



(51) International Patent Classification:

A01N 63/00 (2006.01) A01N 25/34 (2006.01)

A01K 67/033 (2006.01)

(21) International Application Number:

PCT/AU2018/050369

(22) International Filing Date:

23 April 2018 (23.04.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

2017901480 24 April 2017 (24.04.2017) AU

(71) Applicant: MONASH UNIVERSITY [AU/AU];

Wellington Road, Clayton, Victoria 3800 (AU).

(72) Inventors: LIN, Ya-Hsun; Wellington Road, Clayton, Vic-

torial 3800 (AU). JOUBERT, Albert; Wellington Road,

Clayton, Victoria 3800 (AU). GILLES, Jeremie; Well-

ington Road, Clayton, Victoria 3800 (AU).

(74) Agent: SPRUSON & FERGUSON; GPO Box 3898, Syd-

ney, NSW 2001 (AU).

(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: MOSQUITO EGG CAPSULE

(57) Abstract: A mosquito egg capsule comprises a closed capsule; a filler; and a plurality of mosquito eggs; wherein the filler typically comprises a food source for the mosquito eggs when hatched and an inert carrier and/or bulking agent. The mosquito eggs may be infected with or otherwise comprise a mosquito-adapted bacterium capable of modifying one or more biological properties of mosquitoes hatched from the eggs. The mosquito egg capsule provides an improved nutrient environment for the storage and hatching of mosquito eggs to produce mosquitoes useful in biocontrol methods.



TITLE

MOSQUITO EGG CAPSULE

FIELD OF THE INVENTION

THIS INVENTION relates to environmental control of mosquito-transmitted
5 diseases. More particularly, this invention relates to capsule for storing, hatching
and/or rearing mosquitoes for introduction into an environment to act as an agent
for biocontrol of diseases transmitted by mosquitoes.

BACKGROUND OF THE INVENTION

Mosquitoes are a source of, or transmit, many diseases and conditions in
10 humans and other animals. Mosquitos are responsible for the transmission of
disease-causing pathogens such as arboviruses, flaviviruses and protozoa. These
disease-causing pathogens are responsible for a variety of different diseases of
humans and other animals including malaria, Dengue fever, Eastern Equine
encephalitis, Western Equine encephalitis, Venezuelan equine encephalitis,
15 Japanese encephalitis, Murray Valley encephalitis, West Nile fever, Yellow fever,
LaCrosse encephalitis, Asian spotted fever, Q fever, Chikungunya fever, Zika
fever and Ross river fever. More recently, in addition to Zika fever, Zika virus has
been identified as capable of causing microencephaly and Guillain-Barre
syndrome in humans.

20 In recent years, there has been a resurgence in developing sustainable
disease control strategies that target the mosquito host. These include sterile
insect techniques, (SIT) and Release of Insects with Dominant Lethality (RIDL).
A different and particularly successful strategy has employed a strain of the
obligate intracellular bacterium *Wolbachia pipientis*, wMelPop to target
25 mosquitoes such as *Aedes aegyptii*. *Wolbachia* are maternally-inherited bacteria
that use mechanisms such as cytoplasmic incompatibility (CI), a type of
embryonic lethality that results from crosses between infected males with
uninfected females, to rapidly spread into insect populations (Hoffmann &
Turelli, 1997, S. L. O'Neill, A. A. Hoffmann, J. H. Werren, Eds. (Oxford
30 University Press, Oxford, UK), pp. 42-80).

Such life-shortening *Wolbachia* strains do not naturally occur in

mosquitoes, but have been developed *in vitro* to become able to infect mosquitoes such as *Aedes aegypti*. As originally described in United States Patent 9090911 and in McMeniman *et al.*, 2009, supra, *Wolbachia pipientis*-infected mosquitoes have a shorter life-span, reduced fecundity, altered feeding behaviour, reduced pathogen transmission and/or a lower susceptibility to flavivirus pathogens such as Dengue virus. As more recently described, *Wolbachia pipientis*-infected *Aedes aegypti* mosquitoes displayed lower Chikungunya virus and Zika virus prevalence and intensity, decreased disseminated infection and blocked viral transmission (Aliota *et al.* 2016 PLoS Negl Trop Dis **10** e0004677; Dutra *et al.*, 2016, Cell Host & Microbe **19** 771).

