

FINAL REPORT

Insect Resistant Textiles for Disease Vector Protection

SERDP Project WP19-1096

AUGUST 2020

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Luna Innovations Incorporated

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List of Acronyms

Symbol	Meaning
MI	Mechanical Insecticide
NCSU	North Carolina State University
WHO	World Health Organization
DE	Diatomaceous Earth
VOC	Volatile Organic Content
DEET	Diethyltoluamide
DOD	Department of Defense
EPA	Environmental Protection Agency
PPE	Personal Protective Equipment
NYCO	50/50 Nylon Cotton Blend
ACU	Army Combat Uniform
IRB	Institutional Review Board
ATP	Adenosine Triphosphate
HVLP	High Volume Low Pressure

Keywords

1. Vectors
2. Insect Resistance
3. Mechanical Insecticide
4. Diatomaceous Earth
5. Bioassay Testing
6. Blocked isocyanate
7. Permethrin

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1.0 Abstract

1.1 Introduction and Objectives

Vectors such as mosquitoes, flies, and ticks can spread disease with serious short and long term effects on soldiers' health, including permanent scarring, and possibly death. Since permethrin is the only insect repellent product labeled for clothing application by the Environmental Protection Agency, permethrin-treated uniforms are a key component of the Department of Defense Insect Repellent System. However, vector species are developing a resistance to pyrethroid-based repellents, significantly diminishing protection of service members. In addition, permethrin wears off with laundering and is highly toxic to fish and other wildlife. Luna proposed to replace current permethrin-treated fabric treatment with a non-toxic mechanical insecticide fabric treatment that possesses resistance to mosquito bites.

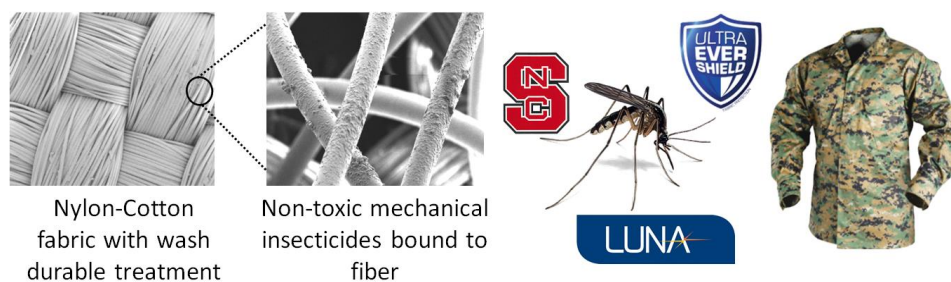


Figure 1. Representative fabrics with Mechanical Insecticides for Disease Vector Protection

1.2 Technical Approach

Luna explored established fabric treatment methods including dip-pad, spray, and blade application while developing the mechanical insecticide fabric treatment. NCSU Entomology Department performed mosquito bioassay tests to evaluate the insecticidal, repellency, and bite resistance of the fabric treatments. In conjunction with mosquito bioassay testing, Luna tested the fabric treatments for wash durability (AATCC 135), total weight (ASTM D776), and air permeability (ASTM D737).

1.3 Results

Luna developed a water-based polyurethane dispersion with reactive cross linker to generate a durable, non-toxic textile with high bite resistance. The program demonstrated that mosquito bite resistance was demonstrated, however the testing showed that the textiles were not insecticidal nor were they insect repellent. [Note although the treated fabric is not insecticidal, the additive used is still referred to as a Mechanical Insecticide (MI) for consistency from the initial proposal and reports.] The treated fabrics demonstrated bite resistance to at least 10 washes, increased the breaking strength of the fabric (ASTM D5034), and maintained breathability and drape after treatment.

The total weight increase was dependent on the fabric selection and textile application method. Future development efforts would enable application optimization and testing against permethrin treated fabrics, allowing for a direct comparison of mosquito bioassay performance.

1.4 Benefits

The absence of harmful chemicals from Luna's MI fabric treatment adds significant environment benefits while maintaining the user safe from pesticides. Luna took measures to ensure the

application method was scalable, ensuring an easy transition to larger, industrial scale setting. In addition to uniforms, the technology may also be applied to t-shirts, socks, and caps. As such, the technology is applicable to commercial markets where there is a need to replace permethrin treated clothing for civilian use.

2.0 Objective

Luna's technical effort focused on developing mechanical insecticide textiles and validating their performance through mosquito bioassay testing, and textile physical property evaluation. The main objective of the SEED project was to develop a mechanical insecticide (MI) textile treatment without the use of chemical pesticides as a proof-of-concept for insect resistance. There is a need to replace chemical-based insect repellent fabrics with a non-toxic alternative for the user and the surrounding environment. As such, there is a need in both the DOD and civilian markets. Additional follow-on funding for Luna would be utilized to i) optimize and scale up the proven MI textile treatment and application process, ii) prototype textile and uniform production with partners in textile mills and uniform manufacturing, and iii) field validation of the prototypes through opportunistic collaboration with DoD colleagues.

2.1 SEED Program Technical Objectives

The objective of the program was to demonstrate the feasibility of producing a wash durable MI fabric treatment with inherent insect resistance. In addition, the MI fabric treatment maintain the critical physical properties for military uniforms, including comfort, air and moisture vapor permeability, good drape, and minimal changes to the tensile and flexibility of the treated fabric. Luna's team utilized extensive formulation and textile treatment expertise to produce a viable treatment process with good adhesion to several selected textiles of relevance to the US military. Luna's partners in the Entomology Department at North Carolina State University (NCSU) acquired the data necessary to demonstrate proof-of-concept via validation testing (e.g. mosquito bioassay testing) to prove efficacy for a possible follow-on SERDP project focused on coating optimization, scale-up, and transition to other DOD agencies.

2.1.1 Objective 1 – Formulate durable insect resistant textile treatment employing non-toxic mechanical insecticides

Durable mechanical insecticide fabric treatments were formulated using water-based polyurethane dispersions with wash durable crosslinkers. The polyurethane chemistry covalently bound the mechanical insecticides to the fabric treatment without compromising kill efficacy. Luna varied the cross linker concentration to optimize the durability of the MI to the fabric treatment. The goal was to develop a wash durable MI fabric treatment while maintaining fabric physical and mechanical properties, while maintaining comfort and flexibility. Luna prepared numerous MI fabric treatments and utilizing industry standard fabric coating application methods to develop an optimal application method specific to Luna's MI fabric treatment. Additionally, Luna's non-toxic insect resistant fabric treatment has excellent potential to be scaled into large industrial scale manufacturing processes.

2.1.2 Objective 2 – Demonstrate the MI fabric treatment meets relevant fabric performance specification

Down selected MI fabric treatments and application methods were screened by NCSU Entomology Department with mosquito bioassay testing. Two distinct fabric treatments and application methods were tested with the most durable MI textile treatment. Additionally, textile properties

were evaluated at Clemson University (ASTM D737) and at Luna Innovations for water vapor transmission rate (ASTM E96), wash durability (ASTM D776), drape (subjective), and mechanical testing (D5034). Comparison testing to permethrin could not be performed under the scope of this program. While none of the Luna coatings could be tested directly against permethrin, Luna's MI insect resistant fabric treatment performed optimally in mosquito bioassays at NCSU Entomology Department.

2.1.3 Objective 3 – Demonstrate the non-toxic MI fabric treatment performs favorably via mosquito bioassay testing

Durable MI fabric treatments and application methods were evaluated by NCSU Entomology Department with select mosquito bioassay testing. NCSU recommended testing for mosquito resistance using Tier 2, Tier 3, and Tier 4 bioassay testing. Tier 2 bioassay is known as the standard WHO funnel test. The approach requires minimal time and cost, allows for high throughput testing and is specific for mortality. Tier 3 bioassay evaluates repellency and mortality; using ATP doped porcine blood instead of human test subjects. The number of landing sites and number of blood fed mosquitos determine the resistance of the fabric against the vector species. Tier 4 bioassay test fabrics with high levels of bite resistance as screened in Tier 3 testing. Tier 4 bioassay tests MI treated fabrics in arm in cage studies using human subjects. Protocol to use human subjects in research was approved by the NCSU Institutional Review Board (IRB Protocol Number 16897). Luna's MI insect resistant fabric treatment performed optimally in mosquito bioassays at NCSU Entomology Department.

3.0 Background

The following sections provide a detailed background on Luna's MI insect resistant fabric treatment and the primary technology focus area during the SERDP Exploratory Development (SEED) program.

3.1 *DOD Insect Repellent System for Vector Protection*

Permethrin is the only insect repellent product labeled for clothing application by the Environmental Protection Agency (EPA). Wearing permethrin-treated uniforms is a key component of the DOD Insect Repellent System. The DOD Insect Repellent System includes application of permethrin to uniforms, use of DEET repellent on exposed skin, and proper wear of uniform (trousers tucked into boots, sleeves worn in down position). In addition, permethrin is applied onto bed nets. In some cases, anti-malarial drugs are taken by troops before, during, and after deployment to prevent the contraction of malaria; a vector disease transmitted by mosquitos. Using permethrin for vector protection against mosquitos generated unexpected adverse effects on vector species. It is suspected that vector species are developing resistance to pyrethroid-based repellents like permethrin, significantly diminishing protection of service members. In addition, permethrin wears off with repeated use and laundering. Lastly, permethrin and other pyrethroid-based repellents are highly toxic to fish and other wildlife. The DOD Insect Repellent System relies on the user for proper usage and efficacy of the treatment kit. It is known that improper tucking trousers into boots and neglecting to roll down sleeves leads to ineffective protection.

Current permethrin treated uniforms comply with MIL-DTL-44411C for permethrin application procedures on uniforms and MIL-DTL-44436A for Nylon/Cotton camouflage blends, among others. MIL-DTL-44411C requires permethrin solutions of 40 to 42 percent by weight permethrin in 58 to 60 percent by weight petroleum distillates. The hazard listed includes warnings for irreversible eye damage and PPE requirements, including the use of protective gloves and face shield when applying the concentrated permethrin solution. In addition, permethrin may not be applied to under garments or cap. The permethrin treatment is expected to last the life of the uniform, which is equivalent to 50 wash cycles.

The permethrin solution used to treat uniform trousers and jacket is concentrated at 40 weight percent in organic petroleum distilled. As such, the current insect repellent employed by the DOD is harmful to apply, with many safety considerations. Any runoff from application that enters water streams is extremely toxic to fish and wildlife. In addition, the solution can only be applied to outer garments, leaving areas of exposure with other garments that require direct contact to the skin, including t-shirts, socks, and caps. Luna's MI insect resistant fabric treatment is water-based, does not contain Volatile Organic Compounds (VOCs), does not require extreme PPE to apply, and is not detrimental to the surrounding wildlife and environment. Most importantly, Luna's MI insect resistant fabric treatment is not limited to the trousers and jacket. T-shirts, socks, and caps can safely be treated and worn without health concerns to the user. Treating previously untreated garments adds significant vector protection to the user.

3.2 Mechanical Insecticides

Our team has shown that diatomaceous earth (Figure 2) and other non-chemical insecticides possess high efficacy for killing mosquitos on fabrics.^{1,2} Mosquito mortality of >70% at 4 hours and >90% at 5 hours was observed.³ These diatomaceous earth based insecticides have been used extensively to control internal and external parasites in animals⁴ and their use is considered safe.⁵ These materials have significant advantages over the current use of pyrethrum based insecticides for uniforms, which are highly toxic and mosquito species known for spreading disease vectors are becoming resistant to these chemicals. Since the MI kills via a physical mechanism, there is minimal concern for insects to build up a resistance to this technology. Diatomaceous Earth (DE) is the fossilized skeletal remains of microscopic microalgae called diatoms that lived in the sea and in freshwater lakes millions of years ago.



