NATULAR™ G AND NATULAR™ G30 AERIAL LARVICIDE INTERVENTIONS IN CENTRAL MASSACHUSETTS 2021 UPDATE

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ABSTRACT

Following unparalleled Eastern Equine encephalitis levels in central Massachusetts during the summer of 2019, the Central Massachusetts Mosquito Control Project was directed to address the potential for a similar situation in 2020. Through discussions with other mosquito control districts, as well as Commonwealth officials, it was decided that expanded larval control for both *Coquillettidia perturbans* and *Culiseta melanura* was the appropriate course of action. Two different formulations of spinosad were chosen, one for each species and their specific larval habitat. Natular™ G30 was selected for *Cs. melanura* crypt habitats, while Natular™ G was designated for *Cq. perturbans* emergent vegetation habitats. After proceeding with the application in 2020, it was determined that a narrowed version of this operation would be planned for 2021. Following the Natular™ G30 application for *Cs. melanura*, water samples were collected from the target areas for larval bioassays and to determine whether the granular product had reached the isolated larval habitat. Once the application of Natular™ G was conducted for *Cq. perturbans*, emergence traps and larval surveillance was conducted within the treatment area and a neighboring untreated area to observe the relative efficacy of the operation.

BACKGROUND

In 2019 Massachusetts experienced extraordinary levels of Eastern Equine encephalitis in the local mosquito population, leading to numerous cases of human infection. For CMMCP specifically, the 2019 season resulted in twelve district communities to be categorized as being of “Critical” risk for EEE infection, with another eleven as “High” risk by the Massachusetts Department of Public Health (MDPH 2019). Following the season, Commonwealth officials gathered and discussed possible interventions to reduce the potential for another year of human infection. It was determined that early season aerial larvicide operations in these “Critical” and “High” risk communities would most appropriately address two important mosquito vectors of EEE.

The two specific mosquito species targeted in this operation, both considered to be significant factors in EEE amplification and transmission, are *Cs. melanura* and *Cq. perturbans*. *Cs. melanura* overwinters as larvae in very specialized habitats, the root systems of white cedar and red maple swamps. These “crypts” are traditionally difficult to treat due to their protective structure. This species has been indicated primarily as an amplification vector of EEE, contributing increasing virus levels within the local avian population (Andreadis 2005).

Emerging in significant numbers every season in central Massachusetts, *Cq. perturbans* is another unique mosquito species. Overwintering as larvae, this single generation species attaches themselves to the root systems of emergent vegetation, breathing through it using a specialized siphon tube (Andreadis 2005). This special larval characteristic of this species creates difficulty when trying to apply traditional control measures because they do not have to surface to obtain air (Johnson 2017). Being a somewhat indiscriminate feeder, and long lived as an adult, *Cq. perturbans* have been implicated as a potential transmission vector of EEE (Andreadis 2005). This pestiferous species may acquire EEE from infected birds and later transmit it to "dead end" hosts such as humans or horses.

With these target species identified, CMMCP staff decided to use the active ingredient spinosad to reduce adult emergence. Created from the fermentation of the soil bacteria *Saccharopolyspora spinosa*, spinosad has been shown to control developing mosquito larvae. Natular® G and Natular™ G30 are currently available commercial formulations of spinsoad. The Environmental Protection Agency has identified spinosad as a "Reduced Risk" pesticide and both of these commercial products are listed by OMRI (Organic Materials Review Institute) as certified organic pesticides (CMMCP 2021). Although Clarke Mosquito Control Products, Inc., has designed Natular® G to release immediately, Natular™ G30 has been formulated for granules to provide larval control for up to 30 days, as implied by the product name. Natular® G could be used on *Cq. perturbans* larvae and their open habitats, while Natular™ G30 would be better utilized in and around the protected crypt habitat of *Cs. melanura*.

