Final report NO BUG – Publishable Summary October 2009 - October 2013

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Author of the document: Benny Malengier
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Dissemination Level		
PU	Public	
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including Commission Services)	
CO	Confidential, only for members of the consortium (including Commission Services)	✓

Document History

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1. Status of Deliverable

Integrated reports of all partners.

This is part A of D8.5 with the publishable content.

2. Main S&T results

2.1. Introduction

The No Bug project operates within the 7th Framework Programme of the EC for research, technological development and demonstration. The project is a collaborative project for SMEs. The duration of the project was 48 months.

No Bug: Novel release system and bio-based utilities for mosquito repellent textiles and garments

In several applications of professional textiles and clothes mosquito repellency is an important issue. Two major problems arise: repellents currently in use are harmful, resistance to conventional repellents increases, and the lifetime of release systems is too short. Solving these two problems are the main goals of the No Bug project.
Novel biorepellents have been selected. They have been combined with four release systems (multilayer coating, textile bio-aggregates, masterbatches and micro-capsules). This allows to repel mosquitoes transmitting malaria or dengue. The novel release concepts are multilayer coatings and in-situ release of the active compounds. Targeted prototypes are textiles for health workers and bed nets (mosquitoes).

2.2. No Bug Methods

Four work packages of the project were aimed at creating specific methods to achieve the No Bug goals. The aim of WP1 was on deciding what natural compounds or bio-aggregates to use as repellents on textiles. The result of this work is still confidential. The second work package concentrated on improving the layering technique used by Utexbel in existing products. The third work package involved developing techniques to embed bio-aggregates in textiles, while a fourth work package aimed to improve the manufacturing techniques.

Natural Compounds and Bio-aggregates
After extensive testing, 2 natural compounds have been selected to use in the final No Bug prototypes. The selection was done based on the effectiveness of the compound, the cost, and the influence of the compound on humans. The aim is to phase out synthetic compounds gradually, so as to replace them by natural compounds.

Apart from the natural compounds, No Bug partner CNRST screened for bio-aggregates showing repellent activity against mosquitoes. This was done in close collaboration to the Laboratory of Entomology of Wageningen University. This could create new opportunities for the development of a novel release system and bio-based utilities for mosquito repellent textiles and garments.

The set up used to evaluate host-seeking responses of *Anopheles gambiae sensu stricto* and *Aedes aegypti* by the bio-aggregates is shown in following Figure. As controls we used distilled water and sterile broth media.

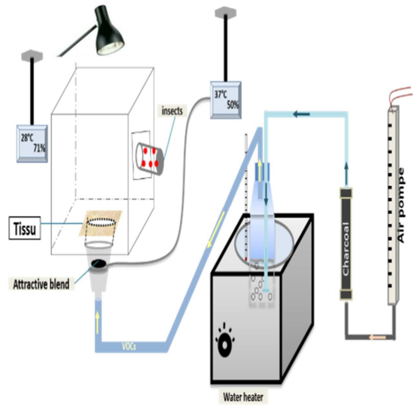


Figure: Set up used in the repellency bioassay

A selection of obtained results are shown below.

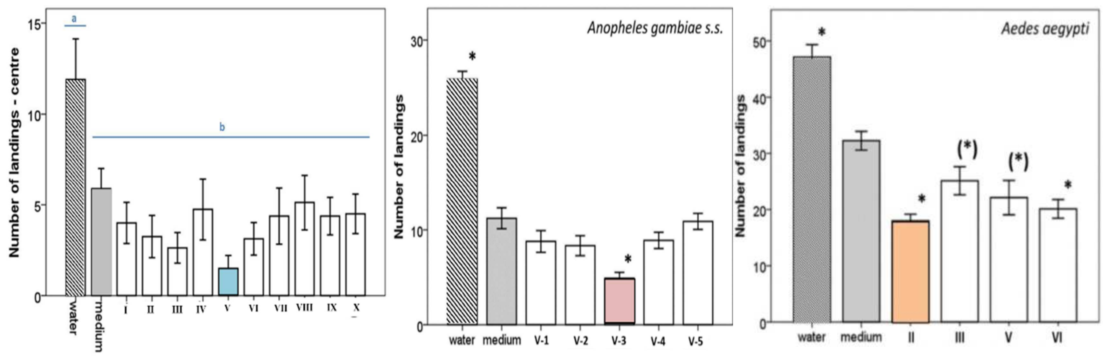


Figure: Comparison of landing numbers obtained with An. gambiae s. s and with Ae. aegypti

Blends II, III, V and VI showed significantly decreased values as compared to the controls. They could be considered as repellent blends against *An. gambiae s. s.* In addition, blend V was the most active. The repellent effects of the five bio-aggregates making up this blend are shown in the second Figure. The bio-aggregates V-3 resulted in significantly fewer landings as compared to the controls, it could have a potential repellent action, but should be more investigated. The four active blends (II, III, V and VI) against *An. gambiae s. s.* were also tested against *A. aegypti.* Blends II and VI elicited significantly fewer landings compared to the control and hold promise as repellent blends against *Aedes aegypti*.