SUMMARY OF THE INVENTION

The inventors have realized that the effectiveness and widespread adoption of mosquito biocontrol measures, such as *Wolbachia*-infected mosquitoes, can be assisted by improving the ability to store, hatch and/or raise *Wolbachia*-infected mosquitoes for subsequent release into the environment.

An object of the invention is therefore to provide an improved environment for mosquito eggs. One particular object of the invention is to provide an improved nutrient environment for the storage and hatching of mosquito eggs.

In a first aspect, the invention provides a mosquito egg capsule which comprises: a closed capsule; a filler; and a plurality of mosquito eggs; wherein the filler optionally comprises a food source for the mosquito eggs when hatched.

In a second aspect, the invention provides a method of producing a mosquito egg capsule including the steps of: combining a plurality of mosquito eggs and a filler that optionally comprises a food source for the mosquito eggs when hatched; delivering at least a portion of the combined mosquito eggs and filler into one or a plurality of capsules; and closing the capsules.

This aspect also includes a mosquito egg capsule produced according to the method.

In a third aspect, the invention provides a method of producing mosquitoes, including the step of introducing one or more mosquito egg capsules according to the aforementioned aspects, or mosquitoes produced therefrom, into an environment under conditions that facilitate hatching of mosquito eggs
5 into mosquito larvae.

The environment may be an artificial environment such as a mosquito rearing system or may be a natural environment.

In a fourth aspect, the invention provides a method of releasing mosquitoes into an environment, including the step of introducing one or more
10 mosquito egg capsules according to the aforementioned aspects, or mosquitoes produced therefrom, into said environment under conditions that facilitate hatching of mosquito eggs into mosquito larvae.

Accordingly, in a particular aspect the invention provides a method of modifying a mosquito population, including the step of introducing a mosquito
15 egg capsule according to the aforementioned aspect, or mosquitoes produced therefrom, into an environment under conditions that facilitate hatching of mosquito eggs into mosquito larvae, wherein release of the mosquito larvae into the environment modifies one or more biological properties of said mosquito population.

20 In one embodiment, the mosquitoes are wild-type or otherwise naturally-occurring mosquitoes that exist in a local environment, region or habitat.

In another embodiment, the mosquitoes have been modified for use as a biocontrol agent in said environment.

In a particular embodiment, the mosquito eggs are infected with or
25 otherwise comprise a mosquito-adapted bacterium capable of modifying one or more biological properties of a mosquito, wherein said mosquito-adapted bacterium does not normally colonize, inhabit, reside in, or infect said mosquito host.

In an embodiment, said mosquito-adapted bacterium is of the genus
30 *Wolbachia*.

In another preferred embodiment, said isolated mosquito-adapted bacterium is of the species *Wolbachia pipientis*.

In one particularly preferred embodiment, said isolated mosquito-adapted bacterium is wMel.

5 Throughout this specification, unless otherwise indicated, “comprise”, “comprises” and “comprising” are used inclusively rather than exclusively, so that a stated integer or group of integers may include one or more other non-stated integers or groups of integers.

10 As used herein, the indefinite articles “a” and “an” should not be read as stating or implying singularity, but instead may also mean “more than one”. By way of example, “a capsule” may relate to a single capsule or a plurality of capsules.

15 As used herein, “about” is used to refer to a variation of tolerance in a stated amount. Typically, the variation or tolerance is no more than $\pm 10\%$, $\pm 9\%$, $\pm 8\%$, $\pm 7\%$, $\pm 6\%$, $\pm 5\%$, $\pm 4\%$, $\pm 3\%$, $\pm 2\%$ or $\pm 1\%$ from a stated amount.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

20 The present invention is predicated on the discovery that mosquitoes may be hatched from eggs that are stored in a capsule comprising a filler that provides a suitable food source for the mosquito larvae. Accordingly a particular form of the invention relates to a capsule comprising mosquito eggs that may be used as a source of mosquitoes for biocontrol of mosquito-borne diseases. In one particular embodiment, the mosquito eggs are infected with a mosquito-adapted *Wolbachia* bacterium into the environment to thereby modify mosquito populations and
25 control mosquito-borne diseases such as dengue fever.

For the purposes of this invention, by “isolated” is meant material that has been removed from its natural state or otherwise been subjected to human manipulation. Isolated material may be substantially or essentially free from components that normally accompany it in its natural state, or may be
30 manipulated so as to be in an artificial state together with components that normally accompany it in its natural state.

