

METHOD FOR MOSQUITO CONTROL

CROSS REFERENCE TO RELATED APPLICATIONS

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This application claims the benefit of U.S. Provisional Application No. 61/477,781, filed April 21, 2011, herein incorporated by reference.

FIELD OF THE INVENTION

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The present invention relates to a method and a formulation for mosquito control and, in particular, a method and a formulation for mosquito control which includes, but is not limited to, using mosquitoes or other insects for delivering agents, e.g., insecticides such as a larvicide, to an insect population to thereby control the insect population.

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BACKGROUND OF THE INVENTION

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Malaria, dengue and dengue haemorrhagic fever, West Nile Virus (WNV) and other encephalites, human African trypanosomiasis (HAT), human filariasis, dog heartworm and other pathogens important to animals are on the increase. These diseases are transmitted via insects and, in particular, mosquitoes. Methods for controlling mosquito populations include the use of pesticides and vector control methods.

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Existing insecticidal control methods rely upon field technicians, who fail to find and treat many breeding sites, which can be numerous, cryptic and inaccessible. Additional methods consist of area-wide treatment via airplane or wind-assisted dispersal from truck-mounted foggers. Unfortunately, the latter fail to treat many breeding sites and are complicated by variable environmental conditions. Barrera et al., "Population Dynamics of *Aedes aegypti* and *Dengue* as Influenced by Weather and Human Behavior in San Juan, Puerto Rico," PLoS Neglected Tropical Diseases, 5:e1378, 2011, describing the effects of various breeding sites on disease.

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Surveys of natural and artificial water containers demonstrate mosquitoes and other arthropods to be highly efficient in finding, inhabiting and laying eggs in variously sized, cryptic water pools, including tree holes and gutters high above ground level.

One prior formulation or method for treating mosquito populations includes the use of dissemination stations which are deployed in a target environment. The dissemination stations may be laced with a pesticide, including, but not limited to, a juvenile hormone analog. The dissemination station may include a box or other structure which attracts female mosquitoes. The mosquitoes enter the dissemination station, become exposed to the pesticide or hormone, and carry that hormone back to affect other mosquitoes by mating. An example of this mosquito control is described in the article by Devine et al., entitled "Using adult mosquitoes to transfer insecticides to *Aedes aegypti* larval habitats," PNAS, vol. 106, no. 28, July 14, 2009.

In tests of another dissemination station, researchers showed that males in the wild that acquire the pesticide from a station can transfer the pesticide to females during copulation. The females receiving pesticide particles via venereal transfer were then shown to cause a significant inhibition of emergence in larval bioassays. This was reported in the article by Gaugler et al, entitled "An autodissemination station for the transfer of an insect growth regulator to mosquito oviposition sites," *Med. Vet. Entomol.* 2011.

In view of continuing mosquito problems, as noted, additional tools are required to control mosquitoes that are important as nuisance pests and disease vectors.

SUMMARY OF THE INVENTION

The present invention is directed to a novel, self-delivering, insecticidal formulations and delivery techniques. The formulations, in one form, are larvicide treated insects, such as male mosquitoes. The insecticidal formulations can control medically important mosquitoes. These medically important mosquitoes include mosquitoes having an economic or medical importance to animal or human health. Medically important mosquitoes include those listed in the Appendix to this disclosure.

One aspect of the present formulations and delivery techniques relates to a new larvicide treatment for males, as a formulation which can be used to control a mosquito population. The formulation can be generated by exposing adult insects, such as mosquitoes and, in particular, male mosquitoes, to a pesticide, such as a juvenile hormone which affects juvenile survival or interferes with metamorphosis of juvenile mosquitoes and has relatively little impact on adult mosquitoes. Advantageously, the adult insects are exposed to the pesticide in a controlled, factory environment. The factory-reared or captured from the wild adult insects which have been exposed to the

pesticide are referred to as direct treated individuals (DTI). The DTI are then released into an environment in which one wishes to control the mosquito population. The DTI control a mosquito population by interacting with untreated individuals (e.g., mating), such that the pesticide, e.g., a larvicide, is communicated to other individuals (known as
5 Indirectly Treated Individuals; (ITI)).

In specific further embodiments, the control method uses compounds that affect immature/juvenile stages (eggs, larvae, pupae) more than adults. A list of larvicidal compounds is maintained at the IR-4 Public Health Pesticides Database. Examples of compounds include (1) insect growth regulators such as juvenile hormone mimics
10 or analogs, including methoprene, pyriproxyfen (PPF), and (2) Microbial larvicides, such as *Bacillus thuringiensis* and *Bacillus sphaericus*, herein incorporated by reference. Exemplary compounds are provided in Tables 1-3, below, in the Detailed Description section.

The present invention, in one form thereof, relates to a method for insect control.
15 The method includes introducing insects which carry one or more insecticides comprising at least one larvicide, to an insect population, to thereby control the insect population. In one specific embodiment, the insects are adult males and the method further includes exposing the adult male insects to a pesticide which affects juvenile survival or interferes with metamorphosis of juvenile insects to adulthood, and which
20 pesticide has little impact on adult insects.

In one further, specific embodiment, the insect population is a mosquito population. Further, the juvenile active insecticide (i.e. larvicide) may be within a chemical class (Table 1) or biological class (Table 2). Examples within the chemical class include insect growth regulators, such as juvenile hormone analogs or
25 compounds which mimic juvenile hormones. For example, the larvicide may be pyriproxyfen or methoprene. Examples within the biological class include viruses, bacteria, protozoa, fungi and crustacean organisms or toxic compounds that they produce.

