

optimal trap density of three traps per home, with a mean capture rate of 1.4 *Ae. aegypti* females per trap per day. We also evaluated a synthetic polymer that is highly attractive to gravid *Ae. aegypti* as an oviposition substrate, but prevents development of their progeny. Our results indicate that a gravid-ovitraps incorporating these components (adhesive, supplemental hay and artificial oviposition substrate) could be an effective tool for the surveillance and/or control of *Ae. aegypti*.

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### FIELD TRIALS OF A NEW GRAVID-OVITRAP FOR INTEGRATED AREA-WIDE CONTROL OF *Aedes aegypti* IN PUERTO RICO

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A new gravid-ovicidal trap was used in conjunction with source reduction and larviciding for integrated area-wide control of *Aedes aegypti* in an isolated urban community (295 houses) in southern Puerto Rico (trap intervention area; TIA). Mosquito population post-intervention was compared with pre-intervention value in TIA and with change in a nearby isolated urban community (423 houses) where only source reduction and larviciding were concurrently applied (no trap intervention area; NTIA). The trap used hay infusion and color contrast as attractants for gravid females that were then captured on internal adhesive surfaces. The trap also used a synthetic polymer as a substrate for egg laying instead of water, where hatching larvae fail to develop. Three traps were deployed for two months in each home in TIA. Source reduction consisted of a clean-up campaign and turning over containers that could not be disposed of or treated with a larvicide (three formulations of spinosad). Containers holding animal or human drinking water were left untreated. Water storage containers were not common in the study areas. The number of adult female *Ae. aegypti* in each community was monitored using 28 BG-Sentinel™ mosquito traps in TIA and 40 in NTI. These traps used BG-lure and were operated for three days a week during eight weeks, before and after the intervention. Average *Ae. aegypti* female post-intervention reduction was 43% in TIA and 21.7% in NTIA. Mosquito population reduction due to the gravid-ovicidal traps was 21%. Three gravid-ovicidal traps captured an average of 0.54 *Ae. aegypti* females per house per day (>95% gravid or parous). The number of eggs per captured female in the traps was 14.9. Increasing trap attractiveness is a next step in the development of this low-maintenance, inexpensive device that targets the epidemiologically important, gravid/parous *Ae. aegypti* females.

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### THE CARTAGENA PROTOCOL AND GENETICALLY MODIFIED MOSQUITOES

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The Cartagena Protocol on Biosafety is the fundamental document of the United Nations on the responsible use of genetically modified (GM) organisms. The Protocol applies to GM mosquitoes; however its terms were negotiated primarily with concerns over the safety and trade of GM crops in mind. We argue that, while the Protocol may be adequate for strains of GM mosquitoes intended for population suppression, it is inadequate for strategies intended to replace entire mosquito populations with disease-resistant varieties. In this latter strategy, gene drive systems are being considered that are capable of propagating transgenes within and across national borders. In its current form, the Protocol provides inadequate protection against an accidental release, notably due to the exemption of GM mosquitoes in transit or destined for contained use from the Advance Informed Agreement. At the same time, the conditions for an intentional release are almost impossible to satisfy, requiring unanimous approval from every country that the species inhabits. Furthermore, mosquitoes infected with non-transgenic *Wolbachia* bacteria are exempt from the Protocol, despite unknown consequences for the environment

and human health. We encourage future regulation that addresses the unique biosafety concerns of modified mosquitoes and seeks a balance between the precautionary principle, respect for the sovereignty of states, and the ethical mandate to prevent disease on a global scale.

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### SUITABLE MATING COMPETITIVENESS OF INCOMPATIBLE *Aedes polynesiensis* MALES SUPPORTS LYMPHATIC FILARIASIS ELIMINATION STRATEGY FOR THE SOUTH PACIFIC

