Autodissemination of insecticides for Mosquito control_

Review of current R&D status, and feasibility for widespread operational adoption.

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1 ABBREVIATIONS

AD	Autodissemination
ADS	Autodissemination Station
AI	Active Ingredient
ATSB	Attractive Targeted Sugar Bait
Ha	Hectare
IGR	Insect Growth Regulator
IRS	Indoor Residual Sprays
ITNs	Insecticide Treated Nets
JHA	Juvenile Hormone Analogue
L	Litre
PCO	Pest Control Operator
PPF	Pyriproxyfen
RCT	Randomized Cluster Trials
SBS	Sentinel Breeding Site
SIT	Sterile Insect Technique
TPP	Target Product Profile
VCAG	Vector Control Advisory Group
WHO	World Health Organization

2 Executive summary

- Autodissemination (AD) may offer a way to significantly increase the efficiency of larviciding, using mosquitoes to seek-out and treat aquatic breeding sites more effectively than human operators. Especially where breeding sites are many and cryptic, this technique has the potential to render larviciding operations more feasible, and make them a more important part of vector borne disease control programmes. Additionally, the use of new active ingredients (AI) in mosquito control for AD will potentially help with resistance management against established AIs used in Insecticide Treated Nets (ITNs) and Indoor Residual Sprays (IRS), especially in Africa. ITNs/IRS are minimally effective against certain *Anopheles* such as *An arabiensis* due to their behaviours, and AD offers a potential tool for controlling such species, and by extension controlling residual malaria transmission.

- Research in AD has mostly been undertaken with pyriproxyfen (PPF) and has produced mixed results. *Aedes* species are in principle well-suited to the technique although there is little published evidence of population reduction at scale. The commercially available In2Care system and a new system to be launched by Springstar are both targeted at *Aedes*. Other commercial companies working on AD against *Aedes* are MosquitoMate and ISCA Technologies.

- The In2Care system against *Aedes* is the only commercialized ADS to date, registered in over 30 countries, with sales having been made in over 20. In2Care are targeting the professional market, which can include abatement districts in the US and municipalities elsewhere. It is planned to launch also in the consumer sector in the US. Commercialization in the US began in early 2018. In Asia, the first launch in Thailand is underway. Limited sales have been made in the Pacific, and in the Caribbean.

- For *Aedes*, the experience thus far with the In2Care system is that the large numbers of autodissemination stations (ADS) required for control, together with the frequent servicing needs, hampers feasibility for large area adoption, and this is recognized by the companies working on *Aedes* systems. In2Care are optimizing the system to reduce ADS density, targeting to *Aedes* hotspots, and integrating with other methods. Trials are ongoing in a number of locations. Spingstar have an *Aedes* ADS which is close to launch in the US, based on an oviposition ADS, which aims to reduce the number of ADSs and servicing frequency significantly compared to the In2Care system.

- For *Aedes*, a World Health Organisation (WHO) approval is not necessary for sales, as witnessed by In2Care. However, national vector control organizations are less likely to use the system in the absence of WHO approval than municipalities, abatement districts or the Pest Control Operator (PCO) sector, which are the main target customers at present. External funding for *Aedes* control programmes is negligible, and so the usual WHO approval requirement of such funding organizations is not relevant. A full WHO approval would nevertheless allow for more widespread usage of the In2Care *Aedes* system, especially by national vector control programmes.

- For *Anopheles*, a WHO approval is almost certainly necessary. PQT-approved larvicidal IGRs (PPF, diflubenzuron, novaluron) are the most logical candidates for possible development of an ADS system for *Anopheles.* However, use of any larvicide in an ADS would be considered as a new paradigm by WHO's Vector Control Advisory Group (VCAG), requiring two epidemiological studies.

- For *Anopheles,* there is no commercial system developed. There is very scant evidence from the field that AD is a feasible control tool for *Anopheles,* beyond some initial proof of concept work. With the exception of *An stephensi, Anopheles* are not container breeders and so will not readily enter an oviposition-type trap as exploited in the In2Care and Springstar *Aedes* systems. Also, the extent to which *Anopheles* practice skip oviposition is poorly understood. Breeding sites of *Anopheles* tend to be larger on average than for *Aedes* and so the dilution effect of the disseminated larvicide becomes more crucial. A critical point regarding feasibility of adoption is whether direct larviciding of larger *Anopheles* breeding sites is realistic alongside AD, as these may be unlikely to receive an adequate dose via AD.

- In Africa, control of *An arabiensis* is an unmet need. An AD system against *Anopheles* as we have defined in our Target Product Profile (TPP), should include control of *An arabiensis* as a minimum, when used as a primary vector control measure. *An gambiae* is an additional African target especially as resistance to AIs used in ITNs/IRS is growing. Against *An gambiae*, we consider AD would at best be a supplementary measure to IRS/ITNs, providing increased control and mitigating against resistance development. We do not consider *An funestus* to be a realistic target for AD mainly because of the larger size of its breeding sites. *An stephensi* is a potential target because it is an urban container breeder, and is therefore potentially well-suited. This species transmits malaria in urban areas throughout the Indian sub-continent, and has been implicated in an urban malaria outbreak in Africa (Djibouti), having earlier invaded.

- There are other key *Anopheles* species elsewhere which also cannot be adequately controlled by ITNs and/or IRS because of their behaviours. These would therefore be potential targets for an AD system, although these have been excluded from the TPP. These include *An darlingi* in S.America, *An furauti* in Oceania, *An culicifacies, An fluviatilis, An lesteri and An minimus* in Asia. These have been identified as a next challenge for malaria control, contributing to residual transmission in their respective regions. The malaria burden associated with these species has not been analysed.

- A major challenge of research is to find a way to concentrate *Anopheles* adults so as to contaminate them in large numbers with larvicide, for subsequent dissemination. There is little or no attraction of *Anopheles* (with the possible exception of *An stephensi*), to an artificial oviposition-type ADS of the type developed by In2Care and Springstar against *Aedes*. So targeting adults at host-seeking is being pursued. However, PPF sterilizes adult mosquitoes if they are contaminated closely to (with ~24 hr before/after) blood-feeding, although there is some evidence this sterilization effect may differ for alternative IGRs. Sterilization as a control strategy does not seem logical. It appears that females which have been sterilized by PPF do not practice AD to much extent, although this is not fully clear. Targeting adults at host-seeking rather than oviposition, increases the period between contamination and dissemination, requiring a formulation which is much more resistant to grooming/shedding.

- The alternative approach is to target mosquitoes for pick-up of larvicide near to the time of oviposition, as with approaches targeting *Aedes*. Not only would this avoid sterilization, but the time period between larvicide pick-up and dissemination would be much shorter, resulting in less larvicide being shed from the body of the adult in the interim. However, an ADS based on oviposition-timing would need to be made very attractive to ensure sufficient adults visit. Resting sites associated with oviposition sites could be targeted, but these are not well-defined. The incorporation of attractive volatiles and sugar-baits in ADS design has not been in explored yet in depth.

- Using adults of other insect species (which may be more abundant or easier to target) to autodisseminate a larvicide to *Anopheles* breeding sites has been suggested, and some passive AD of PPF by *Culex* may have occurred in some of the *Aedes* studies published. Purposely mass-rearing and releasing larvicide-treated adult mosquitoes has been postulated, and some work in this area has taken place (MosquitoMate/Univ Kentucky, Rutgers Univ) or will start (IAEA/CIRAD), albeit with *Aedes* at present.

- For *Anopheles*, the consensus is that improved formulations and attractiveness of an ADS are key. Significantly improved formulations are needed to maximize load per adult mosquito, and ensure improved adhesion, which seems critical for effectiveness and operational feasibility.

- Springstar (collaborating with Rutgers University) are seeking funding to develop a system for *Anopheles*, focussing on improved formulations and ADS design, although this system is at an early stage. ISCA have experience with attractants and sugar-feeding and are currently in early stage development of a system potentially for both *Aedes* and *Anopheles*. Some university researchers are working on AD without industrial partners, examining variations of ADS design, density, etc, but without much involvement in formulation development.

- For *Anopheles,* we consider a low-tech AD solution requiring no ADS would likely be much more feasible to deploy than a system which required high numbers of AD stations requiring expert decision on where and how they are locally deployed, and requiring frequent monitoring and servicing.

- Risk of resistance development is a factor which will likely influence adoption, as larger breeding sites which are dosed with AI via AD may receive sub-lethal doses. There is some evidence of cross resistance between OPs and PPF and pyrethroids and PPF. Generally, the likelihood of resistance/cross resistance developing rapidly in the field and negating the value of a PPF-based AD system is difficult to predict.

- We sought the opinions of mosquito experts regarding feasibility of adoption. Most focused rather on the feasibility of developing a workable system, and what type of use settings might be more appropriate, rather than commenting on the feasibility of large scale operational adoption within those settings. The consensus was that the applicability of AD for *Anopheles* control is uncertain, and if successful, it would probably only find a niche role, during the dry season.

- Having reviewed the literature and discussed widely with experts, we consider that the chances of developing an *Anopheles* AD system which fits our target TPP is low, and the business case for a for-profit company is almost certainly lacking. Given the pressing need for new solutions, the increasing resistance against *An. gambiae,* and the unmet need against *An arabiensis,* supporting further work (which would eventually involve epidemiological trials) may be considered worthwhile by an external funder, dependent on their competing priorities. If further work is considered worthwhile, a pragmatic way forward would be to clarify some key points on biology as early as possible (sterility and oviposition effects, including alternative AIs) as detailed in the recommendations section. Significant further development on formulation and ADS attractiveness, along two parallel paths initially, targeting both host-seeking and oviposition behaviours, would be required. This dual path would increase chances of success. Investigations would first involve lab/semi-field work, and only if significant improvements are realised should field evaluation be undertaken.

3 INTRODUCTION – OBJECTIVES

AD is an approach to amplify insecticide deployment by co-opting insects to transfer insecticides to other insects via either oviposition, mating, resting or host-seeking. AD offers potential advantages in efficiency and precision for insecticide application compared with conventional approaches.

For mosquito control, AD was first demonstrated by Itoh [1] who showed that *Ae aegypti* transferred PPF from treated artificial resting sites to larval breeding sites, resulting in significant insecticidal activity on immature mosquitos. Further development of AD has largely been limited to additional proof of principle studies in the laboratory, semi-field and limited scale field studies. The majority of AD studies have focused on Aedine species and to a lesser extent *Anopheles* species. A variety of factors including novel ADSs, alternative AIs and improved formulations have been evaluated (Table 1 and Appendix 1). One large longitudinal field study against *Ae aegypti* has been conducted in a Brazilian suburb with a population of 60,000[2] providing tantalizing promise that technology could become an operational control tool. However, its applicability and efficacy in range of environments is still to be ascertained.

The purpose of this study is to inform decision-making at IVCC on whether there is sufficient potential in the auto-dissemination approach to support further development with a view to eventual operational deployment.

[1] Itoh, T. (1993) Control of DF/DHF Vector, Aedes Mosquito, with Insecticides. *Tropical medicine* 35**,** 259-267.
[2] Abad-Franch, F., E. Zamora-Perea & S. L. Luz (2017) Mosquito-Disseminated Insecticide for Citywide Vector Control and Its Potential to Block Arbovirus Epidemics: Entomological Observations and Modeling Results from Amazonian Brazil. *PLoS Med,* 14**,** e1002213.

The study covers the potential and feasibility for AD for control of *Anopheles* and *Aedes* species, and includes recommendations on next steps.

A literature review was undertaken, and discussions were conducted with a number of key experts in mosquito control (Appendix 3), including those with past and current involvement in AD.

4 LITERATURE REVIEW

4.1 METHODOLOGY

A systematic literature review was conducted to identify relevant literature from databases using search teams listed below:

- Databases searched included:
  - PubMed
  - LILACS[3]
  - Google Scholar
- Search terms included:
  - "Auto dissemination" AND Anopheles OR Aedes OR Mosquito OR Mosquitoes
  - "Auto dissemination" AND Malaria OR Dengue OR Zika OR "vector control"

Articles identified from the database search were screened for relevance to inclusion criteria before detailed review. Additional articles were selected through screening of the reference list of literature identified through the initial database search.

Inclusion criteria

Peer review publications and grey literature where included. The detailed review focused on semi-field and open field evaluation of AD. In addition, relevant literatures were included such as review articles and laboratory studies that were relevant to the assessment of field efficacy and feasibility for widespread operational adoption of the AD approach.

Exclusion criteria

AD not related to mosquito control. Studies on active ingredients associated with AD, eg PPF, that are not directly related to AD.

[3] http://lilacs.bvsalud.org/en/

4.2 Results

Literature identified with relevance to AD is provided in Table 1 summarizing; target species, AI, study type and literature category. Semi-field studies were defined as confined studies in cages > $8m^3$.

The majority of published work has focused on *Ae aegypti* and *Ae albopictus* arbovirus vectors (30/49), with only 12/49 studies on *Anopheles* malaria vectors. The clear majority (40/49) of studies also focused on the highly potent Insect Growth Regulator (IGR), PPF, as the AI.

From these literature, 20 studies reported semi-field and open-field evaluations which underwent detailed review. A synopsis of these studies is provided in Appendix 1 providing the following for each: study type (semi-field/open-field); location; target species; AD method; AI; formulation; application rate; and summary of objectives, methods, key results and conclusions.

A patent search using the term "autodissemination" was also conducted using PATENTSCOPE, accessed on 24/5/2018. 37 matches were reported, and following review 13 were identified as relevant to AD for mosquito control. These only represented 5 unique inventions after considering multiple submissions of the same invention to different territories. A table of these patents and summary of inventions are provided in Appendix 2.

A number of recent reviews were identified that cover AD for mosquito vector control: Devine (Devine 2016) reviewed AD of PPF for container breeding mosquitoes. Maoz (Maoz et al. 2017) includes review of AD in a systematic review of PPF effectiveness as a dengue vector control method. Faraji (Faraji and Unlu 2016) also reviewed AD as part of wider review of current control methods for *Ae albopictus* in the USA.

Table 1: Summary of literature identified referring to AD for mosquito control with details of: target species, AI, study type and literature category. Semi-field studies were categorized as in cages > $8m^3$.

Reference	Target species	AI	Study	Peer Review (PR), Grey Literature (GL)
(Suman et al. 2018)	*Ae albopictus*	PPF	Open Field	PR
(Swale et al. 2018)	*Anopheles quadrimaculatus*	PPF Novaluron Triflumuron	Lab	PR
(Abad-Franch et al. 2017)	*Ae aegypti*, *Ae albopictus*	PPF	Open Field	PR
(Buckner et al. 2017)	*Ae aegypti*, *Ae albopictus*	PPF + *B. bassiana*	Semi-Field	PR
(Lloyd et al. 2017)	*Ae albopictus*	PPF	Open Field	PR
(Maoz et al. 2017)	*Ae aegypti*, *Ae albopictus*	-	REVIEW	PR
(Mian, Dhillon and Dodson 2017)	*Culex quinquefasciatus*	PPF	Open Field	PR
(Scott et al. 2017)	*Ae albopictus*	PPF	Lab	PR
(Unlu et al. 2017)	*Ae albopictus*	PPF	Open Field	PR
(WHO 2017)	*Ae aegypti*, *Ae albopictus*	PPF + *B. bassiana*	Background	GL

(Lwetoijera et al. 2017)	*An arabiensis*	PPF	Semi-Field	GL
(Lwetoijera 2016)	*An arabiensis*	PPF	Semi-Field	GL
(Bibbs, Anderson and Xue 2016)	*Ae albopictus*	Methoprene	Caged	PR
(Bouyer et al. 2016)	*Ae aegypti, Ae albopictus*	Densovirus	REVIEW	PR
(Chandel et al. 2016)	*Ae albopictus*	PPF	Open Field	PR
(Devine 2016)	*NA*	PPF	REVIEW	PR
(Faraji and Unlu 2016)	*Ae albopictus*	PPF	REVIEW	PR
(Kartzinel et al. 2016)	*Ae aegypti, Ae albopictus*	PPF	Open Field	PR
(Tuten et al. 2016)	*Ae japonicus*	PPF	Caged	PR
(Abad-Franch et al. 2015)	*Ae aegypti, Ae albopictus*	PPF	Open Field	PR
(Kiware et al. 2015)	*Anopheles*	PPF	REVIEW	PR
(Mains, Brelsfoard and Dobson 2015)	*Ae albopictus*	PPF	Open Field	PR
(Mbare 2015b)	*An gambiae*	PPF	Semi-Field	GL
(Bouyer and Lefrançois 2014)	*NA*	PPF	REVIEW	PR
(Fulcher et al. 2014)	*Ae albopictus*	PPF	Lab	PR
(Killeen et al. 2014)	*Anopheles*	-	REVIEW	PR
(Lwetoijera et al. 2014a)	*An arabiensis*	PPF	Semi-Field	PR
(Lwetoijera et al. 2014b)	*An arabiensis*	PPF	Semi-Field	PR
(Mbare, Lindsay and Fillinger 2014)	*An gambiae*	PPF	Lab	PR
(Snetselaar et al. 2014)	*Ae aegypti*	PPF + *B. bassiana*	Semi-Field	PR
(Suman et al. 2014)	*Ae albopictus*	PPF	Open Field	PR
(Wang et al. 2014)	*Ae albopictus*	PPF	Semi-Field	PR
(Burkett et al. 2013)	-	-	REVIEW	PR
(Mbare, Lindsay and Fillinger 2013)	*An gambiae*	PPF	Lab	PR
(Ohba et al. 2013)	*Ae albopictus*	PPF	Semi-Field	PR
(Ponlawat et al. 2013)	*Ae aegypti*	PPF	Open Field	PR
(Caputo et al. 2012)	*Ae albopictus*	PPF	Open Field	PR
(Lwetoijera 2012)	*An arabiensis*	PPF	Semi-Field	GL
(Gaugler, Suman and Wang 2011)	*Ae albopictus*	PPF	Caged	PR
(Garcia-Munguia et al. 2011)	*Ae aegypti*	*Beauveria bassiana*	Lab	PR
(Reyes-Villanueva et al. 2011)	*Ae aegypti*	*Metarhizium anisopliae*	Lab	PR
(Devine and Killeen 2010)	*Anopheles*	PPF	REVIEW	PR
(Devine et al. 2009)	*Ae aegypti, Culex spp.*	PPF	Open Field	PR
(Carlson, Suchman and Buchatsky 2006)	-	Densovirus	Lab	PR
(Sihuincha et al. 2005)	*Ae aegypti*	PPF	Lab	PR
(Scholte, Knols and Takken 2004b)	*An gambiae*	*Metarhizium anisopliae*	Lab	PR

(Dell Chism and Apperson 2003)	*Ae albopictus, Ochlerotatus triseriatus*	PPF	Lab	PR
(Itoh 1994)	*Ae aegypti*	PPF	Open Field	PR
(Itoh 1993)	*Ae aegypti*	PPF	Open Field	PR

4.3 DISCUSSION

The AD method is an approach to amplify insecticide deployment by co-opting insects to perform transfer of insecticides to other insects via different behavioral activities including oviposition, mating, resting and host seeking.

AD offers several potential advantages in efficiency and precision for insecticide application compared with conventional approaches. By co-opting mosquitoes in the dissemination of a larvicide (or pupicide), the most-visited breeding sites will likely receive the greatest transfer of AI, providing a highly targeted dissemination tailored to the mosquito ecology and behavior within any given environment. In theory this could significantly improve the effectiveness and adoption of larviciding, reducing the amount of AI needed, opening the possibility of using an AI that would otherwise be too costly, and providing operational cost savings compared to current larviciding practices. Furthermore, broadening the number of AIs available for vector control is highly desirable in the ongoing fight to manage insecticide resistance.

Targeting aquatic larval habitats of mosquitoes has been the mainstay of many urban vector control programs. A fundamental problem of the strategy is the near impossibility of identifying and appropriately treating all mosquito breeding sites. The importance of cryptic breeding sites in control of *Aedine* species is well documented (Chandel et al. 2016). Targeting breeding sites for control of malaria vectors has been less widely used or recommended in rural settings as the abundance and transient nature of breeding sites makes them even more difficult to target compared to urban environments (WHO 2012). AD has the potential to improve the larviciding approach, by utilizing mosquitoes to target and transfer insecticide to the myriad breeding sites within an environment, many of which are cryptic and not easily detectable or accessible by conventional methods.

The AD approach for mosquito control was first outlined by Itoh (Itoh 1993) who demonstrated *Ae aegypti* would transfer PPF from treated artificial resting sites to larval breeding sites in a house in Bangkok, Thailand, resulting in significant insecticidal activity on immature mosquitoes. The idea was not seriously revisited in the field until 2009 when Devine et al (Devine et al. 2009) showed the potential of the technique in a field study conducted in a graveyard in Iquitos, Peru. It was demonstrated that treatment of ~5% of available breeding/resting sites with PPF resulted in contamination of > 95% of available breeding sites at sufficient concentration to induce 49-85% mortality of juvenile mosquitoes in sentinel breeding sites (SBS). This demonstrated the potential of local mosquito populations (in this case *Ae aegypti* and *Culex* spp) to amplify the coverage of an IGR treatment by AD. Furthermore Devine et al (Devine et al. 2009) make the case that the impact of using an AD approach should achieve greater mortality in the long run, compared with a conventional lure and kill approach. Models based in part on empirical data from the Iquitos field study suggested this may be true, fueling interest in further development of this novel approach.

Following this, ongoing development of AD has largely been limited to further proof of principle studies in the laboratory, semi-field and limited scale field studies (Appendix 1 and Table 2). The notable exception being a recent large longitudinal intervention study conducted in a Brazilian suburb with a population of 60,000 (Abad-Franch et al. 2017). A variety of factors including novel AD stations (ADS), alternative AIs and improved formulations have been evaluated. The majority of AD studies have focused on Aedine species and to a lesser extent targeting *Anopheles* malaria vectors (Appendix 1 and Table 1).

Most AD approaches are based on deployment of an ADS designed to attract and contaminate target mosquitoes, before subsequent dissemination. The exception are methodologies that directly treat artificially reared insects and release them into the environment (Devine 2016, GAUGLER et al. 2017).

The success of ADS depends on three criteria: 1) attraction of mosquitoes to the stations, 2) transfer of larvicide to the mosquitoes, and 3) dissemination of larvicide to target habitats (Gaugler et al. 2011). Attractiveness of the ADS can be mediated by taking advantage of one or more natural behaviors of mosquitoes. Once attracted, the design of an ADS and the formulation of AI will both contribute to successful transfer of AI load to mosquitoes. Subsequent dissemination will be related to AI formulation (durability) and mosquito behavior following contamination. The total population of mosquitoes is also critical, as they carry and transfer the insecticide.

4.3.1 Targeting behaviors

A variety of approaches and designs of ADS have been developed exploiting different behaviors/physiological states of mosquitoes at points where they can become contaminated with larvicide before subsequent dissemination:

4.3.1.1 Oviposition

The majority of AD studies/approaches have revolved around targeting oviposition behavior of gravid females to develop oviposition-based ADS. These are effective in exploiting skip oviposition behaviors exhibited by some mosquito species such as *Ae aegypti* (Day 2016) where they distribute their eggs over multiple sites rather than laying all at once. Given that PPF insecticidal activity is principally mediated though pupicidal effect, targeting AD to breeding sites is key to efficacy. Consequently, targeting females, specifically gravid females, represents the most effective life stage to transfer to breeding sites. Furthermore, the time between contamination and subsequent transfer is minimized by targeting gravid females, an important consideration given the rapid loss over time of load of AI following contamination (Kartzinel et al. 2016, Swale et al. 2018, Mbare 2015b). In addition to the AD component, oviposition based ADSs can also act as an egg sink, and thereby contribute to population control (Buckner et al. 2017).

Attractiveness of oviposition sites may be enhanced with visual cues and odor lures that can borrow from the existing body of knowledge and research in optimization of attractiveness of ovitraps for monitoring and control (Day 2016). Many AD studies have used a variation of hay infusion (Ohba et al. 2013, Suman et al. 2018, Unlu et al. 2017, Chandel et al. 2016, Abad-Franch et al. 2015) to improve attractiveness of oviposition-based ADSs. The In2Care AD system provides yeast pellets as an oviposition attractant (Buckner et al. 2017, Snetselaar et al. 2014), and Mbare (Mbare 2015b) evaluated soil infusion and a sesquiterpene alcohol (cedrol) to enhance attractiveness of an oviposition-based ADS for the malaria vector *An gambiae*.

4.3.1.2 Resting sites

Resting sites can target both sexes and all physiological states of adults. However, the location of resting sites may favour specific sex and physiological states, for example resting sites associated with breeding sites will preferentially target gravid females, whereas proximity to hosts (eg bed nets) would target host-seeking/recently blood-fed females. An ADS described by Itoh consisted of a bamboo basket lined with PPF-treated netting targeting *Ae aegypti* mosquitoes (Itoh 1994, Itoh 1993). Lwetoijera used a similar apraoch tageting *An arabiensis* using artifical resting sites consiting of clay pots lined with PPF-treated black cloth (Lwetoijera et al. 2014a).