The present invention, in another form thereof, relates to a formulation for insect
30 control which comprises an artificially generated adult insect carrier of a larvicide. The larvicide has minimal impact on the adult insect and the larvicide interferes with metamorphosis of juvenile insects to adulthood. In one specific formulation, the adult insect is a male mosquito and in an alternative form, the larvicide is pyriproxyfen,

methoprene and microbial larvicides, including, but not limited to, *Bacillus thuringiensis* and *Bacillus sphaericus*.

BRIEF DESCRIPTION OF THE DRAWING

- 5 The sole figure is a graph showing survival of treated (black) and untreated (white) adults, with bars showing standard deviation, in accordance with the present invention.

DETAILED DESCRIPTION

- 10 The present invention is directed to a method and a formulation for mosquito control. The formulation, in one advantageous form, is larvicide treated males. The treated males are generated from medically important adult male mosquitoes obtained via factory-rearing or captured from the wild. As a demonstration of the chemical class of juvenile active insecticides (Table 1), the adult male mosquitoes are exposed to a
15 larvicide, such as pyriproxyfen (PPF), advantageously in a controlled laboratory or factory environment. PPF is a juvenile hormone mimic which interferes with metamorphosis of juvenile mosquitoes and has relatively little impact on adult mosquitoes. Thus, PPF is commonly used as a mosquito larvicide, but is not used as an adulticide.

- 20 The treated males are subsequently referred to as the Direct Treated Individuals (DTI), and this is the insecticidal formulation. The DTI are released into areas with indigenous conspecifics. Male mosquitoes do not blood feed or transmit disease. Accordingly, male mosquitoes provide unique advantages in the present control method as couriers of the larvicide. The DTI interact with untreated individuals (e.g., mating),
25 such that PPF is communicated to the other individuals to produce Indirectly Treated Individuals (ITI). The PPF is delivered by both the DTI and ITI in the wild/in the environment, into the breeding areas, where the PPF accumulates to lethal doses and acts as a larvicide. It is noted that the PPF would impact additional mosquito species that share the same breeding site, providing control of additional mosquito species.

- 30 In an alternative control method, female mosquitoes can be used as the DTI. However, female mosquitoes blood feed and can vector disease. The use of female mosquitoes are applicable when the females are incapacitated prior to deployment in the environment and the females have limited procreation ability, bite and vector diseases.

In a further alternative method, other larvicidal active ingredients can be used which include, but are not limited to, compounds that affect juvenile survival or affect immature/juvenile stages of development (eggs, larvae, pupae) more than adults. A list of larvicidal compounds is maintained at the IR-4 Public Health Pesticides

- 5 Database. Examples of compounds include (1) insect growth regulators such as juvenile hormone mimics or analogs, including methoprene, pyriproxyfen (PPF), and (2) Microbial larvicides, such as *Bacillus thuringiensis* and *Bacillus sphaericus*. Tables 1-3 provide abridged, exemplary lists of suitable compounds.

Table 1. Juvenile Active Insecticide - Chemical*

Azadirachtin
Diflubenzuron
Methoprene
Neem Oil (<i>Azadirachta indica</i>)
Novaluron
Pyriproxyfen
S-Methoprene
S-Hydropene
Temephos

* A list of Public Health Pesticides is maintained at the IR-4 Public Health Pesticides Database

Table 2. Juvenile Active Insecticide - Biological*

Ascogregarine spp.
<i>Bacillus sphaericus</i>
<i>Bacillus thuringiensis israelensis</i>
Baculoviruses
Copepoda spp.
Densovirinae spp.
<i>Lagenidium giganteum</i>
Microsporida spp.
Spinosad

Spinosyn

* A list of Public Health Pesticides is maintained at the IR-4 Public Health Pesticides Database

Table 3. Public Health Pesticides from the IR-4 Database*

		Oil of Basil, African Blue (Ocimum kilimandscharicum × basilicum)
(-)-cis-Permethrin	Cyfluthrin	Oil of Basil, Dwarf Bush (Ocimum basilicum var. minimum)
(-)-trans-Permethrin	Cyhalothrin	Oil of Basil, Greek Bush (Ocimum minimum)
(+)-cis-Permethrin	Cyhalothrin, epimer R157836	Oil of Basil, Greek Column (Ocimum × citriodorum 'Lesbos')
	Cyhalothrin, Total (Cyhalothrin-L + R157836 epimer)	Oil of Basil, Lemon (Ocimum americanum)
(±)-cis,trans-Deltamethrin		Oil of Basil, Sweet (Ocimum basilicum)
(1R)-Alpha-Pinene	Cypermethrin	Oil of Basil, Thai Lemon (Ocimum × citriodorum)
(1R)-Permethrin	Cyphenothrin	Oil of Bay Laurel (Laurus nobilis)
(1R)-Resmethrin	DDD, o,p	Oil of Cajeput (Melaleuca leucadendra)
(1R, cis) Phenothrin	DDD, other related	Oil of Cassumunar Ginger (Zingiber montanum)
(1R, trans) Phenothrin	DDD, p,p'	Oil of Fishpoison (Tephrosia purpurea)
(1S)-Alpha-Pinene	DDE	Oil of Ginger (Zingiber officinale)
(1S)-Permethrin	DDE, o,p	
(E)-Beta-Caryophyllene	DDT	

