers were placed within the study site to record temperature and relative humidity for the duration of each study season in an attempt to detect any low moisture events which might negatively impact tick survival (Berger et al. 2014).

Treatment Preparations and Applications. The materials evaluated at labeled field rates were commercially available and/or experimental materials (Table 1) and included the following products: Met52® EC (Novozymes Biological Inc., Salem, VA), Essentria™ IC^3 (Envincio LLC, Cary, NC), EcoPCO® EC-X (Prentiss Inc., Alpharetta, GA), Talstar® Professional (FMC Corp, Philadelphia, PA), CedarCide PCO Choice (CedarCide Industries Inc., Spring, TX), EcoEXEMPT® IC^2 with EcoADJUVANT™ (EcoSMART Technologies Inc., Franklin, TN), Tick Stop (Wildflower Farm, Delhi, NY), Tick Killz (Natural Repellents LLC, Newtown, CT), UP-Star Gold (United Phosphorus Inc., Trenton, NJ) and EcoSMART® Organic® Insecticide (EcoSMART Technologies Inc, Roswell, GA). Three experimental formulations included nootkatone with d-Limonene (provided by the CDC), Tick Guard (East End Tick Control, Southampton, NY), and Progaea (Pure Pest Solutions, LLC, Weston, MA). Some of the products were tested more than once because they were either (a) a positive control representing the industry standard (bifenthrin), (b) our sampling timeline differed from the product instructions (Met 52® EC), (c) we were testing different "batches" of the same product (Met52® EC, Essentria™ IC^3) and/or (d) we were testing different concentrations of the same product (Talstar® Professional, Essentria™ IC^3) (Table 2).

Liquid formulations of MRNPs were prepared according to label specifications, mixed in 1 gallon plastic containers and poured into Solo backpack sprayers (Solo Inc, Newport News, VA), where they were hand-pumped to 620.5 kPa. A 0.91 m^2 piece of plastic was used to create a 0.3 m diameter cylindrical "spray shield" which was placed inside of the arenas to prevent over spray beyond the arenas. The sprayer wand was placed inside of the spray shield, just above the leaf litter, and 30 milliliters of product was applied in a circular motion, in an attempt to have even distribution of product. Dry formulations were weighed into plastic portion cups (one per arena) prior to application. Post product application, arenas were covered with 3.2 cm^2 hardware cloth secured with stakes until ready for sampling to prevent disruption from wildlife.

Sampling. Arenas were evaluated for 2 weeks at 3-4 day increments after treatment (Fig. 2) using a round 0.3 m diameter pressboard wrapped in a flannel "bonnet". Each arena was continuously sampled by pressing the board into the leaf litter for 5 second increments to collect questing nymphs, until 3 consecutive samples revealed no ticks attached. Using fine-pointed tweezers, all ticks were placed into vials after each press and results recorded. Care was taken to keep separate pressboards for each treatment and to launder the flannel bonnets between sample days to avoid cross contamination.

Nymphal ticks. Ticks for these experiments were reared from wild-caught host-seeking females, then fed on rabbits, and fed as larvae on hamsters in the laboratory (Mather and Mather 1990)(URI/IACUC approval AN08-04-017, originally dated June 2008). Engorged larvae were held under 23.5°C/>95% RH and 14L/10D until molting. Molted nymphs were held in the incubator for an additional 2-3 months to ensure active questing behavior before being used in the field experiment. Sixty lab reared nymphal blacklegged ticks were seeded into each arena, for a total of 360 per treatment.

Statistical Analysis. Repeated measures analysis of variance (ANOVA) was used for statistical comparisons of nymphal tick counts among treatments and plots using SigmaPlot®11 (Systat Software, Inc., San Jose, CA). Knockdown efficacy (KD) and residual activity (RESID) experiments were treated as two separate analyses. Mean differences were analyzed, for both components of the analysis, using Tukey's Honestly Significant Difference (HSD).

Efficacy of pesticide treatments was evaluated by comparing percent control of nymphal tick densities in each treatment plot against water control plots. Percent control was calculated using Abbott's formula (Abbott 1925):

$$\textbf{Corrected Percent (\%) control} = \left[1 - \frac{n \text{ in } T \text{ after treatment}}{n \text{ in } C \text{ after treatment}}\right] * 100$$

where *n* is nymphal tick density, *T* is treated plots and *C* is water control plots. The Henderson and Tilton formula (1955) wasn't needed for this study because all of the arenas, both treatment and control, contained the same number of ticks at the beginning of the study, therefore making the two formulas equivalent.

RESULTS

2012 Trials. The positive control treatment, bifenthrin (Talstar® Professional) was highly effective as both a knockdown and 2 week residual treatment (0 nymphs recovered, 100% KD, $P < 0.001$; 0 nymphs recovered, 100% RESID, $P < 0.001$, respectively).(Table 2, Figure 3). Among the MRNPs, only the EcoPCO® EC-X was found to have a statistically significant impact as both a knockdown (1 nymph recovered, 99.6% KD, $P < 0.001$), and residual treatment (76 nymphs recovered, 72.4% RESID, $P < 0.001$) relative to water-only control plots two weeks post application. There was no significant difference between the abundance of nymphs in either the knockdown or residual treatments using Essentria™ IC^3 when compared to the water-only control plots (229 nymphs recovered, 15.2% KD, $P = 0.193$; 288 nymphs recovered, 0% RESID, $P = 0.988$, respectively). The Met52® EC treatment also had no significant impact as a knockdown or residual application (243 nymphs recovered, 10% KD, $P = 0.574$; 277 nymphs recovered, 0% RESID, $P = 1.000$, respectively) relative to the water-only controls.