4.3.1.3 Blood/Host seeking

Blood/host seeking behavior can also be exploited in the design of an ADS. This has the advantage of targeting females, although males may often be attracted to the same cues, presumably to seek females. For example, BG-Sentinel traps utilize a host mimic lure and can be equally effective at capturing males as females. PPF treated bed nets have been evaluated targeting *Ae aegypti* in a semi-field setting by Ohba (Ohba et al. 2013) showing PPF transfer to SBSs resulting in increased immature mortality. Resting sites near hosts can be used to contaminate host-seeking and recently blood-fed females. This approach was demonstrated by Lwetoijera (Lwetoijera et al. 2014b) in semi-field settings with *An arabiensis*. A blood meal was provided in the form of a tethered cow housed in a small shed which was lined with PPF-treated black cloth. This resulted in 100 % sterility of females and > 90% juvenile mortality bioassays in SBSs. Direct treatment of non-human hosts is another approach reported by Lwetoijera, who directly treated cattle within a semi-field setting with an oil based PPF formation and showed significant AD by *An arabiensis* resulting in increased immature mortality in SBSs (Lwetoijera 2012).

4.3.1.4 Sugar feed seeking

Targeting sugar feeding behavior is a promising approach actively being developed as a lure and kill system for vector control. The Attractive Targeted Sugar Bait (ATSB) approach utilizes insecticide treated sugar bait and associated odor lures and phagostimulants to induce feeding leading to mortality. Exploiting similar approaches may provide an avenue to contaminate target mosquitoes with PPF for subsequent dissemination. Sugar feeding can be used to attract both males and females. Fulcher (Fulcher et al. 2014) has investigated ATSBs treated with PPF and subjected to simulated rain-wash experiments, and showed significant mortality of mosquito larvae coming into contact with the wash-off.

4.3.1.5 Mating

Contact between opposite sexes during mating can provide an avenue for transfer of AI. This may disseminate AI through a population following initial contamination via an ADS. For example, males contaminated by a resting site ADS could transfer AI to females, thereby eliciting insecticidal activities: be it by a sterilizing effect (PPF), subsequent transfer to breeding sites, or infection and dissemination in the case of biologicals. AD of the entomopathogenic fungi, *Metarhizium anispliae* was demonstrated in a laboratory study of *An gambiae* from infected females to males via mating (Scholte et al. 2004b). Female *Ae aegypti* have been shown to have almost total loss in fecundity following mating with *M anispliae* infected males in laboratory cage studies (Reyes-Villanueva et al. 2011). Mains (Mains et al. 2015) determined that PPF-dusted males could contaminate females during mating with sufficient PPF to confer pupicidal activity in larval rearing bioassays. Transfer via mating of a biological (densovirus) or PPF have also been proposed as a means of AD by treating mass-reared males before release to boost the efficacy of the Sterile Insect Technique (SIT) for mosquito control (Bouyer et al. 2016, Bouyer and Lefrançois 2014).

4.3.1.6 Combinations

May AD approaches have targeted multiple behaviors/life stages to contaminate the mosquito population. Surfaces in and around oviposition sites can represent attractive resting sites, not only for gravid females, but males and non-gravid females, as they offer sheltered, cool and humid micro environments. Indeed, many ovitrap-based ADSs do not apply insecticide directly to water, rather treat adjacent resting surfaces (Buckner et al. 2017, Kartzinel et al. 2016, Abad-Franch et al. 2015, Ponlawat et al. 2013, Caputo et al. 2012, Devine et al. 2009). The In2Care system harnesses this approach, but also includes a slow acting adulticide in the form of *Beauveria bassiana* spores. Kartzinel (Kartzinel et al. 2016) developed an ADS targeting multiple life stages which comprised a rain shelter housing a PPF-treated resting site baited with a known host-seeking lure, and a breeding site lined with PPF-treated treated cloth.

4.3.1.7 Selection of appropriate target behaviour/resource

The ecology of the mosquito species should be considered when selecting behaviours to target mosquitoes for contamination. Oviposition type ADSs (eg In2Care) are suited for pick-up of insecticide by *Aedes*, due to their readiness to enter a man-made container to oviposit. *Aedes* skip-oviposition behavior ensures it spreads the insecticide to multiple breeding sites (Devine et al. 2009). By contrast, the complex hydrology, transient nature and greater geographical distribution of natural breeding sites favored by *Anopheles* makes it more challenging to develop an ADS that can compete with natural sites (WHO 2012, Kiware et al. 2015, Majambere et al. 2008). For these reasons, developing an ADS targeting host-seeking, rather that oviposition behavior, has been proposed as more viable for *Anopheles.*

The effect of PPF on *An gambiae* females treated at different times through a gonotrophic cycle was evaluated by Mbare (Mbare et al. 2014). Females were nearly completely sterilized when exposure occurred close to a blood meal (≤ 24h before and after blood feeding) but were ineffective at transferring PPF to breeding sites. By contrast, females that were exposed while gravid and close to egg-laying time (<24hr) were effective at transferring PPF to breeding sites but were not sterilized. Similar effects have also been reported for *An arabiensis*, and *Ae aegypti* where timing of contamination impacts the degree of sterilization (Harris et al. 2013, Itoh 1994). Transfer of PPF to breeding sites from ADSs targeting host-seeking has been observed in semi-field studies using *An arabiensis* by Lwetoijera (Lwetoijera et al. 2017, Lwetoijera et al. 2014b, Lwetoijera et al. 2014a, Lwetoijera 2016). The principal impact of exposure to PPF was the sterilizing effect on the female. It should be noted that the density in some of these studies was equivalent to ~0.5 million females/ha – so the opportunity of transfer by multiple visits was maximized, as is the real possibility the some females had died in an SBS transferring their full PPF load, which has been estimated to be ~ x5000 that transferred during oviposition (Mbare 2015b).

In addition to differences in preferred breeding site habitats, the ecology of vectors can be categorized in terms of preferred host (anthropophilic/zoophilic) and habitat (endophilic/exophilic) (Dia, Guelbeogo and Ayala 2013, Killeen et al. 2014). These preferences should be considered when developing an appropriate ADS to target mosquitoes. *Ae aegypti*, and to a lesser extent *Ae albopictus*, are highly anthropophilic in host preference and endophilic in terms of habitat and resting site location. Of the *Anopheles* malaria vectors, *An gambiae* is similarly anthropophilic and endophilic. For such species, developing ADS based on indoor resting sites is logical. For the more exophilic species with wider zoophilic host preference (eg *An arabiensis*), identifying and targeting their outdoor resting sites associated with non-human hosts may be a more appropriate approach. Semi-field evaluations by Lwetoijera have shown success in direct treating cattle (Lwetoijera 2012), and utilizing treated resting sites in cattle sheds (Lwetoijera et al. 2014b).

4.3.1.8 Sterilization vs AD larviciding

Evidence from the literature would suggest targeting gravid females would be most effective to maximize AD to breeding sites, where population control can be mediated by insecticidal activity on juveniles. However, if population control is primarily though the sterilizing effect on contaminated females, targeting the host seeking females (and males that could transfer larvicide via mating) might be a better approach. Hence there will be a divergence in strategy based on target mosquito ecology; either focus on AD as mechanism of improved larviciding, or focus on sterilizing impact of PPF where contact with PPF happens around blood feeding behaviours.

If the impact on populations is via sterilization (targeting host-seeking), with negligible or no transfer to juveniles in breeding sites, the approach will be another variant of a lure and kill strategy. This is a significant point raised in a recent review of AD by Devine (Devine 2016) which states "*the only justification for releasing dusted female mosquitoes is to effect control over the larger population. If only the offspring of the exposed females are affected, then a "trap and kill" device would deliver the same modest impact.*" Indeed, an attract and kill device with rapid acting insecticide such as in an ATSB (Maia et al. 2018, Fiorenzano, Koehler and Xue 2017) or eave tubes (Snetselaar et al. 2017) could reduce risk of disease transmission by intercepting infected females, which would not be true for PPF.

4.3.2 Deterministic models

Several publications have sought to define key factors pertinent to AD and incorporate them in deterministic models. These have been used to provide insight into factors and situations important to successful deployment of AD. Devine et al (Devine et al. 2009) first outlined a simple deterministic model that could demonstrate how AD could achieve high coverage of available breeding sites from a small proportion of available resting sites:

"The model proposes that the relationship between the coverage of adult resting sites (Cr) and the larval habitats that the JHA is disseminated to (Ch) can be crudely described as a simple exponential function of the duration for which habitats remain unproductive after contamination (U), the number of ovipositions by the vector population (O) relative to the number of habitats (H), and the mean number of contaminated ovipositions required to render a single habitat unproductive (Ω):

$$C_h = 1 - \exp(C_r U[O/H\Omega])$$

In a follow-on publication Devine and Killeen (Devine and Killeen 2010) utilized the same model to explore applicability of the AD approach to malaria vectors. Amongst other insights, they suggest that AD is best suited to dry seasons when the breeding sites are few and more stable. Kiware et al (Kiware et al. 2015) describes a comprehensive model, expanding that previously outlined by Devine, incorporating additional factors.

4.3.3 Active ingredients

4.3.3.1 Pyriproxyfen

PPF has been synonymous with development of the AD approach from its inception by Itoh (Itoh 1993). Although several other synthetic and biological insecticides have been evaluated to a lesser extent, the properties of PPF seem to make it ideal for the AD approach, and most published work on AD has used PPF as the AI (Table 1). PPF is a juvenile hormone analogue (JHA) which has limited effect on contaminated adults in terms of lethality (Mains et al. 2015), or repellency (Devine et al. 2009, Mbare 2015a). This is key as reduced longevity and repellency would compromise efficacy co-opting target insects for dissemination. Although commercially marketed as a larvicide, PPF could more accurately be described as a pupicide as mortality in principally observed in the pupal stage. PPF is phenomenally potent at preventing development of juveniles to adults with a LC_{50} of 0.012 ppb and can persist for many months (Sihuincha et al. 2005). The high potency means even tiny particles carried by mosquitoes can be sufficient to contaminate breeding sites with an active dose. Mains (Mains et al. 2015) estimates that 1/1,000th the dry weight of an adult mosquito is sufficient to contaminate a 200ml breeding site at concentrations requited to inhibit juvenile development.

In addition to pupicidal activity, PPF can have a sterilizing effect on contaminated females manifest by reduced egg yield and reduced hatch of eggs that are produced. Adults emerging from immatures exposed to sub-lethal concentrations of PPF can also exhibit reduced fecundity (Sihuincha et al. 2005, Harris et al. 2013). Lwetoijera (Lwetoijera et al. 2014b) achieved almost 100% sterility of female *Ae arabiensis* in semi-field studies by exposure to PPF-treated resting sites. *An gambiae* females exposed to PPF close to blood-feeding had significantly reduced egg production compared with treatment of gravid females (Mbare et al. 2014). Timing of exposure was also found to be important in *An arabiensis*, with exposure 1 day before blood feeding resulting in ~100% sterility, but this effect was not observed for females exposed at other times (Harris et al. 2013).

In field studies on a novel resting site ADS, observed mosquito population suppression was attributed primarily to *Ae. aegypti* exposure to pyriproxyfen, shortly after adult emergence or taking a blood meal, resulting in decreased egg production (Ponlawat et al. 2013).

PPF has a relatively good environmental profile, and is considered safe for many non-target organisms with the noted exception of some invertebrates. Its use as a larvicide in drinking water has been approved by the World Health Organization (WHO 2008).

4.3.3.2 Alternative AI

4.3.3.2.1 Synthetic AI

Several other IGRs have been evaluated as they offer similar properties to PPF, being highly potent at disrupting the development of juveniles but remaining relatively innocuous to adult survival and behavior enabling effective dissemination:

- **Methropene** is a JHA with similar activity to PPF and has successfully demonstrated AD with *Ae albopictus* under laboratory conditions (Bibbs et al. 2016). However, in comparative evaluations with several mosquito species (*Cx pipiens*, *An stephensi* and *Ae aegypti*) the inhibition of adult emergence LD_{50} for PPF was approximately an order of magnitude lower than that for methoprene (Itoh 1993).
- **Novaluron** in another promising AI with potential application for AD. It is a benzoylphenyl urea, developed by Makhteshim-Agan Industries Ltd, and is an insect growth regulator that interferes with chitin synthesis.[4]
- **Triflumuron** is another benzoylurea compound with the same mode of action as novaluron and is marketed by Bayer.

[4] http://apps.who.int/iris/bitstream/handle/10665/68898/WHO_CDS_WHOPES_2005.10.pdf;jsessionid=D1426FF5539BC8970DC8ED15260DED5E?sequence=1

Novaluron and triflumuron along with PPF were included in a recent evaluation of potential AIs for AD against *An quadrimaculatus* (Swale et al. 2018). Laboratory dose-response for larval Inhibition of Emergence (IE_{50}) showed triflumuron (IE_{50} = 0.3 ppb) to be the most potent, closely followed by PPF (IE_{50} = 0.8 ppb), with novaluron (IE_{50} = 10 ppb) the least potent. Authors exposed recently blood-fed *An quadrimaculatus* to these AIs for 4 hours, maintained them for 48 hours, then provided oviposition sites in which number of eggs produced, hatch rate and larval development was assessed. Significant sterility (reduced egg yield) was observed for PPF (~50% reduction) and triflumuron (~25% reduction), but not for novaluron. Hatch rate was reduced marginally but statistically-significantly for PPF and novaluron, but not triflumuron. Only novaluron treatments conferred significant inhibition of emergence of 4th instar larvae added to oviposition sites, although this effect was marginal. Given these studies were under controlled laboratory conditions with small (2ml) oviposition sites, results corroborate other studies that have observed AI is rapidly lost following contamination and unlikely to be transferred to oviposition sites if exposure occurs close to blood feeding (Itoh 1994, Mbare et al. 2014). The authors speculate that novaluron may be a better AI choice for ADS targeting recently blood fed females (eg resting sites) as it provided the best transfer of insecticidal activity to breeding sites, and it did not affect egg production so is less likely to inhibit dispersal via oviposition. However, these larvicidal effects were only marginal in a laboratory setting and unlikely to translate to meaningful activity in field settings, unless there are considerable improvements. The more potent PPF would be better for ADS targeting gravid females, where reduced egg production is negligible. Although not evaluated experimentally and reported in literature, additional IGRs with similar properties to PPF may also be applicable to AD.

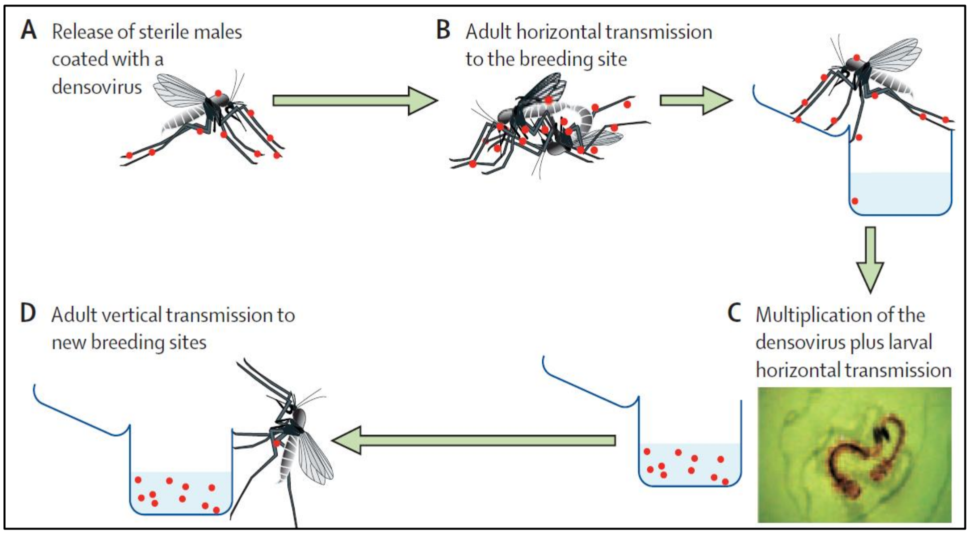
4.3.3.2.2 Biological insecticidal agents

Biological insecticides have potential to be self-propagating within an environment through vertical and horizontal transmission between target species and may persist in the environment. Both densoviruses and entomopathogenic fungi have been proposed as potential biologicals for AD:

4.3.3.2.2.1 Densoviruses

Mosquito densoviruses (MDV) are parvoviruses that replicate in the nuclei of mosquito cells and cause nuclear hyper-trophy (densonucleosis). In a review of mosquito densoviruses, Carlson (Carlson et al. 2006) concluded they have potential as biological control agents for mosquito control as *"They are non-enveloped and relatively stable in the environment. They are highly specific for mosquitoes and they infect and kill larvae in a dose dependent manner in the aqueous larval habitat. Infected larvae that survive to become adult mosquitoes exhibit a dose-dependent shortening of lifespan and many do not survive longer than the extrinsic incubation period for arboviruses. Thus they may have a significant impact on transmission of pathogens. Infected females can transmit the virus vertically by laying infected eggs in new oviposition sites. Population cage studies suggest that they will persist and spread in populations and limited field studies have shown similar preimaginal mortality in wild populations to that seen in laboratory studies."* As densoviruses can persist and accumulate in larval habitats and disseminate to other habits via vertical transmutation of infected females, infected breeding sites can become natural ADSs. Extensive field evaluations of densoviruses (AeDNV) as microbial insecticides targeting *Aedes* and *Culex* species have been conducted with promising results in Ukraine, Russia and Tajikistan in the 1980-90's (Carlson 2006 and references therein). Bouyer (Bouyer et al. 2016) has proposed augmenting Sterile Insect Technique (SIT) programs by contamination of sterile males with densoviruses as a vehicle to propagate the densoviruses into the wild population through horizontal and subsequent vertical transmission as illustrated in Figure 1.

Figure 1. Principle of Augmented Sterile Insect Technique using a densovirus (*Image courtesy of Boyer 2016)*



4.3.3.2.2.2 Entomopathogenic fungi

Entomopathogenic fungi have successfully been deployed as biopesticides targeting a range of insects and have potential for use for mosquito control (Scholte et al. 2004a). Entomopathogenic fungi have been isolated with activity against a wide range of mosquito species including *Aedes* and *Anopheles* (Scholte et al. 2004a). They are typically active against larvae rather than adults, and where they have adulticidal properties they tend to be slow acting, providing opportunity for dissemination into the environment following contamination before mortality. They are highly species-specific, and have a benign environmental profile. *Metarhizium anisopliae* and *Beauveria bassiana* have undergone the most extensive evaluation as potential fungal biocides for mosquitoes with evidence that they can be dispersed in part by AD:

- ***Metarhizium anisopliae* -** In laboratory studies Scholte (Scholte et al. 2004b) demonstrated that AD of *M anisopliae* fungal inoculum between *An gambiae* mosquitoes occurs during mating activity. AD via mating was also demonstrated in laboratory studies with *Ae aegypti* which showed exposure to a *M anisopliae* fungal-contaminated males was also an effective method of infecting female mosquitoes (Reyes-Villanueva et al. 2011).
- ***Beauveria bassiana* –** Has been developed commercially for use in the In2Care ADS (Buckner et al. 2017). It is included in a proprietary mix including PPF 74% and *B bassiana* spores 10% (In2Mix). *B bassiana* has been demonstrated to shorten the life of contaminated adults, but is slow acting enabling adults to disseminate PPF to breeding sites (Snetselaar et al. 2014). However, it is not clear that on its own it would be able to disseminate and have impact beyond mosquitoes that came into direct contact with the In2Care traps.

- ***Leptolegnia chapmanii*** **-** A recent review of *L chapmanii* concludes this species has potential to be developed as a mosquito larvicide for *Aedes* mosquitoes: having high host specificity to family level; viable in wide range of conditions; infecting larvae in domestic and peridomestic environments (Gutierrez et al. 2017). However, little is known about dispersal mechanisms or if it could be formulated for AD.

4.3.3.1 Formulation

Formulation of AI can have significant impact on efficacy. Formulations specific for AD should: promote stability of AI prolonging longevity and correspondingly minimizing time between reapplication in an ADS; contain as high a concentration of AI as possible to increase transfer; have the appropriate 'sticking' properties to adhere to the insect and subsequently transfer to the aquatic habitat.

Powder-based formulations have predominantly been used to contaminate mosquitos, although several other approaches including emulsifiable oil, and soluble solutions have also been evaluated. Powder formulations have mostly been made by pulverizing commercially available larvicide pellets developed by Sumitomo (SumiLarv) with a range of PPF concentrations (0.5-10% AI). The AI concentrations are typically low as larvicide pellets were designed for a different purpose: slow release in aqueous environments. A wettable powder formulation with 35% AI (Esteem 35 WP IGR – Valent Biociences) has been used in AD studies in its powdered from by Kartzinel (Kartzinel et al. 2016). Technical grade PPF has also been used in many studies including: Itoh (Itoh 1993) who treated nets at 1.5g/m² with technical grade PPF, and Ohba (Ohba et al. 2013) who treated bed nets soaked in technical grade PPF diluted in isopropynol (0.1 and 1% w/v) and dried overnight, resulting in 35 and 350 mg/m² PPF.

Formulations with high concentrations designed specifically for an AD approach have more recently been developed and evaluated. A powder formulation with 74% PPF AI (In2Mix) has been developed and marketed for use with In2Care traps (Buckner et al. 2017). A dual formulation comprising 20% PPF AI in emulsifiable oil and 60% PPF AI powder formulations designed specifically for AD use, have been evaluated in several studies of AD by Rutgers University (Gaugler 2013, Gaugler et al. 2011, Wang et al. 2014, Unlu et al. 2017, GAUGLER et al. 2017, Suman et al. 2018). The formulations are designed such that insects are first contaminated with oil providing greater 'sticking' properties for powder, allowing higher total PPF load to be carried by contaminated insects. Bibbs et.al. evaluated a liquid vs powder formulation of methoprene in laboratory studies and concluded a powder formulation was substantially better for AD (Bibbs et al. 2016). In comparative studies conducted in cages containing ADSs and SBSs, powder formulations PPF-treated ADSs resulted in x5 more juvenile mortality bioassays compared with an oil-based formulation when tested against *An gambiae* (Mbare 2015b). Liquid solutions of PPF have been applied to breeding sites as direct spray applications, although the subsequent AD of PPF by the local mosquito population has been poor at best (Suman et al. 2014, Lloyd et al. 2017).

4.3.4 Efficacy

The principle of AD has been well established with extensive laboratory, semi-field and open field evaluations demonstrating contaminated mosquitoes are able to disseminate to other insects via a range of behavioral activity. A summary of semi-field and open field results is provided in Table 2, with more detailed results provided in Appendix 1. There has been little if any field evidence that AD can achieve and maintain sustained control of a vector mosquito population in the field for *Aedes* species, and there is no published field evaluation demonstrating this against *Anopheles* species. Where field efficacy evaluations have been conducted, they have mostly been limited in scale and duration with the primary objective to demonstrate AD, rather that population suppression. As such they have relied on evidence from SBSs to evaluate the transfer and dispersal of AI from ADSs. Although they provide good evidence of autodissemination, one cannot assume reported mortality in SBSs will be representative of impact on population breeding as the majority of SBSs used represent small to medium-sized breeding sites. The volume of water ranging from 0.1-7 L with most < 0.5 L (Table 2). SBSs are evaluated in terms of % contaminated (evidence from bioassay and/or chemical residue analysis) and are used for bioassays to assess impact of larval development. These may be conducted in-situ by observing development of naturally arising larvae and/or introduced larvae. Alternatively, water is sampled and transferred to the laboratory where larval development bioassays are conducted. In some studies, the SBSs are additionally used to indirectly measure adult populations, by assessing the number of immatures (eggs, larvae, pupae) collected. These results may be confounded by the sterilizing effect of PPF in short term studies so should be interpreted with caution. Only a handful of studies attempted to directly assess adult populations, for example with BG-sentinel trapping, to assess impact on populations.

The most compelling evidence of AD controlling a population are the studies authored by Abad-Franch targeting *Aedes* species using a simple oviposition-based ADS (Abad-Franch et al. 2017, Abad-Franch et al. 2015). The later study represents the largest and longest running evaluation of AD, deployed on an operational scale utilizing local city vector control agents. The field site comprised 650 ha with a population of 60,000 in a dense urban dengue endemic environment in Manaus, Brazil. Following 12-month baseline monitoring, there was a 5-month intervention with 1000 ADSs. This represented a density of 1.54 ADS/Ha which is one of the lowest densities of the evaluations reviewed (Table 2). Impact of AD was assessed using SBSs to conduct larval mortality bioassays and provide indirect evidence of adult population control in terms of naturally occurring immatures found in SBSs. Following PPF dissemination, there was an 80%-90% decrease in *Aedes* juvenile catch, while *Aedes* juvenile mortality increased from 2%-7% to 80%-90% over a 5-month intervention phase. Although the % juvenile mortality may not be representative of all breeding sites due to small water volumes of SBSs, the substantial reduction of juvenile catch is likely to be representative of impact on population. Even if egg reduction is in part owing to reduced fecundity due to sterilising impact (unlikely given gravid females are being targeted), sustained reduced number of eggs laid over time would impact total population. Furthermore, the formulation used was pulverised Sumilarv pellets with a 5% AI concentration, suggesting better results may be achieved with higher concentration AI formulations specifically developed for AD. It should be noted the success may be due to the high population of mosquitoes, including conspecific *Culex* species.

