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Use of LYMESIM 2.0 to assess the potential for single and integrated management methods to control blacklegged ticks (Ixodes scapularis; Acari: Ixodidae) and transmission of Lyme disease spirochetes

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ABSTRACT:

Annual Lyme disease cases continue to rise in the U.S. making it the most reported vector-borne illness in the country. The pathogen (*Borrelia burgdorferi)* and primary vector (*Ixodes scapularis;* blacklegged tick*)* dynamics of Lyme disease are complicated by the multitude of vertebrate hosts and varying environmental factors, making models an ideal tool for exploring disease dynamics in a time- and cost-effective way. In the current study, LYMESIM 2.0, a mechanistic model, was used to explore the effectiveness of three commonly used tick control methods: habitat-targeted acaricide (spraying), rodent-targeted acaricide (bait boxes), and white-tailed deer targeted acaricide (4-poster devices). Work was done to evaluate their effectiveness when used alone and in combination with one another. Optimized application strategies were also identified. Additionally, pilot work was done to incorporate prescribed fire into the model and compare its efficacy to the acaricide-based approaches. It was determined that any singular use or combination of methods that included spraying were most effective amongst acaricide-based treatments, suppressing the density of *I. scapularis* nymphs (DON) by >80%. Furthermore, the best time to apply treatments was between January and mid-April, and mid-September to early December. Optimized treatment strategies identified by the model include application of treatment twice annually, every other year at a minimum effectiveness of 25%, which achieves 80% DON suppression and no increases in *I. scapularis* nymphs once treatments are complete. Interestingly, preliminary work to integrate prescribed fire in the model indicated that it achieved 93-100% efficacy in burn years and one-year post burn, making prescribed fire more effective than all acaricide-based treatments. Overall, this study illustrates the value in using models to identify the best method of blacklegged tick population control that is both time- and cost-effective. Future field research should be done to validate the findings of this model.

INTRODUCTION:

The blacklegged tick, *Ixodes scapularis* Say was implicated as the primary vector of the Lyme disease spirochete *Borrelia burgdorferi* sensu stricto (s.s.) (heron, *B. burgdorferi)* in the eastern United States in the early 1980's (Burgdorfer et al. 1982, Spielman et al. 1985). *Ixodes scapularis* fall within the family Ixodidae, e.g., "hard ticks," known for the presence of a scutum (Oliver 1989) and belong to the Acari subfamily, a subclass of the Arachnida family which includes all species of mites and ticks. Meanwhile, *Borrelia burgdorferi* falls within the family Spirochaetaceae and is one of three genospecies to be clearly associated with Lyme disease (Rosa 1997).

As an obligate blood-feeder, *I. scapularis* ticks typically take two to three years to complete the four-stage life cycle consisting of the egg, larvae, nymph, and adult (Oliver 1989). These ticks require a single blood meal before molting to the next life stage and hence are labeled as 3-host ticks. *Ixodes scapularis* ticks are host generalists, meaning that they are relatively indiscriminate in host selection and feed on animals relative to their size at each tick life cycle stage (Oliver 1989, Kocan et al. 2015). Larvae and nymphs, or immature ticks, typically take blood meals from small and medium sized hosts, mainly white-footed mice (*Peromyscus leucopus),* as well as squirrels, voles, shrews, birds, and domestic animals (e.g., dogs, cats, hogs, and cattle) (Ostfeld et al. 1995, LoGiudice et al. 2003). Comparatively, adult ticks typically parasitize medium and large-sized mammals, including white-tailed deer (*Odocoileus virginianus*), raccoons (*Procyon lotor*), coyotes (*Canis latrans*), and humans (Ostfeld et al. 1995, Kocan et al. 2015, Eisen and Eisen 2018).

In efforts to monitor disease trends and evaluate prevention and control efforts in the U.S., Lyme disease was designated as a nationally notifiable disease in 1991 by the Center for Disease

Control and Prevention (CDC) which means that all diagnosed Lyme cases in the U.S. must be reported to the CDC (Mead 2015). Since 1991, the number of annually reported Lyme disease cases have tripled, while the number of counties reporting high incidence of cases has increased 320% (Kugeler et al. 2015). Additionally, *I. scapularis* can also transmit the relapsing fever spirochete, *Borrelia miyamotoi*, as well as the causative agents of anaplasmosis, *Anaplasma phagocytophilum,* and babesiosis, *Babesia microti* (Piesman and Eisen 2008, Krause et al. 2015, Hahn et al. 2016). Importantly, *I. scapularis* can carry *B. burgdorferi, A. phagocytophilum, and B. microti* as a co-infection, as all three share the same reservoir host, white-footed mice, though the prevalence of the coinfection is yet to be established though tick surveillance (Eisen and Eisen 2018).

The general distribution of *I. scapularis* covers most of the eastern United States, whereas majority of Lyme disease cases are restricted to the northeast, mid-Atlantic, and upper Midwest (Eisen and Eisen 2018). However, the geographic range of Lyme cases and *I. scapularis* has steadily expanded in the last two decades, with *B. burgdorferi* and Lyme disease notably expanding southward (Lantos et al. 2015). Furthermore, the incidence of Lyme disease and other diseases vectored by *I. scapularis* have become more prevalent (Eisen et al. 2017). Notably, the high incidence of Lyme disease in the northeastern and upper-midwestern states is believed to be in part due to more aggressive questing behaviors exhibited by northern populations of *I. scapularis,* particularly the nymphs (Arsnoe et al. 2019). Indeed, few *I. scapularis* nymphs are collected in the south (Gleim et al. 2014, Mackay and Foil 2005). Hence, the steadily expanding range of *I. scapularis* and increasing incidence of Lyme disease cases clearly underscores the need for effective methods for controlling tick populations and reducing human Lyme disease risk.

The first reported cases of Lyme disease in 1975 in Lyme, Connecticut were originally thought to be a new form of inflammatory arthritis (Steere et al. 1977). It was not until 1982, that the etiological agent, later named *B. burgdorferi,* was shown to be transmitted by *I. scapularis* ticks (Burgdorfer et al. 1982). Eventually, in 1987, white-footed mice were recognized as the most competent reservoir for maintaining *B. burgdorferi* infection in the eastern U.S. (Donahue et al. 1987). Although insect control measures existed well before this, starting in the 1980's, efforts to identify effective tick control methods intensified in conjunction with the progression of scientists' understanding of the then newly emerging, Lyme disease. These approaches particularly focused on (and continue today to focus on) controlling ticks within residential settings (e.g., people's yards, playgrounds, etc.) since most Lyme disease cases are acquired in people's backyards (Eisen and Dolan 2016). Furthermore, most human Lyme disease cases are the result of bites by infected *I. scapularis* nymphs. Specifically, nymphs' small size makes them difficult for people to detect and remove in a timely manner, thus leading to the successful transmission of the pathogen. Consequently, because nymphs are the life stage most associated with human Lyme cases, many tick controls studies have used densities of host-seeking nymphs and host-seeking infected nymphs pre- and post-treatment to determine whether the control treatment was effective (Spielman et al. 1985).

One treatment approach that has been extensively examined is the use of synthetic chemical acaracides (Eisen and Dolan 2016). Field studies have tested organophosphate (chlorpyrifos and diazinon which are no longer in residential use), pyrethroid (deltamethrin, bifenthrin, and cyfluthrin), and carbamate (carbaryl) chemical pesticides. These acaracides can be applied to an environment in a granule form or broadcast sprayed. The effectiveness of each synthetic chemical agent is influenced by the intensity of acaracide spraying, the concentration of the active agent in the acaracide, the length of time the agent is environmentally persistent, and the weather (Eisen and Dolan 2016). In terms of weather, the impact of rainfall is unclear as it has been suggested to be either beneficial, driving an already applied acaracide deeper into the leaf litter, or determinantal, as rainfall could result in acaracide run-off after treatment. Run-off of acaracides is particularly concerning as several synthetic acaracides have been shown to be highly toxic to aquatic invertebrates and fish. For example, amitraz and permethrin are toxic to fish and aquatic arthropods, with the latter also being highly toxic for beneficial terrestrial and aerial arthropods like bees (George et al. 2008).

Though restricted for use near open water and wetlands, many studies have repeatedly demonstrated the effectiveness of a single synthetic acaracide application both in residential and woodland forest settings. For example, the application of chlorpyrifos, an organophosphate, was shown to effectively reduce host seeking *I. scapularis* nymphs between 84-100% when sampled weekly for up to 6 weeks post-treatment (Curran et al. 1993, Allan and Patrician 1995). Even though organophosphates have been shown to be an effective tick population control strategy, they are no longer in use as they affect non-target species (George et al. 2008). For example, organophosphates are toxic to birds, like Bobwhite Quail; *Colinus virginianus,* should they feed on ticks killed by the acaricide and can also be toxic to fish if exposed via runoff (Van Wieren et al. 2016). Additionally, while this acaracide is still used in certain agricultural settings, it has been banned from use in residential settings due to it being found to cause cancer and other illnesses in humans (Gray and Hammitt 2002). Thus, an alternative to organophosphates is carbaryl, which when tested in a residential setting at a high concentration (1.5-2.1 kg AI/ha) as a single high-pressure barrier spray, achieved at least 90% reduction in questing nymphs for 7-8 weeks after treatment (Stafford 1991a).

An alternative to synthetic acaracides are natural acaricides (Gould et al. 2008). It is possible that homeowners may be more open to using natural versus synthetic acaracides in a residential capacity. Such acaracides include nootkatone, pyrethrin, carvacrol, garlic oil, and other plant oils like rosemary, peppermint, and spearmint (Eisen and Dolan 2016). Unfortunately, unlike synthetic acaricides, natural acaracides may not be effective for long periods because of their nonpersistent nature. Specifically, they readily break down in the environment following exposure to light and oxygen (Allan and Patrician 1995). The lower environmental persistence of natural acaracides may be favored in a residential setting, as this would reduce the chance of mammalian toxicity for pets and acute pesticide poisoning in humans (Quadros et al. 2020). However, higher concentrations of natural acaricides are often used to assist with longevity. For example, the low-pressure spraying of nootkatone, an essential oil extract from the heartwood of cedar, reported an 82-84% reduction after weeks 1 and 2, but dropped to 40-61% by weeks 4 and 5 (Dolan et al. 2009). However, the application of a high-pressure spraying of nootkatone was very effective at reducing *I. scapularis* nymphs by at least 98% for up to 6 weeks after spraying. However, these promising findings were contradicted in a subsequent study where a single high-pressure spraying of nootkatone yielded 0% reduction in nymphal ticks after the third week post-treatment, following an initial 100% reduction success in the first week (Bharadwaj et al. 2012). Like inconsistencies in effectiveness observed in different studies using synthetic acaricides, these inconsistencies are likely explained by variable weather conditions and further research into the impact of weather on the efficiency of natural acaracides for *I. scapularis* nymph control is needed.

Lastly, entomopathogenic fungal biological control, or fungal acaracides, have gained interest in more recent years as fungal acaricides have not been shown to have adverse effects on other beneficial arthropod species such as honeybees (Ginsberg et al. 2017, Zimmermann 2007a, 2007b). However, like other habitat-targeted acaricides, the application and success of fungal acaricides depends on multiple conditions including environmental factors, how a treatment is applied, and its concentration. For example, Stafford and Allan (2010) simultaneously tested *Beauveria bassiana* (Bals. -Criv.; Hypocreales: Clavicipitaceae) strains ATCC 74040 and GHA and pyrethroid bifenthrin, a synthetic acaracide, in a residential setting and both yielded similar reductions in questing *I. scapularis* nymphs. Specifically, the *B. bassiana* strains ATCC 74040 and GHA yielded 83% and 74% respectively, and the pyrethroid bifenthrin yielded an 86% reduction of questing *I. scapularis* nymphs 4 weeks after treatment. In that same study, however, Stafford and Allan (2010) tested both *B. bassiana* strains the following year and only achieved 38% and 55% reductions, respectively. This difference was thought to be the result of the variable mode of application (the original application was a high-pressure spraying whereas the second year was a low-pressure spraying) and/or due to the mild and wet environmental conditions the second year which are more favorable to tick survival.

The discovery of white-footed mice as a principal reservoir of *B. burgdorferi*, in conjunction with the desire to decrease the adverse environmental effects of habitat-wide broadcast acaricide spraying, lead to the development of rodent-targeted acaricides in the late 80's (Levine et al. 1985, Donahue et al. 1987, Mather et al. 1989). It was hypothesized that because white-footed mice are the primary reservoir of *B. burgdorferi,* that targeting white-footed mice could not only reduce *I. scapularis* ticks, but also reduce pathogen prevalence in the *I. scapularis* tick population (White and Gaff 2017). Since then, two primary approaches for rodent-targeted treatments have been explored: bait boxes and tick tubes. Bait boxes contain bait that sometimes contains doxycycline (to clear any infections with tick-borne pathogens). The box has two entry points and hanging over each entrance is a fipronil or permethrin-treated wick. Thus, the rodent brushes up against the acaracide-treated wick, therefore killing any attached ticks and ticks that may attach in the future for up to 4 weeks (Dolan et al. 2017). Alternatively, tick tubes are cardboard tubes containing permethrin-treated cotton. Mice will self-treat with acaricide by harvesting the cotton to be used as nesting material and consequently kill any immature ticks on the mice (White and Gaff 2017).

Field studies using bait boxes alone reported a reduction around 77% for questing adult blacklegged ticks and 68% in questing nymphal tick abundance one year post treatment (Dolan et al. 2004, 2017). Another field study showed effectiveness against larval blacklegged tick abundance (Schulze and Jordan 2006). Specifically, they reported a reduction of larval and nymphal tick burdens on small mammals by 77% and 76%, respectively, while also reducing the *B. burgdorferi* infection prevalence in questing *I. scapularis* nymphs by 93% and reducing the percentage of infected small mammals in the intervention areas by 96% after 17 weeks of bait box deployment. Though field application results are promising, bait boxes can potentially increase rodent survival and reproduction by readily providing food via the bait being provided (White and Gaff 2017). Ultimately, the increase in rodent food sources could cause an increase in rodent populations. Thus, an increase in the rodent population could offset any reduction in tick population abundance in subsequent years as a larger rodent population provides more opportunities for ticks to feed on competent reservoirs, ultimately bolstering tick abundance and pathogen prevalence (Boutin 1990).

Therefore, it has been proposed that tick tubes may be a preferred approach to rodent-targeted acaricides since they do not provide bait. However, the effectiveness of tick tubes remains unclear. Some studies found no significant decrease in blacklegged ticks even after 2

(Daniels et al. 1991) or 3 years (Stafford 1991a, 1992) of control treatment. Contrastingly, when used seasonally, other studies found that tick tubes reduced tick infestation on rodents to nearly zero for the duration of two seasons (Mather et al. 1988, Deblinger and Rimmer 1991). Mather et al. (1988), following 1-year of treatment, reported an 89% reduction in host-seeking nymphs, a 97% reduction in infected host-seeking nymphs, and a 72% percent reduction in prevalence of *B. burgdorferi* in white-footed mice. Though Mather et al. (1998) reported percent reduction in infected questing nymphs and prevalence of *B. burgdorferi,* several studies have reported increases 1-3 years post treatment after the use of tick tubes in both residential (Stafford 1991b, 1992, Daniels et al. 1991) and woodland settings (Deblinger and Rimmer 1991).

While white-footed mice are important hosts of immature stages of blacklegged ticks, white-tailed deer are a principal host for adult blacklegged ticks and therefore an important reproductive host (Rand et al. 2003). However, unlike white-footed mice, white-tailed deer are incompetent hosts of *B. burgdorferi*. Despite white-tailed deer being poor Lyme disease reservoirs, some studies have found that white-tailed deer are an important amplifier for *I. scapularis* populations (Piesman et al. 1979, Spielman et al. 1994). These findings lead to the hypothesis that tick abundance could be suppressed when deer were culled or excluded from an area (Spielman 1988, Wilson et al. 1988). To examine this, Deblinger et al. (1993) reduced deer to <25 deer / km^2 and yielded 78%, 35%, and 41% reductions in infestation of rodents by nymphs after 2 years, 3 years, and 4 years, respectively, following deer removal. Another study yielded 100% reduction in infestation of rodents by nymphs after 3 years and 4 years following deer removal (Rand et al. 2004).

