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Evaluation of a Methoprene Aerial Application for the Control of *Culiseta melanura* (Diptera: Culicidae) in Wetland Larval Habitats

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Abstract

Eastern equine encephalitis virus (EEEV) is an arbovirus endemic to the eastern United States. Human cases are rare but can be serious. The primary enzootic vector is *Culiseta melanura* (Coquillett) (Diptera: Culicidae), an ornithophagic mosquito. We conducted an aerial application of a granular methoprene formulation in Hockomock Swamp (Massachusetts), which represents a focus of EEEV transmission. Water collected from inside and outside *Cs. melanura* crypts was evaluated in bioassays of early fourth instar *Cs. melanura* larvae using treated and untreated water. Adult eclosion rates were 36% significantly lower in treated compared with untreated water ($P < 0.05$). Eclosion rates for water collected from inside crypts were significantly higher (62%) than rates from outside crypts (30%) ($P < 0.05$), indicating higher efficacy outside crypts. We tested whether reduced methoprene efficacy inside the crypts was due to reduced chemical penetration into this habitat. Chemical water analyses confirmed that methoprene concentrations were lower inside the crypts (0.1 ± 0.05 ppb) compared to water from outside crypts (1.79 ± 0.41 ppb). The susceptibility of *Cs. melanura* to methoprene was also determined to allow for comparison against concentrations observed in water collected from the field (LC-95: 1.95 ± 0.5 ppb). Overall, methoprene-treated water prevented mosquito development for up to 4 wk, but with a reduction in efficacy between 4- and 6-wk post-application. Our results suggest that aerial methoprene applications can effectively treat open water in wetlands but may not provide efficacious control of *Cs. melanura* due to an inability to penetrate larval habitats.

Keywords

mosquitoes; eastern equine encephalitis; mosquito control

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Eastern equine encephalitis (EEE) is a vector-borne viral disease endemic to the eastern United States. The majority of human and equine cases in the United States are reported in states east of the Mississippi River. Between 2010 and 2018 the states with the highest human case counts were Florida, Michigan, and Massachusetts (Lindsey et al. 2018). Although there is a commercially available equine vaccine, EEE virus (EEEV) can cause high mortality in unvaccinated horses. The virus has also been a problem for horses in the United States for decades, with the first cases formally described in 1933 (Broeck and Merrill 1933, Komar and Spielman 1994). Human cases are relatively rare but are becoming more frequent (Armstrong and Andreadis 2013, Morens et al. 2019) and can be neuroinvasive. Mortality rates often exceed 30% and EEEV infection can cause lifelong disability for those who survive; with the cost of treatment and care often outweighing costs associated with vector control measures (Villari et al. 1995).

The enzootic cycle of EEEV is primarily maintained in avian populations. Several mosquito species are suspected vectors, but *Culiseta melanura* (Coquillett) (Diptera: Culicidae) is considered the primary sylvatic vector for EEEV (Chamberlain et al. 1958, Vaiyanathan et al. 1997, Hachiya et al. 2007, Skaff et al. 2017). Other mosquito species, including *Coquillettidia perturbans* (Walker) and *Aedes sollicitans* (Walker) are bridge vectors but likely play a less important role in sylvatic transmission of EEEV than *Cs. melanura* (Armstrong and Andreadis 2010). Considered an ornithophagic species, *Cs. melanura* rarely bites humans and is not often targeted in larval control evaluations despite being widespread in the United States, east of the Mississippi River. Early research demonstrated that *Cs. melanura* larvae survived dichlorodiphenyltrichloroethane (DDT) misting, while the spread of granular formulations of heptachlor and dieldrin reduced larval populations. Bioassay results showed that *Cs. melanura* larval habitats (crypts) within the root systems of wetland tree species were not evenly treated using the granular formulations deployed (Hayes 1962). Aerial applications of an organophosphate (Naled) targeting adult *Cs. melanura* have also been evaluated. Naled application caused high adult mortality in cage trials, but the effect on trap counts of wild *Cs. melanura* adults was difficult to determine as trap counts were highly variable (Rathburn et al. 1971). Furthermore, Naled applications in central New York over an 11-yr period did not negatively impact natural *Cs. melanura* populations or EEEV risk (Howard and Oliver 1997). These early studies deployed active ingredients that are currently restricted or prohibited in many states (EPA 2020) and did not always account for the ecology of *Cs. melanura*.