As used herein “*mosquito*” and “*mosquitoes*” include insects of the family *Culicidae*. Preferably, mosquitoes are of the sub-families *Anophelinae* and *Culicinae*. Even more preferably, mosquitoes are capable of transmitting disease-causing pathogens, including viruses, protozoa, worms (*e.g.* nematodes) and bacteria to a host. Non-limiting examples include species of the genus *Anopheles* which transmit malaria pathogens, species of the genus *Culex*, and species of the genus *Aedes* (*e.g.* *Aedes aegypti*, *Aedes albopictus* and *Aedes polynesiensis*) which transmit nematode worm pathogens, arbovirus pathogens such as *Alphaviruses* (*e.g.* Eastern Equine encephalitis, Western Equine encephalitis, Venezuelan equine encephalitis and Chikungunya virus), *Flavivirus* pathogens that cause diseases such as Japanese encephalitis, Murray Valley Encephalitis, West Nile fever, Yellow fever, Dengue fever and Zika virus-associated conditions such as Zika fever, microencephaly and Guillain-Barre syndrome, and *Bunyavirus* pathogens that cause diseases such as LaCrosse encephalitis, Rift Valley Fever, and Colorado tick fever, although without limitation thereto. Non-limiting examples of worm pathogens include nematodes (*e.g.* filarial nematodes such as *Wuchereria bancrofti*, *Brugia malayi*, *Brugia pahangi* or *Brugia timori*), which may be transmitted by mosquitoes.

Disease-causing pathogens transmitted by mosquitoes also include bacteria (*e.g.* *Yersinia pestis*, *Borellia* spp, *Rickettsia* spp, and *Erwinia carotovora*).

Non-limiting examples of pathogens that may be transmitted by *Aedes aegypti* are dengue virus, Zika virus, Yellow fever virus, Chikungunya virus and heartworm (*Dirofilaria immitis*).

Examples of pathogens that may be transmitted by *Aedes albopictus* include West Nile Virus, Yellow fever virus, St. Louis Encephalitis, dengue virus, Zika virus and Chikungunya virus although without limitation thereto.

Pathogens frequently transmitted by the mosquito vector *Anopheles gambiae* include malaria parasites of the genus *Plasmodium* such as, but not limited to, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium berghei*, *Plasmodium gallinaceum*, and

Plasmodium knowlesi.

In one embodiment, said mosquito is of a genus selected from the group consisting of *Culex*, *Aedes* and *Anopheles*.

5 In a preferred embodiment, said mosquito of a species selected from the group consisting of *Aedes aegypti*, *Aedes albopictus*, and *Anopheles gambiae*.

In a particularly preferred embodiment, said mosquito of the species *Aedes aegypti*.

10 A “host” may be any animal upon which a mosquito feeds and/or to which a mosquito is capable of transmitting a disease-causing pathogen. Non-limiting examples of hosts are mammals such as humans, domesticated pets (*e.g.* dogs and cats), wild animals (*e.g.* monkeys, rodents and wild cats) livestock animals (*e.g.* sheep, pigs, cattle, and horses) and avians such as poultry (*e.g.* chickens, turkeys and ducks), although without limitation thereto.

15 As previously described, an aspect of the invention provides a mosquito egg capsule which comprises: a closed capsule; a filler; and a plurality of mosquito eggs; wherein the filler optionally comprises a food source for the mosquito eggs when hatched.

20 The capsule disclosed herein may be any closed or sealed container of preferably spherical, ovoid or cylindrical shape that is of suitable dimensions to encapsulate a plurality of mosquito eggs and sufficient filler to provide a food source for the mosquito eggs and/or larvae hatched therefrom. Typically, the capsule is biodegradable and has a generally flexible, pliable, colloidal or gel-like structure. Suitably, the capsule is or comprises a polymer (inclusive of copolymers), which may be of natural, semi-synthetic or synthetic origin, or a mixture thereof. The polymer may comprise protein, carbohydrates inclusive of
25 mono-, di-, oligo- and poly-saccharides, lipids and/or other organic polymers, or mixtures of these. Non-limiting examples include gelatin, cellulosic polymers, lipid or lipid-containing encapsulating substances.