Table 2. Summary of semi-field and open-field evaluations of AD. Where study includes semi-field and open-field, only open-field data is presented.

Study : 1st author year	Study type (SF = Semi-Field, OF = Open Field)	Target species (Aedes = AE, Anopheles = AN, Culex = CU)	AI (%) / formulation (P = powder, O = oil, Net = treated net, ES = Emulsifiable solution)	ADS density / Ha	Study site size (Ha)	Duration (Weeks)	SBS volume (L)	% immature mortality in SBS	Population suppression in Open Field	
									Indirect assessment: % reduction in egg/larva/adults in SBS	Direct assessment of adult population
Suman 2018	OF	AE	60/P, 20/O	2.5-12	0.5	8-12	0.25	20-50	-	-
Abad-Franch 2017	OF	AE	0.5/P	1.5	650	92	0.2	80-90	80-90	-
Buckner 2017	SF	AE	74/P	473	0.002	<1	0.4	81-80	NA	NA
Lloyd 2017	OF	AE	10/P	0.02	50	6	0.25	ns	-	-
Mian 2017	OF	CU	0.5-5.0/P	1.5	650	92	0.65	80-90	80-90	-
Unlu 2017	OF	AE	60/P, 20/O	35	50	9	0.25	12	80	ns
Gaugler 2017	OF	AE	60/P, 20/O	67	0.03	<1	0.25	74	-	-
Chandel 2016	OF	AE	60/P, 20/O	10-20	1.6	8-12	0.25	11-22	-	-
Kartzinel 2016	OF	AE	35/P	-	-	2	0.1	ns - 45	ns	-
Abad-Franch 2015	OF	AE	0.5/P	14 (2)	7 (50)	16	0.2	>85	>90	-
Mains 2015	OF	AE	10.5/P	1	1	4	0.25	40-70	-	sd
Mbare 2015	SF	AN	10/P	139	0.007	1	7	8-75	NA	NA
Lwetoijera 2014	SF	AN	10/P	109	0.009	4	2	34-79	NA	NA
Lwetoijera 2014	SF	AN	-	109	0.009	2	2	ns	NA	NA
Suman 2014	OF	AE	10 ES	NA	105	6	0.25	3-14%	-	ns
Ohba 2013	SF	AE	0.35g/m²/net	142	<0.001	3-6	1.2	sd	NA	NA
Ponlawat 2013	OF	AE	0.5	7	7	24	3	ns	sd	sd
Caputo 2012	OF	AE	0.5-5	10-2270	0.004-1	2	0.7	28-71	-	-
Devine 2009	OF	AE	5	500	0.018	2	0.2	49-84	-	-
Itoh 1993	OF	AE	1.5g/m²/net	333	0.012	2	-	sd	-	-

ns: not significant, sd: significant difference, NA: Not Applicable

4.3.4.1 Breeding site size and structure

Dilution of AI will be critical to achieving active concentrations and is directly proportional to volume of the water body. When most breeding sites are of equivalent small sizes to SBSs, bioassay results may be representative of impact on the overall breeding population. However, where a significant proportion of the mosquito population develops in larger breeding sites, for example water storage tanks, although the sites may be contaminated, the concentration of PPF could be insufficient to cause lethality of developing mosquitoes. Indeed, it is well documented for *Aedes* species that large breeding sites such as domestic water storage tanks, although numerically fewer, can be the most productive breeding sites contributing to the bulk of breeding populations (Romero-Vivas, Arango-Padilla and Falconar 2006). Clearly larger breeding sites may well attract more visits from PPF-contaminated females, nevertheless the volume of water, and resulting dilution of transferred PPF may negate increased contamination from multiple visits.

It is interesting to note that in the few semi-field and open-field evaluations in which larger volume SBSs were used (>2L), the evidence of larvicidal active concentration was limited. In field studies conducted by Ponlawat (Ponlawat et al. 2013) on *Ae aegypti*: 3L SBSs were used and although there was evidence of adult population control, this was attributed to the sterilizing effect of PPF rather than larvicidal activity, as bioassay from SBSs showed negligible insecticidal activity. Lwetoijera (Lwetoijera et al. 2014a) demonstrated transfer of active concentrations of PPF into 2L SBSs from resting site ADSs in large cage semi-field studies, however the density of *An arabiensis* females used was very high (equivalent to 0.54 million/Ha). In similar large semi-field studies by Mbare (Mbare 2015b) on *An gambiae*, larger 7 L SBSs were used with significantly lower (and more realistic) density of female mosquitoes (equivalent to 27,000/Ha). Results were underwhelming with only the closest SBSs (4m from ADS) showing substantial larvicidal activity (~74% adult emergence inhibition) and the furthest (10m from ADS) exhibiting no significant inhibition of larval development. This does not bode well for field efficacy if ADS evaluations within a small semi-field setting with relatively high mosquito density is not overwhelmingly efficacious. Mbare also quantified the amount of PPF picked up by *An gambiae* from contact with netting dusted with 10% AI pulverized SumiLarv pellets, and subsequently transferred to oviposition sites (Mbare 2015b). Results showed a single female could carry 112ug of PPF, although only ~1/5000th of this load was transferred to the aquatic environment during oviposition and resulted in inhibition of larval development. Based on laboratory lethal dose-response data (Mbare et al. 2013), Mbare estimates a 1 L volume breeding site would require visits from approximately 5 females to inhibit larval development to adults. Although these results are a testament to the potency of PPF, it also highlights that a relatively high population of mosquitoes that may be needed to transfer sufficient PPF to larger breeding sites. For example, a typical 500 L water storage drum would need 2500 visits from female mosquitoes to achieve a lethal concentration. It should be noted the formulation used contained 10% AI, but even a theoretical 100% pure AI formulation would require 250 visits. In *Anopheles*, the breeding sites can be more complex, and include larger natural water bodies as well as transient, mainly sunlit, rainwater pools, such as borrow-pits, drains, car-tracks, hoof prints around pond and water-holes (Kiware et al. 2015, Scholte et al. 2004a, Majambere et al. 2008). The transient nature of these breeding sites makes it less likely that an AI such as PPF can accumulate over time compared to artificial container-breeding sites favored by *Aedes* species. This will be compounded by more rapid degradation of AI from higher exposure to sunlight in natural open breeding sites compared to may container breeding sites. The number of breeding sites compared to the mosquito population will also be a key factor in the efficacy of AD. The challenge of attracting and exposing female *An gambiae* to PPF within 24 hours of oviposition coupled with the limited success in ideal conditions of small semi-field cages studies, and the transient and diverse nature of natural breeding sites led authors to conclude AD is not a feasible strategy for *An gambiae* control (Mbare 2015b). A possible solution proposed by some authors is to deploy AD during the dry season where breeding sites are fewer and more stable compared with the rainy season when myriad transient breeding sites proliferate (Kiware et al. 2015, Lwetoijera 2016). This dynamic may also be true to a lesser extent for *Aedes*.

(Chandel et al. 2016) demonstrated a preference of *Ae albopictus* to lay eggs in Cryptic SBSs (cups with 250 ml oak leaf infusion housed within short section of pipe) compared with open SBSs, in both large cages and open field evaluations. This was corroborated by field evaluation of AD, where they found evidence that there was greater contamination with PPF, and corresponding mortality of larvae/pupae in cryptic SBSs. Conversely, they also demonstrated conventual larvicide application by truck and backpack application was partially successful at targeting open SBSs, but totally ineffective against cryptic sentinel sites. this supports the approach for combining AD with conventional larviciding to achieve total coverage of breeding sites.

4.3.4.2 Mosquito population density

A critical limitation of the AD approach is the dependence on density of target mosquitoes, because these are the agents of the dissemination and there will be a point when the population is too low to transfer sufficient AI to breeding sites. This has been raised by several authors and is examined in most detail in (Kiware et al. 2015).

Several authors have proposed heterospecific species sharing a similar habitat niche to target mosquitoes may augment the dissemination of larvicide to breeding sites (Kiware et al. 2015, Devine 2016). For example *Culex* mosquitoes species are often found in similar breeding sites to *Anopheles* (Majambere et al. 2008) and *Aedes* (Abad-Franch et al. 2017, Devine et al. 2009), and may occur in higher numbers than target mosquito populations providing a mechanism for maintaining AD to breeding sites when target mosquito populations are low. Although breeding sites may be shared, the ADS would have to be attractive to the heterospecific species for this approach to work, furthermore the larvicidal activity would have to impact the heterospecific species to lesser extent than the target species.

Mass-rearing and release of insects dosed with PPF (or other AIs) provides a different mechanism of dissemination of AI into the environment that is not dependent on the density of the target mosquito population. These include approaches that mass-rear target mosquito species and release males (Mains et al. 2015, Bouyer et al. 2016, Bouyer and Lefrançois 2014), or a heterospecific species that shares the same environmental niche and can deliver larvicide to breeding sites (GAUGLER et al. 2017).

4.3.4.3 Trap density

ADS attractiveness and numbers relative to natural resources (resting sites/host/breeding sites) will be critical to efficacy (Kiware et al. 2015). Flight range of mosquitoes will also have implications on density required. For example, *Ae aegypti* will disperse only tens of meters where resources are not limited, whereas some *Anopheles* will disperse kilometers. Table 2 summarizes the ADS density/Ha in the semi-field and open field studies reviewed. To date most studies have been semi-field or small proof of principle studies with densities that would make operational deployment unfeasible. All semi-field and many field evaluations exceed the density of conventional “lure and kill” traps which can achieve control if deployed at sufficiently high density. For example, the Adulticidal Gravid Ovitraps (AGO) in a conventional “lure and kill” trap that has been demonstrated to provide substantial control of *Aedes* population in Puerto Rico, when deployed at 3/house (with ~ 80% compliance) at a density of 50/Ha. In2Care ADSs have a recommended deployment of approximately half this at 1/400m2 (~25/Ha), Ponlawat demonstrated population control with density of 7/Ha (Ponlawat et al. 2013) and Abad-Franch had success at an operational scale with an ADS density of 1.5/Ha (Abad-Franch et al. 2017).

4.4 Conclusions

- AD has the potential to improve the standard larviciding approach, by utilizing mosquitoes to target and transfer AI to the myriad breeding sites within an environment, many of which are cryptic and not easily detectable or accessible by conventional methods.

- In theory AD could dramatically reduce the amount of insecticide needed. Broadening the number of AIs available for vector control is highly desirable in the ongoing fight to manage insecticide resistance.

- The properties of PPF make it ideal for the AD approach. PPF is primarily a pupicide rather than a larvicide. Other synthetic and biological insecticides have been evaluated to a lesser extent.

- Dust/powder formulations (sometimes ground granules) have predominantly been used to contaminate mosquitoes in research settings. High concentration formulations are most logical because of the need for less total quantity of formulation to be transferred. The commercially available In2Care system uses a high concentration (74%) PPF coated on an electrostatic netting inside the station. Dual formulations comprising 20% PPF AI in emulsifiable oil and 60% PPF AI powder formulations designed specifically for AD use, have been evaluated in several studies of AD by Rutgers University

- The majority of AD studies have focused on Aedine species and to a much lesser extent targeting *Anopheles* malaria vectors.

- There is still scant evidence that AD can achieve and maintain control of vector mosquito populations in the field for *Aedes* species, and there is no published evidence against *Anopheles* species.

- A critical limitation of the AD approach is the dependence on an adequate density of mosquitoes, because that are the agents of the dissemination.

- Species other that target mosquito, sharing similar breeding habitat (eg *Culex*), may contribute to AD, mitigating the dependence on target mosquito population density.

- The success of autodissemination stations depends on three criteria: 1) attraction of mosquitoes to the stations, 2) transfer of larvicide formulation to the mosquitoes, and 3) dissemination of the larvicide to target habitats.

- Targeting skip-ovipositing females is the most effective approach for AD for *Aedes*. This requires an ADS which gravid females will visit. An ADS which contains water and is therefore a potential oviposition site attracts females, as is exploited in the In2Care system.

- Targeting gravid females is less applicable to *Anopheles* for the following reasons:
    - less pronounced skip oviposition.
    - harder to develop highly attractive oviposition-based traps that can compete with natural sites.

    - preferred breeding sites are less amenable to accumulation of sufficient AI to achieve larvicidal effect.

- The challenge of attracting and exposing female *An gambiae* to PPF within 24 hours of oviposition, coupled the limited success in ideal conditions of small semi-field cage studies, and the transient and diverse nature of natural breeding sites led some authors to conclude AD in not a feasible strategy for *An gambiae* control.

- PPF contamination can result in significant sterilisation of females. The level of sterilisation is highly dependent on the time of contamination through a gonotrophic cycle; contact close (< 24 hr before and after) to blood feeding resulting in greatest effect, whereas gravid females are unaffected.

- Transfer of AI is optimised when females come into contact with the formulation close to the time of subsequent contact with breeding sites (ie during oviposition), as particles are rapidly shed from contaminated mosquitoes. Correspondingly, mosquitoes contaminated during host seeking/blood feeding are comparatively ineffective at transferring active concentrations of larvicide to breeding sites.

- This leads to two alternative strategies: focus on AD as the mechanism of improved larviciding/pupiciding, requiring exposure of adults to the formulation close to before oviposition; or focus on the sterilising impact of PPF where contact close to blood feeding is required.

- If sterilization rather than AD is the basis for population control, this raises the question of whether a fast-acting adulticide, rather than PPF, is more logical, if a suitable one is available.

- Where breeding sites are few and fixed in nature, transfer by the adult population will be more concentrated, and the AI would have more opportunity to accumulate in these fewer water bodies.

- AD may be best be suited to the dry seasons for *Anopheles*, despite lower mosquito populations. This is because breeding sites are few and more stable in nature, and mosquito populations tend to be in steady state and consequently more amenable to control. This dynamic may also be true for *Aedes*.

- Targeting sugar feeding behaviour is a promising approach being developed as a lure and kill system for vector control. This might be utilized to increase the attractiveness of an ADS.

- Contact between opposite sexes during mating might provide an avenue for transfer of larvicides. Transfer via mating has been proposed as a means of AD, by treating mass reared males with PPF or densovirus before release, to boost the efficacy of SIT for mosquito control.

5 Ongoing Research

From the interviews conducted, the following ongoing and planned research was mentioned.

- In2Care (*Ae.aegypti*)
    - Field studies (In2Care ADS)
        - Malaysia
            - 200 trap mini trial ongoing. Collaborator: Institute of Medical Research (IMR).
            - Thereafter, planned trial of In2care trap plus Bayer outdoor residual spraying (to start early 2019). Involving a few thousand traps. Randomised Control Trials (RCT). Collaborator: IMR, Malaysia.
        - Manatee County Florida. Ongoing. 700 traps. 70 acres.
        - Laos. 600 trap trial in Vientiane. Ongoing. Collaborator: Pasteur Institute.[5]
        - Cambodia. 300 trap trial involving schools. Ongoing. Collaborator: Institute Pasteur (and possibly Queensland Institute of Medical Research)
    - Adaptation of existing trap (but not formulation) for expansion into consumer market. Specific field studies on this not mentioned.

- IAEA/CIRAD (*Ae.albopictus*)
    - Field studies
        - Reunion. In May 2019 it is planned to start adding PPF to male releases (radiation SIT) to try 'boosted SIT'. Developing formulation with higher AI concentration and better 'sticking' properties. Evaluation of densoviruses as biological AI in laboratory look promising, however planned evaluation in field was shelved due to estimated €400K for necessary regulatory work. Project funded by EU[6].

- Entente Interdépartementale pour la démoustication du littoral méditerranéen (EID). (*Ae.albopictus*)
    - Field studies
        - Montpelier. An AD PPF field trial in 2015 (unpublished) showed population effect. To be followed with further field work in 2018.
        - Looking at improved trap attractiveness.

- QIMR/Pasteur Institute (*Ae.aegypti*)
    - Field studies

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[5] http://ecomore.org/2018/07/17/innovative-vector-control-now-operational-in-vientiane/

[6] https://cordis.europa.eu/project/rcn/204738_en.html

        - Madeira and mainland Portugal. Investigating a different type of ADS at differing mosquito densities. 2 year project finished and awaiting publication, reported statistically significant population suppression, although not substantial.

- USAMC-AFRIMS Thailand
    - Evaluating effect of 15 commercially available IGRs fed to *Aedes* mosquitos in sugar solution. Focusing on the sterilising properties of IGR rather than larviciding.
    - Unpublished field evaluation of AD with PPF determined larger water containers were not receiving larvicidal concertation of AI and was a limitation to AD for population control.

- Rutgers University/Springstar/IHI (*Anopheles,* Africa)
    - Field studies
        - Planned further development of ADS design and PPF formulation, with associated field studies, dependent on funding. Rutgers also looking for funding to develop approach with direct dusting of adult midges with PPF for mass release.

- MosquitoMate/Univ Kentucky (*Ae.albopictus and Ae.aegypti*)
    - Field studies
        - NIH-funded field studies. ADAM approach (dusting of males with 35% PPF). *Ae.aegypti and Ae.albopictus.* California and Florida.
    - Also working on operational components (transport of adults etc) for facilitating ADAM approach

- ISCA Technologies Inc.
    - ISCA technologies is an agricultural biotechnology company (established 1996) specialising in exploiting semiochemicals for insect control. ISCA has developed an attract and kill sugar bait formulation (Vectrax) for vector mosquitoes incorporating volatiles associated with sugar feeding. This is being developed as an ATSB system and has undergone field testing in Tanzania targeting *Anopheles* with promising results claimed at village scale by application to eaves of homes.

    - ISCA recently received a national science award [7] to support work adapting this technology for AD by incorporating IGRs as AIs. This work is evaluating a range of IGRs with the aim to achieve sterility while maintaining oviposition behaviour to maximise AD to breeding sites. Formulation development at ISCA has the goal of ensuring durability/rain fastness and availability of AI. AI transfer is anticipated via contact and ingestion (with subsequent pass-through the gut for dissemination at breeding sites). Formulations to be developed may be appropriate for spraying including aerial application. A preliminary field study in the US consisted of spraying vegetation in a $30m^2$ yard and monitoring dispersal in SBSs placed every 50m up to 1 mile. Chemical residue analysis showed good dispersal up to 1 mile, according to ISCA. The target species was *Aedes.*

[7] https://www.nsf.gov/awardsearch/showAward?AWD_ID=1648402

- Philippines (*Ae aegypti*)
    - Field study ongoing to investigate large scale impact and user acceptance – using own ADS. Univ Philippines. Some possible involvement of QIMR.[8]

6 CURRENT COMMERCIALIZATION

The In2Care trap is an ADS, rather than a trap. It targets *Aedes* species, and is the only commercialized ADS to date. The design of the ADS is shown in detail on the In2Care website[9] . Attractants mixed with water in the ADS attract female *Aedes* seeking to oviposit. These become contaminated by PPF which is coated onto a netting within the ADS, and on which the mosquitoes rest. The ADS also incorporates *Beauvaria bassiana* spores, which also contaminate the female mosquitoes and is designed to cause their delayed death, following autodissemination of PPF after exiting the ADS. The ADS also acts an 'egg sink' with eggs laid not able to develop to adults due to PPF. The ADS needs to be replenished at intervals with both the PPF-coated netting and *Beauvaria bassiana* mixed with attractants, and topped-up with water. The materials for replenishment are available in a 50 gram sachet which is known (and registered) as In2Mix. The PPF is 74% concentration. Although In2Care informed that the PPF deposit is active for up to 3 months, the ADS nevertheless needs servicing one per month in the tropics due to the loss of activity of *Beauvaria.*

The In2Care system has registration in over 30 countries including 41 states in the US and In2Care informed that sales have been made in around 20 countries to date. No information on sales volumes was given. The In2Care ADS is in the PQT/VCAG evaluation system, needing epidemiological trials to progress further towards approval and policy recommendation as a public health intervention. It is unclear whether such trials are likely, especially as In2Care are targeting the professional market, which can include abatement districts in the US and municipalities elsewhere, requiring no such WHO approval.

The system currently commercialized by In2Care is a second version product. Further improvements/adaptations may follow, especially for penetration of the consumer market in the US. This may focus on easier changing of the PPF-treated netting, possibly with less user exposure.

The In2Care system is commercialized mainly as a professional product for servicing by PCO operators for residential/business/tourism. The US is a major target market and commercialization with a local distributor, Univar, began there early 2018. Sales are so far in Texas, Florida, Alabama, South Carolina and

8 http://www.registry.healthresearch.ph/index.php?option=com_healthregistry&controller=registry&task=pdfview&id=1578

9 http://www.in2care.org

Georgia. It may be purchased and used directly by mosquito abatement districts in the US, although In2Care consider that it is most suited to abatement districts which outsource control activities to professional PCO companies specializing in mosquito control. US consumer use is planned but not presently approved. There are some consumer approvals in the Dutch Caribbean and it is available for consumer purchase there.

In Asia, In2Care are working with the US company Ensystex as regional distributor for the professional sector, having recently launched in Thailand, to be followed by Hong Kong and Singapore. Commercialization in Laos and Cambodia is pending regulatory approval. Some sales have been made in the Pacific region (Fiji, Tonga) through an Australian-based regional distributor, AustralAsian Biosecurity.

Some sales have been made to the Insect Vector Control Division in Trinidad, although In2Care state that national vector control programs are not a primary target. A reluctance of the national vector control sector to invest in preventative measures was cited, along with staff availability/costs for regular servicing. If the number of stations required per unit area could be significantly reduced, perhaps by better targeting to hotpots, then there would be more interest from vector control organizations, according to In2Care.

Information on pricing and cost of development was not made available.

7 OUTLINE OF REGULATORY ISSUES AND POLICY STATUS

Most research work on autodissemination has been carried out using PPF.

Products containing PPF are registered and sold widely throughout the world for use in crop protection, public health and animal health. PPF is manufactured by Sumitomo and Tagros Chemicals India.

7.1 TECHNICAL MATERIAL AND FORMULATIONS

7.1.1 WHO specifications

The WHO Specification for Public Health Pesticides (May 2016) covers PPF from both manufacturing sources, as Tagros material has been deemed by WHO/FAO to be equivalent to that of Sumitomo, based on Evaluation Reports provided to WHO/FAO by Tagros (2015) and Sumitomo (2005). PPF Technical Material (97%) and a PPF Granule (0.5%) both have a WHO specification[10].

7.1.2 EPA

In2Mix is the refill sachet for the In2Care system, containing a formulation with 74.03% PPF and 10% *Beauvaria bassiana*. In2Mix received a section 3 registration in July 2017, conditional upon fulfilling certain additional data requirements.

[10] http://www.who.int/whopes/quality/Pyriproxyfen_eval_specs_WHO_May_2016.pdf

Additionally, PPF-containing mosquito larvicides are registered in the US, for example Sumilarv 0.5G (0.5% granule)[11].

7.2 PQT/VCAG

7.2.1 PPF larvicides

In December 2017, based on applications from Sumitomo, the WHO Prequalification Team issued decisions relating to the products Sumilarv 0.5G (0.5% Granule) and Sumilarv 2MR (2% Matrix Release) used as mosquito larvicides. PQT states that each product has.. *'met the criteria for conversion to prequalification and is acceptable in principle for procurement by UN and other international agencies and countries'.* [12]

The above-approved PPF products are for use as larvicides (actually they act primarily as pupicides, but fall into the PQT classification as larvicides).

7.2.2 In2Care system

The In2Care AD system, which incorporates PPF (in addition to *Beauvaria bassiana*) has been considered by VCAG and in their 7th Meeting (October 2017) [13] issued the following conclusions and recommendations:

Conclusions

• 'While the In2Trap is already largely commercialized for use as a professional pest control product, the available entomological evidence suggests this trap may also have potential to reduce diseases caused by Aedes-transmitted viruses. This justifies carrying out trials with epidemiological outcomes to assess its public health value. The In2Trap should not be considered as a standalone intervention, but as part of an integrated vector management approach.

• The In2Care Mosquito Trap fits within the product class of auto-dissemination traps for disease management. Further entomological evidence should be generated to advise the design of largescale epidemiological trials'.

Recommendations

• 'The innovator recommends a trap density of 1/400 m2 (10 traps per acre). Data should be provided to estimate the contamination of breeding sites in relation to the distance from In2Trap, and to demonstrate the duration of efficacy that can be expected from the trap, specifically with respect to impact on mosquito populations from re-dissemination of pyriproxyfen.