Culiseta melanura lay egg rafts in thermally stable microhabitats (crypts) in acidic wetlands. The crypts are often located within the root systems of Atlantic white-cedar (*Chamaecyparis thyoides*), red maple (*Acer rubrum*), tupelo (*Nyssa sylvatica*), or swamp cypress (*Taxodium distichum*) (Siverly and Schoof 1962, Mahmood and Crans 1994, Skaff 2017). *Culiseta melanura* is a relatively long-lived multivoltine species which overwinters as larvae (Joseph and Bickley 1969, Morris et al. 1976). Therefore, early season larvicide applications may be an effective method for their control. Woodrow et al. (1995) tested the efficacy of three larvicides: the insect growth regulator methoprene, the organophosphate temephos, and a bacterial biopesticide *Bacillus thuringiensis israelensis* (*Bti*), in Toad Harbor Swamp near Syracuse, NY. The temephos and *Bti* formulations were short-lived and not found to successfully penetrate the larval habitats where *Cs. melanura* oviposit. The application

of the granular methoprene formulation resulted in the active ingredient being detected in the crypts, but the distribution among crypts was uneven. After an aerial application of methoprene, the adult eclosion rate of pupae collected from crypts was 58% lower than those from control crypts. This research demonstrated that an aerial application of granular methoprene in May might be an efficacious method to control *Cs. melanura* in their natural larval habitats.

In 2019 there was an EEE outbreak in Massachusetts with 12 human cases reported and six fatalities (Lindsey et al. 2020). In response, six aerial adulticide spray operations using sumithrin (Anvil 10 + 10) commenced between August and September. The application area covered over 800,000 ha and cost the state over US$5 million. The efficacy of these adulticide applications in reducing populations of adult *Cs. melanura* and *Cq. perturbans* varied widely depending upon the month and duration of application. Furthermore, due to the ad hoc nature of the efficacy evaluations, the results of some spray events were inconclusive (MDAR 2020). The variable control efficacy observed for *Cs. melanura* may have been partly due to their use of protected habitats which could not be directly sprayed with insecticides.

Considering the financial cost of these applications and the variation in efficacy, alternative targeted control methods are needed. In 2020, we tested the efficacy of a spring aerial application of a granular methoprene formulation (Altosid P35), to control *Cs. melanura*. Building on the research conducted by Woodrow et al. (1995), the efficacy of this application was tested over 6 wk, using a laboratory colony of *Cs. melanura* in treated water bioassays with robust sample sizes. Chemical analysis of methoprene concentrations from water collected from inside and outside of treated crypts was performed to determine penetration of the applied chemical into larval habitats. We also determined the susceptibility of *Cs. melanura* to methoprene. These data were paired with chemical analyses of the crypt water to determine whether the methoprene concentrations were high enough to prevent *Cs. melanura* eclosion. The data presented here will assist mosquito control operators in making important spring season management decisions, while also providing information regarding the penetration of an aerial granular methoprene application into open water and larval habitats.

Methods

Description of Field Sites and Study Design

Sampling areas were located in forested Atlantic white-cedar (*C. thyoides*) wetlands in southeastern Massachusetts. Treatment sites were situated in the northern end of the Hockomock Swamp in Easton, MA (41°59′24.00″N, 71°04′48.00″W). Control sites were located 19.5 km southeast in the Little Cedar Swamp in Middleborough, MA (41°56′52.80″N, 70°51′18.00″W). This area of southeastern Massachusetts experiences an annual average rainfall of 125.6 cm. Conditions during the study were dryer than normal: during the 40-d study period from May into June, rainfall was measured at 9.7 cm. Rainfall in May can range from 3.6 to 19.5 cm and from 1.4 to 35.7 cm in June. The 2020 season had lower than average rainfall and was approximately 30% less rainfall than the 10-yr average

of 13.6 cm at the nearest National Weather Service (NWS) location in Taunton, MA (NOAA 2020).

Areas selected for this study were associated with long-term presence of *Cs. melanura* adults and frequent positive detections of EEEV according to surveillance data collected by the Bristol and Plymouth County Mosquito Control Projects. The sites were all dominated by *C. thyoides* with an understory of hydrophyte shrubs and sphagnum moss atop saturated hydric soils or peat muck to at least 1 m depth (Laderman 1989, Little and Garret 1990, USDA 2020). *Chamaecyparis thyoides* root systems are typically shallow, 1–2 m in depth, growing on hummocks. The hollow spaces in the hummock create the crypts sampled in this study. Surface water, from open pools, was collected within 1.5 m of each crypt.