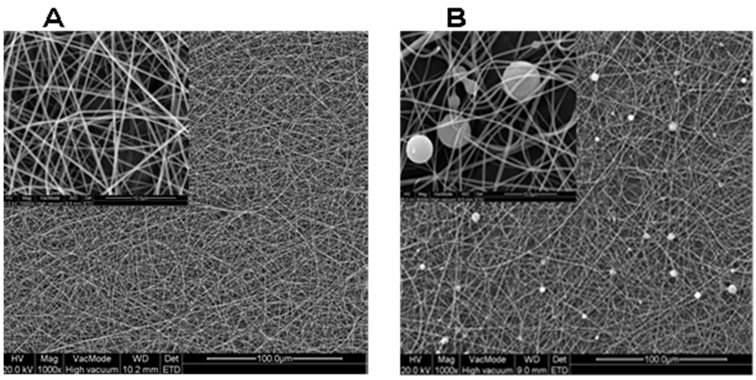
The findings revealed that of the ten tested blends, four blends (II, III, V and VI) show potential repellent activity against *Anopheles gambiae sensu stricto* and *Aedes aegypti*. Of five bio-aggregates making up the blend V (most active against *Anopheles gambiae s. s.*), the bio-aggregate V-3 was significantly active as compared to the controls and to other bio-aggregates.

Functionalized Fibers

Within the manufacturing research, much attention was given to functionalizing fibers, instead of using a finishing step on the fabric to create repellency.

Following the current demand for textiles with new functionalities and improved properties, there has been a continuous effort to modify conventional textile materials. The common trend in the modification of textile materials has been by applying the functionalizing compounds on the material surface as a post-treatment process. However, when the additives are added at the finishing stage, they are normally physically or chemically bonded to the textile surface which does not guarantee their resistance to washing and abrasion. Additionally, some finishing techniques like spraying can cause spills which may be detrimental to the environment whereas others give partial loss during use, care and storage. Thus, taking fibers as the basic units from which complicated textile structures are developed, adding compounds at the fiber production stage means the compounds will interact with the polymer matrix before the fibers are spun resulting in a strong bond between the fiber structure and the functionalizing compounds. Moreover, no chemicals will be needed to bind the compounds on the fiber hence the environmental pollution and the cost of production is reduced as the embedding of the compounds and the production of the fibers are done in a one stage process. Therefore, the textile department of Ghent University in collaboration with Biology department and Devan chemicals investigated the feasibility of adding functionalizing compounds in fibers during melt extrusion and electrospinning.

Melt extrusion: A new BPD registered compound has been fully imbedded in a masterbatch for polyester. Trials have shown good spinability and excellent repelent performances. Bioagrregates (see below) processed in a masterbatch were successfully added during melt extrusion. This sustainable biocompound shows excellent repellency too. Further investigation to develop an industrial process is undergone.
Electrospinning: Functionalizing compounds were successfully electrospun into nanofibers. Results shows that the fiber morphology of the resulting nanofibrous structure was similar to the reference sample. It was also observed that the nanofibers covered the added compounds that were randomly distributed in the nanofibrous structure, see following Figure.



Therefore, these results shows that there is a great potential in the use of melt extrusion and electrospinning techniques to add novel functions into textiles materials which can not only contribute to the laboratory research but also in large-scale production of functionalized textile materials.

Layering Technique and micro-capsules
The layering technique has been optimized in several ways: improved binder techniques, methods for combining layers, addition of micro-capsules, laundry resistance, optimization of repellency time. Also the use of micro-capsules themselves were likewise optimized.
Repellency above 80% was achieved.

The focus of No Bug was on repellent products, as opposed to existing toxic layers. Research has shown that combining the repellent with a toxic gives best results, as mosquitos that do land, have less chance to remain on the textile. As the toxic does not deplete as fast as a repellent, this also means a base functionality remains after the repellent is finished.

2.3. No Bug Testing

The textile products made need extensive testing to validate them. An entire work packages was dedicated to devising testing methods for repellent textiles, as well as doing the actual testing work. Testing was divided over laboratory testing and field testing.