• Semi-field experiments should be carried out to determine whether the killing effect on adult Aedes aegypti mosquitoes will be high enough to impact vectorial capacity.

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[11] https://www3.epa.gov/pesticides/chem_search/ppls/010308-00034-20100819.pdf
[12] http://www.who.int/pq-vector-control/prequalified-lists/soc_001-002.pdf?ua=1

[13] http://apps.who.int/iris/bitstream/handle/10665/259743/WHO-HTM-NTD-VEM-2017.11-eng.pdf

• The residual activity after application of a refill sachet (In2Mix) is reported to be 4–6 weeks. Additional data should be provided to demonstrate this interval is appropriate under variable external factors, such as temperature.

• For optimal efficacy, the innovator recommends that natural larval development sites be removed as much as possible. This should be quantified; that is, determining what effort is required to accompany use of this product and what the estimated density is of natural container sites with which the product can effectively compete.

• Before undertaking epidemiological studies, larger entomological intervention trials with randomized clusters are needed to demonstrate entomological efficacy.

• The assessment of risk to humans of handling and application of the product and environmental risks (including non-target organisms) should be done based on the data provided by the innovator'.

It is unclear (and doubtful) whether there is a sufficient business case for generating further data to satisfy the requirement for epidemiological trials for this ADS. If not, as is assumed, the use of this system will be confined to non WHO-approved uses as highlighted in the *Current Commercialization* section of this report.

7.2.3 Future AD systems and PQT/VCAG

Organizations wishing to develop/commercialize an AD system, in particular for *Anopheles,* will be expected to face a similar requirement for epidemiological data, requiring a similar business case evaluation by the developer. The TPP for an AD system which we propose in this report does not anticipate how wide the adoption of such a system will eventually become. An *Anopheles* product as defined in the TPP is somewhat niche in its potential applications, and therefore there may be expected to be insufficient commercial incentive to develop this to the point where they can be recommended by WHO. Crucially, for *Anopheles* control, the need for such a WHO approval is more important than for an *Aedes* system, because it tends to be a prerequisite for organizations purchasing *Anopheles* control products. Such organizations include national vector control programs, UN agencies, donors, philanthropic organizations. These are substantially different purchasing groups than for *Aedes* products.

If the business case does not support full development, then this suggests the need for external funding towards development of AD products, if there is consensus that the potential value of such product(s) as a health intervention warrants this.

A question arises whether the requirement by VCAG for epidemiological data to support an AD system could potentially be waived. PPF technical and 0.5% granules have WHO/FAO specifications and the 0.5% granule and 2% matrix-release formulations are WHO-approved larvicides. It could be considered that AD represents 'just' an alternative method of application of PPF larvicides. The chances of a special treatment for AD by VCAG seems very unlikely, however, and the currently-approved larvicide formulations are almost certainly not applicable for future widespread use in AD. (Some AD field researchers have taken the commercially available granule and ground it to a powder for pick-up by mosquitoes, although it is generally acknowledged that a much higher concentration formulation is needed for AD). The In2Care system uses a 74% PPF powder formulation. The In2Care system does not have WHO-approval but does have regulatory approval in over 30 countries including EPA approval. It may seem reasonable that high concentration formulations of PPF for use in future *Anopheles* AD systems would gain national-level approvals independent of any WHO approval. But that alone is unlikely to be sufficient to satisfy organizations purchasing products for malaria control, who would require full WHO approval.

7.3 OTHER CONSIDERATIONS

It is important to consider how PPF might be used in a future ADS for *Anopheles*. The high concentration of PPF in the In2Care system is safely enclosed within the ADS. But an enclosed oviposition-based station of this general design is not especially attractive to *Anopheles* mosquitoes[14], and some experts point out that a physical ADS may not be required, and that more widespread application of PPF is potentially relevant for AD, for example in and around homes or animal shelters or near breeding sites, dependent on the mosquito target species.[15]

The appropriateness of concentrated formulations of PPF in unenclosed systems in such locations is a matter for regulatory experts.

Regarding non-target organisms: PPF generally has a high margin of safety but has marked toxicity to some aquatic invertebrates[16]. For this reason, the EPA explicitly forbids application of PPF containing larvicides to natural water bodies[17]. The EPA Section 3 registration for In2Mix states on the label:

"This product is toxic to fish and aquatic invertebrates. Do not apply directly to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water by cleaning of equipment or disposal of equipment wash-waters. Application of this product is prohibited directly into sewers or drains, or to any area like a gutter where drainage to storm sewers, water bodies, or aquatic habitat can occur. Do not allow the product to enter any drain during or after application."

The purpose of AD is to transfer deposits of PPF to numerous undefined water bodies at such concentrations to cause mortality to developing mosquitoes. For a future system for *Anopheles* control we suggest that such concentrations might also be toxic also to non-target aquatic invertebrates in natural water bodies into which PPF has been autodisseminated, although this point has not been raised by others. The WHO approvals for PPF as a larvicide do not forbid application to natural water bodies. *Aedes*, transfer of PPF (or another AI) is likely to be limited to container breeding sites, thereby minimizing exposure to the natural environment.[18]

Other IGRs could potentially be developed for use in AD and the development of a second AI may be particularly desirable from a resistance-development perspective: there is broad agreement that *Anopheles* will be subjected to sub-lethal concentrations of PPF in a portion of water bodies located in areas where AD is adopted. Methoprene, novaluron and triflumuron have been suggested as candidates[19],

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[14] G. Devine, personal communication
[15] G.Devine, personal communication
A. Mafra-Neto, personal communication
[16] http://pesticideinfo.org/List_AquireAll.jsp?Rec_Id=PC35792
[17] https://www3.epa.gov/pesticides/chem_search/ppls/010308-00034-20100819.pdf
[18] S. Dobson, personal communication.
[19] M.Banfield, personal communication

and have been evaluated to a limited extent experimentally[20] although one key expert is firmly of the opinion that PPF is the only really suitable candidate due to its potency[21].

Additional to PPF, the IGRs diflubenzuron and novaluron are pre-qualified by PQT as mosquito larvicides.

8 ECONOMICS CONSIDERATIONS

8.1 MAIN V SUPPLEMENTARY INTERVENTION, AND APPLICABILITY TO URBAN AND RURAL SETTINGS

The use of larvicides for *Anopheles* control, particularly in sub-Saharan Africa is recommended by WHO only under certain circumstances (WHO 2012) [22]

WHO's position is that in areas where there is a significant risk of malaria for a substantial fraction of the population, there is insufficient evidence to support using larviciding as a single intervention as it is not as cost-effective as ITNs or IRS. According to WHO, larviciding may be used as a supplementary measure to core stand-alone interventions of IRS and ITNs, but should only be used in situations where breeding sites are "*few, fixed, and findable, and where there is the opportunity to eliminate all or a large portion of the breeding sites with little effort".* These situations are more likely to be found in urban areas. This points to the limitations of larviciding as a technique especially in rural areas; that it is very difficult to find and treat sufficient breeding sites.

Potentially AD could be much more cost-effective than traditional larviciding techniques, making larviciding a more widely applicable tool, potentially even as a stand-alone intervention. But this would require an extraordinary improvement in effectiveness.

As a stand-alone against *Anopheles*, larviciding would need to demonstrate equal or superior cost-effectiveness to IRS and ITNs, and it is unclear whether AD has the potential to deliver this.

WHO advises that operational funding should be directed towards covering as many people as possible with the most cost-effective single intervention. For WHO, a national strategy of *"universal coverage with the locally-most-cost-effective single intervention"* is normally to be preferred over a strategy of *"double protection to some of the at-risk population, but no protection to others equally at-risk".*

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[20] Swale, D. R., Z. Li, J. Z. Kraft, K. Healy, M. Liu, C. M. David, Z. Liu & L. D. Foil (2018) Development of an autodissemination strategy for the deployment of novel control agents targeting the common malaria mosquito, Anopheles quadrimaculatus say (Diptera: Culicidae). *PLoS Negl Trop Dis,* 12**,** e0006259.
[21] G.Devine, personal communication
[22] http://www.who.int/malaria/publications/atoz/interim_position_statement_larviciding_sub_saharan_africa.pdf

The focus on the single most cost-effective solution does not consider the economic consequences of resistance development. Theoretically, supplementary use of larvicides (for example by AD) alongside IRS or ITNs could additionally be a resistance management tool.

WHO does, however, point out that larviciding as a stand-alone intervention may be applicable in certain niche circumstances: areas with low or no transmission, especially if these have a high transmission potential, for example. Such areas may be found at the fringes of malaria transmission often in close proximity to areas of high transmission. According to WHO, "*In such settings, general coverage with ITNs or IRS is not cost-effective and not justified. In these circumstances, larviciding may be used to consolidate elimination and reduce receptivity, and hence to prevent the re-appearance of malaria outbreaks. This is especially appropriate in settings where hotspots of high transmission risk are known to be associated with breeding sites – for example urban cultivation in the centers of large African cities or irrigated rice in otherwise arid areas. In such situations, larviciding (or other anti-larval measures) targeted at these hotspots may be used as a stand-alone intervention, in order to reduce the risk of resumption of transmission".*

For now, established methods of larviciding are not as cost-effective as core tools, but this does not mean that they are not cost-effective in their own right, according to WHO criteria. WHO has proposed that interventions with a CER per DALY averted[23] which is less than a country's per capita GDP could be regarded as '*very cost-effective*'. Those for which the cost-effectiveness is less than three times the country's per capita GDP could be regarded as '*cost-effective*'[24]. The cost-effectiveness is dependent on the transmission setting.

The first study to examine the cost effectiveness of larviciding for urban malaria control was carried out in Dar Es Salaam[25].

Their conclusion was that according to commonly used WHO GDP thresholds, urban larviciding (alone) in Dar Es Salaam is very cost-effective in most transmission settings, and they supported scale-up of larviciding to further urban settings in the country. Their recommendation was that decision-makers should still prioritize scaling-up ITN and IRS in rural areas, because larviciding interventions have been shown to be more costly when the density of breeding habitats is high and/or the population density is low. It is questionable whether an AD system can be developed which is sufficiently cost-effective (and logistically feasible) for use in rural situations.

8.2 Further considerations

The pricing of an AD system and its total user cost will determine the cost-effectiveness and its adoption. Economies of scale in production and use will be specific to the product or technique involved, and might be significant.

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[23] Cost Effectiveness Ratio per Disability-Adjusted Life Year averted.

[24] http://www.who.int/choice/publications/p_2003_generalised_cea.pdf

[25] Maheu-Giroux, M. & M. C. Castro (2014) Cost-effectiveness of larviciding for urban malaria control in Tanzania. *Malaria Journal,* 13**,** 477.

The number of dissemination stations required, and the frequency of replenishment, are critical. The In2Care ADS for example needs visiting for replenishment every month in the tropics (change of water, attractant, and insecticides). This, plus the large number of stations that are needed (~25/Ha), will likely limit the main usage of that system to middle and high income countries. There is virtually no external funding available for purchase of tools for operational control of *Ae. aegypti.* The in2Care system will likely remain as a product for professional use, with further consumer use to be added.

For *Anopheles*, an ADS for areawide use would need to be simple to deploy and replenish. Cost effectiveness of AD for *Anopheles* species is unknown for systems not yet developed, although larviciding in general can be a cost-effective intervention in some situations according to WHO criteria, although not comparable with ITNs or IRS, except in specific circumstances mentioned above. To minimize overall costs, AD products (or techniques), if developed, would need to require as few annual visits as possible, and the density of AD stations would need to be optimized/minimized. If the replenishment frequency was reduced to 6 months for example, this could potentially be carried out by existing IRS spraymen, in situations where both techniques would be used alongside one another. But this would need the optimal timing of both interventions to be identical, which would be unlikely especially if the AD system were to be targeted for dry season use only[26]. There may be chances to save costs in servicing traps if the local communities can be involved in some way, at least to replenish the water in the case of an oviposition-based ADS.

The extent to which total cost influences widescale adoption will depend on a number of factors, including whether the system will be purchased by NMCPs, by external funders (wholly or partly), PCO companies, or consumers. Whether the AD system is primarily for malaria control, or elimination, or in post-elimination, will also have a bearing on what price purchasers are prepared to pay. A higher cost may be justified as part of an elimination effort. The value of the system will depend on how easy it is to deploy and how effective it is in a variety of settings, whether it has merit as a stand-alone or a supplementary tool to established approaches, and its potential a resistance management tool for ITNs and IRS. The availability and cost of alternative techniques or products is also relevant.

For developers, an adequate return on investment for development of an ADS is likely to be difficult if not impossible, especially if it requires epidemiological trials to be conducted, which at present is anticipated.

8.3 Conclusions on cost-effectiveness

Stand-alone: larviciding seems widely applicable in urban settings where malaria transmission is ongoing or there is a risk. AD, as an improved larviciding technique, would be expected to be even more widely applicable than standard larviciding in urban settings. In situations where transmission is low and widespread use of ITNs or IRS is not justified, the use of larviciding as a stand-alone technique especially targeted to known mosquito breeding hotspots, can be applicable, even in certain rural settings. If AD is more cost-effective than established larviciding procedures, it would potentially displace and expand such

[26] Kiware, S. S., G. Corliss, S. Merrill, D. W. Lwetoijera, G. Devine, S. Majambere & G. F. Killeen (2015) Predicting Scenarios for Successful Autodissemination of Pyriproxyfen by Malaria Vectors from Their Resting Sites to Aquatic Habitats; Description and Simulation Analysis of a Field-Parameterizable Model. *PLOS ONE,* 10**,** e0131835, Lwetoijera, D. 2016. Exploitation of adult Anopheles arabiensis behaviour and ecology for the dissemination of pyriproxyfen, a novel technique for malaria vector control in Tanzania. In *School of Tropical Medicine*. University of Liverpool.

use. In rural areas where the use of ITNs or IRS is justified, AD would need to demonstrate equal or superior cost-effectiveness to ITNs or IRS to be used as an alternative stand-alone intervention, unless there is a strong case for its added value in resistance management.

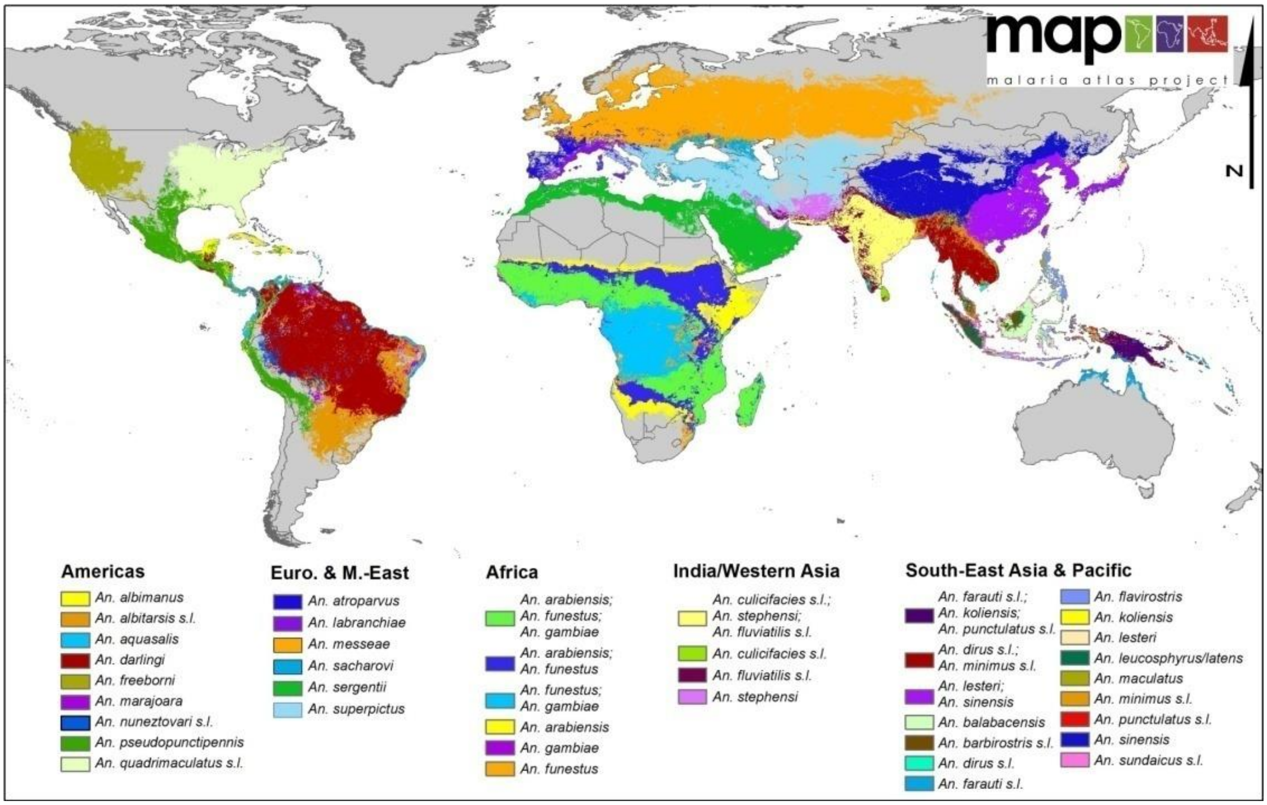
Supplementary: AD can potentially increase the number of settings where larviciding is an appropriate supplementary tool. For example, where a significant portion of breeding population is derived from small cryptic breeding sites inaccessible to direct treatment with conventional insecticides.

9 PROPOSED TARGET PRODUCT PROFILE

9.1 SUITABILITY OF AD TO KEY VECTOR SPECIES.

Worldwide distribution of major vector species is available from the Malaria Atlas Project[27] and reproduced below (Figure 2).

Figure 2. Map of malaria vector distribution



Mosquito behaviour can be categorized in terms of resource utilization[28]: host-seeking preferences, feeding and resting preferences, and preferred breeding sites (types, spectrum, etc).

Opportunities for optimizing contact with mosquitoes for AD is dependent on their behaviours. With *Ae albopictus* and *Ae aegypti*, behaviours are similar to the extent that AD approaches for these species have a high overlap.

[27] https://map.ox.ac.uk/

[28] Killeen, G. F., S. S. Kiware, A. Seyoum, J. E. Gimnig, G. F. Corliss, J. Stevenson, C. J. Drakeley & N. Chitnis (2014) Comparative assessment of diverse strategies for malaria vector population control based on measured rates at which mosquitoes utilize targeted resource subsets. *Malar J,* 13**,** 338.

For malaria vectors, the range of behaviours is greater, and focusing on one species for AD may be to the exclusion of another. The challenge of developing an ADS which is able to control multiple *Anopheles* species is likely to be very considerable.

We suggest the focus should be on unmet need, targeting especially outdoor biting mosquitoes which are difficult to control with ITNs/IRS, and being responsible for residual malaria transmission.

An arabiensis is the key African species responsible for residual malaria transmission, and our TPP includes control of this species as a minimum. There are other key *Anopheles* species worldwide which cannot be adequately controlled by ITNs and/or IRS because their outdoor and polyphagic behaviours, and would therefore be potential targets for an AD system, although these have been excluded from the TPP. These include *An darlingi* in S.America, *An furauti* in Oceania, *An culicifacies, An fluviatilis, An lesteri and An minimus* in Asia. These have been identified as a next challenge for malaria control, being responsible for residual malaria transmission. [29] To assess the individual suitability of these plus additional potentially suitable *Anopheles* species would require very detailed consultation with multiple local entomologists and further in-depth literature review, and is beyond the scope of this study. However, the tables reproduced in Appendix 4 provide an overview of basic information on the preferred host, resting site, and biting site of key *Anopheles* species worldwide.

An.gambiae, whilst amenable to ITNs/IRS, is also included in our TPP due to its major role in Africa, and the pressing need for new tools due to resistance development.

An. funestus is excluded from the TPP because its preferred breeding sites are considered as too large to be amenable to AD.[30]

An.stephensi, which is an important urban vector primarily in India, is included as a target in the TPP. Being a container-breeding species, it is potentially suited to AD as it may be able to disseminate PPF to multiple cryptic breeding sites. Additionally, the urban environment may be particularly suitable for an AD approach for various reasons discussed (see *Economic Considerations* and *Feasibility Of Adoption* sections). In Africa, *An.stephensi* has invaded the continent to some extent and has been implicated in urban malaria transmission[31]. There exists the threat of further geographical spread of *An.stephensi* in Africa, with an associated increase in urban malaria.

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[29] Killeen, G. F., S. S. Kiware, F. O. Okumu, M. E. Sinka, C. L. Moyes, N. C. Massey, P. W. Gething, J. M. Marshall, C. J. Chaccour & L. S. Tusting (2017) Going beyond personal protection against mosquito bites to eliminate malaria transmission: population suppression of malaria vectors that exploit both human and animal blood. *BMJ Global Health,* 2**,** e000198..

[30] Kelly-Hope, L. A., J. Hemingway & F. E. McKenzie (2009) Environmental factors associated with the malaria vectors Anopheles gambiae and Anopheles funestus in Kenya. *Malaria Journal,* 8**,** 268, Dia, I., M. W. Guelbeogo & D. Ayala. 2013. Advances and Perspectives in the Study of the Malaria Mosquito Anopheles funestus. In *Anopheles mosquitoes: New insights into malaria vectors,* ed. S. Manguin, 197-220. IntechOpen.

Pers. Comm. Gerry Killeen.

[31] Faulde, M. K., L. M. Rueda & B. A. Khaireh (2014) First record of the Asian malaria vector Anopheles stephensi and its possible role in the resurgence of malaria in Djibouti, Horn of Africa. *Acta Trop,* 139**,** 39-43.

9.2 TPP Anopheles

Autodissemination system for use in large scale vector control programmes, primarily in Africa		
Disease target: malaria transmitted by *Anopheles gambiae*, *An. arabiensis*, *An.stephensi.*		
Measure	**Minimum criteria**	**Optimistic criteria**
EFFICACY		
Target species	*An arabiensis*	*An gambiae, An arabiensis, An stephensi*
Mosquito population suppression	Capable of achieving 50% reduction of *An arabiensis* population, supplementary to any other control in place, demonstrated by village scale studies.	Capable of achieving 70% reduction of all target species, supplementary to any other control in place, demonstrated by village scale studies. For urban settings, capable of achieving 70% reduction of populations of *Anopheles* vectors when evaluated as a stand-alone intervention.
Resistant strains	Effective against local resistant strains including Kdr, Mace, Rdl, Metabolism (oxidative, esterase, GST)	
Usage settings	Effective as a primary intervention in settings where *An arabiensis* contributes significantly to residual malaria transmission. Primarily dry season intervention Control, pre-elimination, elimination, and post-elimination settings Use as a component of an IVM program.	Additionally, effective as a supplementary intervention against other species in settings where control with IRS/ITNs is poor. Additionally, for wet season intervention. Effective as a primary intervention in urban malaria settings. Effective as the primary intervention in settings where widespread use of IRS/ITNs is not cost-effective or warranted (low transmission/risk areas or post elimination)

Long-lasting effect	For systems needing access to private property, ADS servicing interval once per 4 months or less frequently. For systems without need for access to private property: 2 months.	For systems needing access to private property, ADS servicing interval once per 6 months or less frequently. For systems without need for access to private property: 4 months.
WHO VCAG	Epidemiological impact proven in at least 2 RCT trials.	
MANUFACTURING		
Supply chain	AI available to product developer. Reliable formulator and ADS manufacturer.	
SAFETY AND REGULATORY		
International standards	WHO specification for the technical material and formulation(s) required	
WHO PQ	AI and product(s) must pass WHO PQ risk assessment.	
Toxicology	For all major categories of toxicological importance, risks are understood and considered to be manageable.	
Non-targets	Non-significant population-effects on non-target including aquatic organisms in natural water bodies or in aquaculture systems which receive insecticide from disseminating mosquitoes.	
IMPLEMENTATION COST		
	Total cost of implementation acceptable in low income countries.	
END USER SUITABILITY		

Product form	ADS design and use settings ensure that accidental direct contact of formulation by humans and animals is avoided. If AD involve direct treatment in or around houses, livestock shelters or targeted outdoor sites (eg breeding sites) the formulation should be suitable for this use.	
Irritancy and skin sensitization	No classification of the AI formulation for irritancy or skin sensitivity.	
Odour and staining	If the AD involves formulation application directly to surfaces within houses, the formulation should have non-offensive odour and non-staining.	
IP		
FTO	Manufacturer has access to all ADS components.	

