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John R. Wallace
Millersville University, john.wallace@millersville.edu

Calen D. Wylie
Millersville University

Ryan L. Wagner
Millersville University

* Corresponding Author: John R. Wallace, Department of Biology, Millersville University, Millersville, PA 17551; tel: 717-871-4318. Acknowledgements We would like to thank the Niemeyer-Hodgson Undergraduate Research Board, the MU Student Research Board, and The Millersville University Biological Student Investigators grant for funding this project. We would also like to thank Elizabeth Miller and John Malachowski for their assistance in the field. JW would like to acknowledge two anonymous reviewers for comments on the manuscript.

Plant Extract Efficacy on Mosquito Mortality: Preliminary Studies on the Effect of *Ailanthus altissima* Extract on Adult *Aedes aegypti* and *Culex quinquefasciatus*

John R. Wallace*, Calen D. Wylie, Ryan L. Wagner

Department of Biology, Millersville University, Millersville, PA 17551
* Corresponding author: (e-mail: john.wallace@millersville.edu; tel: 717-871-4318, Fax 717-871-7964).

Abstract

Due to the negative environmental impact and resistance to synthetic insecticides, the development of biological control has increased significantly over the past half century with the potential of plant extracts only recently attracting attention. The purpose of this preliminary study was to examine the potential of *Ailanthus altissima* (Mill.) Swingle extract as a botanical insecticide on adult mosquitoes. Two species of mosquitoes (*Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* Say) (Diptera: Culicidae) and a non-target lepidopteran species, Painted Lady butterfly (*Vanessa cardui* (Linnaeus) (Lepidoptera: Nymphalidae) were treated with *A. altissima* extract from new, mature, and senesced leaflets using serial dilutions (0, 25, 50, 75, 100%) of extract via two application methods (aerosol and sugar feeding). We found that application method and leaf age had significant effects on mosquito mortality at high concentrations. These findings indicate that while mortality was not comparatively high to commercial products, there may be potential to use an invasive plant extract as a bio-control tool for mosquito vectors of human disease pathogens.

Keywords: *Ailanthus altissima*, Tree of Heaven, *Culex quinquefasciatus*, *Aedes aegypti, Vanessa cardui, plant extract*

Since the early work done by Roark (1947), more than 1200 plant species have been listed as having potential insecticidal value. Bioactivities from plant species against insects, parasites, bacteria and fungi used for pest control have been abundantly reported in the literature (Ganjian et al. 1983, Pereira et al. 1997, Adedire and Akinneye 2004, Koona et al. 2007, Ambrósio et al. 2008, Chukwujekwu et al. 2009, Chenniappan et al. 2011, Tesch et al. 2011, Chagas-Paula et al. 2012, Nhamo et al. 2013, Utono et al. 2014, Green et al. 2017). Despite this emergence of plant products used for pest control, there has been an increased need for an environmentally safe insecticide because of the rise of pestilent disease vectors and the emergent disease pathogens they transmit (Isman and Greineisen 2013, Tembo et al. 2018). In addition to being a significant biting pest species, mosquitoes are well-known vectors of pathogens that cause diseases such as malaria, yellow fever, dengue, and many types encephalitides that affect millions of people worldwide (World Health Organization 1996, CDC 2007, Ghosh et al. 2012, Rohring 2013, Mudin 2015).

Due to the multi-pronged anti-herbivory chemical defense systems plants possess to deter phytophagous insects that include feeding deterrent toxins, growth regulators as well as repellents, many of these primary defense functions are also effective against controlling or repelling mosquitoes and other biting Diptera (Pichersky and Gershenzon 2002). The use of plants to repel mosquitoes, specifically anopheline mosquitoes has been thoroughly reviewed by Maia and Moore (2011) and more recently by Asadollahi et al. (2019) where they concluded that knowledge on traditional practices using plants as repellents may be a significant resource from which new natural products could be utilized as alternatives to synthetic chemical repellents. Since the 1920's, the application of phytochemicals has been used to control mosquito populations (Shaalan et al. 2005). Much of this research has been a bio-prospecting exploration into which plants repel mosquitoes through air-borne volatiles from these plants without controlling them (Maia and Moore 2011). However, the attractive toxic sugar bait (ATSB) approach has been well developed and tested on *Anopheles*, *Aedes* and *Culex* adult and larval mosquitoes (Müller and Schlein 2006, 2008; Müller et al. 2008; Müller et al. 2010a,b,c).