Aerial Methoprene Application

A 120-ha polygon was created using ArcGIS Desktop 10.6.1 with land classification and orthoimages provided by the Massachusetts Bureau of Geographic Information (MassGIS 2020). The polygon was then converted using NavViewW 10.37, so it could be used by the airplane's navigation system (AgNav Guia). The product used for the application was Altosid P35 (EPA Reg. No. 89459–95) (Zoecon, Schaumburg, IL). Altosid P35 is a uniform pellet with a 35-d residual activity containing 4.25% (S)-methoprene (CAS #65733-16-6). This product was selected because of its long residual activity and similarities to the product used in a previous *Cs. melanura* larval control experiment (Woodrow et al. 1995).

The pesticide was applied using a 1973 Cessna Ag Wagon 188B (Cessna Aircraft Company, Wichita, KS) with an attached venturi spreader. Prior to the application, the spreader was calibrated for the minimum label application rate of 5.6 kg/ha with an effective swath width of 24.4 m. The AgNav Guia navigation system (AG-NAV Inc., Barrie, ON, Canada) recorded the location, speed, and altitude of the aircraft. The application occurred on 14 May 2020. During the application, the weather was calm with no precipitation and temperatures were between 5 and 8°C. Deciduous trees had not begun to leaf out, but the targeted areas were dominated by a coniferous species (*C. thyoides*). The pesticide was applied in a racetrack pattern with passes running north to south through the block (Fig. 1). The aircraft applied the chemical at a height of 61.9 m above sea level and a ground speed of 185.1 km/h (airspeed 193.1 km/h).

Within the treated area of Hockomock Swamp, four treatment sites were selected. Two untreated reference sites were located in Little Cedar Swamp. Within each treatment site, 10 total crypts were identified a priori for water sampling; five crypts were selected in each of the two reference locations. Water from inside and outside of these crypts was sampled for both analytical chemical analysis and bioassays described below.

Analytical Chemistry

Using glass serological pipettes, 50 ml subsamples from each crypt were combined into a single sample for each site and each collection period. The water was collected and sealed inside polyethylene glycol (MilliporeSigma, Burlington, MA)-treated glass jars to prevent methoprene from binding to the glass. Water for analysis was collected from inside and outside of the 10 crypts selected in each treatment site and five crypts in the reference sites

once a week between 11 May 2020 and 23 June 2020. We collected water for baseline data 3 d before the application. The first posttreatment collection occurred 1 d after the application. Separate samples were taken from inside and outside each crypt. In total, 84 water samples were tested from inside and outside the crypts, evenly divided among the six sites and five collection dates. Water samples in jars were stored at 4°C and delivered within 24 h to the Massachusetts Pesticide Analysis Lab (MPAL) at the University of Massachusetts in Amherst, MA, for analysis.

At MPAL, samples were held to equilibrate to room temperature before extraction. For the extraction, samples were shaken vigorously with 100 ml of methanol. Oasis PRiME HLB (6 ml × 200 mg) (Waters Corp., Milford, MA) solid phase extraction cartridges were washed with 6 ml methylene chloride ($MeCl_2$) and dried under vacuum for 10 min. The cartridges were then conditioned with 16 ml of 3:5 ddwater:methanol. Samples were loaded using Teflon transfer lines (10 ml/min) under a vacuum. Cartridges were dried under full vacuum for 30 min. The analytes were eluted with 10 ml of $MeCl_2$ into a 15-ml calibrated centrifuge tube. The solution was reduced under nitrogen in a 40°C water bath until dry and reconstituted with 1.0 ml 100% MeCN and filtered using a glass syringe and 0.22-nm Nylon Acrodisk filter (Pall Corp, Port Washington, NY). The extract was analyzed with a Waters H class UPLC and Acquity TQD LC/MS/MS using an Atlantis T3 column (Waters Corp.). The detection limit for methoprene was 0.004 ppb.