9.3 TPP *Aedes*

Autodissemination system for use in large scale vector control programmes.		
Disease target: Arboviral diseases transmitted by *Aedes aegypti* and *Ae. albopictus.*		
EFFICACY		
Target species	*Ae aegypti*	*Ae aegypti plus Ae albopictus*
Mosquito population suppression	Capable of achieving 50% reduction of *Ae aegypti* population, supplementary to any other control in place, demonstrated by field studies.	Capable of achieving 80% reduction of both target species, supplementary to any other control in place, demonstrated by field studies.
Resistant strains	Effective against local resistant strains including Kdr, Mace, Rdl, Metabolism (oxidative, esterase, GST)	

Usage settings	Effective supplementary tool targeting residual (subset) population breeding in small cryptic breeding sites inaccessible to primary larviciding/source reduction campaign.	Effective as a primary intervention for urban transmission capable of contributing significantly to arboviral disease transmission reduction and epidemic prevention.
Long-lasting effect	ADS servicing interval once per 2 months or less frequently Systems without need for access to private property 1 months.	Insecticide replenishment needed once per 6 months or less frequently Systems without need for access to private property 3 months.
Regulatory standard	Entomological endpoints sufficient to meet leading national regulatory agencies (eg EPA, EU)	
WHO VCAG standard		Epidemiological impact proven in at least 2 RCT trials.
MANUFACTURING		
Supply chain	AI available to product developer. Reliable formulator and ADS manufacturer.	
Shelf life, packaging, and packability	Minimum 1 year in ambient conditions. Compact packaging suitable for transport.	Minimum 2 years in ambient conditions. Compact packaging suitable for transport.
SAFETY AND REGULATORY		
International standards	Full EPA registration achievable	WHO VCAG approval achievable
Toxicology	For all major categories of toxicological importance, risks are understood and considered to be manageable.	
Non-targets	Non-significant population-effects on non-target including aquatic organisms in natural water bodies or in aquaculture systems which receive AI from disseminating mosquitoes	
IMPLEMENTATION COST		
	Incremental cost of adding ADS to existing public sector areawide *Ae aegypti* program: $25 per household per year in low and middle income countries.	

END-USER SUITABILITY		
Product form	ADS design and use settings ensure that accidental direct-contact of formulation by humans and animals is avoided.	
Irritancy and skin sensitization	No classification of the AI formulation for irritancy or skin sensitivity.	
IP		
FTO	Manufacturer has access to all ADS components.	

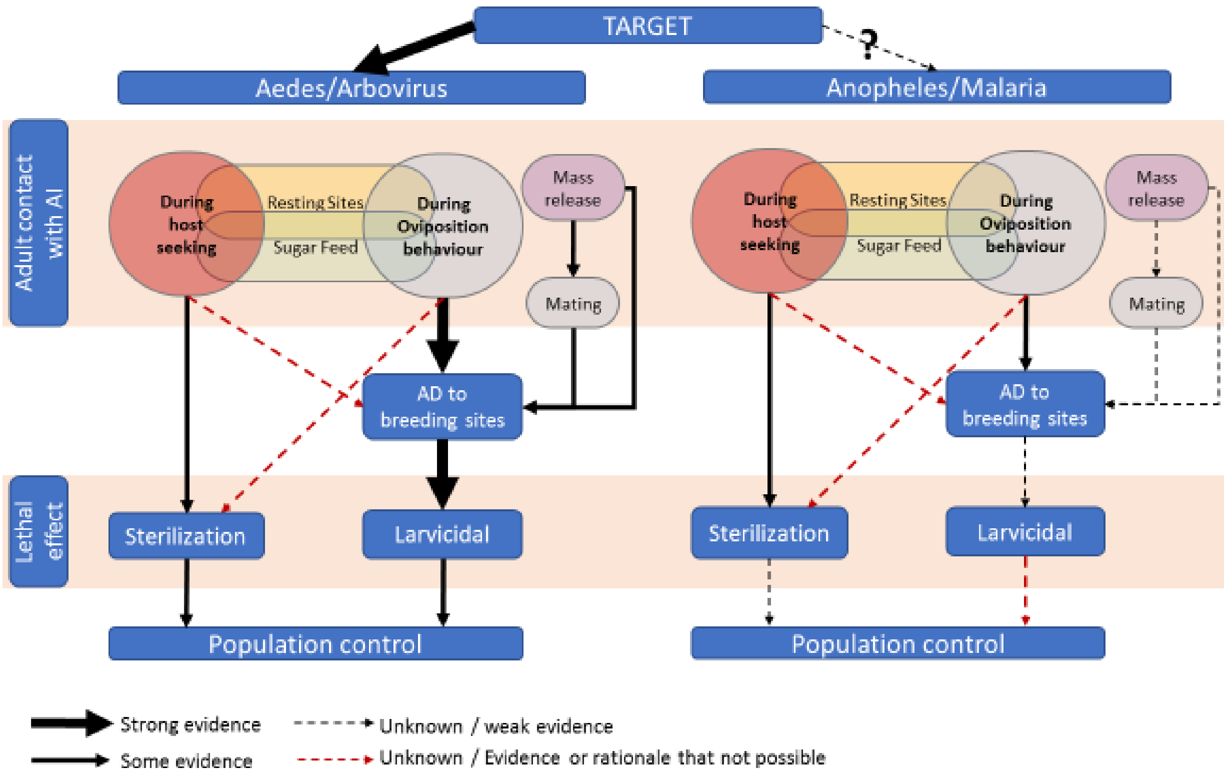
10 GAP ASSESSMENT AND SOLUTIONS ANALYSIS

This section is concerned with bridging the gaps between current knowledge and an aspirational AD system as outlined in the TPP. *Aedes* and *Anopheles* mosquitoes are treated separately due to the differences in their behavior and ecology, however some fundamental aspects of AD are relevant for both.

Figure 3 provides our analysis of the current knowledge of the AD approach for both *Anopheles* and *Aedes* mosquitoes. This knowledge is primarily from studies conducted with PPF. Figure 3 represents the key adult mosquito behaviors that can be targeted for contact with larvicide, with corresponding pathways for dissemination, and impact on populations. Hence, Figure 3 can be used to expose gaps in the current knowledge of AD.

The weight of evidence and likely success for a pathway in Figure 3 is indicated by arrows connecting the different components involved in AD from initial contact through to population control, with thicker arrows indicating strongest evidence and dotted black arrows where there is weak or no knowledge. Dotted red arrows indicate pathways for which there is some experimental evidence or rational that the pathway is not viable.

Figure 3. Schematic overview of the current knowledge of AD approach for both *Anopheles* and *Aedes* mosquitoes illustrating: adult behaviors that can be targeted for contact with AI; pathways for dissemination; effect of AI on insects directly or indirectly contaminated; impact on population.



Adult contact with larvicide

For both *Anopheles* and *Aedes*, host seeking, and oviposition are the two principal behaviours which concentrate adult mosquitoes and can be targeted for delivery of AI to adults in an ADS. Resting sites are associated with either host seeking or oviposition behaviour and have been treated with PPF in experimental AD systems.

AD systems targeting *Aedes* (In2Care and Springstar), exploit the fact that *Aedes* are container breeders and readily enter an oviposition-based ADS. *Anopheles* on the other hand are not generally container breeders (with the obvious exception of *An stephensi*) and so the host-seeking behaviour of these species seems more appropriate as a route to adult contamination. Ways to exploit this host-seeking behaviour for subsequent AD in *Anopheles* are unclear.

Sugar feeding is known to occur throughout the lifecycle of females (host seeking and oviposition) and is also important to males, however, its role in attracting adults for contamination has not been explored for the development of AD.

The need to attract and contaminate naturally occurring populations is avoided by approaches where insects are mass reared, directly contaminated and released where they subsequently contaminate breeding sites or adult populations via mating. This has not been tried in the field, but its feasibility of adoption is considered to be low. Developments in mass rearing and distribution technology, associated release of sterile, GM and Wolbachia infected mosquitoes, may change this in the future.

Lethal effect

Th*e lethal effect* can be broadly categorized as *larvicidal activity* (= inhibition of adult emergence by inhibiting juvenile development; egg, larvae, pupae) or *sterilizing effect* (reduced egg yield and hatch rate) of contaminated females.

For PPF, the lethal effect on adult females is dependent on time of exposure. Contamination with PPF during host-seeking (before/during/after blood feeding) results in partial or full sterility. There is no clear rationale for a system which only sterilizes adults without AD, as it does not amplify lethal effect, and it allows potentially infective females to continue to live. It is not understood whether alternative AIs (in particular IGRs) sterilize adults to a lesser extent as PPF during host-seeking, and whether these might be better exploited for AD.

Other factors

- Skip oviposition

Contrary to *Aedes*, for *Anopheles* there is a lack of robust evidence regarding skip oviposition, and so this needs to be checked.

- Mosquito population density/breeding sites/seasonality

For *Aedes*, where AD is clearly achievable in the field, a critical factor is the density of the target mosquito population: a higher density will increase the number of contaminated females and corresponding transfer of AI to breeding sites, and a lower density will bring about the opposite. Related to this is the volume of water in the breeding sites and its replenishment rate, and AI potency and concentration. The optimal timing of AD where seasonality is pronounced, is also of importance. The above factors which have not been adequately examined in the field yet.

- Formulation

For both *Aedes* and *Anopheles*, improved formulations could increase the amount of AI which can be carried and disseminated by each mosquito, and could reduce the need for such large numbers and frequent ADS servicing, as well as increasing the time period that a contaminated adult retains a biologically significant dose for dissemination. This may be particularly useful for *Anopheles* if these are targeted for contamination at host-seeking (assuming that there is a useful AD effect when adults are contaminated at this early stage).

GAP ASSESSMENT	SOUTIONS ANALYSIS
Aedes	
Need for reduced ADS servicing frequency in existing systems	Develop long-lasting formulations delivering more AI with improved adhesion to adults. (In2Care, Springstar)
Need for reduced ADS density in existing systems	Develop ADS with increased attractiveness exploiting for example, sugar, odours, visual attractants, trap placement. (In2Care, Springstar) Develop long-lasting formulations delivering more AI with improved adhesion to adults. (In2Care, Springstar)
Need for reduced ADS density in new systems	Develop a system that does not involve a physical ADS and therefore does not need operator access to houses and time-consuming servicing of physical ADSs. For example, an effective mosquito-attractive formulation which is rain-fast if outdoor application is envisaged. (ISCA)
Improved systems need to be effective under a wide range of field conditions	Validate effectiveness and determine use recommendations of promising systems by field trials in multiple settings. Also assessing the impact on effectiveness of key variable factors including mosquito populations (high density, low density, effect of heterospecific species, breeding site structure)

GAP ASSESSMENT	SOLUTIONS ANALYSIS
Anopheles **1. Exploiting host seeking behaviour**	
Uncertainty regarding extent of skip-oviposition	Seek further expert opinion / literature. Ecological study if unknown in existing knowledge
Uncertainty regarding sterility-effects induced by other IGRs at host-seeking contamination timing	Seek expert opinion (including manufacturers). Conduct specific laboratory evaluation where needed
Uncertainty on induced sterility effects, in particular whether partial sterilisation results in corresponding reduction in oviposition behaviour, compromising transfer of AI to breeding sites	Undertake studies to evaluate sterilization effects of PPF, novaluron, triflumuron, diflubenzuron, and compare transfer rates, under ecologically representative conditions (semi field initially)
Contamination of adults at host-seeking results in loss of larvicide in the interim period before oviposition.	Develop a formulation which allows for high load of larvicide resistant to grooming for extended periods (>48 hours) during egg maturation between host seeking and oviposition
To attract adults during host-seeking requires a very attractive ADS	Explore visual and olfactory cues associated with host seeking, perhaps exploiting sugar-feeding
Uncertainty regarding optimal ADS location for adult attraction during host-seeking	Explore strategic placement of ADS during host-seeking – optimise encounter, specific to ecology (eg treating animal shelters for zoophilic species)
Anopheles **2. Exploiting oviposition behaviour**	
Uncertainty regarding extent of skip-oviposition	Seek further expert opinion / literature. Ecological study if unknown in existing knowledge

To attract adults during oviposition requires a very attractive ADS	Explore visual and olfactory cues associated with oviposition, perhaps exploiting sugar-feeding.
Uncertainty regarding optimal ADS location for adult attraction during oviposition	Explore strategic placement of ADS during oviposition – optimise encounter, specific to ecology.

11 FEASIBILITY OF ADOPTION

The enthusiasm to adopt AD at operational scale will depend on its ability to contribute to controlling transmission (or elimination) within IVM programs, either as a primary or more likely a supplementary tool. It is too early to say whether AD systems can be developed which deliver benefit in multiple ecological settings, or whether their successful deployment is feasible in terms of cost and operational logistics.

For *Aedes*, the experience thus far with the In2Care system is that the sheer numbers of ADSs required for control, together with the frequent servicing needs, hampers feasibility for large area adoption. This is recognized by In2Care as well as others who have direct experience of deploying this system at relatively small scale in Manatee County. Spingstar's aim with its new *Aedes* ADS to be launched, is to reduce the number of stations per unit area to one third of that needed by the In2Care system. They also plan to reduce the frequency of servicing the ADS. There is also potential to make AD systems for *Aedes* more attractive to mosquitoes.

It remains to be seen whether the Spingstar system for *Aedes* will meet the requirements for large-area use, or whether the In2Care system will be further improved. Until and unless the current In2Care system is significantly improved, and its approval by WHO, its use by national vector control organizations for *Aedes* control may be largely restricted to trials and perhaps targeting to known hotspot areas, in the hope that it may bring some additional benefit. The use by some municipalities in Latin America and by mosquito abatement districts in the US is possible without WHO approval, and if such organizations experience some local success with commercially available *Aedes* AD systems, a growth in uptake by such organizations is to be anticipated. The lack of published efficacy data on the In2Care system at this time also hampers further adoption.

For *Anopheles,* the TPP defines a target system which is feasible to deploy. At present there is scepticism amongst experts that such a product can be developed because of a lack of an obvious fit of AD with *Anopheles* ecology and behaviour and a general lack of data, as analyzed in the literature review. There are two elements here. Firstly, can a system be developed that works at least under certain conditions? And secondly, would that system be logistically feasible for operational adoption? A low-tech AD solution for *Anopheles* such as infrequent dusting of animal shelters would likely be much more feasible to deploy than a system which required high numbers of oviposition/resting site- based AD stations, requiring expert decision on where and how they are locally deployed, and requiring frequent monitoring and servicing. Obviously, the operational adoption of any new tool will require training.

Risk of resistance development is a factor which will likely influence adoption, especially due to exposure to sub-lethal doses of PPF. This has been a point of discussion with experts consulted although it was not spontaneously raised as a major concern by many. There was no consensus on whether this is potentially a major or minor issue, but it could significantly affect adoption. The use of PPF in settings where it is a supplementary tool to IRS or ITNs could contribute to resistance management where resistance to the AIs already in use is evident. This potential value as a resistance management tool may increase the readiness to adopt AD, assuming that a workable system can be developed. However, it should be noted that there appears to be cross-resistance potential between the larvicide temephos, and pyriproxyfen[32]. The value in introducing a PPF-based AD system in areas of temephos resistance may be questionable therefore.

Furthermore, pyrethroid resistance in *An. gambiae* has been associated with elevated levels of P450 expression including CYPs 6M2, 6P2, 6P3, 6P4, 6P5, 6Z2 and 9J5. Researchers have found cross resistance to PPF[33].

Generally, the likelihood of resistance/cross resistance developing rapidly in the field and negating the value of a PPF-based AD system is difficult to predict.

Regarding the cost of ADS required for operational adoption, this is addressed in the section on *Economics*, and in the TPP. Experts consulted did not generally offer insights into cost requirements. Availability of adequate funding is obviously a pre-requisite for the adoption of any new tool.

We sought the opinions of mosquito experts regarding feasibility of adoption. Most focused rather on the feasibility of developing a workable system, and what type of use settings might be more appropriate, rather than commenting on the feasibility of large scale operational adoption within those settings. The number of experts expressing opinions are recorded below.

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[32] Marcombe, S., F. Darriet, P. Agnew, M. Etienne, M. M. Yp-Tcha, A. Yebakima & V. Corbel (2011) Field efficacy of new larvicide products for control of multi-resistant Aedes aegypti populations in Martinique (French West Indies). *Am J Trop Med Hyg,* 84**,** 118-26, Andrighetti, M. T. M., F. Cerone, M. Rigueti, K. C. Galvani & M. d. L. d. G. Macoris (2008) Effect of pyriproxyfen in Aedes aegypti populations with different levels of susceptibility to the organophosphate temephos..

[33] Yunta, C., N. Grisales, S. Nasz, K. Hemmings, P. Pignatelli, M. Voice, H. Ranson & M. J. Paine (2016) Pyriproxyfen is metabolized by P450s associated with pyrethroid resistance in An. gambiae. *Insect Biochem Mol Biol,* 78**,** 50-57.

In total 29 people were interviewed. These have been categorized primarily as AD Key Researchers, ADS Developers, and Others, according to Appendix 3.

Table 3. Interviewee opinions on key feasibility criteria

	Number of respondents expressing opinion		
	No	Uncertain	Yes
Considered AD has promise for use against *Anopheles*	2 2 Others	9 4 ADS Researchers 2 ADS Developers 3 Others	3 1 ADS Researchers 1 Developer 1 Other
Expressed concern for feasibility at scale due to likely complexity	1 1 Developer	2 1 ADS Researcher 1 Other	3 3 Others
Considered that AD would find only niche setting usage	1 1 Developer	1 1 Other	7 2 ADS Researchers 5 Others
Considered AD would be unlikely to become more than a supplementary tool	2 1 Developer 1 Other	2 2 Developers	9 5 ADS Researchers 4 Others
Considered larviciding of larger breeding sites to be necessary in addition to AD		1 1 Other	8 3 ADS Researchers 1 ADS Developer 4 Other
Expressed concern over density dependence of AD		4 2 ADS Researchers 1 ADS Developer 1 Other	1 1 ADS Researchers

Expressed concern that ADS can be made attractive enough	1 1 Developer	1 1 ADS Researchers	4 1 ADS Researchers 3 Others
Considered dry season use more promising		1 1 Other	6 4 ADS Researchers 1 Developer 1 Other
Considered urban setting as more suitable than rural		5 2 ADS Researchers 1 Developer 2 Others	4 4 Others

12 Recommendations for Taking Technology Forward

12.1 Aedes

At least 2 commercial companies (In2care and Springstar) are developing an oviposition-based ADS, and one commercial product is already on the market. These companies are aware of limitations of this approach and the need for further optimization (reducing trap numbers and service frequency) and are actively working to achieve this. Furthermore, large fields trials are underway and/or planned which address remaining questions regarding efficacy at scale across a range of environments. ISCA Technologies is also developing a different approach based on attractive sugar baits, which has potential to dispense with need for a physical ADS but is at an earlier stage of development. Additionally, research groups are developing systems to directly contaminate mass reared mosquitoes or heterospecific species as agents for AD. Optimizing mass rearing and distribution systems remain the main technical challenge for this system. Much research effort is currently being invested in this area as part of other projects utilizing mass release for vector control (GM, Wolbachia, SIT), so advances in this area may make AD using mass reared insects a viable option in future.

- The points identified in the section *Gap Assessment and Solutions Analysis* are actively being addressed by research and commercial operators, so our recommendation is that IVCC should remain informed of ongoing developments although additional focus by IVCC should not be a priority.

12.2 *Anopheles*

The ecology of *Anopheles* makes them significantly less amenable than *Aedes* to development of a viable AD system. In our assessment the likelihood of developing a robust AD system is low. As there is an acknowledged need for new tools and AIs for combating malaria vectors, a decision whether to invest further in the AD approach depends on the perspective and competing priorities of funding organizations. There is no clear business case for *for-profit* developers.

If such a system were to be developed, it would have limited applicability, serving mostly as a supplementary control tool and may be restricted to limited ecological conditions such as dry season application.

Some positive results from the field have been predominantly due to the sterilizing effect of PPF. Ongoing research to optimize sterilization should be considered as a separate approach to AD, as it would appear that it does not involve any or much dissemination to breeding sites. In fact, maximizing the numbers of mosquitoes that are sterilized is likely to simultaneously reduce the numbers of AD-capable mosquitoes. Adult sterilization by PPF is an attract and kill strategy, and needs to be considered and optimized alongside alternative approaches and AIs that are being developed for that purpose.

Further development, if decided upon, should address three key areas of enquiry: oviposition behavior, formulation, and attractive ADS design, as outlined in the section *Gaps Assessment and Solutions Analysis* and elaborated below:

Broad *Anopheles* ADS development pathway

This pathway describes development and evaluation for efficacy. Any additional regulatory studies which may be triggered at any stage are not captured here.

Steps 1-3 to be undertaken concurrently.

1. Evaluate alternative AIs in addition to PPF, including impact of AI dose on sterility and oviposition (laboratory). Confirm candidate AI(s) for formulation work.

   Check known oviposition behaviour of key *Anopheles* species (literature, expert consultations).

2. Develop/test/optimize formulations (laboratory) for maximum transfer of AI to female adults, long-lasting resistance to grooming, and deposition at breeding sites.

3. Develop/test/optimize highly attractive ADS prototypes (laboratory), targeting concurrently:
   a. host seeking females.
   b. oviposition females.

   Selection of most promising AI(s), formulation(s), attractive ADS design(s), for first field evaluations.

Go/no-go decision for semi-field and small-scale field trials.

4. Evaluate efficacy of AI/formulation(s) and ADS design(s) individually and combined in prototype system(s) in semi-field and small-scale field trials.

    a. Measure dissemination of AI and larvicidal impact in both SBS and natural breeding sites (bioassay and chemical analysis).
    b. Confirm preferred system for evaluation in village scale trials.

    Go/no-go decision for village-scale field trials.

5. Village scale efficacy evaluation
    a. Evaluate mosquito suppression at large scale.
    b. Develop/refine usage recommendations.
    c. Evaluate suitability for operators and public at operational scale.

    Go/no-go decision for epidemiology trials

6. Large scale epidemiology studies: two Randomised Cluster Trials (RCT) evaluating entomological endpoints and epidemiological impact. Confirm effectiveness. Confirm usage recommendations. Confirm suitability in use for operators and public.

13 RECOMMENDED OUTLINE PROTOCOL FOR NEXT STAGE TRIALS WORK.

It is premature to propose detailed protocols beyond step 3 in the development pathway above, given that these will be dependent on outcomes of initial steps.

Evaluation of AI(s) and formulation(s) (steps 1-2) should be addressed in laboratory studies initially as described by Mbare[34]:

1) Expose mosquitoes to AI formulation (fixed time ~30min) at different times relative to the gonotrophic cycle.
2) Provide females access to oviposition site (100ml cup)
3) Evaluate impact of sterilization and oviposition behaviour by assessing
    i. Proportion not laying eggs (totally sterile)
    ii. Change in egg yield
    iii. Change in hatch rate of eggs
    iv. Transfer of AI to oviposition site
        1. Larval development bioassay

[34] Mbare, O., S. W. Lindsay & U. Fillinger (2014) Pyriproxyfen for mosquito control: female sterilization or horizontal transfer to oviposition substrates by Anopheles gambiae sensu stricto and Culex quinquefasciatus. *Parasites & Vectors,* 7**,** 280.

2. Chemical residue analysis

Experts and literature should be consulted in step 1 to better understand the extent of skip oviposition practiced by key *Anopheles* species. Some local investigations may eventually need to be initiated if there is insufficient understanding. A lack of pronounced skip oviposition might not be operationally significant for AD, and would be unlikely alone to trigger a no-go decision to semi-field and field testing.

Development of an attractive ADS (step 3) is an iterative process. Having identified the target mosquito behavior/life stage to be targeted, the individual components of the system (visual, olfactory, tactile cues etc) can be isolated and optimized initially in the laboratory. ADS development work authored by Snetselaar [35] and Kartzinel [36] provide examples of the iterative process of isolating and optimizing components of an ADS before bringing them together in a prototype for efficacy evaluation.

In semi-field and small-scale field environments (step 4), evaluation of prototype system(s) should focus on proportion of mosquito population attracted and contaminated. This may be facilitated with markers (eg fluorescent powders), and/or candidate AI formulations. Semi-field evaluations should be conducted following methodologies adapted from those described by Lwetoijera[37] and Mbare[38]: release females in a large cage with ADSs and SBSs, and subsequently assess transfer of AI to SBSs by bioassay and chemical residue analysis. Small-scale field studies may additionally be conducted to verify results under more representative open field conditions with naturally occurring wild mosquito populations.

Village scale studies (Step 5) will be dependent to a large extent on the outcome of previous steps and system being evaluated. Transfer and corresponding larvicidal activity should be assessed in natural breeding sites in addition to SBSs. Studies should be run at scale and duration to assess impact on the mosquito population. Use recommendations should also be developed/refined during this phase including service intervals, timing and spatial deployment of ADS. Acceptability to operators and public should be assessed during all fieldwork, and any unintended consequences noted. These will need to be considered alongside efficacy in making the decision to move to large scale epidemiology studies.

Large scale epidemiology studies (Step 6) need to be conducted according to WHO VCAG guidelines. In addition to demonstrating mosquito control and corresponding epidemiology impact these studies should confirm usage recommendations and suitability in use for operators and public.

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[35] Snetselaar, J., R. Andriessen, R. A. Suer, A. J. Osinga, B. G. Knols & M. Farenhorst ibid.Development and evaluation of a novel contamination device that targets multiple life-stages of Aedes aegypti. 200.

