NATULAR™ G AND NATULAR™ G30 AERIAL LARVICIDE INTERVENTIONS IN CENTRAL MASSACHUSETTS- 2020

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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. Between the two applications types, approximately twenty-five hundred acres were treated over 21 CMMCP member communities. Following the Natular™ G30 application for *Cs. melanura*, water samples were collected from the target areas 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 were placed 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 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*.

MATERIALS & METHODS

As this was a completely novel operation, target sites needed to be identified, field verified, and projected into suitable formats for the aerial application contractors, North Fork Helicopters (Cutchogue, NY). Using several different GIS programs, CMMCP staff proceeded to identify potential habitat for field technicians to verify as suitable for treatment. Attention was focused in all 12 CMMCP member communities of "Critical" EEE level designation at the end of the 2019 season, and another 9 categorized as "High" risk by the Massachusetts Department of Public Health. 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.

With all the targets identified, verified, and prepared for the aerial contractors, on May 26th, Natular™ G30 was applied to 551 acres of *Cs. melanura* habitat in six CMMCP member communities. With the Natular™ G30 portion of the operation complete, on May 31st and June 1st, 1937.5 acres of *Cq. perturbans* habitat were treated with Natular™ G in twenty-one CMMCP member communities (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 two treated locations and one untreated (control) area to attempt to gauge the effectiveness of the operation (Appendix 2). Effort was made to sample from these emergence traps twice a week until collections of new adult *Cq. perturbans* 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.

RESULTS

Weekly water analysis conducted prior to and following the application of Natular™ G30 showed that spinosad was successfully able to be delivered outside of the *Cs. melanura* crypts, but decreased over time. Conversely, spinosad had very little penetration into the crypts themselves from either direct application of the granules or through the crypt substrate over time. These results were relatively similar for both Site 1 and Site 2 (Figure 1; Figure 2).

Figure 1: Spinosad Concentrations Inside and Outside of Site 1 Crypt

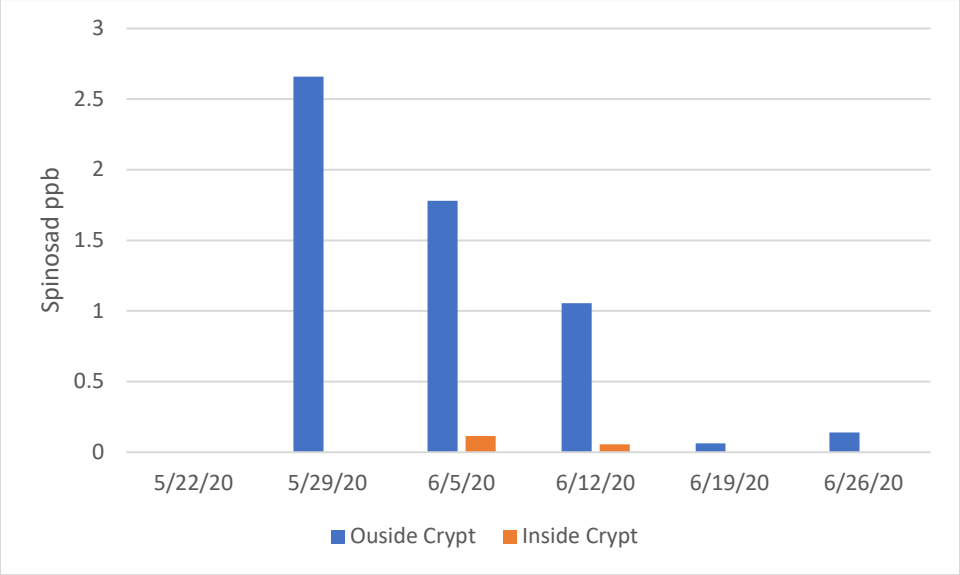
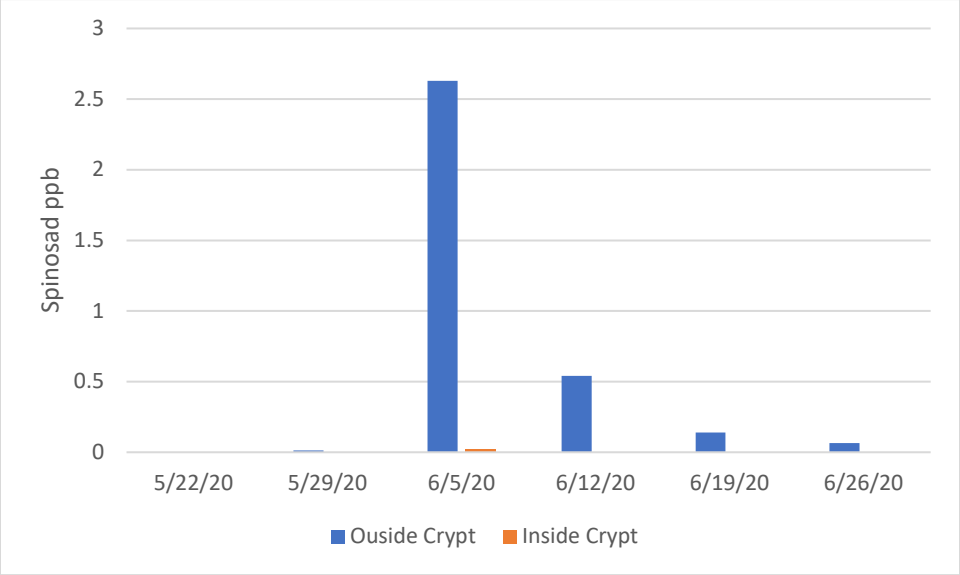
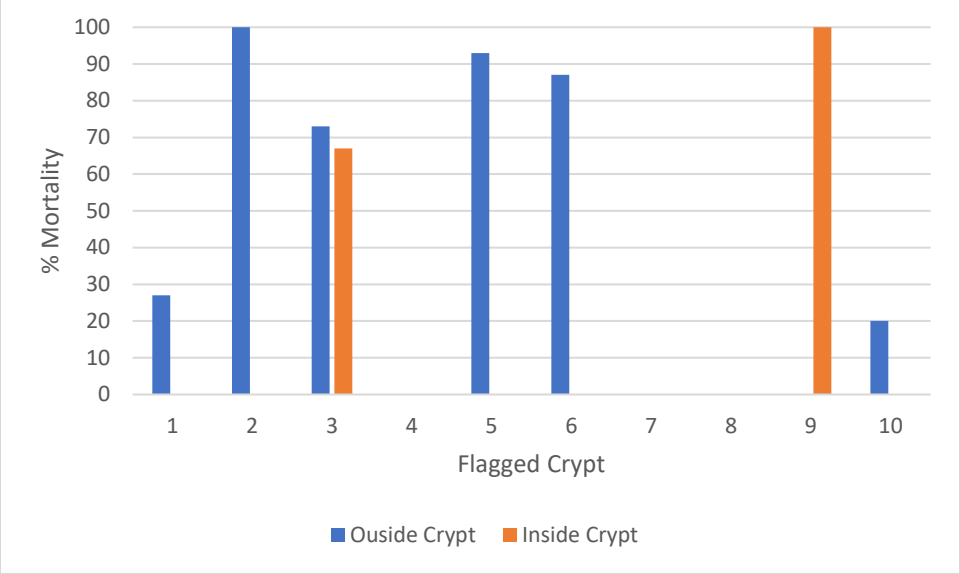


Figure 2: Spinosad Concentrations Inside and Outside of Site 2 Crypt



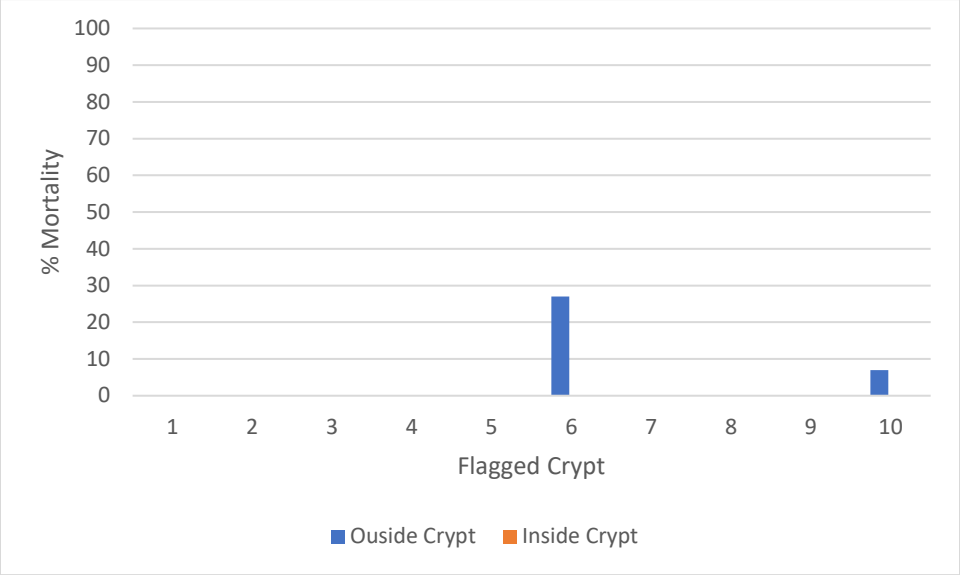
Water samples from inside and outside of the flagged crypts, obtained within 24 hours of the application, were used in larval bioassays and indicated some control, but primarily from outside the crypts. Both the outside and inside crypt samples ranged from 100% mortality to 00.0%, but the bioassays using the samples from the outside averaged 40.0% larval mortality compared to just 16.7% for the inside crypt samples (Figure 3).

Figure 3: Larval Bioassays from Natular™ G30 Application Samples 5.26.2020



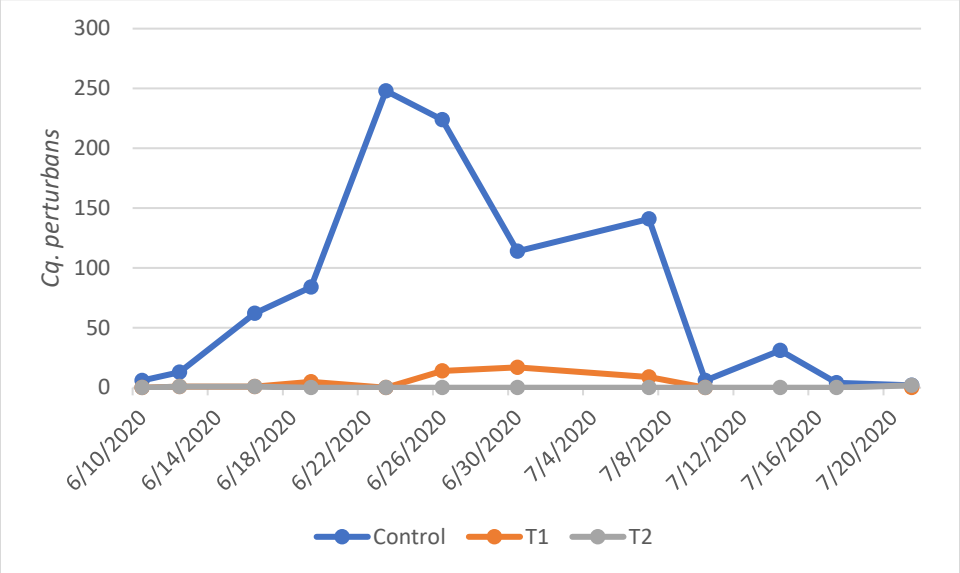
Water samples from inside and outside of the flagged crypts, obtained 2 weeks after the application, were used in larval bioassays and indicated very little control, both outside and inside of the crypts. The bioassays using the samples from the outside averaged just 3.40% larval mortality compared to 00.0% for the inside crypt samples (Figure 4).

Figure 4: Larval Bioassays from Natular™ G30 Application Samples 6.9.2020



There were twelve emergence trap collections made at each of the three emergence trap sites. The first *Cq. perturbans* emergence trap collection was on June 10th, 2020 with the final collections occurring on July 21st. At this point there were not enough newly emerging adults to support further collections. The emergence trap placed in the untreated "control" area produced a traditional *Cq. perturbans* population curve, while the two emergence traps placed in areas treated with Natular™ G, had small, sporadic emergence during the collection period. These figures were significantly lower than those in the untreated emergence trap (Figure 5).

Figure 5: *Cq. perturbans* Emergence Trap Collections



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 decreased weekly. 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. All the sampling was conducted under worsening drought conditions, and so the lack of water in and around the crypts may have influenced the absence of active ingredient detected. The act of obtaining samples itself was difficult due to this lack of water around the sampling sites.

Results from the *Cq. perturbans* emergence traps 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 through a traditional *Cq. perturbans* population curve, whereas the two emergence traps from treated locations were significantly lower and did not produce similar curves. If larval surveillance of *Cq. perturbans* could be achieved it would help evaluate these control measures in addition to the emergence traps already being utilized. A similar operation to reduce *Cs. melanura* and *Cq. perturbans* and the risk for EEE may be performed in 2021. Further evaluation of the potential for this type of product against these vectors would be beneficial to the mosquito control community at large. Ample water in and around the *Cs. melanura* crypt habitat may help reveal whether or not spinosad can penetrate and effectively control the larvae present.

REFERENCES

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APPENDIX

Appendix 1: Acres Treated by Town and Product

Town	Natular G30 *(Cs. melanura)*	Natular G *(Cq. perturbans)*
Ashland		207
Auburn		27
Boylston		10
Grafton		101.5
Holliston	55	249.5
Hopedale		17
Hopkinton	32.5	146
Hudson		75
Marlborough		145
Milford		150.5
Millbury		55
Millville		12
Northborough		217.5
Northbridge	8	83
Sherborn		69
Shrewsbury	10	71
Southborough		50
Stow		30
Sturbridge	12.5	92
Webster		39
Westborough	433	90.5
Total	**551**	**1937.5**

Appendix 2: *Cq. perturbans* Adult Emergence Trap