Culiseta melanura Rearing

Larvae were reared from a colony originally collected from Cape May, NJ, in 1994 (Mahmood and Crans 1994). This colony has been maintained at the Connecticut Agricultural Experiment Station (CAES) since April 2003. Adults are blood fed on buttonquail (*Coturnix chinensis*) (L). Larvae were reared in an environmental chamber (Model 845, Lab-Line, Melrose, Park IL) at 25.5°C and 70% RH with a 16:8 (L:D) h light cycle supplied by a single 25-watt incandescent bulb. Larvae were reared to fourth instar in shallow pans with a minimum density of one larva per 5 ml of purified (RO) water. Larval diet consisting of two parts (by weight) ground tropical flakes (TetraMin, Tetra GMBH, Melle, Germany), two parts ground rabbit chow (Sweet Meadow Farm, Sherborn, MA), and one part liver powder (catalog 900396, MP Biomedicals, Solon, OH) was prepared as a 2% suspension in water and provided every other day. The surface of the larval pans was skimmed with a paper towel prior to feeding to remove any surface growth that formed. As necessary, the rearing temperature was lowered incrementally to 13°C to slow larval development to match the collection dates for water from the field. Fourth instar larvae were transferred into cups (500 or 1,000 ml) containing water with a small amount of larval diet for transport to Cornell University less than 24 h prior to their use in methoprene bioassays.

Bioassay Methods

Water for bioassays was collected from the 50 crypts within the four treatment and two reference sites starting 1 d after the application and then approximately every 2 wk between 15 May 2020 and 23 June 2020, with four total collections. Crypts were sampled with 50-ml serological glass pipettes, and 75 ml was extracted from inside and outside the crypts. The water was sealed inside polyethylene glycol-treated glass jars, to prevent methoprene from

binding to the glass, and stored at 4°C for no more than 24 h before being transported to Cornell University where the bioassays were conducted.

Upon receipt, the glass jars were opened and placed inside 473-ml paper cups and any larvae from the field water were removed, discarded, and 50 mg of fish food and 15 laboratory-reared *Cs. melanura* larvae (fourth instar) were added to each glass jar. In total, 5,305 laboratory-reared *Cs. melanura* larvae were tested in the field bioassays over the duration of this study. The paper cups were topped with fine mesh and placed inside an incubator at 26°C, 70% RH, and a 16:8 (L:D) h cycle. Five positive controls were included with the bioassays. These controls contained fish food (Cichlid Gold, Kyorin Co. Ltd., Teaneck, NJ), deionized (DI) water, and 23.8 ppb of methoprene. The positive control concentration was calculated given the application rate and assuming an average water depth of 1 m. Bioassay containers were checked every 24 h for 9 d and the number of alive eclosed adults was recorded. Once adults began to eclose in a bioassay container, small cotton pads soaked in a 10% sucrose solution were placed on top of the mesh.

To interpret our bioassay results in the absence of prior susceptibility information for *Cs. melanura*, we created a susceptibility curve to methoprene. Susceptibility bioassays were conducted using the same methods described above to determine the lethal concentration (LC) thresholds for the CAES *Cs. melanura* colony to technical grade (>95%) (S)-methoprene (Chem Service Inc., West Chester, PA). Polyethylene glycol-lined glass bioassay jars were filled with 75 ml of distilled water and a gradient of eight methoprene concentrations between 0.002 and 166 ppb. The number of alive adults was recorded every 24 h until no living larvae or pupae remained in the bioassay cups.

Statistical Methods

All data analyses were conducted in R version 4.0.3 using the MASS package. Data from the field bioassays were analyzed using two binomial models. The first model was used to compare the percentage of adults that eclosed in the treatment sites with the percentage eclosed in the control sites, with treatment, and their interaction as fixed effects. The second binomial model compared eclosion rates in water from inside and outside the crypts. This model only included data from the treatment site and excluded the final water collection date (T-41 d) when there was no longer a significant treatment effect. Collection location (inside/outside crypts), collection date, and their interaction were fixed effects. The random effect of site was evaluated for both models, which returned a singular fit, and therefore site was excluded from the final models. A post hoc Tukey's test was used for direct comparisons between groups for both binomial models. A probit analysis was conducted using data from the *Cs. melanura* methoprene susceptibility curve and was used to estimate LC values.

Results

Including the pretreatment testing, a total of 84 water samples were submitted to MPAL for methoprene analysis. Prior to the aerial application, no methoprene was detected in either the treatment or control areas. There was one unexplained outlier (198 ppb methoprene) detected 19 d after the application from an inside crypt sample taken from one of the treatment sites. It is difficult to determine the cause of the high methoprene concentration

found in this collection, but it is possible that a granule was deposited directly into a crypt or the collection container. Additionally, despite measures taken to avoid contamination, including not reusing glassware and ensuring that the formulated methoprene was not stored near collection supplies, we cannot be certain that this sample was not contaminated. Excluding this extremely high value, average methoprene concentrations in the treated sites were approximately 17 times higher in outside crypt water samples (1.79 ± 0.41 ppb) than inside (0.1 ± 0.05 ppb). No methoprene was detected in the untreated reference sites during any of the collection periods (Table 1).