30 In one broad embodiment, the capsule is formed of a cellulosic polymer. Preferably, the cellulosic polymer is a polymer that is formed of glucose and/or glucose-derived monomers. Each glucose repeat unit of the polymer may

comprise three (3) hydroxyl groups at least one of which may be replaced with an alkoxide to form an ether linkage. Suitably, the alkoxide is a C₁-C₄ alkoxide. Included within the scope of the term 'C₁-C₄ alkoxide' are substituted and unsubstituted methoxides, ethoxides, propoxides, isopropoxides, n-butoxides, sec-
5 butoxides and tert-butoxides. The term 'substituted' refers to substitution of the relevant moiety with one or more hydroxy groups. Preferably, one or more of the three (3) hydroxyl groups of the glucose repeat unit is replaced with methoxide, ethoxide or hydroxypropoxide. Preferably, the cellulosic polymer is hydroxypropyl methyl cellulose or hydroxy ethyl methyl cellulose.

10 The filler may comprise a food source for the mosquito eggs. Typically, the food source comprises protein, lipids, carbohydrates, minerals, vitamins, cofactors and/or other energy and nutrient sources that are required by the mosquito eggs. Non-limiting examples of fillers include yeast and yeast extracts, fish meal (*e.g.* Tuna meal), plant materials such as in the form of fish flakes (*e.g.*
15 Veggie flakes, Tetramin) or animal material (*e.g.* liver extracts) although without limitation thereto. Additionally or alternatively, the filler may not comprise a food source but instead comprise a bulking agent, carrier or other inert and/or non-nutritional substance that adds mass or volume to the capsule. Non-limiting examples of fillers include soluble fibres such as guar gum and psyllium husk,
20 carnuba wax, glycerin, beta glucan, mannitol, maltitol, polydextrose, pethylcellulose and activated charcoal. In a particular embodiment a mixture of mosquito egg capsules with and without food sources may be used in combination.

Suitably, the filler is in a granular or powdered form that can be readily
25 combined with the mosquito eggs and placed in the capsule.

Suitably, the ratio of filler to mosquito eggs is about 0.5 to 5 mg per mosquito egg, about 0.8 to 3 mg per mosquito egg or about 1 to 3 mg per mosquito egg, inclusive of about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4 and 2.5 mg per mosquito egg.

30 In embodiments where the filler comprises fish meal, such as tuna meal, the fish meal is preferably about 20-80% of the filler by weight. In certain

embodiments this may be about 30-70% or about 35%, 40%, 45%, 50%, 55%, 60% or 65% of the filler by weight, or any range between these stated values.

In embodiments where the filler comprises animal material, such as bovine liver powder, the animal material is preferably about 10-60% of the filler by weight. In certain embodiments, this may be about 20-50% or about 25%, 30%, 40% or 45% of the filler by weight, or any range between these stated values.

In embodiments where the filler comprises yeast or a yeast extract, this is preferably about 5-50% of the filler by weight. In certain embodiments, this may be about 10-40% or about, 12%, 15%, 20%, 25%, 30% or 35% of the filler by weight, or any range between these stated values.

It will be appreciated that the mosquito eggs and filler may be “batch-prepared” in sufficient quantity to prepare a plurality of capsules, each with substantially the same amount of mosquito eggs and filler. Preferably, each capsule comprises about 50-300 mosquito eggs, about 75-250 mosquito eggs or about 150-200 mosquito eggs. Preferably, each capsule comprises about 150-500 mg filler, about 200-400 mg filler, about 250-450 mg filler or about 285-395 mg filler.

In a particular embodiment, activated charcoal may be combined with eggs and the food source(s) in a batch preparation to prepare a mixture which is then delivered into each of a plurality of capsules. The activated charcoal acts as an inert filler that facilitates delivery of a consistent, measured amount of eggs and food source to each capsule. Preferably, each capsule will contain about 50-200 mg of activated charcoal, about 75-150 mg activated charcoal or about 110 mg activated charcoal.

The mosquito egg capsule disclosed herein may be suitable for storage of mosquito eggs at ambient temperature (*e.g.* 20-25°C) with 70-90% relative humidity. Storage times may be up to 2-3 weeks under these conditions.

In one embodiment, one or a plurality of the mosquito egg capsules may be placed in an artificial mass-rearing facility for mosquitoes, particularly for the mass rearing of modified mosquitoes to be released as a biocontrol agent.