In 2020, with the assistance of North Fork Helicopters (Cutchogue, NY), CMMCP was able to treat approximately 551 acres of *Cs. melanura* habitat with Natular™ G30 in six CMMCP member communities. Another 1937.5 acres of *Cq. perturbans* habitat was treated with Natular™ G in twenty-one CMMCP member communities. After this successful operation, it was determined that these aerial larvicide interventions would continue in the 2021 season, but focus only on towns that were designated as "Critical" EEE risk at the end of 2019 season. Methodology would be similar to 2020, with the addition of *Cq. perturbans* larval surveillance to help observe the impact of Natular™ G treatments.

MATERIALS & METHODS

Attention in 2021 was focused solely on the 12 CMMCP member communities of "Critical" EEE level designation at the end of the 2019 season by the Massachusetts Department of Public Health. Target sites in these towns from the 2020 operation were used as a template. Once again potential targets over 5 acers were included in these operations, while any suitable habitat under 5 acres were held for potential ground treatment by CMMCP staff.

After application targets for 2021 were prepared for the aerial contractors, on May 25th, Natular™ G30 was applied to 538 acres of *Cs. melanura* habitat in five CMMCP member communities. Following the Natular™ G30 portion of the operation, 1526 acres of *Cq. perturbans* habitat were treated with

Natular™ G in twelve CMMCP member communities from May 25th to May 27th (Appendix 1). Both of these applications were conducted at a rate of 10lbs/acre by North Fork Helicopters, with CMMCP providing ground support.

Within 24 hours of the Natular™ G30 applications, water sampling was conducted by CMMCP inside and outside around *Cs. melanura* crypts, at multiple treated areas. These weekly water samples were analyzed for spinosad concentration and with additional samples used in larval bioassays. Sampling from inside the crypts and right outside was performed to help determine whether or not the granular product reached the isolated habitat of the *Cs. melanura* larvae directly or potentially through the crypt substrate. The larval bioassays also allowed for more direct evidence of whether the spinosad concentration was at lethal levels for the larvae. Water analysis for spinosad concentration was conducted by the MA Pesticide Analysis Laboratory in Amherst, MA, with the larval bioassays being conducted at Cornell University.

Following the aerial application of Natular™ G, adult *Cq. perturbans* emergence traps were placed in a treated and untreated "control" area to attempt to gauge the effectiveness of the operation (Appendix 2). Effort was made to sample from these emergence traps weekly until collections of new adult *Cq. perturbans* specimens ceased. Comparing the adult emergence collections from the treated areas to the untreated area would help indicate the level of control achieved from the aerial application of Natular™ G. Larval sampling of *Cq. perturbans* also occurred at these sites and was performed twice a week to assist evaluating the treatments (Appendix 3).

2021 RESULTS

Weekly water analysis conducted after the application of Natular™ G30 showed that spinosad was able to be delivered outside of the *Cs. melanura* crypts in greater concentration than inside. However, this level peaked at approximately 6 ppb, and proceeded to decrease over time. Spinosad had very little penetration into the crypts themselves from either direct application of the granules or through the crypt substrate over time. As with the spinosad concentration outside the crypts, this level also decreased the further away from the application date (Figure 1).