2013 Trials. Following the product label instruction rather than our standard post-application sampling timeline, I extended the time duration between application of Met52® EC and tick sampling to 7 days and observed a statistically significant knockdown effect compared to the water-only control (94 nymphs recovered, 41.3% KD, $P = 0.037$) but the effectiveness of Met52®EC dropped noticeably in the residual study (158 nymphs recovered, 0% RESID, $P = 0.447$) (Table 2, Figure 4). The Essentria™ IC^3 and EcoEXEMPT® IC^2 both exhibited a statistically significant tick knockdown effect, but the Essentria™ IC^3 had a noticeably lesser impact (74 nymphs recovered, 53.8% KD, $P = 0.004$) than the EcoEXEMPT® IC^2 (22 nymphs recovered, 86.6% KD, $P < 0.001$). Neither formulation was effective as a residual treatment (227 nymphs recovered, 0% RESID, $P = 1.000$; 152 nymphs recovered, 30.2% RESID, $P = 0.355$, respectively) when compared to the water-only control. The CedarCide PCO Choice, was neither effective as a knockdown nor residual treatment (152 nymphs recovered, 5% KD, $P = 0.999$; 259 nymphs recovered, 0% RESID, $P = 0.931$, respectively). Talstar® Professional remained highly effective as a knockdown and residual treatment, even after 3 weeks post application (0 nymphs recovered, 100% KD, $P < 0.001$; 0 nymphs recovered, 100% RESID, $P < 0.001$, respectively).

2014 Trials. Essentria™ IC^3 and nootkatone (w. d-Limonene) had significant knockdown effects (186 nymphs recovered, 30.6% KD, $P = 0.003$; 45 nymphs recovered, 83.21% KD, $P < 0.001$, respectively) when compared to water-only controls, but only the nootkatone (w. d-Limonene) remained effective as a residual (229 nymphs recovered, 35.37% RESID, $P = 0.006$). The residual effect of the Essentria™ IC^3 noticeably decreased compared to the knockdown efficacy as in previous trials with this product (275 nymphs recovered, 6.46% RESID, $P = 0.105$) (Table 2, Figure 5). Neither of the other products, Tick Killz and Tick Stop, had an effect on the nymphs as a knockdown treatment (252 nymphs recovered, 5.97% KD, $P = 0.994$; 256 nymphs recovered, 4.48% KD, $P = 0.999$, respectively) nor as a residual treatment (269 nymphs recovered, 8.5% RESID, $P = 0.376$; 285 nymphs recovered, 3.06% RESID, $P = 0.845$, respectively). The bifenthrin product (UP-Star Gold, 7.9% AI) was highly effective as a knockdown and as a residual, this time 4 weeks post application (1 nymph recovered, 99.63% KD, $P < 0.001$; 1 nymph recovered, 99.69% RESID, $P < 0.001$, respectively). Surprisingly, significantly fewer nymphs were recovered in arenas treated with Talstar® Professional in 2013 (1 year residual) when compared to the water-only control (70 nymphs recovered, 78.3% RESID, $P < 0.001$).

2015 Trials. None of the MRNPs tested (Tick Guard, Progaea and EcoSMART® Organic® Insecticide) were found to have a significant effect as a knockdown treatment (253 nymphs recovered, 0% KD, $P = 0.922$; 139 nymphs recovered, 37.1%

KD, $P = 0.099$; 257 nymphs recovered, 0% KD, $P = 0.512$, respectively) when compared to water-only control plots (Table 2, Figure 6), and two of them (Tick Guard and EcoSMART® Organic® Insecticide) had more nymphs recovered than in the water-only control plots. All three products also were found to be ineffective as residual treatments (286 nymphs recovered, 0% RESID, $P = 0.998$; 228 nymphs recovered, 17.9% RESID, $P = 0.475$; 297 nymphs recovered, 0% RESID, $P = 0.954$, respectively), with more nymphs being recovered from the Tick Guard and EcoSMART® Organic® Insecticide plots than from the water-only controls. A one-third lower concentration of Talstar® Professional than used in the previous trials was still highly effective as both a knockdown and residual treatment (1 nymph recovered, 99.55% KD, $P < 0.001$; 0 nymphs recovered, 100% RESID, $P < 0.001$, respectively) when compared to the water-only control plots, and the full-strength application had a similar performance to previous years (0 nymphs recovered, 100% KD, $P < 0.001$; 4 nymphs recovered, 98.55% RESID, $P < 0.001$, respectively).

DISCUSSION

A novel micro-plot system was developed for screening multiple acaricidal products under the same environmental conditions and location in the field as a means of evaluating minimal risk natural products for control of nymphal blacklegged ticks. By compressing large field test sites into single 0.3 m arenas which were seeded with a known number of first generation lab-reared nymphs, my approach saved time by sampling small areas, and also reduced study costs due to more efficient treatment application. Use of spray shields prevented cross-contamination of treatments and allowed me to use less product per treatment. An additional benefit of the novel micro-plot system was that I could use a large and known number of ticks seeded within each test arena, affording decreased variability between seasonal treatments and ease of reproducibility and comparison across years.

Eleven MRNP materials and formulations were assessed for their efficacy as tick control products in comparison to bifenthrin and a water-only control. Results showed that along with the synthetic pyrethroid bifenthrin, only one commercially-available MRNP, EcoPCO® EC-X (pyrethrins) and one experimental (nootkatone with d-Limonene) provided a high level of knockdown control over host-seeking *I. scapularis* nymphs and, although their tick-killing efficacy may have degraded somewhat, acaricidal activity of these MRNPs still persisted to effect a statistically significant level of tick control in the two week residual study.