Figure 2. SEM of diatomaceous earth material

The DE materials have been used mechanical insecticides (MI), yet do not contain any toxic materials. They are highly effective against mosquitos, fleas, ants, and bed bugs. As an insect contacts the DE, the sharp edges of the mineral penetrate the insect's waxy outer epicuticle layer. The high surface area particles then absorb moisture and/or protective lipids from this layer. Without the protective layer, the insect then dehydrates and dies.

3.3 Polyurethane Chemistry

Polyurethane chemistry requires a two-component system with mutually reactive functional groups that are mixed prior to application. The reactions are generally fast and result in excellent performance properties. One of the most common two-component systems is an isocyanate-based polyurethane system. Luna's MI insect resistant fabric treatment consists of a non-hazardous flexible pre-polymer that reacts with the isocyanate. Keeping in line with safe application methods, the reactive isocyanate remains blocked until the oven cure step, where it can react with Luna's flexible pre-polymer. The mechanical insecticides selected for the insect resistant textile coating have naturally reactive hydroxyl (-OH) functionality on the surface. The MI hydroxyl surface functionality can be exploited to covalently attach the MI particles to the textile fiber surface and fabric treatment, generating a durable fabric treatment.

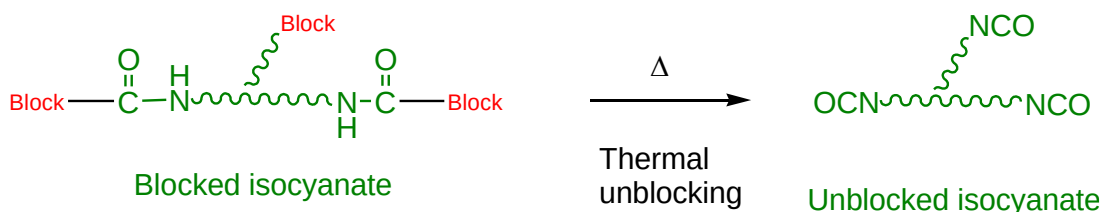


Figure 3. Luna crosslinker for wash durable textile treatment.

As mentioned previously, the diatomaceous earth materials are largely silica-based with surface hydroxyls. These hydroxyl groups are active toward crosslinking with the unblocked isocyanate and generate a crosslinked network around the textile fiber (Figure 4).

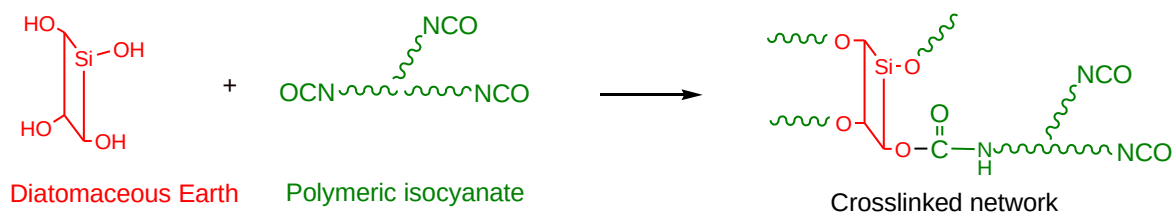


Figure 4. Diatomaceous earth crosslinked within polymeric textile treatment network.

This crosslinking technology was originally developed as part of a chemical warfare resistant textile treatment under Army SBIR funding, commercially available worldwide under the tradename Luna's EverShield®.⁶ The treatment also includes the use of micro particles containing silica (Figure 2), which have proven covalent attachment in similar manner as Figure 4. Using a blocked isocyanate for crosslinking allows the development of an aqueous polyurethane dispersion (PUD) which yields important implications such as a safety for factory workers and users, a long pot life, and fast cure conditions once heated. The polyurethane forms a flexible coating around the fiber while covalently bonding to the mechanical insecticide.

3.4 Luna's Mechanical Insecticide Fabric Treatment

Mechanical insecticide materials were selected to be compatible with Luna's flexible cross linker and Luna's water-based polyurethane dispersion. In addition to having the proper surface chemistry for reactivity into the fabric treatment, the mechanical insecticides had to have surface morphology conducive to generating insect resistance in bioassay testing at NCSU. The mechanical insecticides selected are non-toxic to the user, inexpensive to purchase, react into Luna's fabric treatment, and provide protection against mosquitos. In addition, Luna's MI insect resistant fabric treatment contains no VOCs. The coating was produced in three simple steps and required an oven cure. The application techniques evaluated on this program include dip-pad, spray, and doctor blade (Figure 5). These processes used under laboratory conditions are directly scalable to those currently employed in US based textile mills and can be applied to fabrics at speeds of 1000's of yards per hour. Materials produced using each application technique was evaluated at NCSU for mosquito resistance, at Clemson University for air permeability, and at Luna for mechanical testing and total weight uptake.



Figure 5. Textile application methods.

The coating technique had a direct impact to textile physical properties including total weight, air permeability, and influenced mosquito bioassay results. Luna explored and evaluated how the different textile application techniques influenced the performance of the MI textile treatment with mosquito bioassays, while keeping track of the textile properties previously mentioned.

4.0 Materials and Methods

Combat uniforms are traditionally made from 50/50 Nylon/Cotton blend known as NYCO. Initial mosquito bioassay testing of Army NYCO yielded a fabric with inherent bite resistance. Due to the nature of the bite resistant Army Combat Uniform (ACU) NYCO, Luna selected highly porous mercerized cotton with very low bite resistant towards mosquitos. Luna chose mercerized cotton to prove the efficacy of the MI fabric treatment, which could increase the bite resistance of the highly porous fabric to a near 100 % bite resistant material. The mercerized cotton used throughout the duration of the program was previously procured by Luna. The mechanical insecticides were sampled and provided by our partner Imerys, a commercial developer of mechanical insecticides. The mosquito bioassay testing was developed and performed by the subcontract with NCSU Department of Entomology (Professor Michael Roe). Luna evaluated application techniques commonly used in industry and include dip-pad, spray, and doctor blade. In addition to mosquito bioassay testing, MI treated fabrics were also evaluated for total weight (ASTM D776), breaking strength (ASTM D5034), air permeability (ASTM D737), water vapor transmission rate (ASTM E96), and wash durability (AATCC 135).

Table 1. Test Methods for Textile Evaluation of NYCO

Test	Method	Description	Target
Total Weight	ASTM D3776	Mass per Unit Area	5.5 oz/yd ²
Wash Durability	AATCC 135	Laundryability	5, 10, 25, 50 cycles
Water vapor transmission	ASTM E96	Moisture breathability	≥ 700 g/day/m ²
Breaking strength	D5034	Tensile Grab Test	200 warp x 125 fill lbf
Air Permeability	ASTM D737	Air breathability	25 ft ³ /min/ft ²
Drapability	In house	Easy of fabric to drape or bend	Qualitative

4.1 Insect Bioassay Testing

Luna developed several MI fabric treatments during the program. Luna's MI fabric treatments were evaluated for bite resistance, repellency, and mortality of vector species, with a focus on mosquitos. Dr. Roe at NCSU Entomology Department tested all treated samples with mosquito specific bioassays, here referred to as: Tier 2, 3, and 4. Tier 4 bioassay required IRB #16897, using human test subjects to test for mosquito resistance under the harshest conditions. Tier 4 bioassay test results were very indicative of real-life results. Tier 3 bioassay utilized warm porcine blood doped with ATP as a substitute for human subjects. The results from Tier 3 bioassays generally correlated to Tier 4 results and so, were used to screen fabrics prior to testing on humans. Tier 2 bioassay was used for mortality and indicated repellency. Tier 2 testing required minimal number of mosquitos and testing lasts 30 minutes, allowing for several tests to be run in a single day. Tier 2 testing was used to screen MI materials to determine the best candidate for repellency and mortality. While most of the program was focused on selecting, evaluating, and generating a wash durable MI fabric treatment for mosquitoes, other vector species such as ticks and blow flies (*Cochliomyia macellaria*) were also considered.

4.1.1 Tier 2 Bioassay Funnel Test (Static Cone Test, no host cues).

The Tier 2 bioassay funnel test is the standard WHO funnel test (Figure 6). As previously stated, the funnel test requires minimal time and cost, allowing for high throughput testing. While the test is specific to mortality, it also provides insight into repellency. Tier 2 testing was primarily used at the beginning of the program to screen mechanical insecticide materials. For most of the tests, the fabric was placed underneath the cone. Half the fabric was covered in 300 mg of the selected mechanical insecticide while the other half exposed the bare fabric without any mechanical insecticide. Five mosquitoes (*Aedes aegypti*) were introduced through the top of the funnel. The top of the funnel was plugged to prevent mosquitoes from resting inside the neck of the funnel. The mosquitoes were observed every five minutes and their location was recorded. The locations recorded included the funnel wall, the bare fabric, and the MI covered fabric. The average and standard deviation was recorded. Observations regarding mortality and repellency were noted. The best mechanical insecticide samples for repellency and mortality were down selected for the more representative Tier 3 and Tier 4 bioassay tests.

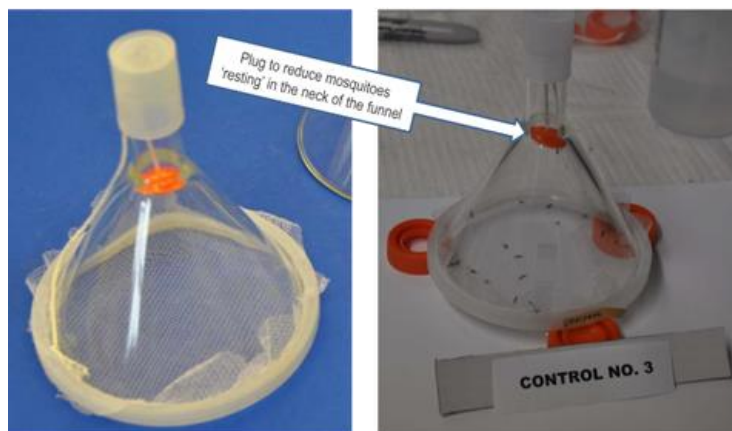


Figure 6. Tier 2 bioassay funnel test.

4.1.2 Tier 3 Bioassays (repellency and mortality; in vitro assay with host cues).

The in vitro blood-feeding device (Figure 7 to Figure 9) is constructed from Plexiglas®. A reservoir for blood (16.5 cm length x 3.5 cm width x 0.5 cm depth) was produced by a hand-held router in a rectangular piece of Plexiglas (28 cm length x 5.5 cm width x 1 cm thickness). A hole (4 mm dia.) was drilled at the center of the top and bottom edge through the plastic into the blood reservoir. A tap was used to cut threads into the plastic so that a valve could be screwed into the top and bottom holes. Two holes (each 4 mm dia.) were drilled from the bottom edge of the device through the plastic to the blood reservoir. A loop of stainless steel tubing (3 mm dia.) was placed into the blood reservoir, and the tubing was inserted through the holes so that the cut ends protruded out of the plastic. Epoxy cement was used to seal the tubing in place inside the blood reservoir of the device. The ends of the tubing were connected to a circulating water bath to heat the porcine blood.