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[37] Lwetoijera, D., C. Harris, S. Kiware, S. Dongus, G. J. Devine, P. J. McCall & S. Majambere (2014a) Effective autodissemination of pyriproxyfen to breeding sites by the exophilic malaria vector Anopheles arabiensis in semi-field settings in Tanzania. *Malar J,* 13**,** 161.

[38] Mbare, O. 2015a. Chapter 7 - Developemnt of an auto-dissemiation station for gravid *Anopheles gambiae sensu stricto* for use in attract and kill stratergies. In *PhD Thesis - Novel insecticides and application strategies for malaria vector control*, 170-192. London School of Hygiene & Tropical Medicine.

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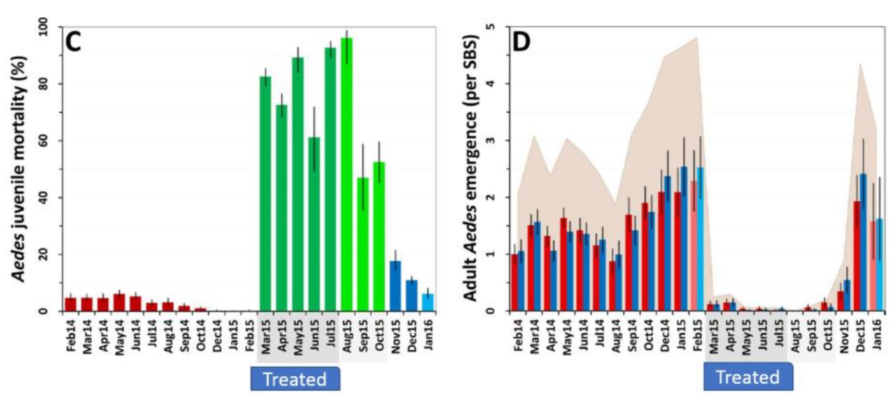
15 Appendix 1 - Synopsis of published semi-field and open field studies

Published semi-field and open field studies on AD underwent detailed review. For each study the following data was captured: study type (semi-field/open-field); location; target species; AD method; AI; formulation; application rate; and summary of objectives, methods, key results and conclusions.

Suman, D. S., Y. Wang, A. Faraji, G. M. Williams, E. Williges & R. Gaugler (2018) Seasonal field efficacy of pyriproxyfen autodissemination stations against container-inhabiting mosquito *Aedes albopictus* under different habitat conditions. *Pest Manag Sci,* 74**,** 885-895.	
-Study type -Location -Target Species	• Open Field • USA • *Ae albopictus*
Objectives	Season-long efficacy evaluation of ADS under a range of environmental conditions
Autodissemination Methods	Adapted oviposition station: ADS contained a reservoir of water (1L) including oak leaf infusion attractant. A unidirectional cone guides mosquitoes to walk over a duel band of oil, followed by powder formulations. Designed for single deployment for whole season ~ 12 weeks.
-Active -Formulation -Application rate	• PPF (technical grade) • Dual formulation: 20% emulsifiable oil & 60% wettable powder • 2.5 to 12 ADS/Ha
Methodology	Evaluation of ADS efficacy against a range of parameters in urban, peri-domestic and junkyard field sites in NJ, USA. **Study 1)** ADS (5 & 20 / Ha) vs ranges of densities of competitor oviposition sites (SBS) in vegetated urban setting (0.4 Ha plots). **Study 2)** ADS deployed at different densities (2.5 - 10 / Ha) in vegetated urban setting (0.4 Km2 plots). **Study 3)** ADS under peridomestic habitat (University campus, density not given) **Study 4)** Dispersal distance: x6 ADS at the centre of orthogonal transects of SBS spanning up to 200m in a residential area. **Study 5)** Tyre pile x6 ADS deployed around 100 used tyres **Study 6)** ADS in Junkyard at a density of 6/0.5 ha SBS (cup with 250 ml oak leaf infusion) were deployed to assess autodissemination to breeding sites. Pupal mortality bioassays were conducted on water samples form SBS and treated tyres over 8-12 weeks.
Results	Study 1) Evidence of reduced efficacy with an increased number of competitive oviposition sites. Overall 15-30% pupal mortality, 50-60% SBS contamination Study 2) Higher densities resulted in significant increase in pupal mortality vs lowest density treatment and control. Overall 20 % pupal mortality, 60% SBS contamination for highest ADS density. Study 3) Mean of 50% pupal mortality and 80% SBS contamination Study 4) Significantly higher pupal mortality was observed compared to controls in SBS at all distance up to maximum tested SBS 200m. Study 5) Overall 15% pupal mortality, 40% SBS contamination Study 5) Overall 20% pupal mortality, 50% SBS contamination
Conclusion	Good evidence of dispersal mechanism (up to 200m) although results for pupal mortality from SBS were modest, generally in the 20-30% range, with exception of one study in the peri-domestic site where 50% was observed (Rutgers Campus), although the density of ADS was not given. Percentage contamination of SBS

	averaged ~50 % (ranging 30-80%). Robust demonstration of the AD in a variety of field settings, although results (pupal mortality generally < 50%) were underwhelming as a robust control method.
Abad-Franch, F., E. Zamora-Perea & S. L. Luz (2017) Mosquito-Disseminated Insecticide for Citywide Vector Control and Its Potential to Block Arbovirus Epidemics: Entomological Observations and Modeling Results from Amazonian Brazil. PLoS Med, 14, e1002213.	
-Study type -Location -Target Species	• Open Field • Amazonas, Brazil • *Ae aegypti, Ae albopictus, Culex* spp., *Limatus* spp.
Objectives	Evaluate the efficacy of simple ADS at transferring PPF to breeding sites in treated and wider surrounding area.
Autodissemination Methods	Adapted oviposition site: 2L plastic cups with 600-700 ml of tap water and the inner wall lined with black, cotton cloth dusted with PPF. Serviced fortnightly.
-Active -Formulation -Application rate	• PPF • SumiLarv 0.5 G granules (Sumitomo) 0.5% ai, ground to powder. ADS cloth dusted with 5 g/m2 • 1.5 ADS/Ha
Methodology	Field site comprised of a 650 ha site with a population of 60,000 in dense urban, dengue endemic environment in Manaus, Brazil. 100 dwelling distributed throughout the site were monitored with x2 SBS (plastic cup with 200 ml water) each. Monitoring was conducted twice/moth for ~2 years: 12 baseline months, 8 months of PPF dissemination (5 months citywide and 3 months focal), and 3 months post-dissemination. SBS was set monthly for 6 days before recovery to a laboratory where the subsequent development of any larval present was assessed to calculate: % conidiation of SBS, juvenile mortality and adult emergence. Treatment – • 5 months city wide:1000 ADS (=1.54/ha) distributed and services fortnightly to reapply PPF and top-up water. • 3 months focused treatment; ADS deployment limited to households identified as *Ae aegypti* positive (29 homes).
Results	Following PPF dissemination, there was an 80%-90% decrease in *Aedes* juvenile catch, while *Aedes* juvenile mortality increased from 2%-7% to 80%-90%. Adult *Aedes* emergence dropped by 96%-98% from SBS. 

Mean Juvenile mortality and adult emergence/month in all SBS for all species

	Before treatment	During Treatment
House infestation	85%	33%
Juvenile mortality	1.9%	79.7%
Adult emergence/month	1177	56

Conclusion

Compelling evidence of autodissemination as a vector control method at operational scale, with city vector control agents used to deploy and service ADS. ADS was simple and used standard larvicidal formulation, with 0.5% AI, suggesting increase in efficacy possible with higher concentration. ADS was relatively low compared to many other AD field evaluations.

Direct adult population was not assessed, although 80-90% reduced juvenile catch in SBS provided proxy for lower adult population This study represents the most convincing evidence to date for the operational use of AD for vector control.

Buckner, E. A., K. F. Williams, A. L. Marsicano, M. D. Latham & C. R. Lesser (2017) Evaluating the Vector Control Potential of the In2Care(R) Mosquito Trap Against Aedes aegypti and *Aedes albopictus* Under Semifield Conditions in Manatee County, Florida. J Am Mosq Control Assoc, 33, 193-199.

-Study type
-Location
-Target Species

- Semi-Field
- Florida, USA
- *Ae aegypti, Ae albopictus*

Objectives

Assess In2Care trap's attraction, and it's larvicidal, autodissemination and adulticidal impact on invasive container-breeding *Aedes* mosquitoes under semi-field environment.

Autodissemination Methods

In2Care trap: modified ovitrap consisting of a black flowerpot holding reservoir of water (3.5 L) including an odor lure to attract gravid Aedes. The trap incorporates a lid and a floater placed on water surface which holds a 5cm wide gauze strip that is treated with PPF, and *B. bassiana* spores.

-Active
-Formulation
-Application rate

- PPF and Beauveria bassiana spores
- In2Mix : PPF 74%; Beauveria bassiana strain GHA spores 10% W/W; yeast tablets attractant lure added to water.
- 473 ADS/HA

Methodology

Evaluations conducted in netted screened rooms (4.6 x.4.6 x.2.4 m) exposed to prevailing climate conditions.

- Larvicidal activity of In2Care traps was assessed by adding larvae and assessing subsequent development vs untreated controls.
- Autodissemination was evaluated by assessment of emergence inhibition of larvae introduced to 4 SBS (400ml water + alfalfa attractant + 20 L2 larvae) placed around a single In2Care trap in each room.
- The adulticidal impact was evaluated by recapturing adults after 48 hr in rooms with and without In2Care traps and subsequently monitoring longevity.

Results

Significant inhibitions of emergence were observed for larvae from within traps and in SBS resulting from autodissemination:

Location	Control	In2Care trap treatment
Within Traps	20-30%	100%
In SBS	20-31%	81-94%

	Exposure to In2Care traps for 48 hr significantly reduced the longevity of adults compared to controls
Conclusion	In2Care traps effective killing multiple life stages of *Ae aegypti* and *Ae albopictus* under semi-field conditions, including by autodissemination transfer of larvicidal concentrations of PPF to SBS.
Lloyd, A. M., M. Farooq, A. S. Estep, R. D. Xue & D. L. Kline (2017) Evaluation of Pyriproxyfen Dissemination via Aedes albopictus From a Point-Source Larvicide Application in Northeast Florida. *J Am Mosq Control Assoc,* 33**,** 151-155.	
-Study type -Location -Target Species	• Open-Field • Florida, USA • *Ae albopictus*
Objectives	Investigate the possibility of pyriproxyfen dissemination from a targeted hotspot treatment site (spray application) to nontreated oviposition sites via the skip oviposition behavior of *Ae. albopictus*.
Autodissemination Methods	PPF spray application to targeted breeding site hotspot (e.g. tyre pile) and rely on natural dispersal by mosquitos
-Active -Formulation -Application rate	• PPF • NyGuard (10% ai) spray application • 0.41 L/ha
Methodology	A tyre pile (n=100, 20m^2) was treated with spray formulation of FFP. SBS (vase with 250 ml oakleaf infusion) were placed in 4 orthogonal transects up to 400m. Control SBS placed at 1500m. SBS were recovered 4h, 1,2,4 and 6 weeks post-treatment. Waters samples from tyre pile and SBS were tested for PPF residue and bioassays evaluating inhibition of larval development.
Results	There was no evidence of autodissemination to SBS observed. Only the directly treated tyre pile showed significantly higher juvenile mortality compare to control, effective for up to 4 weeks.
Conclusion	There was no evidence of autodissemination of PPF beyond the directly treated breeding sites.
Mian, L. S., M. S. Dhillon & L. Dodson (2017) Field Evaluation of Pyriproxyfen Against Mosquitoes in Catch Basins in Southern California. *J Am Mosq Control Assoc,* 33**,** 145-147.	
-Study type -Location -Target Species	• Open Field • CA, USA • *Culex quinquefasciatus*
Objectives	Efficacy evaluation of pyriproxyfen (Sumilarv 0.5% G) and S-methoprene (Altosid XR briquet 2.1%) larvicidal treatment in catch basins
Autodissemination Methods	PPF application to targeted breeding site hotspot (e.g. catch basins) and rely on natural dispersal autodissemination by mosquitos
-Active -Formulation -Application rate	• PPF • Sumilarv 0.5% G: 0.5% ai gradual formulation • Applied at 10 g and 50 g per catch basin
Methodology	Catch basins were treated with granular larvicidal formulation of PPF and subsequent inhibition of adult emergence was assessed over 8 weeks in treated and untreated control catch basins
Results	PPF provided 100% inhibition of adult emergence in treated sites up to 3 weeks. After 4 weeks results were masked by the high level of mortality in control sites.
Conclusion	Authors attributed high mortality in control catch basins to an AD of pyriproxyfen by mosquitoes from treated to untreated catch basins. Although not designed specifically as an AD evaluation, this study provides evidence that PPF may be

	autodisseminated beyond treated breeding sites, sufficient to impact larval development in untreated sites.
Unlu, I., D. S. Suman, Y. Wang, K. Klingler, A. Faraji & R. Gaugler (2017) Effectiveness of autodissemination stations containing pyriproxyfen in reducing immature Aedes albopictus populations. Parasit Vectors, 10, 139.	
-Study type -Location -Target Species	• Open Field • NJ, USA • *Ae albopictus*
Objectives	Evaluate the efficacy of ADS in urban sites of PPF autodissemination and corresponding suppression of *Ae albopictus* population
Autodissemination Methods	Adapted oviposition station: ADS contained a reservoir of water (1L) including oak leaf infusion attractant. A unidirectional cone guides mosquitoes to walk over duel band of oil, followed by powder formulations. Designed for single deployment for whole season ~ 12 weeks.
-Active -Formulation -Application rate	• PPF (technical grade) • Dual formulation: 20% emulsifiable oil & 60% wettable powder • 32-35 ADS/Ha
Methodology	Field studies were conducted in an urban neighbourhood (50ha) in Trenton NJ, USA, from which six city blocks (approx. 0.8 ha each) were selected as hotspots with historically high *Ae albopictus* populations. Half the plots received treatment of 26-28 ADS/plot, with the remainder serving as controls. Surrounding areas received intensive treatment with conventional vector control to mitigate the impact of immigration. The assessment was via BG Sentinel traps (x1), Oviposition cups (x5), and SBS (x10) deployed in each treated and control plot. SBS consisted of the cup with 250 ml water. from which samples were taken for larva development bioassay. Adult Population was assessed via eggs recovered form oviposition cups and adult counts in BG Sentinel traps. Number of larvae in SBS was also assessed. Bioassays on larval development in water samples from SBS were conducted to evaluate contamination with larvicidal active concentrations of PPF. The evaluation was monitored for 9 weeks following installation of ADS.
Results	Autodissemination: Bioassay - pupal mortality: 12.4% Treated / 0.5% Control (sig.diff) Adult Population: Mean Egg/ovicup: 1.4 Treated / 6.9 Control (sig.diff) Mean larvae in SBS : 0.5 Treated / 4 Control (sig.diff) BG Sentinel traps: no significant effect of treatment
Conclusion	A clear demonstration of autodissemination in an urban setting, most evident in ~ 80% reduction in eggs and larvae recovered from SBS, which could be the result of the reduced fecundity of females due to PPF exposure and/or reduced population. Corresponding results not observed in adult capture by BG-Sentinel traps, although this was unsurprising given low traps numbers (1/plot) and likely interference from immigration due to small plat size in relation to dispersal ability of *Ae albopictus*.
GAUGLER, R. R., D. S. SUMAN, B. TAO & Y. WANG. 2017. Methods and apparatus for management of mosquito populations with habitat sharing heterospecific insects carrying insect growth regulators. PATENT# WO 2017/096381	
-Study type -Location -Target Species	• Open Field • USA • *Ae albopictus*

Objectives	Demonstrate heterospecific species (midge) can disseminate PPF to mosquito breeding sites resulting in increased *Ae albopictus* juvenile mortality
Autodissemination Methods	Adult midges (*Chironomus decorus*) treated with PPF used as vehicle to disseminate PPF to mosquito breeding sites
-Active -Formulation -Application rate	• PPF (technical grade) • Treated with 20% AI oil formulation followed by 60% powder formulation • 800 treated midges / back yard (67 release points/Ha)
Methodology	Field site consisted of a fenced back yard (~300m2) with ten SBS (250 ml oak infusion). PPF treated Chironomid midges were released from 2 points and after 3 days SBS were returned to the laboratory where bioassay on juvenile *Ae albopictus* were conducted to evaluate transfer of PPF.
Results	There was a 74% mortality of juvenile *Ae albopictus* compared to only 1% in the control.
Conclusion	Results following a single release of treated Chironomid midges clearly demonstrates they successfully contaminated mosquito breeding sites with sufficient PPF to induce significant mortality of immatures.
Chandel, K., D. S. Suman, Y. Wang, I. Unlu, E. Williges, G. M. Williams & R. Gaugler (2016) Targeting a Hidden Enemy: Pyriproxyfen Autodissemination Strategy for the Control of the Container Mosquito Aedes albopictus in Cryptic Habitats. PLoS Negl Trop Dis, 10, e0005235.	
-Study type -Location -Target Species	• Semi-Filed & Open Field • NJ, USA • *Ae albopictus*
Objectives	Evaluate the efficacy of PPF autodissemination at penetrating cryptic breeding sites difficult to treat with conventional insecticidal sprays
Autodissemination Methods	Adapted oviposition station: ADS contained a reservoir of water (1L) including oak leaf infusion attractant. A unidirectional cone guides mosquitoes to walk over duel band of oil, followed by powder formulations. Designed for single deployment for whole season ~ 12 weeks.
-Active -Formulation -Application rate	• PPF (technical grade) • Dual formulation; 20% emulsifiable oil & 60% wettable powder • 10 & 20 ADS/Ha for 2013 & 2104 studies respectfully
Methodology summary	Semi-Field Netted field cage 3mx2mx50m. Gravid females were released into the cage and provided a choice of cryptic and open SBS. Numbers of eggs recovered from SBS was used to assess oviposition preference. Open Field Field evaluations were conducted in consecutive years in two sites in New Jersey, USA: 2013 - a suburban environment with low mosquito population; 2014 - a dense urban environment with high mosquito population. For each study one control and 3 treated plots of ca. 4000m^2 were evaluated. Treated plots contained 12 open SBS (cups with 250 ml oak leaf infusion) and 12 cryptic SBS (cups with 250 ml oak leaf infusion housed within a short section of pipe). Controls received 5 open SBS. Four and eight ADS were applied to treated plots in 2013 and 2014 respectively. Pupal mortality and % of contaminated SBS were obtained from bioassays on larval survival in water sampled from SBS for 8 and 12 weeks post-deployment of ADS for 2013 and 2014 studies respectively.

<table>
<tr><td>Results</td><td>Semi-Field
Gravid females showed a strong preference for cryptic (53 eggs/SBS) over open (10 eggs/SBS) sites for oviposition.

Field Evaluation:
<table>
<tr><th>Assessment</th><th>Year</th><th>Open SBS</th><th>Cryptic SBS</th><th>All SBS</th></tr>
<tr><td>Mean % pupal mortality</td><td>2013
2014</td><td>8.4
13.4</td><td>13.4
29.7</td><td>10.9
21.6</td></tr>
<tr><td>Mean % SBS contaminated</td><td>2013
2014</td><td>46
58.2</td><td>59
84.6</td><td>52.6
71.4</td></tr>
<tr><td>PPF contamination (µg/L)</td><td>2013
2014</td><td>0.87
0.0046</td><td>1.64
0.0103</td><td>1.26
0.0075</td></tr>
</table></td></tr>
<tr><td>Conclusion</td><td>ADS effectively exploit the oviposition behaviour of females to deliver PPF to target oviposition sites. Preference for cryptic sites observed in caged studies was confirmed by field autodissemination studies demonstrating higher PPF residue, % SBS contamination and pupal mortality from cryptic SBS. However, overall pupal mortality was low at 11% and 22% in the consecutive field studies.</td></tr>
<tr><td colspan="2">Kartzinel, M. A., B. W. Alto, M. W. Deblasio, 2nd & N. D. Burkett-Cadena (2016) Testing of Visual and Chemical Attractants in Correlation with the Development and Field Evaluation of an Autodissemination Station for the Suppression of Aedes aegypti and Aedes albopictus in Florida. J Am Mosq Control Assoc, 32, 194-202.</td></tr>
<tr><td>-Study type
-Location
-Target Species</td><td>• Open Field Study
• Florida, USA
• Ae aegypti, Ae albopictus</td></tr>
<tr><td>Objectives</td><td>Develop and field evaluation of a novel ADS targeting multiple physiological states including host-seeking, resting site-seeking, and oviposition site-seeking adult mosquitoes.</td></tr>
<tr><td>Autodissemination Methods</td><td>ADS consisting of a rain shelter covering a PPF treated resting site (red velvet cloth lined tube + host seeking odour lure) and adapted ovitrap (black cup with 100 ml water) lined with PPF treated red velvet cloth.</td></tr>
<tr><td>-Active
-Formulation
-Application rate</td><td>• PPF
• Esteem 35 WP IGR (Valent Biosciences) is a wettable powder formula containing 35% PPF AI. 1g and 0.25g in host-seeking resting sites and oviposition sites respectively. Misted cooking oil applied to velvet to aid retention of Esteem powder.
• ADS deployments density/area not provided – 5 per property lot</td></tr>
<tr><td>Methodology summary</td><td>
ADS were deployed in conjunction with SBS; consisting of three 450 ml black plastic cups with 100 ml water in each. Each ovicup was seeded with ten 2^{nd}-3^{rd} instar Ae albopictus larvae, larvae food and a strip of germination paper oviposition substrate. One ovicup in each SBS group was covered in mesh to prevent mosquito entry and served as the control reference for subsequent evaluation of larval development. All SBS were deployed for 4-5 days and contents (water and lava) transferred to the laboratory maintained for 14 days to assess larval development. All SBS were located <5 m from ADS.

Study 1 – Field site: abandoned vacant lot in urban areas in Indian River County. Five ADS and 13 SBS were deployed for 5 days.
Study 2 – Field sites comprising x3 residential lots and a wooded lot adjacent to a restaurant, located in Main County (Study 2). Study sites were vegetated and had an abundance of breeding sites and healthy Ae aegypti and Ae albopictus populations. Half the sites received treatment and the remaining half serving as an</td></tr>
</table>

<table>
<tr><td></td><td>untreated reference. Two replicates were conducted with treated and control sites reversed in the second replicate. In each replicate ADS were deployed for 2 weeks during which 3 sets of SBS were deployed for 4-5 days, enabling durability of ADS over time to be evaluated.</td></tr>
<tr><td>Results</td><td>Study 1
Significant mortality of 45% was observed in SBS.

