2.a. PROJECT SUMMARY

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Development of Novel Application Technology for the Control of the Asian Tiger Mosquito in Urban Environments

This is a Community IPM, Joint Research-Extension project to address the control of the invasive vector mosquito *Aedes albopictus* (the Asian tiger mosquito) in urban residential environments. The Asian tiger mosquito is responsible for most complaints to mosquito control programs. These programs rely on controversial and ineffective area-wide applications of broad-spectrum adulticides to combat this mosquito. While larvicides work effectively, it is impractical if not impossible to identify and treat all sources by hand. Without any efficient means of control, many mosquito control programs ignore this pest. The area-wide application of larvicides would solve the problem, but the technology has not been developed sufficiently for use in urban residential environments. This project will develop novel technologies for the area-wide low volume application of the bacterial larvicide *Bacillus thuringiensis* ssp. *israelensis* to control *Ae. albopictus*. We will develop this new biocontrol strategy by adapting low-volume truck-mounted adulticide equipment commonly in use by mosquito control programs to apply liquid *Bti* in an outdoor urban residential setting. After application the larvicide will drift and settle in containers of water where the larvae reside, providing quick, efficient, cost effective control. This IPM approach will reduce the abundance of this pest, the dependence on broad-spectrum adulticides, non-target impacts, and the development of resistance through the use of novel application technology, host-targeted applications of biological larvicides, and training programs for stakeholders. Efficacy of the program will be evaluated by monitoring larval mortality, adult presence, droplet size and distribution, and pesticide persistence. We will also prepare workshops and materials to train mosquito control personnel throughout the northeast and beyond in the use of this technology. The proposed project addresses the priorities of the Northeastern Integrated Pest Management Center's Public Health IPM Working Group, which include evaluation of novel IPM methodologies for vector-borne diseases, and the dissemination of IPM guidelines and educational programs for the control of insect vectors and vector-borne diseases. We are requesting $48,100.00 of P.L. 89.106 funds to support the research component and $11,900.00 of Smith-Lever funds to support the Extension component of the 3-year project.

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Development of Novel Application Technology for the Control of the Asian Tiger Mosquito in Urban Environments

2.b. PROJECT NARRATIVE

(i) Problem, Background and Justification

Problem: The Asian tiger mosquito, *Aedes albopictus*, is among the most invasive of all species, and without question the most invasive of all mosquitoes. Establishment in the continental United States originated from an introduction into Texas in 1985 via used tires shipped from Japan[1]. Within a decade, the species had spread to twenty states including New Jersey where it was collected from a coastal tire dump in 1995[2]. From central New Jersey, the mosquito spread across most of the state. In the northeast, it has been found in CT, DE, MD, NJ, NY, PA, and WV[3]. Today, thirty U.S. states are infested and the species continues to expand its range.

Adult *Ae. albopictus* are known as tiger mosquitoes due to their black bodies with distinctive white stripes. Naturally found in tree holes, this mosquito has adapted to utilize artificial containers as larval habitats in urban areas. Larvae will develop in almost any small vessel that holds standing water including discarded tire piles, catch basins, birdbaths, storm drains, flowerpots, rain gutters, cemetery urns, and cans and other trash. These habitats are especially abundant in urban neighborhoods. Adults in urban areas are found in shade near vegetation. The tiger mosquito is a day-biting mosquito that prefers attacking large mammals including humans and livestock, but also feeds on birds[4]. This vicious and persistent day-biting mosquito inflicts painful bites, induces dermatological and allergic manifestations, and significantly affects the quality of life and human well being[5]. In the U.S., it is regarded as the most important nuisance mosquito[6].

The introduction of *Ae. albopictus* into the U.S. was one of the most significant public health events of the past quarter-century. It has been found naturally infected with several serious human pathogens[3]. *Aedes albopictus* has been implicated as a bridge vector of West Nile virus, constituting the largest outbreak of mosquito-borne meningoencephalitis in the history of the Western Hemisphere. It can act as principal vector of dengue virus, dog heartworm, and chikungunya virus[7, 8]. It was responsible for a major epidemic of chikungunya, on Reunion Island in 2005 that resulted in more than a quarter of a million cases and 237 deaths. This painful and debilitating disease subsequently spread to India where 1.2 million cases were reported[9]. Most recently, 166 endemic cases were reported in northeast Italy in 2007[10]. The 2001-2002 outbreak of dengue in Hawaii, with 95 infections, demonstrates the potential of *Ae. albopictus* to transmit this disease in the U.S. via imported cases[11]. From 1986 to 2000, 516 confirmed and 2,128 suspected dengue cases were imported into the U.S.[12], mostly into areas of *Ae. albopictus* activity.

Unfortunately, conventional mosquito abatement methods do not affect *Ae. albopictus.* Many control programs that rely on ultra-low volume (ULV) fogging, apply materials in the evening or at night because that is the only time when temperature conditions are appropriate. The Asian tiger mosquito is not active during these periods, so they are unlikely to contact these insecticide droplets. Direct treatment of larval sources is impractical due to the innumerous microhabitats where *Ae. albopicuts* larvae can be found. Local mosquito control agencies across the nation mount aggressive control campaigns against salt marsh, floodwater, and many other rural-based mosquito pest species, but they rarely target the Asian tiger mosquito. Their efforts tend to be limited to distribution of leaflets to residents, placing the responsibility for control on unqualified citizens. The urban nature of this mosquito, where individual homeowners bear considerable responsibility for source reduction, has hampered control efforts. Individual residents are not usually able to coordinate their efforts with enough of their neighbors to eliminate the larval sources that contribute biting *Ae. albopictus* to a neighborhood. Currently, there is no effective means to control *Ae. albopictus* on an area-wide basis.

<u>Background</u>: *Bacillus thuringiensis* (*Bt*) is a gram-positive, spore forming, aerobic bacterium. Highly toxic to insects, many serovarieties of *Bt* are used for agricultural pests. *Bacillus thuringiensis* serovariety *israelensis* (*Bti*) was discovered in 1976 in dead *Culex pipiens* larvae found in a small riverbed pond in Israel[13]. Since that time, the efficacy of *Bti* has been demonstrated for many mosquito species in a variety of habitats. Today, formulations of *Bti* are the primary products used for larval mosquito control in the U.S. and other countries[14]. After ingestion by mosquito larvae, proteinaceous toxins are released which cause lysis in the epithelial cells of the larval midgut. *Bti* contains four different larvicidal proteins. These four proteins, all acting in slightly different ways, make the development of resistance to *Bti* very difficult[15-18]. In addition, the specificity of these proteins to mosquito larvae results in very few non-target impacts. With the exception of several families of Nematocera (many of them pest species), *Bti* has no effect on the vast majority of insects, other invertebrates, and vertebrates[19-27].

Normally applied as a solid directly to larval sources, several studies have demonstrated that *Bti* liquid can be applied as a low volume (LV) formulation to successfully control mosquitoes. However, no studies have provided data to suggest that this technique can be used to control *Ae. albopictus* in an outdoor, urban residential setting. VectoBac® WDG has been shown to give better control than VectoBac® 12AS when applied by ULV[28]. Most studies used 12AS[29-34] while only a few studies were conducted with WDG[28, 35-37]. This project will use the superior VectoBac® WDG product. While the majority of the studies focused on container mosquitoes, most used *Ae. aegypti* or other species in their studies[29, 30, 32, 33, 38] and only a few used *Ae. albopictus*[28, 31, 34-37]. In addition, the mosquitoes used in these studies were from laboratory colonies[28-34, 38] that may have much different toxicological profiles than natural populations. This study will use field strains of *Ae. albopictus*. Previous studies only utilized staged field trials where containers of larvae were artificially placed at the study sites[31, 37]. In addition, most of the previous studies were conducted in open fields or inside structures[28-30, 32-34, 38], not in our area of interest, around the outside of homes[31, 35, 37]. This study will utilize staged trials in open fields to collect preliminary data, but those data will be extrapolated to conduct field trials under real-world conditions around the exterior of homes in urban neighborhoods. The equipment used in the previous studies also presents a problem as many of the studies were conducted with thermal ULV units[28, 33], portable ULV machines[32, 35, 37], or obsolete truck mounted units[30]. This study will focus on truck mounted cold ULV machines that are in common use by mosquito control programs in the northeast. The majority of this work was conducted in other countries where differences in the strains of *Ae. albopictus*, housing construction and condition, and accepted pest management practices confound the data[28-36, 38]. Finally, none of the studies measured the impact on adult mosquito populations. Larval control is meaningless unless it can confer a reduction in the population of adult mosquitoes. This project will measure the effect of larval control on the adult populations. While many of the parameters in the present study have been explored before, none of the previous studies have brought all of the pieces together in one comprehensive project to determine the efficacy of the LV application of *Bti* in an outdoor urban residential setting for the control of *Ae. albopictus*. This project will evaluate what was learned in the other studies and combine it into one project specifically designed to address the unique problem that faces the public and mosquito control programs in the northeastern U.S.

This study addresses several documented needs-assessment evaluations listed below.

General Priorities for the Northeast – Biocontrol

- Research on biological control of diseases, arthropods, and weeds; extension of this research into production systems of horticultural crops
- Research/extension demonstrations of biocontrol methods for growers and private pest control operators

NEREAP-IPM

- Urban pest issues including insects and rodents.

Pest Management Alternatives Program

- Develop IPM tactics for critical or emerging pests of regional or national magnitude.

Community IPM Working Group

- Develop a PSMP for residential IPM particularly for suburban outdoor IPM and indoor urban IPM.
- Outreach: Develop and create an outreach campaign for residential IPM (radio, TV, and other creative forums). Develop material and distribute to end-users. Measure success of project.

Justification: Existing methods of *Ae. albopictus* control do not work. The area-wide application of larvicides would provide efficient, cost effective, lasting control that could be easily implemented by mosquito control programs. Although the material and equipment already exists, this technique has not been used in the U.S. due to a lack of research demonstrating efficacy for this specific problem, and a lack of knowledge by mosquito control programs that adulticiding equipment can be used to apply larvicides on an area-wide basis. Without this project, at worst, many mosquito control programs will continue to ignore this pest because of the difficulties associated with controlling it. Control of this mosquito will be left in the unqualified hands of the public and they will continue to suffer. At best, mosquito control programs will continue to rely on repeated area-wide applications of broad-spectrum adulticides for *Ae. albopictus* control. The limited success of this method will undoubtedly lead to wasted resources and the unnecessary use of pesticides with probable non-target impacts. With only two classes of pesticides commonly available for adult mosquito control, there is a great potential for the development of resistance problems. In either scenario, successful *Ae. albopictus* control is unlikely, people will continue to be bitten, and the risk of disease transmission from this mosquito will go unabated. This project will provide a significant benefit to the public and mosquito control programs in the northeast and beyond that are plagued by *Ae. albopictus*. This project will provide a significant benefit to the public and mosquito control programs in the northeast and beyond that are plagued by *Ae. albopictus*.

(ii) Objectives and Anticipated Impacts

The research component of the proposed study will develop and test the efficacy of LV applications of *Bti* to rapidly control *Ae. albopictus* in urban residential environments. First, we will conduct staged field trials where equipment is tested and optimized to deliver the *Bti* to containers of larvae at various distances. Then, we will test the method in actual mosquito control programs under real world conditions. We will demonstrate reductions in larval and adult populations of *Ae. albopictus*, a reduction in the amount of chemical adulticides applied, and an overall reduction in the cost of area-wide control of *Ae. albopictus*.

The extension component of the proposed study will develop training workshops as well as printed and web-based materials to disseminate the knowledge generated in this study to the stakeholders. The extension component will begin with the training of local mosquito control agencies in NJ. These training sessions will be used to develop a formal workshop and training materials that will be made available to mosquito control agencies throughout the northeast. All of the impacts listed below will be compounded across the northeast and beyond as various stakeholders adopt this technology.

<u>Impacts</u>:

1. Reduction in the populations of *Ae. albopictus*. The reduction in larvae will lead to a subsequent reduction in adult populations. The reduction in the adult populations will address *Safeguarding human health and the environment* by lowering the biting frequency of this pest thereby reducing the risk of exposure to diseases transmitted by this pest. Successful suppression of *Ae. albopictus* will also help to prevent the spread of this invasive pest.
2. Reduction in the use of chemical adulticides. The availability of a biorational larvicide will eliminate the dependence on broad-spectrum adulticides such as malathion and the synthetic pyrethroids for *Ae. albopictus* control. The residual nature of *Bti* will also reduce pesticide use because chemicals can be applied less often. This impact addresses *Safeguarding human health and the environment* by reducing human and other non-target exposure to toxic agents.
3. Reduced risk to non-target species. This impact addresses *Safeguarding human health and the environment* by substituting a highly specific biocontrol agent for a broad-spectrum chemical adulticide, resulting in lower mortality in non-target species.
4. Reduction in the potential for the development of pesticide resistance. The availability of a larvicide for area-wide *Ae. albopictus* control will eliminate the repeated applications of adulticides which can lead to the development of resistance[39, 40]. This impact encourages the *implementation of IPM* by promoting biological control strategies.

5. Reduction in costs associated with the control of *Ae. albopictus*. Adulticides provide very temporary control and must be reapplied to be effective. *Bti* is capable of providing lasting control[41-43]. The persistence of *Bti* will accrue *economic benefits* by reducing the frequency of applications required for *Ae. albopictus* control resulting in lower material costs and labor expenses. These savings will free funding for other environmentally conscious mosquito control efforts such as open marsh water management.

(iii) Approach and Procedures

Research Component

Staged Field Trials: Phase I of the study will involve the evaluation, modification and optimization of application equipment. In order for the *Bti* to reach all of the potential larval sources around a building, the droplets must go over and around the structure. Truck-mounted LV applications will be made in an open field at the Rutgers Snyder Research and Extension Farm. Known amounts of field collected larvae will be placed in containers with seasoned water. The containers will be placed in lines perpendicular to the spray path spaced 10 m apart. Within each line, containers will be placed 3 m apart from 1-60 m from the application path. *Bti* will be applied with a truck mounted ULV machine down the center of the plot. Containers will be retrieved and returned to a laboratory at the Rutgers Center for Vector Biology and larval mortality will be measured for 5 days at 24 hr intervals. Persistence of the pesticide will be tested at 24 hr intervals after all of the original larvae have died. The size and distribution of the droplets will be measured. Colony forming units (CFU) of *Bti* will be measured to determine the amount of material delivered to the containers and confirm application rates. Equipment will be adjusted to yield optimum size and dispersion of droplets for maximum larval mortality. This experiment will be repeated several times with different types of equipment under various conditions to determine the best circumstances for maximum efficacy.

Implementation: During Phase II of the project, urban neighborhoods with a high abundance of *Ae. albopictus* will be identified. A thorough inspection will be made of each property to identify and catalog as many larval sources as possible. Pre-trial larval counts will be conducted for each larval source identified to establish the existing larval population. Adult populations will also be measured with traps 24 hrs prior to the application and for 14 days after the application at 48 hr intervals. An area-wide LV application of *Bti* will be made in the area based on the protocol determined from Phase I of the study. Larval mortality, *Bti* persistence, CFUs, and droplet size and distribution will be measured as in Phase I.

Larval Mortality: In Phase I, a known number of larvae will be placed in each container. Mortality will be determined at 24 hr intervals from 24-120 hrs post application. Mortality will be calculated by dividing the number of dead larvae by the total number of larvae in the container. In Phase II, larval abundance of field populations will be measured with a standard 350 ml mosquito dipper. Three dip samples will be taken from each container, the larvae will be counted and the average number of larvae per ml will be calculated. Samples will be taken pre-application, and at 24 hr intervals from 24-120 hrs post application. Mortality will be calculated as above. Statistical differences in mortality between the treated and untreated control containers will be determined by using repeated measures of analysis of variance (ANOVA).

Adult Abundance: Larval mosquito control is ineffective if it does not result in a reduction of adult mosquitoes. Adult abundance will be measured with BG-Sentinel™ mosquito traps. This trap utilizes two lures specifically designed to attract *Ae. albopictus* adults. Studies have demonstrated this trap to be highly efficient and superior to other standard methods for sampling *Ae. albopictus* adults[44]. Traps will be placed approximately 100 m apart in the field for a 24 hr period immediately prior to *Bti* application. Adult mosquitoes will be collected, identified, enumerated and an average adult abundance will be calculated by dividing the total number of *Ae. albopictus* collected by the number of traps. This process will be repeated at 48 hr intervals for 2 weeks following an application to measure the effect of larval control on the adult population. Statistical differences between the pre and post-treatment averages will be determined with a Student's t-test.

Pesticide Persistence: Although not a residual product, *Bti* has been shown to give extended control in containers[41-43]. In the Phase I trials, following the application of *Bti*, containers will be returned to the lab. Additional larvae will be added to the containers that exhibited significant larval mortality, and the subsequent mortality will be scored. This process will be repeated until there is no longer any sign of significant larval mortality in the container. In the Phase II trials, samples of water will be collected from the field containers and returned to the lab to be analyzed by the method described above. Statistical differences between the treated and untreated controls will be determined with a Student's t-test.

Droplet Size and Distribution: Droplet size and distribution will be measured during both phases of the field trials. In Phase I, Kromekote® cards will be placed in lines perpendicular to the spray path spaced every 10 m. Cards within a line will be spaced 3 m apart from one to 60 m from the spray path. In Phase II, one card will be placed in the center of each 3 m^2 plot around the house as well as next to each container of larvae. Droplets deposited on the paper will be scanned into a computer and analyzed using Stainalysis software (REMSpC, Ontario, Canada). The software will determine drop density, deposit volume, and droplet size.

Colony Forming Units of *Bti*: To determine the amount of *Bti* in the containers, water samples will be collected from all of the containers in all of the trials and analyzed by a previously described method[30]. In short, aliquots from each sample will be placed on *Bt* selective media, incubated, and enumerated to determine the CFUs/ml. Samples will be collected at 1 hr, 7 days and 14 days post application. The 1 hr samples will indicate the coverage of the LV application, while the 7 and 14-day samples will serve as an additional measure of persistence. Mortality data will be combined with the CFU data to determine the optimal lethal dose for *Ae. albopictus* larvae using a Probit analysis.

Extension Component

Training of Local Mosquito Control Agencies: Training sessions will be held with the county mosquito control agencies in NJ. These sessions will focus on rapidly disseminating the information learned during the research phases to encourage the quick adoption of the developed methods. These sessions will include centralized seminars, staged trials as in Phase I, and field demonstrations as in Phase II. Researchers will also travel to the county agencies to personally set up equipment and demonstrate techniques directly to the mosquito control agencies.

<u>Training Workshop</u>: A training workshop will be developed and administered through the Rutgers University, Office of Continuing Professional Education. The workshops will contain both classroom and field exercises. The workshop will provide instruction on how to adapt existing equipment to apply the material, how to apply the material for maximum efficacy, and how to measure the success of the application. Field exercises will also be conducted similar to the staged trials in the Phase I to demonstrate the effectiveness of the technique. Stakeholders will be encouraged to bring their own equipment to the class to facilitate the adoption of this novel application technology.

<u>Training Materials</u>: All of the information learned in the course of this project will be assembled into a booklet that will be sent to all of the stakeholders in the northeast. The booklet will provide all of the information necessary to allow interested agencies to adopt this technology in their programs. Concurrently, all of the information in the booklet will be posted on the Rutgers Center for Vector Biology website (http://vectorbio.rutgers.edu/index.php), along with supplemental materials, to educate stakeholders in other parts of the country that were not reached by the workshop or training booklet.

<u>Timetable</u>: The Center for Vector Biology will manage the research component while the Rutgers Cooperative Extension Office and the Office of Continuing Professional Education will take the leadership role in this Joint Research-Extension project. The proposed multidisciplinary and multi-organizational study will be coordinated through quarterly meetings of key personnel to discuss projected research and extension activities to be completed during the period and to discuss results from the previous periods. Additional meetings will convene as needed. Specific activities are summarized in the following timetable.

Objective	Task	Complete by (quarter)											
		2008				2009				2010			
Phase I	Staged Field Trial	■	■	■									
Phase II	Site Identification					■	■	■					
	Implementation					■	■	■					
Extension	Train Local Agencies									■			
	Conduct Workshops									■	■	■	
	Develop Booklet									■	■		
	Develop Website									■	■		
	Distribute Materials											■	
Evaluation	Develop Survey										■	■	
	Survey Stakeholders												■
Analysis					■				■			■	■

(iv) Evaluation Plans

Research Component

Phase I: Success of the staged field trials will be evaluated in several areas. Larval control will be evaluated by the mortality rates in the containers. The goal is to achieve an overall mean mortality greater than 90% within 96 hrs of the application. Product persistence will be evaluated by the length of time that larval mortality continues in the containers. Because adulticides provide very temporary control, persistence of 96 hrs will be judged a success. The size and distribution of droplets will also be evaluated to judge the success of the application equipment. A successful application will contain a uniform distribution of properly sized droplets containing a lethal dose of *Bti* as confirmed by the CFU counts.

Phase II: Success of the actual implementation will also be evaluated in several areas. Larval control will be evaluated by the mortality rates in the natural larval population. Physical boundaries are expected to impede the distribution of the *Bti* and lower the mortality rates. The goal is to achieve an overall mean mortality greater than 75% within 96 hrs in all of the identified larval habitats. Control of the adult population will also be evaluated. Many larval sources may not be identified and adult mosquitoes may migrate into treated areas from distant sources. The goal is to reduce the trap counts of adult *Ae. albopictus* by 50% from pre-application levels within 2 weeks. Because larvae take approximately 2 weeks to develop, an effect on the adult population is not expected prior to this time. Product persistence will be evaluated as above with a goal of 72 hr persistence to allow for environmental degradation of product. The size and distribution of droplets will be evaluated to determine the travel of the product. The goal is to distribute the product around all of the sides of a structure to at least 75% of the identified larval habitats. Distribution of the product will be confirmed with the CFU counts.

Cost analysis: This project is expected to reduce the costs associated with the control of *Ae. albopictus*. The persistence of the product will lead to fewer applications requiring less labor and material. Elimination of the larval population will crash the adult population which will take longer to rebound. The object is to reduce the cost of controlling *Ae. albopictus* by 50% compared to traditional area-wide adulticide applications. Success will be evaluated through a comparison of historical costs for *Ae. albopictus* control versus the costs of our method.

Extension Component

Training: The objective is to train local and regional mosquito control agencies in the methods developed in this study. The goal is to train 75% of the mosquito control agencies in NJ by the end of the first year. Additionally we hope to provide classroom training to agencies from at least two other states in the region by the end of the second year. The success of the training sessions will be determined by the number and geographical distribution of participants, through class evaluations distributed to participants, and by a subsequent survey designed to measure the utilization of the developed methods by agencies that received training.

Dissemination of Information: The objective is to promote the adoption of this method by providing information to stakeholders beyond the reach of our workshops. The goal is to provide written training manuals to 100% of the public mosquito control agencies in the northeast region by the end of the third year. We will also distribute notices to agencies in other regions about the availability of printed and on-line training materials. Success of this effort will be measured by requests for additional workshops or seminars, requests for printed training materials, and visits to the website.

Analysis: The overall success of the extension component will be measured with a survey developed in cooperation with the Bloustein Center for Survey Research at Rutgers. Surveys will be sent out to all of the stakeholders in the region to determine how many of them received information from the project in one form or another, how many actually used the technique for *Ae. albopictus* control, how successful they thought the technique was at controlling *Ae. albopictus*, how much time and/or money was saved by the use of this technique, how much less adulticide was used, as well as other pertinent information.

Because this technology uses existing equipment and materials it can be quickly and easily adopted by most any mosquito control program. Additionally because this method provides a cost effective solution to a difficult problem, it is likely to be adopted throughout the northeast and in other parts of the U.S. where *Ae. albopictus* is prevalent.

REFERENCES CITED

1. Sprenger D, Wuithiranyagool T. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. *J Am Mosq Control Assoc* 2: 217-9.

2. Crans WJ, Chomsky MS, Guthrie D, Acquaviva A. 1996. First record of *Aedes albopictus* from New Jersey. *J Am Mosq Control Assoc* 12: 307-9.

3. Benedict MQ, Levine RS, Hawley WA, Lounibos LP. 2007. Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. *Vector Borne Zoonotic Dis* 7: 76-85.

4. Savage HM, Niebylski ML, Smith GC, Mitchell CJ, Craig GB, Jr. 1993. Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae) at a temperate North American site. *J Med Entomol* 30: 27-34.

5. Madon MB, Mulla MS, Shaw MW, Kluh S, Hazelrigg JE. 2002. Introduction of *Aedes albopictus* (Skuse) in southern California and potential for its establishment. *J Vector Ecol* 27: 149-54.

6. Madon MB, Hazelrigg JE, Shaw MW, Kluh S, Mulla MS. 2003. Has *Aedes albopictus* established in California? *J Am Mosq Control Assoc* 19: 297-300.

7. Gratz NG. 2004. Critical review of the vector status of *Aedes albopictus*. *Med Vet Entomol* 18: 215-27.

8. Paramasivan R, Thenmozhi V, Hiriyan J, Dhananjeyan K, Tyagi B, Dash AP. 2006. Serological and entomological investigations of an outbreak of dengue fever in certain rural areas of Kanyakumari district, Tamil Nadu. *Indian J Med Res* 123: 697-701.

9. Charrel RN, de Lamballerie X, Raoult D. 2007. Chikungunya outbreaks--the globalization of vectorborne diseases. *N Engl J Med* 356: 769-71.

10. Centers for Disease Control. 2007. In the News: Chikungunya fever in Italy. http://wwwn.cdc.gov/travel/contentChikungunyaFeverItaly.aspx (last accessed 11/19/07).

11. Effler PV, Pang L, Kitsutani P, Vorndam V, Nakata M, Ayers T, Elm J, Tom T, Reiter P, Rigau-Perez JG, Hayes JM, Mills K, Napier M, Clark GG, Gubler DJ. 2005. Dengue fever, Hawaii, 2001-2002. *Emerg Infect Dis* 11: 742-9.

12. Anonymous. 2000. Imported dengue–United States, 1998 and 2000. *Morb Mortal Wkly Rep.* 51: 281-3.

13. Margalit J, Dean, D. 1985. The story of *Bacillus thuringiensis* var. *israelensis* (Bti). *J Am Mosq Control Assoc* 1:1-7.

14. Lacey, L.A. 2007. *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control. *J Am Mosq Control Assoc* 23:133-163.

15. Becker N, Ludwig M. 1993. Investigations on possible resistance in Aedes vexans field populations after a 10-year application of *Bacillus thuringiensis israelensis. J Am Mosq Control Assoc* 9:221-224.

16. Georghiou GP, Wirth MC. 1997. Influence of exposure to single versus multiple toxins of *Bacillus thuringiensis* subsp. *israelensis* on development of resistance in the mosquito *Culex quinquefasciatus* (Diptera: Culicidae). *Appl Environ Microbiol* 63:1095-1101.

17. Goldman IF, Arnold J, Carlton BC. 1986. Selection for resistance to *Bacillus thuringiensis* subsp. *israelensis* in field and laboratory populations of the mosquito *Aedes aegypti*. *J Invertebr Pathol* 47:317-324.

18. Regis L, Nielson-LeRoux C. 2000. Management of resistance to bacterial vector control. In: Charles J-F, Delecluse A, Nielsen-LeRoux C, eds. *Entomopathogenic Bacteria: From laboratory to field application*. Dordecht, The Netherlands: Kluwer Academic Publishers. p 419-438.

19. Boisvert M, Boisvert J. 2000. Effects of *Bacillus thuringiensis* var. *israelensis* on target and nontarget organisms: a review of laboratory and field experiments. Biocontr *Sci Technol* 10:517-561.

20. Dejoux C, Elouard JM. 1990. Potential impact of microbial insecticides on the freshwater environment, with special reference to the WHO/UNDP/World Bank Onchocerciasis Control Programme. In: Laird M, Lacey LA, Davidson EW, eds. *Safety of Microbial Insecticides*. Boca Raton, FL: CRC Press. p 66-83.

21. Glare TR, O,Callaghan M. 2000. *Bacillus thuringiensis: Biology, Ecology and Safety*. Chilchester, United Kingdom: J. Wiley and Sons, Ltd. 350 p.

22. Lacey LA, Merritt RW. 2003. The safety of bacterial microbial agents used for black fly and mosquito control in aquatic environments. In: Hokkanen HMT, Hajek AE, eds. *Environmental Impacts of Microbial Insecticides: Need and Methods for Risk Assessment*. Dordecht, The Netherlands: Kluwer Academic Publishers. p 151-168.

23. Lacey LA, Mulla MS. 1990. Safety of *Bacillus thuringiensis* (H14) and *Bacillus sphaericus* to non-target organisms in the aquatic environment. In: Laird M, Lacey LA, Davidson EW, eds. *Safety of Microbial Insecticides*. Boca Raton, FL: CRC Press. p 169-188.

24. Lacey LA, Siegel JP. 2000. Safety and ecotoxicology of entomopathogenic bacteria. In: Charles J-F, Delecluse A, Nielsen-LeRoux C, eds. *Entomopathogenic Bacteria: From laboratory to field application*. Dordecht, The Netherlands: Kluwer Academic Publishers. p 253-273.

25. Saik JE, Lacey LA, Lacey CM. 1990. Safety of microbial control agents to domestic animals and vertebrate wildlife. In: Laird M, Lacey LA, Davidson EW, eds. *Safety of Microbial Insecticides*. Boca Raton, FL: CRC Press. p 115-132.

26. Seigel JP, Shadduck JA. 1990. Mammalian safety of *Bacillus thuringiensis*. In: de Barjac h, Sutherland D, eds. *Bacterial Control of Mosquitoes and Black Flies: Biochemistry, Genetics, and Applications of Bacillus thuringiensis israelensis and Bacillus sphaericus*. New Brunswick, NJ: Rutgers Univ Press. p 202-217.

27. Seigel JP, Shadduck JA. 1990. Safety of microbial insecticides to vertebrates – Humans. In: Laird M, Lacey LA, Davidson EW, eds. *Safety of Microbial Insecticides*. Boca Raton, FL: CRC Press. p 101-113.

28. Yap HH, Lee YW, Zairi J. 2002. Indoor thermal fogging against vector mosquitoes with two *Bacillus thuringiensis israelensis* formulations, VectoBac ABG 6511 water-dispersible granules and VectoBac 12AS liquid. *J Am Mosq Control Assoc* 18:52-56.

29. Eritja R, Aranda C. 1995. Efficacy of ground ultra-low volume larvicidal treatments using temephos and *Bacillus thuringiensis* var. *israelensis* against *Culex pipiens* larvae. *J Am Mosq Control Assoc* 11:491-494.

30. Lee HL, Gregorio ER Jr., Khadri MS, Seleena P. 1996. Ultralow volume applications of *Bacillus thuringiensis* ssp. *israelensis* for the control of mosquitoes. *J Am Mosq Control Assoc* 12:651-655.

31. Seleena P, Lee HL. 1998. Field trials to determine the effectiveness of *Bacillus thuringiensis* subsp. *israelensis* application using an ultra low volume generator for the control of *Aedes* mosquitoes. *Israel J Entomol* 32:25-31.

32. Seleena P, Lee HL, Chiang YF. 1999. Compatibility of *Bacillus thuringiensis* serovar *israelensis* and chemical insecticides for the control of *Aedes* mosquitoes. *J Vect Ecol* 24:216-223.

33. Seleena P, Lee HL, Chiang YF. 2001. Thermal application of *Bacillus thuringiensis* serovar *israelensis* for dengue vector control. *J Vect Ecol* 26:110-113.

34. Yap HH, Chong ASC, Adanan CR, Chong NL, Rohaizat B, Malik YA, Lim SY. 1997. Performance of ULV formulations (Pesguard 102/VectoBac 12AS) against three mosquito species. *J Am Mosq Control Assoc* 13:384-388.

35. Lee HL, Chen CD, Mohd Masri S, Chiang YF, Chooi KH, Benjamin S. 2007. Impact of larviciding with a *Bacillus thuringiensis israelensis* formulation of VectoBac WG, on dengue mosquito vector population in a dengue endemic site in Selangor State, Malaysia. *Southeast Asian J Trop Med Public Health* (accepted).

36. Patrick HY, Ng YY. 2006. Evaluation of *Bacillus thuringiensis israelensis* for mosquito control in Singapore Armed Forces training areas.

37. Valent. 2004. Evaluation of ground aerosol applications of VectoBac WDG (*Bti*) for control of container breeding mosquitoes.

38. Seleena P, Lee HL, Nazni WA, Rohani A, Kadri MS. 1996. Microdroplet application of mosquitocidal *Bacillus thuringiensis* using ultra-low volume generator for the control of mosquitoes. *Southeast Asian J Trop Med Public Health* 27:628-632.

39. Khoo BK, Sutherland DJ, Sprenger D, Dickerson D, Nguyen H. 1988. Susceptibility status of *Aedes albopictus* to three topically applied adulticides. *J Am Mosq Control Assoc* 4:310-313.

40. Robert LL, Olson JK. 1989. Susceptibility of female Aedes albopictus from Texas to commonly used adulticides. *J Am Mosq Control Assoc* 5:251-253.

41. Batra CP, Mittal PK, Adak T. 2000. Control of *Aedes aegypti* breeding in desert coolers and tires by use of *Bacillus thuringiensis* var. *israelensis* formulation. *J Am Mosq Control Assoc* 16:321-323.

42. Mulla MS, Tharavara U, Tawatsin A, Chompoosri J. 2004. Procedures for the evaluation of field efficacy of slow-release formulations of larvicides against *Aedes aegypti* in water-storage containers. *J Am Mosq Control Assoc* 20:64-73.

43. Vilarinhos PTR, Monerat R. 2004. Larvcidal persistence of formulations of *Bacillus thuringiensis* var. *israelensis* to control larval *Aedes aegypti*. *J Am Mosq Control Assoc* 20:311-314.

44. Meeraus W, Johnson J, Arias J. 2007. Field comparison of novel and gold standard traps for collecting Aedes albopictus in northern Virginia. Fairfax County Health Department. Presentation at the 32nd Annual Conference of the Mid-Atlantic Mosquito Control Association.

2.c.(iii)RELEVANCE STATEMENT

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Development of Novel Application Technology for the Control of the Asian Tiger Mosquito in Urban Environments

Joint Research-Extension Project

<u>Project Summary</u>: This project addresses the control of the invasive vector mosquito *Aedes albopictus* (the Asian tiger mosquito) in urban residential environments. The Asian tiger mosquito is responsible for most complaints to mosquito control programs. These programs rely on area-wide applications of broad-spectrum adulticides to combat this mosquito. This controversial method is ineffective for *Ae*. *albopictus* control. Without any efficient means of control, many mosquito control programs ignore this pest. While larvicides work effectively, it is impractical if not impossible to identify and treat all sources by hand. Area-wide applications of larvicides would solve the problem, but the technology has not been developed sufficiently for use in urban residential settings. This project will develop a novel technology for the area-wide low volume application of the bacterial larvicide *Bacillus thuringiensis* ssp. *israelensis* to control *Ae. albopictus*. We will develop this new biocontrol strategy by adapting low-volume truck-mounted adulticide equipment commonly in use by mosquito control programs to apply liquid *Bti* in an outdoor urban residential setting. After application the larvicide will drift and settle in containers of water where the larvae reside, providing quick, effective control. This IPM approach will reduce the abundance of this pest, the dependence on broad-spectrum adulticides, non-target impacts, and the development of resistance through the use of novel application technology, host-targeted applications of biological larvicides, and training programs for stakeholders. Efficacy of the program will be evaluated by monitoring larval mortality, adult presence, droplet size and distribution, and pesticide persistence. We will also prepare workshops and materials to train mosquito control personnel throughout the northeast and beyond in the use of this technology.

<u>Problem</u>: The Asian tiger mosquito, *Aedes albopictus*, is among the most invasive of all species, and without question the most invasive of all mosquitoes. It was introduced to the U.S. in 1985 and quickly spread. Today, thirty U.S. states are infested and the species continues to expand its range. In the northeast, it has been found in seven states. Naturally found in tree holes, this mosquito has adapted to utilize artificial containers as larval habitats in urban areas. The tiger mosquito is a day-biting mosquito that prefers attacking large mammals including humans. This vicious and persistent day-biting mosquito inflicts painful bites, induces dermatological and allergic manifestations, and significantly affects the quality of life and human well-being. In the U.S., it is regarded as the most important nuisance mosquito. *Aedes albopictus* has also been found naturally infected with several serious human pathogens. It has been implicated as a bridge vector of West Nile virus as a principal vector of dengue virus, dog heartworm, and chikungunya virus. It was responsible for several major epidemics of chikungunya, constituting millions of cases and hundreds of deaths. Most recently, 166 endemic cases were reported in northeast Italy in 2007. The 2001-2002 outbreak of dengue in Hawaii, with 95 infections, demonstrates the potential of *Ae. albopictus* to transmit this disease in the U.S. via imported cases. From 1986 to 2000, 516 confirmed and 2,128 suspected dengue cases were imported into the U.S., mostly into areas of *Ae. albopictus* activity. Unfortunately, conventional mosquito abatement methods do not affect *Ae. albopictus.* Local mosquito control agencies across the nation mount aggressive control campaigns against salt marsh, floodwater, and many other rural-based mosquito pest species, but they rarely target the urban Asian tiger mosquito. Their efforts tend to be limited to the distribution of leaflets to residents, placing the responsibility for control on unqualified citizens. Currently, there is no effective means to control *Ae. albopictus* on an area-wide basis.

Background: *Bacillus thuringiensis* (*Bt*) is a gram-positive, spore forming, aerobic bacterium. Highly toxic to insects, many varieties of *Bt* are used for agricultural pests. The efficacy of *Bacillus thuringiensis* serovariety *israelensis* (*Bti*) has been demonstrated for many mosquito species in a variety of habitats. Today, formulations of *Bti* are the primary products used for larval mosquito control in the U.S. and other countries. Development of resistance to *Bti* is very difficult and there is almost no impact on non-target species. Normally applied as a solid directly to larval sources, several studies have demonstrated that *Bti* liquid can be applied as a low volume (LV) formulation to successfully control mosquitoes. The majority of this work was conducted in other countries where differences in the strains of *Ae. albopictus*, housing construction and condition, and accepted pest management practices confound the data. Additionally, none of the studies measured the impact on adult mosquito populations. Larval control is meaningless unless it can confer a reduction in the population of adult mosquitoes. While many of the parameters in this project have been explored before, none of the previous studies have brought all of the pieces together in one comprehensive project to determine the efficacy of the LV application of *Bti* in an outdoor urban residential setting for the control of *Ae. albopictus*. This project will evaluate what was learned in the other studies and combine it into one project specifically designed to address the unique problem that faces the public and mosquito control programs in the northeastern U.S. This study addresses several documented needs-assessment evaluations. General Priorities for the Northeast – Biocontrol; 1. Research on biological control of diseases, arthropods, and weeds; extension of this research into production systems of horticultural crops and, 2. Research/extension demonstrations of biocontrol methods for growers and private pest control operators. NEREAP-IPM; Urban pest issues including insects and rodents. Pest Management Alternatives Program; Develop IPM tactics for critical or emerging pests of regional or national magnitude. Community IPM Working Group; 1. Develop a PSMP for residential IPM particularly for suburban outdoor IPM and indoor urban IPM and, 2. Outreach: Develop and create an outreach campaign for residential IPM (radio, TV, and other creative forums). Develop material and distribute to end-users. Measure success of project.

Justification: Existing methods of *Ae. albopictus* control do not work. This project will provide environmentally responsible, efficient, cost effective, and lasting control that can be rapidly and easily implemented by mosquito control programs. Although the material and equipment already exists, this technique has not been used in the U.S. due to a lack of research demonstrating efficacy for this specific problem, and a lack of knowledge by mosquito control programs that adulticiding equipment can be used to apply larvicides on an area-wide basis. Without this project, at worst, many mosquito control programs will continue to ignore this pest and the problem will be left in the unqualified hands of the public who will continue to suffer.

At best, mosquito control programs will continue to rely on ineffective methods leading to wasted resources, the unnecessary use of pesticides with probable non-target impacts, and potential resistance problems. In either scenario, successful *Ae. albopictus* control is unlikely, people will continue to be bitten, and the risk of disease transmission from this mosquito will go unabated. This project will provide a significant benefit to the public and mosquito control programs in the northeast and beyond that are plagued by *Ae. albopictus*.

Objectives: The research component of the proposed study will develop and test the efficacy of LV applications of *Bti* to control *Ae. albopictus* in urban residential environments. First, we will conduct staged field trials where equipment is tested and optimized to deliver the *Bti* to containers of larvae at various distances. Then, we will test the method in actual mosquito control programs under real world conditions. We will demonstrate reductions in larval and adult populations of *Ae. albopictus*, a reduction in the amount of chemical adulticides applied, and an overall reduction in the cost of area-wide control for *Ae. albopictus*. The extension component of the proposed study will develop training workshops as well as printed and web-based materials to disseminate the knowledge generated in this study to the stakeholders. The extension component will begin with the training of local mosquito control agencies in NJ. These training sessions will be used to develop a formal workshop and training materials that will be made available to mosquito control agencies throughout the northeast. All of the impacts listed below will be compounded across the northeast and beyond as various stakeholders adopt this technology.

Anticipated Outcomes: There are several anticipated outcomes from this study. First will be a reduction in the larval populations of *Ae. albopictus* leading to a subsequent reduction in adult populations. The reduction in the adult populations will lower the biting frequency of this pest thereby reducing the risk of exposure to diseases transmitted by this pest. Successful suppression of *Ae. albopictus* will also help to prevent the spread of this invasive pest. The availability of a biorational larvicide will eliminate the dependence on broad-spectrum adulticides such as malathion and synthetic pyrethroids for *Ae. albopictus* control. The residual nature of *Bti* will reduce pesticide use because it can be applied less often than short-lived adulticides. This will reduce human and other non-target exposure to toxic agents. The availability of a larvicide for area-wide *Ae. albopictus* control will eliminate the repeated applications of adulticides which can lead to the development of resistance. This impact encourages the use of IPM by promoting biological control strategies. Adulticides provide very temporary control and must be reapplied to be effective. The persistence of *Bti* will accrue economic benefits by reducing the frequency of applications required for *Ae. albopictus* control resulting in lower material costs and labor expenses. These savings will free funding for other environmentally conscious mosquito control efforts such as open marsh water management. Because this technology uses existing equipment and materials it can be quickly and easily adopted by most any mosquito control program. Additionally because this method provides a cost effective solution to a difficult problem, it is likely to be adopted throughout the northeast and in other parts of the U.S. where *Ae. albopictus* is prevalent.