Due to dry conditions, some locations did not contain enough water to obtain bioassay samples, particularly during the fourth (T-41 d since application) collection period. In total, 95 samples were obtained for the first collection (T-1 d) and 1,495 *Cs. melanura* larvae from the CAES colony were tested, 92 for the second (T-13 d) with 1,380 larvae tested, 94 for the third (T-27 d) with 1,410 larvae tested, and 68 for the final collection (T-41 d) with 1,020 larvae tested. In total, 5,305 larvae were used in bioassays. When we compared adult eclosion in the bioassays using water from the treated and untreated sites, we detected a significantly lower eclosion rate in the treated sites than the untreated sites. Compared to the untreated control groups, there was a 57% reduction in adult eclosion rate at T-1 d, 51% at T-13 d, and 39% T-27 d. There was no significant difference in the adult eclosion rate at T-41 d (Fig. 2; Table 2). When we compared eclosion rates from water collected inside and outside crypts over the first three collections at treatment sites, adult eclosion rates were higher in water from inside the crypts, indicating less control. Overall, a higher level of control was observed from water outside compared to inside the crypts, with only 30% (± 2, $n = 112$) of adults eclosing from water outside the crypts compared with 62% (± 3, $n = 109$) from inside crypt treatments. This pattern was true for all three collection periods with methoprene treatment significantly reducing adult eclosion rates relative to the untreated sites (Fig. 3; Table 3).

In total, 1,080 fourth instar *Cs. melanura* larvae were used to construct the methoprene susceptibility curve. The results of the probit analysis (df = 70, $t = 25$, $P < 0.001$) and LC values are presented in Table 4.

Discussion

The results of our efficacy bioassays and chemical analyses suggest that an aerial granular methoprene application can effectively treat open water but does not sufficiently penetrate the protected and cryptic *Cs. melanura* larval habitats, highlighting the difficulty in controlling this enzootic EEEV vector. The LC-95 methoprene concentration for *Cs. melanura* was 1.95 ppb. The average methoprene concentration in the water collected from outside of treated crypts was 1.79 ppb, demonstrating that methoprene levels were likely high enough to inhibit *Cs. melanura* development in these open water habitats. Compared against the untreated sites, we observed a 61% reduction in adult eclosion rates in bioassays containing treated water from outside the crypts. In contrast, excepting one high outlier value, the average methoprene concentration of the water from inside treated crypts was lower at 0.1 ppb. There was only a 28% reduction in adult eclosion rates in this water compared against the untreated water. Eclosion rates were low in some bioassays containing

treated crypt water and high in others. Very low concentrations of methoprene were detected inside the crypts, with those in treatment site 3 being the highest throughout the study period (Table 1.) This may be due to uneven penetration of methoprene into the crypts across the treatment area, as has been observed previously (Hayes 1962, Woodrow et al. 1995). Based upon methoprene concentrations observed outside crypts, aerial granular methoprene application may be effective in controlling species that inhabit open water but does not appear to be efficacious in treating protected *Cs. melanura* larval habitats during dry conditions.

In a previous study, Woodrow et al. (1995) also detected uneven treatment of the crypt water. Measurable concentrations were found in two of the 10 crypts they sampled, and residue was detected in an additional six. Despite the patchy distribution of methoprene in the crypts, they recorded a 58% reduction in adult eclosion compared against their untreated control. We can attribute this to the high levels of methoprene detected in their samples (20.9 to 233 ppb). The observed rate of methoprene applied was also estimated to be 2.92 ppb (Woodrow et al. 1995), a concentration 67% higher than the LC-95 that was determined from our probit analysis. In their study, wild larvae were collected from crypts accessible from swamp edges, which may have allowed for better crypt penetration due to reduced vegetative cover. We had relatively consistent sampling across collections and our crypts were under relatively dense coniferous vegetation. In both our study and Woodrow et al. (1995), the sampling locations were spatially clustered along access routes (Fig. 1). A broader collection within the target area may reveal whether vegetation affects the treatment of crypts. Collections on some dates by Woodrow et al. (1995) yielded low sample sizes, with only three larvae collected in their final collection in June. We had relatively consistent sampling across collections, but water levels were also low due to dry conditions (NOAA 2020). This may have isolated the water inside the crypts from the open water, ultimately reducing the capacity of methoprene to leach into the crypts. The effect of drought and crypt location are important to consider in efficacy evaluations for the field control of *Cs. melanura* moving forward.