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1,1-dichloro-2,2-bis-(4-ethyl-phenyl) ethane	DDT, o,p'	Oil of Gurjun Balsam (Dipterocarpus turbinatus balsam)
1,8-Cineole	DDT, p,p'	Oil of Lemon Eucalyptus (Corymbia citriodora)
1H-Pyrazole -3-carboxamide, 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]	DDVP	Oil of Lemon Mint (Monarda citriodora)
1-Naphthol	DDVP, other related	Oil of Melaleuca (Melaleuca spp.)
1-Octen-3-ol	DEET	Oil of Myrcia (Myrcia spp.)
2-(2-(p-(diisobutyl) phenoxy) ethoxy) ethyl dimethyl ammonium chloride	Deltamethrin	Oil of Nutmeg (Myristica fragrans)
2-butyl-2-ethyl-1,3-propanediol	Deltamethrin (includes parent Tralomethrin) Deltamethrin (isomer unspecified)	Oil of Palmarosa (Cymbopogon martinii)
2-Hydroxyethyl Octyl Sulfide	unspecified)	Orange Oil (Citrus sinensis)
2-Isopropyl-4-methyl-6-hydroxypyrimidine	Deltamethrin, other related	Oregano Oil (Origanum vulgare)
2-Pyrroline-3-carbonitrile, 2-(p-chlorophenyl)-5-hydroxy-4-oxo-5-	Desmethyl Malathion	Ortho-Phenylphenol
3,7-dimethyl-6-octen-1-ol acetate	Desulfinyl Fipronil	Ortho-Phenylphenol, Sodium Salt
3,7-dimethyl-6-octen-1-ol acetate	Desulfinylfipronil Amide	Oviposition Attractant A
3-Phenoxybenzoic Acid	Diatomaceous Earth	Oviposition Attractant B
4-Fluoro-3-phenoxybenzoic acid	Diatomaceous Earth, other related	Oviposition Attractant C

Table 3. Public Health Pesticides from the IR-4 Database

Absinth Wormwood		
(<i>Artemisia absinthium</i>)	Diazinon	Oviposition Attractant D
Absinthin	Diazoxon	Oxymatrine
		Paracress Oil (<i>Spilanthes</i>
		<i>acmella</i>)
Acepromazine	Dibutyl Phthalate	
	Didecyl Dimethyl Ammonium	
Acetaminophen	Chloride	P-Cymene
Acetamiprid	Dieldrin	Penfluron
		Pennyroyal Oil (American
		False Pennyroyal, <i>Hedeoma</i>
		<i>pulegioides</i>)
Acetic Acid	Diethyl Phosphate	Peppermint (<i>Mentha ×</i>
		<i>piperita</i>)
AI3-35765	Diethylthio Phosphate	Peppermint Oil (<i>Mentha ×</i>
		<i>piperita</i>)
AI3-37220	Diflubenzuron	
Alkyl Dimethyl Benzyl		
Ammonium Chloride		
(60%C14, 25%C12, 15%C16)	Dihydro Abietyl Alcohol	Permethrin
Alkyl Dimethyl Benzyl		
Ammonium Chloride		
(60%C14, 30%C16, 5%C12,	Dihydro-5-heptyl-2(3H)-	
5%C18)	furanone	Permethrin, other related
Alkyl Dimethylethyl Benzyl		
Ammonium Chloride		
(50%C12, 30%C14, 17%C16,	Dihydro-5-pentyl-2(3H)-	
3%C18)	furanone	Phenothrin
Alkyl Dimethylethyl Benzyl		
Ammonium Chloride		
(68%C12, 32%C14)	Dimethyl Phosphate	Phenothrin, other related
Allethrin	Dimethyldithio Phosphate	Picaridin
Allethrin II	Dimethylthio Phosphate	Pine Oil (<i>Pinus pinea</i> = Stone

Table 3. Public Health Pesticides from the IR-4 Database*

		Pine)
Allethrins	Dinotefuran	Pine Oil (Pinus spp.)
	Dipropyl Isocinchomeronate	Pine Oil (Pinus sylvestris =
Allicin	(2, 5 isomer)	Scots Pine)
	Dipropyl Isocinchomeronate	
Allyl Caproate	(3, 5 isomer)	Pine Tar Oil (Pinus spp.)
Allyl Isothiocyanate	Dipropylene Glycol	Pinene
Alpha-Cypermethrin	d-Limonene	Piperine
Alpha-Ionone	d-Phenothrin	Piperonyl Butoxide
		Piperonyl Butoxide,
Alpha-Pinene	Dried Blood	technical, other related
Alpha-Terpinene	d-trans-Beta-Cypermethrin	Pirimiphos-Methyl
Aluminum Phosphide	Esfenvalerate	PMD (p-Menthane-3,8-diol)
Amitraz	Ester Gum	Potassium Laurate
		Potassium Salts of Fatty
Ammonium Bicarbonate	Estragole	Acids
Ammonium Fluosilicate	Etofenprox	Potassium Sorbate
	Eucalyptus Oil (Eucalyptus	
Anabasine	spp.)	Prallethrin
Anabsinthine	Eugenol	Propoxur
Andiroba Oil (Carapa		
guianensis)	Eugenyl Acetate	Propoxur Phenol
Andiroba Oil (Carapa		
procera)	Extract of Piper spp.	Propoxur, other related
Andiroba, African (Carapa	Extracts of Common Juniper	
procera)	(Juniperus communis)	Putrescent Whole Egg Solids
Andiroba, American (Carapa		
guianensis)	Fenchyl Acetate	Pyrethrin I
Anethole	Fenitrothion	Pyrethrin II
Anise (Pimpinella anisum)	Fennel (Foeniculum vulgare)	Pyrethrins
Aniseed Oil (Pimpinella	Fennel Oil (Foeniculum	Pyrethrins and Pyrethroids,