Successful mass rearing of mosquitoes requires standardization of each step of the mass-rearing process. An important “first step” is mosquito egg production. Typically, mosquito eggs are placed in mass rearing trays or racks, ideally at a suitable density so that consistent numbers of eggs are hatched. This avoids
5 overcrowding, delayed development, undersized pupae and adults and increased mortality. Furthermore, by utilizing a suitable ratio of mosquito eggs:filler, wastage of food resources can be avoided.

In a particular aspect the invention therefore provides a method of modifying a mosquito population, including the step of introducing a mosquito
10 egg capsule into an environment (such as a natural environment) under conditions that facilitate hatching of mosquito eggs into mosquito larvae, wherein release of the mosquito larvae into the environment modifies one or more biological properties of said mosquito population.

The one or more biological properties may include fecundity, tolerance of
15 eggs to desiccation, pathogen susceptibility, viability of offspring, average life-span of a mosquito population and/or the ability to transmit vector-borne diseases such as, but not limited to, malaria, dengue fever, and lymphatic filariasis, although without limitation thereto.

In one particular form of the invention, the modified mosquito eggs are
20 infected with, or otherwise comprise, a mosquito-adapted bacterium.

By “*mosquito-adapted*” bacterium is meant a bacterium that has been taken out of its native host environment and adapted to a mosquito host, in which environment said bacterium does not naturally reside. Accordingly, a non-limiting example of a mosquito-adapted bacterium is a *Wolbachia* bacterium that
25 has been isolated from its native host (*e.g. Drosophila melanogaster*) and adapted to infect, colonize or reside in, a mosquito.

In a preferred embodiment, said isolated mosquito-adapted bacterium is of the genus *Wolbachia*.

In one particular embodiment, said isolated mosquito-adapted bacterium is
30 *Wolbachia pipientis*.

Wolbachia includes strains such as wMel, wMelPop, wMelPop-

CLA, wAlbB, wAu, wMelCS, wNo, wHa, wMau and wCer2, although without limitation thereto.

Suitably, the mosquito eggs are infected with the mosquito-adapted bacterium as described in United States Patent 9090911 and in McMeniman *et al.*,
5 2009, Science **323** 141.

In some other embodiments, the eggs obtained by the method may be used to screen for *Wolbachia* infected mosquitoes to determine the spread of *Wolbachia*-infected mosquitoes in release areas.

In another embodiment, the eggs may be of mosquitoes for release to
10 decrease local mosquito populations through a sterile breeding or “Sterile Insect Technique” (SIT). The sterile males may be released and compete with healthy males to mate. Matings with modified males doesn't result in offspring and the mosquito population is reduced or "crashed".

In one embodiment, the sterile insect technique (SIT) includes radiation
15 sterilization of male mosquitoes subsequently released into the environment.

In another embodiment of SIT, mosquito biocontrol is achieved through an RNAi-based approach where an essential gene for male reproductivity is knocked out or otherwise inhibited using a small interfering RNA molecule to create “sterile males”.

20 Another embodiment relates to a mosquito control system known as Release of Insects with Dominant Lethality (RIDL), which is- a modified version of SIT. In this embodiment, male mosquitoes are genetically modified to contain a homozygous pair of lethal genes. One copy of these genes is passed on to offspring, so that without an antidote (*e.g* tetracycline), these offspring do not
25 survive. Thus, genetically modified males compete with unmodified males to mate, resulting in non-viable offspring and the mosquito population is reduced. By way of example, reference may be made to Massonet-Bruneel *et al.*, 2013, PLOSOne DOI: 10.1371/journal.pone.0062711 for an example of RIDL control of dengue fever.

30 In a still further embodiment, the mosquito egg capsule may be used for maintaining and/or supplementing laboratory colonies of uninfected

(e.g. *Wolbachia* free) mosquitoes.

So that the invention may be fully understood and put into practical effect, the skilled reader is directed to the following non-limiting detailed Examples.

5

EXAMPLES

Preparation of mosquito eggs

Mosquito eggs are initially laid by mosquitoes on a substrate such as a surface-patterned box-board substrate as originally described in International
10 Publication WO2017/083933. After the mosquito eggs have matured and dried, they are gently brushed off from the egg substrate upon which they have been laid, using a paint brush. Brushed eggs are first sieved through a fine mesh to remove non-egg materials and then quantified by weight.