Because of the logistical constraints associated with deer exclusion and the ecological and ethical concerns associated with culling a large proportion of a deer population, the 4-poster device was developed as an alternative deer-targeted approach to blacklegged tick control. The 4-poster device dispenses bait adjacent to topical acaricide-soaked rollers which deer brush up against as they eat the bait, thus applying the acaricide to the deer's head, neck, and ears (Pound et al. 2000). This method is particularly effective given that the acaracide treats the area of the deer where ticks primarily attach, e.g., the head, neck, and ears (Sonenshine et al. 1996). In addition to killing ticks on contact, the acaricide will continue to protect those treated areas on the deer for a month after initial application. The applied acaracide is usually 2% amitraz with devices being distributed at a density of 1 per 20-25 ha. Studies on 4-poster devices have reported an increase in *I. scapularis* nymph suppression with each subsequent year that the devices are deployed (Brei et al. 2009, Daniels et al. 2009, Stafford et al. 2009, Schulze et al. 2009). For example, most studies reported that reduction in questing nymphs ranged between 55-64% for years 4 and 5 of device deployment in residential and woodland settings (Daniels et al. 2009, Stafford et al. 2009, Schulze et al. 2009). These same studies went on to document the most reduction in questing nymphs 6 years after treatment, with suppression ranging from 70-80%.

However, despite showing some effectiveness with long-term use, the 4-poster control method is not without its drawbacks. Like bait boxes, 4-posters potentially increase the cervid population by providing bait and decreasing competition (Eisen and Dolan 2016). This may adversely affect tick populations by providing a larger number of available hosts and essentially offset any population reduction that may result from using 4-poster devices. Additionally, the use of 4-posters leads to topsoil disruption and ground cover damage in the area surrounding the device due to high deer traffic (White and Gaff 2017). Furthermore, such devices can also help facilitate pathogen transmission of infectious diseases like chronic wasting disease (CWD) and tuberculosis (Wong et al. 2017). There are also several logistical constraints that can impact the effectiveness of these devices: variable homeowner acceptance of residential use leading to patchy deployment, the inability to place devices in an optimal time or location due to regulatory issues, interference by non-target mammals e.g., squirrels and raccoons, the availability of alternative food sources, a light and therefore less effective application of acaracides on treated deer, and availability of untreated deer as tick hosts (Carroll et al. 2008, 2009a; Miller et al. 2009; Stafford et al. 2009).

One overarching concern with all the acaracide-based tick control methods (e.g., habitat-wide spraying and host-targeted acaricides) is the development of acaracide resistance. One study found that by applying a chemical acaracide more than 6 times in a year, the treatment selects for resistant individuals by killing all ticks that were once susceptible (Rodrígues-Vivas et al. 2006a). Though *I. scapularis* has yet to show signs of acaracide resistance, elsewhere in the world, other tick species have developed resistance to acaricides. For example, *Boophilus microplus,* the cattle tick, was reported to have developed resistance to organophosphates, synthetic pyrethroids, amitraz (formamidines) and ivermectin in Mexico (Fernandez-Sala et al. 2012). Another study has reported similar acquired resistance of *B. microplus* in India (Vatsya and Yadav 2011) and cited a major concern as the passing of the acquired resistance from parent to offspring.

One promising alternative approach to tick control that does not involve acaricides is prescribed burning. Prescribed burns are normally used to enhance wildlife habitat and the ecosystem health of fire-adapted ecosystems, control invasive species, and/or to reduce the risk of wildfires. In the 80's and 90's, prescribed fires began to be studied as potential tools for tick control. However, most studies focused on *Amblyomma americanum* Linnaeus, the lone star tick, as it is the most common tick in the southeastern United States. Studies examining burning in relation to *I. scapularis* tick control are comparatively sparse. Of the studies that have been conducted, results have been mixed. For example, Mather et al. (1993) reported only a 49% suppression of *I. scapularis* nymphs after a light to moderate intensity burn on Shelter Island, NY. However, most studies have found notable reductions in tick populations post-fire. For example, Stafford et al. (1998) tested two intensities of burning: light to moderate and moderate to severe. They reported 74% and 97% suppression of *I. scapularis* nymphs two weeks after a single burn, concluding that a more intense burn was more effective. Comparatively, Wilson (1986) reported 71%, 76.9%, and 82.8% suppression of *I. scapularis* adults 2 weeks, 11.5 months, and 12 months after an April burn. He also reported 88.3% suppression in *I. scapularis* adults 4 months after a November burn. However, like what had been reported in previous studies with lone star ticks (Barnard 1986, Davidson et al. 1994), these early studies generally found that blacklegged tick abundance was reduced after a burn, but usually returned to pre-burn levels or higher within one to two years (Wilson 1986, Mather et al. 1993, Stafford et al. 1998).

Notably, these early studies had some key flaws which may have prevented a complete understanding of the impacts of prescribed fire on tick populations. Some of these issues included small burn areas (Mather et al. 1993), using sites that had never been burned before (Wilson 1986), and/or only performing a single burn (Stafford et al.1988). This contrasts with the way in which prescribed fire is typically utilized. Specifically, prescribed burning is consistently done every 2-5 years and usually over large acreage, e.g., anywhere from 25 to several hundred acres. More recent studies have examined areas in which long-term prescribed fire had been used for longer periods of time and found more promising results. For example, Gleim et al. (2014) reported 78% suppression of *I. scapularis* adults at sites in Georgia where burning had occurred for at least 10 years. Notably, these reductions were sustained for the entirety of the two-year study. Similarly, Hodo et al. (2020) reported 82% suppression in *I. scapularis* adults 12 months after burning. Again, the location where this study was conducted in east Texas had a history of prescribed burns which had occurred every 1-2 years since 1993. These more recent studies have indicated that long-term burning may be an effective treatment for controlling *I. scapularis* populations.

Thus, despite being studied for decades, the outcomes of studies examining one or more tick control method are often inconsistent. These inconsistencies are likely due to multiple factors including the tick species, tick abundance, phenology, climate, weather, and host dynamics amongst other factors (White and Gaff, 2017). These findings have underscored the challenge of identifying a single, universally effective control method and indicates that approaches may need to be customized to the specific regions, ecosystems, and/or weather conditions from year-to-year. However, large scale, long-term, comprehensive field studies examining either single or integrated management` strategies are logistically challenging and costly. Thus, the use of a validated simulation model may be a cost-effective yet accurate tool for predicting the epidemiological outcomes of various intervention methods based on these many factors.

Several models seeking to predict the population dynamics of *I. scapularis* ticks and transmission of *B. burgdorferi* within the population have been made over the years. Ginsberg employed a discrete-time model predicting infection rate at each tick life stage (1988), while Porco (1991) constructed a mathematical model to evaluate rate of infection dynamics of *B. burgdorferi* in *I. scapularis* ticks specific to the eastern United States. Additionally, Sandberg et al. (1992) employed a modified Leslie matrix methodology to build a model specific to predicting the dynamics of *I. scapularis* populations on Nantucket Island, Massachusetts. All three models offered valuable insight to understanding the population dynamics of *I. scapularis* and *B. burgdorferi* infection, however, no single model included density-dependent variables or biological and environmental variables such as weather.

The first model developed that incorporated density-dependent variables, biological, and environmental variables in the context of *I. scapularis* ticks was LYMESIM (Mount et al. 1997a), a field-validated, mechanistic model originally developed in the 1990's. The original LYMESIM model (Mount et al. 1997a) was built based on previously published tick life history models including: *Amblyomma americanum* (L.) lone star tick *(*Haile and Mount 1987, Mount et al. 1993*), Dermacentor variabilis* (Say) American dog tick *(*Cooksey et al. 1990), and *Rhipicephalus (Boophilus) microplus* (Canestrini) and *Rhipicephalus annulatus* (Say) cattle ticks (Mount et al. 1991). Specifically, LYMESIM simulates the life-history dynamics of *I. scapularis* ticks and enzootic transmission of *B. burgdorferi* within an ecosystem (Mount et al. 1997a, (Figure 1). The objective use of the model initially was to understand the effects of differing host densities on tick abundance and pathogen dynamics (Mount et al. 1997a). Later, it was also used to simulate and predict the outcomes of various tick control strategies to determine their effectiveness at reducing questing ticks and ticks on hosts (Mount et al. 1997b).

The model includes 5 major model structures (Figure 1): (1) temperature-dependent development for each tick life stage, (2) temperature-dependent fecundity rates for female adults when engorged, (3) survival rate of ticks off-host based on temperature, habitat, precipitation, and saturation, (4) host-finding rates for each tick life stage, and (5) density-dependent survival rates for each life stage of ticks by hosts (Mount et al. 1997a). The categories of hosts included 1) white-footed mice, 2) white-tailed deer, 3) medium-sized mammals, 4) reptiles, 5) small mammals and birds, and 6) other large mammals (i.e., domestic livestock). Though field validated, LYMESIM has since become outdated, and no longer functional on modern computing software as it was originally built on Microsoft BASIC version 7.1 (Mount et al. 1991). Furthermore, advances in the understanding of Lyme ecology since the 1990's indicated a need for the model to be updated to improve accuracy. Thus, in 2020, the model was updated as LYMESIM 2.0 (Gaff et al. 2020) and transferred to R statistical software (R Development Core Team 2008).

The updated LYMESIM 2.0 model now includes statements limiting the number of ticks allowed to simultaneously feed on a host (Gaff et al. 2020). Prior to this update, the original model allowed thousands of ticks on a single organism. Additionally, the original model did not account for variable day length, which would change depending on geographic location and time of year. Another important update involved the distinction that *B. burgdorferi* is only passed transstadially within *I. scapularis* (Scoles et al. 2001). Previous research evaluating *Borrelia* infection in larvae most likely failed to distinguish *B. burgdorferi* from *B. miyamotoi,* the latter which is passed transovarially (i.e., adult to offspring). So, this subsequent model iteration set the transovarial transmission of *B. burgdorferi* to zero. As for hosts, all previous categories were included except large mammals were replaced by insectivores and other *B. burgdorferi* reservoir competent hosts including shrews (Figure 1).

Finally, LYMESIM 2.0 updated the survival function of host-seeking ticks. Simplified mathematics were used to modify the assumptions about the physical cost of questing. This allows for a more realistic phenology curve representative of various regions across the United States. Lastly, the updated model now includes outcomes such as density of host-seeking *I. scapularis* nymphs (DON) and density of *B. burgdorferi*-infected *I. scapularis* nymphs (DIN).

These values are commonly reported by field studies as measures of success of tick control treatments (Eisen and Dolan 2016, Gaff et al. 2020).

As with the original model, LYMESIM 2.0 was also field validated, this time using weather data from 2007-2016 and flagging data including reported densities of questing *I. scapularis* nymphs and densities of mice at three locations: Cary, New York; Itasca, Minnesota; and Norfolk, Virginia (Gaff et al. 2020). The model was run using weather data respective to each site taken from the National Oceanic and Atmospheric Association's Comparative Climate Data Center and North American Land Data Assimilation System (NLDAS). The latter data source was specifically chosen as it provides a continuous spatiotemporal record of meteorological conditions over the United States. These values were used in tandem with host dynamics to estimate DON and DIN values for the three sites. To evaluate biological realism, the model's predictions were compared to the field data to identify whether the timing and abundance of peaks at each life stage were similar. By comparing the predicted DON and DIN for all three sites to the field data, the model was found to be consistent with the field reported observations.

Given that LYMESIM 2.0 has been validated using field data, the objectives of the current study were to use LYMESIM 2.0 to provide updated predictions regarding the effectiveness of acaracide spraying, bait boxes, and 4-poster devices when used both singularly and in combination to control *I. scapularis* populations and reduce the densities of *B. burgdorferi*-infected nymphs in the northeast, upper Midwest, and Mid-Atlantic regions of the U.S. Additionally the model was used to determine the most effective ways to apply tick control treatments (e.g. evaluating the best time of year for application, optimal duration and frequency of treatment, and what percentage of an area/host population must be treated). Additionally, pilot work was done to incorporate prescribed burning as a possible treatment option within the model. It was hypothesized that the most effective treatment would be using an integrated approach (e.g., using multiple types of treatments together) annually when *I. scapularis* nymphs are active. Furthermore, it was predicted that prescribed burning would be as effective if not more effective than the treatment methods being tested.

METHODOLOGY:

General Model Overview:

LYMESIM 2.0, a mechanistic model, was used to evaluate the effectiveness of various *I. scapularis* tick control methods to identify optimized tick control strategies. The streamlined equations for each of the following model structures: life cycle, weather inputs, temperature-dependent development and fecundity rates, survival rates off-host, activity-dependent maximum survival, host-finding rates, density-dependent survival on hosts, and infection dynamics can be found in Gaff et al. (2020). Additionally, several assumptions about the model were previously covered in Mount et al. (2017b) and Gaff et al. (2020), but briefly: (1) all three life stages may survive up to 80 weeks, but is reduced 3 weeks for every week spent questing; (2) white-footed mice, shrews, small mammals, birds, and medium sized mammals can be infected; (3) reptiles and white-tailed deer are poor reservoirs and thus cannot infect ticks but act as blood meals; (4) all infected ticks are equally likely to transmit the infection, while infected hosts of the same species are all equally likely to infect a tick; (5) once a host is infected, it remains infected; and (6) survival and reproduction rates are the same for infected and uninfected ticks and hosts.

The model used meteorological data from the years 2007-2016 from three different locations: Itasca, Minnesota; Cary, New York; and Norfolk, Virginia. The model output measures evaluated in this study were the number of questing blacklegged nymphs per hectare (DON) and number of questing *B. burgdorferi*-infected blacklegged nymphs (DIN) per hectare (which accounts for both changes in DON and infection prevalence) (Gaff et al. 2020). To compare the derived output values from comparable iterations of the model, the maximum DON (hereon, DON) and average DIN (hereon, DIN) per year were taken for every treatment year and the first two years post-treatment. To evaluate the effectiveness of any treatment, the percent reduction of DON and DIN, hereon suppression, was calculated as follows:

$$[1 - (\text{treatment DON or DIN} / \text{control DON or DIN})] \times 100$$

Treatments were deemed effective if DON suppression was at least 80% for every year treatment was applied and did not exceed DON or DIN values at control sites in the years following treatment.

Efficiency & Optimization of Spraying, Bait Boxes, and 4-Poster Devices

The effectiveness of three commonly used tick control treatment methods were evaluated. Specifically, the three treatment methods examined were: habitat-targeted acaricide (spraying), rodent-targeted acaricide (bait boxes), and white-tailed deer-targeted acaricide (4-poster). Initial simulations were run evaluating the effectiveness of each treatment as well as all combinations of the three treatment methods. These simulations were run given an efficiency of 25% and two years of control starting in 2007. Here, efficiency refers to the percent of an area covered via spraying and the percent of the host population that is receiving topical acaracide from a host-targeted treatment, e.g., bait boxes and 4-posters. In the model, host-targeted treatments, were deployed year-round during treatment years, while spraying was applied once and assumed effective for 4 weeks which is conservatively the minimum duration that spraying is known to be effective (Eisen and Dolan 2016). To account for potential differences in effectiveness of spraying depending on the time of year it was applied, the model was programmed such that it would test the outcome of spraying every possible week of the year and then provide the lowest DON and DIN across all iterations.