Few studies have investigated the susceptibility of *Cs. melanura* to insecticidal active ingredients. To understand the effect of methoprene on this species, we created the first susceptibility curve for this species, using a colony that has been maintained without pesticide exposure since 1994. Our bioassay results provide evidence that methoprene can be an effective tool for the control of this species. Comparing the susceptibility of *Cs. melanura* to other common control targets, this species falls between the susceptibilities exhibited by *Culex pipiens* L. and *Aedes albopictus* (Skuse) (Burtis et al. 2020). Methoprene can be deployed to effectively control these other mosquito species (Knepper et al. 1992, Bibbs et al. 2016), indicating that methoprene is a good choice for *Cs. melanura* control at face value. However, isolated crypts may be difficult to penetrate with any aerially applied pesticide, potentially resulting in control failures. Other persistent and stable methoprene formulations should be tested in the future to determine if they more effectively penetrate the crypts.

Difficulty in entering cedar swamps also greatly limits the capacity to distribute larvicide by hand in this environment. Other formulations could be explored that may more effectively penetrate the crypts. A small-scale aerial application in Norfolk, MA, concurrent with ours,

deployed an alternative pellet methoprene formulation (MetaLarv SP-T) via helicopter at 11.2 kg/ha, which was twice the rate of our application (5.6 kg/ha). The resulting average methoprene concentration inside crypts was approximately twofold higher than what we detected (K. O'Donnell, personal communication). The average methoprene concentration detected inside the Norfolk crypts (0.15 ± 0.03 ppb) was still below the LC-95 for *Cs. melanura* (1.95 ppb). Higher application rates also significantly increase costs and may not be scalable to cover larger areas. Ultimately, more research is needed to test and determine effective deployment methods and pesticide formulations to target the habitats inhabited by *Cs. melanura* larvae.

Our spring aerial application of granular methoprene targeting the Hockomock Swamp was unlikely to control *Cs. melanura* populations due to the lack of penetration into larval habitats. The results of our bioassays, along with chemical analyses of the crypt water, show that *Cs. melanura* is susceptible to methoprene but an alternative application approach is required. Aerial application during a wet year may increase efficacy, although confirmation of this effect requires robust evaluation. Control and prevention of EEEV outbreaks remains difficult, in part because the domestic and sylvatic vector species are not well-studied. Although we have demonstrated that methoprene is an effective active ingredient for the control of *Cs. melanura*, aerial methoprene and likely other larvicide applications, may not be an optimal investment for vector control districts seeking to reduce immature populations of *Cs. melanura* and, ultimately, EEEV transmission risk later in the season.

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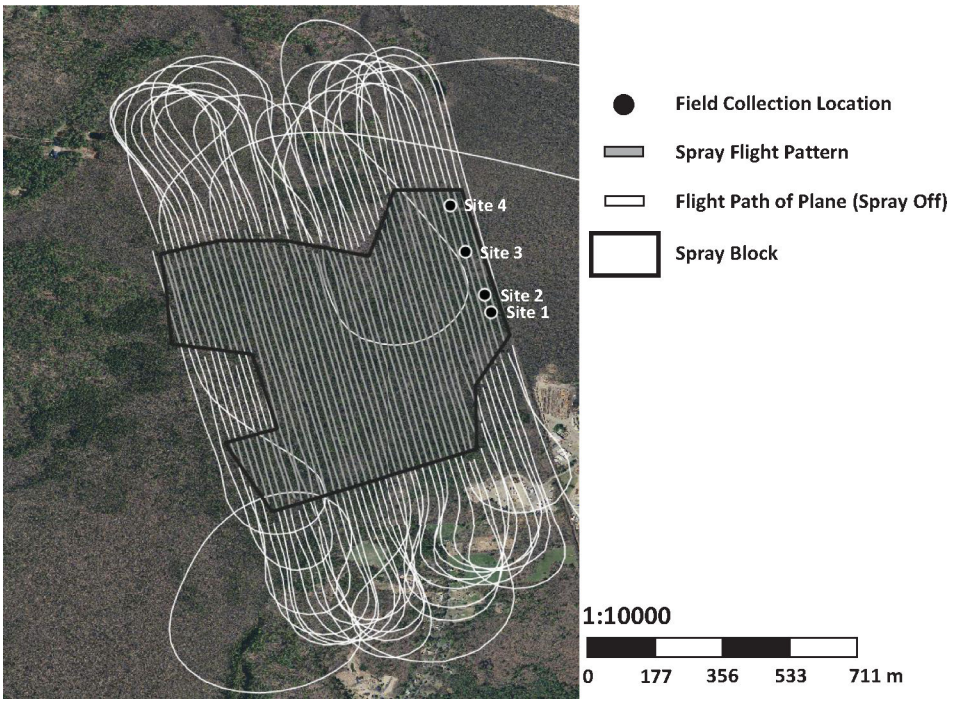

Fig. 1.