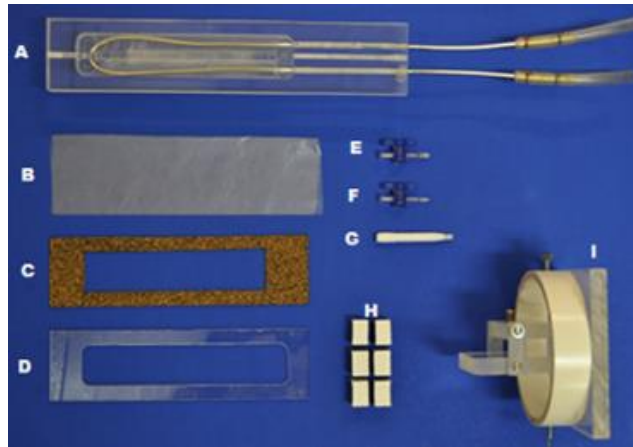


Figure 7. Components of in vitro blood feeding device. A) body of the device showing the blood reservoir and stainless steel heating coils; B) collagen film; C) cork gasket; D) plastic frame; E-G) valves and stem; H) clips; I) PVC collar and clamp for holding the device.

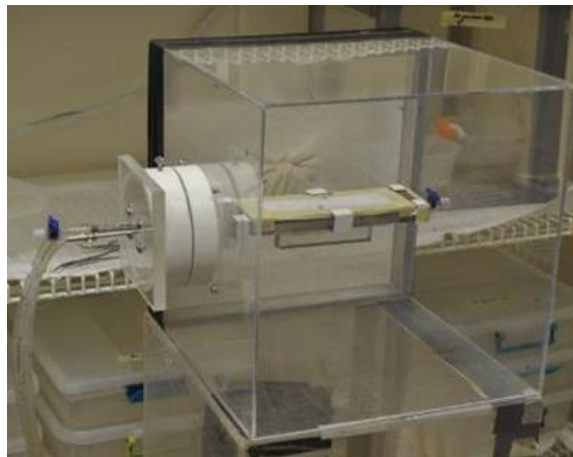


Figure 8. In vitro blood-feeding device, inserted into a bioassay cage.

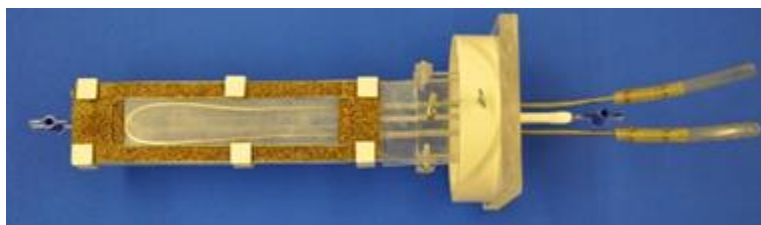


Figure 9. Blood-feeding device with components assembled.

To setup for an in vitro blood feeding bioassay, a transparent collagen film is hydrated in distilled water and stretched over the top of the device. A gasket cut from a sheet of cork-rubber composite is placed on top of the collagen film. A rectangular piece of plastic (3 mm thick) the size of the blood-feeding device is then placed on top of the gasket. The central area of both the rubber gasket and plastic frame was removed so that the collagen film is fully exposed. Metal binder clips hold the gasket and frame in place on top of the blood-feeding device, preventing leakage of blood. A 30 mL syringe filled with blood is then attached to the valve that is screwed into the top hole of

the blood-feeding device. With the device tilted at a slight downward angle the blood is slowly transferred into the reservoir. The valve attached to the bottom of the device is opened to allow air displaced by the blood to escape. When the device is filled, both valves are closed, and the circulating water bath is turned on to warm the porcine blood to 35 °C.

Textiles to be evaluated for bite resistance are cut to exactly fit over the collagen film within the plastic frame. Masking tape, placed around the inner edges of the plastic frame, slightly overlaps the fabric. Mosquitoes are prevented from gaining access to the collagen film by probing around the edges of the fabric. The blood-feeding device is inserted into a Plexiglas bioassay cage containing starved female mosquitoes. Depending on the severity of the test, 25-50 female mosquitoes (*A aegypti*) were transferred to the in vitro bioassay cage. The female mosquitoes were starved overnight (sugar water removed from their rearing cage; females not blood fed) prior to testing. Female mosquitoes from 4-10 days of age (post emergence) were used for bioassays. Porcine blood obtained from a local abattoir is used in our bioassays. At the time of collection, sodium citrate is added as an anticoagulant. Just prior to initiating the bioassay, ATP was added to the blood (2.5 mg/mL blood) as a phagostimulant. Each bioassay was conducted for 10 min., during which the number of times females land and probe the fabric was counted. A single event was recorded if a female landed and inserted or attempted to insert her proboscis into the fabric. At the end of the exposure period, mosquitoes were removed and crushed individually on a sheet of white paper to determine if it was able probe through the fabric and obtain a blood meal. For each fabric sample, the in vitro bioassays were repeated three times, generating an average and standard deviation. In addition, control fabric samples were tested. These included the bare untreated fabric and the fabric with Luna's textile treatment not including MIs.

4.1.3 Tier 4 Bioassays (repellency and mortality; arm in cage).¹

Fabrics treated with Luna's MI fabric treatment showing high levels of bite resistance during in vitro assay were tested in arm in cage studies. The results were compared to in vitro assay and control fabrics. Arm in cage assays were carried out with informed consent using a protocol for use of human subjects in research that has been approved by the NCSU Institutional Review Board (IRB Protocol Number 16897). A novel sleeve device was used by NCSU (Figure 10) to bioassay textiles that were found to be bite resistant in in vitro assays. The sleeve was cut into a trapezoidal shape with a 16.5 cm x 3.5 cm opening in the center that corresponded to the size and shape of the opening in the feeding device. A plastic frame was riveted to the sleeve to keep the exposure area from deforming when the sleeve is attached to the forearm of the study participant.⁷



Figure 10. Left: Sleeve device used for arm-in-cage bioassays; Right: arm-in-cage sleeve attached to study participant.

Consistent with the in vitro bioassay, 25-50 female mosquitoes (*A aegypti*) were transferred to the in vitro bioassay cage. The female mosquitoes were starved overnight prior to testing. Female mosquitoes from 4-10 days of age were used for the bioassays. Female mosquitoes are transferred to the bioassay cage 10-30 minutes before bioassays began. A candidate fabric is laid on the underside of a forearm of a study participant. The sleeve was laid on top of the fabric and attached to the participant's forearm with Velcro® straps. The hand of the participant was covered with a nitrile glove. The bioassay started when the participant inserted his/her arm through the sleeve into the bioassay cage. An observer counted the numbers of mosquitoes landing on the fabric and probing during a 10 min. exposure period. Once the bioassay concluded, mosquitoes were examined for blood feeding as described in section 4.1.2.

4.2 Overview of Textile Properties Evaluation Tests

4.2.1 Total Weight

Total weight, or mass per unit area, was determined following ASTM D3776. Luna selected a mercerized woven cotton fabric to develop the mechanical insecticide textile treatment. The woven cotton was highly porous and thin, which translated to a material with little to no bite resistance. The total weight was compared to initial mass per unit area of the untreated cotton sample. The total weight of the MI fabric treatments was evaluated in connection to the coating application method. During the technical period of the program, Luna evaluated three different application methods with the intention of reducing the total weight of the MI fabric treatment while maintaining favorable bioassay results.

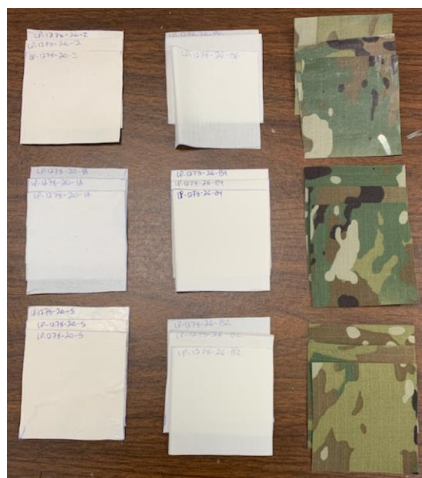


Figure 11. Cotton and NYCO samples cut from template for total weight measurements.

4.2.2 Mechanical Testing

Control and MI treated fabric samples were evaluated using an Admet eXpert 2611 dual column testing machine for breaking strength, (Figure 12). Fabrics submitted for mechanical testing were evaluated for breaking strength using a modified grab test as per ASTM D5034, section 9.3.

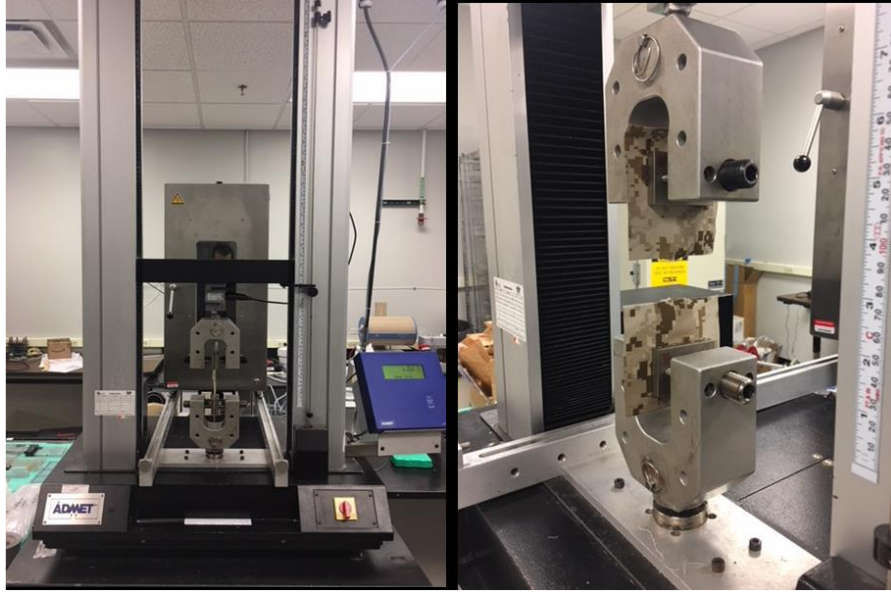


Figure 12. Luna's Dual Column Admet eXpert 2611, running a representative ASTM D5034 sample.

4.2.3 Air Permeability

Control and MI treated fabric samples submitted for mosquito bioassays and yielded bite resistance were submitted for air permeability following ASTM D737. The samples were evaluated by Clemson University, using a Testex FX 3300. The MI treated fabric samples were compared to untreated cotton to determine the percent change in the air permeability.



Figure 13. Representative Testex FX 3300 used at Clemson University for ASTM D737 air permeability testing.

4.2.4 *Moisture Vapor Transmission Rate*

Control and MI treated fabric samples submitted for mosquito bioassays and resulting in high bite resistance were tested for WVTR following ASTM E96. The selected samples were exposed to Luna's environmental chamber and tested at 32.2 °C and 50% relative humidity. The MI treated fabric samples were compared to untreated cotton to determine the percent change in the water vapor transmission rate.



Figure 14. Holders utilized for WVTR test inside Luna's environmental chamber.

4.2.5 *Wash Durability*

Control and MI treated samples were laundered according to AATCC 135. The samples were exposed to a cold cycle with detergent, followed immediately by a dry cycle in low heat. The cycles were consecutively repeated until the samples reached the desired amounts of wash-dry laundering cycles. The initial and final weights were observed to determine coating loss during washing. Samples with less the 5 percent weight loss were submitted to NCSU for mosquito bioassay testing in addition to Clemson University for air permeability testing.

5.0 Results and Discussion

Progress on the SEED program was enabled by the development of the MI fabric treatment in conjunction with evaluating textile properties such as total weight and air permeability to yield a wash durable bite resistance MI fabric treatment. The technology was based on previous work developed on textiles to generate a wash durable fabric treatment.^{8,9} The primary focus of the program was to utilize non-toxic materials, here mechanical insecticides, to provide protection against vector species while eliminating the need for chemical pesticides in clothing. In previous efforts, Luna developed a mechanical insecticide treatment for bed nets. Luna collaborated with NCSU Entomology Department for bioassay testing and established Imerys as a partner. Professor Michael Roe at NCSU Entomology Department had previous experience using mechanical insecticides for repellency.^{1,2} During the course of the program, Luna screened and down selected several mechanical insecticide candidates while optimizing the fabric treatment for wash durability and comfort. Bioassay testing focused on mosquitoes as the primary vector species with the goal of developing a bite resistant fabric. While the screening and down selection of mechanical insecticides was a key focus of the program, developing the proper application method to generate bite resistance of the fabric treatment was equally important.