Table 3. Public Health Pesticides from the IR-4 Database

anisum)	vulgaris)	manufg. Residues
Atrazine	Fenoxycarb	Pyrethrins, other related
Avermectin	Fenthion	Pyrethrum
Azadirachtin	Fenthion Oxon	Pyrethrum Marc
		Pyrethrum Powder other
Azadirachtin A	Fenthion Sulfone	than Pyrethrins
Bacillus sphaericus	Fenthion Sulfoxide	Pyriproxyfen
		Pyrrole-2-carboxylic acid, 3-
Bacillus sphaericus, serotype		bromo-5-(p-chlorophenyl)-4-
H-5A5B, strain 2362	Ferula hermonis	cyano-
		Pyrrole-2-carboxylic acid, 5-
Bacillus thuringiensis		(p-chlorophenyl)-4-cyano-
israelensis	Ferula hermonis Oil	(metabolite of AC 303268)
Bacillus thuringiensis	Finger Root Oil	
israelensis, serotype H-14	(Boesenbergia pandurata)	Quassia
Bacillus thuringiensis		
israelensis, strain AM 65-52	Fipronil	Quassin
Bacillus thuringiensis		
israelensis, strain BK, solids,		
spores, and insecticidal		
toxins, ATCC number 35646	Fipronil Sulfone	R-(-)-1-Octen-3-ol
Bacillus thuringiensis		Red Cedar Chips (Juniperus
israelensis, strain BMP 144	Fipronil Sulfoxide	virginiana)
Bacillus thuringiensis		
israelensis, strain EG2215	Fragrance Orange 418228	Resmethrin
Bacillus thuringiensis		
israelensis, strain IPS-78	Gamma-Cyhalothrin	Resmethrin, other related
Bacillus thuringiensis		
israelensis, strain SA3A	Garlic (Allium sativum)	Rhodojaponin-III
Balsam Fir Oil (Abies	Garlic Chives Oil (Allium	
balsamea)	tuberosum)	Rose Oil (Rosa spp.)

Table 3. Public Health Pesticides from the IR-4 Database*

Basil, Holy (<i>Ocimum tenuiflorum</i>)	Garlic Oil (<i>Allium sativum</i>)	Rosemary (<i>Rosmarinus officinalis</i>)
		Rosemary Oil (<i>Rosmarinus officinalis</i>)
Bendiocarb	Geraniol	
	Geranium Oil (<i>Pelargonium graveolens</i>)	Rosmanol
Benzyl Benzoate		
Bergamot Oil (<i>Citrus aurantium bergamia</i>)	Glyphosate, Isopropylamine Salt	Rosmaridiphenol
Beta-Alanine	Hexaflumuron	Rosmarinic Acid
Beta-Caryophyllene	Hydroprene	Rotenone
	Hydroxyethyl Octyl Sulfide, other related	R-Pyriproxyfen
Beta-Cyfluthrin		
Beta-Cypermethrin	Imidacloprid	R-Tetramethrin
Beta-Cypermethrin ([{(1R)- 1a(S*), 3a] isomer)	Imidacloprid Guanidine	Rue Oil (<i>Ruta chalepensis</i>)
Beta-Cypermethrin ([{(1R)- 1a(S*), 3b] isomer)	Imidacloprid Olefin	Ryania
Beta-Cypermethrin ([{(1S)- 1a(R*), 3a] isomer)	Imidacloprid Olefinic- Guanidine	Ryanodine
Beta-Cypermethrin ([{(1S)- 1a(R*), 3b] isomer)	Imidacloprid Urea	S-(+)-1-Octen-3-ol
Beta-Myrcene	Imiprothrin	Sabinene
	Indian Privet Tree Oil (<i>Vitex negundo</i>)	Sabinene
Beta-Pinene		
Betulinic Acid	Ionone	Sage Oil (<i>Salvia officinalis</i>)
	IR3535 (Ethyl Butylacetylaminopropionate)	Sassafras Oil (<i>Sassafras albidum</i>)
Bifenthrin		
Billy-Goat Weed Oil (<i>Ageratum conyzoides</i>)	Isomalathion	Schoenocaulon officinale
Bioallethrin = d-trans- Allethrin	Isopropyl Alcohol	S-Citronellol

Table 3. Public Health Pesticides from the IR-4 Database*

	Japanese Mint Oil (<i>Mentha</i>	
Biopermethrin	arvensis)	Sesame (<i>Sesamum indicum</i>)
Bioresmethrin	Jasmolin I	Sesame Oil (<i>Sesamum indicum</i>)
Bitter Orange Oil (<i>Citrus aurantium</i>)	Jasmolin II	Sesamin
Blend of Oils: of Lemongrass, of Citronella, of Orange, of Bergamot; Geraniol, Ionone Alpha, Methyl Salicylate and		
Allylisothioc	Kerosene	Sesamolin
Boric Acid	L-(+)-Lactic acid	S-Hydroprene
Borneol	Lactic Acid	Silica Gel
		Silver Sagebrush (<i>Artemisia cana</i>)
Bornyl Acetate	Lagenidium giganteum	Silver Sagebrush Oil
	Lagenidium giganteum (california strain)	(<i>Artemisia cana</i>)
Bromine		
Butane	Lambda-Cyhalothrin	S-Methoprene
Butoxy Poly Propylene Glycol	Lambda-Cyhalothrin R ester	Sodium Chloride
Caffeic Acid	Lambda-Cyhalothrin S ester	Sodium Lauryl Sulfate
		Solvent Naphtha
Camphene	Lambda-Cyhalothrin total	(Petroleum), Light Aromatic
Camphor	Lauryl Sulfate	Soybean Oil (<i>Glycine max</i>)
	Lavender Oil (<i>Lavendula angustifolia</i>)	
Camphor Octanane		Spinosad
	Leaves of Eucalyptus	
Canada Balsam	(<i>Eucalyptus</i> spp.)	Spinosyn A
	Leech Lime Oil (<i>Citrus hystrix</i>)	
Carbaryl		Spinosyn D
		Spinosyn Factor A
Carbon Dioxide	Lemon Oil (<i>Citrus limon</i>)	Metabolite