Once the most effective treatment was determined (combination of bait box + 4-poster + spraying), further work was done to identify optimized strategies for the frequency and duration of treatment. Assuming 25% efficiency, the model was run for 10 years applying the treatment for a variable number of years including no treatment (control) and then treatment for 1 year, 2 consecutive years, 8 consecutive years, and alternating application every odd year for 3 and 5 years. Next, the most effective treatment (combination of bait box + 4-poster + spraying) was modeled assuming two consecutive years of control at various levels of efficiency including 0% (control), 10%, 15%, 20%, 25%, and 30% to determine the minimum efficiency required to consistently attain 80% suppression. Then, to evaluate that the model simulations were consistent regardless of what year treatment was started, e.g., regardless of weather, the most effective treatment (bait box + 4-poster + spraying combination) was simulated varying the starting year of treatment between 2007-2013. The treatment was applied for 2 consecutive years of control at 25% efficiency.

The treatment that was deemed most effective (bait box + 4-poster + spraying combination) was further explored to determine what time of year a single application of spraying yielded the most effective results. To do this, simulations were run testing application every week of the year at each site to determine which weeks yielded DON suppression greater than 80% for at least 2 consecutive years. Treatment weeks that resulted in at least 80% DON suppression across all three sites were included in the recommended timeframe for the most effective method of treatment. Finally, because most pest control companies apply treatments 2-4 times per year, (Jordan and Schulze 2020) scenarios were run to evaluate the effectiveness of the

4-poster + bait box + spraying combination when spraying was applied twice a year (as with previous runs, bait boxes + 4-posters were out year-round) for a total of two years at 25% efficiency. Specifically, 1) the time of year in which treatments are most commonly applied by pest companies was examined (late May, weeks 17-21, and early June, weeks 24-28; Jordan personal communication), and 2) Because the time frames commonly used by pest control companies for dual application were not within the previously identified ideal time windows for singular treatment, alternative dual treatment timings were modeled to attempt to find the most effective times of year to apply twice annual treatments. Specifically, the alternative dual treatment time windows proposed were: (1) late March, weeks 9-13, and early June, weeks 24-28, applied annually and (2) every other year, and (3) late March, weeks 9-13, and late November, weeks 43-47.

The reasoning behind alternative treatment options 1 and 2 is to apply the first treatment earlier in the year which is the time frame that the model predicted to be the most effective, while keeping the second treatment the same as what is commonly employed by pest control companies. By keeping the second treatment within the time window currently used by pest control companies (e.g., when *I. scapularis* nymphs are most active), consumers and pest control companies may be more open to the recommended change based on perceived effectiveness during that time of year. Alternative treatment option 3 reflects the two-time windows that the model predicted to be the most effective based on ideal time windows for singular treatments. Lastly, these dual treatments were also compared to a single application of the 4-poster + bait box + spraying combination treatment for two consecutive years at 25% efficiency, to determine whether the second application of treatment was truly necessary for reducing DON and DIN.

For certain comparisons, i.e., when comparing all treatment options and combinations and when comparing duration and frequency of treatment, heat maps were used to illustrate the effectiveness of the various treatments/scenarios being tested. To make these, the DON and DIN suppression values, respectively were grouped, e.g., "binned", into the following categories: 1 = $x < 0\%$, 2 = $0\% < x < 15\%$, 3 = $15\% < x < 50\%$, 4 = $50\% < x < 80\%$ and 5 = $80\% < x < 100\%$. The reasoning behind these bin categories was as follows: category 1 includes any treatment that exceeded the control indicating an increase in DON or DIN potentially because of the treatment. Category 2 included treatments that had no to minimal effectiveness. Category 3 included treatments that showed some effectiveness but still far from the 80% threshold. Category 4 included treatments that achieved notable suppression but were less than the 80% threshold goal, and lastly, category 5 included all treatments that achieved the target of 80% suppression or greater.

Pilot Work to Adapt LYMESIM 2.0 to Evaluate the Effectiveness of Prescribed Fire

Lastly, pilot work was done to adapt LYMESIM 2.0 to evaluate the effectiveness of prescribed burning. To begin to build an accurate predictive model, a literature review was conducted to analyze the reported effectiveness of prescribed burning at reducing *I. scapularis* tick populations (Table 1). As most literature reported at least a 71% reduction of *I. scapularis*, the model was set to assume that all *I. scapularis* would be reduced by 70% after a burn.

Additionally, because prescribed fire is known to impact host dynamics, work was done to account for the impacts of prescribed fire on host dynamics, including white-footed mice (Greenberg et al. 2006), white-tailed deer (Meek et al. 2008), medium-sized mammals, reptiles (Perry et al. 2012), and small mammals and birds (Adams et al. 2013). To parameterize the model as it related to white-footed mice and small mammal responses to fire, a 15-year, unpublished, mark-recapture data set (Conner, personal communication) on cotton rats (*Sigmodon hispidus*) and cotton mice (*Peromyscus gossypinus*) was used. This data set came from eight sites in southwestern Georgia which were trapped quarterly and burned every other year for the duration of the data set. This was supplemented with two years of telemetry data from cotton rats (n = 70) which were tracked after fires at those same sites. Importantly, it was assumed that cotton mice would respond to fire in the same way that white-footed mice would and collectively that this represented how most small mammal populations would respond to fire.

Table 1. Prescribed burning literature that reported suppression of various life stages of *I. scapularis* ticks in a woodland setting.

Author & Year	*Ixodes scapularis* Life Stage	Fire Intensity	% Reduction at Time Post- Treatment
Wilson 1986	Adult	Moderate to severe	76.9% after 11.5 months
		Moderate to severe	82.8% after 12 months
		Moderate to severe	88.3% after 4 months
		Moderate to severe	86.7% after 7 months
		Moderate to severe	71% after 0.5 months
Stafford et al. 1998	Nymphs	Moderate to severe	97% after 0.5 months
		Low to moderate	74% after 0.5 months
Mather et al. 1993	Nymphs	Low to moderate	49% after 2 months
Gleim 2014	Nymphs	Low to moderate	78% after 12 months
Hodo et al. 2020	Nymphs	Moderate to severe	82% after 12 months

For this initial pilot work, parameters for other host groups being considered, i.e., birds, reptiles, white-tailed deer, and medium-sized mammals, were developed based off the expert knowledge of a wildlife scientist that has studied the impacts of fire on wildlife for over twenty years (Conner, personal communication). Through this work, the model was coded such that the populations of white-footed mice, small mammals, birds, reptiles, and shrews would all be reduced by 95% for two months post-fire. Additionally, white-tailed deer and medium-sized mammals would see no change in abundance post-burning. Also, the model was updated to include a switch for a prescribed burn called "fire" and additional variables "fire_control", “fire_begin” and “fire_end” were added to define what weeks the prescribed burn was completed and impacting tick populations.

The updated model was run applying the prescribed burning in years 1 and 3 (e.g., 2007 and 2009) at 5% efficiency and assumed treatment was effective for 8 weeks. Here, efficiency refers to how much of an area is burned. A low efficiency value was chosen to represent the small proportion of area that would safely be allowed to burn in a residential setting. The application of prescribed burning was chosen to be every other year, as this is commonplace for forest management (Williams et al. 2012). The assumption that the treatment would be effective for 8 weeks is a conservative estimate, as studies have shown effective suppression of DON after treatment for at least 2 months (Wilson 1986, Gleim et al. 2014, Hodo et al. 2020). The model predicted DON and DIN given the application of the treatment in the first week of the first year only. This was compared to the DON and DIN values reported for the first week of applied treatment for the previously tested acaracide treatments which were applied for two consecutive years (2007 and 2008) at 25% efficiency. The preliminary model’s predicted DON and DIN suppression was calculated for a total of 5 years (2007 – 2011).

RESULTS:

Comparing Spraying, Bait Boxes, and 4-Poster Devices

The combination of all three treatments (4-poster, bait boxes, and spraying) was found to be the most effective at decreasing DON at all locations with suppression values ranging across all sites (Norfolk, VA; Itasca, MN; and Cary, NY) from 84-89% during the first year of treatment and 91-100% during the second year of treatment (Figure 2). Additionally, suppression remained greater than the desired 80% suppression threshold during the first-year post-treatment, with suppression ranging between 84-91% across sites before returning to the pre-treatment levels two years post-treatment. Though the combination of all three treatments was found to be the best at suppressing DON, it was only marginally more effective than spraying alone, spraying + bait boxes, and spraying + 4-posters. In short, all treatments that involved spraying were found to effectively suppress DON by more than 80% for the two years of treatment and the first-year post-treatment before returning to pre-treatment levels two years post-treatment (Figure 3).

However, no singular treatment or combination of treatments was found to achieve 80% reduction in DIN (Figure 2). Nevertheless, the combination of all three treatment methods reduced DIN the most across all sites. Notably, all three sites showed the greatest DIN suppression in the second year of treatment. Specifically, given the application of all three treatment methods (spraying + bait box + 4-poster), all three sites reported DIN values ranging from 30-32% during the first year of treatment, 73-79% during the second year of treatment, and 46-75% during the first-year post-treatment, indicating high variation in percent DIN suppression in the first-year post-treatment. In the second-year post-treatment, DIN suppression was reported to return to pre-treatment levels at Itasca, MN. While the DIN suppression values were reported as negative for each treatment at Cary, NY and Norfolk, VA, except for treatments of bait boxes alone, 4-posters alone, and bait boxes + 4-posters in combination at the Cary, NY location. Notably, negative DIN values meant that DIN exceeded that of the control treatment in the second-year post-treatment.

Across all three sites, the treatments of bait boxes alone, bait boxes + 4-posters, spraying + bait boxes, and spraying + bait boxes + 4-posters saw a minimal decrease in prevalence of *B. burgdorferi* in the first-year post-treatment. For example, the reported prevalence for the control ranged between 40.3-43.5%, across all sites whereas, the treatment bait boxes + spraying (the treatment reporting the greatest decrease in prevalence) had reported prevalence ranging between

38.5-42.7%, 36.9-41.9%, and 39.4-42.4% for the sites Cary, NY; Itasca, MN; and Norfolk, VA, respectively.

The Best Time of Year to Apply Control Methods

The best time of year to apply the combination of all three treatments (spraying + bait boxes + 4-poster) was found to be between weeks 1-16, e.g., January through mid-April, and 38-48, e.g., mid-September to December (Figure 4). When applied between weeks 1-16, DON suppression ranged between 84-91% during the two years in which treatment was applied. The effectiveness of DON suppression drops 1-year post-treatment, however, with effectiveness being less than 25%. Conversely, when applied between weeks 38-48, DON suppression ranges between 83-92% in the second year of control and first-year post control, i.e., 2008 and 2009. However, DON suppression does not exceed 3% for the first year of treatment, as treatment is applied late into the first treatment year. The initial application of treatment between the weeks 20-37, e.g., mid-April through mid-September should be avoided as no treatment or post-treatment DON suppression was achieved. Specifically, the reported DON suppression when applied during this time period in the first year of treatment is 0%, and then ranges from 13-74%, 23-76%, and -7–3% in the second year of treatment, first-year post treatment, and second year post treatment respectively.

Most Efficient Duration and Frequency of Treatment

The model was used to evaluate the effectiveness of varying treatment lengths (one year, two consecutive years, eight consecutive years, and every other year for three and five years). In treatment years, because it was shown to be the most effective, the combination of 4-poster + bait box + spraying was applied at 25% efficiency. The model determined that long-term treatment for eight consecutive years was shown to be most effective at reducing DON and DIN values across all three sites (Figures 5-6). Specifically, the applied combination treatment in consecutive years yielded DON suppression values ranging from 84-100% in every year of treatment and the first-year post treatment, before returning to pre-control values in the second-year post treatment. Also, treatment applied every other year for 3 years and 5 years mostly achieved above 80% suppression of DON, ranging from 77-93% suppression in treatment years and first-year post treatment (Figure 5). The first interim year (2008), where no control was applied, at Itasca, MN was the only observation that reported suppression less than 80%, with a 77% suppression.

Importantly, the only approach found to achieve and sustain fairly high DIN suppression across all sites was when treatments were applied every year for eight years. Generally, DIN suppression was fairly low and equal across all sites (30-31% suppression) during the first year of control. However, following the second year (2008) DIN suppression was found to range between 60-77% at Cary, NY, 79-99% at Itasca, MN, and 79-91% at Norfolk, VA across all treatments tested (Figure 5). From 2009 through 2014, the category in which treatments were applied every year for eight years (e.g., through 2014) had notably higher DIN suppression than all other categories including those receiving treatments every other year for three and five years, respectively. Specifically, when treatments were applied for eight consecutive years, Itasca and Norfolk achieved 80% DIN suppression across all years from 2008 through 2014 except for Norfolk in 2013; whereas, DIN suppression ranged from 60-77% in Cary, NY(Figure 5).

Minimum Required Efficiency

The model was used to determine the minimum efficiency required to reach 80% DON suppression. Again, efficiency refers to the percentage of an area that would be sprayed and the percentage of a host population that would be treated with a host-targeted acaracide treatment.

Ultimately, a minimum effectiveness of 25% was required to achieve 80% DON suppression at all three locations by the first year of treatment, second year of treatment, and first year post-treatment (Figure 7). However, 20% efficiency was also found to achieve 80% suppression in the second year of treatment and first year post-treatment; just not in the first year of treatment.

Impacts of Weather on Treatment Outcome

To determine whether weather had any impact on treatment outcome (e.g., whether treatment outcomes might vary depending on the year), simulations were run given the application of the 4-poster + bait box + spraying combination starting on varying calendar years for two consecutive years at 25% efficiency. Again, varying start year would allow us to test whether natural weather fluctuations seen year to year would impact the effectiveness of the treatments. In treatment years, DON is fairly consistent across all three sites with some variability (Figure 8). Specifically, at Cary, NY the DON suppression values range from 84-92% for all treatment years except for when treatments were started in 2010 which only yielded 78% DON suppression. At Itasca, MN the DON suppression values ranged from 82-100% excluding when treatments were started in 2008 and 2009, which resulted in 74% and 75% DON suppressions respectively. At Norfolk, VA the DON suppression values ranged from 81-93%, across all years.

However, DON suppression is quite variable in the first-year post treatment across all three sites. While the 80% threshold continued to be met in the first-year post treatment for most instances, at all three locations, this was not the case when treatments were begun in 2008 and 2009, respectively. In the first-year post treatment, the range of DON suppression values across all sites ranged from 1-61% and 1-20% when treatment started in 2008 and 2009, respectively (Figure 8).

As for DIN suppression, all values ranged between 28%-32% suppression in the first year of treatment except for Itasca, MN in 2011 (53%) and 2012 (50%). DIN suppression values increased and became more variable following the second year of treatment with values ranging from 39 – 100% across all locations and any given year, with some exceeding the 80% suppression threshold (e.g., Itasca 2007, 2010, 2011, 2012 and 2013; Norfolk 2010 and 2012). DIN suppression was variable in post-treatment years ranging between -4–85% in the first-year post treatment and -8–58% across all three sites. The Itasca, MN site reported > 80% DON suppression in years 2012 and 2013 in the first-year post treatment. Notably, all treatments in the second-year post-treatment for Cary, NY and Norfolk, VA reported DIN suppression < 2%.

Optimizing Timing and Number of Treatments per Year

The most commonly employed time frame of treatment by pest control companies is late May and early June. This dual application was the least effective treatment, reporting effective DON suppression only in the second year of application. With similar suppression values reported across all sites, DON suppression ranged between 57-81%, 91-100%, 30-67%, and -6–34% in the first year of treatment, second year of treatment, first year post-treatment, and second year post-treatment, respectively (Figure 9). Also, DIN suppression ranged between 17-32%, 61-92%, 28-70%, and -8–33% in the first year of treatment, second year of treatment, first year post-treatment, and second year post-treatment, respectively.

When examining more effective time windows, the most effective dual treatment was found to be the annual application of treatment in late March and November. This timeframe yielded the highest DON suppression in both years of treatment and the first-year post treatment across all three sites. The reported DON suppression ranged between 84-89%, 98-100%, 89-92%, and 0–88% in the first year of treatment, second year of treatment, first year post treatment, and second year post treatment, respectively (Figure 9). Notably, the reported percent suppression for the second-year post-treatment was 0%, 86%, and 0% for Cary, NY;, Itasca, MN, and Norfolk, VA, respectively. For DIN suppression, the dual application in late March and November also reported the highest suppression values ranging between 85-89%, 98-100%, 90-92%, and 0–87% in the first year of treatment, second year of treatment, first year post treatment, and second year post treatment, respectively.