For the purpose of standardization, in 2012, the sampling timeline for the Met52® EC was kept the same as all the other products, with sampling beginning 3 days post application, which was contrary to the label instructions. Under these conditions, Met52® EC did not have a significant impact as either a knockdown or residual application as had been previously published (Stafford and Allan 2010). In 2013, the sampling timeline for the Met52® EC was adjusted to label instructions, allowing the fungal spores to establish for a full week prior to tick sampling, and under a longer incubation scenario, this biopesticide did exhibit a knockdown effect on the questing nymphs that was statistically significant when compared to water-only controls, but it did not significantly suppress nymphs in the residual study. The use of *Metarhizium sp.* as a biological control agent has been widely studied against several arthropod pests including blowflies in England (Wright et al. 2004), grasshoppers and locusts in Australia (Hunter 2005), mosquitoes in Mexico and Korea (Garza-Hernandez et al. 2015, Lee et al. 2015) and several species of ticks world-wide (Benjamin et al. 2002, Kirkland et al. 2004, Leemon et al. 2008, Bharadwaj and Stafford 2010, Wassermann et al. 2016) with mixed results. One potential reason for this may be due to variation between several strains of the fungus; each may have a different effect depending on pest species, pest life stage, environmental conditions, spore concentration and formulation. Another reason may have been the sampling technique used in the respective study designs. The results from this study were indicative of fungal growth/tick killing effect under natural field conditions, whereas in some previously published *M. anisopliae* studies, ticks were sampled out of plots and returned to the lab to be maintained under ideal conditions for fungal growth (Benjamin et al. 2002, Bharadwaj and Stafford 2010, Stafford and Allan 2010).

Two additional minimal risk natural products exhibited a significant knockdown effect; in 2013, both the EcoEXEMPT® IC^2 and Essentria™ IC^3 knockdown treatments had significantly fewer ticks recovered than the water-only control. Both of these products contain rosemary and peppermint oils. The newer Essentria™ IC^3 is the replacement formulation of EcoEXEMPT® IC^2 which previously had been shown to be effective against blacklegged ticks (Rand et al. 2010). The EcoEXEMPT® IC^2 required adding an emulsifier prior to dilution and application; in re-formulating the product, an adjuvant was added to Essentria™ IC^3 so that the emulsifier was no longer required to keep the oils in suspension. Although still effective, the original IC^2 formulation had a greater impact on host-seeking nymphs than the newer Essentria™ IC^3, but neither product remained active enough to have a significant impact on the nymphs exposed during the residual trials. It is possible that the greater tick-killing action of the IC^2 formulation could be attributed to the emulsifier than to the botanical oils (Schroer et al. 2001, Mullin et al. 2015).

In 2014, I received a sample of nootkatone crystals from the Centers for Disease Control and made a 2% solution by dissolving them in d-Limonene (a solvent extracted from orange peels) before diluting it in water containing EZ-Mulse (a proprietary blend of nonionic surfactants used to emulsify citrus extracts and natural oils) (Jordan et al. 2011, Bharadwaj et al. 2012). As had been seen in previous studies (Dolan et al. 2009, Jordan et al. 2011, Bharadwaj et al. 2012), this experimental nootkatone formulation exhibited a significant immediate knockdown effect (83.2%) on the host-seeking nymphs, and although its tick-killing efficacy may have degraded slightly, it still remained active for the two week residual study, killing 35% of nymphs released into the arenas two weeks after product application. Essentria™ IC^3 was tested for a third time, but using a less concentrated solution as per label rates, and while this treatment had a significant knockdown effect (30.6%), it had no residual effect.

In 2015, two privately-labelled products, Tick Guard and Progaea,based on the original formulation of EcoEXEMPT® IC^2, and EcoSMART® Organic® Insecticide granules had no significant knockdown or residual effect on host-seeking blacklegged tick nymphs. In fact, more nymphs were recovered from the Tick Guard and EcoSMART® Organic® Insecticide plots than from the water-only control plots.

In total, five formulations of rosemary and peppermint oil were tested and only two of them exhibited any significant knockdown effect. The observed batch-to-batch variability in efficacy raises concerns regarding formulating botanical oil products, and this study provides evidence that there is a need for better quality control.

Minimal risk natural product active ingredients include various botanical oils (such as rosemary, peppermint and cedar oils), biopesticides, and abrasives, or a combination of these. Most or all of this class of product are exempted from Environmental Protection Agency registration and regulation under section 25(b) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (EPA 2016). While individual States may impose a greater degree of oversight and regulation, generally, the 25(b) exemption means that this class of products do not typically undergo the same rigorous testing and analysis that most pesticides do prior to production and distribution. Because of this, there may not be sufficient evidence that they truly work against the list of pests claimed on their labels, and potential environmental side effects, while presumably minimal, remain unknown. Furthermore, while a few of the MRNP materials tested here showed a statistically significant effect when compared to the water-only control treatments, many would not be recommended for use in controlling blacklegged tick populations, as their claim of efficacy still left >40-50% of the original tick population alive following a single knockdown treatment. It should be noted that in September 2012, the Federal Trade Commission (FTC) filed deceptive advertising charges and began litigation against multiple companies including CedarCide Industries, Inc., (makers of CedarCide PCO Choice) challenging their strategies for bed bug and lice treatments (Lordan 2012). The complaint was for making unsubstantiated and false claims about (1) the efficacy of their product, (2) about scientific studies that had been conducted and (3) claiming that their product was invented for the U.S. Army at the request of the U.S. Department of Agriculture. In some cases, my study likely provides some of the first or only efficacy data for these products in controlling blacklegged ticks, and consumers may want to consider this before relying on using a MRNP for residential tick control.