15

Preparation of egg and filler mixture

In one example, the mosquito egg capsules are batch-prepared to achieve average constituents as follows:

1 HPMC (Hydroxypropyl Methyl Cellulose) capsule

150-200 *Aedes aegypti* eggs

20

142mg of Tuna meal

100mg of Bovine liver powder

43mg of brewers yeast

25

In this example, the food components (*i.e* tuna meal, bovine liver powder and yeast) are thoroughly mixed together in a separate container. A known
25 number of mosquito eggs are then added to the food and mixed thoroughly. The egg/food mixture is then aliquoted and dispensed into individual capsules based on weight (285mg filler per HPMC capsule).

30

In another example, the mosquito egg capsules are batch-prepared to achieve average constituents as follows:

1 HPMC (Hydroxypropyl Methyl Cellulose) capsule

150-200 *Aedes aegypti* eggs

142mg of Tuna meal

100mg of Bovine liver powder

43mg of brewers yeast

5 110 mg of activated charcoal

In this example, the food components (*i.e* tuna meal, bovine liver powder and yeast) are thoroughly mixed together in a separate container together with activated charcoal. A known number of mosquito eggs are then added to the food and mixed thoroughly. The egg/food mixture is then aliquoted and dispensed into individual capsules based on weight (395mg filler per HPMC capsule).

Storage and use of mosquito egg capsules

The mosquito egg capsules may then be stored for up to two weeks at ambient temperatures (22°C-24°C) with 85% relative humidity maintained with a saturatated potassium chloride solution and/or water gel crytals in a closed container before being placed in a suitable environment for hatching into larvae.

As previously described, for the purposes of *in vitro* rearing, the mosquito eggs may be placed in mass rearing trays or racks, ideally at a suitable density so that consistent numbers of eggs are hatched. This avoids overcrowding, delayed development, undersized pupae and adults and increased mortality. Furthermore, by utilizing a suitable ratio of mosquito eggs:filler, wastage of food resources can be avoided.

Throughout this specification, the aim has been to describe the preferred embodiments of the invention without limiting the invention to any one embodiment or specific collection of features. Various changes and modifications may be made to the embodiments described and illustrated herein without departing from the broad spirit and scope of the invention.

All computer programs, algorithms, patent and scientific literature referred to herein is incorporated herein by reference in their entirety.

CLAIMS

1. A mosquito egg capsule which comprises: a closed capsule; a filler; and a plurality of mosquito eggs; wherein the filler optionally comprises a food source for the mosquito eggs when hatched.
- 5 2. The mosquito egg capsule of Claim 1 wherein the filler comprises said food source and activated charcoal.
3. A method of producing a mosquito egg capsule including the steps of: combining a plurality of mosquito eggs and a filler that optionally comprises a food source for the mosquito eggs when hatched; locating at
10 at least a portion of the combined mosquito eggs and filler into one or a plurality of capsules; and closing each of the capsules.
4. The method of Claim 3 wherein the filler comprises said food source and activated charcoal.
5. The method of Claim 4, which includes the step of batch preparing the
15 food source, activated charcoal and eggs as a mixture and delivering the mixture to each of said plurality of capsules.
6. A mosquito egg capsule produced by the method of any one of Claims 3-5.
7. The mosquito egg capsule of Claim 1 or Claim 2 or the method of Claim
20 3, wherein the capsule comprises a cellulosic polymer such as hydroxypropyl methyl cellulose or hydroxy ethyl methyl cellulose.
8. A method of producing mosquitoes including the step of providing one or a plurality of mosquito egg capsules of Claim 1, Claim 2 or Claim 6, or mosquitoes produced therefrom, into an environment under conditions
25 that facilitate hatching of the mosquito eggs into mosquito larvae.
9. The method of Claim 8, wherein the environment is an artificial mosquito rearing system.
10. The method of Claim 8, wherein the environment is a natural environment.
- 30 11. A method of modifying a mosquito population, including the step of introducing one or a plurality of the mosquito egg capsules according to

Claim 1, Claim 2 or Claim 6, or mosquitoes produced therefrom, into an environment under conditions that facilitate hatching of mosquito eggs into mosquito larvae, wherein release of the mosquito larvae into the environment modifies one or more biological properties of said mosquito population.

5

12. The method of Claim 11, wherein the mosquitoes are wild-type or otherwise naturally-occurring mosquitoes that exist in a local environment, region or habitat.