Figure 1: Spinosad Concentrations Inside and Outside of Crypts

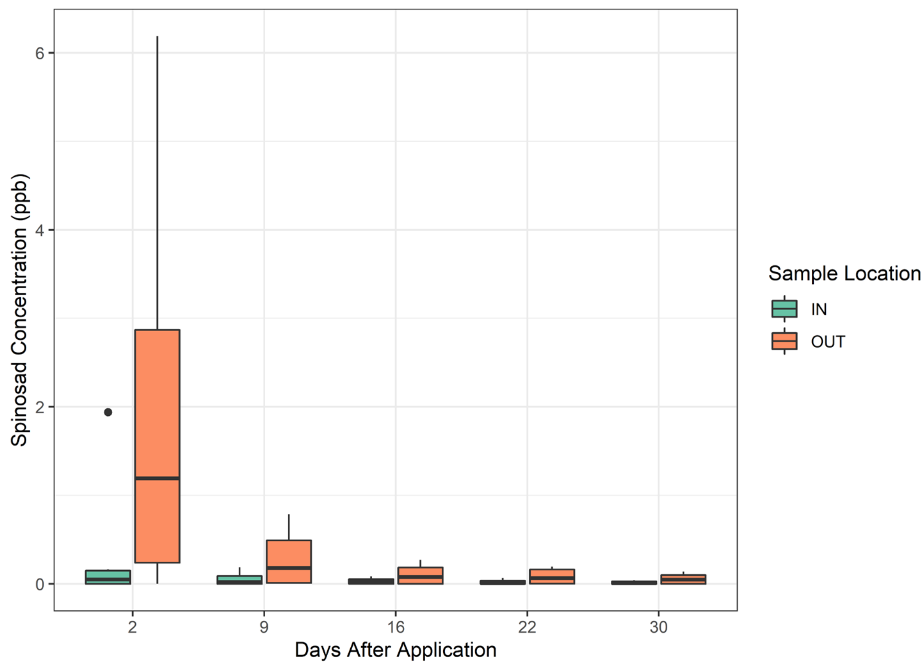
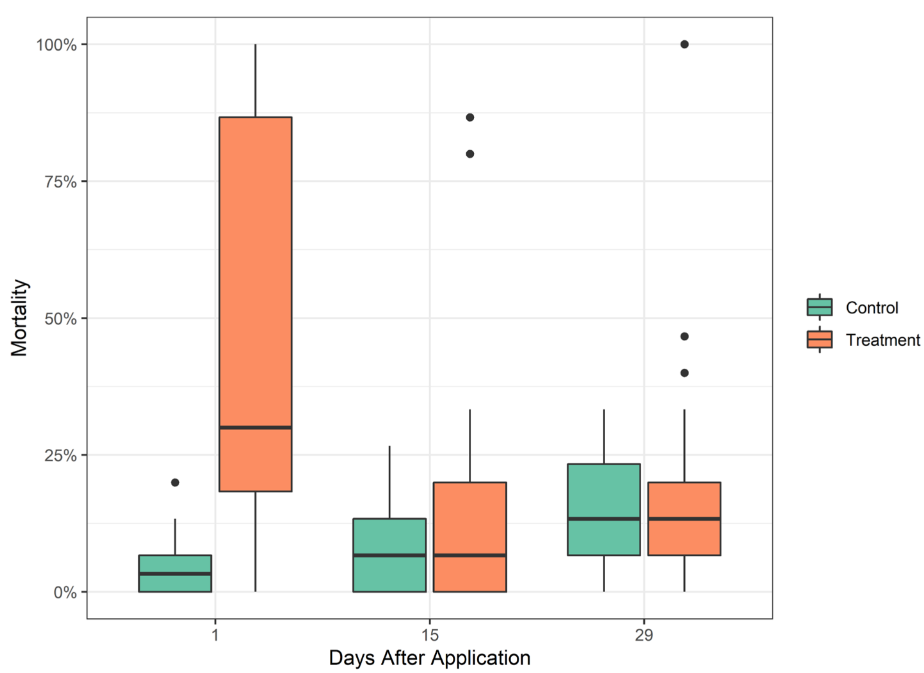


Figure 2: Larval Bioassays from Natular™ G30 Application and Control Samples



Water samples from inside and outside of the flagged crypts, obtained within 24 hours, 2 weeks later, and lastly at 4 weeks after the initial sampling, were used in larval bioassays. Compensating for the larval mortality of control samples, these bioassays indicated some control, but primarily from outside the crypts. As with the spinosad concentration around the crypts, mortality achieved also decreased over time (Figure 3).