Study 2
Overall no significance increases in mortality between treated and control SBS.</td></tr>
<tr><td>Conclusion</td><td>There was some evidence of transfer of larvicidal active concentrations, but overall results were variable and in study 2 were not significant.</td></tr>
<tr><td colspan="2">Abad-Franch, F., E. Zamora-Perea, G. Ferraz, S. D. Padilla-Torres & S. L. B. Luz (2015) Mosquito-Disseminated Pyriproxyfen Yields High Breeding-Site Coverage and Boosts Juvenile Mosquito Mortality at the Neighborhood Scale. PLOS Neglected Tropical Diseases, 9, e0003702.</td></tr>
<tr><td>-Study type
-Location
-Target Species</td><td>Open Field
Amazonas, Brazil
Ae aegypti, Ae albopictus</td></tr>
<tr><td>Objectives</td><td>Evaluate the efficacy of simple ADS at transferring PPF to breeding sites in treated and wider surrounding area.</td></tr>
<tr><td>Autodissemination Methods</td><td>Adapted oviposition site:
Black plastic cups with 400 ml of tap water and the inner wall lined with black, velvet-like cloth dusted with 5 g/m2 of PPF</td></tr>
<tr><td>-Active
-Formulation
-Application rate</td><td>PPF
SumiLarv 0.5 G granules (Sumitomo) 0.5% ai, ground to powder. ADS cloth dusted with 5 g/m^2
ADS density in 7 ha treated area = 14.3/Ha, and density in 50 ha area monitored = 2/ha</td></tr>
<tr><td>Methodology</td><td>Field site comprised of a 50 ha site in dense urban, dengue endemic environment in Manaus, Brazil. 55 dwellings distributed throughout the site were monitored with x3 SBS (plastic cup with 200 ml of hay infusion) each, every month for 20 months. A 7ha subsite was treated with 100 ADS (14.3/Ha) for 4 months coinciding with the rainy season, halfway through a 20-month monitoring period allowing mosquito population before (10 months), during (4 months) and after (6 months) treatment to be evaluated.
SBS was set monthly for 6 days before recovery to a laboratory where the subsequent development of any larval present was assessed to calculate: % contamination of SBS, juvenile mortality and adult emergence.</td></tr>
<tr><td>Results</td><td>SBS contamination - >85% of SBS had evidence of PPF for the period when ADS were deployed. SBS distance from nearest ADS ranged from 3-397m and results demonstrated contamination SBS over the whole site.
SBS larval development – there was a highly significant increase in juvenile mortality following deployment of ADS. Juvenile mortality decreased with distance from ADS, most evident for the period following ADS removal. There was evidence of a greater impact on Ae aegypti than Ae albopictus.
Mean Juvenile mortality and adult emergence/month in all SBS for all species
<table>
<tr><td></td><td>Before treatment</td><td>During Treatment</td></tr>
<tr><td>Juvenile mortality</td><td>4.2% (SE = 0.5)</td><td>75.1% (SE = 1.8)</td></tr>
<tr><td>Adult emergence</td><td>1177 (n=10)</td><td>107 (n=4)</td></tr>
</table></td></tr>
</table>

Conclusion	Compelling evidence of autodissemination as a vector control method at an operational scale. Impact on directly treated 7 ha central area expanding to a wider area of 50 ha resulted in an order of magnitude increase in juvenile mortality and a corresponding reduction in adult emergence observed in SBS for the whole area. Very high mosquito density in this site would likely have facilitated this autodissemination efficacy.
Mains, J. W., C. L. Brelsfoard & S. L. Dobson (2015) Male Mosquitoes as Vehicles for Insecticide. *PLOS Neglected Tropical Diseases,* 9**,** e0003406.	
-Study type -Location -Target Species	• Open Field • Kentucky, USA • *Ae aegypti, Ae albopictus*
Objectives	Evaluate contamination and release of mass-reared males as a mechanism for autodissemination of insecticide by evaluating: • Effect of PPF on males • The ability of treated males to directly contaminate larval breeding sites, without females, • The ability of treated males to transfer PPF to females at dosage adequate to lethally contaminate breeding sites
Autodissemination Methods	Treating and releasing mass-reared males
-Active -Formulation -Application rate	• PPF • 30:70% mix of Esteem and DayGlo powder. Esteem 35 WP IGR (Valent Biosciences) is a wettable powder formula containing 35% PPF ai, and DayGlo is a marker pigment. PPF AI conc. for mix = 10.5%. Applied to 1-2 day adults post-emergence enclosed in 1L cardboard container using powderpuff insufflator. • 1 release point / ha
Methodology	Laboratory studies PPF treatment effect on males was evaluated via longevity studies under laboratory conditions. Caged studies Transfer of PPF to females was assessed in small cages (3 m x 3.6m): 50 treated males + 50 females; 10 SBS (plastic cup with 250 ml water and germination paper insert oviposition substrate). Half the SBS were covered with netting to prevent mosquito access and served as controls. After 5 days, adult males and females were examined for PPF contamination visually and via bioassay. Bioassay consisted of monitoring survival of 2nd instar larvae through to adults in water recovered from SBS. Fields studies Evaluations were conducted in urban residential areas in Lexington, KT, USA. Field Study 1. Mosquito population was monitored weekly for 20 weeks using BG-Sentinel traps in a treated site and two untreated locations. Treatment was applied for 4 consecutive weeks (weeks 9-12) consisting of 4,500 PPF-treated males released/week from a fixed point. Nine SBS were distributed up to ~150 m from male release point. Weekly bioassays were conducted on SBS water samples to assess inhibition of adult emergence from larvae. Field Study 2. The study was conducted in spring before indigenous population emergence to evaluate the potential of males to contaminate SBS without females.

	Six SBS were deployed up to 30 m from release point and in control area > 4 km from the treatment site. Bioassays were conducted weekly for over four weeks. Male were release during weeks 3 and 4 at a rate of 6,300 and 5,100 males/week respectively.
Results	Longevity studies – PPF treatment did not adversely affect treated males survival vs untreated controls Caged studies - demonstrated males transfer PPF to females, at a dose sufficient to induce the lethal effect in the bioassay. Furthermore, bioassays from SBS demonstrated high (>80%) inhibition of adult emergence. Field Study 1: significant PPF contamination of SBS was observed with juvenile mortality in bioassays ranging from 40% to 70% for SBS closest (<25m) and furthest (150 m) from the release point. A significant decline in adult population (BG trapping) was observed after the 4 week treatment period, contrasting with a relatively stable population in control sites. Field Study 2: Following treatment, bioassays demonstrated a significantly reduced survival (~50%) compared with control, demonstrating released males are capable of directly contamination breeding sites.
Conclusion	Laboratory cage studies and subsequent field evaluations clearly demonstrate that release of PPF contaminated males is a viable mechanism for autodissemination of PPF resulting in larvicidal active dose in breeding sites.
Mbare, O. 2015. Chapter 7 - Development of an auto-dissemination station for gravid *Anopheles gambiae sensu stricto* for use in attract and kill strategies. In *PhD Thesis - Novel insecticides and application strategies for malaria vector control*, 170-192. London School of Hygiene & Tropical Medicine.	
-Study type -Location -Target Species	• Semi-Field • Kenya • *An gambiae*
Objectives	Evaluate an autodissemination station targeting gravid *An gambiae* under semi-field conditions
Autodissemination Methods	Adapted oviposition site: Enamel tub artificial ponds (0.42m diameter and 8 cm deep) with 7 L of water and 20 ppm cedrol attractant; covered with gauze netting treated with PPF.
-Active -Formulation -Application rate	• PPF • Powdered Sumilarv 10% AI formulation applied to gauze netting covering breeding site (3.5g = 1.3g/m^2) • 139 ADS/Ha
Methodology summary	Evaluations were conducted in netted semi-field system (10.8m x 6.7 m x 2.4 m high) including a wooden shed, three SBS and one ADS station. SBS consisted of artificial ponds (0.42m diameter and 8 cm deep) with 7 L of water. 200 gravid *An gambiae* were introduced, and the following day 50 insectary late instar larvae were added to each SBS. Pupae development were monitored and removed daily where they were subsequently assessed in the laboratory for % adult emergence. A total of 12 replicates were conducted, each lasting 7 days. Two controls systems were conducted: Control 1) PPF treated with the absence of mosquitoes to evaluate if others factors could transfer PPF; Control 2) With mosquitoes but no PPF treatment.

Results	Eggs were detected in all SBS demonstrating visits by gravid females. No evidence of wind or other mechanism transferring PPF in absence of mosquitoes (control 1). A significant increase in % juvenile mortality was observed in treated vs controls. The distance of SBS from ADS also had an impact, with furthest showing no evidence of lethal concentration of PPF contamination:

Treatment	The distance of SBS from ADS	% juvenile mortality	Sig
Control 1	All	11%	
Control 2	All	16%	
Treated	4.4 m	75%	P < 0.001
Treated	8.4 m	42%	P < 0.001
Treated	10.3 m	8%	NS

Conclusion	Demonstration of autodissemination in the semi-field setting for a malaria vector. However, the likely success in the field is questionable given that only the nearest SBS received sufficient PPF to induce > 50% immature mortality. It should be noted the size of SBS at 7L was substantially larger than most other studies, and would, therefore, require the correspondingly higher transfer of FFP.
Lwetoijera, D. W., C. Harris, S. S. Kiware, G. F. Killeen, S. Dongus, G. J. Devine & S. Majambere (2014b) Comprehensive Sterilization of Malaria Vectors Using Pyriproxyfen: A Step Closer to Malaria Elimination. *The American Journal of Tropical Medicine and Hygiene,* 90**,** 852-855.	
-Study type -Location -Target Species	• Semi-field • Tanzania • *Anopheles arabiensis*
Objectives	Evaluate the reduction of adult production from SBS within the semi-field setting with application of PPF treated resting sites.
Autodissemination Methods	Artificial resting site: Black cotton fabric resting sites, lining walls and ceilings, treated with PPF
-Active -Formulation -Application rate	• PPF • Black cotton cloth dusted PPF to achieve 0.6 – 0.8 g ai/m^2. • 109 ADS / Ha
Methodology	Semi-field studies located in rural Tanzania were conducted in netted chambers (9.6L x 9.6W x 3.9H m) open to elements. Local vegetation grew from a 40cm layer of local soil added to the concrete base. Chambers contained a small hut (1.5 m x 1.5 m x 2m high) which provided shelter for a tethered cow. The inner walls and ceiling were lined with black cloth. Four identical separate chambers were used to evaluate uptake and subsequent transfer of PPF by female *An arabiensis* to SBS. SBS consisted of 2.5 L plastic basins filled with 2L water and 250g soil. A single hut and 4 SBS were added to each chamber. Unfed mated female *An. arabiensis* (5000) were released into the chamber. Studies ran for 11-16 days with cow available for blood feed for first 3 days. 5 replicates for treated and control chambers were conducted. Assessment: pupae were removed from the SBS daily, maintained in laboratory and emergence rates calculated. Additionally, bioassays were conducted in water sampled from SBS by assessing the development of introduced larvae.
Results	PPF treatment of cloth resting surfaces resulted in a 95% reduction in pupae collected and 97% inhibition of adult emergence from SBS. In 4/5 replicate 100% sterility of females was observed as not a single pupa was recovered from SBS. Bioassays showed no difference between treatment and control in juvenile development in water samples recovered from SBS.

Conclusion	In the context of these semi-field studies – PPF treated resting sites had a substantial (>95%) sterilizing effect of *An arabiensis* females. The level and impact of PPF transfer to SBS were hard to assess given the overwhelming level of sterilization, although lab bioassays would suggest insufficient PPF was transferred to cause juvenile mortality.
Lwetoijera, D., C. Harris, S. Kiware, S. Dongus, G. J. Devine, P. J. McCall & S. Majambere (2014a) Effective autodissemination of pyriproxyfen to breeding sites by the exophilic malaria vector Anopheles arabiensis in semi-field settings in Tanzania. *Malar J,* 13**,** 161.	
-Study type -Location -Target Species	• Semi-Field • Tanzania • *Anopheles arabiensis*
Objectives	Assess the potential for *Anopheles arabiensis* to pick up and transfer lethal doses of PPF from treated artificial resting sites to their breeding habitats (i.e. autodissemination of PPF).
Autodissemination Methods	Artificial resting sites: 10 L clay pot lined with black cotton cloth dusted with PPF
-Active -Formulation -Application rate	• PPF • Cloth dusted with 10% AI Sumilarv to achieve 0.2 – 0.3 g AI per pot. • 11-88 ADS/ha
Methodology	Semi-field studies located in rural Tanzania were conducted in netted chambers (9.6L x 9.6W x 3.9H m) open to elements. Local vegetation grew from a 40cm layer of local soil added to the concrete base. A small hut which provided shelter for a tethered cow and possible resting site for mosquitoes. Four identical separate chambers were used to evaluate PPF-treated clay pots for delivering PPF to resting adult female mosquitoes for subsequent autodissemination to SBS within the chambers. SBS consisted of 2.5 L plastic basins filled with 2L water and 250g soil. Unfed mated female *An. arabiensis* (1500-5000) were released in the chamber and study ran for 25 days, with cow available for blood feed for first 3 days. Study 1 – Eight clay pot resting site ADS, and two SBS were added to each chamber. 6 replicates were conducted. Study 2 – Comparable setup to study 1 was conducted without released adult mosquitoes to confirm there was no other mechanism for the AD. Development of larvae introduced to SBS was monitored to assess transfer of PPF. 2 replicates were conducted. Study 3 – Comparable setup to study 1, but only 1, and 2 ADS vs 6 SBS was conducted to evaluate AD from fewer ADS and more breeding sites. 1 replicate conducted for each ADS density. Assessment: pupae were removed from the SBS daily, and emergence rates calculated. Additionally, bioassays were conducted in water sampled from SBS by assessing the development of 2-3rd instar larvae (Bioassay 1). A final bioassay was conducted at end of each replicate, by adding 250 2-3rd instar larvae to SBS and monitoring development (Bioassay 2). Impact of PPF on emergence was determined by comparing treatment with an appropriate control group.
Results	Summary of results from Study 1 and 3 showing number of pupae collected from SBS and % inhibition of adult emergence (IAE):

	<table><tr><td>Assessment</td><td>Study</td><td># ADS/SBS</td><td>Control</td><td>Treated</td><td>Sig Diff</td></tr><tr><td rowspan="3">Pupae collected from SBS</td><td>1</td><td>8 / 2</td><td>590</td><td>711</td><td>NS</td></tr><tr><td>3</td><td>2 / 6</td><td>53</td><td>63</td><td>NS</td></tr><tr><td>3</td><td>1 / 6</td><td>100</td><td>105</td><td>NS</td></tr><tr><td rowspan="3">IAE from SBS</td><td>1</td><td>8 / 2</td><td>5%</td><td>79%</td><td>P<0.001</td></tr><tr><td>3</td><td>2 / 6</td><td>18%</td><td>33%</td><td>P<0.001</td></tr><tr><td>3</td><td>1 / 6</td><td>2%</td><td>34%</td><td>P<0.001</td></tr><tr><td>IAE in lab bioassay 1</td><td>1</td><td>8 / 2</td><td>1%</td><td>38%</td><td>P<0.001</td></tr><tr><td>IAE in SBS bioassay 2</td><td>1</td><td>8 / 2</td><td>3%</td><td>16%</td><td>P<0.001</td></tr></table> Study 2 There was no evidence of PPF transfer to SBS, confirming mosquitoes were a vehicle for dissemination in Study 1
Conclusion	A clear demonstration of the AD in the semi-field setting for a malaria vector utilizing a resting site ADS approach. Inhibition of adult emergence (% morality) approached 80% in SBS where 8 ADS were used but dropped to 34% with only one ADS vs 6 SBS. This demonstrated the need for a high density of ADS even in a confined microcosm with an artificially high density of mosquitoes. This would suggest in open field settings, with a lower density of ADS and mosquito populations, it may not be possible to achieve sufficient AD of PPF to significantly inhibit adult emergence from natural breeding sites.

Suman, D. S., A. Farajollahi, S. Healy, G. M. Williams, Y. Wang, G. Schoeler & R. Gaugler (2014) Point-source and area-wide field studies of pyriproxyfen autodissemination against urban container-inhabiting mosquitoes. *Acta Trop,* 135**,** 96-103.

-Study type -Location -Target Species	• Open Field • NJ, USA • *Ae albopictus*
Objectives	Evaluate direct application of PPF spray to test hypotheses that gravid *Ae albopictus* contaminated directly, or indirectly from contact with contaminated surfaces, would transfer PPF to new larvicidal habitats outside the directly treated areas.
Autodissemination Methods	Dissemination of PPF following direct and indirect contamination with PPF by target mosquitos resulting from direct spray application.
-Active -Formulation -Application rate	• PPF • NyGuard emulsifiable concentrate with 10% AI. • 789 ml/Ha
Methodology summary	• Point source application Study conducted in residential areas of NJ, USA. A used tyre pile (n=100, 20m^2) infested with *Ae albopictus* was treated with spray formulation of PPF. SBS (vase with 250 ml oakleaf infusion) were placed in 4 orthogonal transects up to 200m and 400m in 2010 and 2011 studies respectively. Control SBS were placed at 1.9 Km. Water samples from tyre pile and SBS were sampled for 6 weeks after treatment tested for PPF contamination with bioassays evaluating inhibition of larval development. • Areawide application Field site consisted of a 105 Ha residential area in NJ, USA which received treatment and a nearby 181 area served as a control.

	2010 approximately 50% of parcels were treated in a scattered 'checkerboard' pattern using backpack application to treat only foliar areas, encompassing 3.7% of the total 105ha area. 2011 Truck mounted sprayers applied spray to strips covering 33% to total areas SBS were used to assess PPF penetration in areas spayed and unsprayed areas. BG traps monitored adult population.
Results	• Point source application In 2010 there was some evidence of autodisseminaiton with pupal mortality up to 20% in SBS. Pupal mortality in 2011 was negligible in SBS • Areawide application These was some evidence of higher pupal mortality (3-14%) in SBS outside treated area compared with directly treated areas, suggesting some AD was occurring. Evaluation of population with BG Sentinel traps showed no suppression.
Conclusion	There was some evidence of autodisseminaiton from direct treated areas to untreated assessed by increased larval morality in SBS. However, this effect was moderate at best, and areawide evaluation of population assessed by BG traps showed no significant impact on population compared to untreated comparator site.
Ohba, S.Y., K. Ohashi, E. Pujiyati, Y. Higa, H. Kawada, N. Mito & M. Takagi (2013) The Effect of Pyriproxyfen as a "Population Growth Regulator" against Aedes albopictus under Semi-Field Conditions. PLOS ONE, 8, e67045.	
-Study type -Location -Target Species	• Semi-Field • Nagasaki, Japan • *Ae albopictus*
Objectives	Determine the effects of PPF treated bed nets on *Ae albopictus* populations under semi-field conditions
Autodissemination Methods	Resting sites, blood-seeking: PPF treated bed nets
-Active -Formulation -Application rate	• PPF • Bed nets soaked in technical grade PPF (Sumilarv) diluted in isopropynol (0.1 and 1% w/v) and dried overnight, resulting in 35 and 350 mg/m^2 PPF AI retention. • 142 ADS/ Ha (1 net/cage)
Methodology	Semi-Field study: 6 netted microsomes (2.7L, 2.6W, 2.27H m) were set up in the large greenhouse with half PPF treated and reminder serving as controls. Each microcosm contained a mini bed net (50Lx50Wx50H cm), natural and artificial resting sites (wood rack and plants), sugar water and breeding sites consisting of 10 SBS with 1.2 L water + attractant (0.2 g hay), larval food (3g of 1:1 mix of yeast and mouse pellet) and filter paper oviposition substrate. Blood feeding was provided weekly from mice restrained within bed net which contained artificial holes providing access for mosquitoes. 100 pairs of *Ae albopictus* were introduced at the start of each microcosm study and populations were subsequently monitored for 20 and 44 days for studies evaluating 35 and 350 mg/m2 treated netting respectively. Assessments were conducted weekly: Number of females feeding on mice/time; the number of eggs and pupae in SBS. A subset (20%) of egg were removed and hatch rate evaluated. Bioassays on pupal mortality were conducted in water samples removed from SBS to assess autodissemination of PPF.

<table>
<tr><td></td><td></td></tr>
<tr><td>Results</td><td>Effect of treatment compared with control:
<table>
<tr><td>Assessment</td><td>Treatment 1
35 mg/m^2</td><td>Treatment 2
350 mg/m^2</td></tr>
<tr><td># Females blood feeding</td><td>No effect</td><td>Sig. lower in treated after 4 weeks</td></tr>
<tr><td># eggs laid</td><td>Sig. reduced in PPF treated</td><td>Sig. reduced in PPF treated</td></tr>
<tr><td># pupae</td><td>Sig. reduced in PPF treated</td><td>Sig. reduced in PPF treated</td></tr>
<tr><td>Egg hatch</td><td>Sig. reduced egg hatch (0%T/~50%C) in PPF treated</td><td>Sig. reduced egg hatch (~10%T/~80%C) in PPF treated</td></tr>
<tr><td>Pupal mortality</td><td>Sig. increase in PPF treated</td><td>Sig. increase in PPF treated – only in the first week of evaluation</td></tr>
</table></td></tr>
<tr><td>Conclusion</td><td>Demonstration of PPF impact on multiple life stages of Ae albopictus following contract with treated bed nets leading to reduced population. Evidence of autodissemination via bioassays demonstrated significant pupal mortality, although this effect was only marginal, suggesting most of the effect of PFF on albopictus population observed was as a result of direct contact with PPF treaded bed nets.</td></tr>
<tr><td colspan="2">Ponlawat, A., T. Fansiri, S. Kurusarttra, A. Pongsiri, P. W. McCardle, B. P. Evans & J. H. Richardson (2013) Development and evaluation of a pyriproxyfen-treated device to control the dengue vector, Aedes aegypti (L.) (Diptera:Culicidae). Southeast Asian J Trop Med Public Health, 44, 167-78.</td></tr>
<tr><td>-Study type
-Location
-Target Species</td><td>• Semi-Field
• Rayong Province, Thailand
• Ae aegypti</td></tr>
<tr><td>Objectives</td><td>Field evaluation of a combined resting station/oviposition site ADS at PPF autodissemination and corresponding suppression of Ae aegypti population in a Thailand village</td></tr>
<tr><td>Autodissemination Methods</td><td>Resting station/oviposition site
Open-topped rectangular tube (35x35x55 cm, WxDxH) covered with black cloth (inner surface treated with PPF powder) and a black plastic bucket contained 3 L of PPF treated water.</td></tr>
<tr><td>-Active
-Formulation
-Application rate</td><td>• PPF
• Powder; finely ground Sumilarv 0.5G (0.5% w/w AI) granular formulation (applied at 0.05 g ai/m^2 to cloth)
• 7 ADS / Ha</td></tr>
<tr><td>Methodology</td><td>Semi-Field study
Recently blood fed adults were exposed to ADS resting station (PPF treated water not included) with 60x60x120 cm cage for 24 hr prior to release at one end of an outdoor cage (50 m long x 1.0 m wide x 1.5 m high). 2 L SBS were placed at opposite ends of the outdoor cage and after 6 days, egg laid in SBS were assessed and larval rearing bioassay conducted in water samples. Comparator control cages set up with exception of PPF treatment.</td></tr>
</table>

<table>
<tr>
<td></td>
<td>Field Study
Field sites consisted of two villages in Thailand that were 1.3 Km apart and served as treated and untreated control. They covered ~16Km2 each with populations of 160 and 171 residing in 65 and 71 houses respectively.
Treatment was applied to a central 150m radius circle comprising 12 homes each receiving 4 ADS (=48 ADS/0.07 km^2). ADS efficacy was evaluated in an inner treated circle, and outer circle radiating 150 m beyond inner circle. SBS consisting of buckets containing 3L water were deployed to monitor the population, by a number of larvae collected, and PPF contamination via bioassays on water samples collections. Adult population was monitored with aspiration survey and BG-Sentinel traps. The study was conducted for 30 weeks with monitoring 6 before and 24 after ADS installation.
<table>
<tr><td>Assessment (interval)</td><td>n</td></tr>
<tr><td>BG - traps (8hr/day for x3 days every 2 weeks)</td><td>inner 4 / outer 8</td></tr>
<tr><td>Aspiration (CDC Backpack, 15 min, every 2 weeks)</td><td>inner 8-10 / outer 10-15</td></tr>
<tr><td>Sentinel container (3L, count and remove pupae every 2-3 days)</td><td rowspan="2">inner 10 / outer 10</td></tr>
<tr><td>Bioassay (20 larvae reared in water from SBS, every 2 weeks)</td></tr>
</table>
</td>
</tr>
<tr>
<td>Results</td>
<td>Semi-Field study
PPF treatment resulted in significant reductant in eggs laid, and increased mortality in bioassay form SBS
<table>
<tr><td>Assessment</td><td>Control</td><td>Treated</td><td>Sig diff</td></tr>
<tr><td># Eggs</td><td>2,604</td><td>486</td><td>P < 0.05</td></tr>
<tr><td>Bioassay - % mortality</td><td>9.5</td><td>16.5</td><td>P < 0.01</td></tr>
</table>
Field Study

BG-Sentinel - significantly reduced numbers of adults trapped in the treated area (inner circle) vs control. The outer circle was lower than control but not significantly different.
Aspiration - lower numbers were recovered from the treated village, but the difference was non-statistical.
SBS – no significant difference in pupae yield from SBS was observed
Bioassay – there was no evidence of larvicidal activity (reduced adult emergence) in water sampled from SBS.