Finally, I included bifenthrin, currently considered the industry standard in broadcast tick control treatments, as the positive control in this study. It was highly effective as both a knockdown and residual treatment in all four years, including as a residual 4 weeks post-application. In the final year of testing, we decreased the bifenthrin concentration by a third of its labelled rate and still had <2% recovery of ticks from both the knockdown and residual plots. With such a high rate of efficacy and low rate of application, when combined with its typical use as a perimeter treatment in residential landscapes, it would seem difficult at this time to dismiss the use of bifenthrin as an effective tool in tick control and tick-borne disease prevention.

Table 1. Commercially available and experimental formulations of MRNPs and industry standards evaluated during blacklegged tick seasons

Category	Product(s)	Formulation	Active Ingredient(s)	Application Rate(s), Year(s) Used
Abrasives	Tick Stop	Granule	Organic Fertilizer	45.25 g/ m^2 (2014)
Biopesticides	EcoPCO® EC-X	Liquid	Pyrethrins	25.71 mL/m^2 (2012)
	Met52® EC	Liquid	*Metarhizium anisopliae*	0.96 mL/m^2 (2012); 1.02 mL/m^2 (2013)
Botanical Oils	CedarCide PCO Choice	Liquid	Texas Red Cedar Oil	25.71 mL/m^2 (2013)
	EcoEXEMPT® IC[2] (w. EcoADJUVANT®)	Liquid	Rosemary oil, Peppermint Oil	25.71 mL/m^2 EcoEXEMPT® IC[2], 6.43mL/m^2 EcoADJUVANT® (2013)
	EcoSMART® Organic® Insecticide	Granule	Clove oil, Thyme oil	24.41 g/m^2 (2015)
	Essentria™ IC[3]	Liquid	Rosemary oil, Peppermint Oil, Geraniol	25.71 mL/m^2 (2012, 2013); 19.28 mL/m^2 (2014)
	nootkatone (w. d-Limonene & EZ-Mulse)	Liquid	Nootkatone, d-Limonene, EZ-Mulse	1.02 mL/m^2 Nootkatone; 0.96 mL/m^2 d-Limonene, EZ-Mulse (2013)
	Progaea	Liquid	Rosemary oil, Peppermint Oil, Geraniol	16.07 mL/m^2 (2015)

Table 1 (cont). Commercially available and experimental formulations of MRNPs and industry standards evaluated during blacklegged tick seasons

Category	Product(s)	Formulation	Active Ingredient(s)	Application Rate(a), Year(s) Used
Botanical Oils	Tick Guard	Liquid	Rosemary oil, Peppermint Oil, Geraniol	6.43 mL/m^2 (2015)
	Tick Killz	Liquid	Cedarwood oil	0.64 mL/m^2 (2014)
Chemical Pesticides	Talstar® Professional	Liquid	Bifenthrin	0.33 mL mL/m^2 (2012, 2013, 2015)
	UP-Star Gold	Liquid	Bifenthrin	0.33 mL mL/m^2 (2014)

Table 2. Effect of MRNPs and bifenthrin on *Ixodes scapularis* nymphs after a single application in June 2012-2015

		Knockdown (KD)			Residual (RESID)		
Year	Product	# Ticks Recovered	% Control	*P*-Value	# Ticks Recovered	% Control	*P*-Value
2012	EcoPCO® EC-X	1	99	$P < 0.001$ *	76	72	$P < 0.001$ *
	Essentria™ IC[3]	229	15	$P = 0.193$	288	0	$P = 0.988$
	Met52® EC	243	10	$P = 0.574$	277	0	$P = 1.000$
	Talstar® Professional	0	100	$P < 0.001$ *	0	100	$P < 0.001$ *
2013	CedarCide PCO Choice	152	5	$P = 0.999$	259	0	$P = 0.931$
	EcoEXEMPT® IC[2]	22	87	$P < 0.001$ *	152	30	$P = 0.355$
	Essentria™ IC[3]	74	54	$P = 0.004$ *	227	0	$P = 1.000$
	Talstar® Professional	0	100	$P < 0.001$ *	0	100	$P < 0.001$ *
	Met52® EC	94	41	$P = 0.037$ *	158	30	$P = 0.447$

Percent control was calculated using Abbott's formula.
An asterisk (*) indicates significant differences ($P < 0.05$) between treatment and water negative control plots using Tukey HSD test.

Table 2 (cont). Effect of MRNPs and bifenthrin on *Ixodes scapularis* nymphs after a single application in June 2012-2015

		Knockdown (KD)			Residual (RESID)		
Year	Product	# Ticks Recovered	% Control	*P*-Value	# Ticks Recovered	% Control	*P*-Value
2014	Essentria™ IC[3]	186	31	$P = 0.003$ *	275	7	$P = 0.554$
	nootkatone (w. d-Limonene)	45	83	$P < 0.001$ *	229	35	$P = 0.006$*
	Tick Killz	252	6	$P = 0.994$	269	9	$P = 0.376$
	Tick Stop	256	5	$P = 0.999$	285	3	$P = 0.845$
	UP-Star Gold	1	99	$P < 0.001$ *	1	99	$P < 0.001$*
2015	EcoSMART® Organic® Insecticide	257	0	$P = 0.512$	297	0	$P = 0.954$
	Progaea (Pure Pest)	139	37	$P = 0.099$	228	17	$P = 0.475$
	Talstar® Professional (100%)	0	100	$P < 0.001$ *	4	99	$P < 0.001$ *
	Talstar® Professional (67%)	1	99	$P < 0.001$ *	0	100	$P < 0.001$ *
	Tick Guard	253	0	$P = 0.922$	286	0	$P = 0.998$

Percent control was calculated using Abbott's formula.
An asterisk (*) indicates significant differences ($P < 0.05$) between treatment and water negative control plots using Tukey HSD test.