Figure 3: Larval Bioassays from Natular™ G30 Application Between Crypt Types

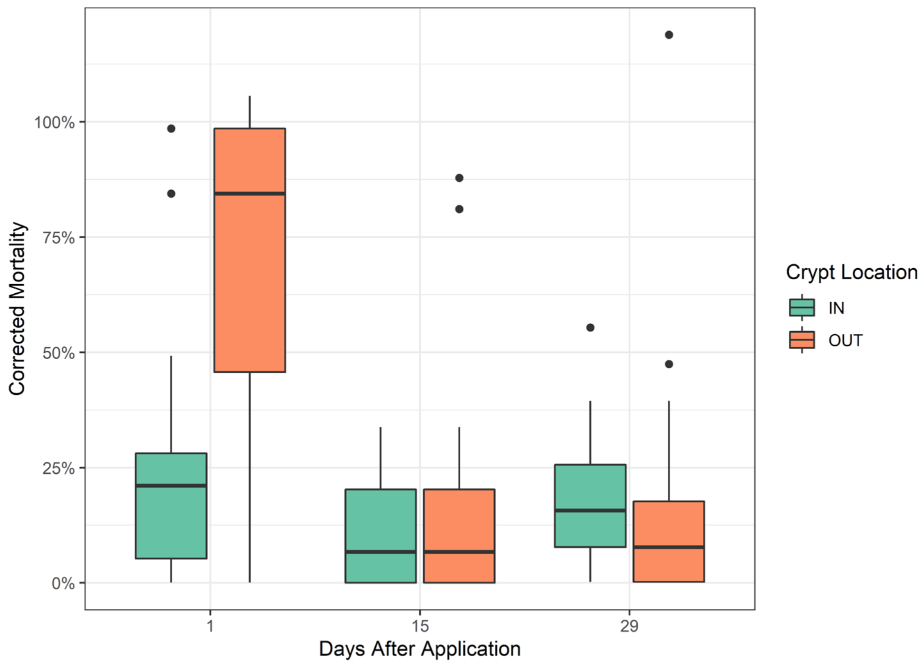
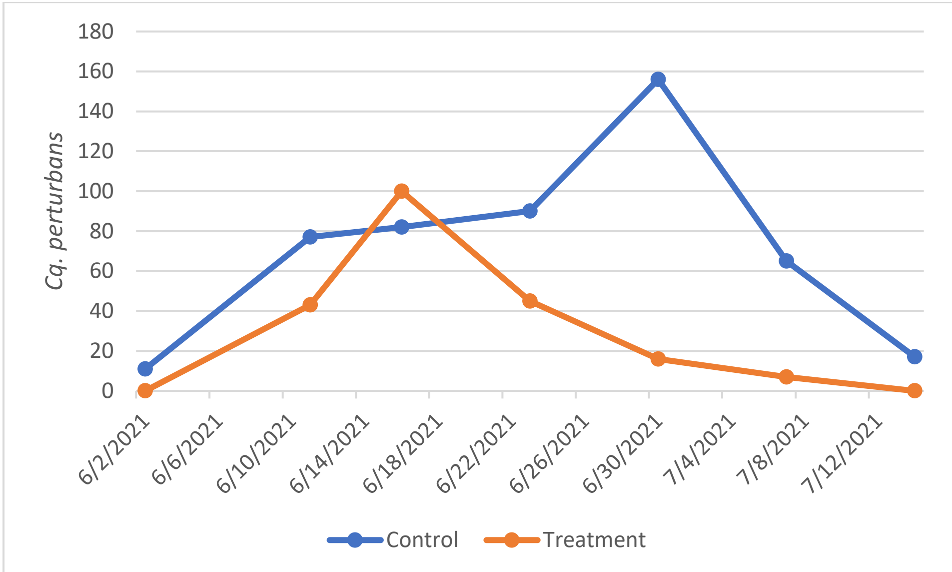
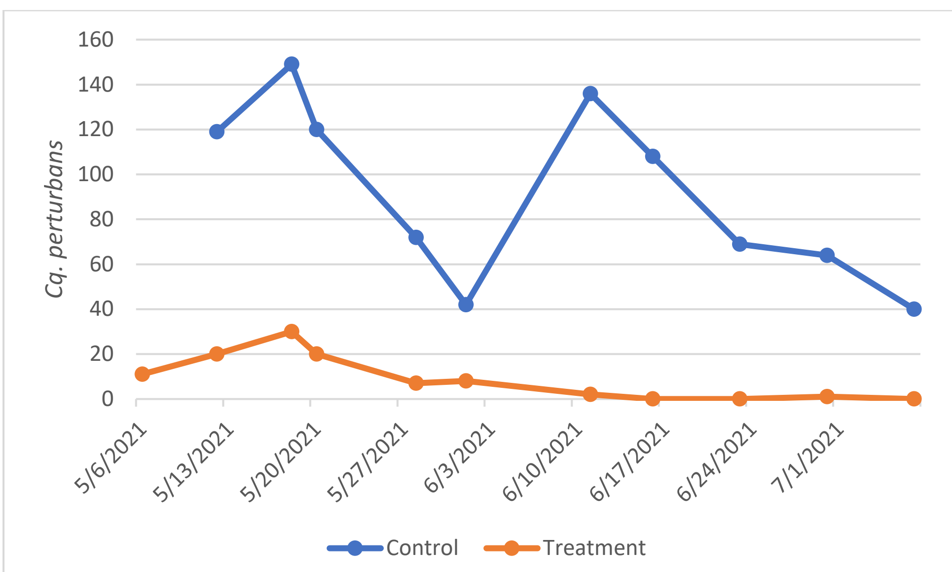


Figure 3: *Cq. perturbans* Emergence Trap Collections



Cq. perturbans larvae sampling began in early May prior to the applications of Natular™ G. These observations continued twice a week until conditions prevented routine surveillance. The untreated "control" area produced significantly more *Cq. perturbans* than the area treated with Natular™ G before advanced stage larvae approached adult emergence. Larvae levels in the treated area remained depressed following the May 25th-27th application (Figure 4).

Figure 4: *Cq. perturbans* Larval Sampling



DISCUSSION

The water sampling around *Cs. melanura* habitats showed that the aerial applications of spinosad were successful in achieving lethal concentrations of active ingredient outside the larval crypts, but this potential was not present after the initial collection. A significantly lower concentration of spinosad was able to be detected directly inside the crypts, and the corresponding larval bioassays had a much lower mortality rate than the samples from outside the crypts had as well. It does not appear that spinosad in this formulation, applied at this rate, can successfully penetrate the crypt systems of *Cs. melanura*. Regardless, the impact of the Natular™ G30 decreases relatively quickly outside the crypts as well.

The *Cq. perturbans* emergence trap surveillance indicate that the Natular™ G treatments impacted the larvae present and reduced the adult hatch. The emergence trap from the untreated "control" area produced adult specimens with a traditional *Cq. perturbans* population curve, whereas the emergence trap from treated locations was significantly lower and did not produce a similar curve. The larval surveillance of *Cq. perturbans* also indicated that the Natular™ G treatments impacted the larvae present and reduced the adult hatch. The untreated "control" area produced significantly more *Cq. perturbans* larvae until adult emergence started to peak. Additional emergence trap collections and larval samples would have been taken but the tremendous rainfall experienced during the season eventually made safe sampling impossible.

If these Natular™ G and Natular™ G30 applications are conducted next year, similar monitoring can take place to further evaluate the impact these treatments have on *Cq. perturbans* and *Cs. melanura*. If these aerial interventions do not continue, resources from the *Cq. perturbans* adult and larval surveillance can be utilized to evaluate ground applications of Natular™ G to their emergent vegetation habitats.

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APPENDIX

Appendix 1: Acres Treated by Town and Product

Town	**Natular G30** ***(Cs. melanura)***	**Natular G** ***(Cq. perturbans)***
Ashland		207
Grafton		101
Holliston	55	249
Hopedale		17
Hopkinton	32	146
Marlborough		145
Milford		150.5
Northborough		217
Northbridge	8	83
Shrewsbury	10	71
Southborough		50
Westborough	433	90
Total	**538**	**1526**

Appendix 2: *Cq. perturbans* Adult Emergence Trap

Appendix 3: *Cq. perturbans* Larvae Sampling