The flight path during the aerial applications in Hockomock Swamp in Massachusetts. The black outlined area is the application area, and the flight path is shown in gray (applicator on) and white (applicator off). The four field collection sites within the swamp are highlighted by as points on the map and the site numbers correspond to those on Table 1. The Orthoimages are from 2013 and were downloaded from the USGS Earth Explorer database.

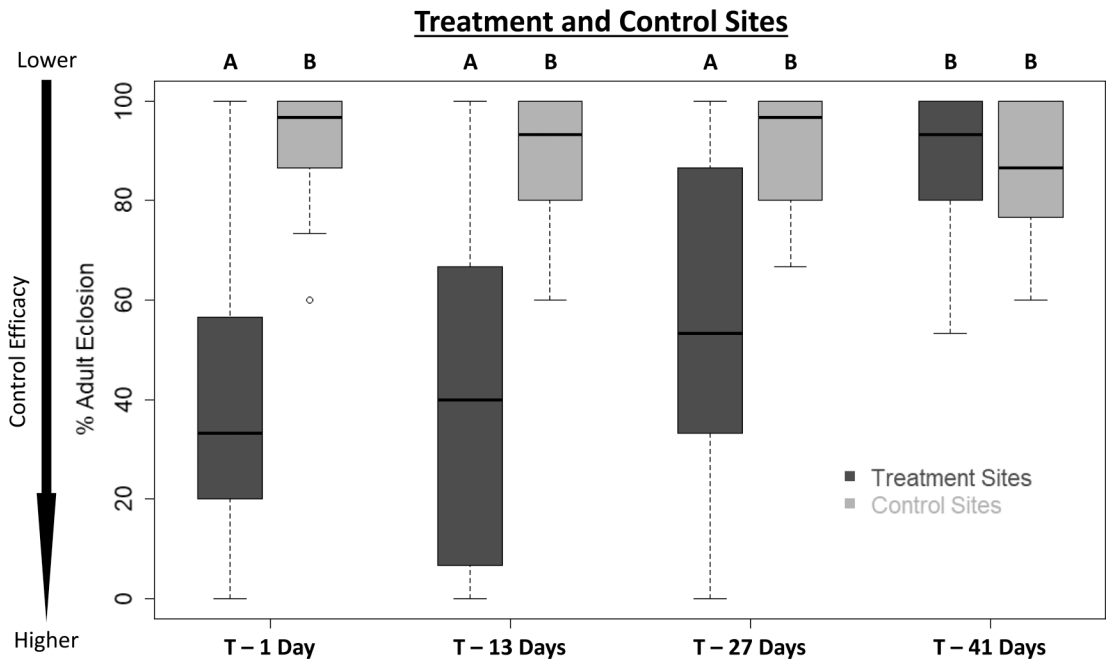

Fig. 2.

Boxplots showing the median and interquartile range, comparing the percentage of adults that eclosed in water collected from treated sites (dark gray) and the control sites (light gray) over the four field collections (T = treatment – days) following the aerial application. The letters above the boxes indicate significance according to a post hoc Tukey's test. Those groups with different letters are significantly different from one another ($P < 0.05$).