Laboratory Testing: The Laboratory of Entomology of Wageningen University developed a standardized bioassay to measure the repellency of impregnated fabrics, see Figure below.

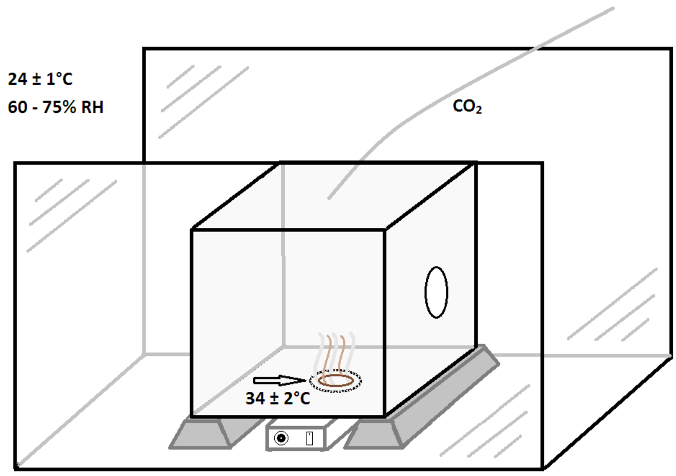
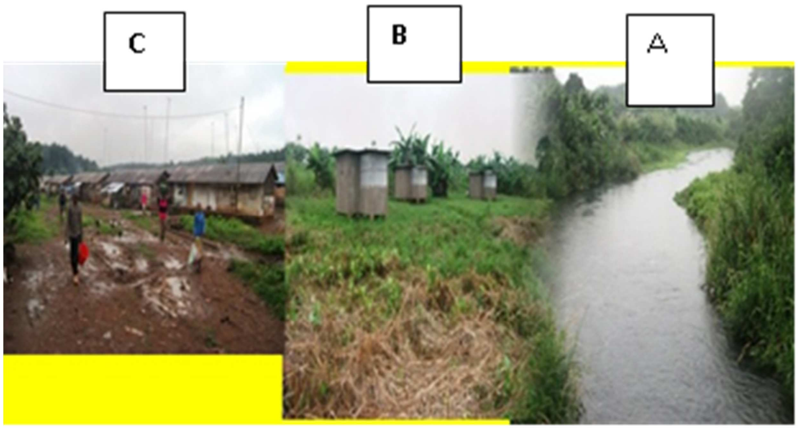


Figure: The vertical landing bioassay, consisting of the flight chamber (shaded) behind glass screens, with the odour bait and treated fabric on the bottom.

This vertical landing bioassay makes use of a synthetic odour bait as attractive as human beings, in combination with pulses of CO_2, to elicit landing by mosquitoes on a warm and moist surface onto which a treated fabric can be applied. Mosquitoes used are *Anopheles gambiae sensu stricto*, the principal malaria vector in Africa.
Wageningen University provided testing of fabrics treated with repellent compounds. One hundred and four different treatments were tested, which encompassed: 15 candidate repellent compounds, applied onto numerous fabrics according to different methods, in various combinations and concentrations, as well as bio-aggregates blends. The untreated and treated fabrics were all prepared by partners in the consortium. To ensure objective evaluations, all fabrics were tested blindly, i.e. the researchers were not informed about the composition of the (repellent) compounds on the fabrics.
Wageningen University delivered input by sharing knowledge and expertise obtained in previous work and suggested novel candidate repellents, some of which proved very successful. Several of the treated fabrics were highly repellent to mosquitoes, depending on the nature of the compound applied, its concentration and formulation. Repellent compounds included “known” chemicals such as DEET, as well as novel compounds that have not been used for this purpose previously.
Repellency was measured quantitatively by comparing the number of landings made on control and treated fabrics and statistically analysed. By bringing together work from the No Bug project and

Figure: NO-BUG Repellent Field Testing station
A: Breeding site, B: Experimental huts, C: Meanja village



other projects we will be able to explore the effectiveness of repellent-impregnated fabrics in the context of public health. This concerns personal protection against mosquito bites over a potentially wide range of mosquito species both indoors and outdoors.

Field Testing: REFOTDE designed an improved field testing system to assess the efficacy of repellent products against vectors of major tropical diseases. Experimental huts were constructed in an environment where *Anopheles*, *Aedes, Mansonia and culex* (malaria, Dengue and lymphatic filariasis vectors) are present and the malaria disease is endemic.
The originality of the design resides in the location of the experimental huts. The huts are constructed in between a prolific breeding site (a damned river with slow flowing water) producing mosquitoes all year round, and a settlement of about 1000 people permanently living in the middle of a huge rubber and banana plantation with extremely few domestic animals available, making humans the main source of blood for mosquitoes.
This location allows the testing to be carried out all year round, unlike other experimental set ups whose functioning depends on rain that conditions the presence of breeding sites.