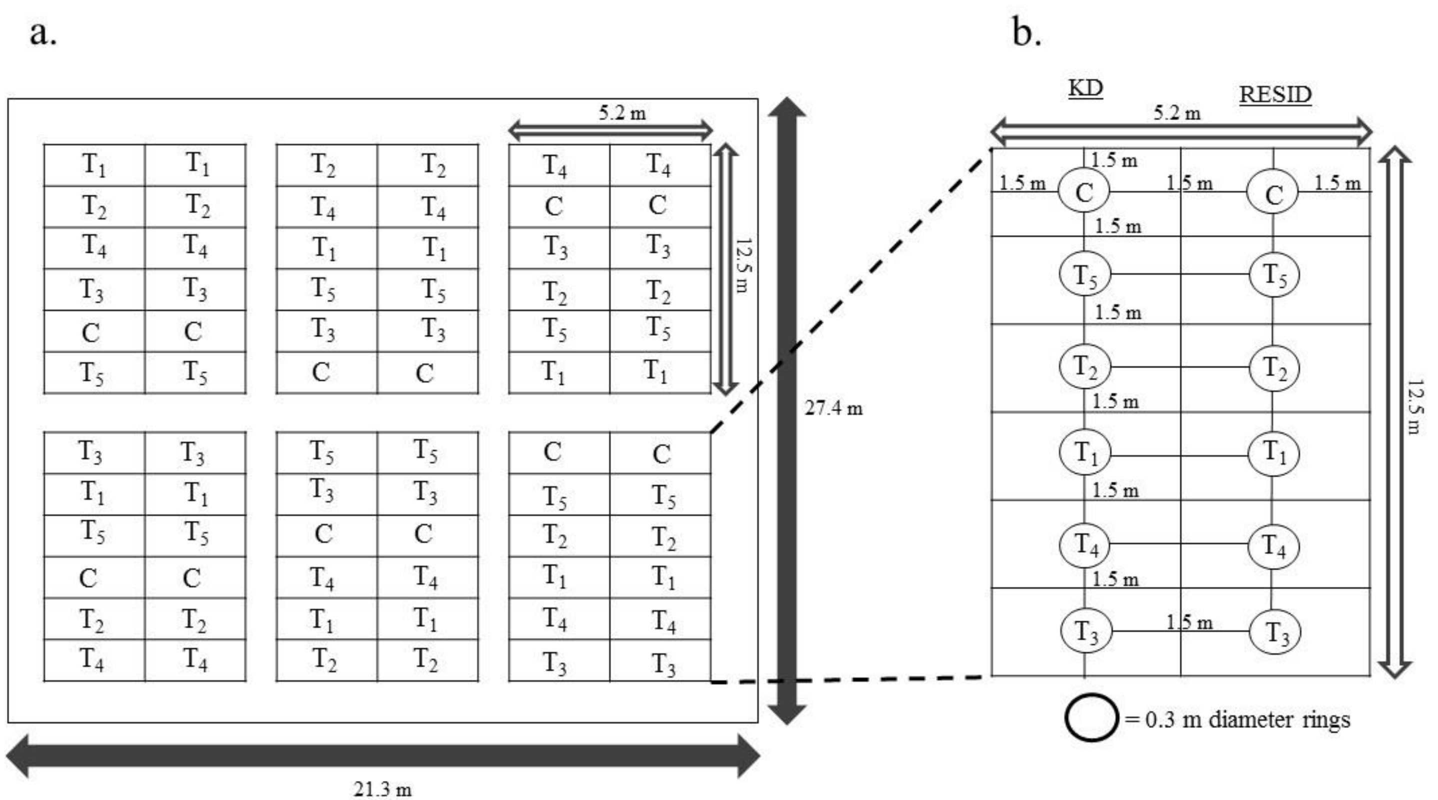

Figure 1. (a) Completely randomized block design for 21.3 m x 27.4 m simulated residential plot. (b) A single block (replicate) consisting of 5 treatments and a single control, the left side and the right side are used for the knockdown (KD) efficacy trial and residual (RESID) efficacy, respectively. Treatment areas are 0.3 m diameter arenas, with 1.5 m wide buffer. Note: Diagram not to scale.