Table 3. Public Health Pesticides from the IR-4 Database*

		Spinosyn Factor D
Carnosic Acid	Licareol	Metabolite
Carvacrol	Limonene	S-Pyriproxyfen
Caryophyllene	Linalool	Succinic Acid
Cassumunar Ginger Oil (Zingiber montanum)	Linalyl Acetate	Sulfoxide
Castor Oil (Ricinus communis)	Linseed Oil (Linum usitatissimum)	Sulfoxide, other related
Catnip Oil (Nepeta cataria)	Lonchocarpus utilis (Cubé)	Sulfur
Catnip Oil, Refined (Nepeta cataria)	Lupinine	Sulfuryl Fluoride
Cedarwood Oil (Callitropsis nootkatensis = Nootka Cypress, Alaska Yellow Cedarwood)	Magnesium Phosphide	Sweet Gale Oil (Myrica gale)
Cedarwood Oil (Cedrus deodara = Deodar Cedar)	Malabar (Cinnamomum tamala)	Tangerine Oil (Citrus reticulata)
Cedarwood Oil (Cedrus spp. = True Cedars)	Malabar Oil (Cinnamomum tamala)	Tansy Oil (Tanacetum vulgare)
Cedarwood Oil (Cupressus funebis = Chinese Weeping Cypress)	Malaoxon	Tar Oils, from Distillation of Wood Tar
Cedarwood Oil (Cupressus spp. = Cypress)	Malathion	Tarragon Oil (Artemisia dracunculus)
Cedarwood Oil (Juniper and Cypress)	Malathion Dicarboxylic Acid	Tarwood Oil (Laxostylis alata)
Cedarwood Oil (Juniperus ashei = Ashe's Juniper, Texan Cedarwood)	Malic Acid	tau-Fluvalinate
Cedarwood Oil (Juniperus macropoda = Pencil Cedar)	Marigold Oil (Tagetes minuta)	Teflubenzuron

Table 3. Public Health Pesticides from the IR-4 Database*

Cedarwood Oil (Juniperus spp.)	Matrine	Temephos
Cedarwood Oil (Juniperus virginiana = Eastern Redcedar, Southern Redcedar)	Menthone	Temephos Sulfoxide
Cedarwood Oil (Oil of Juniper Tar = Juniperus spp.)	Metaflumizone	Terpinene
Cedarwood Oil (Thuja occidentalis = Eastern Arborvitae)	Metarhizium anisopliae Strain F52 Spores	Terpineol
Cedarwood Oil (Thuja spp. = Arborvitae)	Methoprene	Tetrachlorvinphos, Z-isomer
Cedarwood Oil (unspecified)	Methoprene Acid	Tetramethrin
Cedrene	Methyl Anabasine	Tetramethrin, other related
Cedrol	Methyl Bromide	Theta-Cypermethrin
Chevron 100 Neutral Oil	Methyl Cinnamate	Thiamethoxam
	Methyl cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate	Thujone
Chlordane	Methyl Eugenol	Thyme (Thymus vulgaris)
Chlorfenapyr	Methyl Nonyl Ketone	Thyme Oil (Thymus vulgaris)
Chloropicrin	Methyl Salicylate	Thymol
Chlorpyrifos	Methyl trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate	Timur Oil (Zanthoxylum alatum)
Cinerin I	Metofluthrin	Tralomethrin
Cinerin II	MGK 264 (N-octyl Bicycloheptene	trans-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane

Table 3. Public Health Pesticides from the IR-4 Database

	Dicarboximide)	carboxylic acid
Cinnamon (Cinnamomum zeylanicum)	Mineral Oil	Trans-Alpha-Ionone
	Mineral Oil, Petroleum	
Cinnamon Oil (Cinnamomum zeylanicum)	Distillates, Solvent Refined	
	Light	Transfluthrin
cis-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane	Mixture of Citronella Oil, Citrus Oil, Eucalyptus Oil,	
carboxylic acid	Pine Oil	trans-Ocimene
	MMF (Poly (oxy-1,2-ethanediyl), alpha-isooctadecyl-omega-	
cis-Deltamethrin	hydroxy)	Transpermethrin
Cismethrin	Mosquito Egg Pheromone	trans-Resmethrin
cis-Permethrin	Mugwort (Artemisia vulgaris)	Trichlorfon
	Mugwort Oil (Artemisia vulgaris)	
Citral		Triethylene Glycol
Citric Acid	Mustard Oil (Brassica spp.)	Triflumuron
Citronella (Cymbopogon winterianus)	Myrcene	Trifluralin
Citronella Oil (Cymbopogon winterianus)	Naled	Turmeric Oil (Curcuma aromatica)
	Neem Oil (Azadirachta indica)	Uniconazole-P
Citronellal		
Citronellol	Nepeta cataria (Catnip)	Ursolic Acid
Citrus Oil (Citrus spp.)	Nepetalactone	Veratridine
Clove (Syzygium aromaticum)	Nicotine	Verbena Oil (Verbena spp.)
Clove Oil (Syzygium aromaticum)	Nonanoic Acid	Verbenone
CME 13406	Nornicotine	Violet Oil (Viola odorata)