Interestingly, annual treatment in late March and November is only marginally more effective than treatments in late March and early June in alternating years. In fact, this was the only treatment that showed DON suppression in the second-year post treatment and showed effectiveness in nearly every year of treatment and non-treatment years. The reported DON suppression ranged between 84-89%, 89-95%, 87-90%, and 78–97% in the first year of treatment, second year of treatment, first year post treatment, and second year post treatment, respectively (Figure 9). The reported DIN suppression ranged between 85-89%, 89-95%, 87-91%, and 79–97% in the first year of treatment, second year of treatment, first year post treatment, and second year post treatment, respectively.

The dual treatment in late March and November is also only marginally better than a single application of treatment. For the single application of treatment, DON suppression is only effective for the two years of control and first-year post control. The reported DON suppression ranged between 84-89%, 89-95%, 89-92%, and 0–86% in the first year of treatment, second year of treatment, first year post treatment, and second year post treatment, respectively. A single application of the combination treatment reported DIN suppression ranging between 30-31%, 73-79%, 46-75%, and -6–52% in the first year of treatment, second year of treatment, first year post treatment, and second year post treatment, respectively.

The last treatment tested, dual treatment in late March and early June, DON suppression ranged between 84-89%, 91-100%, 29-71%, and -4–48% in the first year of treatment, second year of treatment, first year post treatment, and second year post treatment, respectively. This dual treatment in late March and early June, also has similar DIN suppression as the single treatment ranged between 85-89%, 90-100%, 26-75%, and -6–50% in the first year of treatment, second year of treatment, first year post treatment, and second year post treatment, respectively.

Incorporating Fire into the Model

Finally, pilot work was performed to incorporate prescribed burning into the model. Prescribed burning was applied at 5% efficiency, alternating application in years 2007 and 2009 and assumed the treatment remained effective for 8 weeks. The prescribed burning treatment was compared to all other previous treatments and combinations which were applied at a 25% efficieny for 2 consecutive years of treatment (2007 and 2008). When comparing supression values, prescribed burning was found to be most effective at reducing DON. DON supression ranged from 96-98% in Cary, NY, 97-100% in Itasca, MN and 93-99% in Norfolk, VA in the first 4 years (Figure 10). Comparatively, in that same time frame, the combination of spraying + bait boxes + 4-posters, the second most effective control, reported DON supression ranging from 3-92% in Cary, NY, 76-100% in Itasca, MN and 2-92% in Norfolk, VA for those same 4 years.

It is also notable that higher DIN supression following the application of prescribed burning was observed for Cary, NY and Itasca, MN. Specifically, DIN supression following precribed burning ranged from 96-98% in Cary, NY up until the first year post fire, prior to returning to 0% supression two-years post control. The DIN supression ranged from 96-100% in Itasca, MN for all years of fire and two years post fire. Notably, Itasca, MN was the only site to report effective DIN supression two years post burn. Also all other treatments at this site returned to 0% supression by two years post burn. Meanwhile, in Norfolk, VA, DIN supression ranged from 96-100% in the first three years (control years and interim year), but had poor supression post-treatment years ranging from -42 – 20%. Comparatively, the combination of spraying + bait boxes + 4-posters, the second most effective control, reported DIN supression ranges from -13-73% in Cary, NY, 0-79% in Itasca, MN and -6%-79% in Norfolk, VA in the first three years. Regardless of the treatment and site, the second burn year (i.e., year 3) reported the highest DIN supression.

DISCUSSION:

The purpose of the original LYMESIM model was to not only simulate the natural dynamics of *I. scapularis* ticks and density thresholds required to maintain *B. burgdorferi* within the population, but also to simulate various tick population management strategies to predict their effectiveness (Mount et al. 1997a,b). This original model was updated, as LYMESIM 2.0, to reflect current knowledge of tick and pathogen biology and become a more accurate predictive model to evaluate and recommend various tick control treatments (Gaff et al. 2020). The current study is the first to have examined tick control treatments using the updated LYMESIM 2.0 model and marks the first ever efforts to identify optimized application strategies using any version of LYMESIM. Furthermore, the preliminary work done to begin to integrate and examine prescribed burning has begun to provide important insights into the potential of this often-overlooked tool for the control of *I. scapularis* populations and Lyme disease risk.

Overall, the model predicted that any treatments that involved spraying were most effective, resulting in over 80% suppression of DON. Out of all the treatments involving spraying, the integrated approach of spraying + bait boxes + 4-posters, was marginally more effective than the others. Two studies, conducted by Schulze et al. (2007, 2008b) have tested the integrated strategy employing synthetic acaracide spraying, bait boxes, and 4-posters in a residential setting. They tested the use of a barrier spray (year 1 only), alongside bait boxes (years 1-2) and four posters (years 1-3). They yielded 87%, 95%, and 86% in DON suppression in the first, second and third years, respectively. Thus, this integrated approach effectively reduced the questing tick population which seems to correlate with the current model's findings.

However, according to the model, the combination of all three treatments was only marginally better than spraying alone. Practically speaking, the combination of all three treatments may not be worth the added time and expense for only marginally better suppression compared to spraying alone. In fact, the model indicates that a single acaracide application alone can provide effective tick population control during years of treatment and possibly for the first-year post treatment depending on the timing of acaracide spraying.

The original model also concluded that a singular acaracide application was a cost-effective short-term management option, simulating 80% reduction in DON (Mount et al. 1997b). The use of acaracide spraying has been studied extensively, varying the type of synthetic acaracide used, the concentration of the active ingredient in the acaracide, and time of year applied. This has yielded variable results in the field in terms of percent DON suppression but generally falls in line with the results of our model. Specifically, in residential settings, the reported percent suppression for the use of barrier sprays ranged from 76-100% (Stafford 1991a, Curran et al. 1993, Schulze et al. 1994, 2001b, 2005, Schulze and Jordan 1995, Stafford and Allan 2010, Eisen and Dolan 2016). Notably, the variability seen in the results of these field studies may be attributed to weather. Indeed, when using the current model to evaluate the impact of weather on treatment outcomes, results by site and from year to year usually differed to some degree and in some years, differences were quite notable.

Spraying an area-wide acaracide is likely more effective than host-targeted treatments because this method targets a larger proportion of the tick population. This is because only a small percentage of the population would be on a host at the time of spraying, therefore decimating any host-seeking and quiescent ticks within the application area. Thus, this explains the comparatively low reported effectiveness of both host-targeted treatments within the model. That is, no treatment exclusively involving host-targeted methods reduced DON greater than 42% suppression.

Interestingly, some field studies have shown that the use of host-targeted treatments alone are effective long-term management techniques, although results have been variable. For example, Mather et al. (1993) reported an 89% DON and 97% DIN suppression following 1 year after the deployment of permethrin tick tubes in a residential setting. Similarly, Dolan et al. (2004) reported 97% and 96% DIN suppression following 1 year and 2 years, respectively, after the deployment of fipronil bait boxes. However, other studies have shown lower effectiveness with some even reporting an increase in DON, ranging between -10 – 62%, and DIN, up to two years post treatment (Stafford 1991b, Daniels et al. 1991). As for 4-poster devices, only a few reported a DON suppression of at least 80% (Solberg et al. 2003, Daniel et al. 2009, Carrol et al. 2009b, Schulze et al. 2009). All four of these evaluated DON suppressions yearly, and upwards to 6 years post-treatment. Of note, they all reported their highest DON suppression in the final year they sampled, indicating that 4-posters may became more effective over time when used for several years in a row. This trend was also seen in studies that calculated DIN suppression with the highest suppression seen in year 5 and 6 with 67% and 68% respectively (Brei et al. 2009, Hoen et al. 2009).

Notably, the current study only evaluated the effectiveness of treatments at 25% efficiency after 2 years of deployment with minimal success. This in conjunction with the findings of previous studies seems to indicate that both bait boxes and 4-posters appear to be ineffective unless used on an annual basis for over two years. It is also possible that some previous studies saw higher suppression values because they treated greater than 25% of the targeted host population. Therefore, additional work evaluating the host-targeted acaracides long-term, that is, running the model applying the treatment for three or more consecutive years should be done to determine whether consistent, long-term use of host-targeted treatments could prove effective. Also, testing the effect of increased efficiencies on host-targeted control methods is warranted.

However, if the model did find long-term, yearly use and/or higher efficiencies of host-targeted treatments were effective, there would be several concerns. With increased acaracide application, the potential for development of acaracide resistance within the *I. scapularis* tick population would be a concern. Additionally, 4-poster deployment is concerning as they could become sites for hosts like white-tailed deer to transmit diseases such as CWD and tuberculosis (Wong et al. 2017). Furthermore, consistent access to supplemental food will affect host populations by decreasing resource competition and thus resulting in an increase in host abundance which could lead to an increase in ticks and possibly pathogen prevalence as well. Finally, the ability to maintain 4-poster devices and bait boxes year-round for several years would be logistically challenging given that both the bait and acaricides must be consistently replenished

In terms of host-targeted treatments impacting pathogen dynamics, the model's simulated results after the application of bait boxes alone, 4-posters alone, and bait boxes + 4-posters never exceeded 42% in a single year. This coincides with the original model (Mount et al. 1997b), which found that the percentage of infected ticks were the same in all host-targeted simulations. In terms of field-based studies, only two studies examining rodent-targeted treatments showed effective DIN suppression, e.g., at least 80%, following the first year of treatment in a residential setting (Mather et al. 1988, Dolan et al. 2004). Comparatively, many studies testing rodent-targeted acaracides have shown unsatisfactory results with minimal DIN suppression (Ginsberg 2002) or an increase in DIN (Stafford 1991b, 1992, Daniels et al. 1991, Dolan et al. 2004). As for 4-posters, the few studies that have evaluated the treatment's effectiveness at reducing DIN have shown no field suppression over 80%, which makes sense given that white-tailed deer are not competent reservoirs for *B. burgdorferi* (Brier et al. 2009, Hoen et al. 2009).

The model found that the time of year when spraying is applied is integral for successful tick suppression. Specifically, the model predicted that the best weeks for the application of the integrated bait box + 4-poster + spraying combination in either January to mid-April or mid-September to early December. Notably, late fall through early spring is when adult blacklegged ticks are most active and laying eggs. It's also when larvae overwinter in a quiescent state in the leaf litter. So, by preemptively treating the environment and competent hosts when adults are active and larvae are quiescent appears to be critical points in the life cycle for effective tick control. Specifically, by reducing quiescent larvae, they are then unable to molt and emerge as nymphs the following spring and summer. Additionally, the reduction of bloodmeals for adult ticks would therefore decrease the number of eggs laid, leading to decreased numbers of nymphs the following year. For example, by treating for adults in December of 2007, nymphal reduction, if seen, would occur in 2009.

It is important to note that when examining the ideal time window for a single spraying treatment, when spraying was done early in the year, it yielded effective suppression for only the years that the treatment was applied. For the first-year post treatment, the DON suppression was comparatively low implying that the effectiveness of treatment may not be sustained the year following treatment. Comparatively, when spraying was applied later in the year, it resulted in a delayed suppression, meaning that the treatment was only effective in the second year of treatment and the first-year post-treatment. Notably, this was somewhat in contrast to what was observed when treatment was applied for two consecutive years of control which was done for most of the model runs. When this was done, three years of effective DON suppression was achieved (e.g., both treatment years and post-treatment year one). However, for these runs, the model was programmed such that for each year, it ran multiple iterations of the treatment testing different timings of the single application and only reporting the single, lowest DON value. This was likely leading to an over estimation of the duration of suppression for those model runs. What the results of us testing singular treatments for one year indicate is that two years of treatment with spraying + bait boxes + 4-posters likely would only yield 2 years of suppression. Rather, to get 3 years of suppression as observed in much of the model output would, in reality, require treatment early in the 1st year of treatment and then in the second year, a treatment early in the year and late in the year.

To test the effects of long-term use of spraying + bait boxes + 4-poster devices, the model was used to simulate DON and DIN after varying years of treatments. It was determined that treating long-term every year in perpetuity was only marginally more effective at reducing DON than treating every other year and both were found to suppress DON by at least 80%. However, based on how the model was programmed and insights gained when identifying ideal time windows for singular treatment, the suppression achieved the year after treatment would likely require two treatments per year (one early in the year and one later in the year) to achieve suppression during both the year of treatment and 1-year post-treatment. Regardless, alternating years of treatment application may be a more affordable option for homeowners, while potentially reducing the negative environmental effects associated with spraying on an annual basis.

While it was not imperative to apply treatment in consecutive years to suppress DON, the model did show that treatment in consecutive years is required to reduce DIN. Specifically, the ability to reach 80% suppression for DIN was not previously seen with any of the treatments being applied for two consecutive years. However, when treatments were applied for eight consecutive years, DIN was found to approach / reach and maintain 80% suppression. This notion, that long-term treatment may be necessary for prevalence suppression, is furthered by the less successful DIN suppression following application of treatments in alternating years for five years. Specifically, treatments every other year led to DIN suppression values more comparable to short-term treatment, which was not effective at reducing DIN. This suggests that treating annually in perpetuity may be key to reducing *B. burgdorferi* infection in endemic areas. Notably, this trend was seen with the application of spraying + bait boxes + 4-poster devices. However, further simulations should be run to determine whether spraying alone would be effective in reducing *B. burgdorferi* infection.

Specifically, it is hypothesized that host-targeted treatments would likely be needed to impact DIN. A reduction in the prevalence of infection in the *I. scapularis* nymph population is entirely dependent on reducing the burden of immature ticks on competent rodent reservoirs, i.e., white-footed mice which infect the ticks. To do so, host-targeted acaracides would be necessary to limit immature ticks from taking blood meals from infected hosts. Hence even if a treatment is applied for a year and results in an immediate reduction of infected ticks, this would not continue once a treatment is complete. Without a host targeted treatment, larvae would once again be able to take blood meals from infected hosts and perpetuate the enzootic transmission of *B. burgdorferi*.

Finally, one often overlooked alternative to acaracide treatments for tick control is prescribed burning. As previously mentioned, the overuse of acaracides is concerning for several reasons including the risk of ticks developing acaracide resistance, negative impacts on the environment, and in the case of certain host-targeted treatments, increasing host population sizes by introducing bait and the heightened risk of disease transmission (Carroll et al. 2008, 2009a, Stafford et al. 2009, Eisen and Dolan 2016). By avoiding the use of acaracides, prescribed burning avoids all these issues. In fact, prescribed burning actively benefits most ecosystems when done at the right time, intensity, and frequency. Although preliminary, the initial model results showed that prescribed burning yielded nearly 100% (between 96-100%) suppression for the burns years, the year between burns, and the first-year post-burn across all three sites. Previous field studies have reported 74-97% suppression of *I. scapularis* nymphs when evaluated 4 months to 1 year post burning, thus indicating that the model's predicted results coincide with the higher end of this spectrum. However, additional work still needs to be done to 1) further refine model parameters, and 2) perform field validation of this new addition to the model. Future testing of prescribed fire should explore other burning parameters including burn intensity, as some of the variable efficacy in field studies can be attributed to the severity of the burn (Stafford et al. 1998). Additionally, the model should be run to predict the effects of different timings (e.g., fall versus early spring) and frequencies of burns (e.g., a single year, alternating every other year and every third year and every 5th year) commonly used in management scenarios. Furthermore, the model currently assumes that prescribed burning affects hosts and ticks for the same number of weeks. Additional variables and updates to the model that account for the burn impacting ticks and host populations for different lengths of time may make the model more accurate.