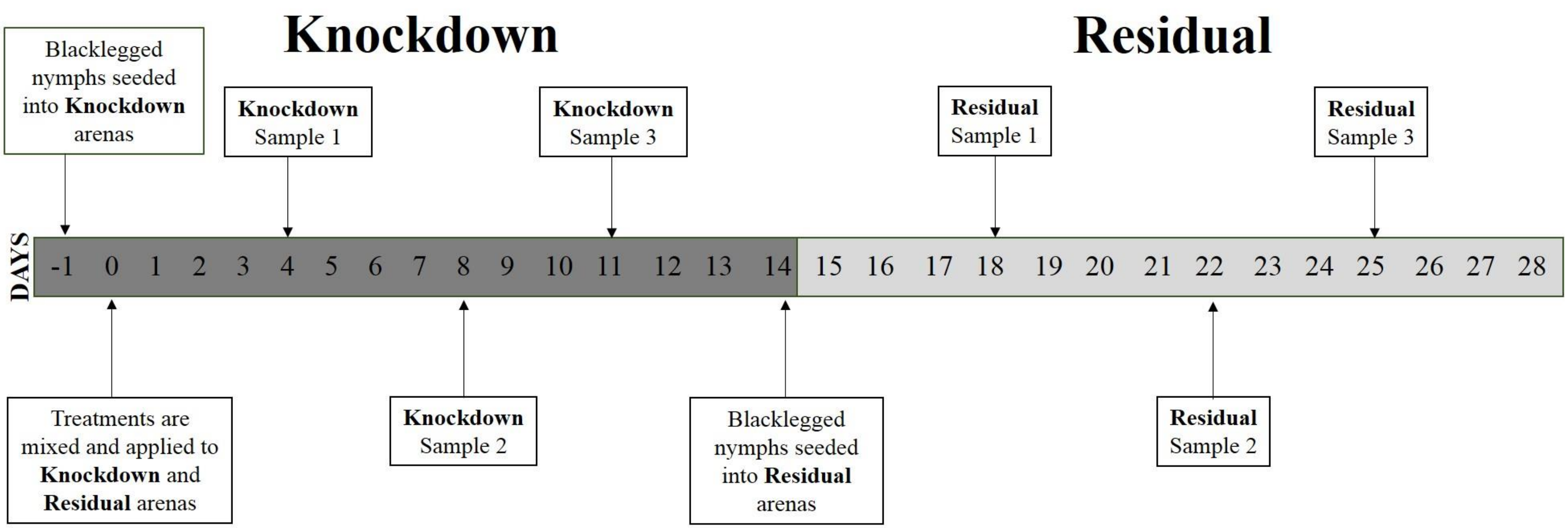

Figure 2. Timeline of events, including treatment and approximate sampling schedules. Due to inclement weather, sampling days may have been shifted accordingly.

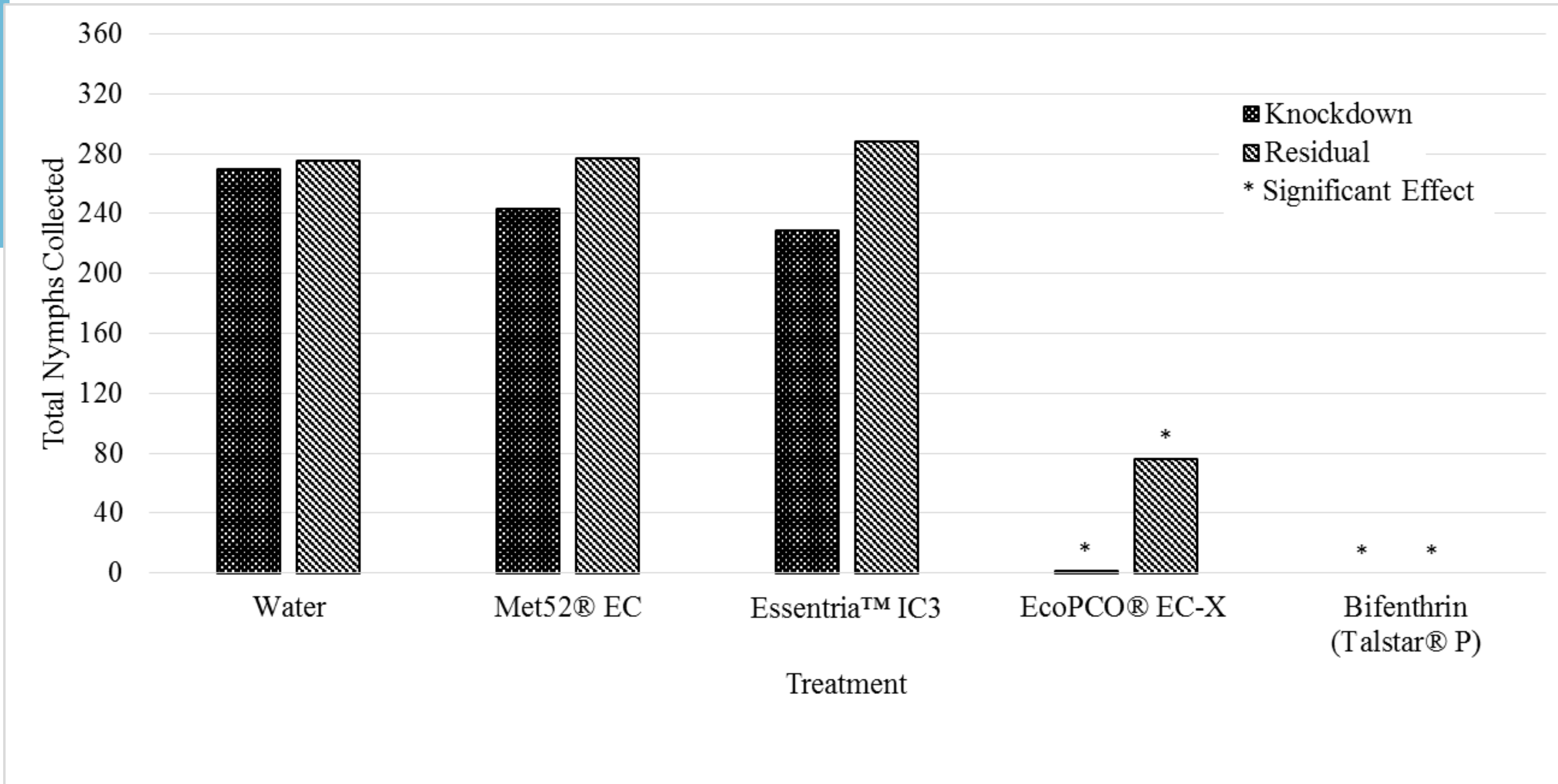

Fig 3. Total number of blacklegged tick nymphs recovered from micro-plots (of 360 total) during 2012 testing.