Table 3. Public Health Pesticides from the IR-4 Database*

Coriander Oil (Coriandrum sativum)	Novaluron	White Pepper (Piper nigrum)
		Wintergreen Oil (Gaultheria spp.)
Coriandrol	Ocimene	
Coriandrum sativum (Coriander)	Ocimum × citriodorum (Thai Lemon Basil)	Wood Creosote
	Ocimum × citriodorum 'Lesbos' (Greek Column Basil)	
Corn Gluten Meal		Wood Tar
Corn Oil (Zea mays ssp. Mays)	Ocimum americanum (Lemon Basil)	Wormwood Oil (Artemisia absinthium)
Corymbia citriodora (Lemon Eucalyptus)	Ocimum basilicum (Sweet Basil)	Ylang-ylang Oil (Canarium odoratum)
Cottonseed Oil (Gossypium spp.)	Ocimum basilicum var. minimum (Dwarf Bush Basil)	Zeta-Cypermethrin
	Ocimum kilimandscharicum × basilicum (African Blue Basil)	
Coumaphos		Zinc Metal Strips
	Ocimum minimum (Greek Bush Basil)	
Cryolite		
Cubé Extracts (Lonchocarpus utilis)	Oil of Balsam Peru (Myroxylon pereirae)	

* A list of Public Health Pesticides is maintained at the IR-4 Public Health Pesticides Database; Version from March 2012

In yet another alternative method, the aforementioned methods can be applied to additional susceptible arthropods, including economically and medically important pests (including animal and human health), where one life stage and/or sex does not cause direct damage.

In other alternative delivery techniques, the present method can be applied using non-targeted, beneficial or non-pest arthropods that utilize the same breeding site as the targeted arthropod. For example, the DTI could be PPF-treated arthropods that

come in contact with the targeted insect's breeding sites. As an example, Oytiscidae adults (Predaceous Diving Beetles) could be reared or field collected and treated with PPF to become the DTI. Additional candidate insects that could serve as the DTI include, but are not limited to: Diptera (e.g., Tipulidae, Chironomidae, Psychodidae, Ceratopogonidae, Cecidomyiidae, Syrphidae, Sciaridae, Stratiomyiidae, Phoridae), Coleoptera (e.g., Staphylinidae, Scirtidae, Nitidulidae, Oytiscidae, Noteridae) and Hemiptera (e.g., Pleidae, Belostomatidae, Corixidae, Notonectidae, Nepidae).

An additional benefit of the latter strategy (i.e. non-Culicid DTI) is that the DTI may be easier to rear, larger size (allowing increased levels of PPF), be less affected by the PPF, or have an increased probability of direct contact with the breeding site of the targeted arthropod (i.e. not necessarily rely on transfer of the PPF via mating, improved location of breeding sites).

It is noted that the species of DTI would vary based upon the specific application, habitat and location. For example, the regulatory issues may be simplified if the species used for DTI were indigenous. However, it is noted that there are numerous examples of exotic arthropods being imported for biological control. Furthermore, different DTI species may be more/less appropriate for urban, suburban and rural environments.

Referring to the following examples for exemplary purposes only, but not to limit the scope of the invention in any way, *Aedes albopictus* were used in experiments from a colony established in 2008 from Lexington, KY. *Callosobrochus maculatus* were purchased from Carolina Biological Supply Company (Burlington, NC) and maintained on mung beans (*Vigna radiata*). Rearing and experiments were performed in ambient conditions (~25°C ; 80% humidity). Larvae were reared in pans with ~500ml water and crushed cat food (Science Diet; Hill's Pet Nutrition, Inc.). Adults were provided with raisins as a sugar source. For line maintenance, females were blood fed by the author.

Sumilarv 0.5G was generously provided by Sumitomo Chemical (London, UK). Liquid PPF was purchased from Pest Control Outlet (New Port Richey, FL). *Bacillus thuringiensis* subspecies israelensis technical powder was purchased from HydroToYou (Bell, CA). For application, Sumilarv granules were crushed into a fine powder and applied using a bellows-type dusting apparatus (J.T. Eaton Insecticidal Duster #530; Do-it-yourself Pest Control, Suwanee, GA). Liquid PPF was applied using a standard squirt bottle (WalMart, Lexington, KY). Treated adults were held in individualized bags with a raisin as a sucrose source until used in larval bioassays.

Larval bioassays were performed in 3 oz. Dixie Cups (Georgia-Pacific, Atlanta, GA) containing ten L3 larvae, 20ml water and crushed cat food.

Adult treatment does not affect survival. Male and female *Ae. albopictus* treated with pulverized Sumilarv showed good survival in laboratory assays, which is indistinguishable from that of untreated control individuals. In an initial assay, 100% survival was observed for adults in both the Sumilarv treated (n=8 replications) and untreated control groups (n=2 replications) during a two-day observation period. In a second comparison, adults were monitored for eight days. Similar to the initial experiment, no difference was observed between the treated and control groups. Specifically, a similar average longevity was observed comparing the Sumilarv treated (6.3 ± 2.0 days; n=4) and undusted control (7.3 days; n=1) groups. In a third experiment, treated and untreated adults were separated by sex and monitored for eight days. Similar to prior experiments, survival was not observed to differ between the treated and untreated groups (Figure).