CONCLUSION:

This research was conducted with the goal of opening dialogue with pest control companies and public health professionals regarding optimized treatment strategies to reduce Lyme disease risk for humans. The model output conveyed that the optimized treatment strategy would be spraying once sometime in January through mid-April and once again sometime between September and December every other year at a 25% effectiveness. However, because synthetic acaricides pose environmental and health concerns, further adapting the model to evaluate alternatives like natural and fungal acaracides is warranted. Indeed, previous studies have shown that fungal acaracides are comparatively less harmful than synthetic acaracides, while attaining the same effectiveness (Stafford and Allan 2010). Additionally, the model should be used to further research optimized host-targeted methods.

Finally, the preliminary model incorporating prescribed burning as a non-acaricidal treatment option has shown fire to potentially be an effective tick control option. Further research needs to be done to further refine the prescribed burning model parameters and explore optimized strategies for use of prescribed fire as a tick control method. Field validation of the new prescribed fire aspect of LYMESIM 2.0 should also be done.

WORKS CITED:

Adams, Jonathan; Edmondson, Chris; Willis, Damien; Carter, Robert. (2013). Effects of prescribed burning on small mammal, reptile, and tick populations on the Talladega National Forest, Alabama. In: Guldin, James M., ed. 2013. Proceedings of the 15th biennial southern silvicultural research conference. e-Gen. Tech. Rep. SRS-GTR-175. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. 123-126.

Allan, S. A., and L. A. Patrican. (1995). Reduction of immature Ixodes scapularis (Acari: Ixodidae) in woodlots by application of desiccant and insecticidal soap formulations. J. Med. Entomol. 32: 16–20.

Arsnoe, I., Tsao, J. I., and Hickling, G. J. (2019). Nymphal *Ixodes scapularis* questing behavior explains geographic variation in Lyme borreliosis risk in the eastern United States. Ticks and tick-borne diseases, 10(3), 553-563.

Barnard, D. R. (1986). Density perturbation in populations of *Amblyomma americanum* (Acari: Ixodidae) in beef cattle forage areas in response to two regimens of vegetation management. J. Econ. Entomol. 79: 122–127.

Bharadwaj, A., and K. C. Stafford III. (2012). Susceptibility of *Ixodes scapularis* (Acari: Ixodidae) to *Metarhizium brunneum* F52 (Hypocreales: Clavicipitaceae) using three exposure assays in the laboratory. J. Econ. Entomol. 105: 222–231.

Brei, B., J. S. Brownstein, J. E. George, J. M. Pound, J. A. Miller, T. J. Daniels, R. C. Falco, K. C. Stafford III, T. L. Schulze, T. N. Mather, et al. (2009). Evaluation of the United States Department of Agriculture Northeast Area- Wide Tick Control Project by meta-analysis. Vector-Borne Zoonotic Dis. 9: 423–430.

Burgdorfer, W., Barbour, A. G., Hayes, S. F., Benach, J. L., Grunwaldt, E., and Davis, J. P. (1982). Lyme disease-a tick-borne spirochetosis?. Science (New York, N.Y.), 216(4552), 1317–1319. https://doi.org/10.1126/science.7043737

Carroll, J. F., D. E. Hill, P. C. Allen, K. W. Young, E. Miramontes, M. Kramer, J. M. Pound, J. A. Miller, and J. E. George. (2009a). The impact of 4-poster deer self-treatment devices at three locations in Maryland. Vector- Borne Zoonotic Dis. 9: 407–416.

Carroll, J. F., J. M. Pound, J. A. Miller, and M. Kramer. (2009b). Sustained control of Gibson Island, Maryland, populations of *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) by community-administered 4-poster deer self-treatment bait stations. Vector-Borne Zoonotic Dis. 9: 417–421.

Cilek, J. E., and Olson, M. A. (2000). Seasonal distribution and abundance of ticks (Acari: Ixodidae) in northwestern Florida. Journal of medical entomology, 37(3), 439–444.

Curran, K. L., D. Fish, and J. Piesman. (1993). Reduction of nymphal *Ixodes dammini* (Acari: Ixodidae) in a residential suburban landscape by area application of insecticides. J. Med. Entomol. 30: 107–113.

Daniels, T. J., Fish, D., and Falco, R. C. (1991). Evaluation of host-targeted acaricide for reducing risk of Lyme disease in southern New York state. Journal of medical entomology, 28(4), 537-543.

Daniels, T. J., R. C. Falco, E. E. McHugh, J. Vellozzi, T. Boccia, A. J. Denicola, J. M. Pound, J. A. Miller, J. E. George, and D. Fish. 2009. Acaricidal treatment of white-tailed deer to control *Ixodes scapularis* (Acari: Ixodidae) in a New York Lyme disease-endemic community. Vector- Borne Zoonotic Dis. 9: 381–387.

Davidson, W. R., D. A. Siefken, and L. H. Creekmore. (1994). Influence of annual and biennial prescribed burning during March on the abundance of *Amblyomma americanum* (Acari: Ixodidae) in central Georgia. J. Med. Entomol. 31: 72–81.

Deblinger, R. D., and D. W. Rimmer. (1991). Efficacy of a permethrin-based acaricide to reduce the abundance of *Ixodes dammini* (Acari Ixodidae). J. Med. Entomol. 28: 708–711.

Deblinger, R. D., M. L. Wilson, D. W. Rimmer, and A. Spielman. (1993). Reduced abundance of immature *Ixodes dammini* (Acari: Ixodidae) following incremental removal of deer. J. Med. Entomol. 30: 144–150.

Dolan, M. C., G. O. Maupin, B. S. Schneider, C. Denatale, N. Hamon, C. Cole, N. S. Zeidner, and K. C. Stafford III. 2004. Control of immature *Ixodes scapularis* (Acari: Ixodidae) on rodent reservoirs of *Borrelia burgdorferi* in a residential community of southeastern Connecticut. J. Med. Entomol. 41: 1043–1054.

Dolan, M. C., R. A. Jordan, T. L. Schulze, C. J. Schulze, M. C. Manning, D. Ruffolo, J. P. Schmidt, J. Piesman, and J. J. Karchesy. (2009). Ability of two natural products, nootkatone and carvacrol, to suppress *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) in a Lyme disease endemic area of New Jersey. J. Econ. Entomol. 102: 2316–2324.

Dolan, M. C., T. L. Schulze, R. A. Jordan, C. J. Schulze, A. J. Ullmann, A. Hojgaard, M. A. Williams, and J. Piesman. (2017). Evaluation of doxycycline-laden oral bait and topical fipronil delivered in a single bait box to control *Ixodes scapularis* (Acari: Ixodidae) and Reduce *Borrelia burgdorferi* and *Anaplasma phagocytophilum* infection in small mammal reservoirs and host-seeking ticks. J. Med. Entomol. 54: 403–410.

Donahue, J. G., Piesman, J., and Spielman, A. (1987). Reservoir competence of white-footed mice for Lyme disease spirochetes. The American journal of tropical medicine and hygiene, 36(1), 92–96.

Eisen, L., and Dolan, M. C. (2016). Evidence for Personal Protective Measures to Reduce Human Contact With Blacklegged Ticks and for Environmentally Based Control Methods to Suppress Host-Seeking Blacklegged Ticks and Reduce Infection with Lyme Disease Spirochetes in Tick Vectors and Rodent Reservoirs. Journal of medical entomology, 53(5), 1063–1092.

Eisen, L. and Eisen, R.J. (2016) Critical evaluation of the linkage between tick-based risk measures and the occurrence of Lyme disease cases. J. Med. Entomol. 53, 1050–1062

Eisen, R. J., Eisen, L., and Beard, C. B. (2016). County-Scale Distribution of *Ixodes scapularis* and *Ixodes pacificus* (Acari: Ixodidae) in the Continental United States. Journal of medical entomology, 53(2), 349–386

Eisen, R. J., Kugeler, K. J., Eisen, L., Beard, C. B., and Paddock, C. D. (2017). Tick-borne zoonoses in the United States: persistent and emerging threats to human health. ILAR journal, 58(3), 319-335.

Eisen, R. J., and Eisen, L. (2018). The Blacklegged Tick, *Ixodes scapularis*: An Increasing Public Health Concern. Trends in parasitology, 34(4), 295–309.

Fernández-Salas, A., Rodríguez-Vivas, R. I., and Alonso-Díaz, M. A. (2012). First report of a *Rhipicephalus microplus* tick population multi-resistant to acaricides and ivermectin in the Mexican tropics. Veterinary Parasitology, 183(3-4), 338-342.

Gaff, H., Eisen, R. J., Eisen, L., Nadolny, R., Bjork, J., and Monaghan, A. J. (2020). LYMESIM 2.0: an updated simulation of blacklegged tick (Acari: Ixodidae) population dynamics and enzootic transmission of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae). Journal of medical entomology, 57(3), 715-727.

George, J. E., Pound, J. M., and Davey, R. B. (2008). Acaricides for controlling ticks on cattle and the problem of acaricide resistance. Ticks: biology, disease and control, 408-423.

Ginsburg, H. S. (1988). A model of the spread of Lyme disease in natural populations. Ann. N.Y. Acad. Sci. 539: 379-380.

Ginsberg, H. S., T. A. Bargar, M. L. Hladik, and C. Lubelczyk. (2017). Management of arthropod pathogen vectors in North America: minimizing adverse effects on pollinators. J. Med. Entomol. 54: 1463–1475.

Ginsberg, H. S., E. Zhioua, S. Mitra, J. Fischer, P. A. Buckley, F. Verret, H. B. Underwood, and F. G. Buckley. 2004. Woodland type and spatial distribution of nymphal *Ixodes scapularis* (Acari: Ixodidae). Environ. Entomol. 33: 1266–1273.

Gleim, E. R., L. M. Conner, R. D. Berghaus, M. L. Levin, G. E. Zemtsova, and M. J. Yabsley. (2014). The phenology of ticks and the effects of long-term prescribed burning on tick population dynamics in southwestern Georgia and northwestern Florida. Plos One. 9: e112174.

Gray, G. M., and Hammitt, J. K. (2000). Risk/risk trade-offs in pesticide regulation: an exploratory analysis of the public health effects of a ban on organophosphate and carbamate pesticides. Risk analysis : an official publication of the Society for Risk Analysis, 20(5), 665–680.

Greenberg, Cathryn H.; Otis, David L.; Waldrop, Thomas A. (2006). Response of white-footed mice (*Peromyscus leucopus*) to fire and fire surrogate fuel reduction treatments in a southern Appalachian hardwood forest. Forest Ecology and Management, Vol. 234: 355-362

Gould, L. H., R. S. Nelson, K. S. Griffith, E. B. Hayes, J. Piesman, P. S. Mead, and M. L. Cartter. (2008). Knowledge, attitudes, and behaviors regarding Lyme disease prevention among Connecticut residents, 1999-2004. Vector Borne Zoonotic Dis. 8: 769–776.

Hahn, M.B. et al. (2016) Modeling the geographic distribution of *Ixodes scapularis* and *Ixodes pacificus* (Acari: Ixodidae) in the contiguous United States. J. Med. Entomol. 53, 1176–1191

Carolyn L. Hodo, David Forgacs, Lisa D. Auckland, Katherine Bass, Chelsea Lindsay, Micah Bingaman, Tijjani Sani, Kara Colwell, Gabriel L. Hamer, Sarah A. Hamer, Presence of diverse Rickettsia spp. and absence of Borrelia burgdorferi sensu lato in ticks in an East Texas forest with reduced tick density associated with controlled burns, Ticks and tick-borne diseases, 11(1), 101310.

Hoen, A. G., Rollend, L. G., Papero, M. A., Carroll, J. F., Daniels, T. J., Mather, T. N., Schulze, T. L., Stafford, K. C., 3rd, and Fish, D. (2009). Effects of tick control by acaricide self-treatment of white-tailed deer on host-seeking tick infection prevalence and entomologic risk for *Ixodes scapularis*-borne pathogens. Vector borne and zoonotic diseases (Larchmont, N.Y.), 9(4), 431–438. https://doi.org/10.1089/vbz.2008.0155

Kocan, K., Fuente, J., and Coburn, L. (2015). Insights into the Development of *Ixodes scapularis*: A Resource for Research on a Medically Important Tick Species. Parasites and Vectors, 8(1).

Kugeler, K. J., G. M. Farley, J. D. Forrester, and P. S. Mead. (2015). Geographic distribution and expansion of human Lyme disease, United States. Emerg. Infect. Dis. 21: 1455–1457.

Lantos, P. M., Nigrovic, L. E., Auwaerter, P. G., Fowler, V. G., Jr, Ruffin, F., Brinkerhoff, R. J., Reber, J., Williams, C., Broyhill, J., Pan, W. K., and Gaines, D. N. (2015). Geographic Expansion of Lyme Disease in the Southeastern United States, 2000-2014. Open forum infectious diseases, 2(4), ofv143. https://doi.org/10.1093/ofid/ofv143

Levine, J. F., Wilson, M. L., and Spielman, A. (1985). Mice as reservoirs of the Lyme disease spirochete. The American journal of tropical medicine and hygiene, 34(2), 355–360.

Lantos, P. M., Nigrovic, L. E., Auwaerter, P. G., Fowler, V. G., Jr, Ruffin, F., Brinkerhoff, R. J., Reber, J., Williams, C., Broyhill, J., Pan, W. K., and Gaines, D. N. (2015). Geographic Expansion of Lyme Disease in the Southeastern United States, 2000-2014. Open forum infectious diseases, 2(4), ofv143. https://doi.org/10.1093/ofid/ofv143

Mackay, A. and Foil, L. (2005) Seasonal and geographical distribution of adult *Ixodes scapularis* say (Acari: Ixodidae) in Louisiana. J. Vector Ecol. 30, 168–170

Mather, T. N., D. C. Duffy, and S. R. Campbell. (1993). An unexpected result from burning vegetation to reduce Lyme disease transmission risks. J. Med. Entomol. 30: 642–645.

Mather, T. N., RIBEIRO, J. M., Moore, S. I., and Spielman, A. (1988). Reducing transmission of Lyme disease spirochetes in a suburban setting. Annals of the New York Academy of Sciences, 539(1), 402-403.

Miller, N. J., W. A. Thomas, and T. N. Mather. (2009). Evaluating a deer-targeted acaricide applicator for area-wide suppression of blacklegged ticks, Ixodes scapularis (Acari: Ixodidae), in Rhode Island. Vector-Borne Zoonotic Dis. 9: 401–406.

Mead, P. S. (2015). Epidemiology of Lyme disease. Infect. Dis. Clin. N. Am. 29: 187–210.

Meek, M. G., Cooper, S. M., Owens, M. K., Cooper, R. M., and Wappel, A. L. (2008). White-tailed deer distribution in response to patch burning on rangeland. Journal of Arid Environments. 72(11): 2026-2033.

Mount, G. A., D. G. Haile, R. B. Davey, and L. M. Cooksey. (1991). Computer simulation of *Boophilus* cattle tick (Acari: Ixodidae) population dynamics. J. Med. Entomol. 28: 223-240.

Mount, G. A., Haile, D. G., and Daniels, E. (1997a). Simulation of blacklegged tick (Acari: Ixodidae) population dynamics and transmission of *Borrelia burgdorferi*. Journal of medical entomology, 34(4), 461-484.

Mount, G. A., Haile, D. G., and Daniels, E. (1997b). Simulation of management strategies for the blacklegged tick (Acari: Ixodidae) and the Lyme disease spirochete, *Borrelia burgdorferi*. Journal of Medical Entomology, 34(6), 672-683.

Quadros, D. G., Johnson, T. L., Whitney, T. R., Oliver, J. D., and Oliva Chávez, A. S. (2020). Plant-Derived Natural Compounds for Tick Pest Control in Livestock and Wildlife: Pragmatism or Utopia?. Insects, 11(8), 490.

Oliver, J. (1989). Biology and Systematics of Ticks (Acari:Ixodida). Annual Review of Ecology and Systematics, 20, 397-430.

Ostfeld, R., Cepeda O., Hazler K., and Miller M. (1995). Ecology of Lyme Disease: Habitat Associations of Ticks (*Ixodes Scapularis*) In a Rural Landscape. Ecological Applications, 5(2), 353-361.

