Integrated pest management and BTi: The future of mosquito control in Hawai'i

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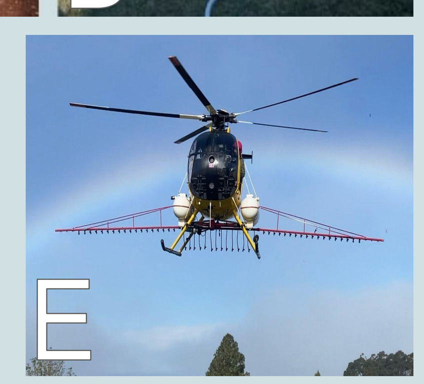
Background

Avian malaria (*Plasmodium relictum*) and its mosquito vector, *Culex quinquefasciatus* (*C. qqx*), are driving Hawaiian honeycreepers to extinction[1]. Mosquito control occurs around the world for diseases of human health concern, but it is seldomly used for conservation. *Bacillus thuringiensis israelensis* (BTi) and *Bacillus sphaericus* (Bs) are highly specific bacterial larvicides that have been used successfully around the world to control mosquitoes[2]. These products could be used to control *C. qqx* and reduce avian malaria transmission in Hawaii. **Our project tested the feasibility of aerially applying BTi in remote Hawaiian forests and BTi's ability to penetrate the forest canopy to reach potential *C. quinquefasciatus* larval pools.**

Methods

We aerially applied a combination of BTi (VectoBac, Valent Biosciences) and Bs (VectoLex, Valent Biosciences) to a remote forest on East Maui (Figs. 1-2). We used an open grassland (4,300 ft elevation) approximately 15.5 kilometers from the core of application area as our mixing site. All supplies, including water, were brought to the mixing site and mixing occurred the day prior to application.

Mixing and application process:



- **A)** At the mixing site, dry VectoLex product was suspended in water using 5 gallon buckets and **B)** combined with Vectorbac liquid in a 50 gal drum. In total, we combined 20 lbs of dry VectoLex product with 7.5 lbs of Vectobac liquid and water to create 40 gals of solution. **C)** The solution was circulated and pumped into a 275 gal tote.
- We repeated the mixing process 6 times to fill the 275 gal tote and 3 batches were made the morning of application for a total of 9-40 gal batches.
- **D)** We pumped the product using 50 ft hosing and a Honda WB20XT 2 in. water pump, **E)** into a spray rig attached to a MD 500 series helicopter.
- We applied 360 gallons of product in 5 rounds of up to 80 gallon loads. Applications occurred 5 times between October-December 2023

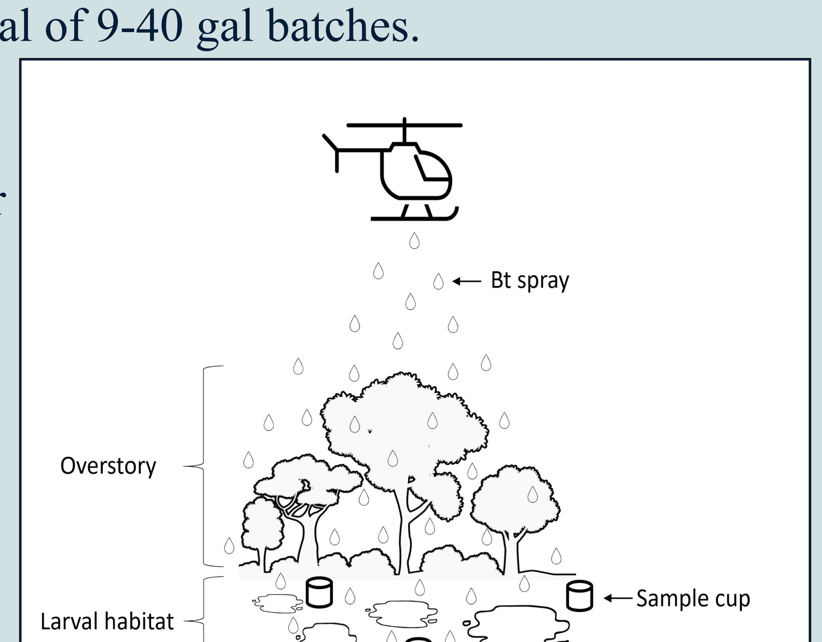


Fig. 1. Aerial Deployment model (Credit: Serena Zhao)