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Despite the sustained mass drug administration (MDA) of anti-filarial treatments over several decades, lymphatic filariasis (LF) remains a leading cause of disability in the South Pacific. Recent epidemiological observations clearly demonstrate that MDA alone will not be enough to end the LF transmission cycle, at least in some Pacific island countries. Supplemental control strategies are thus much needed to ensure the success of the global LF elimination campaign. Obligate vector mosquitoes provide additional targets that can complement existing anti-filariasis approaches. However, due to the ubiquitous nature of mosquitoes, conventional methods have failed to successfully control the vector *Aedes polynesiensis*, the primary LF vector throughout most of the South Pacific. Such paucity in the arsenal of tools available to control *Ae. polynesiensis* has raised interest in the use of evolutionary genetics to fight vector-borne diseases. Replacing the endosymbiont *Wolbachia* present in *Ae. polynesiensis* with that from *Ae. riversi* through interspecific hybridization and introgression has led to the development of a laboratory strain (CP) which is bi-directionally incompatible with its wild counterpart, resulting in egg sterility. Laboratory assays demonstrated the equal competitiveness of CP males and established the proof-of-principle of population elimination following the introduction of incompatible males into wild type *A. polynesiensis* cage colonies. CP male competitiveness was assessed in a field cage trial. This bioassay demonstrated equal CP male mating competitiveness with their wild counterpart under semi-natural tropical conditions. These findings support the implementation of a large field trial to assess the efficacy of the *Wolbachia*-mediated mosquito suppression strategy as a supplemental strategy to curb LF prevalence in endemic regions of the South Pacific.

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### INSECT REPELLENTS: FROM MODE OF ACTION TO NEW APPLICATIONS IN VECTOR CONTROL

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With the spread of pyrethroid resistance in most mosquito vector species and the lack of alternative compounds for public health issues, the search for alternative control strategies effective against resistant vector populations has become a priority. In this weapons race, insect repellents, which have been used for some time via topical application to the skin, are becoming of greater interest for community protection against mosquito borne diseases. Here we used a multidisciplinary approach to investigate whether repellents could be used to limit the contact between mosquito vectors and humans. First, using electrophysiological, biochemical and toxicological methods, we described two modes of action of the gold standard repellent DEET: it is an acetylcholine esterase inhibitor and it exerts neurotoxic effects through an elevation of the intracellular calcium concentration. We also showed that repellents had strong synergistic effects with available insecticides and have great potential for use in insecticide for insecticide treated nets or indoor residual sprayings.

Moreover we showed in both the laboratory and field that different fabrics impregnated with repellents alone and combined with other biocides are highly effective against resistant mosquito vectors. We also showed that the impregnation of clothes with repellents is also a valuable opportunity for personal protection. Moreover repellents could be used for their primary activity, as an insect behavior modifier, in promising strategies like the push-pull strategy. To conclude, repellents are highly promising to better control pyrethroid resistant vectors. Although the volatility of these chemicals limits their immediate use on long lasting fabrics, overcoming this technological problem should be lot more easily achieved than finding insecticides with new modes of action.

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### DRY SEASON PILOT INDOOR RESIDUAL SPRAY (IRS) TARGETING RIVERBANK HAMLETS IN SUDAN SAVANNA AREAS OF MALI

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In the Sudan savanna of Mali there is marked seasonality in malaria transmission, a consequence of very low densities of mosquito vectors during the dry season, and so control efforts are generally focused on the rainy season. However, there are parts of that environment, such as the riparian parts of the Niger River, where there is perennial breeding of *Anopheles gambiae* s.l. together with dry season malaria transmission. Thus control measures targeting adult mosquitoes during the dry season in these areas are of interest, and may decrease the size of mosquito population and transmission in the subsequent rainy season. This study aims to explore the effectiveness and potential impact of a dry season IRS in hamlets along the Niger River where mosquitoes continue breeding in the dry season and describe ways in which such an approach might limit malaria transmission during the rainy season. Entomological parameters of malaria transmission were monitored using PSC before and after the IRS in 3 sets of hamlet-inland villages in 2008 and 2009, respectively. Mosquito density and entomological inoculation rate (EIR) in 2009 (after IRS) were lower than that of 2008 (before IRS) in the 3 hamlets under study (Bozokin [1.5 (0.6\_2.4) vs 0.9 (0.3\_1.5)]; 8.3% in Fourda [1.2 (0.5\_1.8) vs 1.1 (0.6\_1.6)], and 33.3% in Somonosso [1.8 (1.2\_2.5) vs 1.2 (0.5\_1.8)]. The same pattern was observed in EIR, measured as the number of infective bites per person per season, with a reduction rate of 79.4% (0.34 vs 0.07), 36.4% (1.87 vs 1.19) and 42.9% (0.28 vs 0.16) respectively in Bozokin, Fourda and Somonosso. Mosquito density and EIR decreased between 2008 and 2009 in hamlets where the IRS was performed. However excepting in Bozokin, this reduction was < 50%. Additional dry season IRS intervention may be required to observe any significant reduction in malaria transmission in subsequent rainy season.