In a separate experiment, the survival of beetles (*Callosobrochus maculatus*) dusted with Sumilarv were compared to an undusted control group. In both the treatment and control groups, 100% survival was observed during the four day experiment.

To assess the larvicidal properties of treated adults, Sumilarv dusted adults and undusted control adults were placed individually into bioassay cups with larvae. No adults eclosed from the five assay cups receiving a treated adult; in contrast, high levels of adult eclosion was observed from all four control assay cups that received an untreated adult. Chi square analysis shows the adult eclosion resulting in assays receiving a treated adult to be significantly reduced compared to that in the control group ($X^2 (1, N=9)=12.37, p<0.0004$). The bioassay experiment was repeated in a subsequent, larger experiment, yielding similar results; adult eclosion in the treated group was significantly reduced compared to the control group ($X^2 (1, N=24)=13.67, p<0.0002$).

A similar bioassay was used to assess the larvicidal properties of treated beetles. Similar to the prior results, adult eclosion from assays in the treated beetle group was significantly reduced compared to the control group ($X^2 (1, N=14)=13.38, p<0.0003$).

To examine an additional formulation of PPF, an identical bioassay was performed, but a liquid PPF solution was applied to mosquito adults, instead of

Sumilarv dust. Similar to the prior results, adult eclosion in the treated group was significantly reduced compared to the control group ($X^2(1, N=14)=16.75, p<0.0001$).

To examine an example of the biological class of juvenile active insecticides (Table 2) and different active ingredients, an identical bioassay was performed, but a powder formulation of *Bacillus thuringiensis* subspecies israelensis technical powder was applied to mosquito adults, using the same method as the Sumilarv dust. Similar to the prior results, no difference was observed between the longevity of treated versus untreated adults ($X^2(1, N=15)=3.2308, p>0.09$). Upon exposing larvae to treat adults, eclosion was significantly reduced compared to the control group ($X^2(1, N=28)=15.328, p<0.0001$).

The results demonstrate that *C. maculatus* and *A. albopictus* adults do not experience reduced survival resulting from direct treatment with the insecticides. Specifically, the survival of treated mosquitoes and beetles did not differ significantly from that of the untreated conspecifics. The results are consistent with the traits required for the proposed application of treated adults as a self-delivering larvicide. Treated adults must survive, disperse and find breeding sites under field conditions. The results of the feasibility assays reported here provide evidence of an advantageous method of mosquito or other arthropod control.

Bioassays characterizing the larvicidal properties of treated adults show significant lethality resulting from the presence of treated mosquitoes and beetles. Similar results were observed for multiple formulations (i.e. dust and liquid) and multiple active ingredients. Furthermore, representative examples from each of the chemical and biological classes (Tables 1 and 2) of juvenile active insecticides have been demonstrated. This is also consistent with those traits required for the proposed application of treated arthropods as a self-delivering insecticide. Specifically, treated arthropods that reach mosquito breeding sites can be expected to impact immature mosquitoes that are present at the site.

It will now be clear that the present invention is directed to a novel formulation and method for treating insect populations, including, but not limited to, mosquito populations. Unlike prior control methods that disseminate a pesticide using dissemination stations, followed by an insect in the wild entering the dissemination station to become treated with the pesticide, the present formulation and method starts with generating insect carriers in an artificial controlled environment or setting. The insect carriers can either be factory-reared or adults captured from the wild.

Subsequently, the carriers are released into an environment as the control agent or formulation. Thus, the carriers, i.e. the insects with the pesticide, are the formulation for insect control, whereas, in prior methods and formulations, the formulation is a treated dissemination station, not a treated insect.

One of ordinary skill in the art will recognize that the present treatment, which targets insect larvae, offers advantages over prior art techniques of insect control which target adult insects. The present method is a trans-generation insect control technique which targets the next generation of insects, whereas prior techniques target the present generation, i.e. adult insects. For example, García-Munguía et al.,

"Transmission of *Beauveria bassiana* from male to female *Aedes aegypti* mosquitoes," *Parasites & Vectors*, 4:24, 2011 is a paper published on February 26, 2011 describing mosquito control of adults and, thus, the paper describes the killing of the present generation of insects. The García-Munguía paper describes using fungus-treated males to deliver insecticidal fungus to adult females. The fungus shortens the female lifespan and reduces fecundity of adult females. This type of approach (using insects to deliver an adulticide) is not particularly novel, and has been used in several important insect species, including examples described in Baverstock et al., "Entomopathogenic fungi and insect behaviour: from unsuspecting hosts to targeted vectors," *Biocontrol*, 55:89-102, 2009.

As described above, the present technique uniquely uses factory-treatment of adult insects with a larvicide in which the larvicide is chemical or biological in nature. No prior technique includes the manufacturing of larvicidal-treated insects for trans-generational delivery. Further, unlike prior techniques that treat adults with fungi that kills adults, the present technique merely treats adult males with larvicidal compounds which do not kill the adult males; rather, the treatment delivers the larvicidal compounds in a trans-generational delivery to kill the next generation, i.e. larvae.

Advantages which follow from the present technique include using the adults treated with the larvicide to communicate the larvicide to other adults through the lifespan of the initially treated adult insect. As a result, there is an exponential effect of the present technique which delivers a larvicide using treated adults to transfer the treatment to other adults, rather than prior techniques which kill the adult insect.