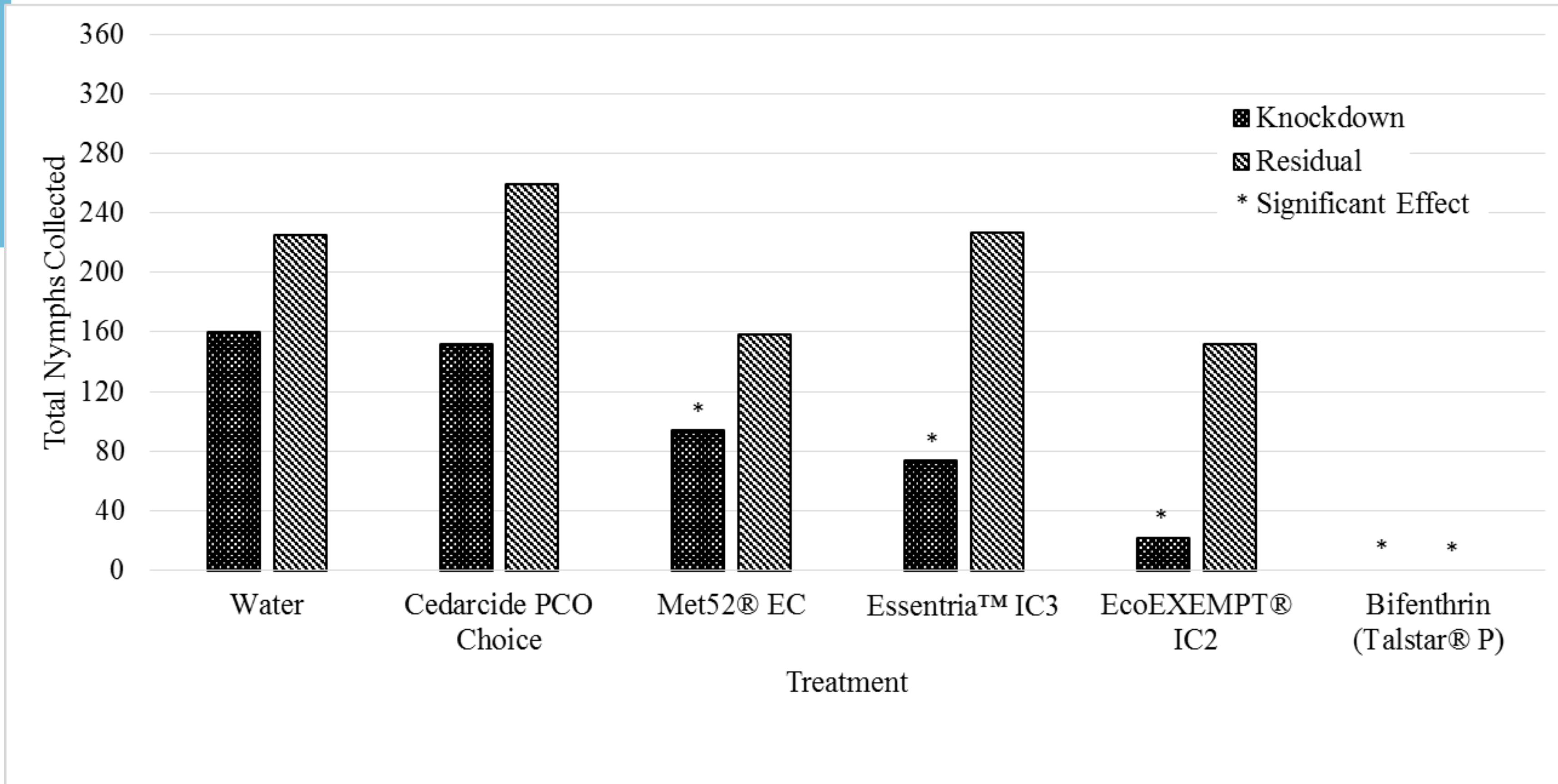

Fig 4. Total number of blacklegged tick nymphs recovered from micro-plots (of 360 total) during 2013 testing.

