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BRIEF REPORT



Acute toxicity of mosquito pesticides on weed biological control agents in south Florida, USA

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ABSTRACT

Mosquito pesticides effectively control vectors of human disease but have numerous unintended consequences. These may include adversely affecting non-target species such as weed biological control agents. Acute toxicity of two mosquito pesticides, naled and permethrin, was studied on three biological control agents released against invasive weeds in south Florida. These were the biological control agent *Oxyops vitiosa* of melaleuca, *Melaleuca quinquenervia*, the agent *Neomusotima conspurcatalis* for Old World climbing fern, *Lygodium microphyllum*, and the agent *Lilioceris cheni* for air potato *Dioscorea bulbifera*. We calculated LD₅₀ values for both pesticides on early and late instars for each herbivore species. The air potato herbivore, *L. cheni* was the most sensitive species tested and all three species were more sensitive to the pesticide permethrin than naled. These results indicate that even low concentrations of these products can have a detrimental effect on these weed biological control agents. The unintended consequences of mosquito adulticide applications should be considered when evaluating biological control agent impacts.

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Adult mosquitoes *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) are primary vectors of several human diseases including chikungunya, dengue, yellow fever, and Zika virus (Kraemer et al., 2015). These mosquito species occur throughout the Caribbean, southeastern US, and in many regions of the world (Kamal et al., 2019), where they can cause regional outbreaks of mosquito-borne diseases (<http://www.cdc.gov/zika/public-health-partners/vector-control-us.html>). The frequencies of these mosquito-borne disease outbreaks are projected to increase, rather than decrease, in the future (Kamal et al., 2019). Although mosquito adulticide applications may offer only temporary reduction of these populations (Fernandes et al., 2018; Stoddard, 2018), they are a common management response, especially in emergency situations against mosquito vectored human diseases (Fonseca et al., 2013). Applications can have a broad range of unintended consequences and may have severe impacts on non-target organisms both within (Milam et al., 2000) and adjacent to treatment areas (Hennessey et al., 1992; Salvato, 2001). Mosquito pesticides may have an adverse effect on federally listed endangered insects (Calhoun et al., 2002; Eliazar & Emmel, 1991; Zhong et al., 2010), non-listed insects

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(Bargar, 2012; Breidenbaugh & de Szalay, 2010; Hoang et al., 2011), and pollinators (Zhong et al., 2003; Zhong et al., 2004). However, the impact of mosquito pesticides on weed biological control agents is not well known.

Invasive weeds, a primary driver of ecosystem degradation, have wide ranging impacts on plant communities (Pyšek & Richardson, 2010). Invasive species contribute to the loss of native plant diversity, the health of natural areas, and agricultural production (Mack et al., 2000). Classical biological control seeks to decrease the impact of these invasive species by reuniting coevolved, host specific, control agents with their natural host in the invaded range. This technique may assist in reducing the aggressiveness of invasive weeds and may be integrated with other control methods (Van Driesche et al., 2010).

Here, we determined the acute toxicity of mosquito adulticides against non-target weed biological control agents. The agents included were those released for biological control of the broad-leaved paperbark tree, *Melaleuca quinquenervia* (Cav.) S. T. Blake (Myrtaceae), Old World climbing fern, *Lygodium microphyllum* (Cav.) R. Br (Lygodiaceae), and air potato or air yam, *Dioscorea bulbifera* L. (Dioscoreaceae). The three established biological control agents were *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae) to control *M. quinquenervia* (Center et al., 2012), *Neomusotima conspurcatalis* Warren (Lepidoptera: Crambidae) to control *L. microphyllum* (Boughton et al., 2012; Smith et al., 2014), and *Lilioceris cheni* Gressitt and Kimoto (Coleoptera: Chrysomelidae) to control *D. bulbifera* (Center et al., 2013; Overholt et al., 2016).

Two pesticides, permethrin and naled, are the most commonly used products to control adult mosquitoes in south Florida municipalities (www.miamidade.gov/global/solidwaste/mosquito/insecticides.page; www.cmcd.org/operations/control-materials/). Permethrin is a broad-spectrum pyrethroid with toxicity either by direct contact or by ingestion of treated leaves. Naled (or dibrom) is a broad-spectrum organophosphate insecticide. These products are typically applied as an ultra-low volume (ULV) mist from a backpack or truck-mounted sprayer, or by aerial treatments (<https://www.epa.gov/mosquitocontrol/controlling-adult-mosquitoes>). Due to widespread mosquito control activities conducted in south Florida, approximately 6 million acres were treated with naled in Florida in 2014 (<https://www.cdc.gov/zika/vector/aerial-spraying.html>), it is likely that weed biological control agents are exposed to these two products. Mosquito control applications are commonly made in urban and suburban neighbourhoods, areas that overlap broadly with the range of all three weed species and the agents released for their control (Center et al., 2012, 2013; Smith et al., 2014). Mosquito insecticide applications were considered a potential factor in the lack of establishment of *L. cheni* on *D. bulbifera* in two south Florida neighbourhoods (Overholt et al., 2016).

Weed biological control insects used in this study were maintained on live plants of their respective weed hosts *M. quinquenervia*, *L. microphyllum*, or *D. bulbifera*. *Neomusotima conspurcatalis* and *L. cheni* were obtained from laboratory colonies, while *O. vitiosa* was collected from garden plants. In all cases, the host plants were grown under garden conditions that were irrigated, fertilised, and pruned to promote foliage suitable for herbivore growth and survival. Laboratory colony insects were fed leaves of live plants or cut foliar bouquets.

Early and late instars of all three biological control agents were tested for acute toxicity. Immediately prior to pesticide applications, larval fresh weights were estimated gravimetrically (Denver [®] balance; SI-215D; ± 0.1 mg; Bohemia, NY, USA). All pesticides were

technical grade purchased from Chem Service® (West Chester, PA, USA) or Sigma/Aldrich (St Louis, MO, USA). Methods of pesticide formulation and insect exposure followed Hoang et al. (2011) and Pridgeon et al. (2008). Permethrin was formulated with 40% *cis*-permethrin and 60% *trans*-permethrin isomers. Technical grade pesticides were dissolved in acetone (Fisher® GC resolv grade; Thermo Fisher Scientific, Waltham, MA, USA) and applied topically to individuals. At least five concentrations of each pesticide were applied in a series of 10x dilutions from 10 µg/insect to 0.001 µg/insect. These concentrations caused 0–100% mortality. A 0.5 µl droplet of pesticide solution was applied to the dorsal thorax of each test insect using a 7000 series syringe and a PB 600 repeating dispenser (Hamilton®, Reno, NV, USA). Each concentration was replicated 10 times on each instar of each insect species. Control treatment included a 0.5 µl droplet of acetone alone for each instar and insect species. Larvae were gently manipulated with a paintbrush or leaf fragment. Insects were maintained in Petri dishes (60 × 15 mm; Fisher® Sterile Polystyrene Petri Dish, Thermo Fisher Scientific, Waltham, MA, USA) lined with filter paper (5.5 cm Filter Paper Qualitative 413, VWR®, West Chester, PA, USA), and provided with an excised leaf of the host weed species. Gloves were worn when handling leaves and changed between leaf treatments. Petri dishes were stored at ambient laboratory conditions, 14 h: 10 h (L:D), 50–60% RH, and 25°C. Following treatment, insects were fed suitable fresh host foliage and enough was provided for unlimited herbivore consumption during 24 h. After a 24 h post-application interval mortality was recorded. An insect was considered dead if it did not move after being gently prodded with a fine paintbrush. All larvae survived the acetone control treatment except 6.25% and 5% of the early and late *N. conspurcatalis* instars, respectively.

The concentration of each naled sample was confirmed with an Agilent® GC/NPD model 6890 (Wilmington, DE, USA). Naled analysis followed a modified version described by Zhong et al. (2010). A DB-5 ms capillary column (Agilent® 30-m length, 0.25-mm inner diameter, 0.25 µm film thickness) bonded with fused silica was used for the naled analysis. Depending on the concentration, a 0.5–1 µl sample was injected either split (35:1) or splitless for each analysis. The injector was operated at 250°C and the detector was maintained at 300°C. Constant flow of helium (4.8 ml/min; 72 cm/sec; 37.5 psi) was used. The initial oven temperature was 60°C, ramped at 20°C/min to 200°C and this final temperature was held constant for 3 min. Under these conditions, the retention time of naled was 6.665 min. A minimum five-point calibration curve covered a naled concentration range from 0.1 to 3,500 ng/µl.

Each permethrin concentration was confirmed by Agilent® GC/MS model 6890/5973. Methods followed a modified version described by Fillion et al. (2000). A DB-5 ms capillary column (Agilent® 30-m length, 0.25-mm inner diameter, 0.25 µm film thickness) bonded with fused silica was used for the permethrin analysis. Target ions for both *cis* and *trans* permethrin were 183 (target), and 163 (abundance ratio Q/tgt: 0.22) and 165 (abundance ratio Q/tgt: 0.18) ions. Depending upon the concentration, either split (20:1) or splitless injection mode was used. Injections were conducted with an autosampler (HP-7683) with volumes that ranged from 0.5 to 1 µL; injector temperature, 250°C; detector temperature, 300°C; and helium served as the carrier gas. The gas chromatograph was operated in constant flow mode (electronic pressure control) at 0.9 mL/min 28 cm/sec. Gas chromatograph temperature programme was held for 2.0 min at 160°C, then increased to 280°C at 3°C/min with a 10 min final hold. Elution time for *cis* and *trans*

Table 1. Acute toxicity (LD₅₀) values for approved weed biological control agents treated with two mosquito pesticides.

Species	Compound	Instar	LD ₅₀ (ng)/ insect	LCI	UCI	Weight (mg)	LD ₅₀ (ng/mg)	LCI	UCI
<i>Lilioceris cheni</i>	Naled	2	160.1	115.5	274.9	18.1	8.8	6.4	15.2
		3	160.6	24.4	299.3	28.4	5.7	0.9	10.5
	Permethrin	2	1.2	0.0	2.5	8.9	0.1	0.0	0.3
		3	3.7	1.6	6.8	28.4	0.1	0.1	0.2
<i>Neomusotima conspurcatalis</i>	Naled	2	3.9	3.1	4.4	0.2	24.4	19.4	27.5
		4	240.3	47.7	354.2	5.6	42.9	8.5	63.3
	Permethrin	2	0.2	0.1	0.2	0.2	0.9	0.8	1.1
		4	22.7	18.6	28.5	5.6	4.1	3.3	5.1
<i>Oxyops vitiosa</i>	Naled	2	707.5	515.2	1199.0	5.0	141.5	103.0	239.8
		4	> 50,000			85.3	> 586.7		
	Permethrin	2	1092	659.1	2060.0	5.0	218.40	131.8	412.0
		4	> 7,500			85.3	> 87.9		

permethrin was 36.9 and 37.4 min, respectively. A minimum five-point calibration curve covered a permethrin concentration range from 0.1 to 1,000 ng/μl (Alder et al., 2006). Data were analysed with SAS® 9.3 (SAS Institute Inc., Cary, NC, USA). Acute toxicity results were subjected to Probit analysis (PROC PROBIT) and LD₅₀ values with upper and lower 95% confidence intervals were calculated.

Acute toxicity values were obtained for both permethrin and naled on three weed biological control agents, *L. cheni*, *N. conspurcatalis*, and *O. vitiosa* (Table 1). Toxicity values and confidence intervals were obtained for both compounds for two larval instars of *L. cheni* and *N. conspurcatalis*, whereas toxicity could be determined only with second instars of *O. vitiosa*. The results indicated that toxicity differed by species and pesticides tested. Permethrin was more toxic (10-fold – 67-fold) for both species and both instars than naled. The *L. cheni* second and third instars were the most sensitive species to permethrin with LD₅₀ values of 0.13 ng/mg larval weight, different than second and fourth instars of *N. conspurcatalis*. *Lilioceris cheni* larvae were also more sensitive in terms of LD₅₀ values to naled than *N. conspurcatalis* larvae. The species most tolerant to these pesticides was *O. vitiosa* with LD₅₀ values for second instars for naled and permethrin greater than the other species and instars tested. Toxicity levels for the *O. vitiosa* fourth instars exceeded 586.7 and 87.9 ng/mg for naled and permethrin, respectively.

Similar acute toxicity results were determined for several non-target species when mosquito pesticides were applied to the thorax of various lepidopteran larvae (Hoang et al., 2011). These studies generally agreed with our results and showed non-target species were also much more sensitive to permethrin compared to naled (Eliazer & Emmel, 1991; Salvato, 2001). Moreover, mosquito pesticide drift results showed that potentially toxic levels of naled and a similar pyrethroid fenitrothion could occur when commercially applied in adjacent areas inhabited by non-target species (Hennessey et al., 1992; Zhong et al., 2010). Due to the high sensitivity of the *L. microphyllum* biological control agent *N. conspurcatalis* and especially the *D. bulbifera* herbivore *L. cheni*, these mosquito pesticides should be applied judiciously in the area where these species are being used for biological control. Additional studies are needed to determine the impact of drift from mosquito adulticide applications on nearby populations of diverse beneficial species, including weed biological control agents. Where possible other means of control should be investigated that do not have the unintended consequences of mosquito adulticide applications.

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