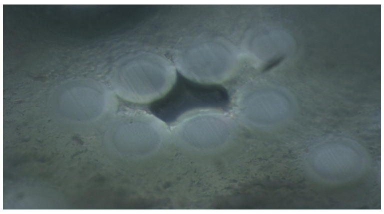
Experimental huts are specially designed to test vector control products against freely entering mosquitoes under natural but controlled conditions. Huts are designed with four window traps that enable mosquitoes to fly into the hut while making sure they can't escape from it. A veranda trap covered with neutral netting material in which exophilic mosquitoes trying to leave the hut at dawn are caught. Huts do not differ between them for their mosquito attractiveness in absence of products to be tested. Adult volunteers well informed on the objective and who consent to participate in the study, enter the hut at dusk (7:00pm) and remained inside until dawn (6:00 am) of the next morning.
Of the three experimental huts used, one is the control, while the net materials with the product to be tested are mounted in the other huts (two test huts).
The average of the results obtained in the two test huts is compared with the results obtained in the control hut.
Indicators of efficacy used to assess the repellency of products: deterrence, feeding prevention, induced exophily, and mortality
- When the product is repelling effectively, less mosquitoes enter the test huts as compared to the control
- When the product repellency is low, there are approximately as many mosquitoes in the test as in the control huts.

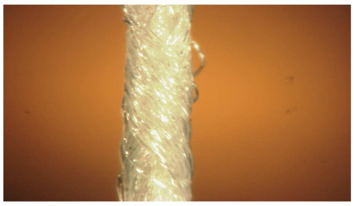
The validity of this system was tested against standard compounds well known to be excellent repellent to Mosquitoes. such as DEET and PMD. DEET and PMD achieved over 97% of prevention of mosquitoes from entering the huts. Results obtained also proved to be reproducible with over 98% consistency in replicate experiments. The system provided satisfactory results comparable to results obtained using standard laboratory test methods (WHO, USEPA), with the advantage that it mimics the natural field situation, though in a controlled setting. This system can confidently be used to field test new repellent products against vectors of major tropical infectious diseases, and has been used for the No Bug prototype products.

2.4. No Bug Prototypes

During the project, several prototypes have been developed and tested, leading to final prototypes which need to undergo final testing. Production methods still need further adaptations to allow industrial production. Only then, actual No Bug products will be on the market.

JMT is processing and improving 2 different kinds of yarns able to contain the repellent products developed. They are using the new technology of bi components synthetic yarns, which allow a larger range of combinations. JMT is also working on micro fibres and multifilament yarns with operations like braiding, and texturization to fix the repellent products in a bundle and increase their efficiency. This kind of yarn is working like a sponge during the process.





Utexbel developed several products that fit in their buzzX line of products, http://www.buzzx.info/. Specifically repellent Leno fabrics and repellent PPE fabrics have been developed.

These fabrics incorporate technologies developed within the No Bug consortium by Utexbel or the partners. Testing focused on pajama's and bed nets, as those allow for targeted testing.

The fabrics developed can however also be used in other applications. To make it possible for the processing chain to react more quickly to market circumstances, repellent yarns have been tested

also. These can then be processed into functional textiles, avoiding environmental stringent measures during the fabrication of the textile.
At the end of the project, Paul Boye and Body Wear made No Bug prototype garments with the Utexbel fabrics. These are now undergoing extensive testing for wear, washing, and comfort.

2.5. Conclusion

At the end of the No Bug project, several prototypes are available with repellency reaching 80%. Some of this is in an academic or early development state (bio-aggregates, masterbatches). Other or in a pre-production state (layering, microcapsules, bi-component yarn). Work remains to be done on upscaling these processes to an industrial production.
A further limitation is the EU Biocides Regulation (BPR). Registration of new repellents is expensive. As a consequence, No Bug products must make clear economic sense before they will reach the market.

3. Impact, dissemination and exploitation

The work done lead to 14 published papers, with several preprints in preparation by the academic partners. Dissemination happened under the guidance of Ro Challenges on TV, Expo's, and conferences, as well as via a newsletter and flyers.
A patent application is in preparation concerning the bio-agreggates.

The immediate impact of the No Bug consortium consists of improved products in the portfolio of Utexbel (BuzzX range) and Devan (Insecta range). JMT considers a new trademark for their line of insect repellent products. Several new generational products are undergoing final testing and investigation (concerning the BPR, market acceptance, …). New innovative products based on the research have been validated, but need further work to allow upscaling to an industrial production.