Approximately 344 plant species have been described as having mosquitocidal activity on biting adult mosquitoes (Sukumar et al. 1991, Tawatsin et al. 2001, Phasomkusolsil and Soonwera 2010) while many others have been reviewed as potential larvicidal properties (Shaalan et al 2005). An example of one of these plants is a non-native tree species, *Ailanthus altissima* (Mill.) Swingle in North America and was originally found in China but introduced to Europe and North America in the mid-late 1700s (Ding et al. 2006). Biochemically, *A. altissima* has been well documented to possess the quassanoid, ailanthone, that is associated with herbidical as well as insecticidal activities (Heisey 1996, De Feo et al. 2003, Alves et al. 2014). Several investigators have noted that leaf and root extract from *A. altissima* has demonstrated variable insecticidal activity against hematophagic and phytophagic insects (Tsao et al. 2002, De Feo et al. 2009). *Ailanthus altissima* has been used as an insecticide to control crop pests such as *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) (Pavela et al. 2014, Pavela 2016). The extract is well documented in terms of an herbicide (Heisey and Heisey 2003) but has not been widely tested as an insecticide against insect vectors of human pathogens (Tsao et al. 2002). Recently, we have conducted evaluations of *A. altissima* food supplements on several species of lepidopteran larvae e.g., *Spodoptera frugiperda* J.E. Smith and also found that *A. altissima* reduced larval relative growth rates and adult size and also affected adult oviposition behavior (Wagner and Card 2020, Wagner et al. 2020). These findings suggested that *A. altissima* might be a source of pesticide metabolites that might be useful to control other pest insect species. The purpose of this preliminary study was to determine the efficacy of *A. altissima* extract on the adult survivorship of two mosquito species, *Culex quinquefasciatus* Say and *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) and a non-target lepidopteran species, *Vanessa cardui* (Linnaeus) (Lepidoptera: Nymphalidae). The objectives for this study included: 1) determine the concentration effect of *A. altissima* extract on target and non-target insect mortality; 2) compare the extract type (leaf age) on insect mortality and; 3) compare application method (aerosol v. sugar fed) on insect mortality.

Materials and Methods

Two adult mosquito species were used in this study, *Cx. quinquefasciatus* and *Ae. aegypti* based on their involvement in vectoring several viral pathogens worldwide (Liu et al. 2017) and were purchased as third instar larvae from Benzon Research, Inc. in Carlisle, PA and maintained at 23°C and 14:10 L:D schedule. Larvae were fed approximately 1oz of Tetramin® fish flakes twice/day until pupation as suggested in Imam et al. (2014). Pupae were transferred to rearing chambers and maintained until adult emergence. Adult mosquitoes were fed on a 10% sucrose solution until assayed with *A. altissima* extract. Adult male and female mosquitoes (n = 25 mosquitoes/cage) were kept in a cage (n = 3 replicate cages per experiment) to avoid crowding. The Painted Lady butterfly, *V. cardui* was selected as the non-target species to represent an adult insect that sugar feeds as a primary food source and has well documented migratory patterns that may overlap with both culicid species chosen for this study (Abbot 1951, Stefanescu et al. 2013). *Vanessa cardui* larvae were purchased from Folk's Butterfly Farm in Nescopeck, PA. Larvae were maintained at the same temperature and light regimes as mosquitoes and fed artificial food provided by the vendor until pupation. Once *V. cardui* adults emerged, they were maintained 10% sucrose solution soaked in cotton balls.

We designed two experiments with a 3 × 2 × 5 factorial design in order to obtain a response to *A. altissima* leaf extract testing three leaf ages (new, mature and senesced leaves) with two application methods (aerosol and sugar fed) across five different concentrations (100%, 75%, 50%, 25%, and 0%). Leaves were collected from live trees between May–October, 2015 representing three different ages (100 leaves/age): newly emerged (May), mature (July) and senesced leaves (October) to assess the effect of leaf age on insect mortality (n = 3 replicate cages/leaf age). Leaves were stored at 0°C until they were used for extractions. Leachate from leaf extract was obtained by using a leaflet pack of 25 g wrapped in cheesecloth that was compressed to a ball-like shape and placed in 1L of distilled water to steep for 24 hours at room temperature (21°C). Dilutions were prepared from a concentrate of 100% extract that was diluted to 75, 50, 25 and 0% (0% represented the control) to assess extract concentration effect insect on mortality (n = 3 replicate cages/treatment with 25 mosquitoes/cage and 12 *V. cardui*/cage). Distilled water was used as a control solution. Two delivery or application methods were tested (aerosol and sugar fed solution). The aerosol application experiment consisted of using a spray bottle to apply three squirts (approximately 3 ml of spray) at the fine spray setting of each extract sprayed evenly across the cage and directly on to the individual mosquitoes and/or butterflies. Aerosol droplet size from the commercial spray bottle was estimated to be approximately 50 microns (Kripena 2020). The sugar fed (ATSB) application experiment was conducted by applying 1 mL of extract solution and 1 mL of 10% sucrose to the cotton ball placing the cotton ball solution inside the each cage. All treated cages were checked 24 hours post extract exposure and mortality was recorded. All percentage data were arcsine transformed for statistical analysis. All comparisons were made using a two-way ANOVA and a Tukey's Range test to compare mean differences.