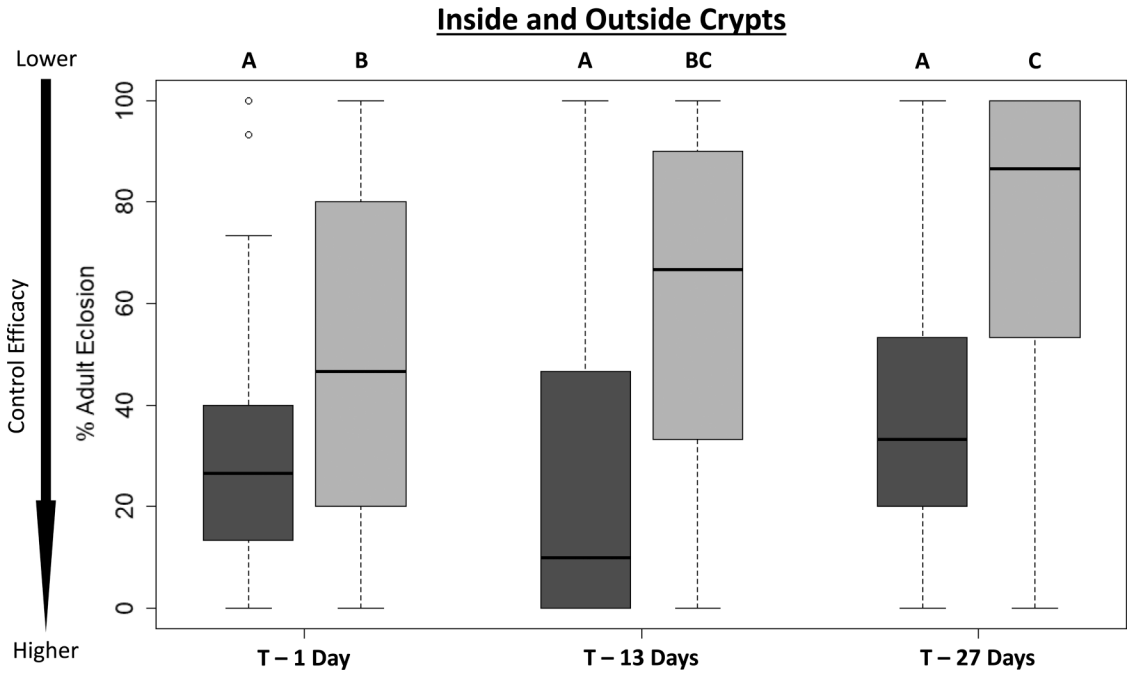

Fig. 3.

Boxplots showing the median and interquartile range, comparing the percentage of adults that eclosed in water collected (T = treatment – days) from outside (dark gray) and inside (light gray) the crypts. The letters above the boxes indicate significance according to a post hoc Tukey's test. Those groups with different letters are significantly different from one another ($P < 0.05$). Only the three collections wherein the treatment had a significant effect were included in this analysis.

Table 1.

Analytical chemistry results from the Massachusetts Pesticide Analysis Laboratory

Site	Crypt	Day 5	Day 12	Day 19	Day 26	Day 33	Day 40
Treat 1	Inside	0*	0	0	0	0	0
	Outside	0.009	1.04	0.96	1.27	0.67	0.14
Treat 2	Inside	0	0	0	0	0	0
	Outside	0.01	0.05	1.05	6.95	0.44	0.14
Treat 3	Inside	0.81	0.68	198	0.24	0.43	0.08
	Outside	0.75	1.06	3.13	1.59	0.52	5
Treat 4	Inside	0	0	0.024	0	0.01	0
	Outside	0.127	5.57	4.84	3.3	1.57	2.71
Ctrl 1	Inside	0	0	0	0	0	0
	Outside	0	0	0	0	0	0
Ctrl 2	Inside	0	0	0	0	0	0
	Outside	0	0	0	0	0	0

Methoprene concentrations are presented in ppb. Methoprene was not detected in the treatment sites in tests prior to the application.

*Zero values are below the detection limit of 0.004 ppb.

Table 2.

Results of the binomial model used to determine the effect of methoprene treatment on the ecolsion of *Cs. melanura* adults in the bioassays

Parameters	df	*F*-value	*P*-value
Treatment	1, 341	106.7	<0.001
Days since treatment	3, 341	27.2	<0.001
Interaction (treatment + days since treatment)	3, 341	12.4	<0.001

Table 3.

Results of the binomial model used to determine the effect of methoprene treatment on the eclosion of *Cs. melanura* adults in water collected from inside and outside of the crypts

Parameters	**df**	***F*-value**	***P*-value**
Crypt (in/out)	1, 215	66.7	<0.001
Days since treatment	3, 215	6.4	0.002
Interaction (crypt + days since treatment)	3, 215	2.3	0.107

Table 4.

Results of probit analysis for the *Cs. melanura* susceptibility curve for methoprene

	Replicates per concentration	df	Slope (SE)	LC-50 (± 95% CI)	LC-95 (± 95% CI)	LC-99 (± 95% CI)	χ^2
Methoprene	9	70	4.75 (0.2)	0.072 (± 0.01)	1.95 (± 0.5)	7.69 (± 2.5)	623

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