10

13. The mosquito egg capsule of Claim 1, Claim 2 or Claim 6, or the method of Claim 3 or Claim 8, wherein the mosquito eggs have been modified to produce mosquitoes for use as a biocontrol agent in said environment.

15

14. The mosquito egg capsule or the method of Claim 13, wherein the mosquito eggs are infected with or otherwise comprise a mosquito-adapted bacterium capable of modifying one or more biological properties of a mosquito, wherein said mosquito-adapted bacterium does not normally colonize, inhabit, reside in, or infect said mosquito host.

15. The mosquito egg capsule or the method of Claim 14, wherein the mosquito-adapted bacterium is of the genus *Wolbachia*.

20

16. The mosquito egg capsule or the method of Claim 15, wherein said isolated mosquito-adapted bacterium is of the species *Wolbachia pipientis*.

17. The mosquito egg capsule or the method of Claim 16, wherein said isolated mosquito-adapted bacterium is *wMel* or *wMelCS*.

25

30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2018/050369

A. CLASSIFICATION OF SUBJECT MATTER

A01N 63/00 (2006.01) A01K 67/033 (2006.01) A01N 25/34 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PATENW (EPODOC, WPIAP and TXTE), Cplus, BIOSOS, CABA, CNFULL, JPFULL, KRFULL, GOOGLE (PATENTS & SCHOLAR) and Espacenet using IPC/CPC: A01N63/00, A01K67/033/LOW, A01M, A01N, A01K and/or Keywords: Mosquito, Mozzie, Insect, Bug, Egg, Capsule, Cellulose, Release, Degrade, Charcoal, Hatch, Wolbachia and similar terms. Applicant (Monash University) and Inventor (LIN, Ya-Hsun; JOUBERT, Albert; GILLES, Jeremie) names searched in Cplus, BIOSIS, CABA, GOOGLE, ESPACENET and IP Australia internal databases.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
22 May 2018

Date of mailing of the international search report
22 May 2018

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
Email address: pct@ipaustalia.gov.au

Authorised officer

Luke Sweetman
AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No. +61262256163

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2018/050369
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 9090911 B2 (MONASH UNIVERSITY) 28 July 2015 Abstract, Example 2	1, 3, 6, 8-9, 11-17
X	US 7868222 B1 (DOBSON) 11 January 2011 Abstract, example 1 (column 9 line 55 - column 10 line 5), example 3 (column 16 lines 15-17)	1, 3, 6, 8-9, 11-17
X	S. RITCHIE, 'Rear and release: a new paradigm for dengue control', Austral Entomology, 2014, Vol. 53, Pages 363-367 Page 364 - left column, final paragraph; Page 366 - right column, paragraph 2	1-17
X	ROSS, P.A. et al., 'Costs of Three Wolbachia Infections on the Survival of Aedes aegypti Larvae under Starvation Conditions', PLOS Neglected Tropical Diseases, 2016, DOI:10.1371/journal.pntd.0004320, Pages 1/22 - 22/22 Rearing regime (lines 2-5)	1, 3, 6, 8-9, 11-17
X	FAQs Eliminate Dengue - A natural method to reduce the spread of dengue [accessed 13/12/2017] - <URL: http://www.eliminatedengue.com/id/faqs >, published on 13/08/2016 as per Wayback Machine Point 3 (What do field trials involve?)	1, 3, 6, 8-9, 11-17
A	TRICHOSAFE® Capsules [Retrieved on 11 December 2017] - <URL: http://biocare.de/trichosafecapsules/ >, published on 22 November 2016 as per Wayback Machine	
A	FR 2583256 A1 (LIMAGRAIN) 19 December 1986 Machine translation of description - see page 1 (paragraphs 2-3)	

Form PCT/ISA/210 (fifth sheet) (July 2009)

INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/AU2018/050369	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
US 9090911 B2	28 July 2015	US 2014298501 A1	02 Oct 2014
		US 9090911 B2	28 Jul 2015
		AU 2009222557 A1	13 Jan 2011
		AU 2009222557 B2	11 Jun 2015
		CA 2707880 A1	17 Dec 2010
		US 2011145939 A1	16 Jun 2011
US 7868222 B1	11 January 2011	US 7868222 B1	11 Jan 2011
FR 2583256 A1	19 December 1986	FR 2583256 A1	19 Dec 1986
		FR 2583256 B1	09 Dec 1988
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2009)			