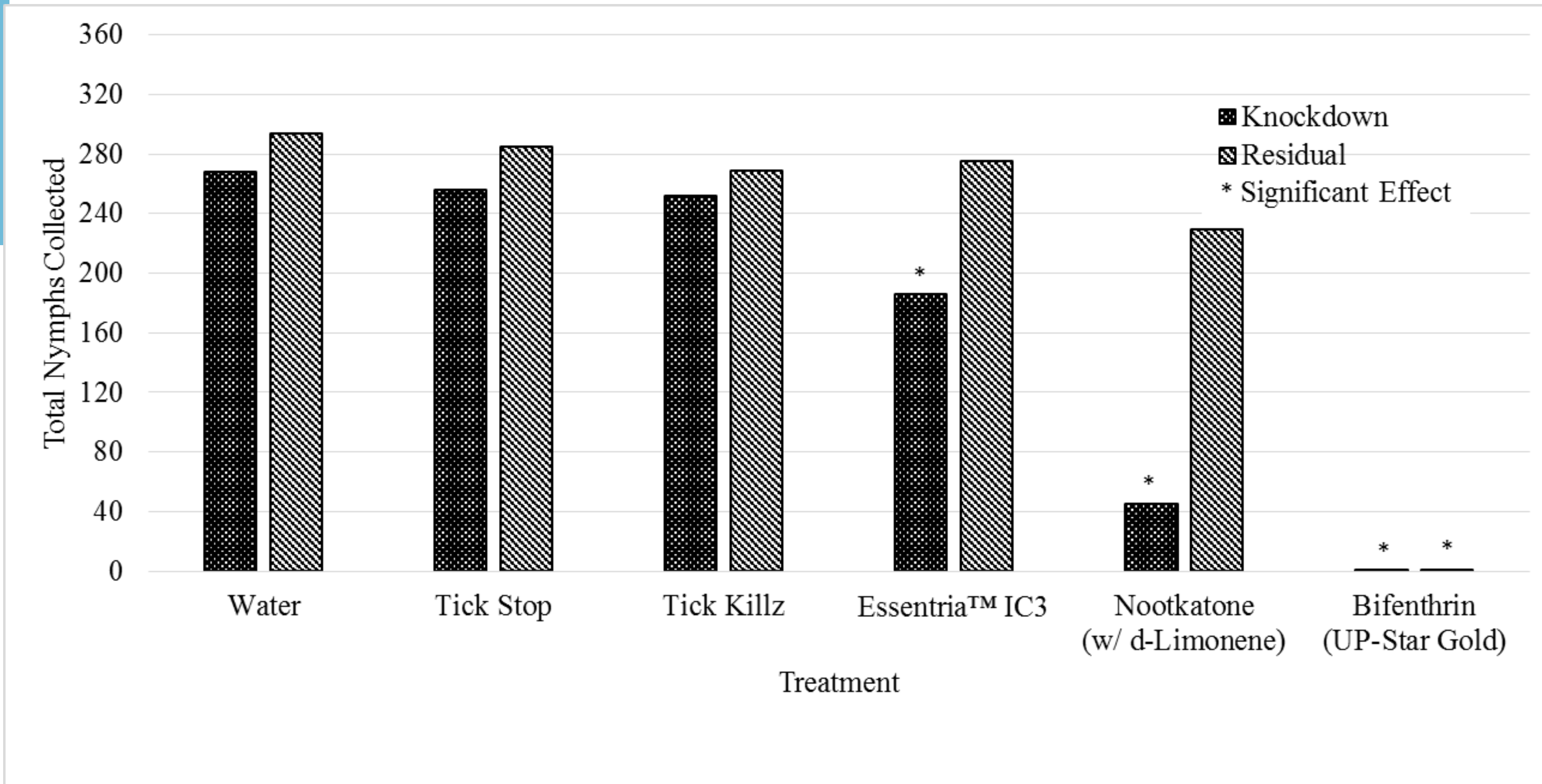

Fig 5. Total number of blacklegged tick nymphs recovered from micro-plots (of 360 total) during 2014 testing.

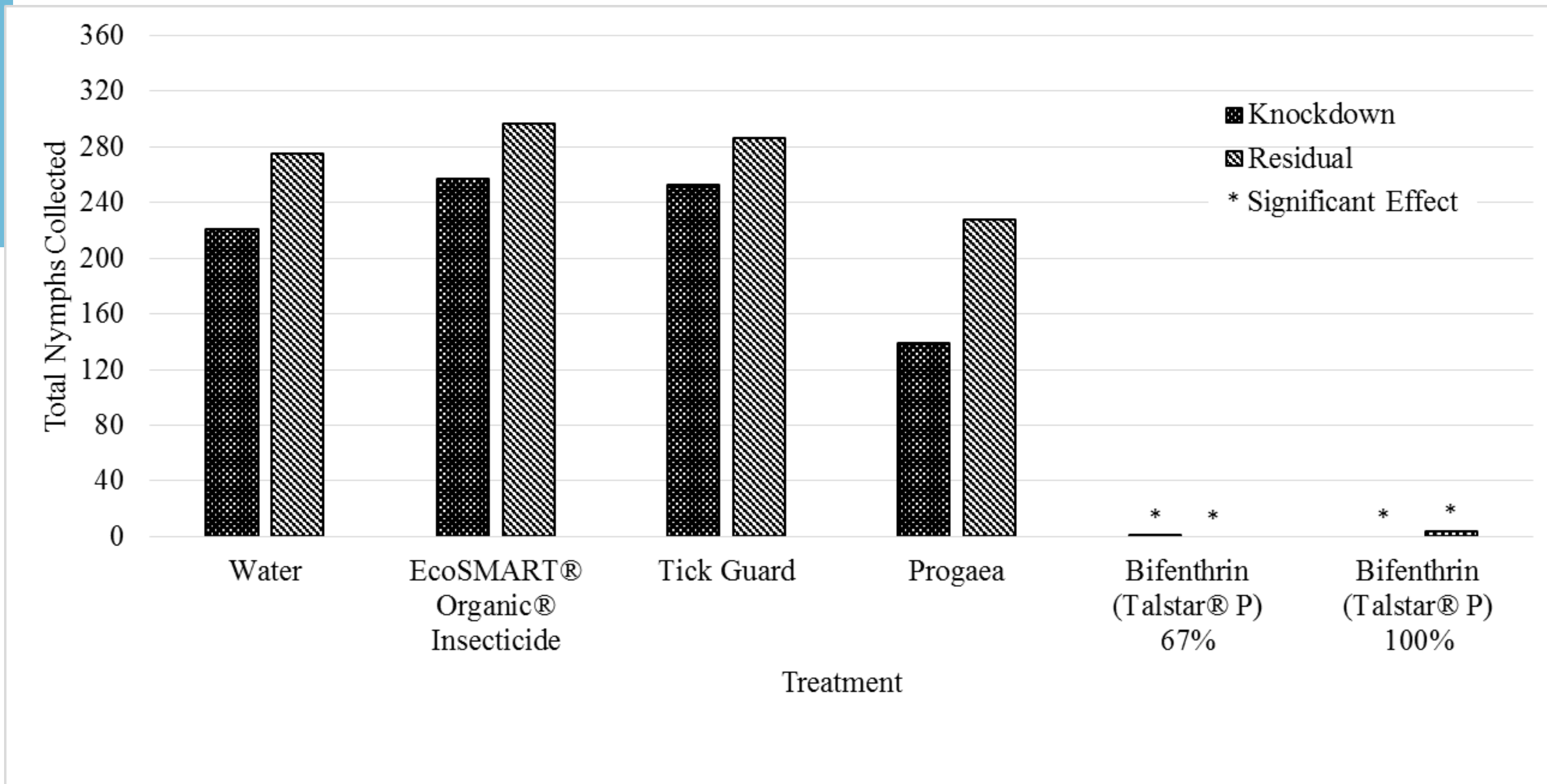

Fig 6. Total number of blacklegged tick nymphs recovered from micro-plots (of 360 total) during 2015 testing.

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