Further, the present technique delivers the larvicide by the treated adults into breeding sites where the larvicide can affect and kill thousands of developing, immature mosquitoes. This technique is unlike prior techniques which merely target the adults

and, thus, only kill the directly affected adults and not thousands of developing, immature mosquitoes, i.e. a next generation of insects.

In addition, the present technique allows for the treatment of insect breeding sites, including cryptic, i.e. previously unknown, breeding sites which prior insect control techniques do not treat.

Further, the present technique allows one to affect insect populations of the species of a treated insect, as well as other species which share a common breeding site. Since the present technique uses adult insects to deliver a larvicide to a breeding site, the present technique allows for the transmission of a larvicide to breeding sites which may be common among more than one insect species. As a result, the present technique can target the species of the treated insect, as well as insects which share a common breeding site.

In addition, in contrast to adulticide methods, the present larvicide technique allows a pesticide to persist in a breeding site after the treated insect has departed or died.

One additional advantage of the present method is that the agents being disseminated are the insects themselves, as carriers of the insecticide which will directly affect an insect population. Prior formulation and methods require indirect dissemination, in which insects of a population in the wild must first find a dissemination station, acquire an appropriate dose of the insecticide, and then return to the population with the larvicide of a dissemination station in order to have an affect on the insect population.

It will now be clear to one of ordinary skill in the art that the present formulation of pesticide carrier insects and the present method for controlling insect populations based on the present experiments. For example, if the insects have a larval stage, adult insects can be used as carriers of larvicides which have minimal affect on the adult insect, but are lethal to the larvae, thereby controlling the insect population.

While the invention has been described in connection with numerous embodiments, it is to be understood that the specific mechanisms and techniques which have been described are merely illustrative of the principles of the invention, numerous modifications may be made to the methods and apparatus described without departing from the spirit and scope of the invention.

WHAT IS CLAIMED IS:

1. A method for insect control, comprising:
rearing insects in a laboratory or artificially controlled environment;
applying an insecticide coating to the surface of the insect to thereby produce insects which carry one or more insecticides comprising at least a larvicide;
and releasing the insects to an insect population, to thereby control the insect population.
2. The method of claim 1, wherein the insects are adult insects, and the method further comprises exposing the adult insects to a pesticide which affects juvenile survival or interferes with metamorphosis of juvenile insects to adulthood, and which pesticide has little impact on adult insects.
3. The method of claim 2, wherein the adult insects are male insects.
4. The method of claim 1, wherein the insect population is a mosquito population.
5. The method of claim 2, wherein the insect population is a mosquito population.
6. The method of claim 3, wherein the insect population is a mosquito population.
7. The method of claim 6, wherein the adult male insects are mosquitoes.
8. The method of claim 2, wherein the pesticide is a chemical agent.
9. The method of claim 8, wherein the chemical agent is selected from the group consisting of Azadirachtin, Diflubenzuron, Methoprene, Neem Oil (*Azadirachta indica*), Novaluron, Pyriproxyfen, S-Methoprene, S-Hydropene and Temephos.
10. The method of claim 2, wherein the pesticide is a biological agent.
11. The method of claim 10, wherein the biological agent is selected from the group consisting of *Ascogregarine* spp., *Bacillus sphaericus*, *Bacillus thuringiensis israelensis*, *Baculoviruses*, *Copepoda* spp., *Densovirinae* spp., *Lagenidium giganteum*, *Microsporida* spp., *Spinosad* and *Spinosyn*.

12. The method of claim 1, wherein the larvicide is a juvenile hormone analog or a compound which mimics a juvenile hormone.
13. The method of claim 12, wherein the larvicide is pyriproxyfen.
14. The method of claim 2, wherein exposing the adult insects to a pesticide comprises exposing the adult males to the pesticide in a controlled environment to produce directly treated individuals and the introducing insects comprises introducing the directly treated individuals into the insect population.
15. The method of claim 14, further comprising allowing the direct treated individuals to interact with untreated individuals of the insect population, to which the directly treated individual insects have been introduced, to thereby produce indirectly treated individuals.
16. The method of claim 15, wherein both the directly treated individuals and the indirectly treated individuals control the insect population by delivering the pesticide to the insect population.
17. The method of claim 1, wherein the insects carrying the pesticide are female.
18. A formulation for insect control, said formulation comprising:
 - a laboratory reared adult insect carrier of a larvicide resulting from having a larvicide coating applied to the adult insect, wherein said larvicide has minimal impact on the adult insect and which larvicide affects juvenile survival or interferes with metamorphosis of juvenile insects to adulthood.
19. The formulation of claim 18, wherein the adult insect is a male mosquito.
20. The formulation of claim 18, wherein the adult insect is a female mosquito.
21. The formulation of claim 18, wherein the larvicide is a chemical agent.

22. The formulation of claim 21 , wherein the chemical agent is selected from the group consisting of Azadirachtin, Diflubenzuron, Methoprene, Neem Oil (*Azadirachta indica*), Novaluron, Pyriproxyfen, S-Methoprene, S-Hdropene and Temephos.

23. The formulation of claim 18, wherein the larvicide is a biological agent.

24. The formulation of claim 23, wherein the biological agent is selected from the group consisting of *Ascogregarine* spp., *Bacillus sphaericus*, *Bacillus thuringiensis israelensis*, *Baculoviruses*, *Copepoda* spp., *Densovirinae* spp., *Lagenidium giganteum*, *Microsporida* spp., *Spinosad* and *Spinosyn*.