Results

We found that both species of mosquitoes were affected by both extract applications, whereas mortality effects on the non-target species was minimal (Fig. 1A,B,C). In general, the highest mean percentage mortality after 24 hours neared 60% for both mosquito species.

For example, *Ae. aegypti* mortality was significantly higher as a result of aerosol applications ($F = 30.8$; df = 1, 4; $P < 0.01$); and concentration ($F = 70.9$; df = 1, 4; $P < 0.01$) compared to ATSB applications (Fig. 1A). There was no interaction effect between leaf age and concentration for the aerosol treatment ($F = 1.61$; $P > 0.05$); however, there was an interaction effect between leaf age and concentration for the sugar fed application ($F = 52.2$; $P < 0.01$). But, leaf age had mixed effects, that is, no significant difference was observed on mortality with aerosol ($F = 2.69$; df = 2, 4; $P > 0.05$) compared to significant effects with ATSB applications ($F = 9.42$; df = 2, 4; $P < 0.01$) (Fig. 1A). Whereas, *Cx. quinquefaciatus* mortality was significantly different for the ATSB application method ($F = 6.91$; df = 1, 4; $P < 0.05$), leaf age ($F = 4.11$; df = 1, 2; $P < 0.05$) and concentration ($F = 18.0$; df = 1, 4; $P < 0.01$) (Fig. 1B). There was an interaction effect between leaf age and concentration for the aerosol application ($F = 5.34$; $P < 0.01$) and no interaction effect between leaf age and concentration for the ATSB application ($F = 1.85$; $P > 0.05$). While there was no difference on the non-target, *V. cardui* mortality between application methods ($F = 0.75$; df = 1, 4; $P < 0.05$) (Fig. 1C), there was significantly greater mortality with 100% concentration ($F = 8.4$; df = 1, 4; $P < 0.01$); however, this mortality % (10.8%) was significantly lower than the mortality for both culicid species (Fig. 1A,B). Leaf age had no significant effect on mortality of *V. cardui* ($F = 0.88$; df = 2, 4; $P < 0.05$). There were no interaction effects between leaf age and concentration with either application method for *V. cardui*.

Discussion

Both aerosol and ATSB application methodologies have been well tested and demonstrated to successfully control several genera of mosquitoes (Müller et al. 2008, 2010b; Maia and Moore 2011). We report in this preliminary study that the method of application of *A. ailanthus* extract had different effects on the two mosquito taxa studied. We found significant effects on *Ae. aegypti* mortality especially when extracts from mature and senesced leaves were used at high concentrations with an aerosol application. Whereas, the ATSB application had a greater impact on *Cx. quinquefasciatus* mortality compared to *Ae. aegypti*. This difference suggests the mode of application may be taxon-specific (e.g., Culicid taxa) suggesting multiple approaches in the use of plant extracts as mosquitocidal agents. While mortality in this study was not as high as commercial synthetic insecticides (not tested in this experiment) to control vectors of major arboviral pathogens, such as dengue viruses, in a region where *A. altissima* is native, it may provide an inexpensive and simple approach to maintain enough sustainable efforts to reduce breeding sites (Mudin 2015). Using *A. altissima*, an invasive plant species in the United States, as a leaflet extract could provide a cost effective alternative to reducing mosquito population and disease cases while at the same time find an application to reduce populations of a noxious invasive tree such as *A. altissima*. Preliminary work on larval and pupal survival indicates that concentration may have similar effects (unpublished data). Future work will address leaf age and other extract sources such as bark and roots on larval survival.

With leaf age having a significant impact on mosquito mortality on both application methods, if *A. altissima* extract were to be used to control culicid vectors, concentrations of the allelopathic compounds would need to be adjusted over the growing season. Publication on the insecticidal attributes of the allelochemistry found within the plant leaf tissue has been sparse to nonexistent (Pavela 2014, Pavela and Sedlak 2018). Our findings suggest a broader ecological selective advantage to maintain such herbicidal compounds as a biocontrol strategy for insect pest species, such as mosquitoes. The leaf extract had a greater impact on mosquito mortality with little to no mortality on Painted Lady butterflies suggesting that this product could be used in field applications where these two families of insects coincide. We did find that the ATSB method of application did have a slightly greater impact on *V. cardui* mortality, this method of application