Perry, Roger W., Rudolph, D. Craig, and Thill, Ronald E. (2012). Effects of short-rotation controlled burning on amphibians and reptiles in pine woodlands. Forest Ecology and Management 271:124-131.

Piesman, J. and Eisen, L. (2008) Prevention of tick-borne diseases. Annu. Rev. Entomol. 323–343

Porco, T. C. (1991). A model of the enzootiology of Lyme disease in the Atlantic Northeast of the United States. Nat. Resour. Model. 5: 469-505.

R Development Core Team. (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org. ISBN 3-900051-07-0.

Rand, P. W., C. Lubelczyk, G. R. Lavigne, S. Elias, M. S. Holman, E. H. Lacombe, and R. P. Smith. (2003). Deer density and the abundance of *Ixodes scapularis* (Acari : Ixodidae). J. Med. Entomol. 40: 179–184.

Rand, P. W., C. Lubelczyk, M. S. Holman, E. H. Lacombe, and R. P. Smith. (2004). Abundance of *Ixodes scapularis* (Acari: Ixodidae) after the complete removal of deer from an isolated offshore island, endemic for Lyme disease. J. Med. Entomol. 41: 779–784.

Rodríguez-Vivas, R.I., Alonso-Díaz, M.A., Rodríguez-Arévalo, F., Fragoso- Sánchez, H., Santamaría, and V.M., Rosario-Cruz, R., (2006a). Prevalence and potential risk factors for organophosphate and pyrethroid resistance in Boophilus microplus ticks on cattle ranches from the State of Yucatan, México. Vet. Parasitol. 136, 335–342.

Rosa, P.A. (1997) Microbiology of Borrelia burgdorferi. Semin Neurol 17: 5– 10.

Solberg, V. B., Miller, J. A., Hadfield, T., Burge, R., Schech, J. M., and Pound, J. M. (2003). Control of *Ixodes scapularis* (Acari: Ixodidae) with topical self-application of permethrin by white-tailed deer inhabiting NASA, Beltsville, Maryland.

Sondberg, S., T. E. A Werbach, and A. Spielman. (1992) A comprehensive multiple matrix model representing the life cycle of the tick that transmits the agent of Lyme disease. J. Theor. Biol. 157: 203-220.

Schulze, T. L., and Jordan, R. A. (1995). Potential influence of leaf litter depth on effectiveness of granular carbaryl against subadult *Ixodes scapularis* (Acari: Ixodidae). Journal of medical entomology, 32(2), 205-208.

Schulze, T. L., R. A. Jordan, and R. W. Hung. 1995. Suppression of *Ixodes scapularis* (Acari: Ixodidae) following removal of leaf litter. J. Med. Entomol. 32: 730–733.

Schulze, T. L., Jordan, R. A., Hung, R. W., Taylor, R. C., Markowski, D., and Chomsky, M. S. (2001). Efficacy of granular deltamethrin against *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) nymphs. Journal of medical entomology, 38(2), 344-346.

Schulze, T. L., Jordan, R. A., and Krivenko, A. J. (2005). Effects of barrier application of granular deltamethrin on subadult *Ixodes scapularis* (Acari: Ixodidae) and nontarget forest floor arthropods. Journal of economic entomology, 98(3), 976-981.

Schulze, T. L., and R. A. Jordan. (2006). Assessment and management of vector tick populations in New Jersey. Freehold Township Health Department, Hightstown, NJ.

Schulze, T. L., Jordan, R. A., Schulze, C. J., Healy, S. P., Jahn, M. B., and Piesman, J. (2007). Integrated use of 4-Poster passive topical treatment devices for deer, targeted acaricide applications, and Maxforce TMS bait boxes to rapidly suppress populations of *Ixodes scapularis* (Acari: Ixodidae) in a residential landscape. Journal of medical entomology, 44(5), 830-839.

Schulze, L., Jordan, R. A., Dolan, M. C., Dietrich, G., Healy, S. P., and Piesman, J. (2008b). Ability of 4-poster passive topical treatment devices for deer to sustain low population levels of *Ixodes scapularis* (Acari: Ixodidae) after integrated tick management in a residential landscape. Journal of medical entomology, 45(5), 899-904.

Schulze, T. L., Jordan, R. A., Hung, R. W., and Schulze, C. J. (2009). Effectiveness of the 4-Poster passive topical treatment device in the control of *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) in New Jersey. Vector-Borne and Zoonotic Diseases, 9(4), 389-400.

Scoles, G. A., M. Papero, L. Beati, and D. Fish. (2001). A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. Vector Borne Zoonotic Dis. 1: 21–34.

Sonenshine, D. E., S. A. Allan, R.A.I. Norval, and M. J. Burridge. (1996). A self- medicating applicator for control of ticks on deer. Med. Vet. Entomol. 10: 149–154.

Spielman, A., M. L. Wilson, J. F. Levine, and J. Piesman. (1985). Ecology of *Ixodes dammini* borne human babesiosis and Lyme disease. Annu. Rev. Entomol. 30: 439–460.

Spielman, A. (1988). Prospects for suppressing transmission of Lyme disease. Ann. N. Y. Acad. Sci. 539: 212–220.

Stafford, III, K. C., J. S. Ward, and L. A. Magnarelli. (1998). Impact of controlled burns on the abundance of *Ixodes scapularis* (Acari: Ixodidae). J. Med. Entomol. 35: 510–513.

Stafford III, K. C. (1991a). Effectiveness of carbaryl applications for the control of *Ixodes dammini* (Acari: Ixodidae) nymphs in an endemic residential area. Journal of medical entomology, 28(1), 32-36.

Stafford K. C. III (1991b) .Effectiveness of host-targeted permethrin in the control of *Ixodes dammini* (Acari: Ixodidae) . J. Med. Entomol. 28 : 611 – 617 .

Stafford III, K. C. (1992). Third-year evaluation of host-targeted permethrin for the control of *Ixodes dammini* (Acari: Ixodidae) in southeastern Connecticut. Journal of medical entomology, 29(4), 717-720.

Stafford, III, K. C., and S. A. Allan. (2010). Field applications of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* f52 (Hypocreales: Clavicipitaceae) for the control of Ixodes scapularis (Acari: Ixodidae). J. Med. Entomol. 47: 1107–1115.

Steere, A. C., Hardin, J. A., and Malawista, S. E. (1977). Erythema chronicum migrans and Lyme arthritis: cryoimmunoglobulins and clinical activity of skin and joints. Science (New York, N.Y.), 196(4294), 1121–1122. https://doi.org/10.1126/science.870973

Van Wieren, S. E., Braks, M. A., and Lahr, J. (2016). Effectiveness and environmental hazards of acaricides applied to large mammals for tick control. Ecology and prevention of Lyme borreliosis. Ecology and Control of Vector-borne diseases, 4, 265-278.

Vatsya S, Yadav CL (2011) Evaluation of acaricide resistance mechanisms in field populations of *Rhipicephalus (Boophilus*) microplus collected from India. Int J Acarol 37:405–410.

Williams, R. J., Hallgren, S. W., and Wilson, G. W. (2012). Frequency of prescribed burning in an upland oak forest determines soil and litter properties and alters the soil microbial community. Forest Ecology and Management, 265, 241-247.

Wilson, M. L. (1986). Reduced abundance of adult *Ixodes dammini* (Acari: Ixodidae) following destruction of vegetation. J. Econ. Entomol. 79: 693–696.

Wilson, M. L., S. R. Telford III, J. Piesman, and A. Spielman. (1988). Reduced abundance of immature *Ixodes dammini* (Acari: Ixodidae) following elimination of deer. J. Med. Entomol. 25: 224–228.

White, A., and Gaff, H. (2018). Application of tick control technologies for blacklegged, lone star, and American dog ticks. Journal of Integrated Pest Management, 9(1), 12.

Wong, T. J., Schramm, P. J., Foster, E., Hahn, M. B., Schafrick, N. H., Conlon, K. C., and Cameron, L. (2017). The Effectiveness and Implementation of 4-Poster Deer Self-Treatment Devices for Tick-borne Disease Prevention: A Potential Component of an Integrated Tick Management Program. Climate and Health Technical Report Series– Climate and Health Program.

Zimmermann, G. (2007a.) Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Sci. Techn. 17: 553–596.

Zimmermann, G. (2007b). Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Sci. Techn. 17: 879–920

FIGURES:

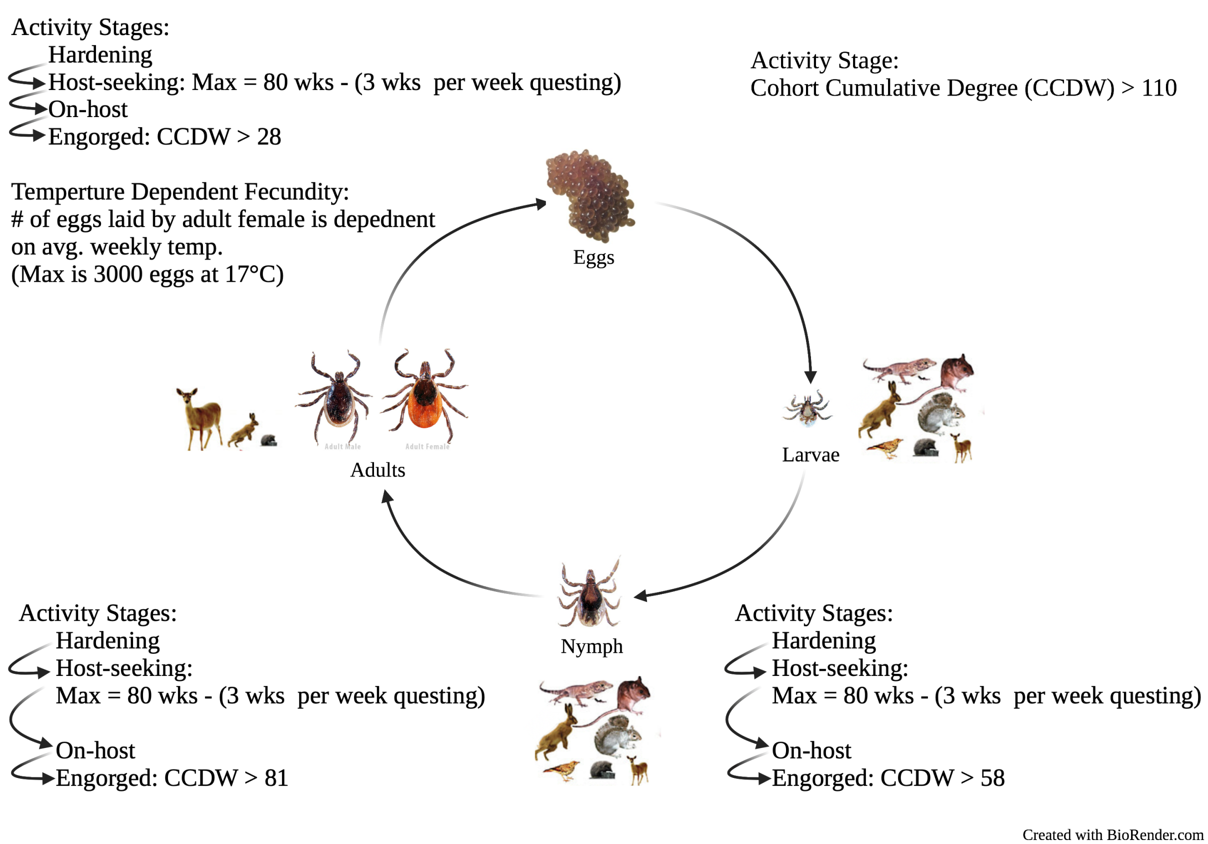

Figure 1. Although too numerous to depict in a single diagram, above depicts some of the core *I. scapularis* natural history factors accounted for in LYMESIM 2.0. All eggs laid in the same week are assigned a single cohort. The progression of each cohort life-stage is dependent on various environmental and biological factors including success rate at each "activity stage", weather conditions, host availability, and temperature. As ticks expend energy when host-seeking, the total number of weeks spent at any given life stage is 80 weeks – 3 weeks for every week spent host-seeking. Wildlife species shown next to each respective tick life stage are host species commonly used by that particular life stage. The cohort cumulative degree (CCDW) threshold is the cumulative number of degrees the weekly average temperature is higher than 6°C and must be met at each life stage to progress to the next. See Gaff et al. (2020) for a comprehensive overview of all mathematical equations and factors considered in LYMESIM 2.0.

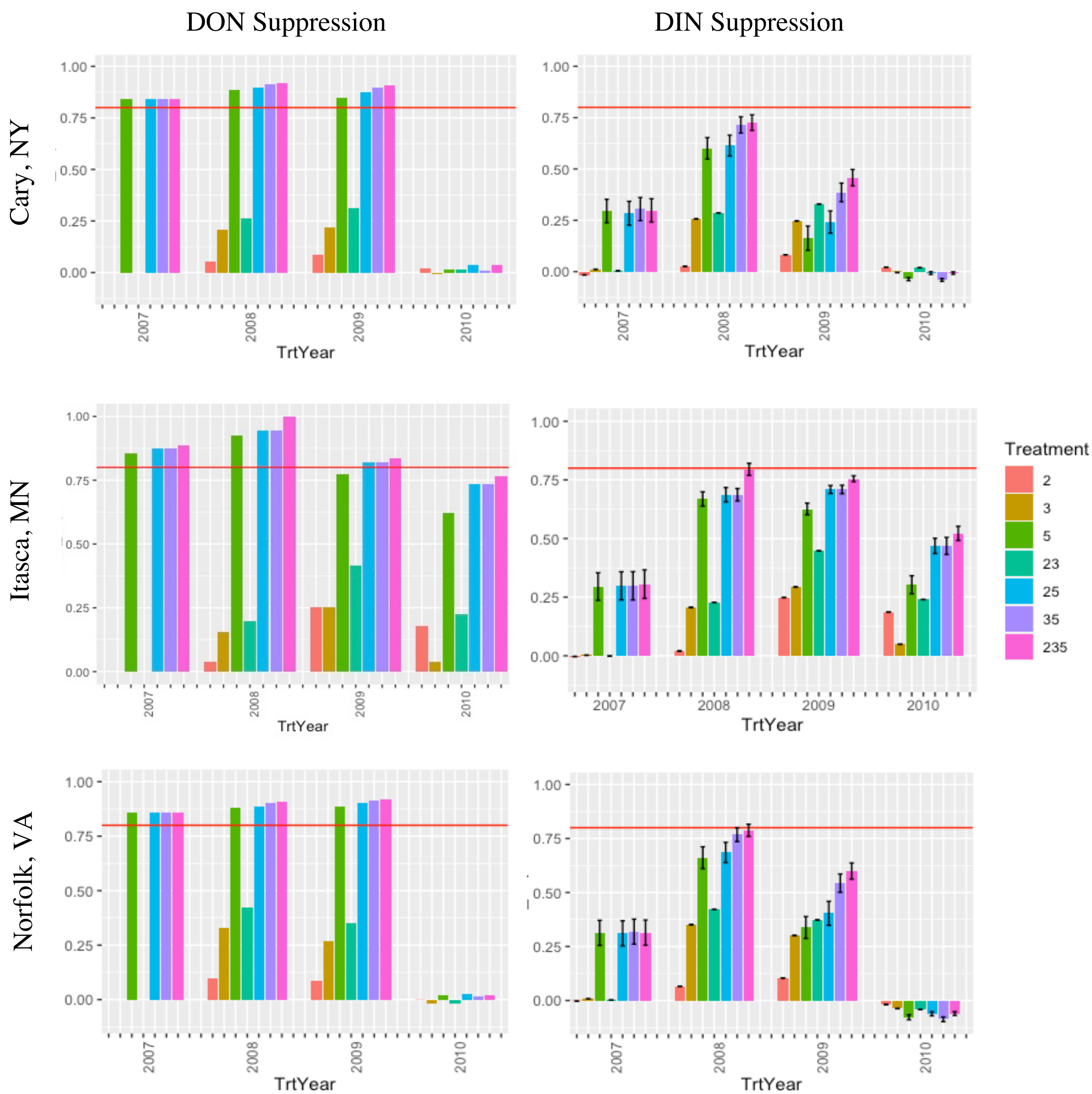

Figure 2. DON (graphs on left) and DIN (graphs on right) supression for each treatment: 4-poster method (2), bait boxes (3), spraying (5) and their combinations. Values were reported for each site (Cary, NY [top], Itasca, MN [middle], and Norfolk, VA [bottom]) given a 25% effectiveness and 2 years of treatment applied in 2007 and 2008. The redline represents the 80% reduction threshold.