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### IMPACT OF LONG-LASTING DELTAMETHRIN-TREATED CONTAINER COVERS ON *Aedes aegypti* OVIPOSITION

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USDA researchers are studying novel methods to control *Aedes aegypti*. One approach focuses on prevention of *Ae. aegypti* oviposition. In collaboration with Vestergaard Frandsen Ltd., factory-treated long lasting

deltamethrin PermaNet® Container Covers (jar lids) were evaluated with 55-gallon drums with and without covers. Exclusion efficacy was measured with sticky ovitraps and oviposition substrates placed on the inner wall of the drums. Tests were performed in 1,800 ft<sup>2</sup> outdoor screened cages (30 ft wide, 60 ft long, 16 ft high gabled to 18 ft) at the USDA CMAVE facilities in Gainesville, Florida. In test 1, there was 1 drum per cage with either an untreated or deltamethrin-treated cover, or an uncovered drum (untreated control). In test 2, there were 4 drums per cage: 3 covered and 1 uncovered drum, 1 covered and 3 uncovered drums, or 4 uncovered drums (untreated control). Test 3 was similar to test 2 but with a different version of the Container Cover. For each test, 200 gravid *Ae. aegypti* were released into each cage. The drum(s) were 2/3 full of well water and lined with absorbent germination papers to detect female oviposition. Container Cover efficacy was measured 24 hrs post-release of females with 5 widely distributed sticky ovitraps (containing a 10% 7 day-old hay infusion) placed in each cage as alternative oviposition sites for gravid females. Sticky ovitraps were examined after 48 hrs and egg (germination) papers were removed after 72 hrs. Drums with untreated covers yielded a similar number of females to those with no cover, whereas treated covers resulted in a 64% reduction in females. With 1 of 4 drums with treated covers, there was a 45-65% reduction in females and a 42-52% reduction in oviposition. With 3 of 4 drums with treated covers, there was a 67-100% reduction in females and a 75-100% reduction in oviposition. The presence of treated Container Covers of either version significantly reduced female oviposition. Container Covers present a potential tool for the control and prevention of dengue virus transmission.

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### MONITORING THE DURABILITY OF LONG-LASTING INSECTICIDAL BEDNETS IN RURAL ETHIOPIA

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Following the distribution of 3 million PermaNet™ 2.0 long-lasting insecticidal nets in Ethiopia in February - May 2007, a total of 609 nets were collected from households at three time intervals: 2007, 2008, and 2009; to assess physical damage and insecticide loss, and to develop practical methods to quantify deterioration. Nets were collected from 19 sites and time-in-use ranged from 3 to 32 months. Collected nets were confirmed as being from the 2007 campaign by using the batch number printed on each net's label. Physical deterioration was first assessed by counting and measuring the size and location of each hole, and later by counting the number of holes falling into each of three size categories. Insecticide retention was measured by x-ray fluorescence spectrometry, and confirmed by testing a subset of nets using high-performance liquid chromatography. Hole formation began early: over 40% of nets used for 3 months had at least one hole >0.5 cm in diameter. The number of holes per net (hole rate) followed a highly skewed distribution, with many nets having few holes and a few nets having many holes. Median hole rate increased from 1 for nets used for 3-6 months to 10.5 for nets used for 17-21 months. Pairwise analysis showed that nets collected from 5 pairs of sites differed significantly (p<0.002) in hole rates after 17-21 months of use. The distribution of hole sizes was highly skewed and, although hole rate increased with time-in-use, the ratio of large to small holes remained unchanged from 3-6 months to 17-21 months. Repairs were rare, suggesting that net lifetime could be increased significantly by improved user care.

Insecticide analysis indicated that 96% (192/200) of the nets retained sufficient (>10mg/m<sup>2</sup>) deltamethrin after 28-32 months of use. The distribution of insecticide level was very broad with 0.5% (1/189) having inadequate deltamethrin after 3-6 months and 6% (12/200) after 17-21