Insecticidal and antifeedant activities of Malagasy medicinal plant (*Cinnamosma* sp.) extracts and drimane-type sesquiterpenes against *Aedes aegypti* mosquitoes

Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

Edna Ariel Alfaro Inocente, B.S.

Graduate Program in Entomology

The Ohio State University

2020

Thesis Committee

Dr. Peter M. Piermarini, Advisor

Dr. Reed M. Johnson

Dr. Liva H. Rakotondraibe
Copyrighted by
Edna Ariel Alfaro Inocente
2020
Abstract

Nearly everyone has been bitten at least once by a mosquito and recognizes how annoying this can be. But mosquito bites are nothing compared to the number of people that have died from and continue to be at risk of contracting arboviruses transmitted by these deadly insects. Female *Aedes aegypti* mosquitoes are the primary vector of the viruses that cause yellow fever, dengue fever, chikungunya fever, and Zika virus in humans. Climate change and globalization have facilitated the spread of these mosquitoes and associated arboviruses, generating new outbreaks and increasing disease risk in the United States. Effective vaccines or medical treatments for most mosquito-borne arboviruses are not available and thereby the most common strategy to control viral transmission is to manage mosquito populations with chemical pesticides and prevent mosquito-human interactions with chemical repellents. Although the use of neurotoxic chemicals, such as organophosphates and pyrethroids, can be very efficient at killing mosquitoes, the limited modes of action of these compounds has strongly selected for individuals that are resistant. Likewise, the chemical arsenal for mosquito repellents is limited. Thus, insecticides and repellents with novel modes of action are needed to improve mosquito control. One source of novel insecticides and repellents is plant secondary metabolites, such as sesquiterpenes. The goal of this thesis is to evaluate sesquiterpenes of the drimane type isolated from the bark, roots, or leaves of Malagasy medicinal plants (genus *Cinnamosma*) as potential insecticides and repellents.
In Chapter 2, we demonstrate that the bark extract of *Cinnamosma* plants possesses the strongest adulticidal activity against *Ae. aegypti* mosquitoes, which is attributed to the abundance of cinnamodial (CDIAL) in the extract. For larvicidal and antifeedant activities the bark and root extracts were equally effective despite lower CDIAL content in the root, suggesting additional active factors in the root extract. As such, we found additional compounds with significant larvicidal and/or antifeedant activities in the root extract, including polygodial (POLYG), cinnfragrin A (CFRAG), capsicodendrin (CPCD), and cinnamolide (CML). Intriguingly, we also discovered a synergistic larvicidal effect between CDIAL and an abundant lactone-bearing drimane sesquiterpene (cinnamosmolide) in the root extract. The testing of aldehyde-bearing drimane sesquiterpenes with similar structures such CDIAL, POLYG, and warburganal (WARB, isolated from an African medicinal plant, *Warburgia ugandensis*) revealed that a hydroxyl (-OH) group plays a key role in modulating the larvicidal and adulticidal bioactivities. Lastly, molecular modeling simulations confirmed that the antifeedant activity of the aldehyde-bearing drimane sesquiterpenes is likely due to agonism of transient receptor potential A1 (TRPA1) channels, but the antifeedant activity of CML is likely due to interactions with a distinct molecular target that remains to be determined. Altogether, the thesis research provides new insights into the structure-activity relationships and potential use of drimane sesquiterpenes from *Cinnamosma* plants as insecticides and antifeedants to control *Ae. aegypti* mosquitoes. The results will facilitate the development of new pesticides and repellents with novel modes of action.
Dedication

In memory of my beloved father Edwin Alfaro. And to my mother Silvia Alfaro, my sisters Edlin and Adriana and brothers Jose and Daniel, with all my love to them.
Acknowledgments

I thank the state of Ohio and federal government of U.S. for the funds and the opportunity of being part of the prestigious university The Ohio State University. Thanks to my committee members Dr. Reed Johnson, Dr. Liva Rakotondraibe and specially to my advisor Dr. Peter M. Piermarini (The Ohio State University) for the helpful support and guidance during the master period and the valuable discussions and feedback of manuscripts and presentations. Thanks to Ms. Elvia A. Alfaro Inocente, Ms. Renata Rusconi, Mr. Erick J. Martinez and Dr. Megha Kalsi and the Department of Entomology (The Ohio State University) for technical support and assisting with my project, friendship and companionship during this process. And all my friends around OARDC and Wooster that support and believe in me, specially to Joe Boreman for his love, patience, and support during this process.
Vita

2008……………………………… General associate Degree
Instituto Nacional Jose Rivera Campos

2012……………………………… Bachelor of science, Food Science & Technology
The Pan-American Agricultural University,
Zamorano. Francisco Morazán, Honduras.

2014……………………………… Production Controller
Sociedad Cooperativa Ganadera de Sonsonate de
R.L. de C.V. Sonsonate, El Salvador.

2017……………………………… Visiting Scholar
Department of Entomology, The Ohio State
University. Wooster, Ohio.

2018- Current…………………… Graduate Teaching & Research Associate
Department of Entomology, The Ohio State
University. Wooster, Ohio.

Publications

Inocente, E.A.; Nguyen, B.; Manwill, P.K.; Benatrehina, A.; Kweka, E.; Wu, S.; Cheng, X.; Rakotondraibe, L.H.; Piermarini, P.M. Insecticidal and Antifeedant Activities of Malagasy Medicinal Plant (Cinnamosma sp.) Extracts and Drimane-Type Sesquiterpenes against Aedes aegypti Mosquitoes. *Insects* 2019, 10, 373. Doi: [https://doi.org/10.3390/insects10110373](https://doi.org/10.3390/insects10110373)

Yang, L.; Turo, K.J.; Riley, C.B.; Inocente, E.A.; Tian, J.; Hoekstra, N.C.; Piermarini, P.M.; Gardiner, M.M. Can urban greening increase vector abundance in cities? The impact of mowing, local vegetation, and landscape composition on adult mosquito populations. *Urban Ecosystems* 2019, 22, 827-839. Doi: [https://doi.org/10.1007/s11252-019-00857-7](https://doi.org/10.1007/s11252-019-00857-7)

Piermarini, P.M.; Inocente, E.A.; Acosta N.; Hopkins C.R.; Denton J.S.; Michel A.P. Inward rectifier potassium (Kir) channels in the soybean aphid *Aphis glycines*: functional characterization, pharmacology, and toxicology. *Journal of Insect Physiology* 2018, 110:57–65. Doi: [https://doi.org/10.1016/j.jinsphys.2018.09.001](https://doi.org/10.1016/j.jinsphys.2018.09.001)

Inocente, E.A.; Shaya, M.; Acosta, N.; Rakotondraibe, L.H.; Piermarini, P.M. A natural agonist of mosquito TRPA1 from the medicinal plant *Cinnamosma fragrans* that is toxic,
antifeedant, and repellent to the yellow fever mosquito *Aedes aegypti*. *PLOS Neglected Tropical Diseases* 2018, 12(2): e0006265. Doi: https://doi.org/10.1371/journal.pntd.0006265

Yang, Z.; Statler, B.-M.; Calkins, T.L.; **Alfaro, E.**; Esquivel, C.J.; Rouhier, M.F.; Denton, J.S.; Piermarini, P.M. Dynamic expression of genes encoding subunits of inward rectifier potassium (Kir) channels in the yellow fever mosquito *Aedes aegypti*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 2017, 204, 35-44. Doi: https://doi.org/10.1016/j.cbpb.2016.11.003

Swale, D.R.; Engers, D.W.; Bollinger, S.R.; Gross, A.; **Inocente, E.A.**; Days, E.; Kanga, F.; Johnson, R.M.; Yang, L.; Bloomquist, J. R.; Hopkins, C.R.; Piermarini, P.M.; Denton, J.S. An insecticide resistance-breaking mosquitocide targeting inward rectifier potassium channels in vectors of Zika virus and malaria. *Scientific Reports* 2016, 6, 36954 Doi: https://doi.org/10.1038/srep36954

**Alfaro, I.E.A.** Castañeda, R.A.E. Desarrollo de un prototipo de torta de huevo evaluando goma xantán y vacío. Bachelor's thesis, Escuela Agrícola Panamericana, Zamorano, 2012. https://bdigital.zamorano.edu/handle/11036/1014

**Fields of Study**

Major Field: Entomology
Table of Contents

Abstract ................................................................................................................................. ii
Dedication .............................................................................................................................. iv
Acknowledgments ................................................................................................................ v
Vita ........................................................................................................................................ vi
List of Tables ........................................................................................................................ x
List of Figures ......................................................................................................................... xi

Chapter 1. Introduction ........................................................................................................ 1

1.1 The dangerous *Aedes aegypti* mosquito ................................................................. 1

1.2 Chemical-based mosquito control (insecticides and repellents) mode of action and resistance .................................................................................................................. 4

1.3 New tools: natural products and mode of action .................................................. 8

1.4 Discovery of bioactive compounds from *Cinnamosma* ........................................ 11

1.5 Research Objective and Hypothesis ........................................................................... 14

Chapter 2. Insecticidal and antifeedant activities of Malagasy medicinal plant *(Cinnamosma* sp.*) extracts and drimane-type sesquiterpenes against *Aedes aegypti* mosquitoes .......................................................................................................................... 15

2.1 Abstract ......................................................................................................................... 15

2.2 Introduction .................................................................................................................... 16

2.3 Materials and methods ............................................................................................... 18

2.3.1 Plant material and isolation of chemicals ............................................................. 18

2.3.2 Estimation of the amount of compounds by $^1$H MNR: .................................... 20

2.3.3 Mosquito colony ....................................................................................................... 20

2.3.4 Toxicology experiments .......................................................................................... 21

2.3.5 Antifeedant experiments ......................................................................................... 22

2.3.6 Computational docking ............................................................................................ 23

2.4 Results .......................................................................................................................... 23

2.4.1 CDIAL and/or CMOS are the major drimane sesquiterpenes in leaves, bark and roots .......................................................................................................................... 23
2.4.2 Relative insecticidal activities of plant extracts and isolated drimane sesquiterpenes against larval and adult female mosquitoes ........................................ 25
2.4.3 Relative antifeedant activity of plant extracts and isolated drimane sesquiterpenes against adult female mosquitoes .................................................. 28
2.4.4 Computational docking of CML and CDIAL to AgTRPA1 .......................... 30
2.5 Discussion ........................................................................................................... 31
  2.5.1 Insecticidal activity of plant extracts and isolated compounds ............... 32
  2.5.2 Insights into the insecticidal SAR of CDIAL-like drimane sesquiterpenes 35
  2.5.3 Antifeedant activity of plant extracts and isolated drimane sesquiterpenes 36
  2.5.4 Insights into antifeedant SAR of CDIAL-like drimane sesquiterpenes ..... 37

Chapter 3. Summary and future directions ................................................................. 39
  3.1 Summary .......................................................................................................... 39
  3.2 Future directions .............................................................................................. 41

Bibliography ............................................................................................................... 43

Appendix A. Extracts raw sucrose consumption ...................................................... 58
Appendix B. Compounds raw sucrose consumption ............................................... 59
List of Tables

Table 1: Relative abundance of compounds 1 and 3 in the extracts of *Cinnamosma* species. Percentages were determined using integration of the $^1$H signals.................. 24
List of Figures

Figure 1. Structures of isolated compounds. Carbons referred to in the text are numbered in CDIAL (1). .......................................................... 14

Figure 2. $^1$H NMR spectra of *Cinnamosma* extracts, CDIAL (1) and CMOS (3). A: bark extract, B: root extract, C: leaf extract, D: CDIAL and E: CMOS ................. 25

Figure 3. Insecticidal efficacy of plant extracts and isolated drimane sesquiterpenes to adult female (A, C) and larval (B, D) *Ae. aegypti* (LVP strain). A, C) adulticidal efficacy was defined as the percentage of adults (after Abbott’s correction) that were incapacitated (dead or flightless) within 24 h when extract (2.5 µg/mosquito) or compounds (5 nmol/mosquito) were applied to the thoracic cuticle of adult females. Values are means ± SEM; N= number of independent replicates of 10 females each. B, D) larvicidal efficacy in 1st instar larvae was defined as the percentage (after Abbott’s correction) that died within 24 h when extract (50 µg/ml) or compounds (100 µM) were added to the rearing water (100 µM). Values are means ± SEM; N= number of independent replicates of 5 larvae each. In all panels, lower-case letters indicate statistical categorization of the means as determined by a one-way ANOVA and Tukey’s multiple comparisons test. (P < 0.05). .......................................................... 27

Figure 4. Synergism between CDIAL (1) and CMOS (3) against 1st instar larvae of *Aedes aegypti*. Larvicidal efficacy in 1st instar larvae was defined as the percentage (after Abbott’s correction) that died within 24 h after adding CDIAL (50 µM), CMOS (75 µM), or the combination of CDIAL (50 µM) and CMOS (75 µM) to the rearing water. Values are means ± SEM; N= number of independent replicates of 5 larvae each................. 28

Figure 5. Antifeedant activity of plant extracts (A) and isolated drimane sesquiterpenes (B) in choice CAFE assays with adult female *Ae. aegypti* (LVP strain) mosquitoes. At the time of feeding, each group of 5 mosquitoes was offered two glass capillaries filled with 10% sucrose and 0.01% trypan blue. The control capillary included 1% acetone (the solvent), and the treatment capillary included 1% acetone and an extract (50 µg/ml) or a drimane sesquiterpene (1 mM). The difference in volume consumed between the capillaries was used to calculate the antifeedant activity. See Figs. 7 and 8 for consumption volumes. Values are means ± SEM; N= number of independent replicates of 5 mosquitoes each. Lower-case letters indicate statistical categorization of the means as determined by a one-way ANOVA and Tukey’s multiple comparisons test (P < 0.05). . 30

Figure 6. Structural models of CML (4) or CDIAL (1) in the putative CDIAL-binding site of AgTRPA1. Potential interactions between ligand (yellow) and residues of AgTRPA1 (cyan) as predicted by computational docking are shown. Several nearby residues are labeled and shown in licorice representation........................................... 31
Figure 7. Volumes of sucrose consumed in control and treatment capillaries during choice CAFE assays in adult female *Ae. aegypti* (LVP strain). The extract (50 µg/ml) used in the treatment capillaries is indicated. As described in the methods, the differences in volume consumed between the capillaries were used to calculate the antifeedant indices in Fig. 5A. Values are means ± SEM; N= 16-24 independent replicates of 5 mosquitoes each. Differences in volumes consumed between control and treatment capillaries were determined by paired t-tests (P < 0.05).

Figure 8. Volumes of sucrose consumed in control and treatment capillaries during choice CAFE assays in adult female *Ae. aegypti* (LVP strain). The compound (1 mM) used in the treatment capillaries is indicated. As described in the methods, the differences in volume consumed between the capillaries were used to calculate the antifeedant indices in Fig. 6A. Values are means ± SEM; N= 10-24 independent replicates of 5 mosquitoes each. Differences in volumes consumed between control and treatment capillaries were determined by paired t-tests (P < 0.05).
Chapter 1. Introduction

1.1 The dangerous *Aedes aegypti* mosquito

Mosquitoes, the biting flies of the family Culicidae (order Diptera), were for early naturalists the subjects of study for metamorphosis in insects, given that the larval stages develop in water and afterward transform into a winged terrestrial adult [1,2]. But ever since the ground-breaking discoveries by Dr. Ronald Ross (recipient of the 1902 Nobel Prize for Physiology or Medicine) and the U.S army officer/physician Dr. Walter Reed in the 1890s, whom respectively demonstrated the mosquito-borne transmission of malaria by *Anopheles* mosquitoes and yellow fever by *Aedes* mosquitoes [3,4], mosquitoes have been recognized as the most dangerous animals in the world. Mosquitoes are now known to transmit numerous pathogens, primarily malaria parasites and arboviruses that cause severe diseases affecting hundreds of millions of people around the globe [5,6]. The study species of this thesis is the yellow fever mosquito *Aedes aegypti*, which is the most medically important mosquito vector of arthropod-borne viruses (arboviruses).

Before becoming a global nuisance, the *Ae. aegypti* mosquito was originally confined to Africa, where its distribution was restricted to latitudes of 45°N and 35°S and altitudes of 6000 to 8000 feet where the temperature did not reach lower than 15°C [1]. However, with the help of humans via travel, migration and global trade, *Ae. aegypti* has spread around the globe in the tropics and sub-tropics. In the Western hemisphere, the distribution of *Ae. aegypti* occurs throughout Latin America and the Caribbean, and is found within 28 states of the U.S., including
the District of Columbia [7]. It is possible that the abundance and range of *Ae. aegypti* will further expand in this hemisphere. For example, a predicted increase of temperature of around 5.8°C due to high carbon emissions over the twenty-first century (2090–2099 relative to 1987–2016) projects that the global abundance of *Ae. aegypti* mosquitoes will increase by more than 30%, especially in Brazil, Mexico and the U.S. However, there may also be corresponding decreases in abundance in parts of Central and South America, Southwest Asia, North Africa, and North Australia [8]. Overall, the preferences of *Ae. aegypti* for urban areas with a moist warm climate and human hosts increases its chances of direct interactions with humans and the possibility of spreading to new areas, thereby adding to its ability to transmit disease around the globe.

Similar to other mosquitoes, *Ae. aegypti* has a holometabolous life cycle that exploits aquatic and terrestrial environments. Eggs are characterized as dark black, small (~0.67 mm in length and 0.17 mm in breadth) and elongate in shape; they are laid on wet surfaces just above the water’s edge, rather than dry surfaces or directly in the water. If the eggs are kept in a moist environment for a period of 24-72 hours after being laid, the embryo will fully develop inside the egg and synthesize a serosal cuticle layer that protects it, conferring the ability to resist desiccation for prolonged periods of time and survive in adverse conditions [1,9-13]. Rehydration of the eggs for at least 3 hours results in 1st instar larvae hatching through the cap-like portions of the eggs by muscular movements of the head [1]. The mosquito larvae are aquatic organisms that swim under water, but they breathe air when at the water surface via a posterior respiratory siphon [1]. The larvae primarily feed on microorganisms and detritus (organic matter suspended in water), increasing in size and weight during four larval instars in less than 5 days [14]. After this last molting, the larvae become active pupae [15]. The behavior of pupae differs from that of larvae by
not feeding, being less responsive to stimuli, and spending most of their time at the water surface. Pupae undergo a metamorphosis for a few days and emerge from the pupal case at the water surface as fully developed flying adults [2].

Adult female *Ae. aegypti* are day-biting mosquitoes. Their mouthparts include an elongate composite proboscis with stylets adapted for piercing human skin to take blood from peripheral vessels. The blood is essential for obtaining protein and other nutrients that allow the eggs to undergo vitellogenesis and produce viable progeny. Females become competent to respond to host cues and take a blood meal within about 30 hours after emergence and this behavior remains constant for 14 days. Specific host cues play an important role as attractants that are detected by the antennal sensory structures, and include: chemical compounds, such as carbon dioxide, lactic acid, and other volatiles that can be produced from human breath, sweat, and urine[16,17]; thermal stimuli, such as body heat emanating from the vertebrate host over a short distance; and visual cues, such as host movements [18,19]. Although feeding on blood is essential for egg production, it also provides opportunities for adult female *Ae. aegypti* mosquitoes to acquire arboviral pathogens from infected humans and transmit these pathogens to an uninfected human host during a subsequent blood meal [20].

Among the emerging and reemerging arboviral pathogens transmitted by *Ae. aegypti*, those of most concern are viruses of the genus *Flavivirus* and *Alphavirus*. Flaviviruses are among the most dangerous and historically significant viruses transmitted to humans by *Ae. aegypti*, such as dengue fever (DEN), yellow fever (YF), and Zika virus (ZIKV) [21]. DEN is the most widely distributed arboviral disease with 3.8 billion people at risk in 128 countries, and a yearly estimate of 390 million infections, resulting in 25,000 deaths around the world [22,23]. YF is distributed
broadly on lowland equatorial Africa and Central and South America comprising 44 countries with 472.9 million people at risk, and an estimate of 200,000 infections and 30,000 deaths annually worldwide [24,25]. ZIKV was originally confined to Africa, but outbreaks in 2007 and 2015 led to its rapid spread to the Western Hemisphere leading to an epidemic of birth defects and neurological problems. ZIKV is now considered a global public health disease with 2.17 billion people at risk in 85 countries [26,27]. Alphaviruses are also emerging concerns. Chikungunya fever (CHIK) is an alphavirus originally restricted to Africa and India but has more recently spread to the Americas and Europe, putting an estimated 1.3 billion at risk of infection in about 106 countries[28-30]. Mayaro virus (MAY) is another emerging alphavirus in the Caribbean and Latin America, where people residing near places with enzootic transmission might be at higher risk [29-31].

Vaccination is the most important preventive measure against arboviruses for potentially providing life-long immunity. However, except for YF, effective vaccines are not available for the other arboviral diseases. Moreover, there are no straightforward methods of therapeutic treatment for these vector-borne pathogens [32]. Thus, methods for controlling the mosquito vector are often the only ways to prevent and mitigate disease outbreaks and thereby are of great interest to human health.

1.2 Chemical-based mosquito control (insecticides and repellents) mode of action and resistance

There are numerous strategies and approaches to control mosquitoes. For example, biological control has been employed using natural aquatic predators such as the mosquito fish (*Gambusia affinis*), killifish (*Fundulus* spp.) and the predator mosquito *Toxorhynchites* [33,34]. In addition, toxic microorganisms such as *Bacillus thuringiensis israelensis (bt)* that produce
crystalline, proteinaceous toxins during sporulation have been used to kill mosquito larvae [35-37]. Recent studies have shown that *Wolbachia* bacteria have the capacity to prevent mosquitoes from becoming infected by a variety of pathogens [38]. Another example of biological control is the release of genetically modified males carrying a lethal transgene that reduces the number of offspring produced by natural populations if they can outcompete wild-type males [39,40]. Despite the promise of these techniques, some are relatively new and in need of more research to demonstrate long-term efficacy and their safety. Thus, the chemical control of mosquitoes using insecticides and/or repellents remains one of the most effective and widely used approaches to mosquito control.

The most common and effective insecticides used for mosquito control have been neurotoxic chemicals, such as organochlorines, pyrethroids, organophosphates, and carbamates. Synthetic organochlorines such as DDT (dichloro-diphenyl-trichloroethane) and dieldrin were widely used in the 20th century, but environmental and human health concerns have led to more restricted global use. The organochlorine DDT disrupts the mosquito nervous system by binding to and activating voltage-gated sodium (Na+) channels in nerve axons, leading to depolarization of the membrane and excess action potentials. Pyrethrum (natural pyrethrins) and synthetic pyrethroids (e.g., permethrin, resmethrin and etofenprox) are presently among the most widely used insecticides for mosquito control. They operate via a similar mode of action as DDT. The organophosphates (e.g., naled, temephos, malathion) are all derivatives from phosphoric acid, and the carbamates (e.g., propoxur and methiocarb) are esters of carbamic acid. Both organophosphates and carbamates mimic the structure of acetylcholine and bind to the active site of acetylcholinesterase (AChE) by phosphorylation, causing either non-competitive inhibition
with an irreversible binding (organophosphates) or competitive inhibition with reversible binding (carbamates). This leads to a buildup of acetylcholine in the synapse, keeping the neuron constantly activated. Thus, all of the above neurotoxic insecticides cause hyperactivation of the nervous system leading to muscle paralysis and death [41].

A consequence of the overuse of neurotoxic insecticides with similar modes of action has been the evolution of insecticide resistance. To date the *Ae. aegypti* mosquito has been reported to have developed resistance to the four major classes of neurotoxic chemical insecticides (carbamates, organochlorines, organophosphates and pyrethroids) [42,43], which includes 35 different active ingredients (Arthropod Pesticide Resistance Database (APRD) (https://www.pesticideresistance.org) [44]. The specific mechanisms of resistance have been divided into behavioral, physiological/cuticular, target site, and metabolic. Behavioral resistance is a change in feeding or resting behavior that causes mosquitoes to avoid contact with insecticide residues that otherwise would be lethal. For example, in Africa the overuse of insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS) has selected for populations of *Anopheles* mosquitoes that are exophilic (prefer to bite outdoors), feed earlier in the day, and/or switch from human to animal hosts [45,46]. Mosquitoes can also develop physiological/cuticular resistance in which the barrier properties of the cuticle are enhanced by increased expression of binding proteins or lipid reservoirs that degrade or sequester insecticides, and/or physical thickening of the cuticle that reduces insecticide penetration [47].

A heritable genetic change to the biochemical target of an insecticide that reduces susceptibility is target site resistance. Typically, mutations that confer target site resistance naturally exist among insect populations in very low frequency due to evolutionary tradeoffs.
However, the use of insecticides selects for individuals with the mutations. For example, the substitution of a single amino acid in the region coding the active site of the acetylcholinesterase gene *ace-1* confers mosquitoes with resistance to organophosphates and carbamates. Likewise, the phenomenon of knockdown resistance (*kdr*) reduces the sensitivity of mosquitoes to DDT and pyrethroids via point mutations in the voltage-gated sodium channel gene [48,49]. Metabolic resistance is caused by the overexpression of specific enzymes that accelerate xenobiotic detoxification. For example, the up-regulation of cytochrome P450 monooxygenases, glutathione S-transferases (GSTs), and hydrolases in resistant mosquitoes has been shown to enhance degradation of insecticides [48,49].

Another chemical-based approach to control mosquitoes is the use of repellents to minimize mosquito-human interactions. The most common repellent types used to date belong to two categories. First are contact repellents that are applied directly to the skin or clothing of the host and require close interactions of mosquitoes with the host. These include relatively non-volatile compounds, such as DEET (N,N-diethyl-meta-toluamide), picaridin, botanical derived IR3535 (ethyl butylacetylaminopropionate from beta-alanine), and PMD (para-methane-3,8-diol from oil of lemon eucalyptus). These repellents can be highly effective against *Ae aegypti* for a few to several hours depending on their relative volatility [50-52]. However, some of these compounds have drawbacks. DEET can cause skin irritation in adults, seizures in babies, and melt plastics [53-55]. It has also been shown that *Ae. aegypti* can develop resistance to DEET [56,57]. Moreover, picaridin can impair the development of aquatic vertebrates that prey on mosquito larvae, such as salamanders [58]. Second are spatial repellents that use passive emanators, coils or fabric impregnated with transfluthrin/ metofluthrin and other volatile chemicals to prevent
mosquitoes from entering spaces occupied by potential human hosts [52]. The specific modes of action for repellents are still under investigation but likely involve the modulation of odorant receptors in olfactory neurons, which lead to changes in mosquito behavior that decrease interactions with human hosts [52]. Given the evolution of resistance to the most common insecticides and the limited modes of action of existing insecticides and repellents used for mosquito control, new insecticides and repellents with novel modes of action are needed.

1.3 New tools: natural products and mode of action

During the mid-1930s to 1950s, synthetic insecticides, such as DDT, were developed to protect military personnel and agricultural crops. Although they were very effective, inexpensive, and in the case of DDT possessed broad-spectrum activity against insect pests with low acute toxicity to mammals [59], the reliance on a few synthetic insecticides led to numerous problems in human health, disruption of wildlife, destruction and contamination of the environment and the evolution of resistance in pests [60-62]. All of these problems and the re-emergence of diseases spread by *Ae aegypti* to new parts of the world in recent years, have increased the interest in plant-based insecticides with possible new modes of action compared to pyrethroids and the other neurotoxic insecticides [32].

Secondary metabolites of plants have been shaped by millions of years of natural selection to protect plants against herbivores, including insects. Thus, they represent a potentially exciting library of bioactive compounds to screen for the discovery of novel insecticides and repellents with a potentially safer use for the environment and human health, offering alternatives to synthetic insecticides and repellents[63-66]. From about sixty families of plants, extracts with insecticidal
The derived extract from the dried flower of *Tanacetum/Chrysanthemum cinerarifolium* Vis. or *C. coccineum* Willd species (Family: Asteraceae/Compositae), denominated pyrethrum, is the most commercially available natural insecticide to date [68-70]. Extractions of the flowers with hexane or other nonpolar solvents contain six active esters of which three (known as pyrethrins I fraction) are formed by one chrysanthemic acid and a pyrethrin I, cinerin I, or jasmolin I alcohol. And the other three (knowns as pyrethrins II fraction) are formed by one pyrethric acid and a pyrethrin II, cinerin II, or jasmolin II alcohol. When the extract is applied to insects it causes hyperactivity, convulsions and rapid knockdown, as a result of the activation of voltage-gated sodium channels at the axon level of the neuron [61,70]. This natural insecticide led to the development of one of the most commercially successful and effective synthetic insecticides, the pyrethroids, which are synthetic derivatives with improved insecticidal and photostability properties [71].

Other examples of insecticides or repellents developed from natural plant products include azadirachtin obtained from seeds of *Azadirachta indica* (Family: Meliaceae) the Indian neem tree [61]. Physiologically it acts by blocking the synthesis and release of ecdysteroid (the molting hormone) from the prothoracic gland and thereby inhibits complete ecdysis as a growth regulator in immature insects and causes sterility in adult females. However, it has also been reported to act efficiently as an antifeedant and is considered nontoxic to mammals, but its production is relatively expensive [71-74]. The extraction with organic solvents of roots, rhizomes and/or stems of tropical and subtropical species of legumes such as *Derris, Lonchocarpus,* and *Tephrosia* contained as major constituents’ rotenone and deguelin (isoflavonoids) [75]. The interference of the electronic transport chain and energy production at the mitochondria level made rotenone an effective
insecticide after ingestion [76,77]. But the acute toxicity to mammals and fish, and the persistence on food crops after application, limit this insecticide for industrial production [61].

Essential oils obtained from the steam distillation of different plants are hydrophobic liquids containing mixtures of volatile compounds that have been used as insecticides and in the case of Ae. aegypti as repellents. Some examples include citronella oil extracted from the leaves and stems of Cymbopogon species (lemongrass) [78,79] The main volatile constituents of this essential oil are monoterpenes (79.8%, citronellal, geraniol, γ-terpineol and cis-sabinene hydrate), sesquiterpenes (11.5%, (E)-nerolidol, β-caryophyllene and germacren-4-ol) and non-terpenic compounds (1.4%) [80]. Another example is the eucalyptus oil (Eucalyptus globus) which is composed of 1,8-cineole (62.5% found also in high abundance in Rosmarinus officinalis), α-pinene (18.5%), limonene (4.0%), and the sesquiterpenes aro-madendrene (3.1%), δ-cadinene (2.9%) and globulol (2.0%) [81,82]. Those essential oils are registered in the U.S. as natural repellents and are considered biopesticides that are non-toxic to humans.

The use of essential oils has increased given the potential environmental benefits, decreased residual actions, and economic benefits to diversify the insecticide and repellent market. The complex mixtures that essential oils possess are naturally organic constituents, which can be predominantly terpenes, terpenoids, oxides, and sesquiterpenes [83]. The specific role that those compounds play in insect-plant interactions is expected to provide a less toxic activity to humans, beneficial insects, and the environment. Preliminary screening of insecticide and repellent properties of different plant extracts and essential oils confirmed their efficacy against all species of mosquitoes, but further studies on identification of active compounds and their mode of action for the development of ecofriendly chemicals is needed [84-86].
1.4 Discovery of bioactive compounds from *Cinnamosma*

In the search for safer alternatives, attention has once again turned to botanicals and the higher plants from the order Canellales are being considered. This order consists of small to medium, aromatic, flowering trees, and comprises two different families: Canellaceae and Winteraceae [87,88]. Five different genera of about 16 different species are from Canellaceae [89], which are widely distributed. *Cinnamosma* is an endemic genus from Madagascar, *Warburgia* occurs in Africa, *Canella* is found in the Caribbean, and *Capsicodendron/Cinnamodendron* and *Pleodendron* are found across the Americas [90-95]. The family Winteraceae consists of five genera *Drimys, Tasmannia, Pseudowintera, Takhtajania*, and *Zygogynum* that respectively occur in the Philippines, Tasmania, New Zealand, South and Central America, and Madagascar [96,97]. Some species are used as aromatic plants, condiments, dietary supplements to prevent scurvy, and traditional medicines to treat fevers, colds, malaria, and various infections (fungal, bacterial and protozoal) [91,98,99]. Moreover, the common uses of the plants in traditional medicine suggest a potentially safe toxicological profile of the bioactive molecules in humans.

Studies on potential insecticides and antifeedants in plants of the order Canellales have demonstrated promising results to date. For example, extracts of different species from the family Winteraceae possess insecticidal and/or antifeedant activities against several lepidopteran, coleopteran, and hemipteran pests, as well as tick vectors [100-107]. And, extracts from species of the family Canellaceae have been shown to possess insecticidal and/or antifeedant activities against lepidopteran, coleopteran, and hemipteran pests [108-112].

The focus of the present thesis are plants of the genus *Cinnamosma* (family Canellaceae), which is comprised of three species (*Cinnamosma fragrans, C. madagascarensis, C. macrocarpa*)
that are used as traditional medicines for treating a wide range of conditions, including malaria, general fatigue, and muscle aches [113-115]. A previous study demonstrated that the essential oils from *C. madagascariensis* stem bark and leaves possessed larvicidal activity against *Culex quinquefasciatus* mosquitoes [116]. And, we recently found that the dichloromethane extract of the bark of *C. fragrans*, which is enriched with drimane-type sesquiterpenes, was larvicidal and adulticidal to *Ae. aegypti*, as well antifeedant and repellent to the adult female *Ae. aegypti* [117]. Thus, we hypothesized that *Cinnamosma* plants might possess a variety of phytochemical compounds with potential insecticidal and/or repellent activities for controlling mosquitoes.

In pursuit of identifying active compounds within the dichloromethane bark extract of *C. fragrans*, we found that the most abundant drimane sesquiterpene, cinnamodial (also known as ugandensidal, CDIAL, I; in Fig. 1) was primarily responsible for the insecticidal, antifeedant, and repellent bioactivities against *Ae. aegypti*. CDIAL is distinguished by the presence of two aldehyde functions, which make it a highly reactive molecule. It bears striking structural similarities to two other aldehyde-bearing drimane sesquiterpenes, polygodial (POLYG 9, first isolated from *Polygonum*) and warburganal (WARB 10, first isolated from *Warburgia*) (Fig. 1) [118-120], which have been shown to have potent antifeedant activity against lepidopteran and hemipteran pests [102,108,109,111,112,121-124], as well as insecticidal activity against lepidopteran and coleopteran pests [110,125,126]. Notably, we found that CDIAL was also insecticidal against larval and adult female *An. gambiae* and *Culex pipiens* mosquitoes and showed similar toxic potency against pyrethroid-resistant and pyrethroid-susceptible strains of *Ae. aegypti*, suggesting broad activity against mosquitoes and a novel mode of action from pyrethroids. We also revealed that CDIAL was an agonist of mosquito transient receptor potential A1 (TRPA1) channels, which
are noxious chemical receptors in animals. The agonism of TRPA1 channels was likely responsible for the antifeedant and repellent actions of CDIAL. However, TRPA1 modulation was not necessary for CDIAL’s insecticidal activity, suggesting distinct modes of insecticidal and antifeedant/repellent activity. [117].

In addition to CDIAL, we evaluated two others abundant drimane sesquiterpenes in the bark extract for insecticidal, antifeedant, and repellent activities against Ae. aegypti. Cinafragrin A (CFRAG, 2; Fig. 1) is a dimeric derivative of CDIAL containing a single aldehyde reducing its electrophilic charge, and cinnamosmolide (CMOS, 3; Fig. 1) is a lactone-bearing derivative of CDIAL (replacing the aldehyde with a γ-lactone ring in the structure). CFRAG and CMOS each exhibited relatively weak dose-dependent topical toxicity in adults compared to CDIAL, and their larvicidal efficacy was limited compared to CDIAL. In addition to CDIAL, CFRAG, and CMOS, the Rakotondraibe lab has identified several other drimane sesquiterpenes from Cinnamosma plants (Fig 1) such as cinnamolide (CML, 4), ugandensolide (UGA, 5) (first isolated from Warburgia species), drimenin (DRIM, 6) (first isolated from Drimys winterii), capsicodendrin (CPCD, 7 first isolated from Capsicodendron dinisii) and cinafragrolide (CFGL, 8), that have not been previously evaluated for insecticidal, antifeedant, or repellent activities against Ae. aegypti [127-131] [132] [133] [134,135]. Moreover, dichloromethane extracts of the roots or leaves of Cinnamosma plants have not previously been evaluated for these activities against Ae. aegypti. Thus, the evaluation of these other isolated sesquiterpenes and extracts is required to fully elucidate the potential of Cinnamosma plants as possible sources of new insecticides and repellents for mosquito control.
1.5 Research Objective and Hypothesis

With the exception of the bark extract, the insecticidal and antifeedant activities of root and leaf extracts of *Cinnamosma* species against *Ae. aegypti* have not been determined. Moreover, the numerous other drimane sesquiterpenes that the Rakotondraibe lab has identified in the bark and leaves of *C. fragrans* have not been previously tested against *Ae. aegypti*. The goal of my thesis research is to fill in these gaps of knowledge with the ultimate objective of identifying botanical-derived molecules that can potentially serve as prototypes for next-generation insecticides or repellents to control *Ae. aegypti* and its transmission of arboviruses. I hypothesize that the root and leaf extracts possess compounds in addition to CDIAL that may have potential for killing or repelling mosquitoes, and/or modulating the activities of CDIAL against mosquitoes.
Chapter 2. Insecticidal and antifeedant activities of Malagasy medicinal plant (*Cinnamosma* sp.) extracts and drimane-type sesquiterpenes against *Aedes aegypti* mosquitoes

2.1 Abstract

The overuse of insecticides with limited modes of action has led to resistance in mosquito vectors. Thus, insecticides with novel modes of action are needed. Secondary metabolites in Madagascan plants of the genus *Cinnamosma* (Canellaceae) are commonly used in traditional remedies and known to elicit antifeedant and toxic effects in insect pests. Here we test the hypothesis that extracts of *Cinnamosma* sp. enriched in drimane sesquiterpenes are toxic and/or antifeedant to the yellow fever mosquito *Aedes aegypti*. We show that the bark and root extracts, which contain a higher abundance of drimane sesquiterpenes compared to leaves, were the most efficacious. Screening isolated compounds revealed cinnamodial to be the primary driver of adulticidal activity, whereas cinnamodial (CDIAL, 1), polygodial (POLYG, 9), cinnafAGRin A (CFRAG, 2), and capsicodendrin (CPCD, 7) contributed to larvicidal activity. Moreover, cinnamosmolide (CMOS, 3), an abundant lactone in the root extract synergized the larvicidal effects of cinnamodial. Antifeedant activity of the extracts was primarily contributed to cinnamodial, polygodial, and cinnamolide. Parallel experiments with warburganal isolated from

---

1This chapter has been published in MDPI, *Insects*: Inocente, E.A.; Nguyen, B.; Manwill, P.K.; Benatrehina, A.; Kweka, E.; Wu, S.; Cheng, X.; Rakotondraibe, L.H.; Piermarini, P.M. Insecticidal and Antifeedant Activities of Malagasy Medicinal Plant (*Cinnamosma* sp.) Extracts and Drimane-Type Sesquiterpenes against *Aedes aegypti* Mosquitoes. *Insects* 2019, 10, 373. Doi: https://doi.org/10.3390/insects10110373
Warburgia ugandensis (WARB, 10, Canellaceae) revealed that aldehydes and a hydroxyl are critical for and modulate insecticidal activity, respectively. Our results indicate that plant drimane sesquiterpenes provide valuable chemical platforms for developing insecticides and repellents to control mosquito vectors.

2.2 Introduction

The mosquito Aedes aegypti (Linnaeus in Hasselquist, 1762) (Diptera: Culicidae) inhabits tropical and subtropical habitats worldwide. It is the predominant vector of numerous medically important arboviruses, such as dengue fever, chikungunya, yellow fever, and Zika, which infect hundreds of millions of people each year. The traditional approaches to combating Ae. aegypti using synthetic pesticides, such as pyrethroids and DDT, have potential side effects on both the environment and human health and their overuse has contributed to the development of insecticide resistance in the mosquito[43,136-138]

In recent years, interest in plant-based insecticides has grown because of this development of resistance and off-target effects of synthetic pesticides [60-62,139] Secondary metabolites of plants have been shaped by millions of years of natural selection to protect plants against herbivores, including insects. Thus, they represent a potentially exciting library of bioactive compounds to screen for the discovery of novel insecticides and repellents. Moreover, they offer alternatives to synthetic insecticides and repellents that are potentially safer for the environment and human health [63-66,139] Examples of insecticides or repellents developed from natural plant products include azadirachtin [73], citronellal [78], geraniol [79], p-menthane-3,8-diol [82], pyrethrum [67,71], nicotine, and ryanodine [140].
Recent studies by our group and others have demonstrated that plants of the genus *Cinnamosma* (Baill) (Canellaceae) (*C. fragrans*, *C. macrocarpa* and *C. madagascariensis*) are a potential source of novel insecticides and repellents for controlling mosquitoes [116,117]. These plants were first described by Henri Baillon as small trees with a pleasant smell that were widely distributed in the northern and eastern parts of Madagascar. The decoctions of the bark and root bark have a distinct pepper-like taste, and essential oils obtained from their different parts have been used by Malagasy people for generations as a traditional medicine for malaria, respiratory problems, muscle aches and gastrointestinal infections [113,114,141-143]. The use of *Cinnamosma* spp. as remedies for a wide range of ailments suggests that plants from this genus produce diverse and highly bioactive phytochemicals [114]. Moreover, the common uses of the plants in traditional medicine suggest a potentially safe toxicological profile of the bioactive molecules in humans.

We previously demonstrated that the dichloromethane extract of the bark of *C. fragrans*, which is enriched in pungent drimane sesquiterpenes, was insecticidal, antifeedant, and repellent to mosquitoes [117]. The most abundant drimane sesquiterpene in the extract, a dialdehyde known as cinnamodial (CDIAL, 1), was insecticidal against larval and adult female mosquitoes (*Ae. aegypti, Anopheles gambiae, Culex pipiens*). Notably, 1 exhibited similar toxic potency against pyrethroid-susceptible and pyrethroid-resistant strains of *Ae. aegypti*, suggesting a novel mode of action from pyrethroids. Our studies also revealed that 1 was an agonist of mosquito TRPA1 channels, which is the likely mechanism of antifeedant and repellent actions. However, TRPA1 modulation was not necessary for CDIAL’s insecticidal activity, suggesting distinct mechanisms of insecticidal and antifeedant/repellent activity. We also tested two other drimane sesquiterpenes in the bark extract: 1) cinafragrin A (CFRAG, 2), a dimeric derivative of 1 containing a single
aldehyde; and 2) cinnamosmolide (CMOS, 3), a lactone-bearing derivative of 1 (Fig. 1). Compounds 2 and 3 showed relatively weak bioactivities against mosquitoes compared to 1 [117].

Due to the promising bioactivities of the dichloromethane extract of *C. fragrans* bark and 1, here we evaluated the relative insecticidal and antifeedant activities of dichloromethane extracts from other parts of *Cinnamosma* plants (roots, leaves), which have not previously been determined. Furthermore, *Cinnamosma* spp. have been reported to produce numerous CDIAL-like sesquiterpenes (Fig. 1), including: lactone-bearing compounds, such as 3, cinnamolide (CML, 4), ugandensolide (UGAN, 5), and drimenin (DRIM, 6); dimers, such as 2, capsicodendrin (CPCD, 7) and the oxidation derivative cinnafragrolide (CFGL, 8); and another dialdehyde, polygodial (POLYG, 9) [114,127]. Here we evaluate the insecticidal and antifeedant activities of these molecules, which that have never been determined against mosquitoes.

Furthermore, to advance the structure-activity relationship (SAR) of 1, we evaluated the insecticidal and antifeedant activity of warburganal (WARB, 10; Fig. 1), a drimane sesquiterpene dialdehyde isolated from the bark of South-eastern African medicinal plant (*Warburgia ugandensis*, Canellaceae) closely related to *Cinnamosma* spp. Compound 10 has previously been shown to possess potent antifeedant activity against lepidopteran pests [100,122], but has not been tested in mosquitoes.

2.3 Materials and methods

2.3.1 Plant material and isolation of chemicals

Bark extract was prepared from *Cinnamosma fragrans* purchased in the market of traditional medicine in Analakely/Antananarivo (Madagascar), while the leaves and the root extracts were obtained from *Cinnamosma madagascariensis* collected in Mangoro region,
Madagascar. Isolation and structure elucidation of compounds 1-9 have been previously described [127-129]. In brief, the air-dried stem bark, roots, or leaves were pulverized and the powder (400 g) was extracted with dichloromethane for 5 days at room temperature. The extract was filtered and concentrated in vacuo to yield a yellow-brown oily residue (80.68 g, 20.2% on dry plant material). The residue was divided into 35 fractions (F01-35) using column chromatography over silica gel, eluting with a gradient system of hexanes–EtOAc (from 4:1 to 0:1). Ugandensolide (5) was crystallized by slow evaporation of F26 and the crystals rinsed with cold hexanes – EtOAc (1:1) to yield orange crystals (67.6 mg, 0.017 % on dry plant material). The structure of 5 was confirmed by spectral comparison to those previously published [132,133]. CDIAL(1) was recrystallized from fraction 15 using hexanes–EtOAc (1:1) as colorless crystals (5.28 g, 1.32 % on dry plant material). The fractions containing CPCD (7) (F22-23) were rinsed with ethyl acetate and the supernatant removed to obtain 7 as a white amorphous solid.

Warburganal (10) was isolated from the bark of Warburgia ugandensis collected in Emariete Village Forest in the Monduli Juu district in Arusha Region of Northern Tanzania and identified by Mr. Emmanuel Mboya of the Tropical Pesticides Research Institute (Arusha, Tanzania). A ground root bark sample of W. ugandensis (Canellaceae) (200.6 g) was macerated in dichloromethane, (3.5 L) over a period of four days, and the obtained extract was dried in vacuo. The extract (5.3 g) was fractionated on a silica gel column (40 cm height × 4.08 cm diameter) and eluted with hexanes: Ethyl acetate (4:1), followed by ethyl acetate (2.9 L), and methanol (1.5 L), affording 13 fractions. Fraction 9 was further applied on normal-phase silica gel (17.5 cm height × 1.7 cm diameter) eluted with hexanes: Ethyl acetate (4:1) and ethyl acetate, resulting in ten
fractions, WU-D-F9.1-10. Fraction Wu-D-F9.6 (2.3 mg) was identified as warburganal based on interpretation of NMR data and comparison with the literature [144].

2.3.2 Estimation of the amount of compounds by $^1$H MNR:

The $^1$H NMR spectra of Cinnamosma extracts were first measured to identify the signals of abundant compounds. The major compound was then selected and each of its proton signals were integrated (and calibrated to 1 for one proton signal). The amount of other compounds in the extract relative to the most abundant compound can thus be obtained by integration of all signals in the $^1$H NMR spectrum (except solvent). The percentage amount of most abundant compound was estimated by using the following equation:

\[
\text{Percentage of the major compound} = (1 - S) \times 100
\]

Where S= Sum of 1-proton signal integrations of the other identified compounds present in the $^1$H NMR.

Signal intensities with integration below 5% of the most abundant compound were not considered.

2.3.3 Mosquito colony

The present study utilized a colony of Ae. aegypti (Liverpool strain) derived from eggs which were obtained from the MR4 as part of the BEI Resources Repository, NIAID, NIH (LVP-IB12, MRA-735, deposited by M.Q. Benedict). Eggs were raised to adults as described previously [145]. 1st instar larvae and adult females (5 to 10 days post-eclosion) were used for bioassays.
2.3.4 Toxicology experiments

The larval toxicities of the extracts and isolated compounds were evaluated using an established assay [117,146,147]. In brief, five 1st instar larvae were placed into each well of a 24-well Falcon Multiwell plate (Becton Dickinson Labware, Franklin Lakes, NJ) containing 985 μl of dH2O and 5 μl of a food solution (13 mg/ml of finely ground fish food flakes in dH2O; Tetramin, Blacksburg, VA). Ten microliters of an extract or compound (all dissolved in 100% acetone) or 100% acetone (solvent control) were added to each well. In some experiments, 100% DMSO was used as the solvent (see Results). The plates were placed in a rearing chamber (28˚C, 80% relative humidity, 12:12 light:dark) for 24 h before larval toxicity was assessed. Larvae were considered dead if they did not move after prodding with a fine needle or pipette tip. Efficacy was defined as the percentage of larvae that died within 24 h after correcting for solvent effects using Abbot’s formula [148].

Adulticidal efficacy was evaluated using an established assay [117,146,149]. After immobilization on ice, groups of 10 adult female mosquitoes (5–10 days post-emergence) were topically treated with 500 nl of an extract or compound (dissolved in 100% acetone) or 100% acetone (solvent control) and transferred to small cages (16 oz. containers) with access to 10% sucrose. The compounds or solvent were delivered to the thorax of mosquitoes with a repeating dispenser (PB600-1, Hamilton, Reno, NV). The cages were provided with cotton wicks soaked in 10% sucrose and held in a rearing chamber (28˚C, 80% relative humidity, 12:12 light:dark) for 24 h before assessing toxicity. Efficacy was defined as the percentage of mosquitoes that were
incapacitated (i.e., dead or unable to fly) within 24 h [117,146,149] after correcting for solvent effects using Abbott's formula [148].

In some experiments the compounds were injected directly into the hemolymph of adult females using a pulled-glass capillary needle and Nanoject II injector (Drummond Scientific Company, Broomall, PA). Each mosquito received an injection of 500 nl of phosphate buffered saline (Thermo Scientific, Waltham, MA) containing 3 mM of a compound (1.5 nmol/mosquito) or 3% DMSO (the solvent control). The efficacy was determined 24 h after injection as described above.

2.3.5 Antifeedant experiments

Antifeedant activity was assessed using a capillary feeding (CAFE) choice assay [117,150]. Adult female mosquitoes (5–10 days post-emergence) were starved for 24 h but provided with water-soaked cotton. Groups of five mosquitoes were transferred to Drosophila vials (28.5 x 95 mm; VWR International, Radnor, PA) and covered with cotton plugs containing two holes to allow for the insertion of 5-μl calibrated glass capillaries (VWR International). The control capillary was filled with 5 μl of 10% sucrose containing 0.01% trypan blue (to provide contrast) and 1% acetone (the solvent). The treatment capillary was filled with 5μl of 10% sucrose containing 0.01% trypan blue and an extract or isolated compound dissolved in 100% acetone. In some experiments, 100% DMSO was used as the solvent (see Results). The capillaries were capped with mineral oil to minimize evaporative losses. Vials with capillaries but no mosquitoes were also included to account for evaporative losses. The vials were held in a rearing chamber (28°C, 80% relative humidity, 12:12 light:dark) for 18–20 h before measuring the volume of sucrose remaining in each
capillary. After correcting for evaporation, the antifeedant index was calculated by subtracting the volume consumed from the treatment capillary from that of the control capillary and dividing by the total volume consumed from both capillaries [151]. The mean antifeedant indices were compared using GraphPad Prism (version 7) software with a one-way ANOVA and Tukey’s multiple comparisons test.

2.3.6 Computational docking

CDIAL (1) and CML (4) were docked to a potential binding pocket centered around Cys684 in a structural model of An. gambiae TRPA1 (AgTRPA1; AGAP004863) that was built and described previously [152] using a combination of homology and ab initio modeling approaches based on the human TRPA1 structure (PDB accession number 3J9P). A 96×68×78 grid box with a grid spacing of 0.375 Å centered around Cys684 defined the region of the protein that ligands would explore. 500 docking runs were performed for each ligand. All docking calculations were performed with the Lamarckian genetic algorithm using Autodock 4.2 [153].

2.4 Results

2.4.1 CDIAL and/or CMOS are the major drimane sesquiterpenes in leaves, bark and roots.

The drimane-sesquiterpene content of the dichloromethane extracts of leaves, bark, and roots of Cinnamosma spp. were profiled using one-dimensional 1H NMR (Fig. 2A-C). Compounds in each extract were identified using our previously isolated compounds CDIAL (1) and CMOS (3) as reference spectra (Fig. 2D&E, respectively). Results showed that the bark extract (Fig. 2A) contained 1 as the major drimane constituent with a small amount (<5%) of 3 (Table 1). CPCD.
(7), and POLYG (9) were also present, but in small amounts. The relative abundance of 1 in the bark extracts was 60% of total detectable/identifiable sesquiterpenes (Table 1). The $^1$H NMR spectrum of the root extract (Fig. 2B) showed the presence of 1 and 3 in a 2:3 ratio, while the leaves (Fig 2C) showed a high amount of 3 (Table 1) and a small amount of a seco-triterpene derivative previously isolated from *C. fragrans* [154]. Leaves did not have detectable amounts of 1 (Table 1).

Table 1: Relative abundance of compounds 1 and 3 in the extracts of *Cinnamosma* species. Percentages were determined using integration of the $^1$H signals.

| Plant extract | Relative abundance of compounds |
|---------------|-------------------------------|
|               | 1    | 3     |
| Bark          | ~60% | < 5%  |
| Root          | ~30% | ~40%  |
| Leaves        | -    | ~30%  |
2.4.2 Relative insecticidal activities of plant extracts and isolated drimane sesquiterpenes against larval and adult female mosquitoes

We first compared the insecticidal activity of the bark, root, and leaf extracts of Cinnamosma plants against adult female and 1\textsuperscript{st} instar larval Ae. aegypti (Liverpool, LVP, strain). As shown in Fig. 3A, at the screening concentration (2.5 µg/mosquito), the bark extract had the strongest adulticidal efficacy within 24 h (90%) followed by the root (52%) and leaf (13%) extracts. As shown in Fig 3B, at the screening concentration (50 µg/ml), the bark and root extracts each had strong larvicidal efficacy within 24 h (~75%) that were significantly more efficacious than the leaf extract (~0.9%).

Figure 2. $^1$H NMR spectra of Cinnamosma extracts, CDIAL (1) and CMOS (3). A: bark extract, B: root extract, C: leaf extract, D: CDIAL and E: CMOS
We next screened drimane sesquiterpenes isolated from the bark and root extracts to determine which compounds contributed to their adulticidal and larvicidal activities. In addition, we tested WARB (10) isolated from W. ugandensis. In adult females (Fig. 3C), at the screening dose (5 nmol/mosquito), WARB (10) and CDIAL (1) exhibited the strongest efficacy, whereas POLYG (9), CFRAG (2), and CFGL (8) were moderately to weakly toxic; CML (4), UGAN (5), CMOS (3), DRIM (6), and CPCD (7) were nominally toxic (Fig. 3C). To determine if the significantly lower topical adulticidal activity of 9 relatives to 1 was due to weaker cuticular penetration, we injected each into the hemolymph of adult females (1.5 nmol/mosquito). The toxic efficacy of 9 (49.86% ± 4.84; N = 7) within 24 h was significantly lower (unpaired t-test; P < 0.05) than that of 1 (84.19% ± 6.34; N = 16) when delivered by injection.

In larvae (Fig. 3D), at the screening concentration (100 µM), WARB (10), CDIAL (1), and CFRAG (2) were among the most toxic, while POLYG (9) and CPCD (7) were moderately efficacious; CML (4), UGAN (5), CMOS (3), DRIM (6), and CFGL (8) were among the least toxic. Previously, we found that 7 dissociated into monomers of 1 in DMSO [155]. Thus, in a parallel experiment we directly compared the larvicidal efficacy of 7 when dissolved as a stock solution in acetone vs. DMSO. The 24 h larvicidal efficacy of 7 from a DMSO stock (100% ± 0.0%; N = 6) was significantly greater (P < 0.01, unpaired t-test) than that from an acetone stock (55.5% ± 11.1%; N = 6).
Figure 3. Insecticidal efficacy of plant extracts and isolated drimane sesquiterpenes to adult female (A, C) and larval (B, D) *Ae. aegypti* (LVP strain). A, C) Adulticidal efficacy was defined as the percentage of adults (after Abbott’s correction) that were incapacitated (dead or flightless) within 24 h when extract (2.5 µg/mosquito) or compounds (5 nmol/mosquito) were applied to the thoracic cuticle of adult females. Values are means ± SEM; N= number of independent replicates of 10 females each. B, D) Larvicidal efficacy in 1st instar larvae was defined as the percentage (after Abbott’s correction) that died within 24 h when extract (50 µg/ml) or compounds (100 µM) were added to the rearing water (100 µM). Values are means ± SEM; N= number of independent replicates of 5 larvae each. In all panels, lower-case letters indicate statistical categorization of the means as determined by a one-way ANOVA and Tukey’s multiple comparisons test. (P < 0.05).

Given that the root extract exhibited similar larvicidal activity as the bark extract (despite a lower abundance of 1), we tested for potential synergy between 1 and 3; the latter is highly abundant in the root extract (Table 1). When tested individually, 50 µM CDIAL (1) or 75 µM CMOS (3) resulted in 18% or 5% larvicidal efficacy within 24 h, respectively (Fig. 4). When tested together at these concentrations they resulted in 37% larvicidal efficacy (Fig. 4), which was
significantly greater than CDIAL and ~1.5-times larger than expected based on the sum of their individual activities (~23%).

![Bar graph showing synergism between CDIAL (1) and CMOS (3) against 1\textsuperscript{st} instar larvae of \textit{Aedes aegypti}. Larvicidal efficacy in 1\textsuperscript{st} instar larvae was defined as the percentage (after Abbott's correction) that died within 24 h after adding CDIAL (50 µM), CMOS (75 µM), or the combination of CDIAL (50 µM) and CMOS (75 µM) to the rearing water. Values are means ± SEM; N=number of independent replicates of 5 larvae each.](image)

Figure 4. Synergism between CDIAL (1) and CMOS (3) against 1\textsuperscript{st} instar larvae of \textit{Aedes aegypti}. Larvicidal efficacy in 1\textsuperscript{st} instar larvae was defined as the percentage (after Abbott’s correction) that died within 24 h after adding CDIAL (50 µM), CMOS (75 µM), or the combination of CDIAL (50 µM) and CMOS (75 µM) to the rearing water. Values are means ± SEM; N=number of independent replicates of 5 larvae each.

2.4.3 Relative antifeedant activity of plant extracts and isolated drimane sesquiterpenes against adult female mosquitoes

To assess the relative antifeedant activity of the bark, root, and leaf extracts against adult female \textit{Ae. aegypti} (LVP strain), we used a capillary feeding (CAFE) choice bioassay [117,150,156]. In brief, this assay compares the consumption of 10% sucrose by mosquitoes from two glass capillaries over an 18-20 h period: a control capillary is treated with the solvent (1% acetone or DMSO) and a treatment capillary is treated with an extract or isolated compound. Mosquitoes consumed significantly less sucrose from the capillaries treated with 50 µg/ml bark or root extract.
vs. control capillaries (Fig. 7), indicative of antifeedant activity. On the other hand, mosquitoes consumed similar volumes of sucrose from the capillaries treated with the leaf extract vs. the control capillaries (Fig. 7), indicative of nominal antifeedant activity. As demonstrated in Fig. 5A, the antifeedant indices of the bark and root extracts were similar to each other and significantly greater than that of the leaf extract.

To determine which compounds contributed to the antifeedant activity, we performed similar assays with the compounds isolated from the bark and roots. In addition, we tested WARB (10) isolated from the bark of W. ugandensis. Mosquitoes consumed significantly less sucrose from the capillaries treated with 1 mM POLYG (9), WARB (10), CDIAL (1), or CML (4) vs. control capillaries (Fig. 8), indicative of antifeedant activity. Mosquitoes consumed similar volumes of sucrose from capillaries treated with 1 mM UGAN (5), CMOS (3), DRIM (6), CPCD (7), CFRAG (2) or CFGL (8) vs. control capillaries), indicative of nominal antifeedant activity (Fig. 8). As shown in Fig. 5B, the antifeedant efficacies of CDIAL (1), POLYG (9), WARB (10), and CML (4) exhibited similar among one another and significantly greater than the other compounds.

In a parallel experiment we directly compared the antifeedant efficacy of 250 µM CPCD (7) when dissolved as a stock solution in acetone vs. DMSO. When using acetone as the solvent, mosquitoes consumed similar volumes of sucrose from capillaries treated with 7 vs. control capillaries (paired t-test; P = 0.98). However, when using DMSO as the solvent, mosquitoes consumed significantly less sucrose from the capillaries treated with 7 vs. the control capillaries (paired t-test; P < 0.05). As such, the antifeedant index of 7 from a DMSO stock (0.20 ± 0.07; N
was significantly greater (\( P < 0.01 \), unpaired t-test) than that of an acetone stock (-0.02 ± 0.07; \( N = 10 \)).

Figure 5. Antifeedant activity of plant extracts (A) and isolated drimane sesquiterpenes (B) in choice CAFE assays with adult female \( Ae. \) aegypti (LVP strain) mosquitoes. At the time of feeding, each group of 5 mosquitoes was offered two glass capillaries filled with 10% sucrose and 0.01% trypan blue. The control capillary included 1% acetone (the solvent), and the treatment capillary included 1% acetone and an extract (50 \( \mu g/ml \)) or a drimane sesquiterpene (1 mM). The difference in volume consumed between the capillaries was used to calculate the antifeedant activity. See Figs. 7 and 8 for consumption volumes. Values are means ± SEM; \( N \) = number of independent replicates of 5 mosquitoes each. Lower-case letters indicate statistical categorization of the means as determined by a one-way ANOVA and Tukey’s multiple comparisons test (\( P < 0.05 \)).

2.4.4 Computational docking of CML and CDIAL to \( AgTRPA1 \)

To test whether CML (4) can potentially bind mosquito TRPA1 channels in a similar manner to CDIAL (1), we computationally docked both to the putative binding pocket centered around Cys684 in \( AgTRPA1 \) that was identified and described previously [152]. The docking results for 1 reveal that both the C-12 and C-11 aldehyde groups are located near Lys678, which makes these sites easy to be attacked by the lysine’s amino group to form a covalent adduct (Fig. 6). In contrast, 4 adopts a different binding pose from 1 (Fig. 6) and binds \( AgTRPA1 \) less effectively than 1 as suggested by their binding scores (\( 4 = -4.9 \) kcal/mol, 1 = -7.2 kcal/mol).
Additionally, although the lactone moiety of 4 stays close to Lys678 and forms a hydrogen bond with the lysine’s amino group, the lactone carbonyl group is less electrophilic than the aldehydes, and its orientation makes it less reactive towards the lysine amino group.

![Structural models of CML (4) or CDIAL (1) in the putative CDIAL-binding site of AgTRPA1. Potential interactions between ligand (yellow) and residues of AgTRPA1 (cyan) as predicted by computational docking are shown. Several nearby residues are labeled and shown in licorice representation.](image)

Figure 6. Structural models of CML (4) or CDIAL (1) in the putative CDIAL-binding site of AgTRPA1. Potential interactions between ligand (yellow) and residues of AgTRPA1 (cyan) as predicted by computational docking are shown. Several nearby residues are labeled and shown in licorice representation.

2.5 Discussion

The present study was the first to compare the insecticidal and antifeedant activities of dichloromethane extracts from different parts of *Cinnamosma* plants and drimane sesquiterpenes isolated from these extracts against mosquitoes. Our results advance our understanding of the potential use of these plants as sources of natural insecticides and repellents for mosquito control and the chemical features of drimane sesquiterpenes that contribute to their insecticidal and/or antifeedant bioactivities against mosquitoes.
2.5.1 Insecticidal activity of plant extracts and isolated compounds

The acute topical toxicity experiments against adult female *Ae. aegypti* revealed that root and bark extracts of *Cinnamosma* spp. were significantly more toxic than leaf extracts (Fig. 3A). This trend extends to insecticidal efficacy against first instar larvae where we found that the bark and root extracts were stronger insecticides than the leaf extracts from these plants (Fig. 3B). Our results are similar to those reported by Pavela *et al.* [116] who found that essential oil from the bark of *C. madagascarensis* was more toxic to larval *Culex quinquefasciatus* compared to essential oil from the leaves. Thus, the bark and roots of *Cinnamosma* sp. appear to be the major sites of insecticidal compound production. However, this trend does not apply to all plants, because in some species leaves have been known to produce insecticidal compounds (e.g., [110,141,157]).

The relative insecticidal activities of the bark, root, and leaf extracts can largely be explained by differences in the relative concentration of CDIAL (1) within the extracts. In the bark, 1 was highly abundant and composed more than 60% of the total sesquiterpenes. On the other hand, 1 only composed ~30% of the total sesquiterpenes in the root extract and was not detectable in the leaf extract. As such, the adulticidal efficacy of the root extract was ~50% lower than the bark extract and the leaf extract was nominally efficacious (Fig. 3A). Previously we demonstrated that 1 was a superior adulticidal compound against *Ae. aegypti* compared to CFRAG (2) and CMOS (3) [117]. In the present study, 1 was the only compound isolated from the bark and root extracts of *Cinnamosma* to elicit >50% efficacy in adult females at the screening dose (Fig. 3D; note WARB was isolated from *W. ugandensis*). Thus, our results suggest that CDIAL is the principal active component of the *Cinnamosma* bark and root extracts responsible for their adulticidal activities.
In contrast, the larvicidal activity of the root extract is more complex. Notably, despite the lower 1 content of the root extract, its larvicidal efficacy was surprisingly similar to that of the bark extract within 24 h. One possible explanation for this result is synergism of 1 with another abundant compound in the root extract, such as 3. Remarkably, when added to the larval rearing water in a similar molar ratio as found in the root extract, we found that the efficacy of 1 and 3 were ~1.5-times greater than the summation of their individual efficacies, suggesting synergy between the two compounds. It is unclear how 3 enhances the efficacy of 1, but it has been found that some essential oils of plants are more active than the isolated major compounds against larval *Ae. aegypti* and that some essential oils are able to synergize the toxicity of established insecticides by inhibiting detoxification mechanisms, such as cytochrome P450s and glutathione S-transferases [158-161]. Thus, one hypothesis to test in future studies is that 3 enhances the insecticidal activity of 1 by inhibiting detoxification systems in a similar fashion as essential oils.

Another potential, and not mutually exclusive, explanation for the greater than expected larvicidal efficacy of the root extract is that other bioactive components in the root extract were toxic to larvae. Consistent with this notion, 1 was not the only *Cinnamosma*-derived compound to exhibit strong larvicidal efficacy within 24 h at the screening concentration. 2 was similarly larvicidal to 1, confirming our earlier study [117]. In addition, POLYG (9) and CPCD (7) exhibited >50% efficacy at the screening concentration. Thus, in addition to 1, other aldehyde-bearing drimane sesquiterpenes in the root extract, such as 9, 7, and 2 may contribute to the greater than expected larval toxicity, especially if their toxicity is also synergized by 3. However, the abundances of these other active compounds in the root extract are very low compared to 1 (Fig. 2).
Previously, the Rakotondraibe laboratory demonstrated that the weakly-bioactive dimer 7 dissociated into highly bioactive monomers of 1 and the presence of DMSO enhanced this dissociation [155]. Consistent with the previous study, we found that 7 was significantly more larvicidal when its stock solution was prepared in DMSO vs. acetone, suggesting 7 is a pro-insecticide that requires dissociation into 1 before becoming toxic. This phenomenon may explain why 7 prepared in 100% acetone was relatively non-toxic when applied topically to the hydrophobic cuticle of adult females. That is, on the cuticle 7 would not have an opportunity to dissociate into 1 unless it was rapidly absorbed into the aqueous hemolymph. Compared to 7, the dimer 2 is more stable and does not dissociate into monomers [128]. Thus, its prominent larvicidal activity suggests it is bioactive in its dimeric form. Consistent with this notion we have previously observed that 2 acutely activates mosquito TRPA1 channels expressed heterologously in Xenopus oocytes [117].

In addition to direct toxic effects of the drimane sesquiterpenes on larval mosquitoes, we cannot rule out that some of the compounds are indirectly toxic to larvae by influencing the microbial composition in their rearing water and/or guts. Previous studies on Ae. aegypti have shown that the gut microbiota, which they acquire from their rearing water, strongly influence mosquito development and survival [162,163]. Moreover, essential oils of C. fragrans and some sesquiterpenes have antibiotic properties [164-168]. Thus, future studies should explore whether 1 and other drimane sesquiterpenes in the bark and root extracts influence the microbial composition of the rearing water and/or gut in a manner that would cause acute larval toxicity or perturb development.
2.5.2 Insights into the insecticidal SAR of CDIAL-like drimane sesquiterpenes

Comparing the structures and relative insecticidal activities of the monomeric drimane sesquiterpenes allows for valuable insights into the insecticidal SAR of these molecules. First of all, only the aldehyde-bearing monomers (1, 9, 10) were insecticidal, whereas all of the lactone-bearing monomers (3-6) were nominally toxic. Notably, 3 and 4 are identical to 1 and 9, respectively, with the exception of the lactone functions replacing the aldehydes (Fig. 1). Thus, the presence of highly reactive aldehydes appears to be essential for the larvicidal and adulticidal bioactivities of these molecules.

Comparing the structures of the insecticidal monomers (1, 9, 10), which are nearly identical with the exception of a hydroxyl (-OH) on C-9 in 1 and 10, and an acetyl (-OAc) on C-6 in 1 (Fig. 1), reveals that the -OH plays a key role in modulating insecticidal activity. That is, the insecticidal efficacies of 1 and 10, which possess the -OH are 1-3 times greater than 9. Given that 9 was still less efficacious than 1 when injected into the hemolymph, the -OH group does not appear to enhance cuticular penetration. Instead, the -OH group likely enhances toxicity of the molecule to mosquitoes by enhancing interactions with its molecular target(s) and/or making it a poorer substrate for mosquito detoxification systems (e.g., cytochrome P450s). Future studies on the mode of 1’s insecticidal action and how it is detoxified by mosquitoes will be required to fully elucidate how the -OH group modulates insecticidal activity. On the other hand, the -OAc on C-6 does not appear to enhance or detract from the insecticidal efficacy against larvae or adults given the similar activities of 1 and 10.
2.5.3 Antifeedant activity of plant extracts and isolated drimane sesquiterpenes

Similar to the trend found for insecticidal activity, the antifeedant efficacies of the bark and root extracts were significantly stronger than that of the leaf extract. Thus, the bark and roots of *Cinnamosma* species also appear to be the major sites of antifeedant compound production. In other plants, the leaves also produce antifeedant compounds [141,169,170].

Similar to the relative larvicidal activity of the extracts, the relative antifeedant efficacies of the extracts cannot completely be explained by differences in the relative concentrations of 1 within the extracts. Previously, and consistent with our current results, we have shown that 1 is a more effective antifeedant than 2 and 3 and is likely the principal antifeedant component of the bark extract [117]. However, the root extract produced a similar antifeedant effect as the bark extract despite an ~50% lower concentration of 1. Thus, other compounds likely contribute to the antifeedant activity of the root extract. Consistent with this notion, 9 and 4 exhibited comparable antifeedant efficacy to 1. However, given the low abundances of these compounds compared to 1, we also cannot rule out potential synergistic effects of the antifeedant compounds with each other and/or the less active lactones and dimers.

In the present study, the dimer 7 was not antifeedant when prepared as a stock solution in 100% acetone, suggesting the compound does not substantially dissociate into monomers of 1 in the 10% sucrose solution over the 18 h course of the experiments or acutely alter feeding behavior after ingestion by the mosquito. However, when prepared as a stock solution in DMSO, the antifeedant activity of 7 was unmasked, suggesting that dissociation into 1 was necessary for bioactivity. These results are consistent with those from the aforementioned larval toxicity experiments that demonstrated DMSO enhanced the larvicidal activity of 7.
2.5.4 Insights into antifeedant SAR of CDIAL-like drimane sesquiterpenes

Comparing the structures and relative antifeedant activities of the monomeric drimane sesquiterpenes allows for valuable insights into the antifeedant SAR of these molecules. Notably, all of the aldehyde-bearing compounds (1, 9, 10) were antifeedant to mosquitoes, whereas all of the lactone-bearing compounds, except for 4, were nominally antifeedant. We have previously demonstrated that the relative antifeedant activities of 1, 2, and 3 were correlated with their relative agonism of mosquito TRPA1 channels and that TRPA1 was essential for the antifeedant activity of 1 [117]. The strong antifeedant activity of 9 and 10 is likely due to agonism of TRPA1 channels, because both are potent agonists of vertebrate TRPA1 channels [171,172] and known antifeedants against a wide variety of insects [108,109,173-175]. The electrophilic aldehyde groups of 1, 9, and 10 are likely critical for interacting with nucleophilic cysteine and lysine residues in the NH2-terminal domain of TRPA1 channels [117,176-178]. The similar antifeedant efficacies of these compounds suggest that the -OH on C-9 (1, 10) and the -OAc on C-6 (1) do not substantially modulate the antifeedant activity and by extension the agonism of mosquito TRPA1. In contrast, as mentioned above, the presence of the -OH on C-9 enhanced insecticidal activity. These divergent results are consistent with the idea that 1 has distinct mechanisms of insecticidal and antifeedant action [117].

Whereas agonism of TRPA1 likely explains the strong antifeedant activities of 1, 9, and 10, the antifeedant activity of 4 is puzzling. 4 is a natural lactone derivative of 9 wherein the electrophilic aldehydes are replaced with a relatively less reactive lactone function (Fig. 1). This substitution would be expected to make 4 an inferior agonist of TRPA1 channels and antifeedant, as we have previously observed for 3, the lactone derivative of 1 [117]. Consistent with this notion,
computational docking simulations of 4 with AgTRPA1 revealed that although it has potential to fit in the pocket near Cys684, its binding pose/strength and reactivity suggest it is weaker agonist compared to 1 (Fig. 6). Furthermore, CML does not induce detectable agonism of heterologously-expressed TRPA1 channels from vertebrates [172]. However, the antifeedant efficacy of 4 was similar to 1, 9, and 10. Moreover, in the hemipteran pests Bemisia tabaci and Myzus persicae, 4 was antifeedant albeit with apparent less potency than 9 [179]. Thus, our results suggest that the mechanism of 4’s antifeedant activity is distinct from 1, 9, and 10, and remains to be elucidated.

In humans, 4 has been shown to interact with acetylcholine receptors [180], and possess cytotoxic activity [154]. Whether 4 can interact with similar receptors or have similar activities in insects to induce antifeedant or repellent behaviors remains to be determined.
3.1 Summary

The *Ae. aegypti* mosquito is expected to expand its range in the Western hemisphere likely resulting in increased transmission of arboviral diseases that it vectors. The control of the transmission of arboviral diseases by mosquitoes is difficult due to the rapid development of resistance to insecticides with limited modes of action. This thesis has been divided into two major sections. Chapter 1 provided a general introduction to the *Ae. aegypti* mosquito and rationale for discovering insecticides with novel modes of action. Chapter 2 focused on the first study and experimental work of evaluating extracts and compounds of Malagasy plants in the genus *Cinnamosma* sp. as potential insecticides and antifeedants to control mosquitoes.

As mentioned in Chapter 1, plant secondary metabolites have evolved as a chemical defense strategy against herbivores. Only a small fraction of plant species has been tested for potential insecticidal and repellent activities against mosquitoes. However, these efforts have discovered and led to the development of some highly effective active molecules. For example, the development of synthetic pyrethroids, one of the most widely used insecticides, was inspired by pyrethrin, which is a natural secondary metabolite produced by flowers of *Chrysanthemum cinerariifolium*. Thus, screening the insecticidal properties of secondary compounds from higher plants can lead to the discovery of new insecticides and repellents to control mosquitoes.
In Chapter 2, I compare for the first time the insecticidal and antifeedant activities of extracts prepared from different parts of *Cinnamosma* plants (order Canellales) against *Ae. aegypti*. The results in this chapter also provide the first insights into the toxicity of several drimane sesquiterpenes extracted from *Cinnamosma* plants against *Ae. aegypti*. The abundances of various sesquiterpenes varied among the dichloromethane extracts of the bark, roots, and leaves, resulting in varying insecticidal and antifeedant activities. It was first demonstrated that the bark extract possessed the highest concentration of CDIAL 1, which was highly toxic to adult and larval *Ae. aegypti*, and was a strong antifeedant to adult mosquitoes. The root extract possessed about 50% less CDIAL than the bark, but a much higher concentration of CMOS 3. This resulted in a 50% lower adulticidal activity compared to the bark, but the larvicidal and antifeedant activities were similar to the bark. Possible reasons for the similar larvicidal and antifeedant activities were attributed to other compounds in the root extract such as CFRAG, POLYG, CPCD and CML. Moreover, we discovered a potential synergistic effect of CMOS on the larvicidal activity of CDIAL, which could explain the higher than expected activities of the root extract.

In Chapter 2, we also compared for the first time the insecticidal and antifeedant activities among three dialdehyde-bearing drimane sesquiterpenes that are very similar in structure: CDIAL, WARB and POLYG. CDIAL and WARB were highly effective insecticidal molecules compared to POLYG, which was attributed to the presence of a hydroxyl group that is present in CDIAL and WARB, but not POLYG. The antifeedant activities of the molecules were similar, which was attributed to strong interactions with TRPA1 channels. Thus, the screening of plant extracts and isolated compounds in Chapter 2 has provided exciting new lead structures for insecticide and repellent development.
3.2 Future directions

The results of this thesis indicate that novel secondary compounds from *Cinnamosma* plants have potential for development into insecticides with new modes of action. But there are additional questions to research to further develop these compounds and evaluate their potential as public health insecticides. Some other considerations to be pursued are described below:

- Is CMOS the only drimane sesquiterpene that synergizes the activity of CDIAL? In Chapter 2 we found that the larvicidal activity of CDIAL was significantly enhanced to a level greater than expected, when tested together with CMOS, suggesting synergism. But what about other weakly insecticidal drimane sesquiterpenes in the root extracts of *Cinnamosma* species, such as CML, UGAN, and DRIM? Do they also synergize the insecticidal activity of CDIAL? And, does CMOS have the potential to synergize conventional insecticides, such as pyrethroids? If CMOS proves to be the only synergistic compound, what is its mode of action? Does it inhibit detoxification and/or excretion by the mosquito? Further addressing these questions may allow for the development of novel synergists for enhancing natural and/or synthetic insecticides for mosquito control and assist in overcoming insecticide resistance.

- Are the larvicidal activities of the drimane sesquiterpenes associated with an indirect effect on the mosquito microbiome? The mosquito gut microbiota are affected by the composition of the aquatic environment and if this natural composition is disrupted by antibiotics then mosquito development and survival can be perturbed [162,163,181,182]. Previous studies have shown that some drimane sesquiterpenes possess antibiotic properties [164-168]. Thus, future studies should explore whether *C. fragrans* extracts and isolated drimane...
sesquiterpenes influence the microbial composition of the rearing water and subsequently the gut microbiota of the mosquito. This could potentially explain the larvicidal mode of action and the similar larvicidal efficacy between the root and bark extracts.

- What is the mode of antifeedant action for the lactone CML? As was shown in Chapter 2, the antifeedant activity of the lactone CML was similar to that of the dialdehyde CDIAL, but CML was predicted to be an inferior agonist of TRPA1 compared to CDIAL. Thus, the mode of CML’s antifeedant activity is current unknown. Future studies that elucidate the binding activity of CML to potential molecular targets that drive antifeedant activity (e.g., odorant receptors) will allow for a better understanding of the mode of antifeedant activity and can potentially lead to the development of novel repellents.

Thus, further elucidation of the structure–activity relationships of these molecules, their potential synergies, and modes/mechanisms of action will facilitate the development and formulation of next-generation insecticides and repellents for mosquito control.
1. Christophers, S. *Aëdes aegypti (L.) the Yellow Fever Mosquito: its Life History, Bionomics and Structure*; London: The Syndics of the Cambridge University Press, Bentley House, 200, Euston Road, N.W.1.: 1960; pp. xii + 739 pp.
2. Clements, A.N. *The biology of mosquitoes: development, nutrition and reproduction*; Vol. 1.
3. Ross, R. Report on a Preliminary Investigation into Malaria in the Sigur Ghat, Ootacamund. *Indian medical gazette* 1898, 33.
4. Reed, W.; Carroll, J.; Agramonte, A. The Etiology Of Yellow Fever.: An Additional Note. *Journal of the American Medical Association* 1901, XXXVI, 431-440, doi:10.1001/jama.1901.52470070017001f.
5. Mullen, G.R.; Durden, L.A. *Medical and veterinary entomology*; Academic press: 2009.
6. Clements, A.N. *The biology of mosquitoes, Volume 3 Transmission of viruses and interactions with bacteria*; Cabi: 2012; Vol. 3.
7. Hahn, M.B.; Eisen, L.; McAllister, J.; Savage, H.M.; Mutebi, J.-P.; Eisen, R.J. Updated Reported Distribution of *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* (Diptera: Culicidae) in the United States, 1995–2016. *Journal of Medical Entomology* 2017, 54, 1420-1424, doi:10.1093/jme/tjx088.
8. Liu-Helmersson, J.; Brännström, Å.; Sewe, M.O.; Semenza, J.C.; Rocklöv, J. Estimating Past, Present, and Future Trends in the Global Distribution and Abundance of the Arbovirus Vector *Aedes aegypti* Under Climate Change Scenarios. *Frontiers in Public Health* 2019, 7, doi:10.3389/fpubh.2019.00148.
9. Bacot, A. The Effect of the Presence of Bacteria or Yeasts on the Hatching of the Eggs of *Stegomyia fasciata* (the Yellow Fever Mosquito). *Journal of the Royal Microscopical Society* 1917, 1917, 173-174.
10. Roubaud, É. Sur l'hibernation de quelques mouches communes. *Bulletin de la Société entomologique de France* 1927, 32, 24-25.
11. Shannon, R.; Putnam, P. The Biology of Stegomyia under Laboratory Conditions: II. Egg-laying Capacity and Longevity of Adults. *Proceedings of the Entomological Society of Washington* 1934, 36.
12. Rezende, G.L.; Martins, A.J.; Gentile, C.; Farnesi, L.C.; Pelajo-Machado, M.; Peixoto, A.A.; Valle, D. Embryonic desiccation resistance in *Aedes aegypti*: presumptive role of the chitinized Serosal Cuticle. *BMC Developmental Biology* 2008, 8, 82, doi:10.1186/1471-213X-8-82.
13. Buxton, P.A. Researches in Polynesia and Melanesia. An Account of Investigations in Samoa, Tonga, the Ellice Group, and the New Hebrides, in 1924,
1925. Parts I-IV. (Relating principally to Medical Entomology). *Researches in Polynesia and Melanesia. An Account of Investigations in Samoa, Tonga, the Ellice Group, and the New Hebrides, in 1924, 1925. Parts I-IV. (Relating principally to Medical Entomology).* 1927.

14. Macfie, J.W.S. Morphological changes observed during the development of the larva of *Stegomyia fasciata*. *Bulletin of Entomological Research* **1917**, *7*, 297-307, doi:10.1017/S0007485300017636.

15. Comstock, J. An Introduction to Entomology [part I]. Comstock, Ithaca, New York: 1920.

16. Eiras, A.E.; Jepson, P.C. Host location by *Aedes aegypti* (Diptera: Culicidae): a wind tunnel study of chemical cues. *Bulletin of Entomological Research* **1991**, *81*, 151-160, doi:10.1017/S000748530001221.

17. Acree, F.; Turner, R.B.; Gouck, H.K.; Beroza, M.; Smith, N. L-Lactic Acid: A Mosquito Attractant Isolated from Humans. *Science* **1968**, *161*, 1346-1347.

18. Clements, A.N. *The biology of mosquitoes. Volume 2: sensory reception and behaviour*; CABI Publishing: Wallingford, 1999; pp. xv + 740 pp.

19. Clements, A.N. *The physiology of mosquitoes: International series of monographs on pure and applied biology: Zoology*; Elsevier: 2013; Vol. 17.

20. Scott, T.W.; Takken, W. Feeding strategies of anthropophilic mosquitoes result in increased risk of pathogen transmission. *Trends in Parasitology* **2012**, *28*, 114-121, doi:https://doi.org/10.1016/j.pt.2012.01.001.

21. Leta, S.; Beyene, T.J.; De Clercq, E.M.; Amenu, K.; Kraemer, M.U.G.; Revie, C.W. Global risk mapping for major diseases transmitted by *Aedes aegypti* and *Aedes albopictus*. *International Journal of Infectious Diseases* **2018**, *67*, 25-35, doi:https://doi.org/10.1016/j.ijid.2017.11.026.

22. Brady, O.J.; Gething, P.W.; Bhatt, S.; Messina, J.P.; Brownstein, J.S.; Hoen, A.G.; Moyes, C.L.; Farlow, A.W.; Scott, T.W.; Hay, S.I. Refining the Global Spatial Limits of Dengue Virus Transmission by Evidence-Based Consensus. *PLoS Neglected Tropical Diseases* **2012**, *6*, 1-15, doi:10.1371/journal.pntd.0001760.

23. Bhatt, S.; Gething, P.W.; Brady, O.J.; Messina, J.P.; Farlow, A.W.; Moyes, C.L.; Drake, J.M.; Brownstein, J.S.; Hoen, A.G.; Sankoh, O., et al. The global distribution and burden of dengue. *Nature* **2013**, *496*, 504-507, doi:10.1038/nature12060.

24. Shearer, F.M.; Moyes, C.L.; Pigott, D.M.; Brady, O.J.; Marinho, F.; Deshpande, A.; Longbottom, J.; Browne, A.J.; Kraemer, M.U.G.; O'Reilly, K.M., et al. Global yellow fever vaccination coverage from 1970 to 2016: an adjusted retrospective analysis. *The Lancet Infectious Diseases* **2017**, *17*, 1209-1217, doi:https://doi.org/10.1016/S1473-3099(17)30419-X.

25. Organization, W.H. Yellow Fever. Available online: [https://www.who.int/mediacentre/factsheets/detail/yellow-fever](https://www.who.int/mediacentre/factsheets/detail/yellow-fever) (accessed on 26. Messina, J.P.; Kraemer, M.U.; Brady, O.J.; Pigott, D.M.; Shearer, F.M.; Weiss, D.J.; Golding, N.; Ruktanonchai, C.W.; Gething, P.W.; Cohn, E., et al. Mapping
global environmental suitability for Zika virus. *eLife* 2016, 5, e15272, doi:10.7554/eLife.15272.

27. Organization, W.H. Fact sheet Zika virus. Available online: https://www.who.int/news-room/fact-sheets/detail/zika-virus (accessed on 28.

28. Nsoesie, E.O.; Kraemer, M.U.; Golding, N.; Pigott, D.M.; Brady, O.J.; Moyes, C.L.; Johansson, M.A.; Gething, P.W.; Velayudhan, R.; Khan, K., et al. Global distribution and environmental suitability for chikungunya virus, 1952 to 2015. *Euro Surveill* 2016, 21, 10.2807/1560-7917.ES.2016.2821.2820.30234, doi:10.2807/1560-7917.ES.2016.21.20.30234.

29. Figueiredo, M.L.G.d.; Figueiredo, L.T.M. Emerging alphaviruses in the Americas: Chikungunya and Mayaro. *Revista da Sociedade Brasileira de Medicina Tropical* 2014, 47, 677-683.

30. Wesula Olivia, L.; Obanda, V.; Bucht, G.; Mosomtai, G.; Otieno, V.; Ahlm, C.; Evander, M. Global emergence of Alphaviruses that cause arthritis in humans. *Infection Ecology & Epidemiology* 2015, 5, 29853, doi:10.3402/iee.v5.29853.

31. Albert, J.A.; Jonathan, L.; Naomi, L.F.; Dileyvic, G.; Maria, M.; Kanya, C.L.; Dulce, M.; Nuris de, M.; Robert, B.T.; Eric, S.H., et al. Evolutionary and Ecological Characterization of Mayaro Virus Strains Isolated during an Outbreak, Venezuela, 2010. *Emerging Infectious Disease journal* 2015, 21, 1742, doi:10.3201/eid2110.141660.

32. Organization, W.H. A global brief on vector-borne diseases; World Health Organization: 2014.

33. Chapman, H.C. Biological Control of Mosquito Larvae. *Annual Review of Entomology* 1974, 19, 33-59, doi:10.1146/annurev.en.19.010174.000341.

34. Floore, T.G. *Biorational control of mosquitoes*; American Mosquito Control Assoc: 2007.

35. Tyrell, D.J.; Davidson, L.I.; Bulla, L.A.; Ramoska, W.A. Toxicity of parasporal crystals of *Bacillus thuringiensis* subsp. *israelensis* to mosquitoes. *Applied and Environmental Microbiology* 1979, 38, 656-658.

36. De Barjac, H. Toxicity of *Bacillus thuringiensis* var. *israelensis* for larvae of *Aedes aegypti* and *Anopheles stephensi*. *Comptes rendus hebdomadaires des seances de l'Academie des sciences. Serie D: Sciences naturelles* 1978, 286, 1175.

37. Tetreau, G.; Grizard, S.; Patil, C.D.; Tran, F.-H.; Tran Van, V.; Stalinski, R.; Laporte, F.; Mavingui, P.; Després, L.; Valiente Moro, C. Bacterial microbiota of *Aedes aegypti* mosquito larvae is altered by intoxication with *Bacillus thuringiensis israelensis*. *Parasites & Vectors* 2018, 11, 121, doi:10.1186/s13071-018-2741-8.

38. Ross, P.A.; Wiwatanaratananabutr, I.; Axford, J.K.; White, V.L.; Endersby-Harshman, N.M.; Hoffmann, A.A. *Wolbachia* Infections in *Aedes aegypti* Differ Markedly in Their Response to Cyclical Heat Stress. *PLoS pathogens* 2017, 13, e1006006-e1006006, doi:10.1371/journal.ppat.1006006.

39. Benedict, M.Q.; Robinson, A.S. The first releases of transgenic mosquitoes: an argument for the sterile insect technique. *Trends in Parasitology* 2003, 19, 349-355, doi:https://doi.org/10.1016/S1471-4922(03)00144-2.
40. Lees, R.S.; Gilles, J.R.; Hendrichs, J.; Vreysen, M.J.; Bourtzis, K. Back to the future: the sterile insect technique against mosquito disease vector. *Current Opinion in Insect Science* 2015, 10, 156-162, doi:10.1016/J.COIS.2015.05.011.
41. Simon, J.Y. The Toxicology and Biochemistry of Insecticides; CRC Press: 2014.
42. Ranson, H.; Burhani, J.; Lumjuan, N.; Black IV, W.C. Insecticide resistance in dengue vectors. *TropIKA.net [online]* 2010, 1.
43. Vontas, J.; Kioulos, E.; Pavlidi, N.; Morou, E.; della Torre, A.; Ranson, H. Insecticide resistance in the major dengue vectors *Aedes albopictus* and *Aedes aegypti*. *Pesticide Biochemistry and Physiology* 2012, 104, 126-131, doi:10.1016/j.pestbp.2012.05.008.
44. Mota-Sanchez, D.a.J.C.W. The Arthropod Pesticide Resistance Database. Available online: [http://www.pesticideresistance.org](http://www.pesticideresistance.org) (accessed on
45. Zalucki, M.P.; Furlong, M.J. Behavior as a mechanism of insecticide resistance: evaluation of the evidence. *Current Opinion in Insect Science* 2017, 21, 19-25, doi:[https://doi.org/10.1016/j.cois.2017.05.006](https://doi.org/10.1016/j.cois.2017.05.006).
46. Sougoufara, S.; Doucouré, S.; Sembéne, P.M.B.; Harry, M.; Sokhna, C. Challenges for malaria vector control in sub-Saharan Africa: resistance and behavioral adaptations in Anopheles populations. *Journal of vector borne diseases* 2017, 54, 4.
47. Balabanidou, V.; Grigoraki, L.; Vontas, J. Insect cuticle: a critical determinant of insecticide resistance. *Current opinion in insect science* 2018, 27, 68-74.
48. Hemingway, J.; Ranson, H. Insecticide Resistance in Insect Vectors of Human Disease. *Annual Review of Entomology* 2000, 45, 371-391, doi:10.1146/annurev.ento.45.1.371.
49. Hemingway, J.; Hawkes, N.J.; McCarroll, L.; Ranson, H. The molecular basis of insecticide resistance in mosquitoes. *Insect Biochemistry and Molecular Biology* 2004, 34, 653-665, doi:[https://doi.org/10.1016/j.ibmb.2004.03.018](https://doi.org/10.1016/j.ibmb.2004.03.018).
50. Katz, T.M.; Miller, J.H.; Hebert, A.A. Insect repellents: Historical perspectives and new developments. *Journal of the American Academy of Dermatology* 2008, 58, 865-871, doi:[https://doi.org/10.1016/j.jaad.2007.10.005](https://doi.org/10.1016/j.jaad.2007.10.005).
51. Leal, W.S. The enigmatic reception of DEET-the gold standard of insect repellent. *Current Opinion in Insect Science* 2014, 6, 93-98, doi:10.1016/J.COIS.2014.10.007.
52. Norris, E.J.; Coats, J.R. Current and future repellent technologies: the potential of spatial repellents and their place in mosquito-borne disease control. *International journal of environmental research and public health* 2017, 14, 124.
53. Samuel Legeaya, N.C., Véronique Apaire-Marchais, Sébastien Faure, Bruno Lapied Unusual modes of action of the repellent DEET in insects highlight some human side effects. *European Journal of Pharmacology* 2018, 825, 92 - 98, doi:[https://doi.org/10.1016/j.ejphar.2018.02.033](https://doi.org/10.1016/j.ejphar.2018.02.033).
54. Sudakin, D.L.; Trevathan, W.R. DEET: A Review and Update of Safety and Risk in the General Population. *Journal of Toxicology: Clinical Toxicology* 2003, 41, 831-839, doi:10.1081/CLT-120025348.
55. Osimitz, T.G.; Murphy, J.V.; Fell, L.A.; Page, B. Adverse events associated with the use of insect repellents containing N,N-diethyl-m-toluamide (DEET). *Regulatory Toxicology and Pharmacology* **2010**, *56*, 93-99, doi:https://doi.org/10.1016/j.yrtph.2009.09.004.

56. Stanczyk, N.M.; Brookfield, J.F.Y.; Ignell, R.; Logan, J.G.; Field, L.M. Behavioral insensitivity to DEET in *Aedes aegypti* is a genetically determined trait residing in changes in sensillum function. *Proceedings of the National Academy of Sciences* **2010**, *107*, 8575-8580, doi:10.1073/pnas.1001313107.

57. Stanczyk, N.M.; Brookfield, J.F.; Field, L.M.; Logan, J.G. *Aedes aegypti* mosquitoes exhibit decreased repellency by DEET following previous exposure. *PloS one* **2013**, *8*.

58. Almeida, R.M.; Han, B.A.; Reisinger, A.J.; Kagemann, C.; Rosi, E.J. High mortality in aquatic predators of mosquito larvae caused by exposure to insect repellent. *Biology Letters* **2018**, *14*, 20180526, doi:10.1098/rsbl.2018.0526.

59. Isman, M.B. Botanical Insecticides, Deterrents, and Repellents in Modern Agriculture and an Increasingly Regulated World. *Annual Review of Entomology* **2006**, *51*, 45-66, doi:10.1146/annurev.ento.51.110104.151146.

60. Silva, W.J.; Dória, G.A.A.; Maia, R.T.; Nunes, R.S.; Carvalho, G.A.; Blank, A.F.; Alves, P.B.; Marçal, R.M.; Cavalcanti, S.C.H. Effects of essential oils on *Aedes aegypti* larvae: Alternatives to environmentally safe insecticides. *Bioresource Technology* **2008**, *99*, 3251 - 3255, doi:https://doi.org/10.1016/j.biortech.2007.05.064.

61. Chaccour, C.; Lines, J.; Whitty, C.J.M. Effect of Ivermectin on *Anopheles gambiae* Mosquitoes Fed on Humans: The Potential of Oral Insecticides in Malaria Control. *The Journal of Infectious Diseases* **2010**, *202*, 113-116, doi:10.1086/653208.

62. Diaz, J.H. Chemical and Plant-Based Insect Repellents: Efficacy, Safety, and Toxicity. *Wilderness & Environmental Medicine* **2016**, *27*, 153 - 163, doi:https://doi.org/10.1016/j.wem.2015.11.007.

63. Maia, M.F.; Moore, S.J. Plant-based insect repellents: a review of their efficacy, development and testing. *Malaria Journal* **2011**, *10*, S11, doi:10.1186/1475-2875-10-s1-s11.

64. Pang, Y.-P.; Brimijoin, S.; W. Ragsdale, D.; Yan Zhu, K.; Suranyi, R. Novel and Viable Acetylcholinesterase Target Site for Developing Effective and Environmentally Safe Insecticides. *Current Drug Targets* **2012**, *13*, 471-482, doi:10.2174/138945012799499703.
67. Benner, J.P. Pesticidal compounds from higher plants. *Pesticide Science* **1993**, *39*, 95-102, doi:10.1002/ps.2780390202.

68. Dev, S. Insecticides of natural origin; *Routledge*: 2017.

69. Casida, J. Pyrethrum: the natural insecticide; *Elsevier*: 2012.

70. Casida, J.E. Pyrethrum Flowers and Pyrethroid Insecticides. *Environ Health Perspect* **1980**, *34*, 189-202, doi:10.2307/3428960.

71. Isman, M.B.; Akhtar, Y. Plant Natural Products as a Source for Developing Environmentally Acceptable Insecticides. In *Insecticides Design Using Advanced Technologies*, Ishaaya, I., Horowitz, A.R., Nauen, R., Eds. Springer Berlin Heidelberg: Berlin, Heidelberg, 2007; 10.1007/978-3-540-46907-0_10pp. 235-248.

72. Rembold, H.; Sharma, G.K.; Czoppelt, C.; Schmutterer, H. Azadirachtin: A potent insect growth regulator of plant origin. *Zeitschrift für Angewandte Entomologie* **1982**, *93*, 12-17, doi:10.1111/j.1439-0418.1982.tb03564.x.

73. Zebitz, C.P.W. Effects of three different neem seed kernel extracts and azadirachtin on larvae of different mosquito species. *Journal of Applied Entomology* **1986**, *102*, 455-463, doi:10.1111/j.1439-0418.1986.tb00945.x.

74. Schmutterer, H. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annual review of entomology* **1990**, *35*, 271-297.

75. Cabizza, M.; Angioni, A.; Melis, M.; Cabras, M.; Tuberoso, C.V.; Cabras, P. Rotenone and Rotenoids in Cubè Resins, Formulations, and Residues on Olives. *Journal of Agricultural and Food Chemistry* **2004**, *52*, 288-293, doi:10.1021/jf034987a.

76. Hollingworth, R.M.; Ahammadsahib, K.I.; Gadelhak, G.; McLaughlin, J.L. New inhibitors of Complex I of the mitochondrial electron transport chain with activity as pesticides. *Biochemical Society Transactions* **1994**, *22*, 230-233, doi:10.1042/bst0220230.

77. Fang, N.; Casida, J.E. Anticancer action of cubé insecticide: Correlation for rotenoid constituents between inhibition of NADH:ubiquinone oxidoreductase and induced ornithine decarboxylase activities. *Proceedings of the National Academy of Sciences* **1998**, *95*, 3380-3384, doi:10.1073/pnas.95.7.3380.

78. Kwon, Y.K., Sang Hoon; Ronderos, David S.; Lee, Youngseok; Akitake, Bradley; Woodward, Owen M.; Guggino William B.; Smith Dean P.; Montell, Craig. Drosophila TRPA1 Channel Is Required to Avoid the Naturally Occurring Insect Repellent Citronellal. *Current Biology* **2010**, *20*, 1672 - 1678, doi:https://doi.org/10.1016/j.cub.2010.08.016.

79. Zahran, H.E.D.M., & Abdelgaleil, S. A. Insecticidal and developmental inhibitory properties of monoterpenes on *Culex pipiens* L. (Diptera: Culicidae). *Journal of Asia-Pacific Entomology* **2011**, *14*, 46 - 51, doi:https://doi.org/10.1016/j.aspen.2010.11.013.

80. Mahalwal, V.S.; Ali, M. Volatile constituents of *Cymbopogon nardus* (Linn.) Rendle. *Flavour and Fragrance Journal* **2003**, *18*, 73-76, doi:10.1002/ffj.1144.
81. Silvestre, A.J.D.; Cavaleiro, J.A.S.; Delmond, B.; Filliatre, C.; Bourgeois, G. The essential oil of eucalyptus globulus labill. from Portugal. *Flavour and Fragrance Journal* **1994**, *9*, 51-53, doi:10.1002/ffj.2730090203.

82. Carroll, S.P.; Loye, J. PMD, a registered botanical mosquito repellent with deet-like efficacy. *J Am Mosq Control Assoc* **2006**, *22*.

83. Koul, O.; Walia, S.; Dhaliwal, G. Essential oils as green pesticides: potential and constraints. *Biopesticides International* **2008**, *4*, 63-84.

84. Ciccia, G.; Coussio, J.; Mongelli, E. Insecticidal activity against *Aedes aegypti* larvae of some medicinal South American plants. *Journal of Ethnopharmacology* **2000**, *72*, 185-189, doi: https://doi.org/10.1016/S0378-8741(00)00241-5.

85. Sutthanont, N.; Choochote, W.; Tuetun, B.; Junkum, A.; Jitpakdi, A.; Chaithong, U.; Riyong, D.; Savolainen, V.; Chase, M.W. Phylogeny of Basal Angiosperms: Analyses of Five Genes from Three Genomes. *International Journal of Plant Sciences* **2000**, *161*, S3-S27, doi:10.1086/317584.

86. Das, N.; Baruah, I.; Talukdar, P.; Das, S. Evaluation of botanicals as repellents against mosquitoes. *Journal of vector borne diseases* **2003**, *40*, 49.

87. Qiou, Y.L.; Lee, J.; Bernasconi-Quadroni, F.; Soltis, D.E.; Soltis, P.S.; Zanis, M.; Zimmer, E.A.; Chen, Z.; Savolainen, V.; Chase, M.W. Phylogeny of Basal Angiosperms: Analyses of Five Genes from Three Genomes. *International Journal of Plant Sciences* **2000**, *161*, S3-S27, doi:10.1086/317584.

88. Soltis, P.S.; Soltis, D.E. The origin and diversification of angiosperms. *American Journal of Botany* **2004**, *91*, 1614-1626, doi:10.3732/ajb.91.10.1614.

89. Kubitzki, K., Rohwer, J.G., Bittrich, V., Eds. Springer Berlin Heidelberg: Berlin, Heidelberg, 1993, Canellaceae. In Flowering Plants Dicotyledons: Magnoliid, Hamamelid and Caryophyllid Families, 10.1007/978-3-662-02899-5_19pp. 200-203.

90. Humbert, H. Flore de Madagascar et des Comores. **1960**.

91. Leonard, C.M.; Viljoen, A.M. Warburgia: A comprehensive review of the botany, traditional uses and phytochemistry. *Journal of Ethnopharmacology* **2015**, *165*, 260-285, doi: https://doi.org/10.1016/j.jep.2015.02.021.

92. Muchugi, A.; Kindt, R.; Muluvi, G.M.; Muge, E.; Kipruto, H.; Jamnadass, R.H. Genetic Variation of Kenyan Populations of Warburgia ugandensis, an Important East African Highlands Medicinal Tree Species. *Journal of Life Sciences* **2012**, *4*, 97-105, doi:10.1080/09751270.2012.11885201.

93. Austin, D.F. Florida ethnobotany; *CRC Press*: 2004.

94. Müller, S.; Salomo, K.; Salazar, J.; Naumann, J.; Jaramillo, M.A.; Neinhuis, C.; Feild, T.S.; Wanke, S. Intercontinental long-distance dispersal of Canellaceae from the New to the Old World revealed by a nuclear single copy gene and chloroplast loci. *Molecular Phylogenetics and Evolution* **2015**, *84*, 205-219, doi: https://doi.org/10.1016/j.ympev.2014.12.010.

95. Hammel, B.E.; Zamora, N.A. Pleodendron costaricense (Canella Ceae), a new species for Costa Rica. *Lankesteriana International Journal on Orchidology* **2005**, *5*, 211-218.
96. Vink, W. The Winteraceae of the old world; Groen: 1970.
97. Vink, W. Kubitzki, K., Rohwer, J.G., Bittrich, V., Eds. Springer Berlin HeidelbergWinteraceae. In Flowering Plants · Dicotyledons: Magnoliid, Hamamelid and Caryophyllid Families, 1993; 10.1007/978-3-662-02899-5_77pp. 630-638.
98. Behra, O.; Danthu, P.; Sarter, S.; Radaniela, R.; Fourcade, C.; Randrianarivelo, R.; Ranaivosoa, B.; Arnal-Schnebelen, B. Saro (Cinnamosma fragrans Baillon) essential oil: Application in Health and Medicine. In African Natural Plant Products: New Discoveries and Challenges in Chemistry and Quality, American Chemical Society: 2009; Vol. 1021, pp. 485-494.
99. Cuppage, F.E.; Peterson, M.; Wagner, J.; Miller, J.B.; Cherikoff, V. James Cook's Eighteenth-Century Prevention of Scurvy by the Use of Indigenous Plants as Dietary Supplements. Terrae Incognitae 1994, 26, 37-47, doi:10.1179/tin.1994.26.1.37.
100. Gerard, P.J.; Perry, N.B.; Ruf, L.D.; Foster, L.M. Antifeedant and insecticidal activity of compounds from Pseudowintera colorata (Winteraceae) on the webbing clothes moth, Tineola bisselliella (Lepidoptera: Tineidae) and the Australian carpet beetle, Anthrenocerus australis (Coleoptera: Dermestidae). Bulletin of Entomological Research 1993, 83, 547-552, doi:10.1017/S0007485300039973.
101. Zapata, N.; Budia, F.; Viñuela, E.; Medina, P. Antifeedant and growth inhibitory effects of extracts and drimanes of Drimys winteri stem bark against Spodoptera littoralis (Lep., Noctuidae). Industrial Crops and Products 2009, 30, 119-125, doi:https://doi.org/10.1016/j.indcrop.2009.02.009.
102. Zapata, N.; Vargas, M.; Medina, P.; Viñuela, E.; Rodríguez, B.; Fereres, A. The activity of a selected extract of Drimys winteri bark and polygodial on settling and probing behavior of the lettuce aphid Nasonovia ribisnigri. Phytoparasitica 2010, 38, 191-199, doi:10.1007/s12600-010-0087-7.
103. Zapata, N.; Smagghe, G. Repellency and toxicity of essential oils from the leaves and bark of Laurelia sempervirens and Drimys winteri against Tribolium castaneum. Industrial Crops and Products 2010, 32, 405-410, doi:https://doi.org/10.1016/j.indcrop.2010.06.005.
104. Muñoz, O.; Gutierrez, M.; Gonzalez, R.; Hammann, S.; Vetter, W. Antifungal and insecticidal properties of the phytoconstituents of Drimys winteri (Winteraceae) growing in Chiloé Island (Chile). Nat. Prod. Chem. Res 2015, 3, 4-9.
105. Anese, S.; Gambarra, W.P.T.; Grisi, P.U.; Gualtieri, S.C.J. Insecticidal action of Drimys brasiliensis Miers on black citrus aphid. Revista Ciência Agronômica 2018, 49, 484-490.
106. Ribeiro, V.L.S.; Rolim, V.; Bordignon, S.; Henriques, A.T.; Dorneles, G.G.; Limberger, R.P.; von Poser, G. Chemical composition and larvicidal properties of the essential oils from Drimys brasiliensis Miers (Winteraceae) on the cattle tick Rhipicephalus (Boophilus) microplus and the brown dog tick Rhipicephalus sanguineus. Parasitology Research 2008, 102, 531-535, doi:10.1007/s00436-007-0799-x.
107. Hilje, L.; Mora, G.A. Chapter 15 Promiscory botanical repellents/deterrents for managing two key tropical insect pests, the whitefly Bemisia tabaci and the mahogany shootborer Hypsipyla grandella. In Advances in Phytomedicine, Rai, M., Carpinella, M.C., Eds. Elsevier: 2006; Vol. 3, pp. 379-403.

108. Kubo, I.; Lee, Y.-W.; Pettei, M.; Pilkiewicz, F.; Nakanishi, K. Potent army worm antifeedants from the east African Warburgia plants. *Journal of the Chemical Society, Chemical Communications* **1976**, 10.1039/C39760001013, 1013-1014, doi:10.1039/C39760001013.

109. Kubo, I.; Ganjian, I. Insect antifeedant terpenes, hot-tasting to humans. *Experientia* **1981**, 37, 1063-1064, doi:10.1007/bf02085009.

110. A Opiyo, S.; OA Manguro, L.; A Okoth, D.; A Ochung, A.; O Ochieng, C. Biopesticidal Extractives and Compounds from Warburgia ugandensis against Maize Weevil (*Sitophilus zeamais*). *The Natural Products Journal* **2015**, 5, 236-243.

111. Messchendorp, L.; Gols, G.J.; van Loon, J.J. Behavioral Effects and Sensory Detection of Drimane Deterrents in *Myzus persicae* and *Aphis gossypii* Nymphs. *Journal of Chemical Ecology* **1998**, 24, 1433-1446, doi:http://dx.doi.org/10.1023/A:1020999430889.

112. Vedovatto, F.; Valério Júnior, C.; Astolfi, V.; Mielniczki, P.A.A.; Roman, S.S.; Paroul, N.; Cansian, R.L. Essential oil of *Cinnamodendron dinisii* Schwanke for the control of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *Revista Brasileira de Plantas Medicinais* **2015**, 17, 1055-1060.

113. Ravoajaharisoa, C. *Cinnamosma fragrans*: une Canellacée médicinale endémique de Madagascar. Université De Limoges, 1986.

114. Quéro, A.; Molinié, R.; Brancourt, D.; Rémy, M.J.; Mesnard, F. Sesquiterpene composition of *Cinnamosma fragrans*: A Malagasy endemic plant used in traditional medicine. *Comptes Rendus Chimie* **2016**, 19, 1056-1061, doi:https://doi.org/10.1016/j.crci.2016.04.006.

115. Randrianarivelojosia, M.; Rasidimanana, V.T.; Rabarison, H.; Cheplogoi, P.K.; Ratsimbason, M.; Mulholland, D.A.; Maucële, P. Plants traditionally prescribed to treat tazo (malaria) in the eastern region of Madagascar. *Malaria Journal* **2003**, 2, 25, doi:10.1186/1475-2875-2-25.

116. Pavela, R.; Maggi, F.; Ngahang Kamte, S.L.; Rakotosaona, R.; Rasoanaivo, P.; Nicoletti, M.; Canale, A.; Benelli, G. Chemical composition of *Cinnamosma madagascariensis* (Cannelaceae) essential oil and its larvicidal potential against the filariasis vector *Culex quinquefasciatus* Say. *South African Journal of Botany* **2017**, 108, 359-363, doi:10.1016/j.sajb.2016.08.017.

117. Inocente, E.A.; Shaya, M.; Acosta, N.; Rakotondraibe, L.H.; Piermarini, P.M. A natural agonist of mosquito TRPA1 from the medicinal plant *Cinnamosma fragrans* that is toxic, antifeedant, and repellent to the yellow fever mosquito *Aedes aegypti*. *PLoS Negl Trop Dis* **2018**, 12, e0006265, doi:10.1371/journal.pntd.0006265.

118. Appel, H.H.; Brooks, C.J.W.; Overton, K.H. 673. The constitution and stereochemistry of drimenol, a novel bicyclic sesquiterpenoid. *Journal of the
119. Brown, G.D. Drimendiol, a sesquiterpene from *Drymis winterii*. *Phytochemistry* 1994, 35, 975-977, doi: [https://doi.org/10.1016/S0031-9422(00)90650-2](https://doi.org/10.1016/S0031-9422(00)90650-2).

120. Cechinel Filho, V.; Schlemper, V.; Santos, A.R.S.; Pinheiro, T.R.; Yunes, R.A.; Mendes, G.L.; Calixto, J.B.; Delle Monache, F. Isolation and identification of active compounds from *Drimys winteri* barks. *Journal of Ethnopharmacology* 1998, 62, 223-227, doi: [https://doi.org/10.1016/S0378-8741(98)00069-5](https://doi.org/10.1016/S0378-8741(98)00069-5).

121. Prota, N.; Bouwmeester, H.J.; Jongsma, M.A. Comparative antifeedant activities of polygodial and pyrethrins against whiteflies (*Bemisia tabaci*) and aphids (*Myzus persicae*). *Pest Management Science* 2014, 70, 682-688, doi:10.1002/ps.3610.

122. Fritz, G.L.; Mills, G.D.; Warthen, J.D.; Waters, R.M. Reimer-Tiemann adducts as potential insect antifeedant agents Reviewing the structure-activity relationship theory of the antifeedant, warburganal. *Journal of Chemical Ecology* 1989, 15, 2607-2623, doi:10.1007/bf01014720.

123. Schoonhoven, L.M.; Fu-Shun, Y. Interference with normal chemoreceptor activity by some sesquerpenoid antifeedants in an herbivorous insect *Pieris brassicae*. *Journal of Insect Physiology* 1989, 35, 725-728, doi: [https://doi.org/10.1016/0022-1910(89)90092-9](https://doi.org/10.1016/0022-1910(89)90092-9).

124. Blaney, W.M.; Simmonds, M.S.J.; Ley, S.V.; Katz, R.B. An electrophysiological and behavioural study of insect antifeedant properties of natural and synthetic drimane-related compounds. *Physiological Entomology* 1987, 12, 281.

125. Ross, D.C.; Brown, T.M. Inhibition of larval growth in *Spodoptera frugiperda* by sublethal dietary concentrations of insecticides. *Journal of Agricultural and Food Chemistry* 1982, 30, 193-196, doi:10.1021/jf00109a045.

126. Gerard, P.; Ruf, L.; Perry, N.; Foster, L. Insecticidal properties of the terpenoids polygodial, 9-deoxymuzigadial and azadirachtin. In Proceedings of Proceedings of the New Zealand Plant Protection Conference; pp. 239-242.

127. He, D.; Slebodnick, C.; Rakotondraibe, L.H. Bioactive drimane sesquiterpenoids and aromatic glycosides from *Cinnamosma fragrans*. *Bioorganic & Medicinal Chemistry Letters* 2017, 27, 1754-1759, doi: [https://doi.org/10.1016/j.bmcl.2017.02.067](https://doi.org/10.1016/j.bmcl.2017.02.067).

128. Harinantenaina, L.; Asakawa, Y.; De Clercq, E. Cinnamacrins A–C, Cinnafragrin D, and Cytostatic Metabolites with α-Glucosidase Inhibitory Activity from Cinnamosma macrocarpa. *Journal of Natural Products* 2007, 70, 277-282, doi:10.1021/np060435l.

129. Harinantenaina, L.; Matsunami, K.; Otsuka, H.; Kawahata, M.; Yamaguchi, K.; Asakawa, Y. Secondary Metabolites of *Cinnamosma madagascariensis* and Their α-Glucosidase Inhibitory Properties. *Journal of Natural Products* 2008, 71, 123-126, doi:10.1021/np070474c.

130. Harinantenaina, L.; Takaoka, S. Cinnafragrins A–C, Dimeric and Trimeric Drimane Sesquiterpenoids from *Cinnamosma fragrans*, and Structure Revision of Capsicodendrin. *Journal of natural products* 2006, 69, 1193-1197.
131. Rakotondraibe, L.H.; Muñoz Acuña, U.; Dai, Y.; Chai, H.; Carcache de Blanco, E.J.; Asakawa, Y. SAR study of drimane sesquiterpenes from three Cinnamosma species of Madagascar. *Planta Med* **2014**, *80*, PD57, doi:10.1055/s-0034-1382478.

132. Kioy, D.; Gray, A.I.; Waterman, P.G. A comparative study of the stem-bark drimane sesquiterpenes and leaf volatile oils of *Warburgia ugandensis* and *W. Stuhlmannii*. *Phytochemistry* **1990**, *29*, 3535-3538, doi:https://doi.org/10.1016/0031-9422(90)85270-P.

133. Brooks, C.J.W.; Draffan, G.H. Sesquiterpenoids of *Warburgia* species—II: Ugandensolide and ugandensidial (cinnamodal). *Tetrahedron* **1969**, *25*, 2887-2898, doi:https://doi.org/10.1016/0040-4020(69)80031-1.

134. Bastos, J.K.; Kaplan, M.A.C.; Gottlieb, O.R. Drimane-Type Sesquiterpenoids as Chemosystematic Markers of Canellaceae. *Journal of the Brazilian Chemical Society* **1999**, *10*, 136-139.

135. Mahmoud, I.I.; Kinghorn, A.D.; Cordell, G.A.; Farnsworth, N.R. Potential Anticancer Agents. XVI. Isolation of Bicyclofarnesane Sesquiterpenoids From Capsicodendron dinisii. *Journal of Natural Products* **1980**, *43*, 365-371, doi:10.1021/np50009a008.

136. Brengues, C.; Hawkes, N.J.; Chandre, F.; McCarroll, L.; Duchon, S.; Guillet, P.; Manguin, S.; Morgan, J.C.; Hemingway, J. Pyrethroid and DDT cross-resistance in *Aedes aegypti* is correlated with novel mutations in the voltage-gated sodium channel gene. *Medical and Veterinary Entomology* **2003**, *17*, 87-94, doi:10.1046/j.1365-2915.2003.00412.x %U https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1365-2915.2003.00412.x.

137. Smith, L.B.; Kasai, S.; Scott, J.G. Pyrethroid resistance in *Aedes aegypti* and *Aedes albopictus*: Important mosquito vectors of human diseases. *Pesticide Biochemistry and Physiology* **2016**, *133*, 1-12, doi:https://doi.org/10.1016/j.pestbp.2016.03.005.

138. Strode, C. Genomic analysis of detoxification genes in the mosquito *Aedes aegypti*. *Insect Biochemistry and Molecular Biology* **2008**, *38*, 113 - 123, doi:https://doi.org/10.1016/j.ibmb.2007.09.007.

139. Ntalli, N.; Koliopoulos, G.; Giatropoulos, A.; Menkissoglu-Spirodi, U. Plant secondary metabolites against arthropods of medical importance. *Phytochemistry Reviews* **2019**, 10.1007/s11101-019-09647-7, doi:10.1007/s11101-019-09647-7.

140. Ujváry, I. Nicotine and other insecticidal alkaloids. In *Nicotinoid insecticides and the nicotinic acetylcholine receptor*, Springer: 1999; pp. 29-69.

141. Asakawa, Y.; Ludwiczuk, A.; Harinantenaina, L.; Toyota, M.; Nishiki, M.; Bardon, A.; Nii, K. Distribution of Drimane Sesquiterpenoids and Tocopherols in Liverworts, Ferns and Higher Plants: Polygonaceae, Canellaceae and Winteraceae Species. *Natural Product Communications* **2012**, *7*, 1934578X1200700601, doi:10.1177/1934578x1200700601.

142. Mohammed, M.S.A. Traditional Medicinal Plants and Malaria in Africa. 10.1021/bk-2009-1021.ch012, 217-230, doi:10.1021/bk-2009-1021.ch012.
143. Randrianarivony, T.N.; Ramarosandratanana, A.V.; Andriamihajarivo, T.H.; Rakotoarivony, F.; Jeannoda, V.H.; Randrianasolo, A.; Bussmann, R.W. The most used medicinal plants by communities in Mahaboboka, Amboronabo, Mikoboka, Southwestern Madagascar. *Journal of Ethnobiology and Ethnomedicine* 2017, 13, 19, doi:10.1186/s13002-017-0147-x.

144. Nakata, T.; Akita, H.; Naito, T.; Oishi, T. A total synthesis of (+)-warburganal. *Journal of the American Chemical Society* 1979, 101, 4400-4401.

145. Piermarini, P.M.; Hine, R.M.; Schepel, M.; Miyauchi, J.; Beyenbach, K.W. Role of an apical K+Cl cotransporter in urine formation by renal tubules of the yellow fever mosquito (*Aedes aegypti*). *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 2011, 301, R1318-R1337.

146. Calkins, T.L.; Piermarini, P.M. Pharmacological and genetic evidence for gap junctions as potential new insecticide targets in the yellow fever mosquito, *Aedes aegypti*. *PloS one* 2015, 10, e0137084.

147. Pridgeon, J.W.; Becnel, J.J.; Clark, G.G.; Linthicum, K.J. A High-Throughput Screening Method to Identify Potential Pesticides for Mosquito Control. *Journal of Medical Entomology* 2009, 46, 335-341, doi:10.1603/033.046.0219.

148. Abbott, W.S. A method of computing the effectiveness of an insecticide. *J. econ. Entomol* 1925, 18, 265-267.

149. Swale, D.R.; Engers, D.W.; Bollinger, S.R.; Gross, A.; Inocente, E.A.; Days, E.; Kanga, F.; Johnson, R.M.; Yang, L.; Bloomquist, J.R., et al. An insecticide resistance-breaking mosquitocide targeting inward rectifier potassium channels in vectors of Zika virus and malaria. *Scientific Reports* 2016, 6, 36954, doi:10.1038/srep36954.

150. Isman, M.B.; Koul, O.; Luczynski, A.; Kaminski, J. Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. *Journal of Agricultural and Food Chemistry* 1990, 38, 1406-1411, doi:10.1021/jf00096a024.

151. Manwill, P.; Kalsi, M.; Wu, S.; Cheng, X.; Piermarini, P.; Rakotondraibe, H.L. Semi-synthetic Cinnamodial Analogues: Structural Insights into the Insecticidal and Antifeedant Activities of Drimane Sesquiterpenes Against the Mosquito *Aedes aegypti*. *bioRxiv* 2019, 536961.

152. Goodsell, D.S.; Morris, G.M.; Olson, A.J. Automated docking of flexible ligands: applications of AutoDock. *Journal of Molecular Recognition* 1996, 9, 1-5.

153. Nomoto, Y.; Harinantenaïna, L.; Sugimoto, S.; Matsunami, K.; Otsuka, H. 3,4-seco-24-homo-28-nor-Cycloartane and drimane-type sesquiterpenes and their lactams from the EtOAc-soluble fraction of a leaf extract of *Cinnamosma fragrans* and their biological activity. *Journal of Natural Medicines* 2014, 68, 513-520, doi:10.1007/s11418-014-0828-x.

154. Karmahapatra, S.; Kientz, C.; Shetty, S.; Yalowich, J.C.; Rakotondraibe, L.H. Capsicodendrin from *Cinnamosma fragrans* Exhibits Antiproliferative and Cytotoxic Activity in Human Leukemia Cells: Modulation by Glutathione. *Journal of natural products* 2018, 81, 625-629.
156. William, W.J.; Carvalho, G.B.; Mak, E.M.; Noelle, N.; Fang, A.Y.; Liong, J.C.; Brummel, T.; Benzer, S. Prandiology of Drosophila and the CAFE assay. *Proceedings of the National Academy of Sciences* **2007**, *104*, 8253-8256.

157. Maheswaran, R.; Ignacimuthu, S. Effect of *Polygonum hydropiper* L. against dengue vector mosquito *Aedes albopictus* L. *Parasitology research* **2014**, *113*, 3143-3150.

158. Dias, C.N.; Moraes, D.F.C. Essential oils and their compounds as *Aedes aegypti* L. (Diptera: Culicidae) larvicides: review. *Parasitology Research* **2014**, *113*, 565-592. doi:10.1007/s00436-013-3687-6.

159. Gross, A.D., Norris, E. J., Kimber, M. J., Bartholomay, L. C., & Coats, J. R. Essential oils enhance the toxicity of permethrin against *Aedes aegypti* and *Anopheles gambiae*. *Medical and Veterinary Entomology* **2017**, *31*, 55-62, doi:10.1111/mve.12197.

160. Norris, E.; Johnson, J.; Gross, A.; Bartholomay, L.; Coats, J. Plant essential oils enhance diverse pyrethroids against multiple strains of mosquitoes and inhibit detoxification enzyme processes. *Insects* **2018**, *9*, 132.

161. Norris, E.J.; Bartholomay, L.; Coats, J. Present and Future Outlook: The Potential of Green Chemistry in Vector Control. In *Advances in the Biorational Control of Medical and Veterinary Pests*, American Chemical Society: 2018; Vol. 1289, pp. 43-62.

162. Dada, N.; Vannavong, N.; Seidu, R.; Lenhart, A.; Stenstrom, T.A.; Chareonviriyyaphap, T.; Overgaard, H.J. Relationship between *Aedes aegypti* production and occurrence of *Escherichia coli* in domestic water storage containers in rural and sub-urban villages in Thailand and Laos. *Acta Trop* **2013**, *126*, doi:10.1016/j.actatropica.2013.02.023.

163. Ponnusamy, L.; Xu, N.; Stav, G.; Wesson, D.; Schal, C.; Apperson, C. Diversity of bacterial communities in container habitats of mosquitoes. *Microb Ecol* **2008**, *56*, doi:10.1007/s00248-008-9379-6.

164. Sarter, S.; Randrianarivelo, R.; Ruez, P.; Raherimandimby, M.; Danthu, P. Antimicrobial Effects of Essential Oils of *Cinnamosma fragrans* on the Bacterial Communities in the Rearing Water of *Penaeus monodon* Larvae. *Vector-Borne and Zoonotic Diseases* **2011**, *11*, 433-437, doi:10.1089/vbz.2010.0069.

165. Randrianarivelo, R., Sarter,S., Odoux, E., Brat, P., Lebrun, M., Romestand, B., Menut, C., Sahondra, H.A., Raherimandimby, M., & Danthu, P. Composition and antimicrobial activity of essential oils of *Cinnamosma fragrans*. *Food Chemistry* **2009**, *114*, 680 - 684, doi:https://doi.org/10.1016/j.foodchem.2008.10.007.

166. Chadwick, M.; Trewin, H.; Gawthrop, F.; Wagstaff, C. Sesquiterpenoids Lactones: Benefits to Plants and People. *International Journal of Molecular Sciences* **2013**, *14*, 12780-12805.

167. Cowan, M.M. Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews* **1999**, *12*, 564-582, doi:10.1128/cmr.12.4.564.

168. Di Pasqua, R.; Betts, G.; Hoskins, N.; Edwards, M.; Ercolini, D.; Mauriello, G. Membrane Toxicity of Antimicrobial Compounds from Essential Oils. *Journal of Agricultural and Food Chemistry* **2007**, *55*, 4863-4870, doi:10.1021/jf0636465.
Govindarajan, M.; Mathivanan, T.; Elumalai, K.; Krishnappa, K.; Anandan, A. Mosquito larvicidal, ovicidal, and repellent properties of botanical extracts against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology research* 2011, 109, 353-367.

Loder, J. Occurrence of the Sesquiterpenes Polygodial and Guaiol in the Leaves of *Drimys lanceolata* (Poir.) Baill. *Australian Journal of Chemistry* 1962, 15, 389-390, doi:https://doi.org/10.1071/CH9620389.

Escalera, J.; von Hehn, C.A.; Bessac, B.F.; Sivula, M.; Jordt, S.-E. TRPA1 mediates the noxious effects of natural sesquiterpene deterrents. *Journal of Biological Chemistry* 2008, 283, 24136-24144.

Mathie, K.; Lainer, J.; Spreng, S.; Dawid, C.; Andersson, D.A.; Bevan, S.; Hofmann, T. Structure–Pungency Relationships and TRP Channel Activation of Drimane Sesquiterpenes in Tasmanian Pepper (*Tasmannia lanceolata*). *Journal of Agricultural and Food Chemistry* 2017, 65, 5700-5712, doi:10.1021/acs.jafc.7b02356.

Asakawa, Y.; Dawson, G.W.; Griffiths, D.C.; Lallemand, J.-Y.; Ley, S.V.; Mori, K.; Mudd, A.; Pezechek-Leclaire, M.; Pickett, J.A.; Watanabe, H., et al. Activity of drimane antifeedants and related compounds against aphids, and comparative biological effects and chemical reactivity of (−)- and (+)-polygodial. *Journal of Chemical Ecology* 1988, 14, 1845-1855, doi:10.1007/bf01013481.

Arc, W.-C.M. Alterations of chemoreceptor function in army worm larvae (*Spodoptera exempta*) by a plant-derived sesquiterpenoid and by sulfhydryl reagents. *Physiological Entomology* 1977, 2, 199-207, doi:10.1111/j.1365-3032.1977.tb00104.x.

Kubo, I.; Nakanishi, K. Some Terpenoid Insect Antifeedants from Tropical Plants. In *Synthesis of Pesticides Chemical Structure and Biological Activity Natural Products with Biological Activity*, GeissbÜHler, H., Ed. Pergamon: 1979; https://doi.org/10.1016/B978-0-08-022349-0.50041-2pp. 284-294.

Hinman, A.; Chuang, H.-h.; Bautista, D.M.; Julius, D. TRP channel activation by reversible covalent modification. *Proceedings of the National Academy of Sciences* 2006, 103, 19564-19568, doi:10.1073/pnas.0609598103.

Macpherson, L.J.; Dubin, A.E.; Evans, M.J.; Marr, F.; Schultz, P.G.; Cravatt, B.F.; Patapoutian, A. Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. *Nature* 2007, 445, 541, doi:10.1038/nature05544.

Peng, G.; Kashio, M.; Morimoto, T.; Li, T.; Zhu, J.; Tominaga, M.; Kadowaki, T. Plant-Derived Tick Repellents Activate the Honey Bee Ectoparasitic Mite TRPA1. *Cell Reports* 2015, 12, 190-202, doi:10.1016/j.celrep.2015.06.025.

Henquet, M.G.L.; Prota, N.; van der Hooft, J.J.J.; Varbanova-Herde, M.; Hulzink, R.J.M.; de Vos, M.; Prins, M.; de Both, M.T.J.; Franssen, M.C.R.; Bouwmeester, H., et al. Identification of a drimenol synthase and drimenol oxidase from *Persicaria hydropiper*, involved in the biosynthesis of insect deterrent drimanes. *The Plant Journal* 2017, 90, 1052-1063, doi:10.1111/tjp.13527.
Arias, H.R.; Feuerbach, D.; Schmidt, B.; Heydenreich, M.; Paz, C.; Ortells, M.O. Drimane Sesquiterpenoids Noncompetitively Inhibit Human α4β2 Nicotinic Acetylcholine Receptors with Higher Potency Compared to Human α3β4 and α7 Subtypes. *Journal of Natural Products* **2018**, *81*, 811-817, doi:10.1021/acs.jnatprod.7b00893.

Coon, K.L.; Vogel, K.J.; Brown, M.R.; Strand, M.R. Mosquitoes rely on their gut microbiota for development. *Molecular Ecology* **2014**, *23*, 2727-2739, doi:10.1111/mec.12771.

Strand, M.R. Composition and functional roles of the gut microbiota in mosquitoes. *Current Opinion in Insect Science* **2018**, *28*, 59-65, doi:[https://doi.org/10.1016/j.cois.2018.05.008](https://doi.org/10.1016/j.cois.2018.05.008).
Appendix A. Extracts raw sucrose consumption

Figure 7. Volumes of sucrose consumed in control and treatment capillaries during choice CAFE assays in adult female *Ae. aegypti* (LVP strain). The extract (50 µg/ml) used in the treatment capillaries is indicated. As described in the methods, the differences in volume consumed between the capillaries were used to calculate the antifeedant indices in Fig. 5A. Values are means ± SEM; N= 16-24 independent replicates of 5 mosquitoes each. Differences in volumes consumed between control and treatment capillaries were determined by paired t-tests (P < 0.05).
Appendix B. Compounds raw sucrose consumption

Figure 8. Volumes of sucrose consumed in control and treatment capillaries during choice CAFE assays in adult female *Ae. aegypti* (LVP strain). The compound (1 mM) used in the treatment capillaries is indicated. As described in the methods, the differences in volume consumed between the capillaries were used to calculate the antifeedant indices in Fig. 6A. Values are means ± SEM; N= 10-24 independent replicates of 5 mosquitoes each. Differences in volumes consumed between control and treatment capillaries were determined by paired t-tests (P < 0.05).