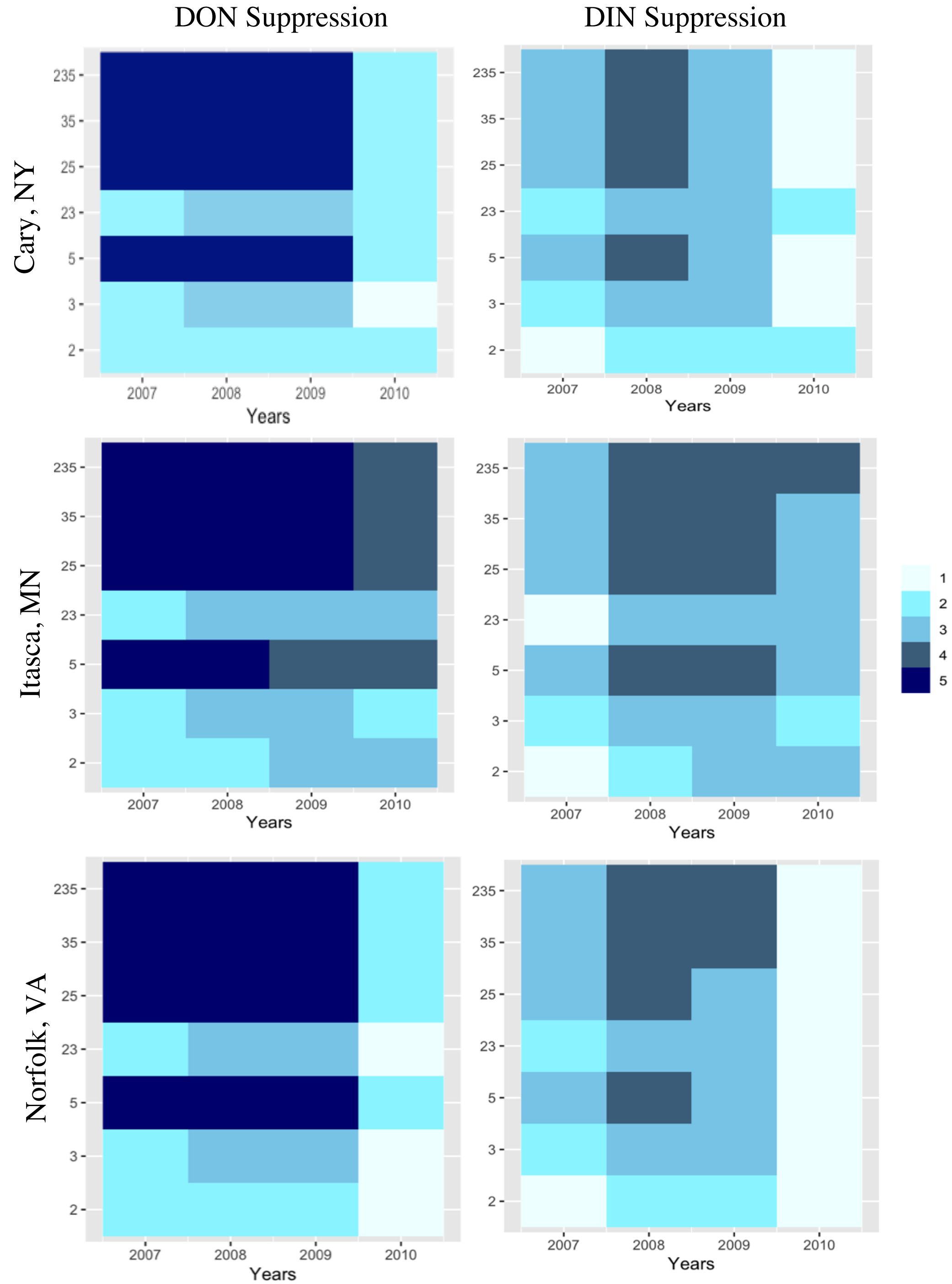

Figure 3. Heat maps represent percent DON (graphs on left) and DIN (graphs on right) suppression for each treatment: 4-poster method (2), bait boxes (3), spraying (5) and their combinations. Values were reported for each site (Cary, NY [top], Itasca, MN [middle], and Norfolk, VA bottom]) given a 25% effectiveness and 2 years of treatment applied in 2007 & 2008. Percent supression is lumped into 5 categories represented in the legend: 1 = x < 0%, 2 = 0 < x < 15%, 3 = 15% < x < 50%, 4 = 50% < x < 80%, and 5 = x > 80%.

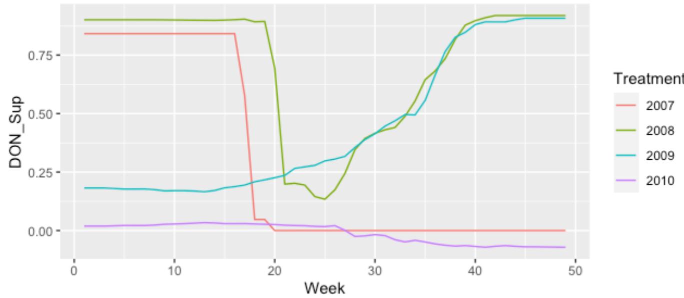

Figure 4. Model simulations were run examining outcomes of different timings of a single application of spraying (in conjunction with bait boxes and 4-posters being out year-round) at a 25% efficiency. Simulations were run for each week of the year. Each week on the above graph depicts the model outcome for application of spraying in that week. DON suppression values represent the second year of treatment, at the Cary, NY.

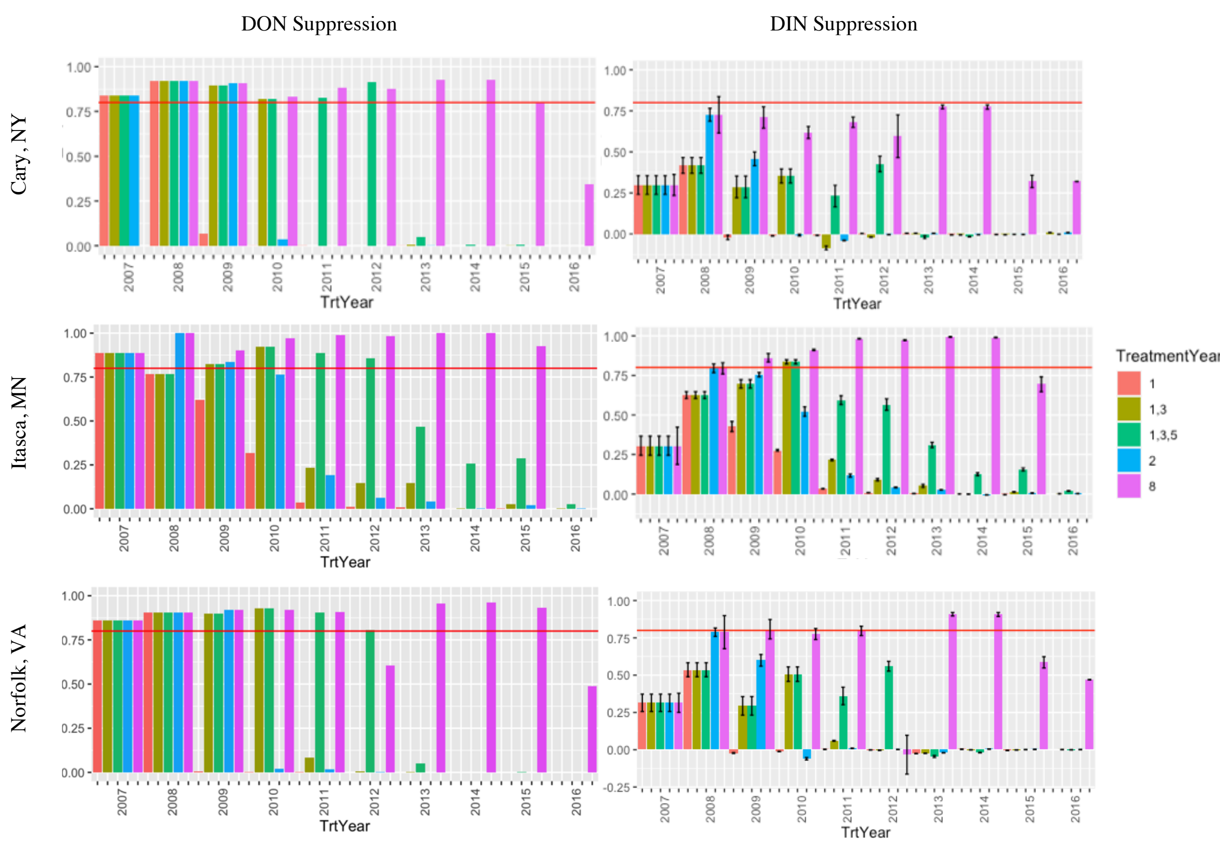

Figure 5. DON (top) and DIN (bottom) suppression when applying the 4-poster + bait box + spraying combination at 25% efficiency for different durations and frequencies. Values were reported for each site. The legend TreatmentYear refers to the varying treatment durations tested. For example, 1 = 1 year of treatment in 2007; 1,3 = treatment applied in years one and three (e.g. in 2007 and 2009); 1,3,5 = treatment applied in years 1,3, and 5 (e.g. in 2007, 2009, and 2011), 8 = treatment applied for 8 consecutive years (e.g. 2007-2014).

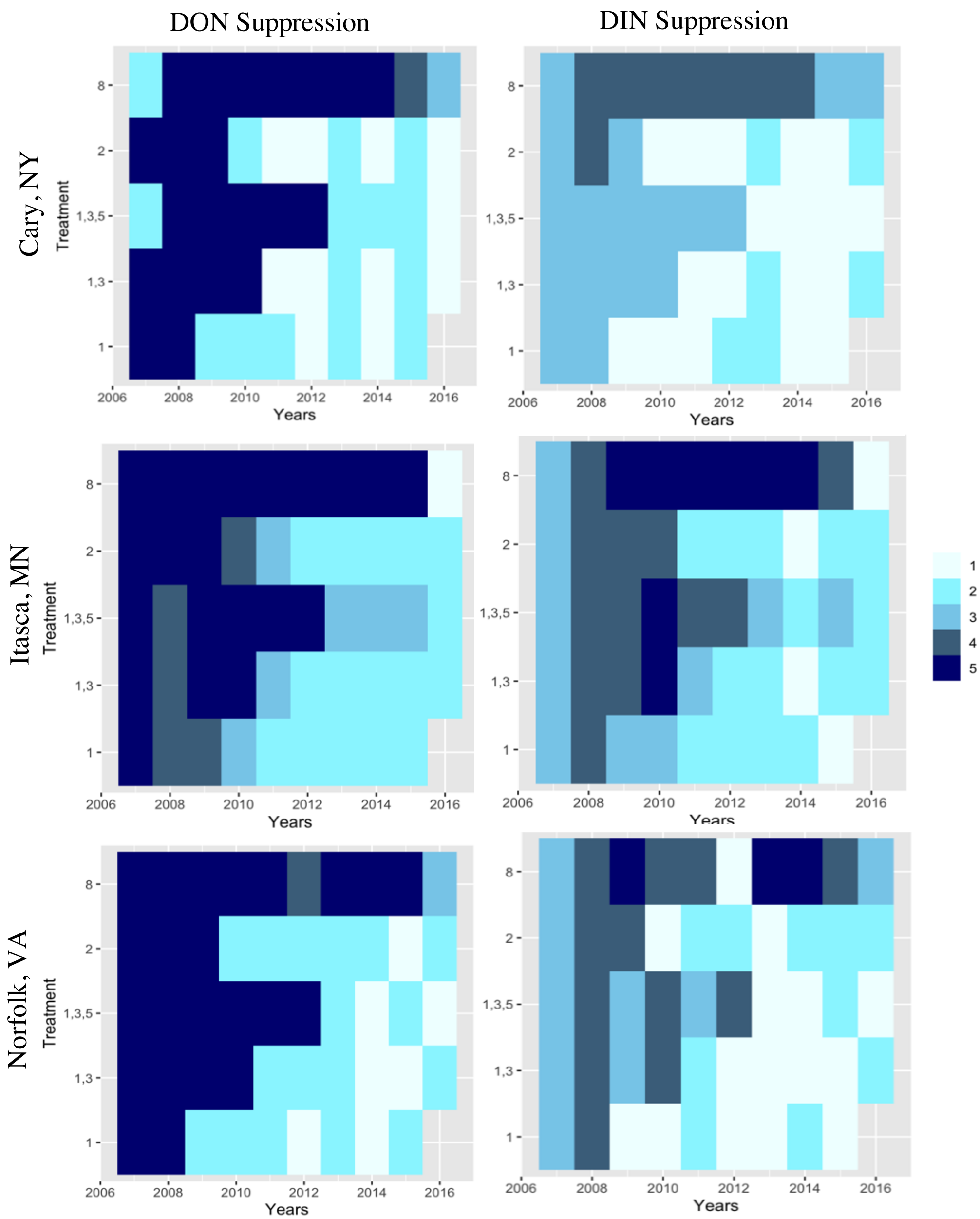

Figure 6. Heat maps represent percent DON (graphs on left) and DIN (graphs on right) supression given an application of the 4-poster + bait box + spraying combination at 25% efficiency for different durations by location. Values were reported for each site (Cary, NY [top], Itasca, MN [middle], and Norfolk, VA [bottom]. Percent supression is binned into 5 categories: 1 = x < 0%, 2 = 0 < x < 15%, 3 = 15% < x < 50%, 4 = 50% < x < 80%, and 5 = x > 80%. Treatment on the y-axis refers to the varying treatment durations tested: 1 = 1 year of treatment in 2007; 1,3 = treatment applied in years one and three (e.g. in 2007 and 2009); 1,3,5 = treatment applied in years 1,3, and 5 (e.g. in 2007, 2009, and 2011), 8 = treatment applied for 8 consecutive years (e.g. 2007-2014).

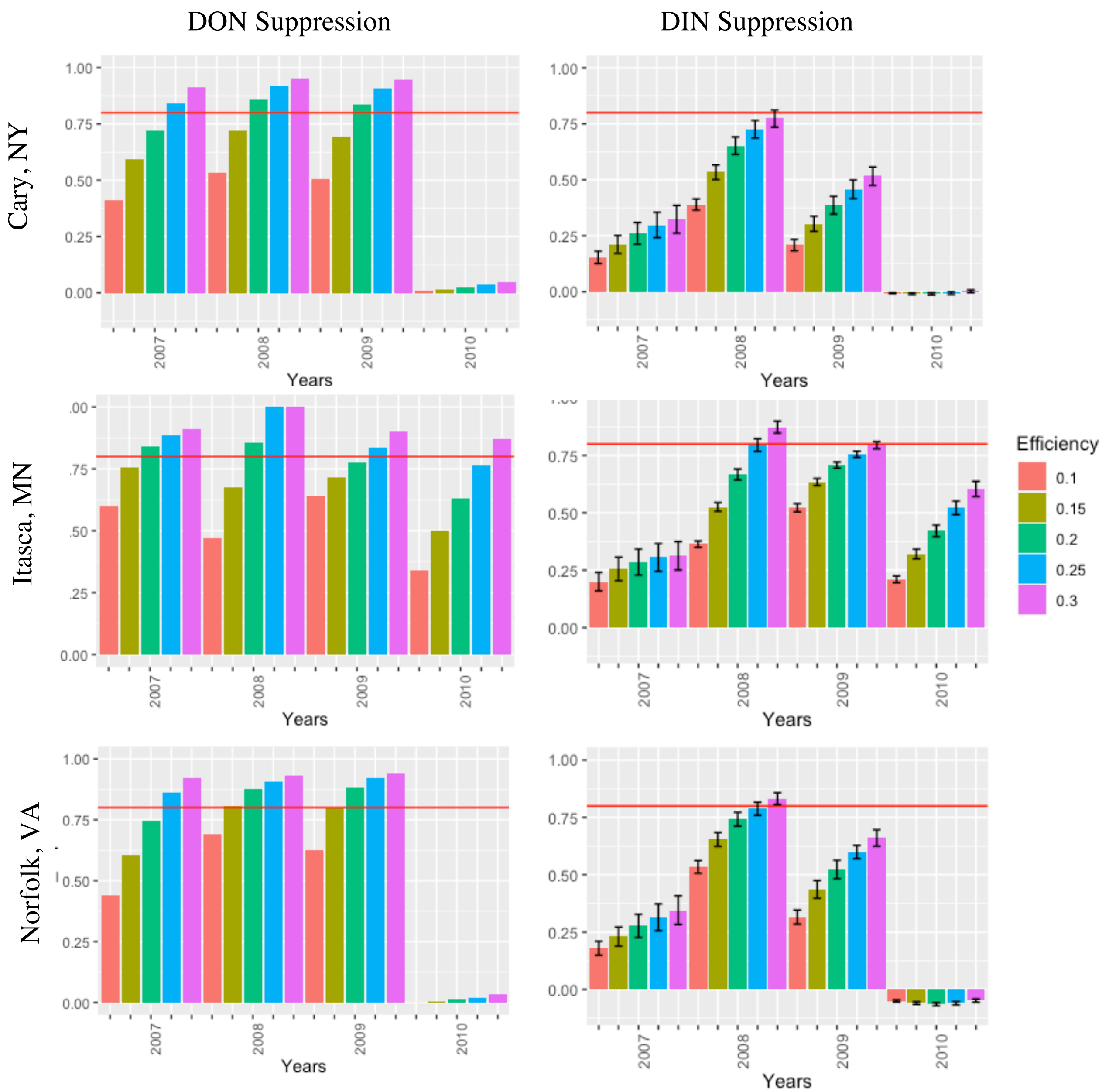

Figure 7. Comparing varying efficiencies on DON (left) and DIN (right) suppression when applying the 4-poster + bait box + spraying combination for two consecutive years starting in 2007. Values were reported for each site (Cary, NY [top], Itasca, MN [middle], and Norfolk, VA [bottom]). The redline represents the 80% reduction threshold.

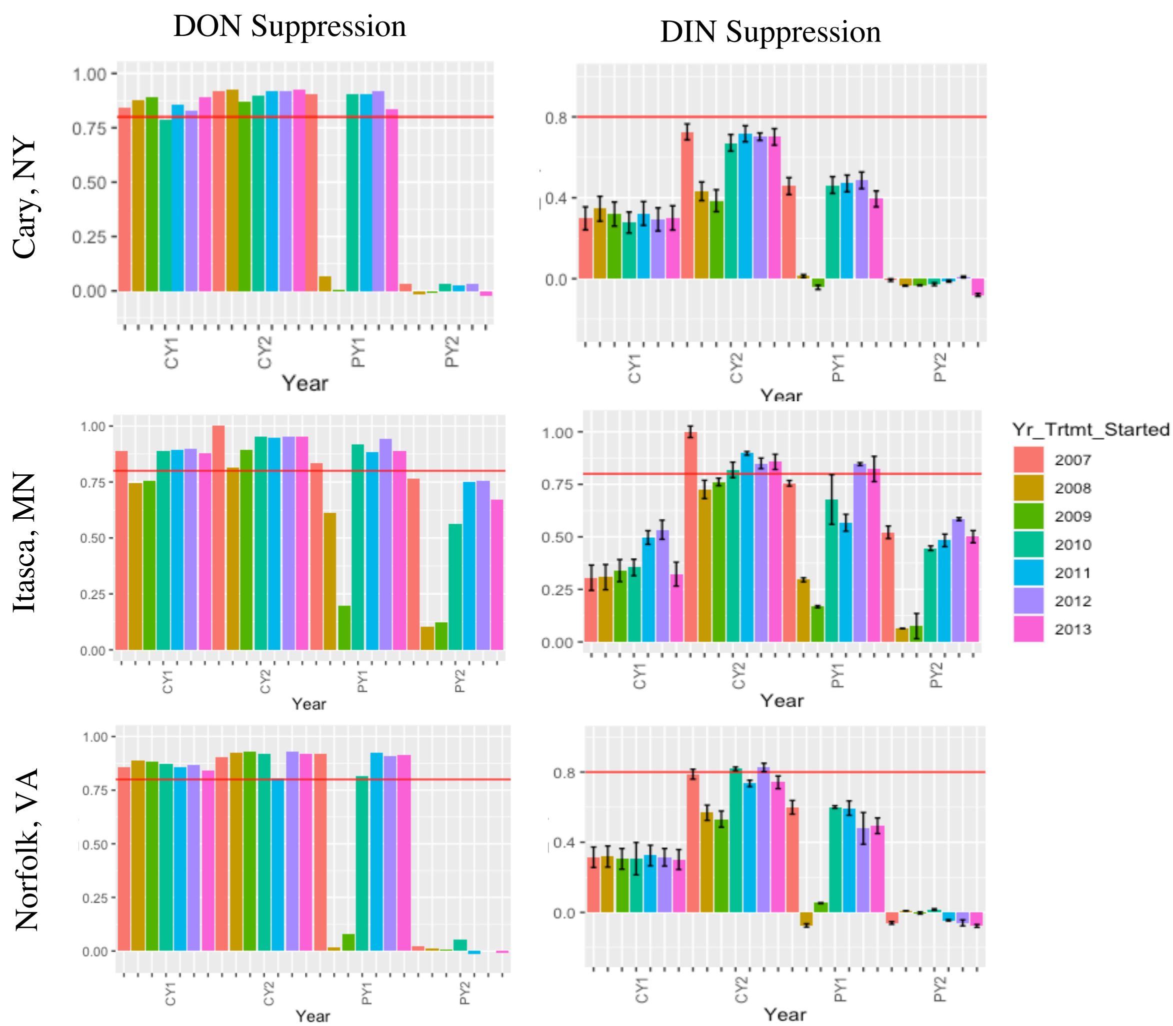

Figure 8. Comparing effects of applying treatment starting in different years on DON (left) and DIN (right) supression given the application of the 4-poster + bait box + spraying combination for two consecutive years at a 25% efficiency. For each bar, the first year of control treatment (CY1) begins in the year it corresponds to on the legend. Treatment is applied in CY1 and CY2 (control treatment year 2). Values were reported for each site (Cary, NY [top], Itasca, MN [middle], and Norfolk, VA [bottom]). PY1 and PY2 corresponds to one year post-treatment and two-years post-treatment, respectively. The redline represents the 80% reduction threshold.

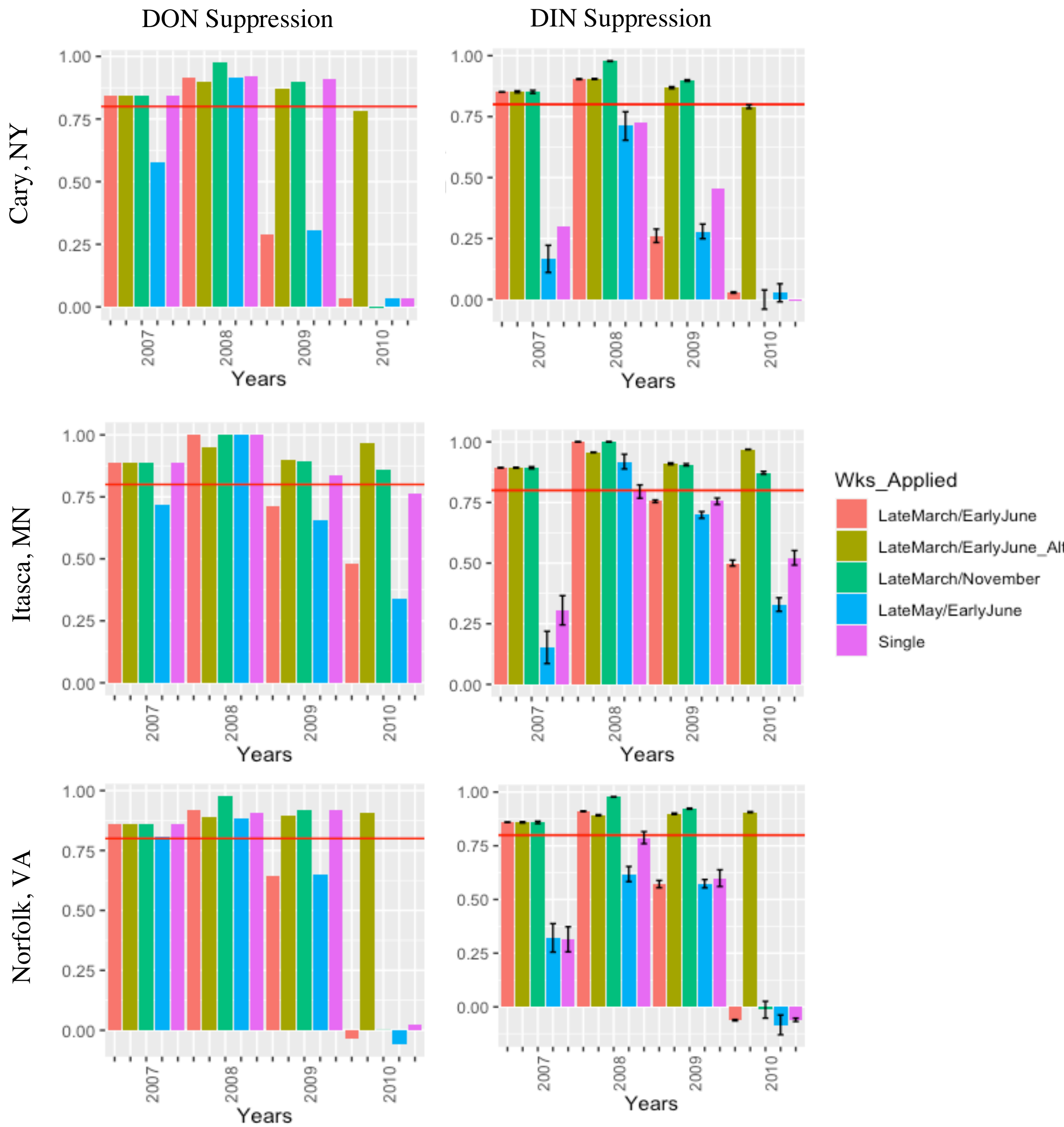

Figure 9. The DON (left) and DIN (right) suppression using bait boxes + spraying + 4-poster devices for the following time windows: LateMarch/EarlyJune (weeks 9-13 and 24-28), LateMarch/EarlyJune_Alt (weeks 17-21 and 24-28, applying treatment in 2007 and 2009), LateMarch/November (weeks 9-13 and 43-47), and LateMay/EarlyJune (weeks 17-21 and 24-28). Graphs presented in the site order of Cary, NY (top), Itasca, MN (middle), and Norfolf, VA (bottom. All treatments applied in 2007 and 2008 unless otherwise noted. The redline represents the 80% reduction threshold.

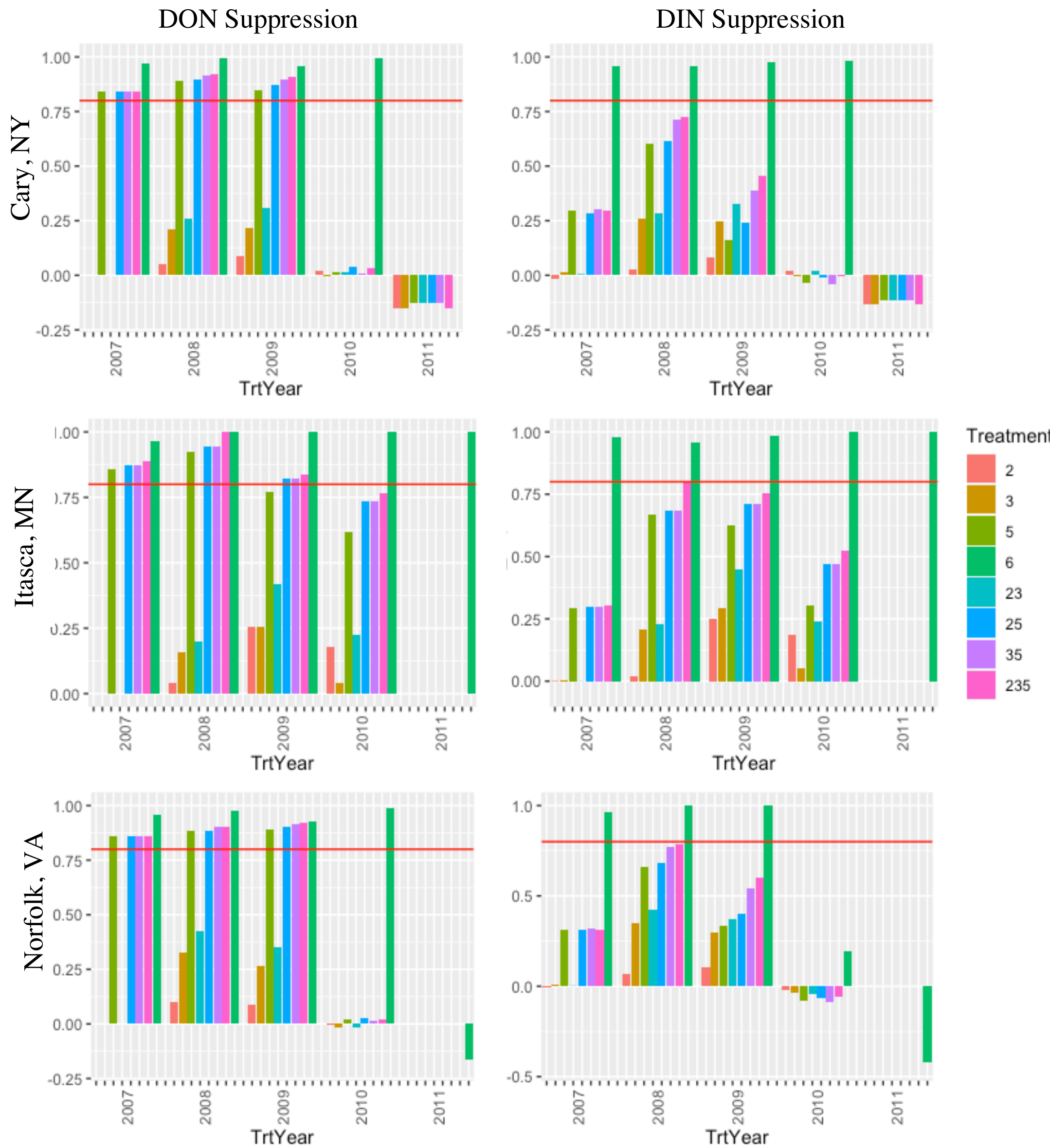

Figure 10. Comparing DON (left) and DIN (right) suppression when applying prescribed burning at a 5% efficiency for two years alternating application in 2007 & 2009. Values were reported for each site: Cary, NY (top), Itasca, MN (middle), and Norfolk, VA (bottom). The prescribed burn treatment is compared to all other previously tested treatments and combinations which were applied at a 25% efficiency for 2 consecutive years (2007-2008). Control treatments tested: 4-poster method (2), bait boxes (3), spraying (5), prescribed fire (6) and their combinations. The redline represents the 80% reduction threshold.