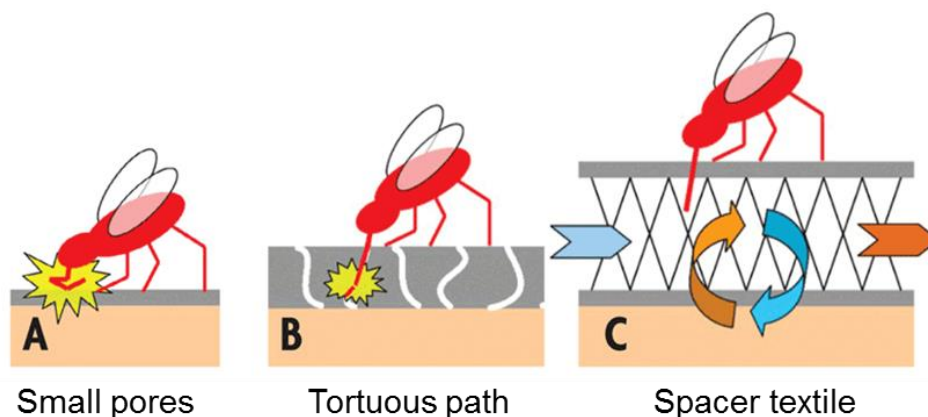


Figure 15 Three main strategies of bite-resistant clothing; strategy A - small pores; strategy B - tortuous path, and strategy C - thick spacer.

Figure 15 illustrates the three different approaches utilized to obtain protection from mosquito bites.¹⁰ The first approach and the most common uses insect repellents that are applied by spraying directly onto the human body or clothing. Thus, it does not consider the specific architecture of a textile substrate for mosquito repellency because in those protections the main factor influencing the level of repellency are chemical additives applied on the surface of these textiles. The second approach uses mosquito repellent coatings (textiles are coated, padded, or immersed), which contain chemical repellents in the form of, e.g., microcapsules.¹¹ This approach also does not require any specific substrate fabric structure, unless chemical substance in coatings would require one. The third approach, which physically blocks the access of mosquitos to the skin, considers textile architecture as a mean of countermeasure the mosquito bites. All the textile structures that are considered in this approach are preventing mosquitos from bites by blocking their access to the skin. The most common and typically used blocking structures are laminates with very small pores, protective layers with tortuous paths, and thicker layers called spacers, as presented in

Figure 15. Typical spacer fabric is too stiff to be considered for clothing due to inserted stiff ribs connecting two layers (top and bottom) of fabrics (woven or knitted). The best path to prevent mosquitoes from penetrating through the fabric is generating a fabric treatment or coating which generates the tortuous path depicted in Figure 15. Luna focused on developing an MI fabric treatment with bite resistance to mosquitoes employing the tortuous path approach while optimizing application methods to yield a comfortable and wash durable textile.

5.1 Task 1 – Establish Performance Criteria

List of Work Plan Tasks

Task 1. Establish Performance Criteria

Task 2. Develop mechanical insecticide coatings

Task 3. Validate textile properties

Task 4. Evaluate Insect resistance

Tier 1 - Bioassays (repellency and mortality in absence of host cues).

Tier 2 - Bioassays (mortality in cone test).

Tier 3 - Bioassays (repellency and mortality; in vitro assay with host cues).

Tier 4 - Bioassays (repellency and mortality; arm in cage).

The objective of this task was to develop the performance criteria necessary to evaluate the mechanical insecticide fabric treatment. While permethrin is applied onto NYCO uniforms, the fabric proved to be inherently bite resistant when tested at NCSU. Thus, Luna developed the technology on a light-weight, porous cotton. Since there are no specifications or metrics for direct comparisons to NYCO, Luna made performance comparisons to untreated cotton.

Luna's main objective was to develop a durable, bite resistant fabric using non-toxic mechanical insecticides. The fabric treatment was developed to maintain or improve the textile properties of the untreated fabric, here mercerized cotton. Luna aimed to evaluate key textile properties including breaking strength, air permeability, MVTR, drape, and wash durability. Luna took measures to reduce the impact the MI fabric treatment the mechanical properties of the fabric and evaluated the breaking strength of the MI treated samples. Results were compared to untreated fabric samples. In conjunction to evaluating the textile and mechanical properties of the MI fabric treatment, Luna targeted 80 percent bite resistance in bioassay testing Tier 3 and Tier 4. NCSU Entomology Department assessed samples with Tier 2, Tier 3, and Tier 4 mosquito bioassays. Tier 2 bioassay was modified to screen the repellency of the MI fabric treatment, resulting in useful allocating of resources. Tier 3 bioassay was conducted to determine the bite resistance of the MI fabric treatment prior to evaluating on a human subject. Tier 4 bioassay tested the MI treated fabrics on human subjects and was indicative of results in the field.

5.2 Task 2 – Develop Mechanical Insecticide Coatings

The objective of this task was to develop an insect resistant textile treatment with non-hazardous mechanical insecticides to provide protection against vector and vector-borne diseases without the use of chemical pesticides. Properties of interest included air permeability, drape, total weight, water vapor transmission rate, durability, tensile strength, and resistance towards vectors with a focus on mosquitoes. IRB Protocol Number 16897 did not allow bioassay testing on permethrin

treated fabrics, so no direct comparison could be made with these. However, NCSU provided results regarding permethrin treated t-shirts from previous work. On the present project, ACU NYCO fabric was initially screened and found to be inherently bite resistant. As a result, Luna developed the MI fabric treatment on a thin cotton fabric, due to its high porosity and inherently low resistance to mosquito bites. Luna took a material lacking vector protection and developed a water-borne non-toxic textile treatment, increasing the bite resistance of the fabric to nearly 100 percent. The following sections provide detailed results from textile development, application, and characterization.

5.2.1 *Selecting Mechanical Insecticides for Luna's MI fabric treatment*

Luna screened four mechanical insecticides recommended by Imerys - our partner and expert in the field, (Figure 16). The MI samples shown on the top row are diatomaceous earth particles with distinctly different shapes, size, and porosity. The MI samples shown on the bottom row are volcanic glass particles known as perlite and processed to yield a coarse material (S3) and a fine material (S4).

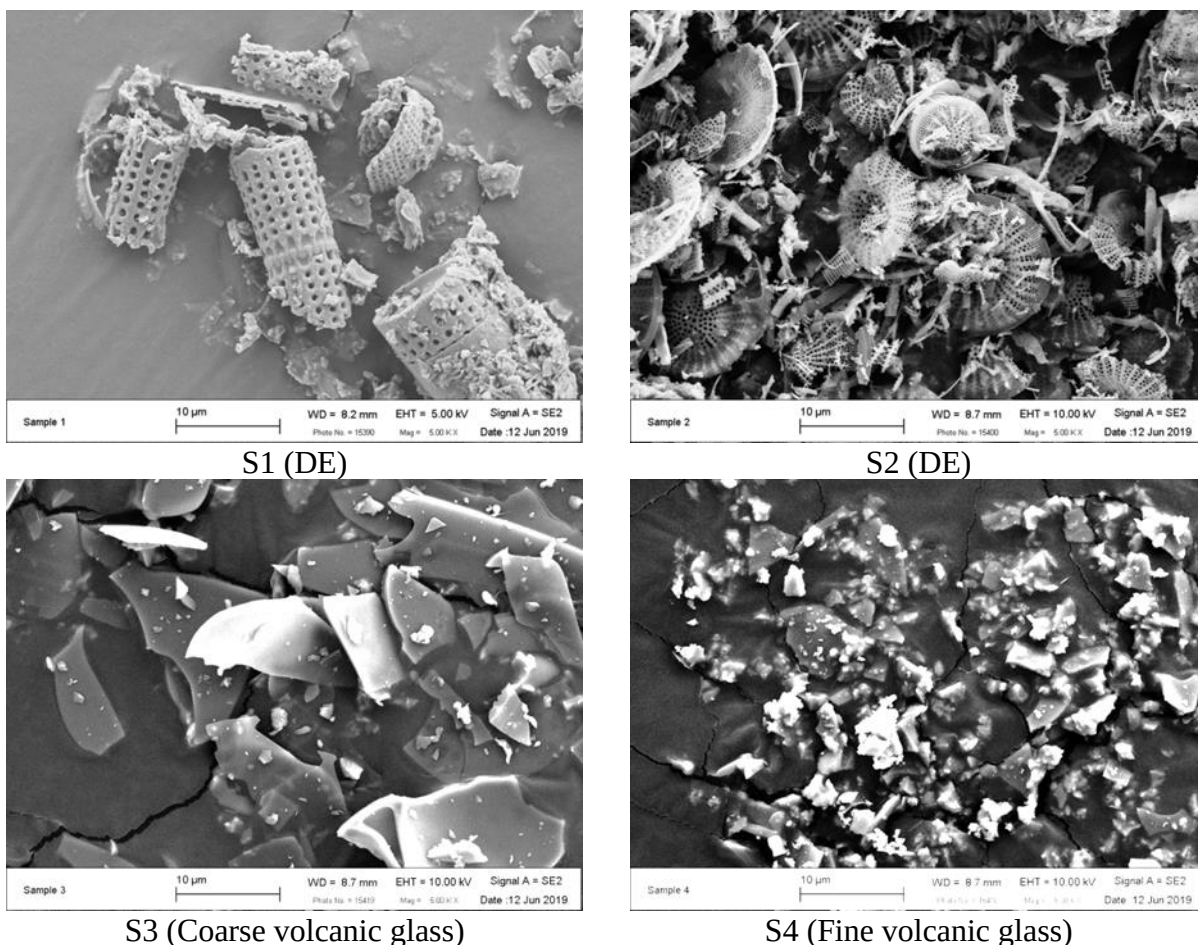


Figure 16. Mechanical insecticides screened for MI textile treatment.

Our collaborators in the NCSU Entomology Department, led by Professor Michael Roe, evaluated the mechanical insecticides via Tier 2 bioassay. The bioassay was set up so that only half the fabric was coated with the mechanical insecticide. Each mechanical insecticide was tested with the Tier 2 bioassay and the mosquito landings were observed to determine which mechanical insecticide

was most repellant. While the mechanical insecticides tested similarly, Luna selected a diatomaceous earth material (S2) and the fine perlite material (S4) to continue the fabric treatment and application development. It was expected that the selected materials would provide the tortuous path required to add mosquito bite resistance to the fabric.

5.2.2 Mechanical Insecticide Textile Treatments and Application Development

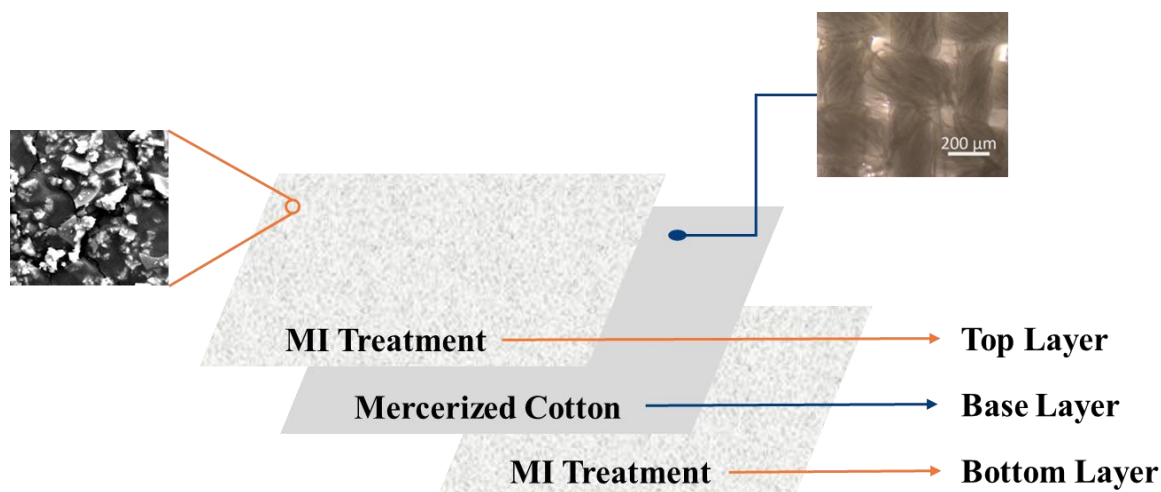


Figure 17. Schematic representing Luna's mechanical insecticide fabric treatment to generate tortuous path for vector protection.

Luna developed a mechanical insecticide textile treatment, focusing on generating a tortuous path for the mosquito's proboscis, thereby hindering the proboscis from penetrating through the fabric to the user, Figure 17. Developing an application method for the textile treatment to yield a successful mosquito resistant fabric was a key objective during the course of the program. Luna investigated standard textile treatment application methods commonly found in textile mills, including dip-pad, spray, and blade application (Figure 5). Each application method was evaluated at NCSU for mosquito bioassay testing to determine the efficacy and reproducibility of the application method provided the fabric. The total weight, air permeability, and tensile strength were tracked in conjunction with bioassay testing.

Table 2. Overview of Luna's MI fabric treatments, application, and general bioassay results

Generation #	Treatment	Application	Bioassay Results (Arm in cage)
1	Luna's Fabric Treatment 1	Dip-pad	Not Reproducible
2	Luna's Fabric Treatment 1	HVLP	Inconsistent
3	Luna's Fabric Treatment 2	HVLP	~90% Bite Resistant
4	Luna's Fabric Treatment 2	Blade	~90% Bite Resistant

Luna's Fabric Treatment 1 is an omniphobic fabric treatment with a blocked cross linker. The cross linker unblocked during the oven cure stage and reacts with the surface functionality of the mechanical insecticide, Figure 18. Fabric treatment 1 was initially applied via the dip-pad method, where the fabric is run through a bath with the textile treatment and padded 30 psi to reveal a

homogenous and even coating. After padding, the fabric was transferred to an oven to cure at 165°C for 8 minutes. While dip-pad is a commonplace in industry, the application method did not yield favorable results. The mechanical insecticides settled in the bath, generating inconsistency between treated samples and differences in weight pick up. The lack of reproducibility quickly led to the evaluation of other application methods: spray and blade.

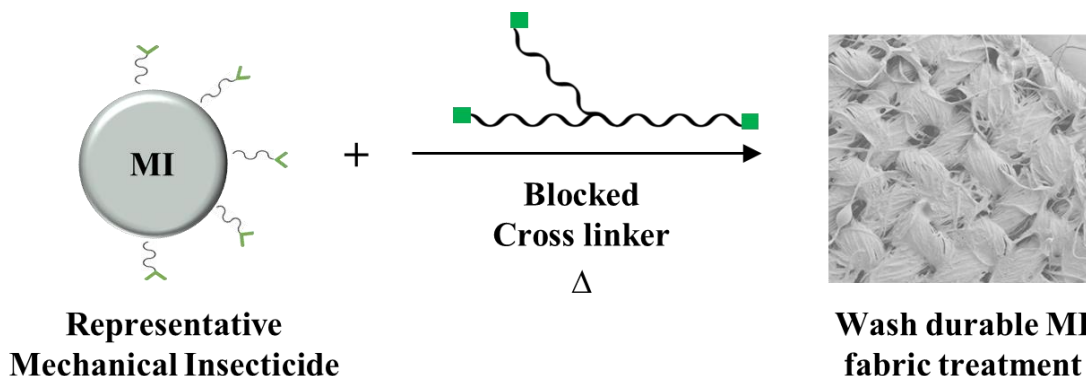


Figure 18. Schematic representation of crosslinking with a mechanical insecticide to yield a wash durable MI fabric treatment.

Luna transitioned Fabric Treatment 1 from dip-pad to application using a High Volume, Low Pressure (HVLP) spray system. The new application method required the evaluation of the spray conditions, including the pressure flowing through the lines to spray the MI fabric treatment, the nozzle tip, and the solids of the fabric treatment to generate a fabric treatment with evenly dispersed MIs. While it was easier to generate fabrics with similar weight uptake using the spray application method, the mechanical insecticides were not evenly dispersed on the fabric, thereby generating inconsistent mosquito bioassay results. In addition, the spray application resulted in fabrics with high weight uptake.

Luna developed a second fabric treatment more compatible with the selected mechanical insecticides (S2 and S4), improving settling of the particles during application. Luna's Fabric Treatment 2 was initially applied using spray application. While the particles were well dispersed and the coating was even, the weight uptake of the fabric treatment was high and affected the air permeability of the treated fabric. To decrease the weight uptake of the fabric treatment, Luna explored doctor blading. Doctor Blading allowed the textile treatment to be spread across the fabric using a blade with set gap to apply an even coating, Figure 5. The samples generated with the latest method were more reproducible and allowed for optimization of Fabric Treatment 2. Luna reduced the MI percent solids of the fabric treatment from 40 percent solids with the dip-pad method to 7 percent solids with doctor blading.



a) HVLP spray

b) Blade

c) Cotton Control

d) Army NYCO

Figure 19. Drape Comparison of Luna Fabric Treatment 2 as applied with HVLP spray and blade methods.

The application method directly impacted the total weight (oz/yd²) and drape of the treated fabric. HVLP spray application generated a thicker coating than blade application methods. While a thicker coating imparted favorable bioassay results and generated a high bite resistant fabric, the textile properties were compromised. As is shown in Figure 19, HVLP spray application affected the total weight and consequently, the drape of the treated fabric. The drape and total weight was improved by applying the MI fabric treatment with a blade. The MI treated fabric has comparable drape to both untreated cotton and Army NYCO. While the mechanical insecticides are a key component to developing a bite resistant material, the particles may generate unfavorable textile properties, such as a reduction in air permeability and increased total weight. Luna optimized the fabric treatment by adjusting the solids of the fabric treatment and optimizing the doctor blading application.

Luna has a commercial product utilizing similar polyurethane chemistry as this project with minimal changes in air transmission since it is a conformal coating around textile fibers. This polymer system (with the addition of MI) was added to the fabric at a higher concentration than the previous system. This was done with the goal to provide more of the MI at the fiber interface presumably where the mosquito proboscis would penetrate. While excellent bite resistance was observed, the system was not optimized for MI loading or fabric weight uptake. It is the goal for future work to achieve better air permeability with lower weight uptake, while maintaining bite resistance.

5.3 Task 3 – Validate Textile Properties

Luna evaluated the effect of the fabric treatments and application methods on important textile properties, including total weight, water vapor transmission rate, and air permeability.

5.3.1 Total Weight

Luna developed several MI fabric treatments on mercerized cotton. The MI fabric treatments were applied with three distinct application methods commonly found in industry: dip-pad, spray, and blade. Luna evaluated all three application methods for total weight, air permeability, and favorable results with mosquito bioassay testing. The most durable and successful MI fabric

treatment developed was Luna Fabric Treatment 2. To this fabric treatment was added mechanical insecticides at different loading levels (weight percent). Luna aimed to narrow down the MI loading level to minimize the total weight of the fabric.

Table 3 A summary of select fabrics treated with Luna Treatment 2 and corresponding total weight results, as per application method. NYCO highlighted in green and mercerized cotton in orange

Sample ID	Treatment	MI Loading (wt. %)	MI	Application	Total Weight (oz/yd ²)	Total Weight (% Increase)
NYCO	None	N/A	N/A	N/A	6.59	N/A
FM-1295-58-A1	Luna Fabric Treatment 2	N/A	N/A	Blade	8.25	25.2
FM-1295-58-A2	Luna Fabric Treatment 2	7	S2	Blade	8.54	29.6
FM-1295-58-A3	Luna Fabric Treatment 2	7	S4	Blade	8.47	28.5
Mercerized Cotton	None	N/A	N/A	N/A	3.28	N/A
LP-1278-26-C11	Luna Fabric Treatment 2	N/A	N/A	Blade	4.39	33.8
LP-1278-26-B2	Luna Fabric Treatment 2	7	S2	Blade	4.79	46.0
LP-1278-26-D2	Luna Fabric Treatment 2	7	S4	Blade	4.99	52.1
LP-1278-26-B4	Luna Fabric Treatment 2	15	S2	Blade	5.67	72.9
LP-1278-26-D4	Luna Fabric Treatment 2	15	S4	Blade	5.40	64.6
LP-1278-21-B	Luna Fabric Treatment 2	15	S2	HVLP (40 psi)	5.61	71.0
LP-1278-23-B	Luna Fabric Treatment 2	15	S2	HVLP (20 psi)	5.62	71.3
LP-1278-20-2	Luna Fabric Treatment 2	15	S2	HVLP (20 psi)	6.78	106.7
LP-1278-20-5	Luna Fabric Treatment 2 (Washed 6x)	15	S2	HVLP (20 psi)	6.76	106.1

The MIs evaluated were S2 (diatomaceous earth) and S4 (fine perlite) (Figure 2). While both mechanical insecticides were added to Luna's base resin fabric treatment solution, the densities of the each were different. The S2 diatomaceous earth sample was less dense than the fine perlite sample, thus, required more volume than sample S4. To apply the MI fabric treatment, Luna explored traditional textile application methods to apply the MI fabric treatment onto mercerized cotton. A comparison to 50/50 nylon/cotton (NYCO) was made for reference. Luna was able to

decrease the weight pick up of Luna Fabric Treatment 2 by switching from HVLP spray to doctor blading as the application method. The total weight of the fabric was influenced by the application method, the selected mechanical insecticides, and the fabric type. Table 3 depicts the dependence of total weight based on the fabric type. NYCO increased in weight by 25% while mercerized cotton increased in weight by almost 34%. The addition of mechanical insecticides influenced the total weight as well, as samples with S2 resulted in slightly higher total weights. During the technical reporting period of the program, Luna was able to reduce the total weight significantly from initial coating applications with HVLP spray (20 psi), to HVLP (40 psi), and ultimately, using a blade for an even, well-dispersed coating. Based on the preliminary results, Luna's team is confident that fabrics with lower add on weight can yield the desired insect resistance and physical properties. More importantly, the samples treated with the lower MI loading of 7 weight percent performed favorably at NCSU. If given the opportunity, Luna would continue to narrow down the optimal window between total weight and successful bioassay results.

5.3.2 Mechanical Testing

Control and MI treated fabric samples were evaluated using an Admet eXpert 2611 dual column testing machine for breaking strength (Figure 12). Fabrics submitted for mechanical testing were evaluated for breaking strength using a modified grab test as per ASTM D5034, section 9.3. The breaking strength of the treated material was established and compared to untreated controls. The control samples included bare mercerized cotton and treated cotton without the presence of mechanical insecticides, giving insight into how the added MI may affect the mechanical properties of the fabric, separate from the binder of the fabric treatment.

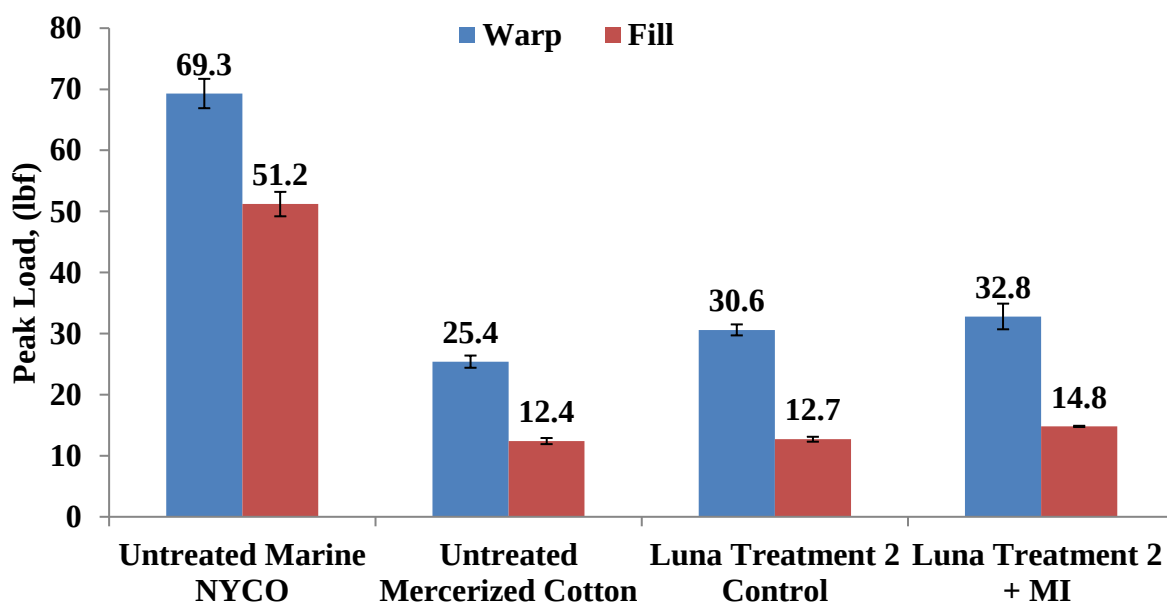


Figure 20. Comparison of breaking strength values of Luna Treatment 2 control to MI (S2) Luna treated cotton sample as applied via HVLP spray.

The mercerized cotton fabric is a woven and is expected to yield higher peak load in the warp direction as opposed to the fill direction. The mercerized cotton was treated via HVLP at 40 psi with Luna Treatment 2 Control, a base resin without the addition of mechanical insecticides. Figure 20 compared Luna Treatment 2 treated samples to Luna Treatment 2 Control samples and

untreated mercerized cotton and Army NYCO to determine if the mechanical insecticides were altering the mechanical properties of the fabric.

Luna MI treated samples displayed an increase in mechanical strength in both the warp and fill direction. Luna Treatment 2 Control increased the mechanical strength in the warp direction by 20.5 percent and in the fill direction by 2.4 percent. The addition of mechanical insecticides (here S2) further increased the mechanical strength, yielding a 29.1 percent increase in the warp direction and 19.4 percent increase in the fill direction. The loading of the mechanical insecticide in Luna's MI Fabric Treatment was 15 wt. %. The treated samples evaluated for breaking strength were all generated using HVLP spray as the fabric treatment application method. While spray applying yields a more homogenous coating, there was a significant weight pick up. Luna optimized the application method to doctor blading to maintain favorable bioassay results while reducing the weight pick up. Luna tested the new application method to HVLP spray at the same MI loading (wt. %) and consecutive lower MI loadings.

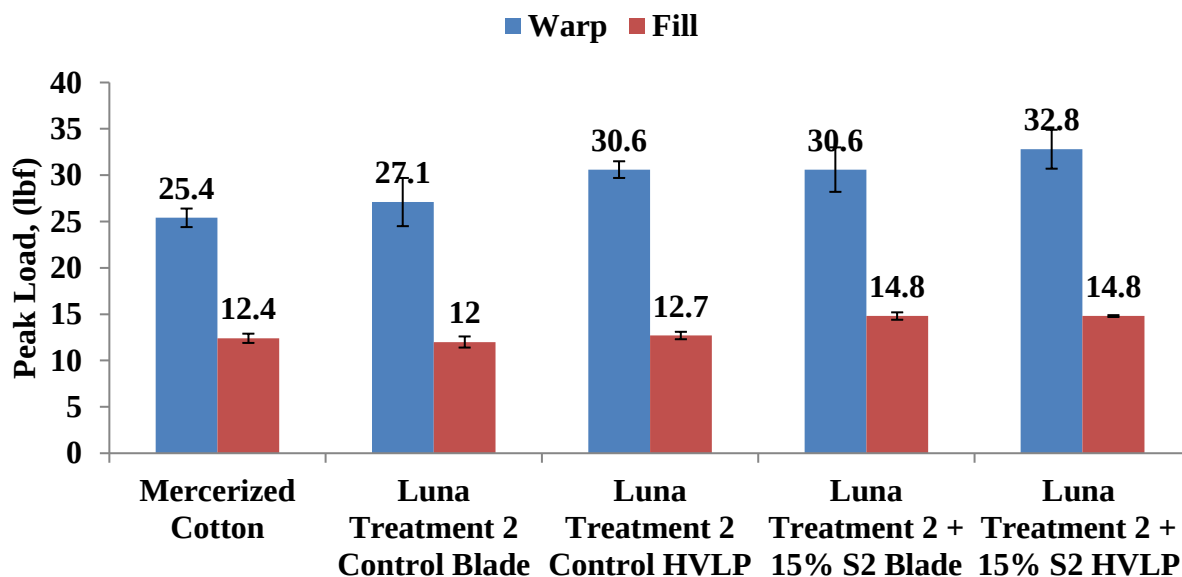


Figure 21. Comparison of breaking strength values of Luna Treatment 2 control to MI (S2) Luna treated cotton samples as applied via HVLP and blade applications.

The correlation between application method to resulting mechanical properties is visible in Figure 21. To decrease the total weight of the fabric treatment, Luna switched application methods from HVLP spray to blade application. Samples applied with a blade have a lower impact on the breaking strength of the bare mercerized cotton than HVLP spray application. It is important to note that regardless of the application method, no loss in mechanical properties was observed. Rather, there was an increase in breaking strength with Fabric Treatment 2.

5.3.3 Air Permeability

Control and MI treated fabrics were evaluated for air permeability at NCSU using a Testex FX 3300. The air flow (cfm) was measured in three different locations, allowing for average and error.

Table 4 Comparison of air permeability (ASTM D737) of Fabric Treatment 2 with mechanical insecticides S2 and S4 loaded at 7% and 15%

Sample	Cotton Control	Fabric Treatment 2 (0% MI)	7 wt. % S2	7 wt. % S4	15 wt. % S2	15 wt. % S4
Application	None	Blade	Blade	Blade	Blade	Blade
Air Flow Average (cfm)	66.6	42.58	4.99	55.12	0.24	26.08
St. Dev.	4.36	11.27	1.61	7.20	0.02	2.90
Total Weight (oz/yd²)	3.3	4.6	4.8	5.0	5.7	5.4

Samples treated with Luna MI Treatment 2 and applied with a blade were submitted for air permeability. The samples were made with different MI loadings (7 wt. % and 15 wt. % of the fabric treatment). The air permeability results were compared to a sample made with unfilled Luna Treatment 2 to determine the effect of the MIs on the fabric treatment, Table 4. Luna's unfilled Fabric Treatment 2 reduced the air flow from 66.6 cfm to 42.6 cfm. The addition of mechanical insecticides further reduced the air flow. However, MI S4 had less of an impact than MI S2, reducing the air flow from 66.6 cfm to 55.1 cfm. The total weight of the samples was shown to show the correlation between total weight and air permeability. Luna demonstrated the total weight was a direct result of application method (Table 3). As such, Luna can further decrease the total weight in future optimization studies to continue to improve the air permeability of the system.

Table 5 Air permeability of spray and blade application methods compared to untreated NYCO and untreated cotton controls.

Sample	Untreated NYCO	Untreated Cotton	15 wt. % S2	15 wt. % S2	7 wt. % S2
Application	N/A	None	Spray	Blade	Blade
Air Flow Average (cfm)	10.3	92.86	1.02	0.24	4.99
St. Dev.	0.61	1.53	0.83	0.02	1.61
Tier 4 Bite Resistance (%)	100	2	98	98	96
Total Weight (oz/yd ²)	6.59	3.28	6.78	5.7	4.8

Fabrics treated with Luna MI Treatment 2 generated different air flow results. The application method impacted both the total weight and the air permeability of the treated fabrics. The samples were tested at NCSU for mosquito bioassays and resulted in high bite resistance ratings, close to 100 percent bite resistance. Untreated Army NYCO is inherently bite resistant with a total weight of 6.59 oz/yd² and air flow average of 10.3. Luna's MI Treatment 2 samples have comparable total

weights. The air permeability was improved from 0.24 cfm to 4.99, while maintaining a high bite resistance rating of 96 percent.

5.3.4 Moisture Vapor Transmission Rate

Moisture vapor transmission was measured according to ASTM E96. Samples were tested in Luna's environmental chamber at 26.7 °C and 50% relative humidity.

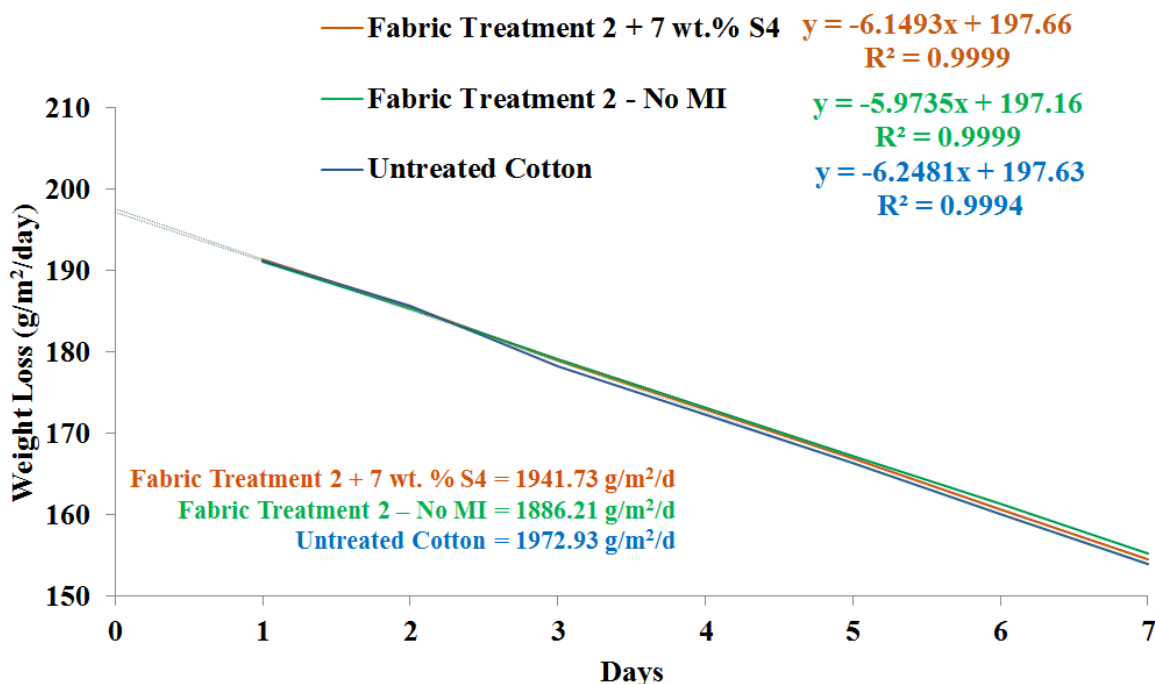


Figure 22. ASTM E96 moisture vapor transmission of Luna's MI Fabric Treatment 2 compared to a fabric treatment without MIs and untreated cotton.

Each sample for moisture vapor transmission was run in triplicates and averaged. The mass loss was observed and plotted against time. All samples experienced a loss in mass, including the untreated control sample, corresponding to the permeability of the fabric. All samples maintained similar moisture transmission values. The base Fabric Treatment 2 reduced the moisture transmission by 4.40 percent. Fabric Treatment 2 with the addition of S4 mechanical insecticide only reduced the moisture transmission by 1.58 percent. Fabric Treatment 2 with the incorporation of S4 mechanical insecticide did not negatively impact the moisture vapor transmission as compared to untreated control sample.

5.3.5 Task 3 – Wash Durability

Luna washed blade-treated samples with Luna MI Treatment 2 to determine the durability of the fabric treatment after washing. The sample labeled control was treated with the base fabric resin without any mechanical insecticides. The samples underwent wash/dry cycles as per AATCC 135 with the following conditions: cold wash cycle with detergent followed by low temperature dry cycle. The samples were washed/dried until the desired number of cycles was attained. After equilibrating for at least 4 hours, the weight uptake was recorded.

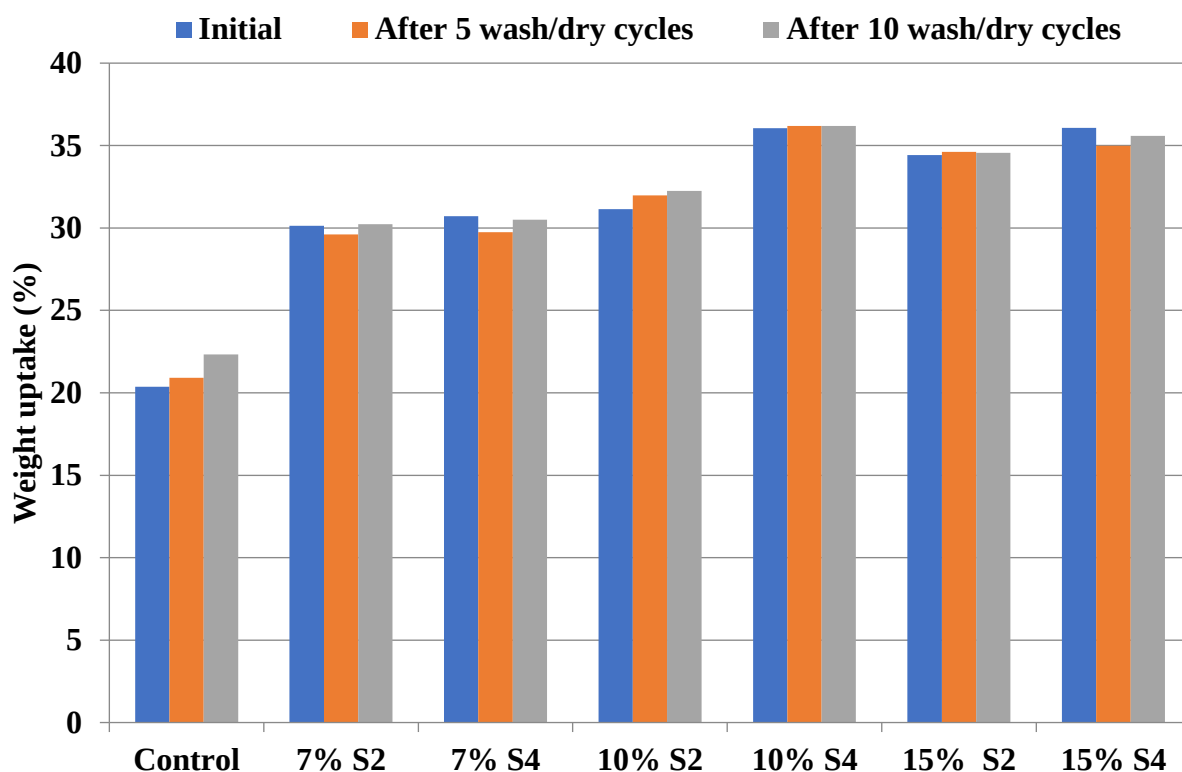


Figure 23. Weight loss comparison of Luna MI Treatment 2 with different MI loadings to control treated fabric without MI loading.

As shown in Figure 23, Luna MI Treatment 2 is wash durable up to 10 wash cycles. To determine the wash durability of the fabric treatment, the treated samples were exposed to consecutive wash cycles. The weight of the washed sample was compared to its initial weight to generate the weight uptake (%) result. The same samples laundered to five wash/dry cycles were laundered to the final 10 wash/dry cycle. Overall, the samples demonstrated remarkable wash durability, with less than four percent weight loss. In most cases, there was an increase in weight. Due to its porous nature, it was expected for mechanical insecticide S2 to absorb moisture from the surroundings more than mechanical insecticide S4. However, both S2 and S4 show stability and durability after washing. The samples were submitted for mosquito bioassay testing at NCSU to evaluate the bite resistance of the laundered samples as compared to unwashed treated samples.

5.4 Task 4 – Evaluate Insect Resistance

5.4.1 Bioassay Tier 2

Traditionally, the funnel test is conducted to evaluate fabric treatments for mortality. When testing for mortality, the cone utilized to entrap the vector species is placed directly over the treated sample. NCSU Entomology Department modified the funnel test to screen for repellency while testing for mortality. In the modified funnel test, only half the sample contains a treatment and the other half is untreated mercerized cotton. Luna utilized the funnel test at the beginning of the program to down select from four possible mechanical insecticides, two diatomaceous earth samples and two perlite samples.

To evaluate the repellency of the four mechanical insecticides, 10 Female *Aedes aegypti* were placed into cones over cotton fabric. One half of the fabric had been treated with a mechanical insecticide at an amount of 50 mg/² in. Location of the mosquitoes were noted and classified as on the untreated, treated, or top sides. The top side was any mosquitoes not on the treated or untreated sides. The cones were tapped after each reading to determine any death. After each replicate, the fabric was rotated 90 degrees clockwise to eliminate any directional bias.

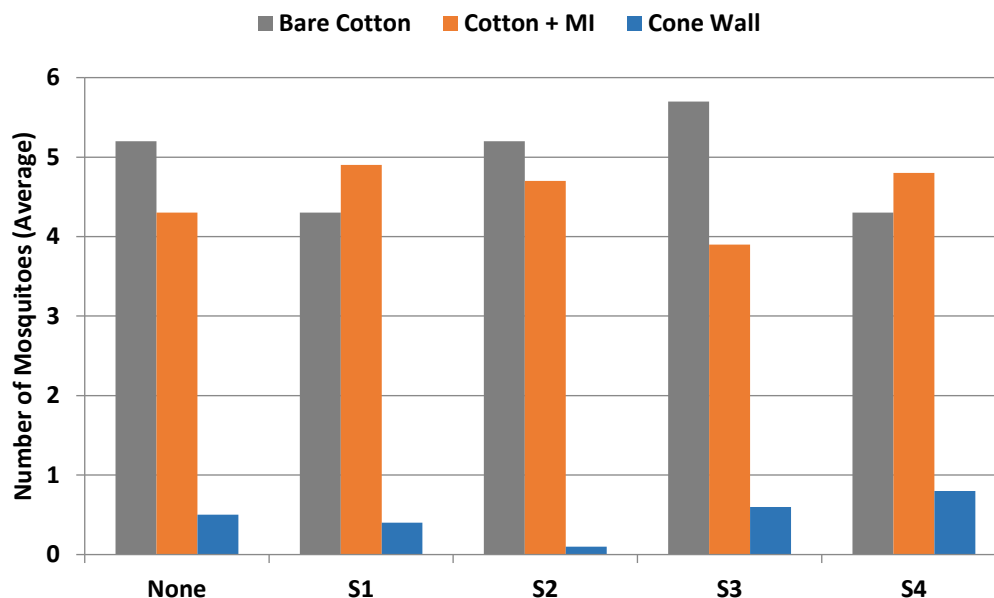


Figure 24. Funnel test results utilized to determine the repellency capabilities of the recommended mechanical insecticides, S1, S2, S3 and S4.

The funnel test results are shown in Figure 24. All ten mosquitoes were tracked during a 30 minute period and their location observed. The number of mosquitoes present at each select location was recorded every five minutes and averaged at the end of the 30 minute bioassay. The results of the funnel test indicate no repellency from the mechanical insecticides. The mosquitoes did not preferentially land on the bare cotton as opposed to the mechanical insecticide covered half. While the results were unexpected, the lack of repellency may be an added value to the MI fabric treatment. Preliminary results at NCSU offered by Professor Roe indicate that while permethrin treated fabrics repel mosquitoes from the fabric. The mosquitoes are re-directed to exposed skin to feed, thereby generating more bites in practice. The lack of repellency of the mechanical insecticide and resulting MI treated fabric keeps mosquito's attention to the fabric, which continually protects the user for the probing proboscis of the mosquito. Since the mosquitoes are not repelled, they are not re-directed to exposed skin, resulting in fewer bites in a practical real-world environment. NCSU Entomology Department continued testing of all four mechanical insecticides in Tier 3 bioassay testing, in continued efforts to study the repellency and bite resistance of the mechanical insecticides.

In addition to testing for mosquitoes, NCSU tested Luna MI Treatment 2 against blow flies to determine mortality. Adult blow flies spread pathogens and food-borne illnesses. Blow flies are known for carrying the bacteria that causes dysentery, typhus, and cholera. A modified cone test was used to test three different types of cloths against blow flies, *Cochliomyia macellaria* : Control;

cloth containing 15% mechanical insecticide S2; cloth with 15% mechanical insecticide S4. Each type of cloth was placed in a tray and ten 8-10-day-old adult flies, mixed sexes were anesthetized for 10 seconds and placed on the cloth. A glass funnel (cone) was then placed up-side-down onto the cloth. The insects were then incubated at 30 °C and 50 ± 5 % relative humidity, and mortality assessed at different times thereafter. Three replicates were run per treatment.

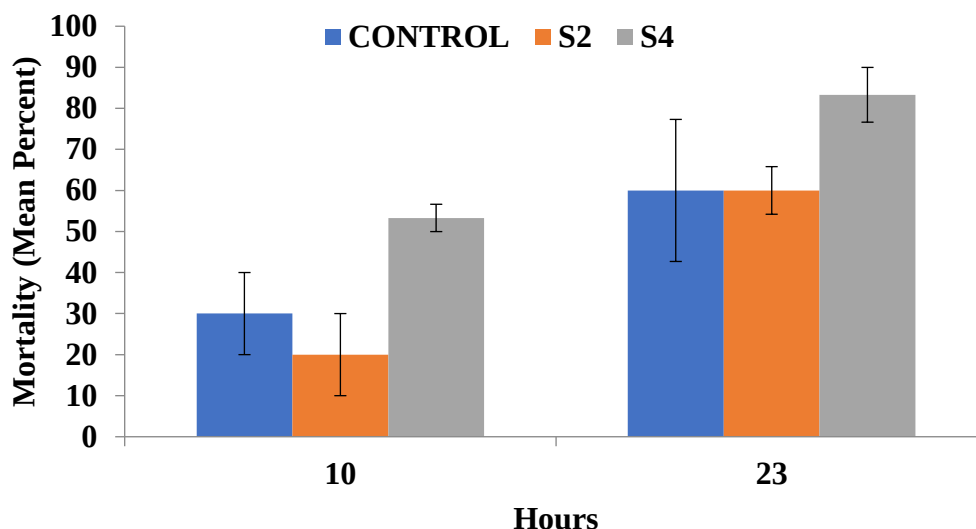


Figure 25. Percent mortality of blow flies *C. macellaria* exposed to three cloths: Control, Luna Fabric Treatment with S2 and Luna Fabric Treatment with S4.

The overall mean percent mortalities (mean ± SE) are shown in the graph below. No mortality was observed after 1h exposure. At 10h, mean percent mortalities were 30 ± 10, 20 ± 10 and 53 ± 3.33 % for Control, Luna Fabric Treatment with S2 and Luna Fabric Treatment with S4. Mortality increased over time for all treatments. At 23h, percent mortalities were 60 ± 17.3, 60 ± 5.8 and 83.33 ± 6.7 % for the same treatments. While the focus of this program was to show resistance against mosquitoes, Luna was able to expand the scope to show evidence for mortality against blow flies. The treatment was not optimized to generate mortality against this particular vector species. However, Luna has the capabilities to yield a fabric treatment with resistance towards several vector species, given the opportunity.

5.4.2 Bioassay Tier 3

Tier 3 bioassay testing is commonly used to screen for the more stringent and telling Tier 4 arm in cage bioassay, which required the use of a human test subject. Tier 3 in vitro bioassay requires the use of ATP-doped porcine blood to flow through a collagen tube, which is warmed to internal body temperature to entice bites from mosquitoes.

Table 6. Tier 3 In vitro results indicating bite resistance of Luna Fabric Treatment 1 with mechanical insecticides applied with a padder, where bite resistance is defined at 80% or higher.

Fabric Treatment 1 + MI	MI Loading (wt. %)	Number Mosquitoes	Number Landings	Bite Resistance (%)
None	None	100	167	2
Control	0	100	190	74
S1	40	100	117	98
S2	40	100	150	100
S3	40	100	90	99
S4	40	100	36	100

Luna tested Fabric Treatment 1 as applied with a padder at 30 psi, in a bath composed of a fabric treatment base resin, Luna crosslinker, and 40 weight percent mechanical insecticide. The intention of the high loading was to find the upper limit of the fabric treatment. Fabric treatment 1 performed optimally in Tier 3 bioassay testing, with all mechanical insecticides generating a tortuous path for the mosquito proboscis. NCSU Entomology Department defines a textile bite resistance at 80 percent or higher. As such, Luna's fabric treatment 1 with both diatomaceous earth and perlite mechanical insecticides are considered bite resistant after the treatment was applied. The base fabric treatment without mechanical insecticides tested at 74 percent bite resistance, increasing the bite resistance from the bare untreated cotton, which is not bite resistance at a mere 2 percent. The addition of the mechanical insecticides to the base fabric treatment provided close to a 30 percent boost in performance. Due to the time and energy required to grow, harvest, and test the fabrics with mosquitoes, Luna down selected the mechanical insecticides to sample S2, a disk-shaped diatomaceous earth, and S4, fine perlite.

During this time, Luna encountered reproducibility issues with the application of the textile treatment using the padder. As a result, Luna evaluated alternate application techniques to yield a homogenous MI treatment of cotton. NCSU tested fabrics with the second application technique to confirm the improvement of bite resistance over the control mercerized cotton.

5.4.3 Bioassay Tier 4

Tier 4 bioassay involved the use of human subjects and is the most accurate representation of mosquito resistance in the field. Tier 3 in vitro testing is representative of the results typically observed with Tier 4 arm in cage, however, more inconsistencies can be seen with in vitro. As such, all samples with ratings above 80 percent resulting in a bite resistant material were verified using Tier 4 arm in cage (Table 7).

Table 7. Comparison of Tier 4 Arm in cage results to Tier 3 In vitro, verifying bite resistance of Luna Fabric Treatment 1 with mechanical insecticides, where bite resistance is defined at 80% or higher. Treatment was dip-pad applied.

Fabric Treatment 1 + MI	MI Loading (wt. %)	Number Landings	Tier 3 Bite Resistance (%)	Tier 4 Bite Resistance (%)
None	None	167	2	2
Control	0	28	74	78
S1	40	16	98	95
S2	40	21	100	92
S3	40	23	99	89
S4	40	24	100	92

Tier 4 arm in cage bioassay testing verified the bite resistance of Luna's MI fabric treatment 1. The dip-pad method was utilized to apply the coating onto mercerized cotton. While the initial results at NCSU Entomology Department were favorable, they were not consistently reproducible. The lack of reproducibility led Luna to explore new application methods known in industry, including spray application and doctor blading. During this time, Luna evaluated the wash durability of MI Fabric Treatment 1, finding poor wash durability results after 5 wash cycles. Luna was able to optimize the wash durability of the fabric treatment by modifying the cross linker, generating Luna MI fabric treatment 2. Luna MI fabric treatment 2 also allowed the mechanical insecticide particles to remain in suspension, generating a better reproducible formulation.

Due to application inconsistencies with the padder, Luna transitioned its application method to HVLP spray. Luna sprayed and evaluated both MI Fabric Treatment 1 and MI Fabric Treatment 2. Overall, MI Fabric Treatment 2 sprayed more consistently, dispersed the mechanical insecticides better, and was more wash durable. The total weight and air permeability of the HVLP samples were surprisingly high.

Luna quickly transition the blade applying the MI fabric treatment. Blade allows for a thinner, more controlled coating. While air permeability requires further optimization, the blade application method led to a natural decrease of the MI loading (weight percent) in the fabric treatment. NCSU tested the improved application method with lower MI loadings of 15 wt. percent, 10 wt. percent, and 7 wt. percent. Both diatomaceous earth and perlite samples were evaluated at the fore mentioned loading levels.

Table 8. Tier 4 bioassay comparison of 15 wt. % MI loaded samples, before and after washing

Fabric Treatment 2 + MI	MI Loading (wt. %)	Wash Cycles	Number Landings	Tier 4 Bite Resistance (%)
Control	0	0	21	71
Control	0	10	61	11
S2	15	0	54	98
S2	15	10	260	90

NCSU evaluated S2 diatomaceous earth samples that had been exposed to 10 wash/dry cycles, under normal wash conditions and dried at low heat, Table 8. The wash cycles included the use of detergent. After 10 wash cycles, the control samples treated with Luna Treatment 2 without mechanical insecticides dropped from 71 percent bite resistant to 11 percent bite resistant. A similar trend was seen with the MI samples. Luna Fabric Treatment 2 with S2 mechanical insecticide dropped from 98 percent bite resistant to 90 % bite resistant. While the fabric samples were still considered bite resistant, there was a noticeable drop after washing, Table 8. Luna will continue to optimize the cross linker and MI loading to improve wash durability in future work.

Table 9. Tier 4 Arm in cage mosquito bioassay evaluation of MC NYCO as compared to a Nylon 6 NW and mercerized cotton.

Fabric	Wash/dry cycles	Number of Mosquitoes	Number Landings	Bite Resistance (%)
Mercerized Cotton (MC)	0	50	167	2
Nylon 6 NW	0	100	223	93
Nylon 6 NW	5	100	119	95
MC NYCO	0	100	52	100
MC NYCO	5	100	447	100

6.0 Conclusions and Implications for Future Research

During the technical effort of the program, Luna was able to demonstrate the feasibility of developing a fabric treatment based on mechanical insecticides that was wash durable at least 10 laundering cycles, and maintained or improved the breathability and mechanical properties of the fabric. The fabric treatment was developed using Luna's water-borne textile treatment, without the use of any chemical pesticides or hazardous solvents. Luna evaluated two MI fabric treatments and optimized the application by testing three different application methods: dip-pad, spray, and blade. Luna was able to reduce the total weight and increase the air flow by controlling the application method.

Luna evaluated four mechanical insecticides at NCSU Entomology Department for repellency and mortality. Luna found the mechanical insecticides did not repel mosquitoes nor did they impart mortality. However, the mechanical insecticides generated a tortuous path for the mosquito proboscis, hindering the mosquito from biting through the fabric and reaching the user. Luna selected mechanical two insecticides to their ability to best hinder mosquitoes from penetrating through the fabric.

Luna tested MI treated samples and corresponding control samples for bite resistance and repellency at NCSU Entomology Department. Luna MI Treatment 2 performed well in Tier 3 and Tier 4 bioassays when spray applied or blade applied. While the total weight of these fabrics is relatively high, future work would include optimizing the total weight and air permeability while still maintaining resistance to mosquito biting. Luna MI Treatment 2 continued to display bite resistance in Tier 3 and Tier 4 testing after 10 laundering cycles when blade applied.

Luna was not able to directly compare MI fabric treatments to permethrin due to concerns of contaminating the bioassay equipment with permethrin, thereby risking future evaluations. Professor Roe offered insight to permethrin treated fabrics, stating that whereas permethrin treated

uniforms will repel mosquitoes, it drives them to exposed skin. Although the lack of repellency of the mechanical insecticide may seem like a negative result, the improved resistance of biting through the fabric actually results in fewer bites than wearing traditional permethrin treated uniforms.

The use of Luna's mechanical insecticide insect resistant fabric treatment offers several advantages: environmentally-friendly solution, REACH compliant, no VOCs are required to apply the treatment, and the application techniques are in line with industrial practices. Unlike permethrin-based insect fabric treatments, Luna's MI insect resistant fabric treatment is not limited to the trousers and jacket. T-shirts, socks, and caps can safely be treated and worn without health concerns to the user. Additional follow-on funding for Luna would be utilized to i) optimize and scale up the proven MI textile treatment and application process, ii) prototype textile and uniform production with partners in textile mills and uniform manufacturing, and iii) field validation of the prototypes through opportunistic collaboration with DoD colleagues.

Literature Citation

1. Stell, F. M., Roe, R. M., Arellano, C. & Apperson, C. S. Innovative Sugar-Insecticide Feeding Bioassay for Adult Female *Anopheles gambiae* (Diptera: Culicidae). *J. Med. Entomol.* **50**, 804–815 (2013).
2. Stell, F. M. *et al.* Proof of concept for a novel insecticide bioassay based on sugar feeding by adult *Aedes aegypti* (*Stegomyia aegypti*). *Med. Vet. Entomol.* **27**, 284–297 (2012).
3. “The application of diatomaceous earth for mosquito vector control to reduce the incidence of vector borne diseases”, Project Livingstone IVCC Meeting, March 9th, 2016
4. <http://www.motherearthnews.com/organic-gardening/pest-control/how-to-use-diatomaceous-earth-zw0z1304zkin.aspx>
5. <http://www.deq.state.or.us/er/docs/LowerBridge/DiatomaceousEarthFactSheet.pdf>
6. Luna. ‘Multifunctional Textiles for Advanced Personal Protective Equipment’ Department of Homeland Security First Responder BAA (2014-ST-108-FRG002).
7. Halbkat L., Luan K., Cave G., McCord M., Roe M., DenHartog E., Travanty N.J., Apperson C.S. & West A.J. Fabric infused with a botanical repellent for protection against mosquitoes. *J. Text. Inst.* **10**, 1468-1474 (2019)
8. Luna. *Multifunctional Textiles for Personal Protective Equipment*, DHS BAA (2014-ST-108-FRG002).
9. Luna. *Omniphobic Textiles for Military Uniforms*, US Army Phase II SBIR (W911QY-10-C-0071).
10. West, A. Mosquito Bite Resistant Maternity Clothing for Zika Virus Protection | Textile World. *Text. World* (2017).
11. Anuar, A. A. & Yusof, N. Methods of imparting mosquito repellent agents and the assessing mosquito repellency on textile. *Fash. Text.* **3**, (2016).