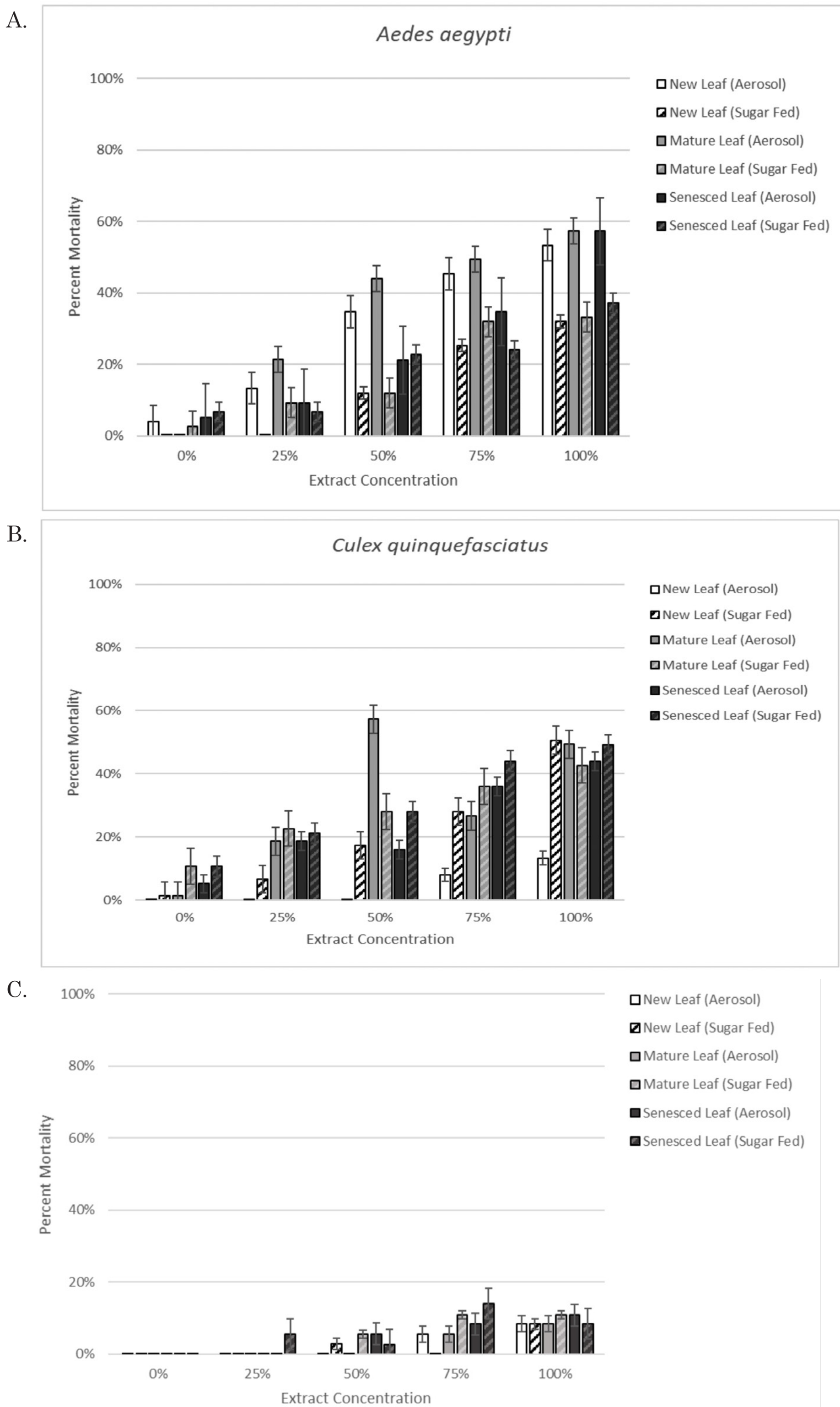

Figure 1. Comparative analysis of A. *altissima* extract concentration and leaf age (A. *Aedes aegypti*; B. *Culex quinquefasciatus*; C. *Vanessa cardui*) from leachate on mean % mortality using the aerosol and sugar fed (ATSB) applications. Error bars represent SEM.

would not benefit those non-targets that nectar feed. While the use of *A. altissima* extract has had detrimental effects on the mortality of other lepidopteran species, e.g., *S. littoralis* and *S. frugiperda* (Pavela et al. 2014, Wagner et al. 2020, Wagner and Card 2020), additional work on the impacts of other non-target taxa is warranted.

The effects of *A. altissima* against target species such as mosquitoes or other dipteran and/or pest species, the safe use, and the overall socio-economic and agro-ecological benefit requires additional work. Because natural compounds are not as effective as synthetic pesticides (Casida 1980), in order to adopt more widespread natural pest control products, the novel use of a readily available resource, such as the invasive *A. altissima* to control pest populations can only be attained through their evaluation under lab as well as field conditions (Tembo 2018). Using an invasive plant such as *A. altissima* to control pest insect species could have significant effects on both invasive species and pest management while having little impact on non-target nectar feeding taxa as long as the ATSB method of application is not employed.

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