Study Area

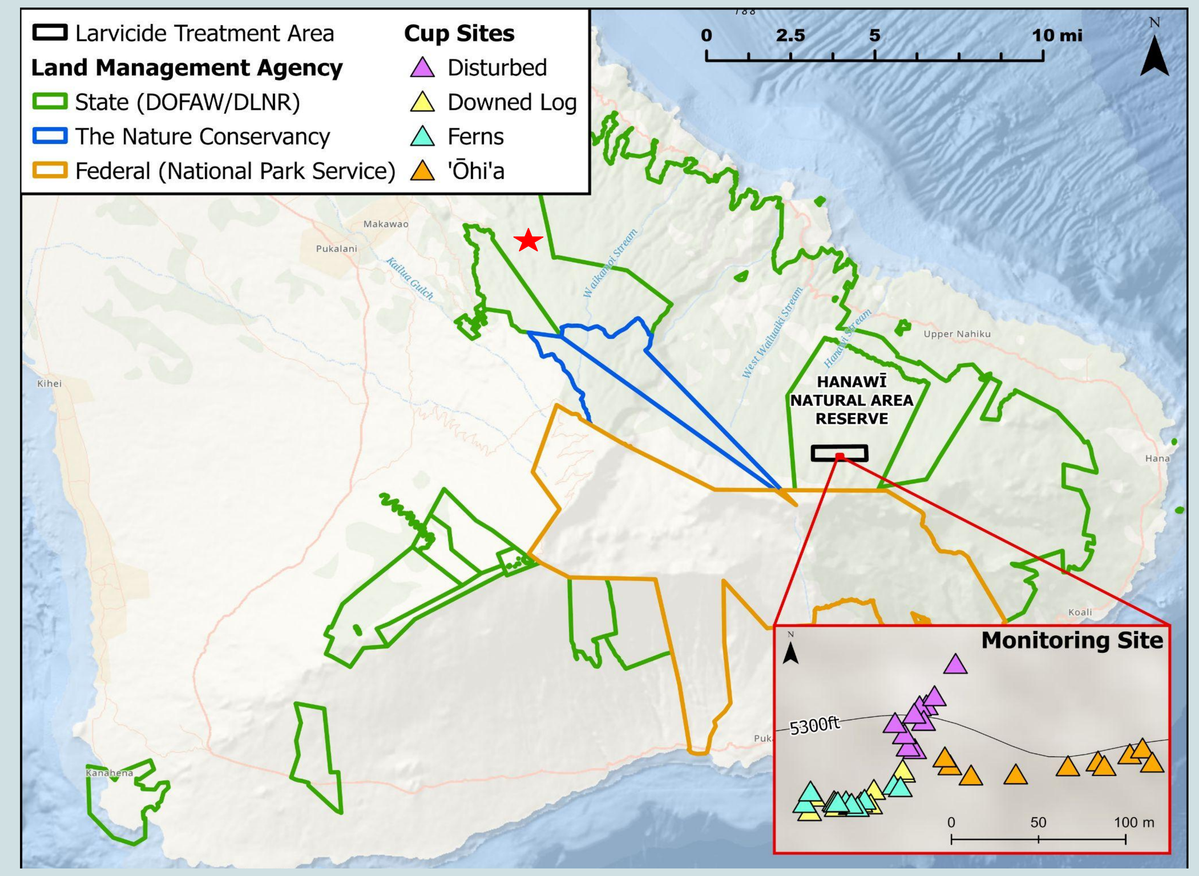


Fig. 2. Application area monitoring site within Hanawi Natural Area Reserve. Red star shows control field site.

BTi cup placement and larval rearing:

We used 6 oz. transparent plastic cups as proxies for larval breeding habitats and tested the extent of product reach into cups using bioassays measuring level of larval kill from 4 habitat types (ohia, ferns, down log, disturbed, Fig. 3). Cups were placed 24 hrs prior in the application area and a control field site and capped immediately following application (Fig. 2) and frozen until processing 1-4 days later. In a lab, 10 *C. qqx* larvae were added to each thawed cup with 100 mL of distilled water. Live larvae were counted at 24 hr, 48 hr, and 1 week intervals after exposure in Bti treated jar or control jars.

<u>Application area</u>: 40 BTi sample cups on 3 transects
<u>Control site</u>: 40 BTi sample cups placed in untreated field site.

Fig. 3. Examples of cup site types. 'Closed' means there are immediate obstructions between the cup and the sky (such as trees or plants), 'Open' means there is little or no immediate obstruction between the cup and the sky.

Results

In all of the applications, larvae in cups from the treated areas had higher mortality than larvae in the control cups (reference area and lab control; Figs. 4-5. Wilcoxon test: Maui 48h post exposure $p < 2.2e-16$, Maui 1 week post exposure $p < 2.2e-16$)

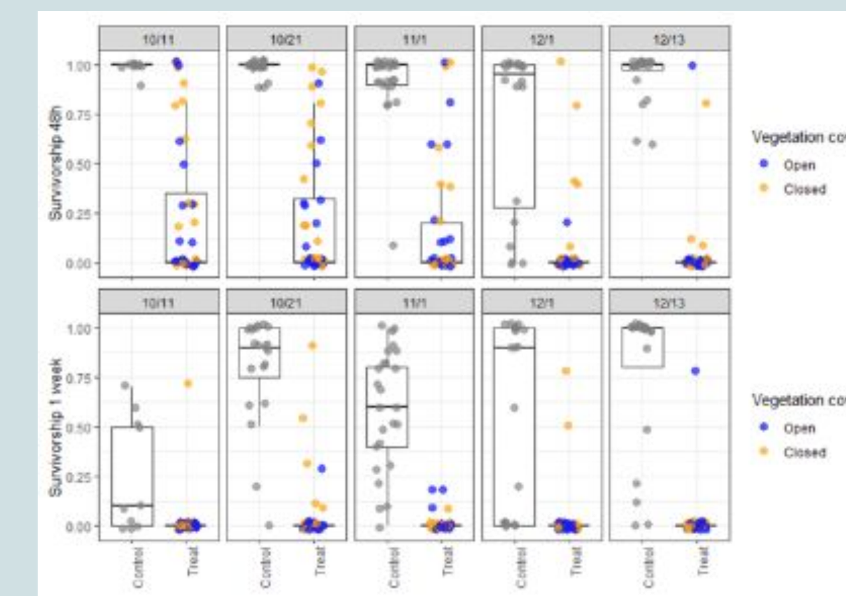


Fig. 4. This study investigates the survivorship of C. qqx larvae using habitat proxy cups in Maui, comparing "open" versus "closed" exposures over the five different applications. The "open" cups were fully exposed to the sky, while the "closed" cups were placed under vegetation cover. The results show the proportion of larvae alive after 48 hours (top panels) and one week (bottom panels) for each application date. The "Control" box plots include field cups from a reference area and lab control cups that were not exposed to the environment. The "Treatment" group includes cups placed in treatment areas.

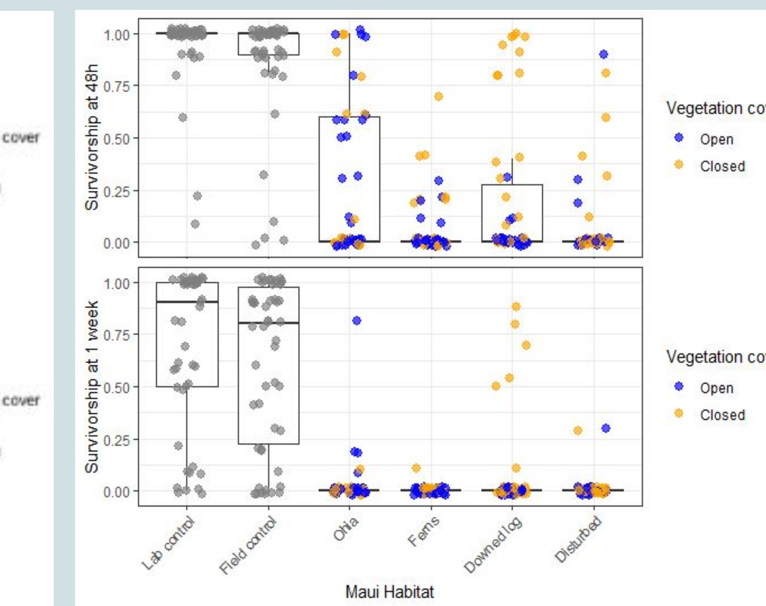


Fig 5. This study examines the survivorship of C. qqx larvae analyzing all habitat proxy cups in different habitat types in Maui. The proportion of larvae alive after 48 hours (top panels) and 1 week (bottom panels) was measured. "Open" cups were fully exposed to the sky, while "Closed" cups were placed under heavy vegetation cover. The "Lab control" box plots represent results from cups unexposed to the environment, and the "Field control" box plots represent results from cups placed in a reference area. The remaining boxplots show results from cups placed in various habitat types within the treatment areas.

Discussion

The results of the BTi applications show that BTi penetrated the forest canopy at levels capable of reducing larval survivorship. In the future, we will further analyze our data to determine whether BTi penetration differed significantly between cups placed in open vs closed vegetation cover. Future applications of BTi combined with mosquito population monitoring are set to occur in the near future on Maui to broaden the assessment of BTi's effectiveness in reducing mosquito populations.

The use of BTi builds on an integrated pest management approach and could complement on-going *Wolbachia* IIT (Incompatible Insect Technique) mosquito releases for conservation benefit in Hawai'i.

Some drawbacks of the mixing process is that it is time intensive, requires multiple 4x4 vehicles, and sufficient staffing with the appropriate helicopter safety training. Application occurs in remote field sites with limiting factors such as weather conditions, time constraints, and special equipment (spray boom).

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Citations: 1 Paxton EH, Laut M, Enomoto S, Bogardus M. Hawaiian forest bird conservation strategies for minimizing the risk of extinction: biological and biocultural considerations. Hawaii Cooperative Studies Unit Technical Report 103. 2022.
2 Lacey LA. Bacillus thuringiensis serovariety israelensis and Bacillus sphaericus for mosquito control. Journal of the American Mosquito Control Association. 2007;23(2suppl.):133–63.