</td>
</tr>
<tr>
<td>Conclusion</td>
<td>This is one of the only field studies that directly assess the impact on adult population rather than rely on evidence of AD from SBS. Results demonstrated a significant impact based on BG traps data, which was reflected in aspiration data although not significant.
Interestingly, data from SBS in the field showed no evidence of transfer of sufficient PPF to have larvicidal activity. Semi-field study demonstrated 80% reduced egg yield confirming the sterilising effect of contamination of females, but only a marginal increase in larval mortality compared to control. The reduced adult population may, therefore, have been mediated by sterilizing effect on females contaminated with PPF. SBS used in this study were substantially larger (3L) that</td>
</tr>
</table>

<table>
<tr><td></td><td>most other studies (<1L) and this may have diluted any PPF transferred resulting in loss of larvicidal activity in the field and may explain the marginal activity in observed in semi-field settings. It is possible smaller breeding sites smaller breeding sites in the field could have been contaminated with sufficient PPF to have larvicidal activity, contributing to impact on adult population observed.</td></tr>
<tr><td colspan="2">Caputo, B., A. Ienco, D. Cianci, M. Pombi, V. Petrarca, A. Baseggio, G. J. Devine & A. della Torre (2012) The “Auto-Dissemination” Approach: A Novel Concept to Fight Aedes albopictus in Urban Areas. PLOS Neglected Tropical Diseases, 6, e1793.</td></tr>
<tr><td>-Study type
-Location
-Target Species</td><td>Open Field
Rome, Italy
Ae albopictus</td></tr>
<tr><td>Objectives</td><td>Evaluate adapted ovitrap autodissemination stations in an open field setting at transferring PPF and conferring larvicidal activity.</td></tr>
<tr><td>Autodissemination Methods</td><td>Adapted sticky oviposition traps: Plastic pot containing 700ml water and net to prevent oviposition served as an attractant. Internal panels lined with cloth (replacing sticky panels) were treated with PPF.</td></tr>
<tr><td>-Active
-Formulation
-Application rate</td><td>PPF
Powder; finely ground (0.5 and 5% AI) Proxilar tablet formulation (applied at ~ 1g powder added to each ADS)
Site 1 – 2270 ADS/Ha; Site 2 – 10 ADS/Ha</td></tr>
<tr><td>Methodology summary</td><td>Field evaluations were conducted in two areas in Rome Italy, with typically high Ae albopictus infestation:
Site 1 –Shallow subterranean crypts (2x22m) in the central graveyard
Site 2 – Enclosed garden (1ha) within an urban setting
Each site was treated with x10 ADS, x10 SBS. SBS consisted of plastic vase outer enclosing a glass beaker containing 200ml water and seeded with x25 3rd instar Ae albopictus larvae and 0.07g cat food. An equal number of control-SBS were also deployed, which had the addition of mesh cover to stop ingress of mosquitoes. Larvae in all SBS were monitored for development and adult emergence.
Two replicates were conducted in each site: 0.5% and 5% PPF formulations were used for site 1; for site 2 only the 5% formulation was used.
ADS treatment was evaluated for 12 days with two replicates were conducted in each site: 0.5% and 5% PPF formulations were used for site 1; for site 2 only the 5% formulation was used.</td></tr>
<tr><td>Results</td><td>A significant increase in mortality was observed for juvenile Ae albopictus in SBS vs control-SBS:
Mortality in SBS
<table>
<tr><td>Site</td><td>PPF conc.</td><td>Control - SBS</td><td>SBS</td></tr>
<tr><td rowspan="2">Site 1</td><td>0.5%</td><td>2.4</td><td>28.8%</td></tr>
<tr><td>5.0%</td><td>1.2</td><td>71.2%</td></tr>
<tr><td rowspan="2">Site 2</td><td>5.0%</td><td>1.2</td><td>50.0%</td></tr>
<tr><td>5.0%</td><td>1.6</td><td>52.4%</td></tr>
</table></td></tr>
<tr><td>Conclusion</td><td>Field demonstration of the potential of autodissemination, with mortality of mosquito juveniles in SBS > 50% where the higher 5% AI PPF formulation was used. ADS density in Site 1 was > 2000/Ha and although density was lower at ~10/Km2 in site 2 autodissemination efficacy over greater distance can’t be inferred as SBS</td></tr>
</table>

	were placed 2m from ADS. These densities would be prohibitive for operational use. The increased in mortality observed in Site 1 corresponding with higher PPF concentration highlights the potential for an improved formulation of PPF to improve efficacy.
Devine, G. J., E. Z. Perea, G. F. Killeen, J. D. Stancil, S. J. Clark & A. C. Morrison (2009) Using adult mosquitoes to transfer insecticides to Aedes aegypti larval habitats. Proceedings of the National Academy of Sciences, 106, 11530-11534.	
-Study type -Location -Target Species	• Open Field • Iquitos, Peru • *Ae aegypti, Culex spp*
Objectives	Evaluate adapted ovitrap autodissemination stations in an open field setting at transferring PPF and conferring larvicidal activity.
Autodissemination Methods	Adapted oviposition/resting station: 1 L plastic pot contain 200ml water and lined with a black cloth treated with PPF.
-Active -Formulation -Application rate	• PPF • Powder; finely ground Sumilarv 0.5G (5% AI) granular formulation (applied at ~ 5g AI/m2 to cloth) • 480/Ha (Site A); 640/Ha (Site B)
Application rate	
Methodology summary	Field evaluations were conducted in graveyards in Amazon region of Peru. Two relatively small sites (Site; A = 52x4m, B = 52 x 3 m) were each treated with x 10 ADS. Autodissemination was evaluated using x40 SBS within each test site. SBS consisted of containers holding 200ml water seeded with x25 3rd instar *Ae aegypti* larvae. Larval development and mortality were evaluated in each site pre-treatment (= experimental control) and post-treatment. ADS treatment was evaluated for 12 days with x3 replicates conducted in each site.
Results	• SBS contamination > 95% • A significant increase in mortality was observed for juvenile *Ae aegypti* in SBS: Mean % *Ae aegypti* juvenile mortality Site / Pre-treatment / Post-treatment A / 8% / 84% B / 7% / 49%
Conclusion	The first reported open field demonstration of the potential of autodissemination, with 3- 5% of breeding sites treated with PPF resulting in contamination of > 95% of available breeding sites and mortality in SBS ranging from 49-84%. However, test sites were small and corresponding density of ADS deployed was very high at approximately 50,000 per Km2, which would be unfeasible for operational deployment.
Itoh, T. (1993) Control of DF/DHF Vector, Aedes Mosquito, with Insecticides. Tropical medicine 35, 259-267.	
-Study type -Location -Target Species	• Semi-Field • Bangkok, Thailand • *Ae aegypti*
Objectives	Determination of potential for *Ae aegypti* adults to transfer PPF from treated artificial resting sites to larval breeding sites in a house in Thailand.
Autodissemination Methods	PPF treated artificial resting sites: Black bamboo baskets lined with black nylon netting
-Active	• PPF

-Formulation -Application rate	• Netting treated with technical grade PPF at 1.5g/m^2 ai. • 333 ADS/Ha
Methodology	A field study conducted in a house (6x10 m) in Bangkok, Thailand. Four resting site ADS we place in the house together with 10 SBS with coarse brown paper for egg collection. The study was maintained for 16 days. New SBS were placed every 4 days with existing SBS removed and transferred to the laboratory where egg numbers were counted. Bioassays of PPF contamination was assessed by adding 4th instar *Ae aegypti* larvae and monitoring their development to adults.
Results	Substantial inhibition of adult emergence was observed in many of the SBS confirming autodissemination of PPF had occurred. Not all SBS showing inhibition of adult emergence had eggs. This, together with observed dead males in SBS suggest both males and females may be involved in autodissemination.
Conclusion	Small study but offers the first demonstration of an AD from a treated resting site to larval breeding site.

16 Appendix 2 – Patent search

A patent search using the term "autodissemination" was conducted using PATENTSCOPE (http://www.wipo.int/portal/en/index.html). Accessed on 24/5/2018. 37 matches were reported, and following review, only 13 were relevant to AD for mosquito control. Many of these represented multiple submissions of the same invention to different territories and represent 5 unique approaches:

- Dobson - *Method for mosquito control*
  - Describes an approach whereby artificially mass-reared mosquitoes are treated with insecticide and released into the environment. Patent also suggests carrier could be a species other than the target species.
- Gaugler - *Autodissemination of an insect-growth regulator for insect management*
  - Autodissemination station based on an oviposition trap to attract gravid females and includes a unidirectional cone which guides mosquitoes to walk over a duel band of oil, followed by powder formulations of AI.
- Gaugler - *Methods and apparatus for management of mosquito populations with habitat sharing heterospecific insects carrying insect growth regulators*
  - Describes a method and apparatus for control of mosquitoes by mass-rearing and treating (with appropriate IGR) different species that share the same habitat as target mosquitoes, and can thereby provide an AD vehicle for IGRs.
- Grasso - *Insect Biocontrol Method and Device: Attract-Infect-Release (AIR) Technology*
  - Describes a solar panel/battery pack powered AD station that attracts target species (eg mosquitoes) and treats them by spraying AI or infectious agent (spores) before release.
- Bertrand - *Insect control formulation with improved autodissemination characteristics*
  - Describes improvements in formulation of PPF specifically for the purpose of AD application.

Summary table of patent search relevant to AD for mosquito vector control

(patent number in blue is hyperlink to URL)

<table>
<tr><th colspan="3">Title</th><th>Ctr</th><th>PubDate</th></tr>
<tr><th>Int.Class</th><th>Appl.No</th><th>Applicant</th><th colspan="2">Inventor</th></tr>
<tr><td colspan="3">1. 3231285 METHOD FOR MOSQUITO CONTROL</td><td>EP</td><td>18.10.2017</td></tr>
<tr><td>A01N 65/00</td><td>17169030</td><td>DOBSON STEPHEN</td><td colspan="2">DOBSON STEPHEN</td></tr>
<tr><td colspan="5">A formulation and method for insect control is provided in the form of insecticide carrying insects which can be introduced in a population to thereby control the insect population. The formulation may include artificially generated adult insect carriers of a larvicide in which the larvicide has minimal impact on the adult insect and which larvicide affects juvenile survival or interferes with metamorphosis of juvenile insects to adulthood. The insects may be either male or female and may include mosquitoes.</td></tr>
<tr><td colspan="3">3. WO/2017/096381 METHODS AND APPARATUS FOR MANAGEMENT OF MOSQUITO POPULATIONS WITH HABITAT SHARING HETEROSPECIFIC INSECTS CARRYING INSECT GROWTH REGULATORS</td><td>WO</td><td>08.06.2017</td></tr>
<tr><td>A01N 43/00</td><td>PCT/US2016/064976</td><td>RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY</td><td colspan="2">GAUGLER, Randy R.</td></tr>
<tr><td colspan="5">Compositions and heterodissemination methods for controlling unwanted target insect populations are disclosed. In preferred embodiments, the target insects are mosquitos or house flies and the heterospecific insects are non-biting midges and soldier flies respectively.</td></tr>
<tr><td colspan="3">8. 20170064952 Insect Control Formulation with Improved Autodissemination Characteristics</td><td>US</td><td>27.10.2016</td></tr>
<tr><td>A01N 43/40</td><td>14693615</td><td>Jacques C. Bertrand</td><td colspan="2">Jacques C. Bertrand</td></tr>
<tr><td colspan="5">An insecticide composition designed for improved autodissemination. The insecticide may be used as a dry powder or a wet composition. The composition allows for better transfer of active ingredient to the target species, as well as improved stability of the active ingredient.</td></tr>
<tr><td colspan="3">9. WO/2016/171753 INSECT CONTROL FORMULATION WITH IMPROVED AUTODISSEMINATION CHARACTERISTICS</td><td>WO</td><td>27.10.2016</td></tr>
<tr><td>A01M 1/20</td><td>PCT/US2015/050856</td><td>THE UNITED STATES OF AMERICA as represented by THE SECRETARY OF THE NAVY</td><td colspan="2">BERTRAND, Jacques, C.</td></tr>
<tr><td colspan="5">An insecticide composition designed for improved autodissemination. The insecticide may be used as a dry powder or a wet composition. The composition allows for better transfer of active ingredient to the target species, as well as improved stability of the active ingredient.</td></tr>
<tr><td colspan="3">10. 20160242403 AUTODISSEMINATION OF AN INSECT-GROWTH REGULATOR FOR INSECT MANAGEMENT</td><td>US</td><td>25.08.2016</td></tr>
<tr><td>A01M 1/20</td><td>15050439</td><td>Rutgers, The State University of New Jersey</td><td colspan="2">Randy Gaugler</td></tr>
<tr><td colspan="5">The described invention provides a gel formulation of a composition comprising at least one insecticide in an amount effective to control an insect larval population, an apparatus for autodissmenination of an insecticide for insect management containing (1) a reservoir (2) a transfer plate and cover, and (3) a mesh component, a method and a system for autodissemination for effectively controlling an insect larval population. Also disclosed is an improvided biphasic autodissemination station for control of undesirable insect populations.</td></tr>
</table>

Title			Ctr	PubDate
Int.Class	**Appl.No**	**Applicant**	**Inventor**	
11. 229001 **METHOD FOR MOSQUITO CONTROL**			IL	31.07.2016
A01N 25//00	229001	STEPHEN DOBSON		
12. 20150359228 **Insect Biocontrol Method and Device: Attract-Infect-Release (AIR) Technology**			US	17.12.2015
A01N 63/00	14272447	Ying Zhang Grasso	Ying Zhang Grasso	
A pest biocontrol method and device for flying insects configured to be used with solar power is disclosed. The device includes a solar panel, a solar rechargeable battery pack, a solar charge controller, an insect-attracting lamp and/or a semiochemical vial, and a biopesticide-spraying device. This biocontrol device can be used with or without a trap fan to draw in insects, and an exit fan that draws out infected insects to be released. The said device accomplishes AIR (Attract-Infect-Release) method of insect biocontrol.				
14. 2699096 **METHOD FOR MOSQUITO CONTROL**			EP	26.02.2014
A01N 65/00	12774208	DOBSON STEPHEN	DOBSON STEPHEN	
A formulation and method for insect control is provided in the form of insecticide carrying insects which can be introduced in a population to thereby control the insect population. The formulation may include artificially generated adult insect carriers of a larvicide in which the larvicide has minimal impact on the adult insect and which larvicide affects juvenile survival or interferes with metamorphosis of juvenile insects to adulthood. The insects may be either male or female and may include mosquitoes.				
15. 20130303574 **Autodissemination of an insect-growth regulator for insect management**			US	14.11.2013
A01M 1/20 Bottom of Form	13863359	Rutgers, The State University of New Jersey	Randy Gaugler	
The described invention provides a gel formulation of a composition comprising at least one insecticide in an amount effective to control an insect larval population, an apparatus for autodissmenination of an insecticide for insect management containing (1) a cap component, (2) a cup component, and (3) a mesh component, a method and a system for autodissemination for effectively controlling an insect larval population. Also disclosed is an improvided biphasic autodissemination station for control of undesirable insect populations.				
16. 2012245837 **Method for mosquito control**			AU	07.11.2013
A01N 25/00	2012245837	Dobson, Stephen		
A formulation and method for insect control is provided in the form of insecticide carrying insects which can be introduced in a population to thereby control the insect population. The formulation may include artificially generated adult insect carriers of a larvicide in which the larvicide has minimal impact on the adult insect and which larvicide affects juvenile survival or interferes with metamorphosis of juvenile insects to adulthood. The insects may be either male or female and may include mosquitoes.				
17. 20130259846 **Method for Mosquito Control**			US	03.10.2013
A01N 63/00	13636889	Dobson Stephen	Dobson Stephen	
A formulation and method for insect control is provided in the form of insecticide carrying insects which can be introduced in a population to thereby control the insect population. The formulation may include artificially generated adult insect carriers of a larvicide in which the larvicide has minimal impact on the adult insect and which larvicide				

Title			Ctr	PubDate
Int.Class	**Appl.No**	**Applicant**	**Inventor**	
affects juvenile survival or interferes with metamorphosis of juvenile insects to adulthood. The insects may be either male or female and may include mosquitoes.				
21. WO/2012/158192 **AUTODISSEMINATION OF AN INSECT-GROWTH REGULATOR FOR INSECT MANAGEMENT**			WO	22.11.2012
A01N 25/00	PCT/US2011/056106	RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY	GAUGLER, Randy	
The described invention provides a gel formulation of a composition comprising at least one insecticide in an amount effective to control an insect larval population, an apparatus for autodissmenination of an insecticide for insect management containing (1) a cap component, (2) a cup component, and (3) a mesh component, a method and a system for autodissemination for effectively controlling an insect larval population.				
22. WO/2012/145145 **METHOD FOR MOSQUITO CONTROL**			WO	26.10.2012
A01N 65/00	PCT/US2012/031437	DOBSON, Stephen	DOBSON, Stephen	
A formulation and method for insect control is provided in the form of insecticide carrying insects which can be introduced in a population to thereby control the insect population. The formulation may include artificially generated adult insect carriers of a larvicide in which the larvicide has minimal impact on the adult insect and which larvicide affects juvenile survival or interferes with metamorphosis of juvenile insects to adulthood. The insects may be either male or female and may include mosquitoes.				

17 Appendix 3 – Table of Interviewees

The following people were identified as experts in AD and/or operational mosquito control, and were interviewed:

Name	Category	Affiliation
Rabindra **Abeyasinghe**	Other	WHO WPRO, Philippines
Mike **Banfield**	AD Developer	Springstar, USA
Jacques **Bertrand**	Other	Navy Entomology Center of Excellence, USA
Haroldo **Bezzera**	Other	PAHO, USA
Jeremie **Bouyer**	Key AD Researcher	IAEA/CIRAD, Austria, France
Chee Seng **Chong**	Other	NEA, Singapore
Lyell **Clarke**	Other	Clarke, USA
Vincent **Corbel**	Other	IRD and WIN, France
Alessandra **della Torre**	Key AD Researcher	Rome Univ, Italy
Gregory **Devine**	Key AD Researcher	QIMR Berghofer Medical Research Institute, Australia
Stephen **Dobson**	AD Developer	MosquitoMate, USA

Marit **Farenhorst**	Other	In2care, Netherlands
Randy **Gaugler**	AD Developer	Rutgers Univ, USA
Joel **Gustave**	Other	MOH, Guadaloupe
Angela **Harris**	Other	CDC, Puerto Rico
Gerry **Killeen**	Key AD Researcher	LSTM/Ifakara Health Institute, Tanzania
Gregory **Lambert**	Other	EID, France
Teresa **Leslie**	Other	Consultant, USA
Steven **Lindsay**	Key AD Researcher	Durham Uni, UK
John 'Luke' **Lucas**	Other	Ex-Sumitomo, UK
Dickson **Lwetoijera**	Key AD Researcher	Ifakara Health Institute, Tanzania
Agenor **Mafra-Neto**	AD Developer	ISCA Technologies, USA
Bhupender **Nagpal**	Other	WHO SEARO, India
Karen **Polson-Edwards**	Other	PAHO, Barbados
Alongkot (Boi) **Ponlawat**	Key AD Researcher	AFRIMS, Thailand
Risinthe **Premaratne**	Other	WHO SEARO, India
Andrew **Saibu**	Other	IVCC - Afrcan regional coordinator
Angus **Spiers**	Other	I2I, UK
Dan **Strickman**	Other	BMGF, USA

18 APPENDIX 4 – KEY VECTOR SPECIES: PREFERRED HOST, RESTING SITE, AND BITING SITE

Species	Larval site characteristics	Host		Biting		Resting		Other
		Anthropo-philic	Zoophilic	Endophagic	Exophagic	Endophilic	Exophilic	
An. arabiensis	Sunlit (occasionally shaded), fresh (occasionally brackish), clear or turbid, still or flowing water with higher plants or algae (occasionally without vegetation)	•	•	o	•	o	•	Bites at dusk/night and occasionally at dawn. Species shows high behavioural plasticity and readily adapts in response to control
An. funestus	Sunlit or shaded, fresh (occasionally brackish), clear, still or flowing water with higher plants or algae (occasionally without vegetation)	•	o	•	•	•	o	Bites at dusk, but mainly during the night and to a lesser extent at dawn
An. gambiae	Sunlit (occasionally shaded), fresh (occasionally brackish), clear or turbid, still or flowing water with or without higher plants or algae	•	o	•	•	•	•	Larval site characteristics are influenced by molecular and/or chromosomal form
An. melas	Sunlit or shaded, fresh or brackish, clear or turbid, still water with higher plants or algae	•	•	•	•	o	•	Unlike other DVS, *An melas* densities tend to link to tides rather than rainfall
An. merus	Sunlit or shaded, fresh or brackish, clear or turbid, still water with higher plants or algae	•	•	o	•	o	•	Despite also being a coastal vector, *An. merus* is not influenced by tides like *An. melas*, nor can it tolerate the same levels of salinity.
An. moucheti	Sunlit (occasionally shaded), fresh, clear (occasionally turbid), still or flowing water with higher plants or algae	•	o	•	o	•	o	Range entirely restricted to equatorial forests. This vector is highly anthropophilic and endophilic.
An. nili complex	Sunlit or shaded, fresh, clear, still or flowing water with higher plants or algae	•	o	•	•	•	•	Behaviour depends on sibling, with *An. nili* being highly anthropophilic and the most important vector of the complex

Species	Larval site characteristics	Host		Biting		Resting		Other
		Anthropo-philic	Zoophilic	Endophagic	Exophagic	Endophilic	Exophilic	
An. aconitus	Sunlit, fresh, dear (occasionally turbid), still or flowing water with higher plants and algae (occasionally without vegetation)	○	●	●	●	○	●	Particularly favours both coast plain and upland rice fields as larval sites
An. annularis	Sunlit, fresh, dear (occasionally turbid), still or flowing water with higher plants and algae (occasionally without vegetation)	○	●	●	●	●	○	Vector role depends on location. Possible complex of two (species A and B) siblings, but these do not appear to be linked to variable vector capacity
An. balabacensis	Shaded (occasionally sunlit), fresh, still water with or without higher plants or algae	●	-	●	●	●	○	Primarily found in forested environments
An. barbirostris complex	Sunlit or shaded, clear or turbid, still or flowing water with higher plants or algae (occasionally without vegetation)	○	●	○	●	○	●	The siblings within the complex are yet to be fully resolved and their distributions are unclear.
An. culicifacies complex	Sunlit, fresh (occasionally brackish) clear (occasionally turbid), still or flowing water with or without higher plants or algae	●	●	●	●	●	○	Bionomics dependent on sibling: Sp E = Anthropophilic; Sp A, B, C , D = Zoophilic)
An. dirus complex	Shaded, fresh, clear or turbid, still water without vegetation	●	○	●	●	-	●	Bionomics dependent on sibling but the two main vectors are *An. dirus* and *An. baimaii*. *Anopheles scanloni* is also anthropophilic but plays more focal role in transmission in Thailand
An. farauti complex	Sunlit or shaded, fresh or brackish, dear or turbid, stagnant (occasionally flowing) water with higher plants or algae (occasionally without vegetation)	●	○	●	●	○	●	*Anopheles farauti*, *An. hinesorum* and *An. farauti* No. 4 are the only siblings considered to be important malaria vectors
An. flavirostris	Shaded, fresh, dear, flowing (occasionally still) water with higher plants or algae (occasionally without vegetation)	○	●	●	●	○	●	Historically confused/misidentified as *An. minimus*. All records of *An. minimus* from the Philippines, Sabah (Malaysia) and Indonesia are now considered to be *An. flavirostris*
An. fluviatilis complex	Sunlit, fresh, flowing (occasionally still), water with higher plants or algae (occasionally without vegetation)	●	●	●	●	●	●	Bionomics dependent on sibling. Species S is the most anthropophilic and endophilic and is the main vector of the complex. Species T and U are primarily zoophilic, exophagic and exophilic and non or poor vectors in India.

Species	Larval site characteristics	Host		Biting		Resting		Other
		Anthropo-philic	Zoophilic	Endophagic	Exophagic	Endophilic	Exophilic	
An. koliensis	Sunlit (occasionally shaded), fresh, clear, still water with higher plants or algae (occasionally without vegetation)	●	○	●	●	○	●	Currently considered a single species but new evidence suggests it may be a complex of two or more species
An lesteri	Shaded, fresh water with higher plants or algae	●	●	?	?	●	-	*Anopheles lesteri* is synonymous with *An. anthropophagus.*
An. leucospyrus/latens	Shaded, fresh, clear or turbid, still water	●	-	●	●	-	●	Most reported information for *An. leucosphyrus* probably actually refers to *An. latens*
An. maculatus (group)	Sunlit (occasionally shaded), fresh, clear (occasionally turbid), still or flowing water with higher plants or algae (occasionally without vegetation)	○	●	●	●	○	●	Vector role of individual species is unclear due to previous misidentifications based solely on overlapping morphological characteristics and due to apparent variability within species depending on location
An. minimus complex	Shaded (occasionally sunlit), fresh, clear, still or flowing water with higher plants or algae (occasionally without vegetation)	●	●	●	●	●	●	Within the complex, only *An. minimus* and *An. harrisoni* are current vectors of malaria
An. punctulatus complex	Sunlit (occasionally shaded), fresh, clear or turbid, still water without vegetation (occasionally with higher plants or algae)	●	○	●	●	○	●	Within the complex, only *An. punctulatus* is a known vector of malaria
An. sinensis complex	Fresh, clear, still (occasionally flowing) water with higher plants or algae (occasionally without vegetation)	○	●	-	●	-	●	Possibly refractory to *P. falciparum* but an important vector of *P. vivax*
An. stephensi	Sunlit or shaded, fresh (occasionally brackish), clear or turbid still (occasionally flowing) water with higher plants or algae (occasionally without vegetation)	○	●	●	○	●	-	One of the few anophelines able to flourish in urban areas
An. subpictus complex	Sunlit, brackish or fresh, clear or turbid, still (occasionally flowing) water with higher plants or algae (occasionally without vegetation)	○	●	●	●	●	○	The complex is currently considered to consist of four siblings: Species A, B, C and D although there is some confusion in the identification of Sp. B in some localities (may be a member of *An. sundaicus* complex)
An. sundaicus complex	Sunlit (occasionally shaded, brackish (occasionally fresh), clear or turbid, still (occasionally flowing) water with higher plants or algae (occasionally without vegetation)	●	○	●	●	●	●	The complex is currently considered to consist of four allopatric siblings: