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Perspective

Major General Russell J. Czerw

Among the more well-known of the ancient Chinese maxims of military principles attributed to Sun Tzu is "Know your enemy as you know yourself," a common sense requirement for anyone planning strategy and tactics for an impending conflict. Of course, one immediately thinks of intelligence operatives gathering information about the character of the enemy's forces, locations, capabilities, and support structure, among other things, and analysts examining that data to identify ways to counter and eliminate the threat. The same approach applies not only to opposing military forces, but also to any adversary with the potential to inflict harm on your force and reduce its combat capability. As we have learned from hard experience over the last 110 years, some of the most pernicious threats to our Soldiers are posed by the smallest of adversaries—mosquitoes, ticks, sand flies, fleas, mites, and other arthropods—which cause problems directly through bites and irritation, and, more importantly, vector some of the most virulent disease pathogens that afflict humans and other mammals.

Ever since MAJ Walter Reed confirmed and extended Dr Carlos Finlay's previously ridiculed theories about the role of an arthropod, the mosquito, as intermediary host in the spread of yellow fever, the need to know this constant, pervasive enemy has been recognized by the military. Further, as understanding of the extent and significance of the threat has grown, the military's organization and application of resources has become increasingly sophisticated, with military entomologists on the point. The US Army first commissioned entomologists into the Sanitary Corps during World War I, and significantly increased those numbers during World War II. Today, there are some 64 active duty and 35 reservist Army entomologists serving in a variety of roles in operational commands, on staffs, in research laboratories, and in training organizations, often jointly with entomologists from our sister services. The senior medical and veterinary entomologist at the Army Medical Department (AMEDD) Center and School, COL Mustapha Debboun, has organized this special focus issue of the *AMEDD Journal* to feature the work of those military entomology professionals, including contributions by Air Force, Navy, and civilian authors. The result is an outstanding issue that is not only a wealth of important, timely medical information, but also provides insight as to the broad scope of roles and responsibilities of medical entomologists in today's military.

Although the role of arthropods as vectors of disease pathogens is, understandably, the best known of the threats they pose, there are other hazards. In the first article, CPT Silas Davidson and his coauthors present an excellent description of the problems caused by beetles that do not sting or bite, but secrete vesicating chemicals which cause blistering in human skin. These blisters are often misdiagnosed, and have sometimes even been attributed to suspected chemical warfare agents. In certain parts of the world, the beetles periodically swarm in numbers large enough to pose a considerable health threat, and have in fact adversely affected military operations. The biology of the production of the irritant, and the physiology of its harmful effects are clearly explained as the authors use an outbreak of dermatitis among US Soldiers in Iraq in

2007 as a case study to illustrate the reality of the threat to readiness. It was discovered that the possibility of vesicating beetles had not been considered as Soldiers and Airmen at Joint Base Balad were being treated for blisters, assumed to be burns from maintenance activities. Sampling at the locations where the affected personnel worked revealed the presence of such beetles, and further collections determined the types of beetles, potential sources, the attraction factors (lights), and the times of activity. Based on the data collected, preventive measures were instituted to reduce human contact with the beetles, and the incidence of dermatitis was significantly reduced. This very informative article is a textbook example of how the teamwork of the healthcare provider and the entomologist effectively identified and resolved a potentially serious public health concern.

By the end of World War II, US military planners understood that learning, or relearning, the particulars of regional disease threats each time US forces deployed was counterproductive, and often proved deadly to military personnel. Overseas medical research laboratories were established in Thailand, Indonesia, Peru, Egypt, and Kenya to minimize such knowledge gaps by developing and using surveillance tools to discover and understand the spread of disease and the vectors involved. MAJ Brian Evans has assembled a team of authors representing all five of those laboratories to present an examination of the most serious disease threats targeted by their research, and describe the ongoing work to address the gaps in our knowledge and capabilities to counter them. In a well-developed, very informative article, they carefully lay out the core questions that pertain to the particular vector of concern, and how the answers drive the approach to developing preventive measures to mitigate the respective threat. This article is detailed and complete in its approach that defines the task necessary, identifies the deficiencies encountered in accomplishing the task, and describes the solutions under development to address those deficiencies. However, even though the information presented is extensive, it is only a snapshot of the fascinating and extremely important work under way in those laboratories by our military and civilian entomology professionals.

One advantage of dealing with a vector-borne disease is that the potential occurrence of the disease in a given area is predictable, as long as the absence or presence of the vector can be determined. Unfortunately, that is often the most difficult aspect of the medical threat assessment, in that surveillance of vectors in the area may be impossible prior to deployment, and even after arrival in a combat environment. MAJ Michelle Colacicco-Mayhugh has contributed a very interesting article which looks at one way technology is providing assistance to planners for this problem. The adaptation of remotely sensed data into a number of modeling techniques is providing basic information about distribution, ecology, and the potential range of species without the collection of actual sampling data. By combining what is known about the vector species' biological and environmental requirements with the environmental and geographic data obtained from remote sensors, ecological niche modeling allows planners to determine if conditions in a geographic area are favorable for a given vector, and to assign probabilities to their occurrence. Obviously, this information is of great value to preventive medicine planners in that it allows them to sharpen their focus in their assessments, reducing the unknowns by a considerable amount. MAJ Colacicco-Mayhugh's article is a concise, clearly presented description of niche modeling as a potentially invaluable tool for those planning not only combat deployments, but also humanitarian assistance missions in remote parts of the world.

Although military entomologists are usually focused on management of the smallest of pests, they often share responsibilities with military veterinarians in dealing with feral and wild animals in deployment areas. LTC Raymond Dunton and MAJ Gerald Sargent have contributed an important article that addresses their experiences in this area while deployed to Iraq. Their article provides detailed insight into an often controversial area of public health, the significant differences in purpose and capabilities between contingency animal control efforts and rabies control programs. Ideally, a military deployment is temporary and localized, contending with environmental and public health concerns which directly affect the readiness of the force. Invariably, there is contact between deployed personnel and feral dogs and cats, as well as wild animals drawn to the deployment area by the increased availability of food, either as predators or foragers. Resulting animal bites take military personnel away from their duties until the

status of potential rabies infection has been determined, and resources are dedicated to locating, capturing, and examining the animal involved. In their explanation of why the military must use capture and euthanasia as animal control measures, LTC Dunton and MAJ Sargent clearly and completely explain the various rabies control measures that are used as part of long-term, national programs designed to first reduce zoonotic disease, but often with reduction in the numbers of animals as only a secondary effect. They explain why such measures are neither practical nor effective in protecting personnel in a localized, temporary situation, and how the military works very hard to minimize animal movement into the bases, thus reducing the requirement for euthanasia as much as possible. The information in this article should be used in predeployment training to expose personnel to the "whys" of the Army's animal control policy, which, to the uninformed, may seem harsh. Such concerns are distracting and counterproductive to accomplishing the military mission of the deployment.

Identification of arthropod species is one of the most important skills that a military medical entomologist must have. Recognition of disease vectors during surveillance is the vital first step in planning and implementing countermeasures to the disease threat they represent. As with every other extensive field of science, entomology has many areas of expertise among its specialists, but those entering the military must be proficient in species recognition and identification—our Soldiers' health depends on their ability. Such training has been expensive and time consuming, involving contracting classes and experts. In their informative article, Navy CDR (Ret) George Schultz and Dr Richard Robbins introduce a project by the Armed Forces Pest Management Board to provide training in species identification using interactive programs available on computer CDs and DVDs. They describe in detail the 3 initial remote teaching programs focusing on ticks and mosquitoes, and include illustrations of their functionality and interactive capabilities. The advantages in accommodation of student schedules, flexibility of use, and cost effectiveness are clearly explained. This is an exciting first step in training in a specific skill set that is undoubtedly adaptable into many other areas that require specialized medical training of smaller student populations.

For most people, perhaps the most common of arthropod pests are the various species of house flies. These ubiquitous insects are not only annoying, but have been recognized for many years as threats to human health as transporters of a considerable array of human pathogens. The presence of house flies is especially problematic in deployed environments, where public health infrastructure to support sanitation and hygiene may not exist, or, in many cases, has never existed. Their large numbers pose significant challenges for our military preventive medicine professionals in reducing both their existing numbers and their reproduction rates. Unfortunately, we have learned that house flies are extremely adaptable, rapidly developing resistance in their breeding cycles to our attempts to control them with insecticides. Two University of California at Riverside scientists, MAJ Alec Gerry (USAR) and Dr Diane Zhang, investigated the rapidly spreading resistance to a common fly bait that house flies in southern California have demonstrated since 2003. They describe their well researched, carefully designed study in a fascinating article that reveals the complexity of attempting to identify whether the generational change is increased physiological resistance to the bait's toxic component, a change in behavior by which the flies increasingly avoid the bait, or a combination of both. Their study is an illustration of the scientific rigor that must be applied when examining a phenomenon that has multiple, perhaps interacting causes which may also be changing over time. Further, this study is a classic example of an area of public health research that clearly has significant value for both military and civilian applications.

The problems of obtaining data and understanding the situation with regard to vector-borne diseases is not limited to remote areas with no advanced medical surveillance and laboratory resources. The US military has had a continuous, significant presence in the Republic of Korea (ROK) for over 50 years, and a tremendous amount of work has been done in improving public health and dealing with vector-borne diseases. However, as LTC William Sames and his coauthors point out in their detailed, very informative article, much is still unknown about the presence and distribution of a number of tick- and rodent-borne pathogens that cause serious illnesses in humans. This is due to a number of factors over the years, and, as pointed out in the article, the ROK government and US military are both placing increased emphasis and

resources into surveillance, tracking, and analysis of disease data, and research into the vectors and pathogens throughout the country. Because the presence of these diseases may not be well known, the potential for misdiagnosis is significant. This well-researched, carefully developed article contains important information for healthcare providers who provide services to military personnel stationed in the ROK, or to those who rotate there on training deployments.

The first line of the individual Soldier's defense against arthropod-borne pathogens is the proper use of personal protective measures, particularly the application of an effective repellent. The most widely produced and used repellent, *N*,*N*-diethyl-3-methylbenzamide (deet), was developed in 1954, but over the last decade has come under increasing critical scrutiny for associated adverse effects and other troubling chemical properties. LTC Van Sherwood and his team have contributed an excellent article reporting a formal study they performed in Kenya evaluating deet in comparison with 4 other commercially produced repellent formulations against mosquitoes. This was a detailed, scientifically rigorous field study involving volunteers and careful data collection and analysis. LTC Sherwood et al point out that although the work performed and data analysis of this study were extensive and did produce solid results, the study should be contributory to additional studies exploring other aspects of repellent effectiveness, under other conditions. This article is another excellent example of the high caliber of work being performed every day by the US military's dedicated scientific professionals in our overseas laboratories.

One method for rapid application of pesticides over large areas is aerial spraying. Although on the surface it may appear to be a straightforward process involving special equipment and skillful flying, in reality it is a much more complex undertaking. USAFR Maj Mark Breidenbaugh and his coauthors have contributed a fascinating article which reports on the test and evaluation trials that the USAF Aerial Spray Unit performed to determine the character and dispersal of the spray patterns generated by a new fuselage-mounted spray boom configuration installed on their C-130H airplanes. Generation of predictable droplet size and prediction of drift are key parameters in planning effective aerial pesticide spray applications, especially for mosquito control. Therefore, the new configuration had to be carefully tested to evaluate the effect of the airplane's turbulence on droplet size and drift, under various atmospheric conditions. This article is an illuminating presentation of the myriad of details, calculations, considerations, and allowances which must be included in planning spray missions, not only considering the weather and coverage areas, but the species of the target pest as well. Different species may require different droplet sizes and dispersals to be effective, and may also have different optimal times of application, which could then affect the configuration of the delivery system. This article gives insight into the high level of expertise and professionalism that is required for this very specialized capability, which is extremely important to both the military and civilian emergency management agencies.

The extent of the serious impact of diseases on combat effectiveness, especially in large-scale, extended hostilities, was fully understood after World War II and the Korean War, and military leadership realized that addressing that threat was a full-time effort, in periods of both peace and war. In addition to establishing the overseas laboratories, a centralized activity was envisioned to coordinate efforts and resources, and ensure the timely availability and exchange of current pest management information across the services. The predecessor of the Armed Forces Pest Management Board (AFPMB) was created in 1956 and chartered to be the US military's clearinghouse in support of US forces worldwide in the prevention of arthropod-borne diseases, as well as losses from other forms of pest attacks. USAF Col William Rogers, Dr Richard Robbins, and Navy CAPT Stanton Cope of the AFPMB have contributed an excellent article describing the history, responsibilities, organization, and functioning of the AFPMB. Among other important resources provided by the AFPMB, the article details the Literature Retrieval System which accesses the AFPMB's extensive reference library, one of the world's most highly respected sources of information in vector-borne diseases and medically related zoological topics, especially entomology. In addition, the authors highlight the important role AFPMB plays in careful management of pest control products, ensuring that military pest control efforts are environmentally sound, and, above all, safe for all concerned.

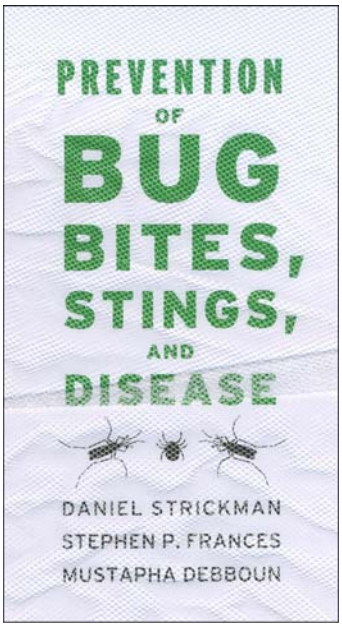
Soldiers in many tactical environments must use camouflage face paints to improve their concealment, especially in jungles or other areas of foliage and trees. Operations in such areas also make Soldiers vulnerable to arthropods, and to the disease pathogens they may carry. As mentioned earlier, the first defense in such situations is proper use of personal protective measures, especially repellents. Application of the separate face paint and repellent is a 2 step process, repellent first, then paint. The Army has developed a formulation of face paint combined with 30% deet repellent to eliminate that second step, and reduce the number of items involved. The development process had to ensure that the efficacy of neither the paint nor the deet was compromised in the blend, and the product had to be field tested before it could be accepted and put into production and distribution. In their article, MAJ Kendra Lawrence and her coauthors present a detailed, well-developed report on the field trials of the combined product. The tests were conducted in rural Belize, using local volunteers, and were designed to be scientifically rigorous and thorough, examining every aspect of product effectiveness and safety. The data was analyzed producing extensive statistical profiles of effectiveness against a number of mosquito species. The article by MAJ Lawrence et al is yet another example of the professional skills and technical expertise of AMEDD professionals that directly benefit our Warriors in every tactical environment and situation.

As the science of identification and analysis of vectors and their pathogens has become increasingly more sophisticated, our ability to plan and counter the threats they impose have markedly improved. However, no matter the capabilities of laboratory science, the first requirement of the process remains basic—specimens of the potential vectors must be collected in the field. In their article, CPT Lee McPhatter, Cara Olsen, and COL Mustapha Debboun describe their study into ways to refine the methodology of collecting specific mosquito species, especially gravid female mosquitoes which provide the best estimate of an infected population. We are all familiar with the commercial types of mosquito control traps, especially the light and CO_2 attractant versions, but research has show that any trap must use a lure to be truly effective. In order to attract and ultimately trap gravid female mosquitoes, research such as that described in this article must determine the type of aquatic site that a mosquito species prefers for depositing eggs. CPT McPhatter et al infused a variety of organic substances in water to examine their respective effectiveness in traps placed in a variety of locations across Fort Sam Houston. Their detailed, clearly written article presents the methodology, results and analysis of a well-researched, carefully planned and executed study project. Their work is another important contribution to the ever-growing body of vital scientific knowledge with which we better know and combat these dangerous and evolving scourges to human health.

OF INTEREST

A new book by an international collaboration of 3 military medical entomologists is another clear example of the significant contributions to worldwide public health made by the research and experience of military medical professionals worldwide. COL (Ret) Dan Strickman of the US Dept of Agriculture Research Service, Dr Stephen Frances of the Australian Army Malaria Institute, and COL Mustapha Debboun of the AMEDD Center & School designed their book as a practical, easy to read resource for those with an interest in the subject, or, more importantly, anyone involved in activities presenting the potential for contact with harmful arthropods.

The Editor



Outbreak of Dermatitis Linearis Caused by *Paederus ilsae* and *Paederus iliensis* (Coleoptera: Staphylinidae) at a Military Base in Iraq

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ABSTRACT

An outbreak of dermatitis linearis caused by *Paederus iliensis* (Coiffait) and *Paederus ilsae* (Bernhauer) occurred at Joint Base Balad in north central Iraq during 2007. It was the first reported incident of *P iliensis* in Iraq. Some *Paederus* species contain the vesicating chemical, pederin, which causes painful lesions when crushed on the skin. At this location, 20 Soldiers and Airmen sought medical treatment for skin blistering, most commonly affecting the neck and hands. All cases presented during May and June. Sampling for *Paederus* began in June after beetles were collected in an area where Soldiers had developed dermatitis and continued until October when no further beetles were collected. *Paederus* beetles were most likely flying in from areas surrounding the base, and were most common near the base's perimeter in close proximity to bright lights. Nighttime sampling showed that *Paederus* beetles were most active from one hour after sunset until midnight. Most of the military personnel affected were Soldiers who worked night shifts near bright lights. The occurrence of dermatitis linearis can largely be prevented by modifying the light sources that attract *Paederus* beetles.

INTRODUCTION

Several families of beetles have the ability to secrete vesicating chemicals. The presumed significance of these chemicals is that they deter predators, and, when passed along to the eggs, improve the likelihood of successful reproduction.[1,2] From a medical standpoint, the vesicating chemicals can injure human skin. From a public health standpoint, some of the beetles swarm during certain seasons and the large numbers of beetles pose a considerable public health threat.

The beetle families with the greatest impact on humans are Meloidae (blister beetles), Oedemeridae (false blister beetles), and Staphylinidae (rove beetles). Although many people use the term "blister beetle" to describe any beetle with vesicating properties, in this article that term will be applied strictly to Meloid beetles. Blister beetles and false blister beetles are medically important because they produce the chemical cantharidin that causes skin blistering. There are approximately 2,500 species within the family Meloidae worldwide, and all produce cantharidin.[3] There are approximately 1,000 species within the family Oedemeridae, but only a few species in the Pacific basin and Caribbean are known to produce cantharidin.[4,5] Cantharidin is synthesized by male beetles and passed to females during mating.[2] It is contained in the hemolymph and is exuded by reflexive bleeding from leg joints of adult beetles as a defensive mechanism or in response to external pressure, such as a person rubbing a beetle against the skin. It is a potent vesicant and causes blisters within 24 hours of skin contact. If a blister beetle is ingested, the consumed cantharidin causes severe irritation of the gastrointestinal tract and inflammation of the kidneys.[6] Cantharidin poses a special hazard for horses which can die after eating Meloid contaminated hay.[7,8] Soldiers may encounter these beetles when working outdoors. Indeed, there are numerous examples of cantharidin affecting military operations. A large number of French Legionnaires were hospitalized in Algeria for cantharidin poisoning after eating frogs that had ingested Meloids,[9] and Oedemerids have caused blistering among troops in New Zealand.[10]

The genus *Paederus* (Staphylinidae) contains more than 600 members with species occurring in all

temperate and tropical continents, north and south of the equator.[11] Most species are slender, about 7 mm to 13 mm long, and are distinctly colored with black heads, orange bodies, black abdominal tips, and metallic blue or green elytra (Figure 1). One or a combination of the vesicating chemicals pederin, pseudopederin, and pederone have been found in 20 species of *Paederus*.[12] Pederin is the most common chemical of the three and is one of the most complex nonproteinaceous insect secretions known.[12] It acts at the cellular level by blocking mitosis and is produced by endosymbiotic bacteria within beetles.[13] Females that are "infected" with these symbiotic bacteria produce eggs that contain the bacteria in the outer shell walls. Subsequent generations of beetles acquire the symbiotes necessary to produce pederin by ingesting eggs shells "infected" by the symbiotic bacteria or by cannibalizing larvae containing the bacteria.[14] Not all *Paederus* contain endosymbiotic bacteria and uninfected beetles do not produce pederin. Outbreaks often contain mixed populations of infected and noninfected *Paederus*.[15]

Paederus beetles do not release pederin as a defensive secretion and people are exposed to the chemical only when a beetle is accidentally crushed on the skin.[12] Therefore, skin blistering most often occurs on exposed skin of body parts such as the neck, head, arms, and legs where a beetle is felt crawling on the skin and subsequently crushed (Figure 2). Mirror-image lesions, commonly called "kissing" lesions, may form when uncontaminated skin is pressed against an opposite area of pederin contaminated skin. Pederin is sometimes transferred to other sensitive areas of the body such as the eyes or genitals by pederin-contaminated hands. Ocular and periocular lesions caused by accidentally rubbing pederin into the conjunctivae and eyelids are commonly known as "Nairobi eye" in east Africa.[16]

Figure 1. Adults of *Paederus ilsae* (top) and *Paederus iliensis* (bottom). The bright coloration may function to warn potential predators that these beetles are toxic.
(Photo courtesy of Mr Lyle Buss, University of Florida)

The skin condition in humans that is caused by vesicating chemicals from *Paederus* is called dermatitis linearis.[17] This eruption usually begins 24 to 72 hours after pederin contacts exposed human skin. Initially, affected areas turn red and then vesicles form a few days later. These vesicles typically coalesce into bullae that last for one or 2 weeks and eventually crust over, dry, and peel off, leaving red marks that can last for months.[6] The lesions are often very uncomfortable and may itch or burn severely.[18] In rare cases, contact with pederin can cause fever, headache, joint pain, and vomiting.[17]

Dermatitis caused by cantharidin is generally less symptomatic than that caused by pederin because cantharidin blisters do not cause as intense burning or itching. Cantharidin signs and symptoms usually begin within 12 to 24 hours, which is sooner than pederin's effects.[18] Another difference is that victims usually remember coming into contact with Meloid or Oedemerid beetles because of their large size (Figure 3). Many victims are not aware that *Paederus* were ever crushed on their skin because of their small size and the delayed onset of blistering.

Figure 2. Blisters caused by *Paederus* on the neck and hand of Soldiers at Joint Base Balad in 2007.

An additional militarily-relevant concern with *Paederus* is that blisters can easily be mistaken for the effects of chemical weapons. Some authors

regard pederin as a natural mimic of vesicating warfare agents such as mustard and Lewisite.[19] When adult beetles are active, a large number of individuals may be affected, which could lead to the false assumption that a chemical agent was used. In 1997, during a training exercise in Arizona, several Marines developed blisters, probably caused by *Paederus*, but believed they had been exposed to chemical weapons.[20]

In the tropics, outbreaks of dermatitis linearis are more common among western expatriates than among indigenous populations because the westerners typically use more lights at night, which then attract *Paederus*.[18] Similarly, outbreaks of dermatitis linearis that have affected military operations often involve the creation of well-illuminated camps in *Paederus* endemic areas. Outbreaks have occurred among British troops conducting operations in northern Kenya,[16] Canadian forces serving in the Central African Republic and in Sierra Leone,[21] and the Indian military during a training exercise in northern India.[22]

Dermatitis linearis was recently observed among US military members serving in central and southwest Asia. From December 2001 to March 2002, 191 cases of dermatitis linearis were observed among military personnel serving at a remote military base in Pakistan.[23] Most of the affected individuals worked near artificial lights at night. In May 2002, more than 30 cases of dermatitis linearis were observed among Special Operations Forces serving in Afghanistan.[24] The majority of affected Soldiers were night shift maintenance personnel.

Figure 3. Size comparison between *Epicauta vittata* (left) at 18 mm, a common cantharind-producing Meloid blister beetle from North America, and *Paederus iliensis* (right) at 7 mm, from Iraq.

There are few reports of *Paederus* species collected in Iraq. *Paederus mesopotamicus* (Eppelsheim) was described near Baghdad in 1889.[25] *Paederus fuscipes* (Curtis) and *Paederus ilsae* (Bernhauer) were included among a list of Iraqi insects published in 1965.[26] *P ilsae* has been found in cotton and clover fields near the city of Mosul in northern Iraq.[27] The first report of dermatitis linearis occurring in Iraq was recently published by an Iraqi doctor.[28] His report described 87 cases among Iraqi civilians that were treated at a hospital in the Najaf province of southern Iraq from April 2006 to April 2007, with the majority of cases occurring in May and June. The Iraqi doctor noted that dermatitis linearis has been a well known skin disease in the area for several years.

This article describes *Paederus* activity that occurred in 2007 at Joint Base Balad in Iraq. Although dermatitis linearis in Iraq is not limited to Joint Base Balad, it is the only location where the species of *Paederus* causing dermatitis linearis was identified. Information presented here can be used to help prevent and identify future outbreaks at other locations.

Materials and Methods

Joint Base Balad is approximately 110 km north of Baghdad in north central Iraq (Figure 4). The base is surrounded by agricultural areas that are supplied with water from the Tigris River through an extensive system of canals. There are no large urban areas in close proximity to the base.

In early May 2007, information was presented at a Multi-National Corps-Iraq Surgeon's Conference held in Baghdad about the possibility of vesicating beetles causing dermatitis in Iraq. Several military dermatologists had observed cases over the previous 4 years that they suspected might have been caused by beetles. At that time, no Meloid blister beetles or *Paederus* beetles had been collected and linked to cases of dermatitis occurring among Soldiers in Iraq. Based on the information presented at the Surgeon's Conference in Baghdad, a short presentation about vesicating beetles was made at a monthly Preventive Medicine Forum hosted at Joint Base Balad by the 133rd Medical Detachment on May 31, 2007. Within 24 hours, the 133rd Medical Detachment was contacted by medical personnel from 2 separate medical treatment facilities on base stating that they had recently treated Soldiers and Airmen with blisters that matched the clinical

signs and locations on the body associated with vesicating beetles. One medical provider noted that he assumed he was treating classical burns since his patients were mechanics and may have accidentally came into contact with hot vehicle parts or had hot fluids drip on their skin while working.

A survey was conducted on June 2, 2007, at a site where 5 Airmen worked and who had sought treatment for blisters on their necks the previous week. Sampling during the day at this site did not identify any beetles, however, 2 *Paederus* beetles were found the next morning on cockroach sticky traps that were placed overnight in the area. These beetles were sent for identification to Dr Howard Frank, an entomologist at the University of Florida who is a leading authority on *Paederus*, along with other specimens collected later during the summer.

During the following week, sampling for *Paederus* was conducted throughout the base and was focused around bodies of water because many species of *Paederus* are known to live in wet places, such as the edges of lakes, marshes, and floodplains.[12] Visual searches for beetles were conducted among plants and rocks surrounding retention ponds and other areas with standing water on base. Also dozens of cockroach sticky traps were placed around the edges of bodies of water.

Starting on June 6, 2007, sampling was conducted near portable light towers because many of the Soldiers and Airmen who developed blisters worked near bright lights at night. More than 100 generator-powered portable light towers were located throughout the base. Portable light towers were set at heights from 6 m to 9 m and had 1,000 watt metal halide lamps. White plastic dinner plates 26 cm in diameter were placed on the ground under the towers. The plates were filled with water and 3 to 4 drops of commercial dishwashing soap were added to each plate to break the surface tension. The plates were collected the following morning and the beetles counted.

Sampling was conducted each night from June 6 through June 9, 2007, at 20 light towers located throughout the base. After June 10 only light towers near the perimeter were used and sampling was conducted 3 times a week until the end of October 2007. An index for nightly beetle activity was determined by dividing the total number of beetles caught per night by the number of plates set out.

Figure 4. Location of Joint Base Balad in Iraq. (Map courtesy of US State Department, 2008, annotated separately.)

Sampling was conducted for 3 nights during the week of June 10 to June 16, 2007, to determine how proximity to the light source affected the number of beetles captured. On each night, 5 light towers were selected and 3 plates were placed at each light tower at fixed distances. One plate was placed directly underneath the light source, another at 2 meters, and a third 6 meters away.

Sampling was conducted on June 5 and June 20, 2007, to determine what times of the night beetles were actively flying. A particular light tower near the base's perimeter was selected based on high beetle collection counts during previous sampling periods. A white bed sheet was spread on the ground and visually observed from dusk and throughout the night. The observers counted beetles that actually landed on the sheet and recorded these tallies in one hour increments.

There were many questions asked by Soldiers and medical personnel as to whether permethrin treated uniforms would function to repel *Paederus*. To answer their questions, 10 live beetles were collected on the

night of June 20, 2007, and placed in clean Petri dishes that were kept inside an air conditioned building until the following day. Three of the beetles were individually placed on an Army Combat Uniform field jacket that was treated with permethrin 2 weeks earlier with an Individual Dynamic Application Absorption Kit. The beetles were left on the jacket for 15 minutes, their activity was recorded, and then they were placed back into Petri dishes and monitored until they died.

Results and Discussion

Twenty Soldiers and Airmen sought treatment at Joint Base Balad in 2007 for symptoms related to dermatitis linearis. It is possible that more individuals were affected but did not seek medical treatment. All cases occurred between late April and early June 2007. The most commonly affected body region was the neck followed by the hands and forearms (Table 1). The neck was the most affected region because it is one of the few exposed areas of skin when a Soldier is wearing a uniform. The hands were less commonly affected although they were probably involved with crushing beetles on the neck. The thick epidermis of the palms often prevents lesions from forming even when a beetle is crushed.[12] There were no reports of conjunctivitis caused by pederin contacting the eye. There was one case where a "kissing" lesion formed on a Soldier's neck and another Soldier developed a blister near the waistline which most likely occurred when a beetle fell down into his shirt and was crushed against his body.

Table 1. Most commonly affected body regions among 20 individuals at Joint Base Balad in 2007. Note: total percentage is greater than 100 because some patients had more than one region affected.

Affected Body Region	Number	%
Face	1	5
Neck	14	67
Torso	1	5
Arm and forearm	2	10
Hand	6	28
Total	24	115

The beetles collected at Joint Base Balad were identified by Dr Frank as *Paederus* (*Heteropaederus*) *iliensis* (Coiffait) and *Paederus* (*Dioncopaederus*) *ilsae* (Bernhauer). *Paederus ilsae* has been identified in 2 previous collections from Iraq.[26,27] This is the first known report of *P iliensis* occurring in Iraq. However, it is possible that this species was misidentified as another species in earlier studies. A report from 1965 listed *P fuscipes* in Iraq.[26] *P fuscipes* is closely related to *P iliensis*, and since *P iliensis* was not described until 1970,[29] it is possible that the earlier described *P fuscipes* was actually *P iliensis*. Both of the identified species are known in other countries to cause blister dermatitis. *P ilsae* has been identified as causing dermatitis in Israel[30] and *P ilsae* and *P iliensis* in southern Iran.[31,32]

The severity of dermatitis and amount of pederin contained by each species was not determined in this study. In Iran, *P ilsae* is known to cause more severe and longer lasting dermatitis than *P iliensis*.[31] In southern Iran, *P ilsae* was also more abundant than *P iliensis*.[32] In 2007, *P iliensis* was the most common species collected at Joint Base Balad. On the nights of 24 July and 25 July, 269 *P iliensis* and 9 *P ilsae* were collected, for a ratio of 30:1.

The period when beetles initially became active was not determined because surveillance did not begin until early June when cases of dermatitis were being noticed. Sampling showed that beetles became less active in mid-July and the last beetle was collected on October 8, 2007 (Figure 5). Since all cases of dermatitis occurred between late April and early June, beetle activity might have been greater during those months. *Paederus* activity at Joint Base Balad was consistent with previous reports showing that *P ilsae* and *P iliensis* were most active in the spring and early summer. *P ilsae* adults have been collected in northern Iraq in early June[27] and in Israel from May to July.[30] *P ilsae* and *P iliensis* were found in southern Iran from late April to early September.[31] Even though the species of *Paederus* were not identified, dermatitis linearis occurred in the Najaf province of Iraq from April to August with a peak incidence in May.[28]

There were no obvious peaks during sampling that indicated distinct generations of beetle emergence. *Paederus* beetles have one to 3 generations per year with more generations occurring in the tropics.[12] *P ilsae* is thought to have 2 generations per year in Iran.[32] A late start of sampling and overlap of generations may have masked generation peaks.

Flight activity by *Paederus* is often considered to follow rain showers or the rainy season.[12] Beetles most likely fly after rain showers because the higher

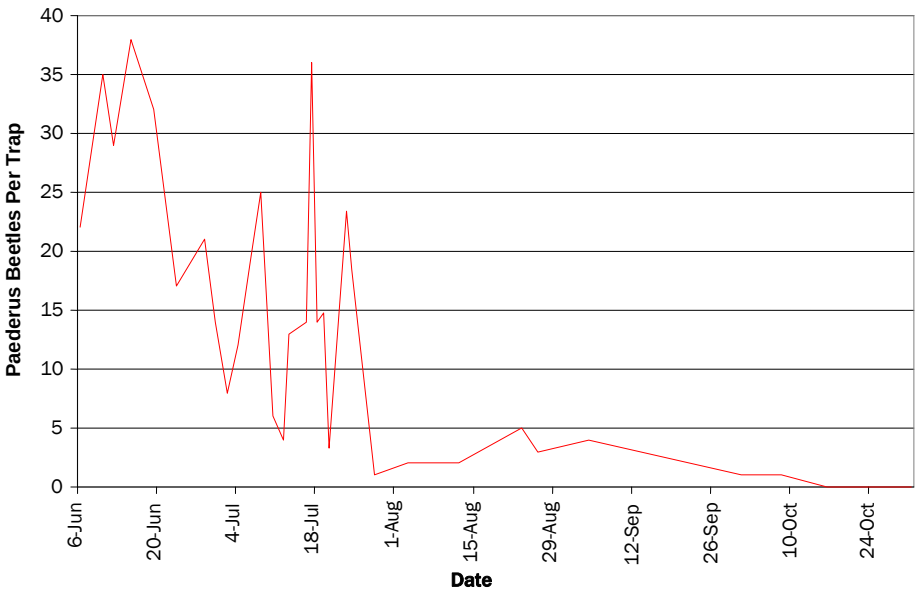

Figure 5. Average number of *Paederus spp* collected in water filled plastic plates under light towers per night at Joint Base Balad in 2007.

humidity helps the beetle avoid becoming desiccated during dispersal flights.[12] Cases of dermatitis in southern Iraq have been attributed to beetles beginning to fly after the rainy season ends in late March or early April.[28] At Joint Base Balad in 2007, there was no significant rainfall that immediately preceded beetle activity. However, it is possible that extensive irrigation in the area could have created conditions favorable for beetle flight.

During the night time sampling, observers first noticed beetles flying one hour after sunset (Figure 6). Beetle activity peaked before midnight and did not continue throughout the night. Reports from Israel indicated that *P ilsae* actively flies after sunset when the sky is completely dark.[30]

Beetles were most commonly collected directly beneath light sources. Placing plastic plates filled with water at fixed distances from light towers showed that significantly more beetles were found directly under lights than at distances only a few meters away (Table 2). Beetles were observed to have a positive phototaxis whether flying or running. Flying beetles fell to the ground as they approached a light source. Once beetles were on the ground, they moved rapidly to the spot of greatest illumination directly below the light source. This strong attraction to light greatly influences which individuals come into contact with the beetles. Many of the Soldiers who developed dermatitis were mechanics who worked at night on vehicles and other equipment that were directly illuminated by overhead light sources. Security guards were also commonly affected, especially those who had placed ultraviolet electrocution devices (Bug Zappers®) near their guard posts. In contrast, other Soldiers who worked at the same time, but not directly under a light source, were not affected.

The following 3 conditions are most often associated with outbreaks of dermatitis linearis[18]:

- People working in agricultural areas where beetles are active
- Beetles entering a building where people are working or sleeping
- People working at night in illuminated areas.

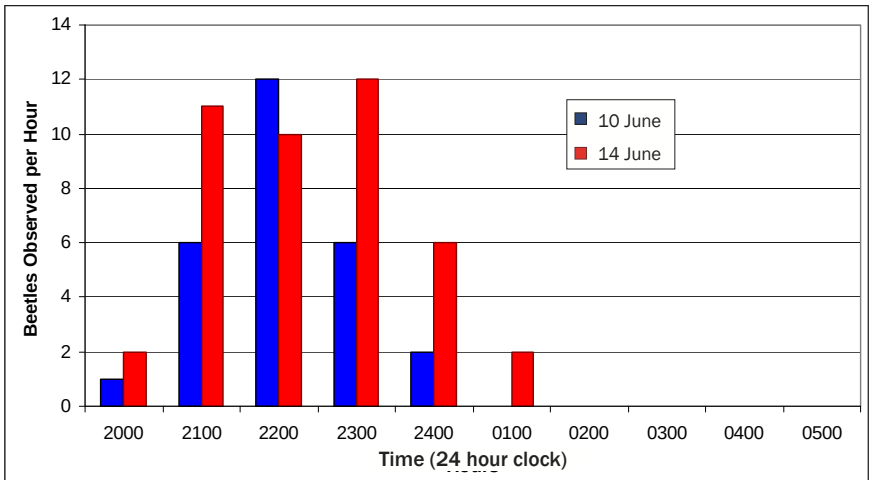

Figure 6. Number of *Paederus spp* observed at hourly intervals at Joint Base Balad in 2007. Nautical sunset was at 2011 hours June 10, 2007 and at 2013 hours on June 14, 2007.

It appeared that all cases in 2007 at Joint Base Balad resulted from Soldiers working under lights at night. Mass aggregations of beetles were never observed inside buildings as sometimes happens during outbreaks. Sometimes *Paederus* can be so numerous inside buildings that white walls appear black.[33]

Observed *Paederus* spp were most likely flying in from fields surrounding the base and were not found breeding around bodies of water on the base. On base, live beetles were found only near light sources at night. Dead beetles were found during the day under rocks or concrete barriers close to light towers. Another indication that beetles were flying onto the base was that they were more common near the perimeter of the base compared to the interior. The majority of Soldiers who developed dermatitis worked or lived near the base perimeter.

Joint Base Balad is an ideal environment for attracting *Paederus* because it has a large quantity of bright lights in a rural area with few competing light sources. It is unclear how common dermatitis linearis is among the communities surrounding the base. In southern Iraq, dermatitis linearis is commonly called phosphorous insect rash due to the beetles' attraction to fluorescent lights.[28]

To answer Soldiers' questions as to whether permethrin-treated uniforms repelled *Paederus*, 3 *P iliensis* were placed on a permethrin treated field jacket (Figure 7). The beetles showed no obvious aversion to being on the jacket and moved to concealed places, such as under the collar, where they remained until removed. We do not know if permethrin-treated uniforms will effectively cause beetles to quickly fly or drop off after they have landed on a Soldier. Nevertheless, we noticed that when a small sample of 3 beetles were placed on the treated uniform, they died sooner than the other beetles, ie, 2 to 3 days sooner, while the other beetles lived from 5 to 12 days in their Petri dishes. It is possible that the exposure to permethrin caused them to die sooner after being placed on the uniform. The efficacy of *N,N*-diethyl-3-methylbenzamide (deet) was not evaluated, although it might be useful, since it repels other types of beetles.[34] More studies are needed to determine if permethrin and deet repel *Paederus*.

Table 2. *Paederus spp* collections at different distances from a light source at Joint Base Balad in 2007.

Distance from Light Source	Date			
	10 June	12 June	16 June	Average
0 meters	35.8 ± 15.6	27.8 ± 15.4	44 ± 21.7	35.9 ± 17.6
2 meters	1.8 ± 1.5	2.2 ± 1.9	3 ± 2.3	2.3 ± 1.9
6 meters	0.2 ± 0.4	0.4 ± 0.9	0.2 ± 0.4	0.3 ± 0.6

Paederus beetles are highly susceptible to insecticides and there are many reports of controlling outbreaks with insecticides.[12] Most reports describe situations where *Paederus* enter buildings or form large aggregations and are clearly visible crawling on the walls. Permethrin was applied with an ultralow volume sprayer to control *Paederus* in a US military fitness center in Pakistan.[23] However, it does not seem practical to use insecticides to control *Paederus* in situations where they are attracted to lights and fly in from unknown distances away. The area that would need to be treated is too large and the sites where the beetles are breeding are often unknown.

Figure 7. *Paederus iliensis* crawling on permethrin treated uniform.

The best strategy to prevent outbreaks of dermatitis linearis when *Paederus* are flying into an area based on their attraction to light is to modify the attracting light sources. Staphylinid beetles are attracted to ultraviolet and white light, but are relatively insensitive to orange and yellow light.[35] An outbreak of dermatitis linearis at a game reserve in Tanzania was quickly halted after all mercury tube lights, which emit ultraviolet light, were replaced by incandescent light bulbs, which emit mainly yellow and orange light.[36] If possible, switch to sodium vapor and halogen lights because they emit only orange and yellow light and are therefore the least attractive to flying insects.[37] Another method of prevention would be to remove unnecessary lights

from an area to reduce the number of beetles attracted. At Joint Base Balad in 2007, *Paederus* were a problem at guard posts where ultraviolet insect electrocution devices were used but ceased to be a problem when those devices were removed. Rest and work areas can be relocated farther away from bright lights. There were several cases of dermatitis linearis among a group of night-shift mechanics who took smoke breaks at a table located directly under a light tower, but new cases of dermatitis ceased once the table was moved.

Sampling for *Paederus* was performed by placing water-filled plastic plates under light towers near the perimeter of base. This sampling method was efficient because it collected a large number of beetles, did not require special equipment, and was easily implemented by preventive medicine personnel during their routine mosquito and sand fly surveillance. Using light towers or other bright lights to determine if *Paederus* are present would be worthwhile at other locations because outbreaks are often unpredictable. When large outbreaks do occur, they can have a major impact on public health. In 1966, more than 2,000 patients sought treatment for dermatitis linearis at a US Army hospital in Okinawa,[38] and outbreaks among Australian aboriginal communities have forced the evacuation of entire villages.[39] Early detection of *Paederus* through sampling will allow implementation of preventive measures to lessen the effects of an outbreak. Notification of medical personnel of the presence of *Paederus* enables prompt education on diagnosis and management of cases of dermatitis and averts or dispels needless concern that chemical weapons have been released.[19] Information from entomologic surveillance can help educate Soldiers about the time periods when beetles are active and what to do if they work in an area infested with *Paederus*. Soldiers should be instructed to try to avoid crushing any insects crawling on their skin, and if a *Paederus* beetle is crushed, to immediately wash the area with soap and water to prevent skin blistering.[12]

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Operational Vector-borne Disease Surveillance and Control: Closing the Capabilities Gap through Research at Overseas Military Laboratories

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ABSTRACT

Malaria, dengue fever, chikungunya virus, leishmaniasis, and a myriad of other vector-borne diseases pose significant threats to the warfighter and to the overall combat effectiveness of units. Military preventive medicine (PM) assets must accurately evaluate the vector-borne disease threat and then implement and/or advise the commander on countermeasures to reduce a particular threat. The success of these measures is contingent upon the biology of the disease vector and on the tools or methods used to conduct vector/pathogen surveillance and vector control. There is a significant gap between the tools available and those required for operational PM assets to provide real-time, effective surveillance and control. A network of US Army and US Navy overseas laboratories is focused on closing the current capabilities gap. Their mission is to develop and field test tools and methods to enhance the combatant commander's ability to identify and mitigate the threat posed by these vector-borne diseases.

INTRODUCTION

Since its inception, the US military has consistently been called upon to wage battles and to conduct peacekeeping and humanitarian relief operations in locations of the world where the most formidable enemy is often a tiny, 6- or 8-legged creature. Indeed, in every war fought by the United States up through the Vietnam conflict, the number of casualties caused by arthropod-borne diseases has significantly exceeded the number of battlefield casualties.[1] It was the pioneering efforts of Walter Reed, William Gorgas, and others that helped to decipher the link between microbes, human disease, and mosquito vectors.[2] Their discoveries led the way in the fight against typhoid fever, yellow fever, malaria, and other diseases that had plagued military and civilian populations for millennia.

In spite of these discoveries, malaria continued to take its toll on US forces. During World War I, almost 17,000 troops acquired malaria.[1] Over 24,000,000 person days were lost during World War II to malaria and other arthropod-borne diseases such as scrub typhus and dengue fever.[1] In 1943, Allied forces averaged 208 new cases of malaria per 1,000 Soldiers stationed in the south Pacific.[3] In the Vietnam conflict, annual case rates of malaria reached as high as 600 per 1,000 troops.[1] In the 1940s, entomologists from the US Department of Agriculture developed methods and equipment to use DDT* to control mosquitoes, lice, and other vectors. In collaboration with military entomologists, they developed insecticide dispersal equipment and implemented malaria eradication programs which together reduced the number of malaria cases in the south Pacific to 5 per 1000 Soldiers by 1945.[3]

Despite these early successes, there has been a dramatic resurgence of mosquito-borne diseases

*Dichlorodiphenyltrichloroethane

during the post-DDT era, and today's military continues to face a significant threat from arthropod vectors. While significant strides have been made in the implementation of personal protective measures, including malaria chemoprophylaxis, bed nets, repellents, and permethrin-treated uniforms, the battle against arthropod-borne disease is far from won. Compliance with such measures remains a problem, as evidenced from a survey of Soldiers returning from Afghanistan.[4] In general, though, the advances in science and medicine have been outpaced by the adaptability of vectors and the evolution of pathogens. Novel more efficient strategies to reduce transmission of vector-borne diseases are required if the military is to effectively combat this threat.

The required tools would allow deployed PM personnel to accurately evaluate the risk of disease transmission through vector/pathogen surveillance, and subsequently implement control measures to break the cycle of transmission. However, there currently exists a gap between what preventive medicine assets have at their disposal for surveillance and control and what they require to more effectively minimize the potential for arthropod-borne disease transmission. Effective control measures are only achievable through the use of real-time, accurate surveillance tools along with a sufficient understanding of the biology and behavior of problem vectors.

US Army, Navy, and Air Force entomologists stationed in 5 overseas laboratories are focused on developing and evaluating tools and methods that would fill this capabilities gap:

- Thailand – the Armed Forces Research Institute of Medical Sciences (AFRIMS)
- Kenya – the US Army Medical Research Unit - Kenya (USAMRU-K)
- Indonesia – the US Naval Medical Research Unit No. 2 (NAMRU-2)
- Egypt – the US Naval Medical Research Unit No. 3 (NAMRU-3)
- Peru – the US Naval Medical Research Center Detachment (NMRCD)

This group of researchers is on the front lines working to address the military's vector/pathogen surveillance and control shortfalls. What follows is a summary of the team's efforts associated with 5 priority arthropod-borne disease threats (malaria, dengue fever, chikungunya virus, visceral leishmaniasis, and cutaneous leishmaniasis).[5]

DISEASE OVERVIEW

Malaria

Annually, 300 to 500 million people are infected with malaria, with 1.5 to 2.7 million fatalities (mostly children).[6] Several hundred cases of malaria, transmitted by night-feeding *Anopheles* mosquitoes, continue to infect military personnel deployed in locations throughout southwest Asia, sub-Saharan Africa, and the Korean peninsula. A total of 425 cases of malaria were diagnosed in US military personnel between the years 2000 and 2005.[7] Most notable was the occurrence of 38 cases of vivax malaria in a 725-man US Army Ranger task force that deployed to Afghanistan between June and September 2002.[4] Another significant malaria outbreak occurred during the deployment of 225 Marines to Liberia in which 80 Marines contracted falciparum malaria.[8] Since the early 1990s, US troops deployed in South Korea have consistently been at risk of exposure to vivax malaria.[9] Although there are significant efforts underway from Department of Defense (DoD) researchers, the Gates Foundation, and private industry to develop antimalarial drugs and malaria vaccines, a licensed vaccine is likely a decade or more away, and drug resistant parasites are continually appearing.[10] In the interim, improved methods to reduce the risk of infection through mosquito control or personal protection are critical.

Dengue Fever

We are currently witnessing a worldwide resurgence (and in some cases emergence) of arthropod-borne viral (arboviral) diseases, and dengue fever leads the list as a public health threat.[11] The 4 dengue virus (DENV) strains are maintained in cycles involving humans and the container-breeding *Aedes aegypti* mosquito, a vector that typically feeds on humans during the daylight hours. The lack of treatment, the explosive nature of this disease, and the potential for acquiring dengue hemorrhagic fever (a deadly form of the disease) are causes for concern among DoD planners. While DoD researchers are working towards a vaccine that is protective against all 4 DENV serotypes, mosquito surveillance and control remain central to prevention and control of dengue fever outbreaks.

Chikungunya

Chikungunya virus (CHIKV) is an alphavirus transmitted to humans by container-breeding *Aedes* mosquitoes. The virus is endemic to Africa and various parts of Asia, including Indonesia and the Philippines.[12] This disease is currently showing a pattern of reemergence. In the last 5 years, explosive outbreaks have occurred in Kenya, the Seychelles, Comoros, Mayotte, Mauritius, Madagascar, India, and Italy.[13] A specific example of the alarming attack rates occurred on the island of La Reunion where almost 40% of the island's total population of 785,000 fell ill in 2005-2006.[14] Recent outbreaks have also been reported in Singapore, Malaysia, and Thailand. Symptoms include fever, incapacitating joint pain, and rash which generally disappear after a few days. While CHIKV is rarely fatal, joint pain can persist for months or even years. Given the lack of a vaccine or treatment and the history of large epidemics, prevention of infection through vector control is paramount.

Leishmaniasis

Leishmaniasis presents itself in 2 main forms: cutaneous leishmaniasis, which often results in skin lesions or attacks mucous membranes; and visceral leishmaniasis, which can lead to liver and other organ damage, and sometimes even death. Leishmaniasis is a parasitic infection caused by a variety of *Leishmania* species. Phlebotomine sand flies are involved in the transmission of *Leishmania*, from rodents and canids to humans. Sand flies are elusive, pinhead-sized insects of which little is understood about their biology and behavior. Consequently, control of these disease vectors continues to be a significant challenge for military entomologists.[15] No prophylactic drugs or vaccines are currently available, and emphasis is placed on preventive measures to break the cycle of transmission.[16]

Defining the Gap

Effective vector-borne disease prevention relies on answering a core set of questions. Once these questions are answered, decision makers can design strategies based on evidence and tailored to the unique dynamics of their specific situation (in space and time). The fewer questions we can answer, and the more assumptions we make, the more likely we are to implement ineffective, one-size-fits-all solutions. At a minimum, the following questions must be answered.[16]

1. Is there disease transmission in the area?
2. Which arthropod species are present and which ones are vectors of disease?
3. Which ones feed on humans?
4. Where does human-vector contact occur?
5. Where do the vector species breed and rest?
6. When and where should a vector control strategy be implemented?
7. What proportion of the vector population is susceptible or resistant to insecticides?
8. What vector control options will likely reduce disease transmission?

Malaria can be used as an example to highlight the importance of addressing the previous questions. Approximately 430 Anopheles species are found worldwide of which only 30 to 40 species transmit malaria in nature.[17] Of these, some feed indoors and others feed outdoors. If mosquito control programs are designed without determining when and where personnel are being exposed, they will likely fail to reduce the threat. However, with answers to the questions above, preventive medicine personnel may advise the use of insecticides inside sleeping areas to control a mosquito species which prefers to feed indoors at night. Although it is paramount that most, if not all, of the questions above be addressed prior to the implementation of a program, the current reality is that we do not yet have the capability to answer all of them. Seven of the major gaps are presented below, along with the measures that are being undertaken by military entomologists working overseas to overcome the recognized deficiencies.

Defining and Closing the Capabilities Gaps

Task: Conduct Vector Surveillance

Gap: Adult mosquito and sand fly collection devices are minimally effective

Problem. Numerous devices have been developed over the years to survey for *Aedes* (dengue/chikungunya vectors) and *Anopheles* mosquitoes (malaria vectors).[18] Sticky traps, visual traps, light traps and backpack aspirators are the most widely used tools for conducting mosquito surveillance,[19-21] with Centers for Disease Control and Prevention (CDC) light traps (Figure 1) often considered the industry standard for mosquito surveillance. However, all of these trap types

Figure 1. A CDC light trap.

have drawbacks.[18] For example: sticky traps can damage sampled mosquitoes, the degree of success with backpack aspirators is highly dependent on the collector/inspector, visual traps are generally of lower efficiency, and CDC light traps lack an olfactory-based attractant and contain a light source that has the unintended effect of repelling some mosquito species.

Ideally, human landing counts (HLCs) can be used to evaluate the efficacy of vector control. This technique involves collecting host-seeking arthropod females that land on an individual human's exposed legs. Data collected using this technique generally correlates well with local vector population densities. The HLCs can also be used to help determine entomological inoculation rates, which are true estimates of the disease risk posed to humans. While surveillance using HLCs is the most effective approach for determining mosquito densities, the ethical issue of placing humans at risk for contracting disease from pathogens originating from mosquito vectors makes this approach less appealing in today's environment. Currently, PM assets depend on the CDC light trap (or a related version) to conduct most adult mosquito surveillance. The main attractant is a 4- to 6-watt incandescent light bulb. As the mosquito approaches the light source, it is drawn downward into a collection bag by a fan mounted just below the light bulb. This surveillance tool has significant limitations with regard to collecting disease vectors:

- The trap is only effective for some mosquito species that feed at night (ie, *Anopheles*). The CDC light trap is an inadequate surveillance tool for mosquitoes which feed during the day, such as DENV and CHIKV vectors.
- Not all night-feeding adult mosquitoes are attracted to the light source of a CDC trap. Mosquitoes only see in the visible light spectra of blue, green, and red. Incandescent light sources emit light most strongly in the infrared spectra and weakly in the visible light spectra of blue, green, and red.[22] Therefore, even though *Anopheles* feed during the night, potentially important vectors are often "repelled" by the light source.
- The trap should be augmented with a carbon dioxide source to enhance trap effectiveness. The carbon dioxide source might come in the form of dry ice, granular CO_2-sachets, or a canister of CO_2. Blood-feeding mosquitoes use a combination of olfactory cues in addition to visual cues to find a suitable host. Carbon dioxide is considered to be a principal olfactory attractant.
- The efficiency of converting electrical current to light using incandescent light bulbs is exceptionally low (approximately 6%). The remainder (94%) is often dispersed as heat or infrared radiation.[22] As a consequence, this device consumes a great deal of energy.
- The effectiveness of these traps for collecting sand flies has not been systematically evaluated.

Solutions

BG-Sentinel Trap (BGS) (Biogents AG, Regensburg, Germany). The BGS (Figure 2) has shown significant promise as a tool for collecting *Ae aegypti*,[23-26] and *Ae albopictus*.[27] To our knowledge, this device has not been evaluated for malaria vectors in Asia. The BGS uses a blend of mosquito attractants consisting of lactic acid, ammonia, and caproic acid, substances all found on human skin. The blend is released in a fixed ratio from a dispenser known as the BG Lure.[23] The efficacy of the BGS (with and without carbon dioxide) relative to other surveillance devices for collecting DENV and malaria vectors is being evaluated in Thailand.

Bed Net Traps. An alternative to HLCs is needed, particularly in areas of high disease transmission. The performance of a self-supporting bed net trap is undergoing evaluation by Navy entomologists at NAMRU-2. Preliminary results show that a lightweight (2 kg), easy-to-assemble bed net trap

Figure 2. The BG-Sentinel trap.

collected significantly greater numbers of *Anopheles* spp and *Culex* spp (vectors of Japanese encephalitis virus and filariasis) than a CDC trap. The trap, shown in Figure 3, is designed to protect the person (the attractant) in the bed net while simultaneously trapping (in an outer tent structure) vectors that can be identified and tested for human pathogens. In areas where disease transmission is very high or drug resistant pathogens occur, collections of mosquitoes could be made while minimizing the risk to the collector.[28,29]

Mass Trapping Techniques for Surveillance/Control. Traps that generate CO_2 by catalyzing propane have demonstrated a reduction in nuisance mosquito or biting midge populations.[30-33] Little is known regarding the effectiveness of these traps on reducing disease vector species in isolated, tropical environments.[33] Manufacturers of commercially available traps claim the ability to control mosquito populations over an area as large as one acre.* Future evaluations of this technology at overseas field sites will determine the efficacy of a commercial mosquito trap in reducing prevalence of mosquito populations in a specific area.

Sand Fly Attractants. Modified CDC light traps were recently tested in southern Egypt by NAMRU-3 researchers. The light traps were modified to accommodate light emitting diodes, and proved to be very effective for sand fly surveillance.[34] At present, field studies are being conducted to determine the efficacy of a wide range of semiochemicals, either repellents or attractants, on the activity of sand flies. Pheromones from *Lutzomyia longipalpis* (leishmaniasis vector) males have shown significant promise.[35] Like many sand flies, *L longipalpis* is a lekking species, one in which males gather for the purposes of a competitive mating display. Females are attracted to displays of male wing-fanning behavior and pheromones released by the male. It is therefore likely that traps baited with male pheromones will attract female sand flies in the field (their potential has already been shown in the laboratory). Efforts are underway in Colombia where scientists from the NMRCD, in collaboration with Rothamsted Research (Harpenden, Hertfordshire, UK)[20] are evaluating the efficacy of these pheromones as baits in a variety of commercial traps placed in jungle encampments in the Colombian Amazon.

Figure 3. The bed net trap.

Task: Identify Vector Species

Gap: Limited availability of country-specific taxonomic keys and limited knowledge of species' bionomics, relative vector status, and distribution

Problem. The Walter Reed Biosystematics Unit has made significant strides in designing user-friendly, regional keys for the identification of mosquitoes and sand flies worldwide. These keys have proven their utility, most especially in southwest Asia. However, regionally relevant identification keys along with descriptions related to the feeding, breeding, and resting behavior, relative vector status, and distribution of species are still lacking for much of the tropics. Without these references, PM planners are not able to answer the relevant questions which drive implementation of sound control programs.

*Example: Mosquito Magnet®, Woodstream Corp, Lititz, PA Information available at http://www.mosquitomagnet.com

Solutions

Sand Fly Biology and Taxonomic Research. The NAMRU-3 has been conducting extensive sand fly research and surveillance in countries throughout Africa and the Middle East for over 50 years. During that time, the NAMRU-3 Vector Biology Research Program (VBRP) has determined the vector status of numerous *Phlebotomus* species found throughout the Middle East and African region,[36-38] and has developed a better understanding of sand fly ecology in various countries within the aforementioned region, including extensive bionomics studies of sand flies in the Sinai[36,39] and in southern Egypt, where sand fly daytime resting sites were recently discovered for the first time.[40] Sand fly species distributions have been determined in Djibouti,[41] and NAMRU-3 is completing compilations for Egypt and parts of Ghana. Further, wide-scale species distribution projects are currently underway in Afghanistan and Libya. Collectively, NAMRU-3 has produced taxonomic keys for the sand flies of Afghanistan, Egypt, Ghana, Sudan (B.D.F., unpublished data, 2009), and Djibouti.[41] To supplement the morphological identification of sand flies, VBRP has a rapidly expanding polymerase chain reaction component which allows for the molecular identification of sand flies.

Mosquito Keys. World-renowned mosquito taxonomist Dr Rampa Rattanarithikul has spent decades at AFRIMS dissecting, classifying, and analyzing mosquitoes.[42] With the assistance of illustrator Prachong Panthusiri, they are on the verge of completing the last volume of a 6-volume publication entitled *Illustrated Keys to the Mosquitoes of Thailand*.[43-47] This tool will be exceptionally valuable for preventive medicine assets deploying to southeast Asia, especially for those associated with the annual Cobra Gold exercises in Thailand and current operations in the Republic of the Philippines.

Task: Conduct Pathogen Surveillance

Gap: Rapid pathogen surveillance devices are lacking

Problem. While a disease vector may be present in a particular area, the broad application of control measures may not necessarily be warranted. Pathogen surveillance tools allow for the effective local targeting of control measures, thereby enhancing the possibility of managing the disease, while reaping the benefits of reduced costs and avoidance of the potential environmental and human health risks often associated with insecticide application. For example, the malaria dipstick assay is a rapid, one-step procedure that uses a test strip capable of detecting and then differentiating between infections of *P falciparum* and *P vivax* in adult mosquito collections within minutes.[48] This particular hand-held device is of lower cost and is very user-friendly relative to polymerase chain reaction methods, and can potentially identify areas where the risk of contracting malaria is high. Such a tool can help to prioritize control efforts and has significant impact when requesting support or the assistance of the command. This type of rapid screening tool is not available for most vector-borne pathogens.

Solutions. The overseas laboratories are actively involved in the field evaluation of hand-held dipstick assays for the detection of leishmaniasis, DENV, Japanese encephalitis virus, and Rift Valley fever virus. In addition, in collaboration with both the US Army Research Institute for Infectious Diseases and the US Air Force 59th Clinical Research Division, entomologists at AFRIMS, NAMRU-3, and USAMRU-K are conducting field evaluations of real-time polymerase chain reaction assays to detect vector-borne pathogens. Many of these assays are designed for the Joint Biological Agent Identification and Diagnostic System using the Ruggedized Advanced Pathogen Identification Device (Idaho Technology Incorporated, Salt Lake City, Utah) as the platform.[49,50]

Task: Provide Personal Protective Measures

Gap: The military has yet to field bed nets, tents, or other materials which have been treated with long lasting insecticides

Problem. The use of insecticide-treated nets (ITNs) and insecticide-treated tents is regarded as one of the most promising measures available to reduce vector-borne disease transmission.[51] However, treatment of ITNs with insecticide is performed in the field (not at the factory) which presents an added burden for PM assets. Application of insecticide to bed nets and tents is labor-intensive and not often a command priority.

Solutions

Long-lasting Insecticidal Net (LLIN) Evaluations. Manufacturers have developed LLINs such as Perma-Net® (Vestergaard Frandsen, Lausanne, Switzerland) which are ready-to-use, factory pretreated nets. Many of these nets are designed to release insecticide slowly so that the nets retain their efficacy after repeated

washings. Some LLINs are said to require no further treatment during their physical lifespan of 4 to 5 years.[51] Currently, AFRIMS is evaluating the long-term efficacy of LLINs on vector densities in villages located in western Thailand. If shown to be effective, LLINs may be a valuable alternative to the field-application of insecticide to bed nets.

Insecticide-impregnated Tent Evaluations. US Army Deployable Rapid Assembly Shelter (DRASH) tents are woven from a noncanvas XYTEX® (DHS Technologies LLC, Orangeburg, New York) fabric, which is polyester-coated with polyvinyl chloride. Studies are underway in southeastern Thailand to determine whether insecticide-impregnated tents are indeed a viable approach for protecting Soldiers from vector-borne disease over the long-term. If this is found to be the case, further studies will then evaluate the utility of insecticide-impregnated DRASH tents (assuming that the technology is developed) in affording protection for Soldiers.

Task: Conduct Vector Control

Gap: Lack of effective and sustainable control methods for *Aedes* mosquitoes (CHIKV and DENV vectors)

Problem. Given adequate personnel, community involvement, money, and time, *Ae aegypti* population control can be achieved through sanitation and the elimination of mosquito breeding sites. This option is often not viable for military pest controllers during deployments. Effective source reduction is difficult when the military has no control of areas surrounding encampments occupied by US personnel. Chemical control using DoD-registered larvicides (larval stage insecticides) such as methoprene, temephos, and *Bacillus thuringiensis israelensis (Bti)* and various adulticides (adult stage insecticides) has proven effective in managing both larval and adult populations respectively of *Ae aegypti*.[52] However, these options have obvious limitations when it comes to deployments and protecting the Warfighter. Indoor adulticide application can effectively reduce *Ae aegypti* populations but must be applied inside occupied structures on a regular basis. This can be difficult to justify as a preventive measure and is often unachievable. In general, the military is more likely to practice indoor adulticide application in response to disease transmission rather than as a preventive measure. Traditional larval control is logistically daunting and is minimally effective if surrounding areas are left untreated, or if coverage in the target area is low. What is lacking is an expedient, low risk, efficient, and sustainable approach to achieving epidemiologically-relevant levels of *Ae aegypti* population suppression.

Solutions

Resting/oviposition Lures Treated with Pyriproxyfen. Pyriproxyfen is classified as an insect growth regulator and a potent inhibitor of embryogenesis, metamorphosis, and adult formation in insects.[53] In addition, it has been shown to decrease the fertility and fecundity of *Ae aegypti* adults that develop from sublethally exposed larvae.[54] Evidence also suggests that adult mosquitoes not killed by contact with pyriproxyfen that is applied to breeding containers can actually carry the chemical to uncontaminated environments. The tiny doses of pyriproxyfen that are moved can then negatively affect the development of susceptible larvae.[55] Pyriproxyfen has long residual efficacy yet has an excellent mammalian toxicity profile. The most promising scenario involves females resting on surfaces treated with pyriproxyfen, picking up tiny doses of chemical that sterilize the females which then transports the pesticide to other breeding sites. Work conducted by the NMRCD, Rothamsted Research, and the Peruvian health authorities suggests that this approach has considerable potential. The NMRCD is designing a variety of simple resting and oviposition lures which will attract *Ae aegypti* and which can be treated with pyriproxyfen. The efficacies of these treated lures on fecundity and on the horizontal transfer of pyriproxyfen will be evaluated under seminatural conditions with future plans for field evaluations to establish the optimal distribution of such treated lures.

Pyriproxyfen-treated Ovitrap/resting Station Device Design/evaluation. Researchers at AFRIMS are designing and evaluating a visually attractive, pyriproxyfen-treated ovitrap (egg trap)/resting station device. It is clear that *Ae aegypti* females tend to spread their eggs among many sites. This behavior should improve the natural transfer of pyriproxyfen. *Ae aegypti* tend to remain relatively close to their larval habitat, with maximum dispersal distances around 100 to 200 m,[56] therefore significant control (or local eradication) via this approach is within reason. This is particularly relevant in the context of a military

camp where sanitation is high and larval habitat can be minimized. Initial lab evaluation of prototypes is underway using field cages/tunnels. Field trials are scheduled to assess the impact of the final product, spatially arranged as a barrier-like treatment, on *Ae aegypti* densities in a simulated military camp within a dengue-endemic village.

ProVector™ Trap Evaluations. A possible tool for the control of adult *Ae aegypti* populations currently being evaluated at USAMRU-K is the ProVector™ trap (MIT Holding Inc, Savannah, Georgia). This low cost trap mimics visual and chemical cues used by mosquitoes in search of a sugar meal. The trap is designed in such a way that only mosquitoes can feed on it, thus reducing the possibility of exposure to nontarget organisms and accidental environmental contamination. These traps, designed at the Biodefense and Infectious Disease Laboratory at Georgia Southern University, use a formulation of *Bacillus thuringiensis israeliensis* bioinsecticide as the active ingredient. This trap will be evaluated not only for its efficacy as a method for DENV and malaria vector control tool, but also as a tool for increasing the efficacy of *Ae aegypti* surveillance.

Push-pull System. Research is underway at NMRCD to develop a novel insecticide treated material (ITM) push-pull system to reduce *Ae aegypti* inside homes where they are most likely to feed on humans and transmit DENV. The system is comprised of ITMs designed to repel *Ae aegypti* from inside homes and an attractive, lethal trap positioned outside the home to pull the mosquitoes from the peridomestic environment. Following proof-of-concept, long-term goals include defining the public health impact of the system through epidemiological studies for operational refinement.

Gap: No effective replacement for the insecticide DDT

Problem. The successful eradication of malaria from the developed world and large portions of tropical Asia and Latin America was due largely to the widespread use of DDT.[57] The effectiveness of DDT is primarily a result of its long lasting persistence. Ironically, this quality also led to the cancellation of its registration by the Environmental Protection Agency.[58] Modern alternatives to DDT are relatively unstable in the environment and therefore have shorter windows of efficacy. Effective vector control operations thus require more frequent spraying with obvious repercussions on sustainability. A DDT alternative with similar durability and effectiveness is desperately needed.

Solution. Pyrethroids have replaced many older insecticides because of their effectiveness and relative safety for the applicator.[58] The chemical structure of pyrethroids resembles a component of the natural botanical insecticide known as pyrethrum. Pyrethroids are highly toxic to most insects at very low rates of application. While pyrethroids do not measure up to DDT in terms of stability, there is evidence that the pyrethroid bifenthrin may be efficacious against mosquitoes for a considerable length of time postapplication (6 weeks) when applied to vegetation relative to other insecticides.[59] TalStarOne™ (FMC Corp, Philadelphia, Pennsylvania) (bifenthrin 7.9%) is EPA-registered and endorsed by the Armed Forces Pest Management Board for use on military installations and during deployments. Recent work at USAMRU-K, in partnership with the US Department of Agriculture Center for Medical and Veterinary Entomology, have shown that bifenthrin-treated camouflage nets provide an effective protective barrier against mosquitoes and sand flies, significantly reducing total trap numbers for over 6 weeks. Additionally, mortality rates were significantly higher for those vectors that made it through the nets, suggesting that treated nets provide protection beyond the initial barrier effect for which they were tested. Evaluations are also being performed in Thailand to determine the extent to which this insecticide is effective for the long-term control of malaria vectors when applied to vegetation along the perimeter of a village (Figure 4).

Gap: No effective sand fly control strategies

Problem. Unlike mosquito control, sand flies pose numerous problems that prevent the coherent development of an effective control strategy. Sand fly breeding habitats are much more cryptic and often impossible to identify, thus preventing the development of any effective larviciding strategy. Sand fly populations are extremely focal, with significant geographical and seasonal variations. Sand fly surveillance is a challenge and without the surveillance data, it is difficult to make sound decisions for targeted sand fly control operations. In

the absence of better alternatives, deployed personnel currently revert to mosquito control methods. Sustainable and evidence-based strategies for targeting sand flies are clearly needed.

Solution. Rodent Pass-through System. The effectiveness of a rodent pass-through system to control sand fly populations is being evaluated at USAMRU-K. Laboratory studies have shown that diflubenzuron can prevent synthesis of chitin (the material composing the outer skeleton of arthropods). Diflubenzuron is nontoxic to rodents and remains active after passage through the rodent digestive tract.[60] Since the larvae of many sand fly species feed on rodent feces,[61] this may provide the first truly effective technique for targeting larval sand flies. Once baits are formulated for the targeted rodent fauna, diflubenzuron will be incorporated and the baits offered on a monthly basis. It is important to note that this would target native rodent populations in areas where troops are deployed, not pest rodent populations that are normally targeted with anticoagulant baits. Also, preliminary work is underway to evaluate the efficacy of using a sugar solution spray as bait for targeting adult male and female sand flies in search of sugar meals.

Figure 4. Application of pyrethroid bifenthrin on the perimeter of jungle village in Thailand in a test of long-term effectiveness.

Conclusion

There is a well recognized gap between the resources required and those available to manage the risks to the Warfighter posed by malaria, dengue fever, chikungunya virus, leishmaniasis, and myriad other vector-borne disease threats. Vector control experts are expected to accurately evaluate the vector-borne disease threat and then make sound decisions to reduce that threat. Following the models of the pioneering work of Reed, Gorgas, and others, US Army, Navy, and Air Force researchers working overseas are helping to close this gap. Their efforts are enhancing the combatant commander's ability to identify and mitigate the threat posed by these vector-borne diseases.

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Sand Fly

Asian Tiger Mosquito

Applications of Ecological Niche Modeling to Enhance Medical Threat Assessment and Disease Control and Prevention Strategies

MAJ Michelle Colacicco-Mayhugh, MS, USA

One of the major challenges for military preventive medicine is in developing medical threat assessments both prior to and during deployment. When it comes to vector-borne diseases, this can be especially problematic. In many areas, it is either impractical or impossible to conduct a thorough predeployment surveillance program. This leads to reliance on products, such as the Disease Vector Ecology Profiles, that may be out of date or too general in nature.

In recent years, the use of remotely sensed data (ie, satellite imagery) has gained popularity in many areas of biology, including epidemiology.[1] Along with this, a number of modeling approaches have been developed to answer basic questions about the distribution, ecology, and potential range of a species in the absence of comprehensive sampling data. These techniques are commonly referred to as ecological niche modeling or species distribution modeling. Niche modeling holds the potential to be a powerful tool for the military preventive medicine community in developing more robust risk assessments for vector-borne disease and better targeting surveillance, control, and education efforts.

BACKGROUND

Joseph Grinell originally presented the idea that species inhabit specific areas (the niche), defined by the species' biological requirements for survival.[2] The ecological niche is determined by 4 interacting factors.[3-5] These are:

- Biotic factors: Interactions with other species including: predators, pathogens, prey, parasites, and vegetation.
- Abiotic factors: The environmental factors that determine whether or not a species can survive in a given location (climate, soil, etc.).
- The ability of a species to disperse to new areas. This includes any physical barriers to dispersion (such as mountain ranges, bodies of water, and deserts) as well as the biological capacity or propensity of a species to disperse.
- The ability of the species to adapt to new environments.

Together, these factors determine the actual and potential geographic distribution of a species.[3] Niche modeling techniques aim to answer a variety of questions based on the knowledge of these factors for a given species.

Figure 1 presents a diagrammatic representation of the factors that determine the niche for a given organism. The area in which all 3 circles overlap is the actual niche of a species. The areas where only 2 circles overlap are the potential niche. For instance, the area labeled "D" in this example is currently uninhabitable by a species due to abiotic factors (such as temperature or precipitation). If the abiotic factors limiting the range of the species are changed (such as through an El Niño or La Niña event, or some other form of climate change) so that they are conducive to that species, the niche could shift to include that area.

Niche modeling has been increasingly applied to disease ecology.[5] Models of monthly predictions of Dengue fever in Mexico have been created based on mosquito activity.[6] Niche modeling has been used to help prevent anthrax in wildlife and livestock, by predicting the expected distribution of the pathogen, *Bacillus anthracis* (Cohn) in the environment.[7] Niche models of malaria vectors in the *Anopheles gambiae* complex have been developed for undersampled regions of Africa.[8] The distributions of *Triatoma* spp vectors of Chagas' disease in the Americas have been examined in an effort to better refine vector control programs.[4,9] A number of studies have focused on both present and potential distributions of *Lutzomyia* spp vectors of cutaneous and visceral leishmaniasis in

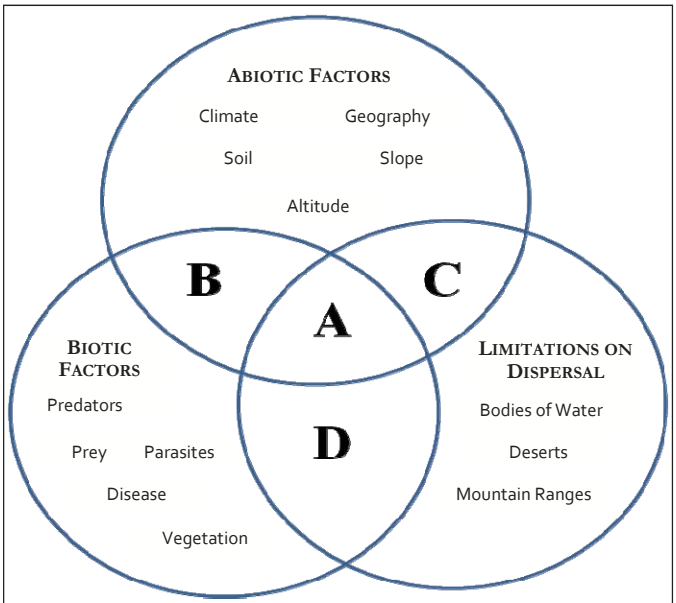

Figure 1. Venn diagram illustrating the factors that determine a species' niche.
A indicates the actual niche area of a species.
B indicates the area where the conditions are right for a species, but barriers to dispersion keep it from reaching.
C indicates areas where the biotic factors are not conducive to the species' survival.
D indicates areas where the abiotic factors are not conducive to survival.[3]

Central and South America.[10-13] Niche modeling allows the user to develop maps showing predicted distribution of an organism based on current and projected data.

One of the reasons that niche modeling has grown in use is the ability to use records from a variety of sources, including museum records, literature reports, and direct sampling. Many of the computer algorithms that have been developed for this modeling can create distribution models without actual absence records. This ability to use only presence records is what enables the creation of models for areas with poor or incomplete sampling. A number of methods have been developed to perform niche modeling. However, the most prevalent are GARP (genetic algorithm rule-set predictor) and MAXENT (maximum entropy) techniques.[14] Software for both applications is available freely on the internet and is simple to install and use. However, knowledge of geographic information system software is helpful in developing and refining the risk maps created by these techniques.

USING NICHE MODELING TO DETERMINE SAND FLY DISTRIBUTION

Niche modeling has been used to develop a map of the predicted distribution of *Phlebotomus papatasi* (Scopoli) which is a vector of sand fly fever virus and *Leishmania major*, a protozoan that causes cutaneous leishmaniasis (M.C-M., unpublished data, 2009). Both sand fly fever and cutaneous leishmaniasis are important disease threats to military operations in the middle east and Mediterranean regions.[15-17] The abiotic factors affecting this species include temperature, precipitation, and elevation. The biotic factors relevant to this species include availability of acceptable blood meal sources, level of predation, and the availability of the appropriate vegetation to provide a sugar source. The limits to dispersal include mountain ranges, bodies of water, and the distance the insect can fly. Using Figure 1 as a reference, the area labeled "A" represents the actual distribution of this species. If military forces were entering an area that did not meet the requirements of area A, the diseases vectored by this species would not be a concern. However, if military forces were operating in this area, preventive medicine personnel could plan appropriately for prevention and control of the vectors, and Soldier training could be adjusted to address the actual threat.

Figure 2 shows the predicted distribution of *P papatasi* across a portion of the Middle East. This map was developed using the niche modeling software Maxent, version 3.2.1. It incorporates sand fly collection records (both from direct collections and records in the

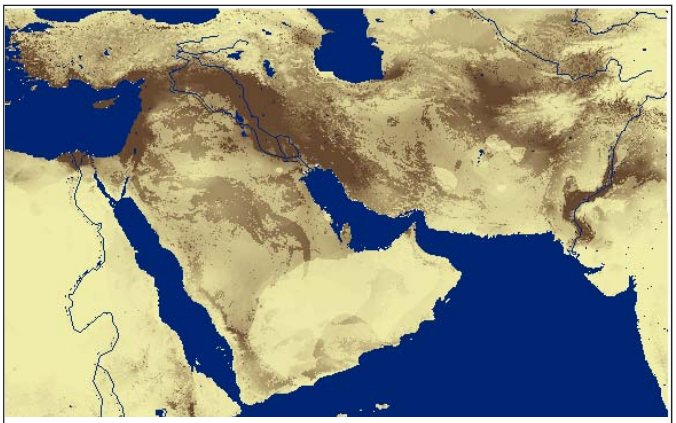

Figure 2. Map developed using niche modeling showing the relative risk that the cutaneous leishmaniasis and sand fly fever vector (*Phlebotomus papatasi*) is present in an area. The light areas have lowest risk and the darkest areas have the highest risk of *P papatasi* presence.

literature), temperature, precipitation, and land use data (M.C-M., unpublished data, 2009). While the map is similar to the one in the Disease and Vector Ecology Profile for cutaneous leishmaniasis in the same area (Figure 3), it provides more refined information about the distribution of a specific vector of concern and is easily updated. Such models could be developed rapidly, targeting specific areas of interest to provide the most realistic picture of the disease risk to deployed or deploying military personnel.

Using Niche Modeling to Determine Mosquito Distribution

Researchers at the Walter Reed Biosystematics Unit are developing a novel online tool called MosquitoMap[19] that provides a clearinghouse of information on mosquito distribution and risk of mosquito-borne diseases worldwide. The application incorporates niche models developed by individuals across the modeling community. Users may use the tool to determine what mosquito species are present, overlay distributional models of specific mosquito species, and/or overlay models of mosquito-borne diseases. Disease models currently incorporated in MosquitoMap are *Plasmodium vivax* and *Plasmodium falciparum* malaria, yellow fever, dengue fever, Japanese encephalitis, and Rift Valley fever. A useful component of this effort is the development of the "Mal-area calculator" which determines the areas where the distribution of human populations, *Plasmodium* spp parasites, and disease vectors overlap in order to better gauge the real malaria risk in a given area.[19] Figure 4 is a representation of the MosquitoMap model for the Republic of Korea, displaying the distribution of *Anopheles sinensis* (Wiedemann), a vector of *P vivax* malaria.

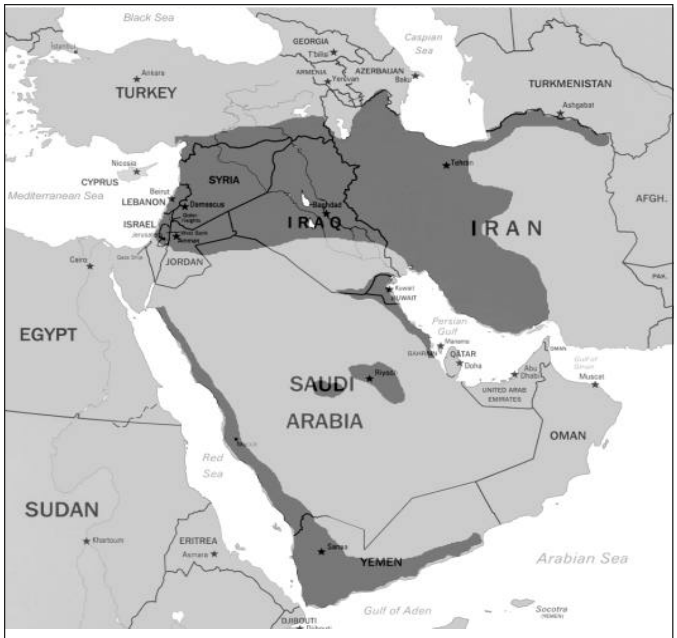

Figure 3. Map from the *Disease Vector Ecology Profile of the Middle East*[18] showing areas in the Middle East where cutaneous leishmaniasis is endemic.

Once complete, this tool will allow public health personnel to better determine the threat of mosquito-borne disease in an area of interest. This sort of global or regional plan could be applied to other vectors and vector-borne diseases. Such a resource could provide preventive medicine personnel targeted maps to use in planning disease control and prevention strategies. Ultimately, this sort of comprehensive effort could help to target use of prophylaxis, allow military entomologists to refine surveillance and control measures, and help emphasize the risk and importance of disease threats to an operation.

Military Applications

Niche modeling tools have potential to help preventive medicine personnel better understand the medical threat to our forces in areas to which they are deployed or may be deploying. Some of the potential applications have already been highlighted.

These tools may be used to assist in the development of the predeployment medical threat assessment. For some operations (such as the movement of combat forces), it may be impossible or impractical to conduct a thorough, on the ground, predeployment site survey of areas in which US military units will be engaged. In addition, many areas in which we operate have incomplete or inaccurate data regarding the threat of disease. In these instances, niche modeling could help fill in the information gaps by modeling the relative risk of key vector species or vector-borne diseases across an area of interest.

Niche modeling can be used to help identify the effects of habitat or climate change on a species or disease. This application would be beneficial in projecting disease threat in areas where the environment is changing (ie, as a result of a hurricane or tsunami). Modeling with the goal of predicting future populations of vector species could assist public health planners in addressing ongoing preventive medicine

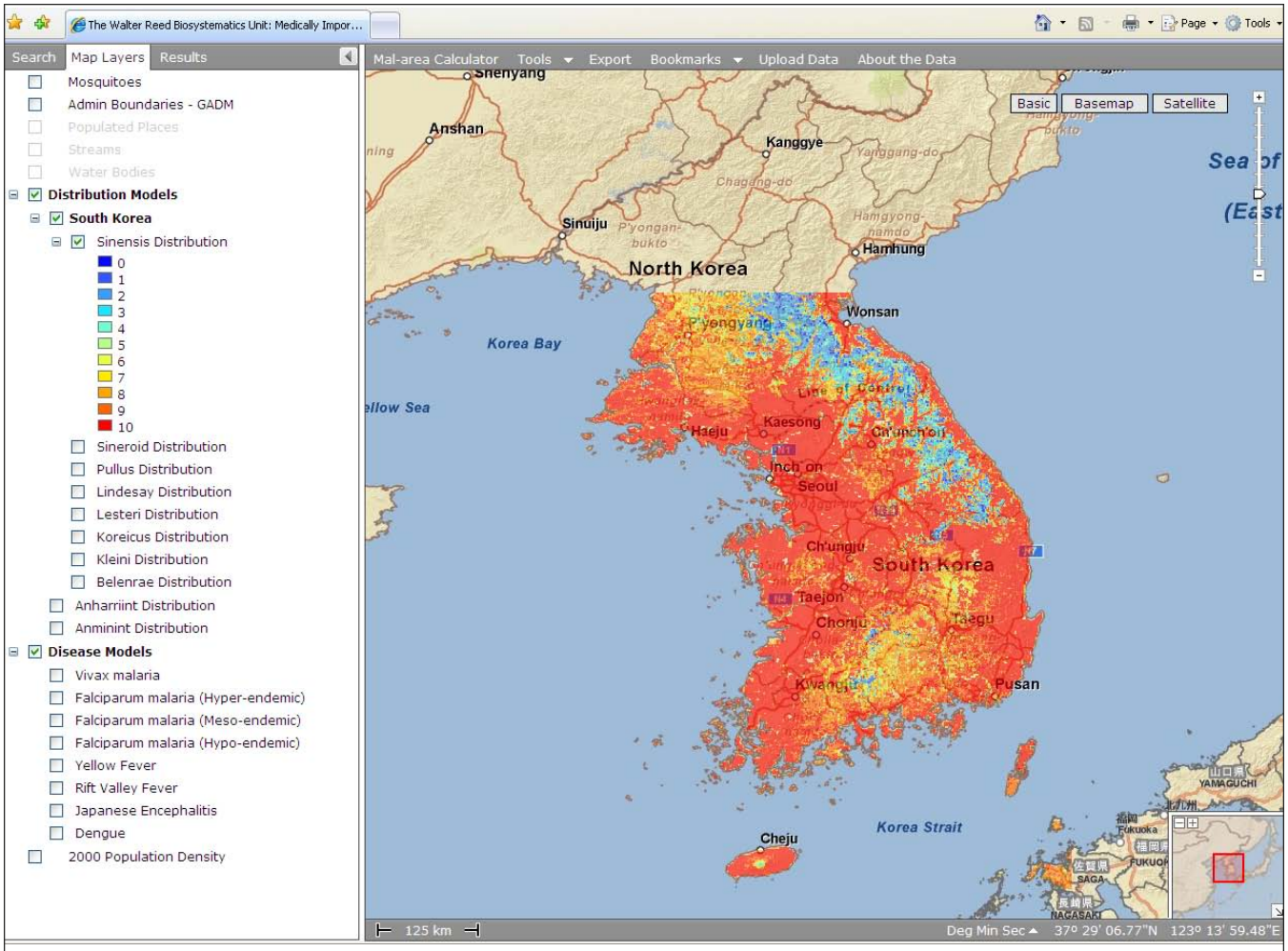

Figure 4. The MosquitoMap[19] niche model for the mosquito, *Anopheles sinensis* (Wiedeman). Map generated Feb 27, 2009.

concerns over weeks or months after such a habitat alteration scenario.

At a finer scale, these techniques could help target vector surveillance efforts, determine the best layout of a base in order to minimize contact with a vector, or identify appropriate engineering controls for a target species. If these tools are available to the military entomologists on the ground, vector control strategies could be enhanced through the use of remote imagery and predictive models to increase the overall efficacy of surveillance and control strategies.

RECOMMENDATION

A great deal of work remains for the military to employ these tools to their full potential. There should be some effort to coordinate public health research utilizing remote sensing and predictive modeling across the military in order to most effectively and efficiently exploit these technologies. Work should be targeted and focused on priority vectors, diseases, and regions in order to create a comprehensive and useful tool for the military public health community. With some forethought and deliberate planning, these tools hold tremendous potential to improve our ability to identify the risk to our forces and develop better disease prevention and control strategies during all phases of deployment.

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Addressing Feral and Wild Animal Threats During Deployment: The Distinction Between Animal Control and Rabies Control

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MAJ Gerald R. Sargent, VC, USA

ABSTRACT

Feral and wild animal control is a critical component of force health protection during troop deployments. The primary goal of animal control during a military deployment is to limit human injury or disease by reducing the likelihood of human-animal contact on US bases. For deployed US military forces, animal control will often be necessary, and trapping and euthanasia will remain the standard. In contrast, a rabies control program focuses on reducing disease incidence in an animal population with a subsequent reduction in rabies risk to military personnel. A successful rabies control program requires several components that typically are not possible in areas where the US Army deploys. The responsibility for animal control during an Army deployment has historically fallen to Army Preventive Medicine Sciences officers (Area of Concentration 67C). Understanding that the deployed US Army conducts animal control to limit human-animal contact, and also that it is generally not possible to mount a successful rabies control program on US contingency bases in the absence of an established, mature national plan will ensure that we are doing all we can to protect Soldiers from animal injury and disease transmission.

BACKGROUND

Preventive medicine and veterinary personnel should be prepared to conduct feral and wild animal control on every deployment—it is critical in the Iraq theater of operations. Animal bites take both service members and military working dogs out of the fight and place a preventable burden on medical care and logistics systems. Feral, as used in this article, refers to ownerless dogs and cats that have reverted to the wild state and lack characteristics associated with domestication.

Iraqi cultural and religious beliefs (dogs and cats are considered dirty; they are seldom kept as pets), the absence of a government-backed animal/zoonotic disease control program, and the new food and water sources on US bases in Iraq led to the establishment of unacceptable animal populations soon after the bases were occupied. This, in turn, led to the development of the Theater Animal Control Program early in Operation Iraqi Freedom.

Military animal control during deployments has neither the same goals nor the same options as a government-sponsored, long-term zoonotic disease control program. The focus of a military preventive medicine contingency animal control program is to reduce animal contact and human injury. A national rabies control program is designed to reduce zoonotic disease.

A successful rabies control program requires a long-term government commitment, either at the regional or national level. In its guidelines for canine rabies control, the World Health Organization (WHO) recommends mandatory vaccination of owned animals, movement restriction, and reducing the number of ownerless animals by euthanasia.[1] The WHO also recommends that nontraditional canine rabies control programs such as the oral rabies vaccine not be started without first collecting at least 5 years of data on dog population parameters and infection rates.[2] As an example of the level of commitment required, it took almost 60 years of national effort to rid the United States of dog-to-dog rabies transmission.[3] While a properly run program of this type will reduce the incidence of rabies over time, it cannot be expected to have any impact on US contingency bases in the absence of a national program.

CANINE RABIES CONTROL

Rabies is an acute viral encephalomyelitis that principally affects carnivores and insectivorous bats, although it can affect any mammal. It is almost

invariably fatal once clinical signs appear; there are only 6 documented cases of humans recovering from rabies following appearance of clinical signs.[4] Reservoirs of rabies vary throughout the world. Canine rabies predominates in Africa, Asia, Latin America, and the middle east.[5] According to the World Health Organization, there are about 55,000 deaths from rabies annually, mostly in Asia and Africa.[6]

The WHO lists 3 basic elements of a successful rabies control program: epidemiological surveillance, mass vaccination, and dog population control.[1] In areas that have large populations of ownerless dogs such as Iraq, the WHO recognizes that mass parenteral vaccination may not be possible[1,2] Control or elimination of canine rabies is possible as demonstrated by successes in the US, Japan, and other countries.[1] These control efforts required long-term commitment, wide-area coverage, local population buy-in, significant managerial investment, and legislation.

The Centers for Disease Control and Prevention's (CDC) program in the US is viewed as a model of success by the WHO.[1] The CDC's 2008 program[7] advocates mandatory vaccination of owned animals and removal of unwanted animals/animal control as cornerstones. In support of the national rabies control program in the US, city and county programs collectively euthanize almost 10 million animals every year.[8] This underscores the fact that there are far more stray animals in the US than can be adopted. The problem is magnified in a country like Iraq where there is no organized rabies control program and people do not keep pets. Most medium-sized cities and counties in the US kill more animals annually than the US does theater-wide in Iraq. Table 1 provides a comparison between animal euthanasia in Iraq and representative US districts.

The CDC plan also emphasizes supporting activities including public education, ongoing surveillance, and identification of vaccinated animals. As a result of many years of national commitment, the US has gone from almost 7,000 rabies-positive dogs per year in the late 1940s to declaring the country free of dog-to-dog rabies transmission less than 60 years later.[3] Sporadic canine infections continue to be reported in the US (71 in 2006).[3] these infections are attributed to spillover from virus types that circulate in wild animal populations.[3] Mass vaccination campaigns combined with euthanization of ownerless dogs has also been used in Malaysia, another country that has succeeded in eliminating canine rabies.[1]

Alternative Approaches to Rabies Control

In addition to mass parenteral vaccination of owned animals and elimination of strays, the WHO has suggested 2 supplementary approaches for consideration in some situations.[1,2]

Oral Rabies Vaccine (ORV). Successful wild animal rabies control programs that incorporate the ORV have been run in several countries.[1] The ORV is a potentially useful tool for canine rabies control in countries where a large percentage of the dogs are ownerless or where the target reservoir is a wild animal species. A successful ORV canine rabies control program would still have to be region- or country-wide and require long-term national commitment. The WHO recommends considering the following criteria before deciding to use ORV for control of rabies in dogs[2]:

- Dog rabies is endemic.
- A monitored dog rabies vaccination program by parenteral vaccination is in place for the last 5 years and is permanently evaluated.
- Commitment of the authorities is demonstrated by allocation of sufficient annual budget for the operation of the rabies surveillance and control program.
- There is a network of biomedical services and diagnostic laboratory capacity established in the

Table 1. Numbers of dogs and cats euthanized by year in the Iraq theater of operations and in 3 representative US locations.

	Iraq*	San Antonio, TX†	Albuquerque, NM†	Ft. Hood, TX†
2005	14,000	37,927	16,306	500
2006	6,500‡	33,785	14,839	981
2007	7,092	32,210	12,003	900

*Numbers for Iraq are from contract vector control records and do not include animals euthanized on bases that did not have permanent contract presence.

†Three United States representative districts are provided for comparison: a large city (San Antonio), a medium-sized city (Albuquerque), and a primarily rural Army installation (Fort Hood). Data supplied by local humane societies.

‡Iraq 2006 data was incomplete.

country and historical data on human and animal rabies cases for at least the 5 previous years are available.

- Dog demography information (population size estimates, density, distribution, age structure, turnover, etc) is available.

A properly managed ORV campaign could eventually reduce the prevalence of rabies in the target population, but it is not appropriate for military contingency purposes. It does not address the numbers of animals on bases, it will not change how we treat bite victims, and it will foster a false sense of security that could lead to increased violation of General Order Number 1, which prohibits the adoption of pets and mascots. The suggestion that we should use ORV to vaccinate animals inside our bases, and that this would allow us to stop euthanizing animals, ignores the primary goal of Army contingency animal control: reducing the number of human-animal encounters. In the absence of an ongoing national program, spreading ORV bait throughout the installations of deployed US forces protects neither military personnel nor the local population.

Trap-Neuter-Return (TNR) or Animal Birth Control. In 1990 the WHO published guidelines for rabies control programs built around the sterilization, vaccination, and release of dogs.[9] It was thought that this would reduce the size of the dog population, the number of animals susceptible to rabies infection, and ultimately the number of human infections. While it may have potential in some situations, a military deployment is not one of them. TNR will not reduce human-animal encounters, does not protect military personnel, is impractical in terms of work load for deployed veterinarians, and therefore should not be considered in a contingency situation.

There is an ongoing debate in the United States on the utility of TNR programs designed to control the size of animal populations,[10-12] (not rabies control programs), with more evidence against than for TNR. Discussions regarding the best way to address feral animal population control in the US typically have an emotional component, with people averse to euthanasia advocating TNR.[13,14] In many instances, this has been the primary impediment to solving a feral animal problem.[15,16]

Focusing strictly on rabies control, there is very little hard evidence that TNR programs are effective. The WHO itself states that, almost 20 years after they issued guidance on conducting this type of program, "…the results are promising, but data are limited and independent evaluation of projects has not yet been undertaken".[1]

Vaccination of owned animals and elimination of strays are the mainstays of WHO rabies control recommendations. An ORV program may be of value for controlling rabies in wild populations or in large populations of ownerless dogs, but ORV and TNR are not good options for the US military on deployment. None of the rabies control approaches address the basic goal of military contingency animal control.

ANIMAL CONTROL IN A CONTINGENCY ENVIRONMENT

The primary goal of military animal control in a contingency environment is to reduce the feral animal population inside US bases. This has at least 5 distinct benefits:

- Reduces human-animal contact and thus the likelihood of human injury by an animal.
- Reduces the disease reservoir population.
- Reduces damage to personal and government property.
- Reduces interference between military or contract working dogs and feral animals.
- Allows us to produce data on zoonotic disease prevalence in the animal populations.

The Army's approach for contingency deployments, euthanization of ownerless animals inside the base perimeter, addresses all of the threats posed to US and coalition forces by animals. Rabies control methods do not. Historically, the chain of command has authorized and advocated very basic means of animal control, such as kinetic force, early in a deployment and on more remote bases, acknowledging the importance of limiting human-animal contact. As the theater matures, animal trapping and chemical euthanasia become the norm, at least on the larger bases.

It is essential that preventive medicine and veterinary services personnel, installation commanders, and contractors in theater work closely together in the

initial development of a comprehensive animal control program. Army Preventive Medicine Sciences officers, and enlisted Preventive Medicine Specialists (MOS* 68S), have primary responsibility for contingency animal control in their pest management role. They build a team to assess the problem, develop a plan to address the problem, and make recommendations to commanders regarding animal control.

Veterinary Officers (Area of Concentration 64A/64B) and enlisted Animal Care Specialists (MOS 68T), consult on the initial development of the animal control plan, provide euthanasia support, collect disease surveillance data using the trapped animals, and provide quality control oversight to the program. This support will vary depending on mission priorities and number of veterinary personnel available. Veterinary services are responsible for euthanasia whenever personnel are available. They should also provide oversight and guidance when others must euthanize trapped animals to ensure that humane methods are being used in accordance with current American Veterinary Medical Association Guidelines.[17]

While preventive medicine has overall responsibility for animal control, installation commanders assume responsibility for implementing trapping programs on their bases. Base programs are developed based on theater guidance and support the theater plan. Both preventive medicine and veterinary service personnel should monitor these programs to ensure the use of proper trapping methods and humane treatment of animals.

Deployment animal control has been conducted during most of Operation Iraqi Freedom (OIF) in much the same way as in other US deployments. Although it is not a viable option as a long-term disease control program, animal trapping combined with humane euthanasia of trapped animals is effective at reducing the threat and frequency of Soldier injury by animals on US bases. The program used by the US Army and other military services deployed in support of OIF is designed to reduce the number of animals "inside the wire."

Table 2. Comparison of the ability of deployment animal control (trapping and euthanasia) and 2 rabies control programs to reduce human-animal encounters. The comparison uses selected parameters of importance in a general deployment setting and specifically in the Iraq theater of operations.*

Parameters of Importance	Control Measures		
	Animal Trapping & Euthanasia†	Oral Rabies Vaccine	Sterilize, Vaccinate, & Release
Protection from animal bites	✓	No Affect	No Affect
Immediate reduction of rabies risk to Soldiers	✓§	No Affect	No Affect
Protection from other diseases (leishmaniasis, cryptococcosis, Q fever)	✓	No Affect	No Affect
Keeps animal populations at acceptable levels	✓	No Affect	No Affect
Reduces rabies risk from jackal & fox‡	✓	✓**	No Affect
Provides rabies prevalence data for risk assessments	✓#	No Affect	No Affect
Reduces threatened animal population††	No Affect	No Affect	No Affect
Makes people uncomfortable or increases stress††	✓	No Affect	No Affect

* The assessment of the rabies control programs assumes no established national program, as is the case in Iraq.

† For the US DoD in a contingency environment, trapping and euthanizing animals on US bases provides the most protection for deployed personnel.

‡ The primary rabies reservoirs in Iraq according to the WHO and NCMI.

§ Some infected animals are removed from the population, but this is not measurable because tests are not available that identify infected animals prior to the time that they become infective. Reducing the number of animals on US installations makes human-animal encounters less likely and thus contributes to reducing rabies risk and damage of personal and government property.

¶ If captured animals are euthanized.

If captured animals are tested.

** Rabies risk reduced from those animals that consume the bait.

†† No Affect is the desirable result for this parameter.

Preventive medicine's focus with regard to animal control has been and must continue to be very limited—protect our military personnel, DoD civilians, contractors, and coalition partners from animal injuries. Euthanizing trapped animals accomplishes this, and in addition reduces the available disease reservoir population. Military and contract working dogs have a proven record of protecting US forces. However, in situations where unrestrained feral dogs exist, these working dogs may be harassed, attacked, and distracted from their duties. Euthanizing animals

*Military occupational specialty

trapped on coalition bases also provides the opportunity to generate data on zoonotic disease prevalence in the theater of operations which currently does not exist.[1,18,19]

While some have suggested that we should not euthanize animals and even that some animals should be released, it is critical that all animals known to be zoonotic disease reservoirs trapped in a contingency environment be euthanized. In Iraq, contract vector control personnel have not trapped an endangered or threatened animal during more than 5 years of animal control operations on US bases. Moreover, the National Center for Medical Intelligence (NCMI, formerly the Armed Forces Medical Intelligence Center) lists fox and jackal as the primary reservoirs of rabies in Iraq.[19] The International Union for the Conservation of Nature (IUCN), referenced in the 2007 Iraq Animal Control Plan, lists no endangered or threatened mammals that are likely to be caught incidentally during the animal control trapping program. The IUCN is currently tracking 5 endangered and 9 vulnerable mammals in Iraq,[20] listed in Table 3.

Iraqi cultural and religious beliefs explain in part why there are so many feral dogs and cats in Iraq. Iraqis do not keep pets such as dogs and cats, because they are considered unclean and are not allowed in the house. The other major factor that has resulted in a large feral animal population is that there was no regional or national program to address this health threat before September 2008.

Animal trapping and euthanizing trapped animals works on deployment in general and on US bases in Iraq in particular because we concurrently do 2 other things: movement restriction and habitat modification.[1] The walls and fences around our bases, while not completely impassable, serve to limit animal movement onto the bases. Refuse on bases is managed in much the same way that it is in the US, which limits food sources for animals. While animal-proof compounds are not a realistic expectation, both waste management and exclusion are continually improved throughout the theater. Holes under walls and in fences were identified and repaired, more incinerators were put into operation, and burn pit management was improved.

The US feral and wild animal control program in Iraq was effective in reducing the number of animals to which coalition forces are exposed. Organized animal control has been conducted continuously on major US bases in Iraq since the beginning of Operation Iraqi Freedom in 2003. Contract vector control personnel trapped approximately 14,000 feral and wild animals, such as those shown on page 39, in 2005; 6,500 in 2006; and 7,092 animals in 2007 (Table 1). This is a good indication that the program is working—reducing the number of feral animals on our bases.

Table 3. Iraq mammals currently listed as Endangered (blue rows) and Vulnerable by the World Conservation Union (http://www.iucnredlist.org).

Common Name	Scientific Name
Blue Whale	*Balaenoptera musculus*
Persian Fallow Deer	*Dama mesopotamica*
Asiatic Wild Ass	*Equus hemionus*
Bunn's Short-tailed Bandicoot Rat	*Nesokia bunnii*
Arabian Oryx	*Oryx leucoryx*
Cheetah	*Acinonyx jubatus*
Long-fingered Bat	*Myotis capaccinii*
Mehely's Horseshoe Bat	*Rhinolophus mehelyi*
Indian Smooth-Coated Otter	*Lutrogale perspicillata*
Brown Bear	*Ursus arctos*
African Lion	*Panthera leo*
Goitered gazelle	*Gazella subgutturosa*
European Marbled Polecat	*Vormela peregusna*
Finless Porpoise	*Neophocaena phocaenoides*

CONCLUSION

For the deployed US military, some type of animal control will often be necessary, and animal trapping and euthanasia will remain the standard. In countries with large feral animal populations such as Iraq, animal control is a critical component of force health protection. In the absence of a functioning national disease control program, it is the only tool we have that addresses injury by animals and effectively reduces the zoonotic disease reservoir population.

If US forces are tasked to assist the Iraq government in the establishment of a sustainable national animal or zoonotic disease control program, it will be critical to understand both the Iraqi culture and the programs that have worked in other countries. Since Iraqis do not keep pets and consider dogs and cats to be unclean, a program that includes euthanasia would probably receive more support than one that advocates sterilization and release of animals. An ORV program is ill-advised for the same reason.

Until about 25 years ago, the Iraq government paid a bounty for dogs and cats, dead or alive. Apparently, this program worked very well and reduced the feral animal populations significantly in Iraq. Resurrecting the program could be the solution to the country's feral animal problem. In November 2008, the government of Iraq initiated kinetic dog control in Baghdad to prevent rabies transmission and stop feral dog packs from killing people. Expanding this initiative to other population concentrations will benefit all Iraqis.

The US must continue to make improvements to installations that will limit the number of animals gaining entrance to our bases. This includes maintenance of barriers such as fences and walls, and also taking steps to exclude animals from dump sites as much as possible. We must continue to improve burn pit management where necessary, and continue efforts to increase the number of incinerators in use in the theater. Finally, our expectations for animal exclusion efforts must be tempered with a clear understanding of the costs versus benefits—neither bases nor burn pits can be made completely animal-proof.

Whenever and wherever our Army deploys, we must be prepared to conduct animal control operations as necessary. In Iraq, the US military must stay the course with its animal trapping and euthanasia program until the Iraqi government is able to establish a program that addresses the feral animal problem throughout the country, and that program begins to show positive results. To do anything else increases health risks to Soldiers.

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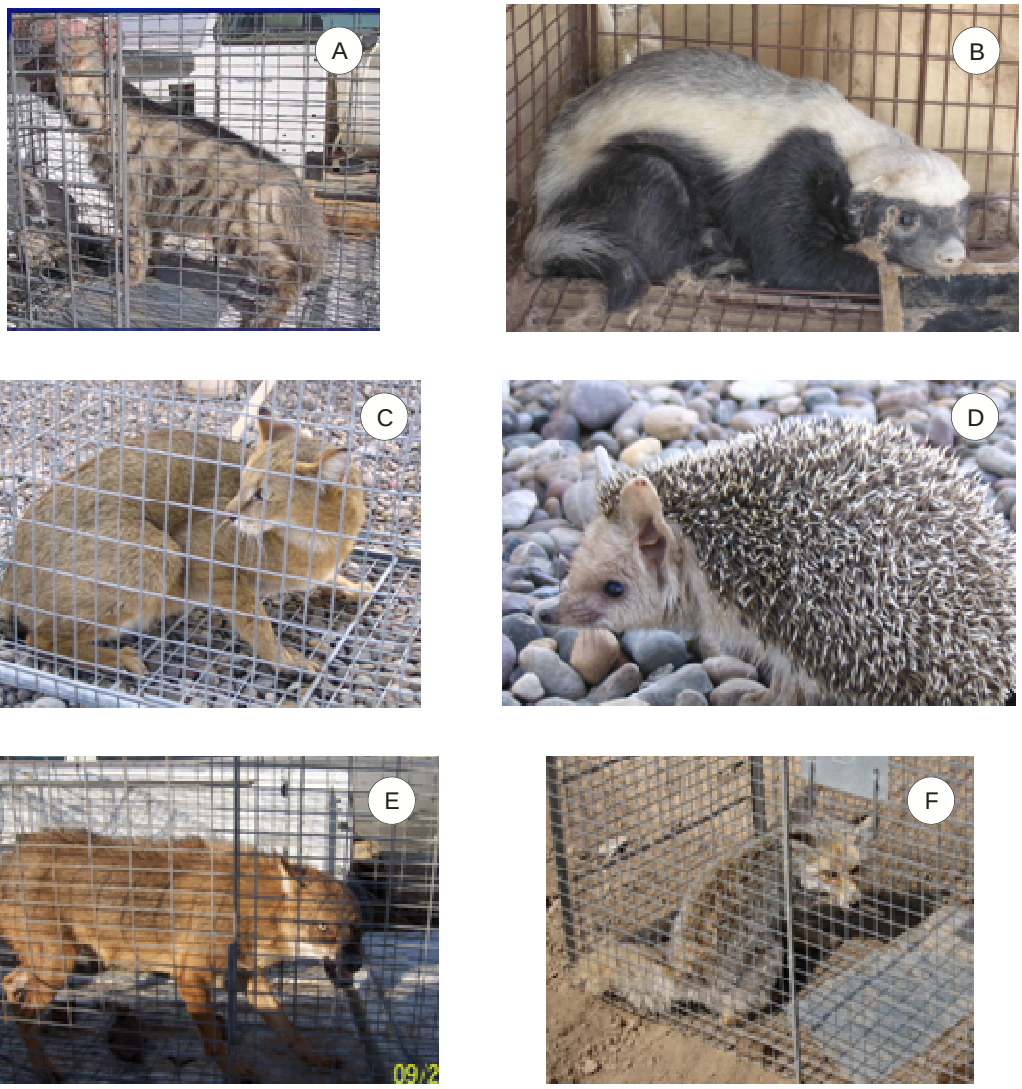

Wild animals captured by contract vector control personnel during animal control operations in Iraq. Although trap locations are selected to maximize capture of feral dogs and cats, wild animals are occasionally trapped. Decisions regarding whether to euthanize or release these animals must take into account command guidance, disease threat, and the additional risk to which personnel are exposed in attempting to release wild animals.
Species: hyena (A), badger (B), reed cat (C), hedgehog (D), jackal (E), fox (F)
(Photos of hyena, badger, jackal, and fox courtesy of Scott Brown, KBR Vector Control Supervisor, Camp Victory, Iraq. Other photos provided by the authors.)

Perspectives on Scrub Typhus, Tick-Borne Pathogens, and Hantavirus in the Republic of Korea

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Introduction

In a previous article,[1] we reviewed the status of malaria and Japanese encephalitis in the Republic of Korea (ROK) with the intent of providing the reader useful information on understanding and dealing with these diseases in the ROK. This article is a continuation of that discussion with a focus on one disease (scrub typhus) and 2 disease groups (tick-borne pathogens and hantaviruses).

Scrub Typhus

Scrub typhus, caused by the rickettsial bacterium *Orientia tsutsugamushi* (Hyashi) that is transmitted by several species of larval mites belonging to the family Trombiculidae, is a disease of major military importance. During World War II (WWII), its presence made such a dramatic impact on US military personnel deployed to southeast Asia and the Pacific islands that the military initiated a research project performed by the US Department of Agriculture on the concept of treating clothing with a repellent to reduce chigger (larval mites) bites.[2-4] This research was conducted in Florida, with field trials in New Guinea, after which it was exported for immediate use on military uniforms of Soldiers deployed to the frontlines. Later, this method was adopted for use against malaria and other diseases caused by vector-borne pathogens, but it was the effects of scrub typhus that eventually led to the development of permethrin uniform treatments used by US military forces and similarly treated clothing made available to nonmilitary consumers.

Scrub typhus was first reported in Korea when 8 United Nations Soldiers were diagnosed with it during the Korean War and shortly after the signing of the armistice (1951-1954).[5] Prior to the Korean War, scrub typhus was not known to occur among the Korean population, although unconfirmed reports based on symptoms of scrub typhus, fever, chills, headache, and rash had been observed during the late autumn to early winter for many years.[6,7] After the Korean War, scrub typhus cases were reported in relatively low numbers in the ROK population. However, reported cases increased dramatically from 1,415 cases in 2003 to 4,698 cases in 2004, and peaking with 6,780 cases in 2005, as shown in Table 1. While mortality rates vary in different areas, the usual fatality rates among untreated patients range from 10% to 20% in the tropics.[8] During WWII, 6,861 cases were reported in Burma, India, and the Philippines, and in some areas more than one of every 4 Soldiers died of the disease during this preantibiotic period. Of those that survived, the disease was often prolonged with an average time loss of 60 to70 days per Soldier.[8-10]

Table 1. Number of scrub typhus, leptospirosis, and hantavirus (hemorrhagic fever with renal syndrome) cases reported by year, 1997-2008.*

Disease	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Scrub Typhus - ROK	277	1,140	1,342	1,758	2,637	1,919	1,415	4,698	6,780	6,480	6,022	6,035
Leptospirosis - ROK	4	90	130	106	133	122	119	141	83	119	208	100
Hantavirus - ROK	104	215	196	203	323	336	392	427	421	422	450	376
Hantavirus – US	2	4	1	2	2	0	3	0	4	0	0	0

*Data from the Korea Center for Disease Control and Prevention (http://dis.cdc.go.kr/english/index.htm).

The Koreans use indirect fluorescent antibody tests for definitive diagnosis of scrub typhus, whereas some US medical laboratories frequently use only enzyme-

linked immunosorbent assay (ELISA) tests. Recent studies suggest the ELISA serological tests are often negative for scrub typhus for up to 30 days following symptoms, as patients may be anergic and not produce antibodies during the early stages of scrub typhus. Further, after 30 days or more, the patients have self-resolved infections, been successfully treated, or died.[11] For example, in Japan, 17 of 1,700 Marines operating in a "scrub typhus endemic" area (Fuji Maneuver Area), demonstrated scrub typhus symptoms, and all were treated with loading doses of doxycycline and recovered uneventfully.[11] All 17 patients tested serologically negative for scrub typhus by ELISA, therefore, either the diagnostic test demonstrated low sensitivity, or another pathogen (eg, spotted fever group (SFG) *Rickettsia*) was the cause for the illness. Either way, the cause of the illness was not definitively determined. Similarly in the ROK, 2 patients with typical scrub typhus symptoms (including eschars), who were also serologically negative, were treated with doxycycline, fully recovered, and returned to duty with no follow-up serology to identify the cause of illness. Eschars, however, may be observed in patients with SFG *Rickettsia* which is present in the ROK, thus SFG *Rickettsia* infections could not be ruled out as the source of illness.[12] Complications with diagnostic tests suggest scrub typhus rates in US military personnel may be underreported and suggests a need to evaluate current and possibly find better, more definitive diagnostic tests.[11]

Investigations to identify transmission rates of scrub typhus in US Soldiers deployed to the ROK were initiated as a result of a comprehensive rodent surveillance program at field training sites supported by funding through the Global Emerging Infections Surveillance and Response System and the National Center for Medical Intelligence. These investigations identified scrub typhus infection rates in rodent populations ranging from 11.1% to 100%.[13] In another study, data indicate approximately 0.2% of US Soldiers seroconverted for scrub typhus while deployed to the ROK.[14] In addition, bites by chiggers in Korea, unlike the Americas, usually do not elicit an "itchy" response, thus, Soldiers do not realize they have been bitten.[10] These data demonstrate the value of conducting vector and reservoir surveillance that determines the threat of known diseases, but also identifies the presence of reemerging and unknown infectious disease threats. Furthermore, these surveillance data provide for the development, implementation, and evaluation of scrub typhus mitigation strategies.

Assessment of the risk of scrub typhus to the US Forces Korea (USFK) population is difficult, as few cases are diagnosed in the USFK population compared to the disease presence in the Korean population (Table 1). The reasons for the number of postdeployment positive sera for scrub typhus and few suspected cases is not understood, but may be related to using diagnostic assays with low sensitivity, lack of provider awareness and education of emerging diseases in the ROK, and perhaps presumptive treatment with doxycycline with no serology follow-up to determine the cause of illness. Even though scrub typhus appears to be having a low impact on current USFK populations, a change to hostilities or a natural disaster requiring humanitarian interventions could quickly change this status, especially in light of the relatively high number of cases observed in the Korean population.

Additional studies of scrub typhus should focus on surveillance of small rodents and serological studies of Soldiers with suspected scrub typhus and other rickettsial diseases, eg, SFG rickettsial pathogens. Surveillance of small mammals is needed to provide baseline disease risk threats for selected habitats and sites where Soldiers train. Serological studies of Soldiers with vector-borne pathogen-like illnesses where presumptive treatment with antibiotics resolves disease symptoms should be thoroughly evaluated to determine the pathogen, so appropriate diagnostic tools and preventive measures can be applied. Since they are similar, preventive measures for scrub typhus will be discussed in the tick-borne pathogen section.

Diseases Caused by Tick-Borne Pathogens

Identified in the ROK are 31 species of ticks, listed in Table 2, and 16 tick-borne pathogens, some of which are zoonotic (animal-arthropod-animal/human), as shown in Table 3. Early tick-borne pathogen studies in the ROK focused on agricultural economic damage. However, with advancements in ectoparasite control, tick infestations in agricultural and pet animals can be severely limited. Studies of tick-borne pathogens as they relate to human health came later, but have primarily been related to identifying the pathogen's presence in the ROK or documenting new or novel cases. The prevalence and incidence rate of human

diseases caused by tick-borne pathogens in Korea is confounded and not clearly understood for a variety of reasons. These include:

- Koreans self-treated with antibiotics prior to a medical prescription requirement implemented in the year 2000.
- Diseases from tick-borne pathogens are not reportable diseases within the ROK.
- Massive urbanization has significantly reduced human-tick interaction.
- Changes in the ecosystem (eg, reforestation) since the Korean War.
- Operational conditions (hence exposure) may be different for military personnel during armistice than during wartime.

Prior to August 2000, a prescription was not required to buy antibiotics in the ROK, allowing the populace to purchase antibiotics at-will from local pharmacies.[13] Since many diseases from tick-borne pathogens range from mild to severe, are treatable with antibiotics (eg, doxycycline), and may be self-limiting, Koreans may have self-treated their symptoms rather than seek professional medical care. Another reason for potential undocumented cases is that Korean medical authorities do not require medical care providers to report diseases from tick-borne pathogens, hence, these diseases are not tracked within the human population. Thus, the health threat of tick-borne pathogens is not fully realized, even though several early retrospective studies of Korean patients with unidentified diseases were serologically positive for spotted fever group *Rickettsia* parasites.[31,32] Surprisingly, recent studies of pre- and postdeployment sera from US Soldiers deployed to the ROK demonstrated a 1.3% seroconversion rate to SFG *Rickettsia*. Thus, some of the eschars observed in US Soldiers that were suspected to be the result of scrub typhus infections may have actually been SFG *Rickettsia* infections.[14] Additionally, studies of associated infections of ehrlichiosis and bartonellosis in rodents and ticks resulted in the identification of tick-borne encephalitis (TBE) virus in the ROK.[16,29] The impact of these findings are not known as no human TBE cases have been reported in Korea.

Since the Korean War, the ROK has shifted from an agricultural society to an industrial and technology dominant society, which has led to massive urbanization of metropolitan areas such as Seoul, Busan, Pyeongtaek, and Daegu. Even though many Koreans spend weekends hiking in mountainous areas, the trails and paths are generally well worn and free of tick harborage like vegetation and leaf litter. However, when hikers go off the beaten path into forested areas covered with leaf litter and patches of grasses, the potential for human-tick interaction greatly increases. Still, urbanization appears to have reduced the overall exposure potential of the Korean population to ticks.

Interestingly, very few ticks were found during the Korean War,[33] when Korea was primarily rural. However, in the late 1960s and early 1970s, the ROK government directed the replanting of trees on hills and mountains, which had been deforested during the Japanese occupation of 1910 to 1945. The environmental changes may have increased tick populations in those areas by increasing tick survival due to increased numbers and density of suitable hosts.

Presently, large numbers of ticks (not necessarily all species) can be collected from selected sites, including short-grass and leafy-forest habitats throughout the ROK. For example, 2 US Army medical tick surveys

Table 2. Tick species reported from Korea.*

Amblyomma testudinarium†	*Haemaphysalis phasiana*†
Argas boueti	*Ixodes acuminatus*
Argas japonicus	*Ixodes cavipalpus*
Argas vespertilionis	*Ixodes granulatus*
Dermacentor marginatus	*Ixodes nipponensis*†
Dermacentor reticulates	*Ixodes ovatus*
Dermacentor silvarum	*Ixodes persulcatus*‡
Haemaphysalis campanulata	*Ixodes pomeranzevi*‡
Haemaphysalis concinna	*Ixodes ricinus*
Haemaphysalis cornigera	*Ixodes signatus*
Haemaphysalis flava†	*Ixodes turdus*†
Haemaphysalis japonensis	*Ixodes vespertilionis*
Haemaphysalis japonica‡	*Rhipicephalus annulatus*¶
Haemaphysalis kutchensis	*Rhipicephalus microplus*¶
Haemaphysalis longicornis†	*Rhipicephalus sanguineus*
Haemaphysalis ornithophila§	

*Sources: Yamaguti et al,[15] Ree,[16] and as individually annotated.
†US Army tick surveys, 2007
‡US Army tick surveys, 2002-2008
§Captured in 2008 on migratory birds on Hong Island, southwestern Korea.
¶ Formerly *Boophilus* spp

in 2007 resulted in the collection of approximately 6,800 ticks (total 6 species, shown in Table 2) from Jeju Province and along the southern coast in Jeollanam and Gyeongsangnam Provinces, all shown in the Figure. US Army tick surveillance data from this area had been lacking, and the surveys resulted in substantial collections and information about an obscure and uncommonly collected and reported tick species, *Haemaphysalis phasiana* Saito, Hoogstraal, and Wassef.[34]

Additional surveys in 2008 led to the collection of another rarely collected species, *Ixodes pomeranzevi* Serdyukova, which was collected in low numbers (4 nymphs, 2 adults) on the striped field mouse, *Apodemus agrarius* Pallas. However, more than 24 larvae, 9 nymphs, and 17 adults were removed from an Asiatic chipmunk, *Tamias sibiricus* (Laxmann), which suggested the need for extensive small mammal surveys to identify the host range, prevalence, and associated tick species.[35] In 2008, collaboration with a Korean Migratory Bird Survey on Hong Island (Jeollanam Province) resulted in the discovery of another tick, *Haemaphysalis ornithophila* Hoogstraal and Kohls, which is at least passing through Korea along the birds' migratory route.[36] Why all the new discoveries? Perhaps, global warming is creating environmental conditions favorable to these species and affecting their distribution in Korea,[37] or perhaps our surveillance activities are just finding what has been present, but not detected earlier due to lack of funding and personnel to conduct vector-borne pathogen surveillance. At any rate, these discoveries demonstrate the need for comprehensive and periodic surveillance of vector populations and reservoir hosts to identify changes in vector populations and disease prevalence that may impact Soldier activities.

Ticks are present in the ROK and researchers are finding a high prevalence of tick-borne pathogens within tick (Table 2) and rodent populations, but where is the corresponding disease? One possibility is that humans are not the preferred host of the primary vectors while other possibilities include human avoidance of tick habitats, unfamiliarity with disease from tick-borne pathogens among the medical community, issues with ordering the correct diagnostic test, and lack of reporting requirements, as discussed earlier.

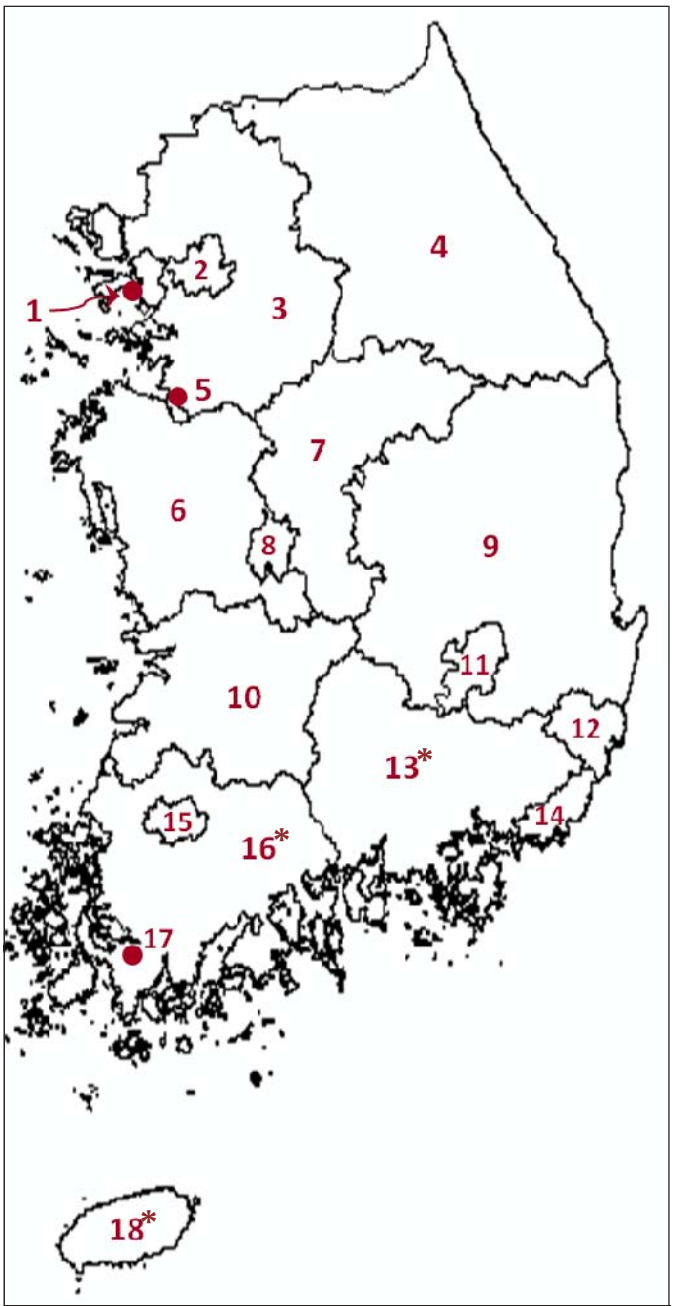
Table 3. Human and other animal pathogen associations with tentative tick vectors in the Republic of Korea.

Pathogen	Vector(s)
Anaplasma bovis	*Haemaphysalis longicornis*[17]
*Anaplasma phagocytophilum**	*Haemaphysalis longicornis, Ixodes persulcatus*[17]
Anaplasma platys	*Haemaphysalis longicornis*[18]
Babesia spp*	*Haemaphysalis longicornis*[19]
*Bartonella elizabethae**	*Haemaphysalis longicornis*[20]
Bartonella spp*	*Haemaphysalis longicornis*[20]
*Borrelia afzelii**	*Ixodes persulcatus*[21]
Borrelia burgdorferi sensu lato*	*Ixodes granulatus, I nipponensis, I persulcatus*[22-24]
*Borrelia garinii**	*Ixodes persulcatus*[21]
*Borrelia valaisiana**	*Ixodes nipponensis*[25]
Coxiella spp*	*Haemaphysalis longicornis*[26]
Ehrlichia canis	*Haemaphysalis longicornis*[18]
*Ehrlichia chaffeensis**	*Haemaphysalis longicornis, Ixodes persulcatus*[17,18,27]
*Ehrlichia ewingii**	*Haemaphysalis longicornis*[18]
Theileria sergenti	*Haemaphysalis longicornis*[28]
Tick-borne encephalitis virus, Western subtype*	*Haemaphysalis longicornis, H flava*[29,30]

*Causes disease in humans.

Operational field conditions for military forces are typically different during a prolonged armistice than intensive combat operations such as during the Korean War. Generally speaking, military personnel operating during noncombat periods can expect to have a clean uniform and perhaps a warm, soapy shower every one to 3 days, and many live or operate out of established base camps, which provide additional opportunities for personal hygiene. These situations allow for frequent body inspections for ticks, plus, the soapy water may injure or kill attached ticks and interfere with or reduce the potential for disease transmission, hence reducing the incidence of tick-borne disease.

In contrast, during major combat operations such as those of World War I, World War II, or the Korean War, military personnel in forward areas were generally not able to sustain the same level of hygiene (even with periodic rotations), due to the extended and austere battlefield conditions, as they would during noncombat operations. Consequently, their exposure to



Provinces and major city areas in the Republic of Korea. Two US Army tick surveys in 2007 identified 6 species among 6,800 ticks collected in Jeju (18*), Jeollanam (16*), and Gyeongsangnam (13*) provinces.

Identification Key

1 Incheon	7 Chungbuk	13 Gyeongsangnam
2 Seoul	8 Daejeon City	14 Busan
3 Gyeonggi	9 Gyeongbuk	15 Gwangju
4 Gangwon	10 Jeollabuk	16 Jeollanam
5 Pyongtaek City	11 Daegu City	17 Haenam City
6 Chungnam	12 Ulsan	18 Jeju

zoonotic tick-borne and other vector-borne pathogens was increased. In addition, they may occupy areas that have higher tick populations, eg, forested areas during hostility operations, and therefore have increased contact with the ground and vegetation harboring questing ticks. Similarly, the civilian population may experience displacement, homelessness, reduced sanitation, increased exposure to vectors and disease, elevated disease levels, and thus increase the risk of spreading the disease through vectors to military forces operating in that region.

These aspects of Korean society and the current hygiene standards of US military personnel make it difficult to estimate the impact of tick-borne pathogens on a military force operating in substandard field conditions during major combat operations in Korea. Hence, the tick-borne pathogen threat to military forces in the ROK is currently an estimate derived from limited reports of the diseases present, the tick population densities and distributions, vector bionomics, potential human exposure rates, disease mitigation actions, season, etc. Increased vector and human disease surveillance would lead to a better understanding of the tick-borne pathogen risks and mitigation strategies required to reduce these risks.

Fortunately, the Department of Defense (DoD) Insect and Arthropod Repellent System, which includes the proper wear of the uniform, wearing permethrin treated uniforms, and applying military formulations of extended duration topical insect and arthropod repellent N,N-diethy-3-methylbenzamide (deet) to exposed human skin, is estimated to reduce virtually all tick exposures in the ROK, significantly mitigating the risk of disease from tick-borne pathogens among US Soldiers. This repellent system also provides protection against other vector-borne pathogens in the ROK, so its implementation to prevent disease from tick-borne pathogens also provides added protection against malaria, Japanese encephalitis, scrub typhus, body lice (epidemic typhus), fleas (murine typhus), and other biting arthropods.

However, the fielding of the flame-retardant Army Combat Uniforms (FRACU) necessitates a reevaluation of the permethrin-fabric properties as they relate to protection against ticks, chiggers, and other blood-feeding arthropods. These evaluations need to determine the protection level of the FRACU compared to the standard (>90% protection after 50

washings) and then determine its effectiveness against blood-feeding arthropods. Currently, permethrin treatment is not approved for use on FRACUs by the military. Permethrin treatments do not meet the Environmental Protection Agency label required permethrin concentration in the uniforms nor provide the required amount of protection against biting arthropods.

General observations during the 2007 surveys in Jeju Province and along the southern coast showed that survey personnel who wore permethrin treated uniforms rarely found a tick on their uniform (in some cases the "found" tick was dead), while those who did not wear permethrin treated uniforms commonly found ticks on their uniform. Of course, personal protective measures have to be implemented and used properly, eg, tucking the trouser leg into the boot and not using blousing garters, for these countermeasures to be most effective.

From an entomological perspective, very little was known about Korean ticks during the Korean War. Ticks were reported as being uncommon, and very few Soldiers complained of tick bites.[33] In 1971, US Army personnel assigned to the 406th Medical Laboratory, Camp Zama, Japan published a comprehensive systematic and bionomical work on ticks in Japan and the ROK, even though limited Korean tick data were available for inclusion.[15] This document, however, remains useful as many of the ticks found in Japan are also found in the ROK, and it is still the primary document for identifying Korean ticks. Since 1971, few US military or ROK tick surveillance field studies have been reported in professional literature, so knowledge about the current status of tick populations and disease prevalence is lacking.

Articles have been published on diseases specifically caused by Korean tick-borne pathogens, but no tick-borne pathogen list or comprehensive discussion of the importance of tick-borne pathogens in the ROK could be found. To prevent surprises during a time of stress (major combat operations) and to develop a better understanding of tick-borne pathogens in the ROK, there is a need for surveillance which targets host and vector species to determine relationships between vectors, pathogens, reservoirs, and hosts. This surveillance should include a peninsula-wide tick collection to better understand tick distributions, habitat, population densities, and the potential for disease transmission by each species, (eg, human attraction, infection rates, and relative abundance, seasonal distribution, environmental factors limiting its distribution, and host-parasite life cycles), and an entire season's collection to show each species' seasonal activity and disease cycle.

Ten US Army studies since 2003, such as Chae et al,[38] Lee et al,[39] and Kim et al,[40] have addressed these deficiencies. A bibliography on the ticks of the ROK is being created based upon an extensive literature search and review for all tick-related information for the ROK and surrounding countries, ie, North Korea, Japan, northeastern China, and southeastern Russia. The bibliography and the accompanying PDF files (currently about 284 citations with about 69% as PDF files) of the articles are being incorporated into the Defense Pest Management Information Analysis Center for access by DoD personnel. Tick collections described within these articles and from recent US Army tick surveys are being compiled as a record of known tick locations and will be used to create graphical tick distribution maps and a list of diseases caused by tick-borne pathogens with vector associations for the ROK. Table 2 represents our initial attempt at extracting vector and disease associations from published literature.

HANTAVIRUS

There are 4 known rodent-borne hantaviruses in the ROK: Hantaan virus (Korean Hemorrhagic Fever (KHF)), Seoul virus, Soochong virus, and Muju virus. Reservoirs of each have primary reservoir hosts with the striped field mouse, *Apodemus agrarius coreae*, vectoring KHF; the common roof rat, *Rattus rattus* Linnaeus, and the Norway rat, *Rattus norvegicus* Berkenhout, vectoring Seoul virus; the Korean field mouse, *Apodemus peninsulae* Thomas, vectoring Soochong virus; and the royal vole, *Myodes regulus* Thomas, vectoring Muju virus.[41,42] A fifth hantavirus (Imjin virus) in an insectivore, *Crocidura lasiura* Dobson, has recently been identified.[43]

Transmission is associated with inhalation of aerosolized particles, such as dusts, that have been contaminated with rodent excreta and/or secreta (feces, urine, and saliva). It also can be contracted through a rat or mouse bite, so precautions should be made when handling them and they should never be kept as mascots or pets.

Hantaan virus, commonly referred to as KHF, is the most common and virulent of the group and fits into the hemorrhagic fever with renal syndrome (HFRS) disease group. These diseases are characterized by leakage of blood from the circulatory system and abnormal kidney function. Conditions during the Korean War led to 2,158 hantavirus cases in US forces.[44] Most of these cases occurred north of Seoul and with a large majority occurring north of the 38th parallel.[45]

While the risk for HFRS is always present in the ROK, cases in the Korean population show a very small transmission peak in May and June, and a much larger transmission peak from September through December. These transmission periods appear to be associated with agricultural practices (planting for May and June, draining/drying and harvesting rice fields for September and October), which results in significant human-rodent interaction and hence exposure. However, more recent evidence suggests that the primary peak is associated with the rodent's reproductive behavior (primary brood production in the early fall preceding the peak transmission) that produces large numbers of naïve rodents.[46] When associated with high local infection rates, movement, and competition for overwintering habitat, the results are acute infections with high viremias and shedding of virus. Consequently, when farmers are actively harvesting their fields or military personnel are training in the field during these periods, there is an increased risk of acquiring hantavirus.[47] Generally, the risk of HFRS in the ROK can be characterized as having a low frequency of occurrence, but a severe outcome if acquired, and field training activities that lead to human-rodent interactions at any time of the year can result in Soldiers contracting HFRS. The incubation period may be as long as 50 days before symptoms occur, so Soldiers should be informed of HFRS symptoms (early symptoms are often flu-like), and if they become ill, they should alert their medical-care providers with information as to their travel or field training history.

Surveillance studies have shown that the reservoir of HFRS is very common throughout the country and seasonal Hantaan virus rodent infection rates were as high as 60% during some trapping periods at some of the field training sites.[48,49] These studies have also shown that a portion of the Hantaan virus genome was descriptive for selected sites and made it possible to identify transmission sites for HFRS case studies for 4 Soldiers that demonstrated incubation periods from a minimum of 6 to 22 days to a maximum of 15 to 35 days.[50]

Studies also have shown that high populations of rodents are present in tall grassy and scrub habitats.[51] In training sites, areas of tall, unmanaged vegetation bordering fighting positions (artillery firing points, trenches, and foxholes) increase human-rodent interactions along the border vegetation, as well as the often barren training area interface that may become potentially contaminated with virus laden rodent excreta. Also, dust produced when firing large weapons such as field artillery may increase the risk of infection as rodent urine and fecal particles become airborne from the muzzle blast of these weapons. Similarly, the dust from vehicles going through dry vegetated areas, trails, or dirt roads bordered by grassy/scrub vegetation may increase hantavirus infection risks as rodents traversing these grassy borders produce virus laden excreta on these trails and roads. These risks can be reduced by discouraging rodents from being attracted to fighting positions through vegetation control (maintaining vegetation cut short to reduce ground cover) and sanitary measures, such as frequent removal of trash and debris. Fighting positions should be located away from heavily traveled roads to prevent exposure to potentially infected dusts. Artillery personnel should use sanitary measures to discourage rodent infestations where they fire their weapons, and should take personal preventive measures to reduce inhalation of dust from the muzzle blast.

Exposures in closed spaces have the greatest risk, thus military personnel should not bring potentially infested materials, such as rice straw for flooring or bedding, into closed areas such as tents, barns, houses, or other buildings. Additionally, military personnel in the field should avoid sleeping on the ground or placing vegetation that was lying on the ground into their helmets.

Conclusion

Scrub typhus, tick-borne pathogens, and hantaviruses present challenges to the protection of military forces in the ROK, and surveillance studies focused on the improvement of our knowledge of these diseases and how to prevent them need continual support. While the distribution of scrub typhus has been well described, the distribution and prevalence of tick-, flea-, and

rodent-borne pathogens affecting man are largely unknown. The rickettsial agents are currently under investigation and are just now being identified to species, which will provide future endeavors to determine their distribution and prevalence in rodent and human populations. As these parasites are identified, appropriate laboratory assays can be developed to identify the incidence of disease among military and civilian populations in the ROK. Similarly, assays to identify Imjin virus, which does not serologically cross react with the other rodent-borne hantaviruses, can be developed to determine if it causes human disease. As an awareness of the presence of tick-, mite-, flea-, and rodent-borne pathogens are developed, the Korea Center for Disease Control and Prevention may place new zoonotic pathogens on the reportable disease list, which, in conjunction with an understanding of the transmission cycle, will enhance our ability to determine health risks among US military and civilian populations in the ROK.

Information gathered as a result of surveillance studies must be prepared and disseminated through channels where actions can be taken by commanders at all levels to reduce risks to Soldiers from often preventable diseases and nonbattle injuries during hostilities, while training, or off-duty.

Indeed, the real value of such surveillance work is reflected in the sentiments of His Royal Highness, Prince Mahidol of Songkla (Thailand, 1892-1929):

> True success is not in the learning, but its application to the benefit of mankind.[52]

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Remote Teaching of Arthropod Species Identification Through Interactive Multimedia

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ABSTRACT

Remote teaching programs are effective means for providing entomologists with the knowledge they need to confidently identify medically important arthropods. Using interactive multimedia, these programs teach the morphology of ticks and larval and adult mosquitoes through tutorials, followed by practice identifications of collected arthropod specimens. Interactive multimedia programs are created in 3 phases: planning, design, and development. Species identification is one of the most important skills that entomologists must have, because recognition of disease vectors during field surveillance is an important component of overall troop protection.

INTRODUCTION

Remote teaching is most appropriate when the subject matter does not change frequently and is of importance to a large audience, or where cost-effectiveness is a concern. The Armed Forces Pest Management Board has recently released 3 diskettes that teach the morphology of ticks, larval mosquitoes, and adult mosquitoes. The lessons emphasize anatomical structures that are most commonly used in arthropod identification keys. The goal is for students to be able to confidently identify unknown arthropod specimens using any arthropod identification key written for that group.

PRODUCTS

The 3 instructional diskettes are:

- Interactive Program for Teaching Tick Morphology
- Interactive Program for Teaching Larval Mosquito Morphology
- Interactive Program for Teaching Adult Mosquito Morphology

They are available free of charge by contacting the Armed Forces Pest Management Board (afpmb-webmaster@osd.mil) and providing a mailing address and the titles of the requested disks. The tick program is distributed on a CD, the mosquito programs are distributed on DVDs.

Each program contains 4 major sections. The first is a tutorial that discusses each morphological characteristic and how its appearance can vary between species and genera (Figure 1). A clear instance and a clear non-instance of the characteristic are usually presented on the same page. In some cases, an unclear instance or an unclear non-instance will follow on the next page to let students know where mistakes commonly occur and how to avoid them.

Following the tutorial, students are able to practice what they have learned by identifying unknown arthropod specimens. It is important for them to use their new knowledge to solve problems within the same context that they will encounter later in their work.[1] A specimen is selected, along with an identification key that is commonly used for that geographic area, and the student begins working through the key's couplets (Figure 2). After reading the descriptions in each couplet and examining the image(s), the student selects an identification key path. If the choice is incorrect, an explanation is provided. A button with a plus symbol is included on some of the images (Figure 3). It presents a magnified image of the area within the box, similar to the action of the zoom feature on a microscope.

The third section of the program is the glossary, which serves as a quick reference. The terms and structures in the identification keys are concisely explained using words and images (Figure 4).

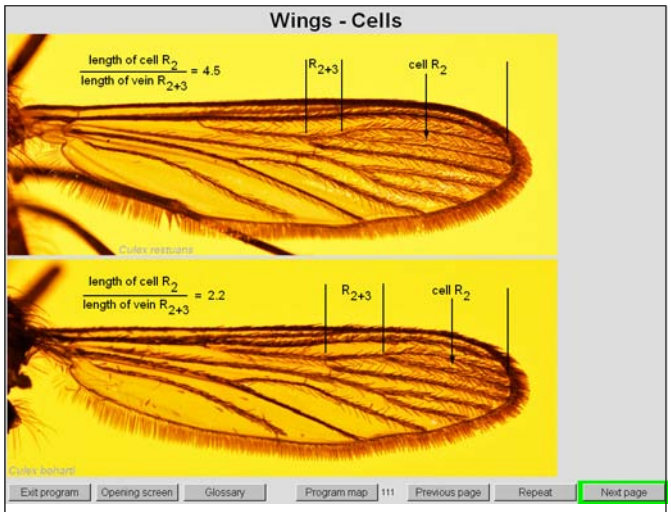

Figure 1. Extract from the adult mosquito tutorial showing variation in wing cell length.

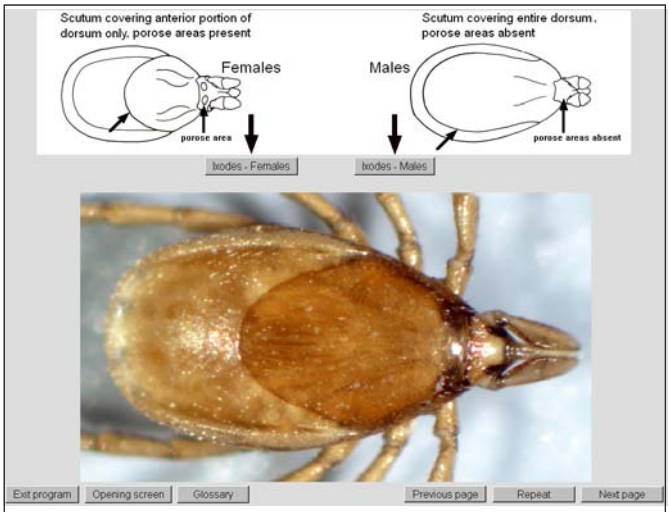

Figure 2. Couplet and images from the student identification practice section of the tick morphology program.

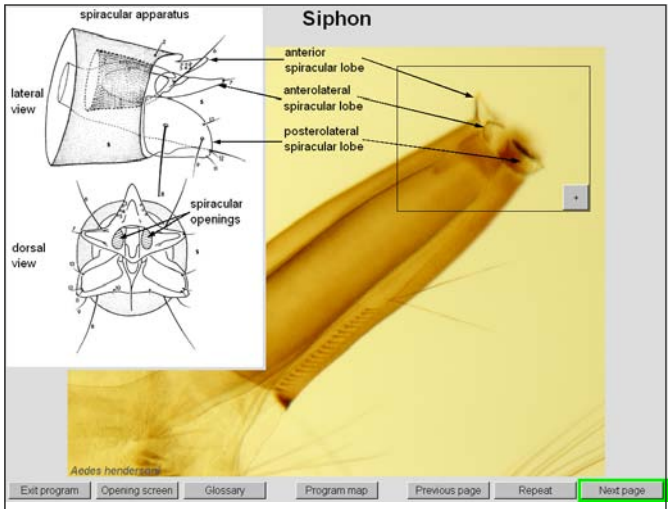

Figure 3. Some images can be enlarged by clicking the plus symbol within the rectangle.

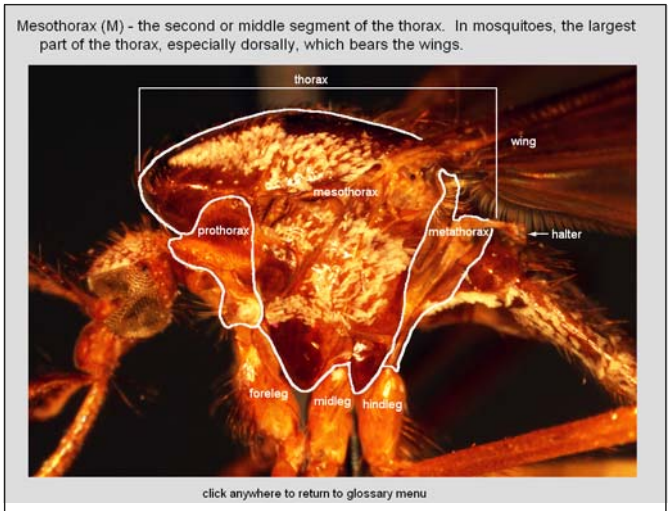

Figure 4. Extract from the glossary of the adult mosquito program.

In the mosquito programs, the fourth section contains diagrams of hundreds of species (Figure 5). These diagrams are taken from the literature. The tick program has a unique feature: a collection of identification keys that can be searched by author, tick genus, or geographic area (Figure 6).

METHODS

The creation of an interactive multimedia program usually follows 3 phases: planning, design, and development.[2] In the planning phase, the scope of the project is defined. The tutorial teaches the characteristics that are used in identification keys, as well as their variations. Next, the project style and standards are determined. The colors, fonts, size of text, and how the student navigates and interacts with the program are established to maintain consistency.[3]

The design phase involves the creation of flowcharts and storyboards that describe the path that the program follows and the lesson to be taught on each page. These programs were developed using Authorware (Adobe Systems Inc, San Jose, California), an icon-based authoring system. The program is designed around a page/chapter metaphor. During this stage, each page describes in words what the images will show and the audio narrative.

The development phase begins with photographing the specimens and then editing the images before placing them into the program. Next, the audio script is

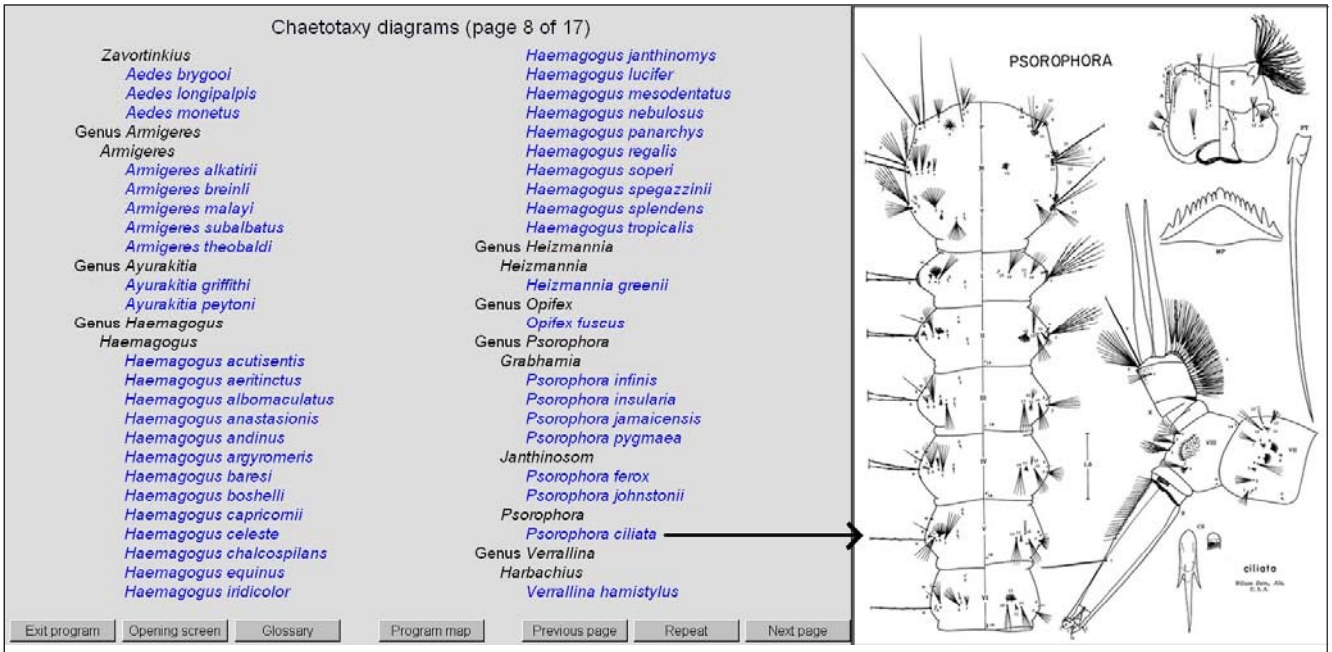

Figure 5. The menu of diagrams for the larval mosquito program and an example of a chaetotaxy drawing of one species which is presented when a link is selected.

recorded, brought into a sound editor, and added to the program. Using audio rather than text to describe the images has the advantage of freeing the screen for larger images, resulting in higher resolution. It also permits more images to be placed on the screen simultaneously for comparison. Another advantage of audio rather than text is that information can be presented to the student at a calculated speed.[4] The timing of the program requires careful attention so that the information is presented at an optimal speed for the average user. The program is then tested by a variety of users, including subject matter experts, before it is released.

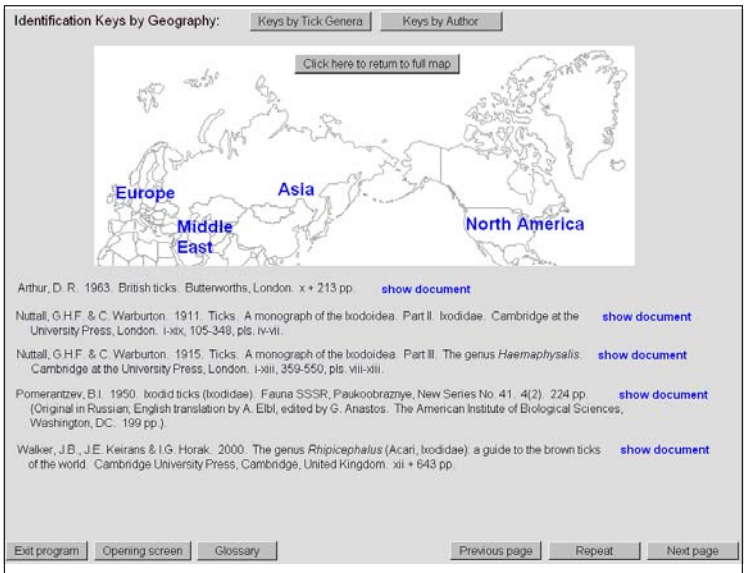

Figure 6. The tick program provides identification keys that can be used to search by genus, author, or geographic area. The above extract illustrates identification keys for the ticks of Europe.

Discussion

Traditional methods of achieving proficiency in arthropod identification, such as with ticks or mosquitoes, require students to attend classes, often at a university. Sometimes they spend a week at a museum, studying under a taxonomic expert. The disadvantages of these methods are numerous, including cost, course availability, and willingness of experts to provide training. Even if the students are able to attend courses and become proficient in a particular group, there are many other orders and families of medically important arthropods. Remote teaching is the ideal means for presenting an expanded knowledge base to large numbers of students over broad geo-graphic areas.

The major disadvantage of a technology-based learning program is the initial cost of development, which is high compared to preparing a traditional

lecture or laboratory identification course. It is important to do a cost-benefit analysis before beginning such a project.[5] Quantifying benefits can be difficult. These programs may also be unsuitable for presenting information that changes frequently or regularly. Continuous changes in a program would be economically unfeasible.

The advantages of using a technology-based learning program to teach arthropod identification are numerous. Students can choose the time and location for training that is convenient for them. They can work at their own pace. Those unfamiliar with the subject can spend as much time as needed on the lessons. Even inexperienced students usually complete the instruction in less time than with traditional, instructor-led methods. All the specimens used in technology-based learning programs are of high quality and come from major museums. As a result, students receive a more comprehensive introduction to taxonomy than might otherwise be possible using locally available arthropod specimens.

Another major advantage of these programs is that their target audience is broad. The lessons described here, although primarily designed for entomologists, can be used by anyone in public health, preventive medicine, pest control, or other related fields. Also, in this particular application, teaching arthropod identification, the life cycle of the product is quite long because, from a human perspective, insect morphology is unchanging. And this teaching tool is inexpensive. At an estimated program production cost of $100,000, if only 2,000 students were to use the program (a very conservative figure), the cost per student would be just $50 over the life of the product.

Medical entomologists entering the military usually have broad knowledge of their subject, but few are experts in more than one or two areas, such as toxicology, ecology, behavior, systematics, or other specialties. Even those who are systematists usually specialize in no more than a few families within an order. After a year or two of training, these entomologists will be deployed, and they will need to make health-related decisions that could impact the success of their mission. Species identification is one of the most important skills that military medical entomologists can possess because recognition of disease vectors during field surveillance is an important component of overall troop protection. Remote teaching of arthropod morphology is a comprehensive, efficient, cost-effective tool that is well suited to the military environment.

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Behavioral Resistance of House Flies, *Musca domestica* (Diptera: Muscidae) to Imidacloprid

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ABSTRACT

House flies captured at a field site in southern California were examined for physiological and behavioral resistance to the insecticidal toxicant imidacloprid using a no-choice and a choice feeding assay, respectively. Relative to a susceptible laboratory colony of house flies, field-captured house flies demonstrated moderate physiological resistance to imidacloprid using a no-choice feeding assay. In contrast, behavioral resistance of field-captured flies was very high with 72% survival of flies at even the highest imidacloprid concentrations tested using a choice feeding assay. Since the introduction of imidacloprid baits in California during 2003, the overuse of imidacloprid baits in southern California has resulted in the rapid selection, over only 5 years, of a house fly population that is highly resistant to imidacloprid. While house fly resistance is shown to be both physiological and behavioral, observed field failures of imidacloprid fly baits are primarily due to behavioral resistance. Field-deployable kits to assess behavioral resistance of house flies and other important insect pests are needed.

INTRODUCTION

House flies (*Musca domestica* L) are a significant pest associated with military operations where proper sanitation of manure and refuse is often limited.[1,2] Developmental sites for house flies include human and animal waste and food waste, all of which may be common at temporary military encampments or in the communities surrounding military encampments. In addition to the considerable nuisance caused by large populations of these flies, they have also been implicated in the transmission of a phylogenetically diverse group of human pathogens including *Escherichia coli* O157:H7, *Salmonella enteritidis*, *Yersinia pseudotuberculosis*, *Helicobactor pylori*, *Vibrio cholerae*, and rotavirus,[3-11] as shown in Table 1. The extent to which house flies may be involved in pathogen persistence and dispersal among troops or neighboring communities remains unclear; however, it is well known that flies may disperse a considerable distance (5 km or more) from breeding sites.[32,33] Flies dispersing from their developmental sites are commonly attracted to human habitations and food preparation sites where they can transmit pathogens.[34-36] Annual increases in human infections with enteric pathogens can demonstrate a distinct seasonality that coincides with increasing abundance of house flies.[13,37] Further, area-wide control of house fly has been associated with a concurrent reduction in human sickness due to enteric pathogens,[16,38-40] providing strong evidence that house flies can be important in the spread of these enteric diseases.

With the advent of DDT (dichlorodiphenyltrichloroethane) for control of insects, house fly control has been largely through use of chemical insecticides.

Table 1. House fly-transmitted pathogens known to have the most serious affect on human health.
Note: The list is not exhaustive for all pathogens carried by house flies.

Bacteria	Viruses
Acinetobacter spp[3]	Coxsackievirus[3]
Campylobacter spp[12,13]	enteroviruses[24]
Chlamydia trachomatis[14,15]	poliovirus[3]
Enterobacter spp[3]	rotavirus[11]
Enterococcus spp[3]	**Parasites**
Escherichia coli O157:H7 [6,16-19]	*Cryptosporidium parvum*[25]
Helicobactor pylori[9]	*Endolimax nana*[26]
Klebsiella spp[3]	*Entamoeba coli*[26]
Proteus spp[3]	*Entamoeba histolytica*[26]
Psuedomonas spp[3]	*Giardia lamblia*[5,27-29]
Salmonella enteritidis[7,20]	*Isospora* spp[26]
Shigella sonnei[16,21,22]	*Sarcocystis* spp[30]
Staphylococcus aureus[22,23]	*Toxoplsma gondii*[31]
Streptococcus[22]	
Vibrio cholerae[10]	
Yersinia pseudotuberculosis[8]	

However, house flies have shown a great adaptability to these insecticides, developing resistance first to DDT,[41] and then to most of the chemicals that have later become available for their control.[42,43] The extent of house fly resistance to any particular chemical appears to be principally determined by the historical use of that chemical regionally.[44-48] However, cross-resistance where selection with one chemical may result in resistance to another has also been shown.[42] In the military environment, rapid assessment of house fly resistance to chemicals approved for management of house flies by the Armed Forces Pest Management Board is needed whenever military units are deployed to a location where sanitation is lacking and house fly populations exceed acceptable numbers.

A rapid assessment of the physiological resistance of an insect population to chemicals is typically accomplished using some form of contact assay, such as the World Health Organization bottle assay,[49] or topical application of the material to the insect dorsum. A standard assay for determining house fly resistance to chemicals has been developed using 230 ml glass jars.[50] For chemicals formulated into fly baits, a standard feeding assay has also been developed.[43] In each of these assays, tested house flies are ensured substantial contact with the chemical. However, under natural field conditions, fly contact with a chemical may be limited due to detection and avoidance of the chemical, or due to irritation following contact with the chemical resulting in movement away from the chemical. Such behavioral resistance to chemicals has been shown in some house fly populations using "choice assays" even where physiological resistance was limited.[44,51]

In this study, we examined both physiological and behavioral resistance of a wild house fly population from southern California to imidacloprid, the active ingredient found in one of the most commonly used fly baits in the United States during the last 5 years.

Materials and Methods

House flies from a southern California dairy (BS Strain) were collected by sweep net in October 2008 and colonized at the University of California at Riverside (UCR) using standard methods.[52] Field collected flies were maintained in colony through 7 generations for use in this study. Susceptibility of this fly population to imidacloprid was evaluated relative to a UCR susceptible strain population of house flies that have been in colony since their collection in 1982 from a dairy in Mira Loma, California.

Feeding Assay

Because imidacloprid is typically formulated into a fly bait material, house fly susceptibility to this chemical was assessed using either a no-choice feeding bioassay[43] or a modification of this bioassay (choice feeding assay), providing flies the opportunity to feed on a food source with or without imidacloprid. Colony -reared house flies were chilled in a freezer for approximately 4 minutes, and then 25 female house flies, 3 to 6 days old, were placed into 230 ml glass jars (VWR International (West Chester, PA) catalog No. 16195-008) containing either one 4 cm cotton dental wick (Richmond Dental Co., Charlotte, NC) soaked in 20% sugar water containing technical grade imidacloprid (Chem Service Inc., West Chester, PA) (no-choice feeding assay), or two 4 cm dental wicks, one with sugar water and imidacloprid and the second with sugar water only (choice feeding assay). Bioassay jars were then covered with mesh netting and kept at 25°C with a 12:12 L:D photoperiod. Dental wicks were hydrated at 24 and 48 hours, and mortality was assessed at 72 hours with house flies considered dead if they were unable to right themselves.

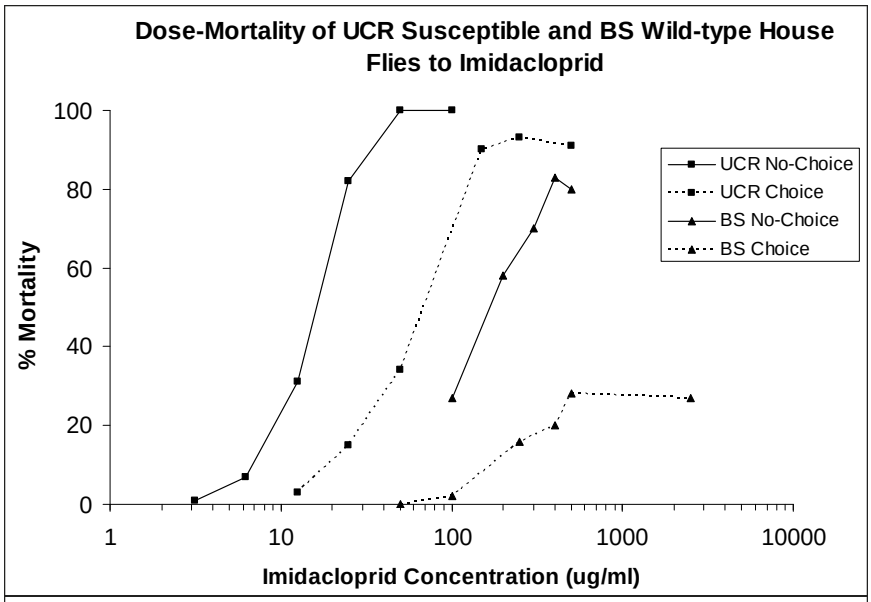
For both the no-choice and the choice feeding assays, a minimum of 5 jars were used for each imidacloprid concentration, with an additional 5 jars provided dental wicks with 20% sugar water only as a negative control. For both the BS strain and the UCR strain flies, a minimum of 5 different concentrations of imidacloprid

Table 2. Physiological and behavioral resistance to imidacloprid demonstrated by wild-type house flies captured from a dairy in southern California relative to a susceptible laboratory strain.

Fly Strain (Assay)	N	Slope (SE)	LD_{50} (95% CI) (µg/ml)	RR
UCR (no-choice)	1,250	4.27 (0.29)	15.1 (13.1-17.5)	–
BS (no-choice)*	750	2.12 (0.22)	155.9 (110-194)	10.3
UCR (choice)	725	2.36 (0.13)	68.2 (38.4-117.3)	–
BS (choice)†	725	0.78 (0.12)	NA	NA

*Significantly different from UCR Susceptible strain based on nonoverlap of 95% confidence intervals in no-choice assay.
†Significantly different from UCR Susceptible strain based on failure to achieve 50% mortality at maximum dose (2,500 µg/ml) of imidacloprid tested.

resulting in >0% and <100% mortality were used. Bioassay data was pooled and analyzed by standard probit analysis[53] with Abbot's correction to adjust for control mortality using POLO-PC (LeOra Software, Petaluma, CA).[54] Resistance ratios (RR) were calculated by dividing the lethal dose (LD) values of the wild house fly population by the corresponding LD value of the UCR susceptible colony for each assay type. A significant difference ($P<0.05$) in the susceptibility of house fly strains to imidacloprid was determined by nonoverlap of the 95% confidence interval of the LD value of each fly strain.



Dose-mortality response of UCR susceptible and field-collected strains of house fly to imidacloprid following choice and no-choice feeding assays.

Results and Discussion

The field-collected BS house fly strain was significantly less susceptible ($P < 0.05$) to imidacloprid as compared to the UCR susceptible strain using both the no-choice and the choice feeding assays, as shown in Table 2. House fly resistance to imidacloprid when measured by the no-choice assay was moderate (RR=10), but was very high when measured by the choice assay. Low field fly mortality at even the highest concentration of imidacloprid tested in the choice assay ($50 \times LD_{99}$ of the susceptible UCR strain) resulted in the lack of a significant regression line to estimate a 50% mortality value (data presented in the Figure). From these data, it is reasonable to infer that the BS strain of house flies would be minimally affected by the use of toxic fly baits containing imidacloprid as part of a fly management program under natural field conditions.

The LD_{50} values for the UCR and BS strain of house flies tested for susceptibility to imidacloprid using the no-choice feeding assay were similar to previously published LD_{50} values for susceptible and resistant fly strains, respectively.[48] The UCR susceptible flies will readily feed on commercial fly bait containing 0.5% imidacloprid (A.C.G., unpublished data, 2006). The 10 $\times$ higher LD_{50} value for UCR susceptible strain flies when tested with the choice assay is a result of altered test conditions providing both a treated and an untreated dental wick on which the flies may feed over the 72 hour assay period. Small changes in the test conditions can result in significant changes in calculated LD values,[44] thus a direct comparison between the LD_{50} value for the no-choice and the choice feeding assays should be avoided.

Fly baits containing imidacloprid were first introduced in California in 2003. Studies conducted in 2003 showed imidacloprid baits to be quite effective at attracting and killing field fly populations in southern California.[47] From 2003 to 2008, fly baits containing imidacloprid dominated the fly management market due to serious house fly resistance to the older baits containing the toxicant methomyl[44,47] and the lack of alternative granular fly baits with quick fly kill characteristics. With nearly year-round use of this single imidacloprid bait product by most facilities or agencies engaged in fly management, it should be no surprise that house fly populations quickly became resistant to imidacloprid. At a diagnostic imidacloprid concentration of $2 \times LD_{99}$ for a susceptible laboratory colony, survival of house flies from field sites in southern California exposed to dental wicks containing imidacloprid in no-choice assays has increased from <40% in 2005 to 45%-51% in 2006,[48] and finally to 73% for BS strain flies in this study. While this physiological resistance is significant, substantial mortality of the BS strain flies could still be achieved

using high concentrations of imidacloprid, as indicated in the Figure.

In contrast to the no-choice assay, the choice feeding assay indicated that 72% of the BS strain flies could not be killed by even the highest concentration of imidacloprid used ($50 \times LD_{99}$ for a susceptible laboratory colony). The choice feeding assay is certainly more indicative of field conditions where flies have many available food options and are not forced to contact or consume toxic bait. Under field conditions, flies may be selected for behavioral responses to avoid or limit contact with toxic bait. The results of the choice feeding assay support field observations during 2008, indicating substantial field failures of imidacloprid baits in southern California (A.C.G., unpublished data, 2008).

The failure to achieve complete control of the BS strain flies at higher imidacloprid concentrations in the no-choice assay may also be indicative of behavioral resistance. In these assays, flies were not evaluated for sugar consumption, and behaviorally resistant flies may have limited sugar contact or consumption over the 72 hour assay period, even in the no-choice assay.

This study showed that house flies can develop resistance to toxic baits through altered behavioral response to a toxicant. These findings are in agreement with earlier studies[44,47,51,55] showing that avoidance behaviors against a chemical toxicant may be selected for in house flies. Most volatile compounds in commercial fly baits are selected for attractiveness to house flies, indicating a priori value to house flies in persisting to express appetitive response to these compounds. It seems unlikely that behaviors would be selected to avoid these attractive volatiles. More reasonable would be the selection for flies exhibiting either an avoidance response to nonattractive toxicant volatiles, or an irritancy response to the toxicant that limits fly contact with the toxicant. If true, then a change of toxicant in the fly bait product may be all that is needed to rescue a failing fly bait.

Further studies are needed to examine whether house fly resistance to toxicants is a result of altered house fly perception and response to volatiles associated with toxicants, or due to irritation and subsequent movement of flies following contact with toxicants. Also needed are studies to develop field deployable kits for rapidly evaluating behavioral resistance of flies, mosquitoes, and other pest arthropods to pesticides formulated into baits or applied as space or surface sprays.

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Field Trial of Five Repellent Formulations Against Mosquitoes in Ahero, Kenya

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ABSTRACT

Twelve volunteers, using one leg for repellent application and the other leg as a control, field-tested 5 insect repellent formulations—Avon's (New York, NY) SS220 Spray, SS220 Lotion, and Bayrepel Lotion, and SC Johnson's (Racine, Wisconsin) Autan© Bayrepel Lotion—against the standard *N,N*-diethyl-3-methyl-benzamide (deet) in a rice-growing district near Kisumu, western Kenya, in 2 trials in May and June 2004. In addition to a control leg for each volunteer, an additional control was introduced into the study by the use of a sixth repellent, a "null repellent," which was literally a treatment application of no repellent at all. The 5 active repellent formulations were uniformly applied at the maximum Environmental Protection Agency recommended dose of 1.5 g per 600 cm^2 in the first trial and half that dose in the second trial, and none of them failed during the nightly 12-hour test period over 6 consecutive days, May 19 through May 24, 2004, and June 14 through June 19, 2004. However, the repellent control legs demonstrated a statistically significant increased landing rate compared to both the null repellent and the null repellent control leg. This suggests that, in this approach, active repellents increased the capture rate on an adjacent control leg compared to null controls. A single human volunteer can act as his/her own control provided null treatment controls are included.

OVERVIEW

Arthropod repellents represent a first line of defense against biting arthropods.[1] The Department of Defense and other agencies are interested in repellent formulations to replace *N,N*-diethyl-3-methylbenzamide (deet) because of deet's chemical properties and safety concerns. Although deet is regarded as safe, registered with the Environmental Protection Agency, and has been in use over 5 decades, there have been incidences of serious adverse effects associated with the use of deet products, especially in infants and young children,[2] its chemical properties are damaging to some synthetic material and plastics,[3] and deet experienced a major public relations hit in the mid 1990s as it was suspected to have contributed to the so-called "Gulf War Syndrome."[4] Effective candidates, then, must be less caustic to the user, a nonplasticizer, and be at least as effective as deet. SS220 (1S, 2'S) 2-methylpiperidinyl-3-cyclohexene-1-carboxamide) and Bayrepel (picardin, 1-methyl-propyl 2-(2-hydroxyethryl)-1-piperidinecarboxylate) are considered such candidates. The purpose of this study was to evaluate 2 formulations each of SS20 and Bayrepel against deet using volunteers acting as both treatment and their own control against *Anopheles, Aedes, Coquillettidia,* and especially *Culex* and *Mansonia*, the 2 most prevalent mosquito species in Kisumu, western Kenya. In order to confirm that the test leg repellent did not have a spatial affect on the other leg (the control leg), we included as one of the repellents a "null repellent", which was an application of no repellent at all. Volunteers treated with the null repellent, then, had an "application" of the "null repellent" on their treatment leg, while their other leg served as the control. In other words, there was no repellent on either leg.

MATERIALS AND METHODS

Study Site

This study was conducted at the homestead of a local inhabitant in the midst of a rice growing region near Kisumu, in the Kamagaga Village, Ahero Irrigation Scheme Sub-Location, Ombeyi Location, Miwani

Division, Nyando District, Nyanza Province, Kenya, Africa (Lat -0.152098°, Long 34.925649°) (Figures 1 and 2). There were ample species of mosquitoes due to the abundant breeding sites and recent seasonal rains in April and May 2004. Two field trials were conducted during May and July 2004.

Test Repellents

The following 5 repellent formulations were used: (1 and 2) SS220 ((1S, 2'S) 2-methylpiperidinyl-3-cyclohexene-1-carboxamide) formulated as a spray (20%) or a lotion (20%) by Avon (New York, NY); (3 and 4) 20% Bayrepel, (1-methyl-propyl 2-(2-hydroxyethryl)-1-pi-peridinecarboxylate) formulated as a lotion by Avon or SC Johnson (Racine, Wisconsin); (5) 33% *N,N*-diethyl-3-methylbenzamide (deet) formulated as a cream by 3M (St Paul, MN). For statistical design, a "null repellent" was introduced as the sixth repellent formulation, and consisted of no application at all. Note, the null repellent must not be confused with the control leg.

Figure 1. Location of The study site was near Kisumu, a town on Lake Victoria in western Kenya.

Procedures

Male volunteers* from the local population were selected and screened for acceptance by a brief medical questionnaire, and blood pressure and pulse check. Each volunteer completed and was given a copy of a Kiswahili and English language human use protocol approved by the Kenya Medical Research Institute and the Walter Reed Army Institute of Research.

Twelve adult male volunteers participated in this study. Prior to repellent application, they washed both legs with clean water and allowed them to air dry. Clothing varied, as long as it prevented mosquito bites anywhere except the intentionally exposed skin area between the calf and ankle of each leg. The clothing usually included a light jacket, pants rolled to the knees, and locally purchased cotton work gloves and sport head nets which covered the entire head and neck. Since many volunteers wore open, slipper style shoes ("flip flops"), each foot was protected in a loosely fitting enclosure of mosquito bed net material which was gathered and taped around the volunteer's leg at the treatment line. As shown in Figure 3, the loose fitting ensured an air space around the foot (except for the sole), allowing olfactory cues to attract mosquitoes but not allowing them to feed. An area of 600 cm^2 was calculated and marked on each leg of each volunteer by averaging 3 equally-spaced circumference measurements between the lower knee and upper calf and dividing into 600 cm^2 in order to get the length. The resulting area was taped off to the pants at the top, and to the feet netting at the bottom. Each repellent was weighed for dose and applied evenly with a gloved-finger over the exposed skin on the treatment leg (Figure 4). To standardize procedure in the study design, the control leg was rubbed with a clean, dry,

Figure 2. The field trials were held in the rice-growing region of western Kenya, near Lake Victoria.

*No female volunteers were used, as it was culturally unacceptable. The village chiefs and elders, whose endorsements were required for the trial to be performed at this site, deemed it unwise to have men and women together throughout the night.

Figure 3. A volunteer and a collector at a collection site with collection cartons. Note the envelopes of net material enclosing the feet below the exposed skin test areas.

gloved finger. Similarly, the null repellent was also "applied," once again using a clean, dry, gloved finger.

The first volunteer's repellent was applied at 4 PM, followed by the other volunteers at 5 minute intervals. Evaluation was conducted on each of 6 nights every 2 hours starting at 6 PM (then 8 PM, 12 midnight, 2 AM, and 4 AM), each volunteer staggered at successive 5 minute intervals based on their application time. At the assigned time, the volunteer left the screened tent and walked the short distance (50 to 60 m) to the assigned test site. The test sites were separated from each other by at least 15 m. Each volunteer sat in an assigned location that was randomly determined, and a "collector/helper" sat opposite the volunteer. The collector/helper was fully clothed and helped monitor the legs of the volunteer for landing mosquitoes in order to aspirate them before biting could occur (Figure 3). Volunteers remained in the test area for 20 minutes. Aspirated mosquitoes were expelled into lidded, pint-sized paper collection cartons. Each collection carton had a screen mesh in the lid for visibility, and a double dental dam portal on the side for the aspirating tube. The collected mosquitoes were immobilized by ether, counted, recorded, and transferred into vials in the field to be identified to species at a later date.

Figure 4. Repellent is applied evenly over the designated 600 cm^2 of skin on a volunteer's leg which will be exposed during the test period.

Totals at each time point were calculated for each repellent group and control. Percentage protection was defined as the number of bites received by a treatment group relative to that of the control ((control - treatment) / control × 100).

Statistical Analysis

Because there were no failures of 100% protection in the treatment group, we did not apply a statistical analysis, although had the protection been less than 100%, we would have performed an arcsine transformation before statistical analysis. We used a Student 2 tailed T test with groups having equal variance to compare the 3 control groups.

Results and Discussion

The goal of this study was to compare repellent activity of deet with potential candidate replacements SS220 and Bayrepel against mosquito species commonly found in Kisumu, Kenya.

Mosquito Populations Collected

In the first trial, a total of 3,137 mosquitoes were collected during these exposures. The main species were *Mansonia uniformis* (Theobald) (59.9%), *Culex pipiens* Linnaeus (18.1%), and *Culex poicilipes* (Theobald) (4.3%). Overall, 4 species of *Aedes*, 2 species of *Anopheles*, 3 species of *Coquillettidia*, 4 species of *Culex*, and 2 species of *Mansonia* were collected, as shown in Table 1. Figure 5 shows that *Mansonia* represented 76.6% of the total collected, and *Culex*

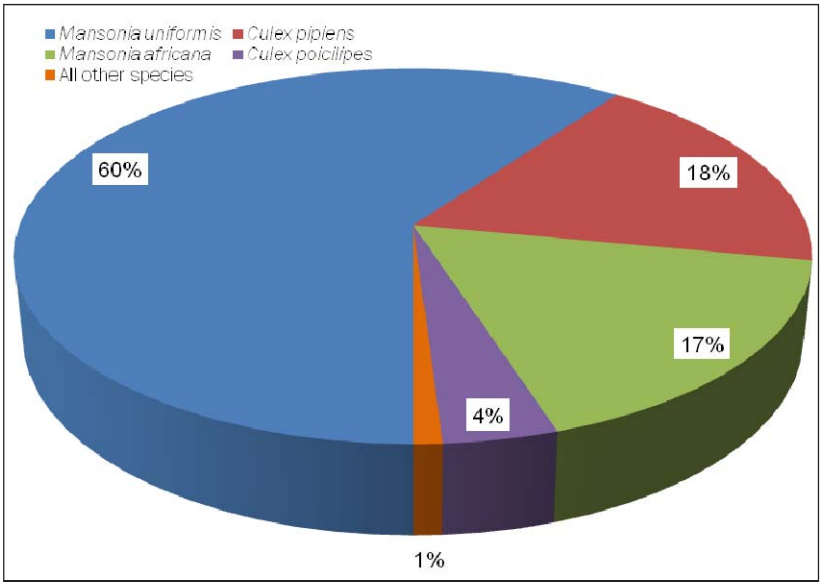

Figure 5. Distribution of species mosquitoes collected during the first trial of the study in the Kisumu area, Western Kenya, May 2004.

represented 22.9%. It was surprising that few *Anopheles* were collected, as malaria transmission is a major problem in Kisumu. It is possible that the time of our study, 6 PM to 4 AM, did not coincide with either the main biting time of *Anopheles*, or the study site was not an optimal location where *Anopheles* may be found.

In the second trial, we focused only on the main species identified in the first trial. A total of 4,495 mosquitoes were collected, and of these, the most frequent were *Culex pipiens* (45.3%), *Mansonia uniformis* (42.2%) *Culex poicilipes* (10.7%) and *Mansonia africana* (Theobald) (1.6%). In this study, *Culex* represented 56.2% of the total collected, and *Mansonia* represented 43.8%. Therefore, the rates of collection varied from season to season, but the main species that predominated were similar, as were the collection rates.

Effect of Each Repellent on Mosquito Biting Rates

In the second trial, 10 of the 12 volunteers were used to test the 5 repellents, with one leg as the repellent, and the other leg as the repellent control. Two of the 12 volunteers were treated with null repellent on one leg and the other leg was the null repellent control.

We found that each repellent tested at the dose specified protected each treated leg 100% from mosquito bites, and there were no failures. Therefore, this trial did not distinguish whether SS220 and Bayrepel were less or more effective than deet. However, total protection lasted at least 8 hours after repellent application. It is clear that SS220 and Bayrepel were equally effective against the main mosquito species collected in Kisumu, *Culex* and *Mansonia*. A previous study indicated that piperidine compounds were less effective than deet in controlling *Culex pipiens*,[5] but the dose may have affected this outcome. Whether these repellents are as active against *Anopheles*, *Aedes*, or other genera would need confirmation in tests in areas where they are more prevalent than in Kisumu.

In a trial in Australia,[6] deet and Bayrepel were relatively much less active against *Anopheles* spp, where both protected volunteers for only up to one hour after application. However, deet and Bayrepel protected volunteers for up to 5 hours after application against *Culex* species,[6] comparable to our results in Kisumu. In another study in Burkina

Table 1. Distribution of mosquito species collected at the Kisumu area, Western Kenya, May 2004.

Mosquito Species	First Trial		Second Trial	
	Total	%	Total	%
Aedes (*Albopictus*) *kennethi*	1	< 0.1	NT*	NT
Aedes (*Neomelanion*) *luteolateralis*	1	< 0.1	NT	NT
Aedes (*Neomelanion*) *circumluteolus*	1	< 0.1	NT	NT
Aedes (*Aedimorphus*) *cumminsii*	1	< 0.1	NT	NT
Anopheles coustani	1	< 0.1	NT	NT
Anopheles funestus	1	< 0.1	NT	NT
Coquillettidia aurites	3	< 0.1	NT	NT
Coquillettidia fraseri	2	< 0.1	NT	NT
Coquillettidia fuscopennata	4	0.1	NT	NT
Culex annulioris	4	0.1	NT	NT
Culex pipiens	567	18.1	2,035	45.3
Culex poicilipes	136	4.3	483	10.7
Culex theileri	12	0.4	10	0.2
Mansonia africana	524	16.7	72	1.6
Mansonia uniformis	1,879	59.9	1,895	42.2
Grand Total	3,137	100.0	4,495	100.0

*Not tested

Faso, Bayrepel performed better than deet against *Anopheles gambiae* Giles, but did not repel *Aedes*.[7] However, Bayrepel and deet were effective against *Aedes taeniorhynchus* (Wiedemann).

A major problem with all such studies is the small sample size. Larger studies may be warranted to more effectively determine the activity of these repellents.

The method used in this study was to use each volunteer as his/her control, by treating one leg and leaving the other leg untreated. At each time, 10 volunteers were treated this way, and 2 volunteers were similarly treated using a null repellent (literally, no repellent at all). Surprisingly, the number of mosquitoes collected from the legs serving as the control for active repellents were statistically higher than either the null repellent ($P = 0.007$) or the null repellent controls ($P = 0.003$) (Table 2). However, there was no significant difference between the null treatment legs and the null treatment controls ($P = 0.4$) (Table 2).

Table 2: Total numbers of each mosquito collected on the repellent treated leg and its control untreated legs compared to null repellent controls at the Kisumu area, Western Kenya, May 2004.

Leg	Mean	SD	SE	*P* Compared with Repellent Control	*P* Compared with Null Control
Repellent control	48.1	20.00	2.65	—	—
Null repellent treatment	29.9	12.10	3.82	0.007	—
Null repellent treatment control	34.4	12.67	3.65	0.003	0.4

NOTE: The Student 2 tailed-T test where groups have equal variance was used to calculate whether more mosquitoes were collected on repellent control legs compared to null treatment and null treatment controls, or between null treatment and its null treatment control.

There is little known about how exactly these repellents influence insect behavior. However, a recent study has suggested that they cause insects to move away from treated skin and bite only skin without the chemical.[8,9] This suggests that insects use a sense of smell to detect the chemicals and avoid biting where the repellent is coated. Our data suggests that insects were repelled from biting the treated skin and moved towards the untreated skin (controls), increasing the biting rates compared to the null repellents. We had no failures with the repellents in our study. However, this behavior effect would increase collections from controls and therefore underestimate the effectiveness of the repellent on the other leg if it is less than 100%. It seems, therefore, if a single volunteer is used as a repellent treatment and a no treatment control, it is essential that null treatment volunteers are also included to detect and correct such underestimation.

Finally, the wide spectrum of arthropods reacting to SS220 and Bayrepel against ticks,[10] biting midges (*Leptoconops*),[11] and the mosquitoes tested in this and similar studies,[12-16] suggest that these compounds may be valuable in controlling vector-borne diseases, in both military and civilian populations.

CONCLUSION

Studies such as reported here are valuable to better define appropriate evaluations of repellents under field conditions and to determine the mean protection periods.[12] Further investigation of the toxicity of repellents to mosquitoes may reveal more effective compounds or ones that may act synergistically,[13] as well as compounds that lack potential adverse effects of deet such as BioUD.[14] Recently, research measuring the toxicity of 8 repellents to female mosquitoes suggested that another piperidine compound, A13-37220,[15] may be more toxic at lower concentrations than deet.[16] Therefore, these single volunteer studies in Kenya and other regions may aid in the practical identification and acceptance of other, more effective repellent regimes.

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Characterization of a New Ultra-Low Volume Fuselage Spray Configuration on Air Force C-130H Airplane Used For Adult Mosquito Control

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ABSTRACT

The US Air Force (USAF) tested a new fuselage boom configuration on the C-130H airplane. We used into-the-wind and crosswind field trials to characterize a BVA oil (BVA Inc, Wixom, MI) droplet spectra produced by fuselage booms with flat-fan nozzles (8001, 8005) at the Air Force Range at Avon Park, FL. Across all trials, median droplet diameter (DV_{50}) for 8001 and 8005 nozzles were 11.4 μm and 54.3 μm, respectively. For 8005 nozzles 22% of droplets collected were 7 μm to 25 μm size range while 75% of droplets from 8001 nozzles were <7 μm. Fuselage configuration parameters and field data were also used as input variables into the Agricultural Dispersal (AGDISP) computer model to predict aerosol deposition and droplet fate. AGDISP predictions were compared with field data from crosswind tests and the model was found to fit reasonably well to empirical data. However, AGDISP predictions were better correlated with empirical findings for larger droplets than smaller droplets and for locations closer to the release point than further downwind.

INTRODUCTION

Ultra-low volume (ULV) mosquito spraying has become increasingly technologically advanced since it was first introduced in the 1960s as a replacement to thermal fogging.[1] Examples of technological advancements are new nozzles designs to produce optimal sized droplets and computer modeling of droplet fate under a variety of metrological conditions. These technologies help reduce spray volume and are important tools to minimize the use of pesticide.

Aerial ULV sprays are the primary method used to interrupt insect-borne epidemics.[2-4] For example, the US Air Force (USAF) used airplanes to control mosquito outbreaks after major hurricanes, and other public agencies have used aerial ULV sprays to decrease disease transmission and control nuisance mosquitoes.[5-7] ULV applications have been used outside the United States to interrupt malaria transmission.[8]

A crucial element of ULV technology is the creation of an effective aerosol cloud that will optimize target pest mortality while reducing pesticide use and nontarget species mortality.[9,10] Maximum efficacy is achieved by dispersing uniform droplets of the correct size. For mosquitoes, the optimum droplet size is 7 to 25 μm in diameter as demonstrated by laboratory and small scale ground ULV studies. Aerial sprays probably need to produce larger droplets in order to reach the target.[11-13] However, many aircraft spray systems create a spectrum of droplet sizes between 1 μm and 150 μm.[14] Extremely small droplets may not be lethal and large droplets (>50 μm) can settle out too quickly and are less likely to contact flying mosquitoes. Larger droplets are also wasteful because they contain more toxin than needed to kill the pest.[15] Because of their potentially high deposit peaks close to the aircraft flight line, large droplets also represent a potential hazard to nontarget organisms and thus create unfavorable environmental effects.[16]

The USAF typically uses a modular aerial spray system (MASS) on the C-130H airplane with ULV flat fan nozzles installed under the airplane wing.[17] The wing boom configuration requires pressurized tubing installed along the length of the wing, which results in

residual pesticide waste when the equipment is cleaned after spray operations. Furthermore, the tubing is located in the interior of the wing, which makes installation and repairs more difficult. The USAF has recently modified the MASS on the C-130H airplane to use fuselage-mounted spray booms, as shown in Figure 1. The fuselage booms do not require auxiliary equipment for installation and reduce pesticide waste because the pressurized tubing is shorter.

Figure 1. The fuselage-mounted spray boom installed on the C-130H airplane.

The fate of droplets released from airplanes is strongly affected by airplane generated turbulence.[18] However, it is not known how airplane vortices affect droplet size and drift when a fuselage boom configuration is used with the C-130H. Effective aerial mosquito control operations also need to predict the drift of the aerosol swath. Although keeping pesticide droplets aloft for a longer time is advantageous to increase the chance that droplets contact the target pest, it also makes it more difficult to predict swath drift patterns. The US Forest Service has developed computer models to predict pesticide drift for agricultural applications (ie, crop dusting).[19] An earlier version, the Forest Service Cramer Barry Grimm model, has been integrated into the Agricultural Dispersal (AGDISP) model which can model the lower volumes and smaller droplets used in ULV mosquito adulticide sprays.[20] Standard parameters for over 173 spray aircraft are available in the AGDISP model's library, including the C-130H airplane with wing booms and flat-fan nozzles. However, parameters for the new fuselage spray boom configuration are not available. Computer model predictions validated by field testing are helpful to estimate the effects of changing parameters (eg, altitude, nozzle type, etc) on droplet dispersal. While the model has been well-validated for large droplet/ low altitude agricultural applications,[21] few field trials have been conducted to confirm its predictive ability for the small droplet/high altitude applications used in ULV mosquito sprays.

This study reports on the characterization of droplet size and drift generated by USAF C-130H aircraft with the newly developed ULV fuselage boom configuration. Droplet size and downwind dispersion were measured using into-the-wind and crosswind field trials, respectively. The resulting droplet size and application parameter data was then input into the AGDISP computer model to compare the model's predictions with the empirical field results.

METHODS

Study Site Description

The fuselage boom tests were performed from December 2004 to February 2005 on the Avon Park Air Force Range (APAFR), an approximately 42,000 ha facility in Highland and Polk counties, Florida. The field site was chosen to minimize disruption of drifting droplets by vegetation.[22] As shown in Figure 2, site vegetation was dominated by shrubs or open woodlands. Primary roads on the APAFR are aligned along cardinal directions, which facilitated realignment of sampling stations when wind direction changed between test dates.

Field Characterization of Droplet Size

Applications were made by USAF C-130H airplane with fuselage mounted booms containing nozzles that were directed towards the ground. The airplane was equipped with a Satloc GPS [global positioning system] Agricultural Navigation System (Hemisphere GPS, Calgary, Canada) to record airplane position and time when the spray system was turned on. Fuselage boom configurations were tested with flat-fan TeeJet® nozzles (Spraying Systems Co, Wheaton, IL) sizes 8001 and 8005, which were rated by the manufacturer to deliver 0.4 liters/min and 1.9 liters/min per nozzle at 276 kPa, respectively.

The airplane flew at 370.4 km/hr and the spray system activated 30 seconds prior to reaching the sampling transect (Figure 2) and was left on until 30 seconds after passing the transect (60 seconds total). Wind speed, direction, air temperature, and humidity were recorded 2.5 m above ground surface using a Swath Kit Weather Station (Droplet Technologies, College

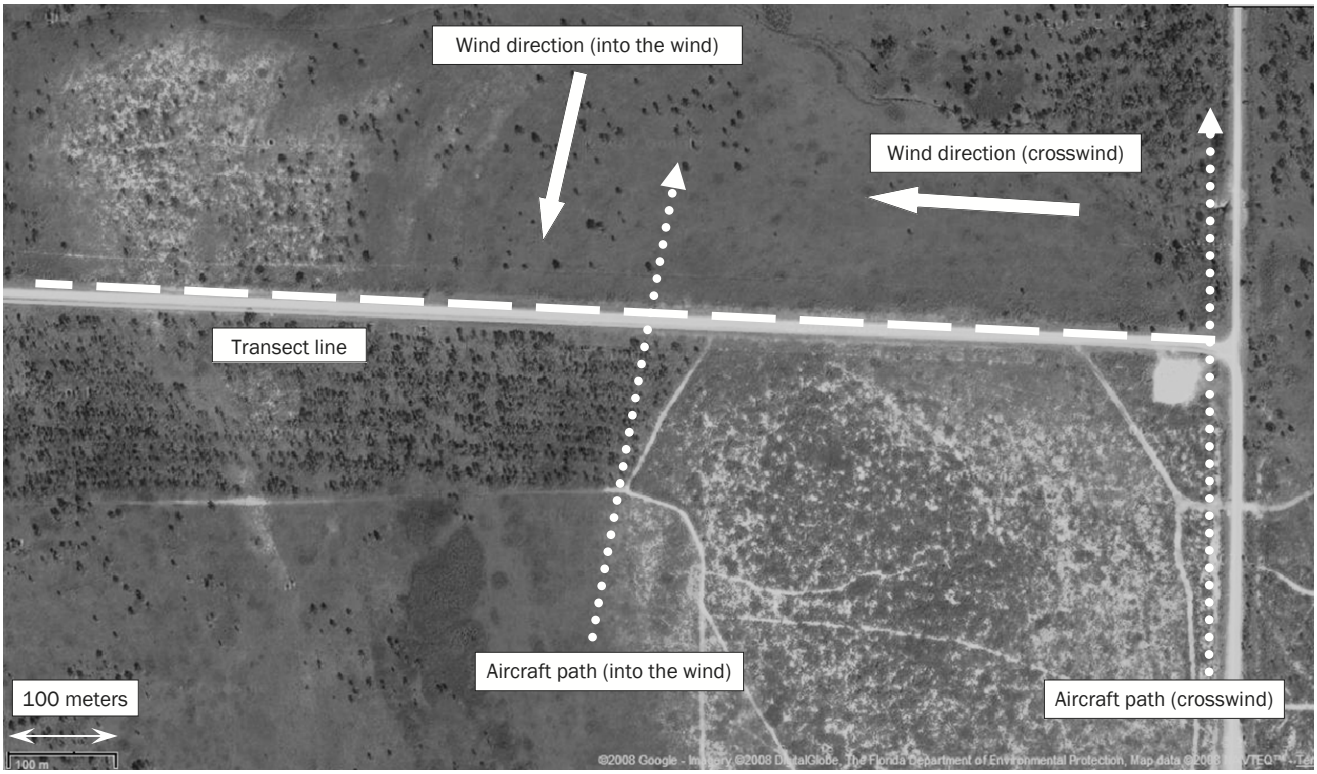

Figure 2. Aerial photograph of the field test area at the Avon Park Air Force Range, Florida. Directions of flight paths and wind for the test flights are shown.

Station, PA) and at spray altitude using the airplane's self-contained navigation system.

The MASS delivered an application rate of 45.3 ml/ha that was based on standard operational practices of public health agencies using a common mosquito control pesticide, Anvil® 10+10 (Clarke Mosquito Control Products, Inc, Roselle, IL; hereafter Anvil). We conducted multiple trials in the same location, which would have caused excessive pesticide accumulation. Therefore, our sprays used only the pesticide carrier, BVA spray oil 13 (BVA Inc., Wixom, MI), without the pesticide.

On December 4, 6, and 8, 2004, and February 15, 2005, the airplane flew directly into the prevailing wind (into-the-wind trials) at 46 m above ground. To quantify the droplet spectra, 9 sampling stations were positioned every 61 m along a 488 m transect perpendicular to the prevailing wind (Figure 2). The airplane flew directly over the center station. Slide rotator devices (John Hock Company, Gainesville, FL) held spinning Teflon® coated glass microscope slides (25 mm by 75 mm, approximately 420 rpm, effective slide speed of 3.6 m/second) that collected

Table 1. Parameters entered into the AGDISP model to predict droplet fate from fuselage boom spray applications. Drop size distribution used field collected data.

Aircraft	Lockheed C-130H airplane, weight 63,047 kg; speed = 371 km/h
Release height	46 m, 91 m; flight lines = 1
Nozzles	8, 8005 flat-fan, positioned at 3.61, 3.71, 3.81, 3.91 m from aircraft centerline, both sides
Drop size distribution	DV_{10} = 22.3 μm; DV_{50} = 54.3 μm; DV_{90} = 104.7 μm
Material	BVA oil (specific gravity = 0.85; nonvolatile fractions = 1; active = 1; rate = 45.3 ml/ha
Swath width	152 m (maximum allowed), Swath displacement = -76
Wind speed	6.4, 7.5 km/hr at 46 m or 91 m above ground level
Temperature and relative humidity	18.3°C and 70% for 46 m and 17.1°C and 92% for 91 m
Stability	day - weak (sunset to 1 hour after sunrise, weak)
Canopy	none
Surface roughness	0.0075 m

the droplet cloud as it passed over the station. Microscope slides were collected 30 minutes after the airplane passed to allow enough time for airborne droplets to drift through the transect. Droplet data from all stations were pooled to determine the characteristics of the droplet spectra.

In addition to characterizing the droplet size spectra with "into-the-wind" tests, crosswind trials were used to compare actual drift in field trials with predictions by the AGDISP model. On February 16, 2005, we conducted a crosswind trial at a release height of 46 m above ground, which is standard for the USAF mosquito control operations. Although we ran 2 trials, a wind shift during the second trial disrupted the application and the data was lost. Also, we only used the 8005 nozzles for the crosswind trials because 8001 nozzles clogged repeatedly during the into-the-wind trials. On February 17, 2005, 2 trials measured spray drift released at 91 m, which is an altitude proposed for potential nighttime mosquito control operations with the C-130H airplane. In all crosswind trials, droplets were collected with rotating microscope slides at 8 stations arranged along the prevailing wind direction. On February 16, 2005, stations were set 154 m apart (154-1,219 m) on a transect downwind from the release point (Figure 2). On February 17, 2005, we spread the stations 457 m apart (457-3,658 m) along the transect in anticipation of greater drift from the higher release altitude.

All slides were processed within 6 hours after the trial was conducted. Droplets on slides were measured under a compound microscope equipped with a reticule. A total of 100 droplets were measured or the entire slide was scanned, whichever came first. Measured drop diameter was converted to airborne drop diameter with a 0.59 correction factor (Anvil 10+10 Resource guide) that accounted for the spread of droplets when they impacted the glass slide.[23] These data were used to determine volume median diameter (DV_{50}) and droplet density at each sampling station.[24] The DV_{50} represents the droplet size which divides the droplet spectrum in half by volume, or in other words, where 50% of the spray volume is contained in droplets smaller than the DV_{50}. Also of interest are the DV_{10} and DV_{90} values which are the points in the droplet distribution where 10% and 90% respectively of the spray volume is in drops smaller than this size.[10]

AGDISP Computer Model

Droplet size data obtained from the into-the-wind field trials with 8005 nozzles were input into the AGDISP computer model (version 8.08, USDA Forest Service) to predict crosswind droplet trajectories and deposition. Operational parameters and meteorological conditions recorded during the trials at Avon Park were used as input values for the AGDISP model (Table 1). To allow placement of the spray boom relative to the trailing edge of the wing, the AGDISP model has a library of aircraft (including C-130H with wing booms) and droplet spectra produced by different nozzle types under varying application scenarios. However, the specific parameters of the C-130H fuselage spray system are not included as a standard. Consequently, where possible we used values measured in our field trials (eg, boom placement, nozzle position, application parameters, meteorology, and DV_{10}, DV_{50}, and DV_{90} droplet sizes) to test the model's accuracy. Predictions for downwind deposition of BVA oil released from spray altitudes of 46 m and 91 m were modeled. Output values regarding droplet deposition and trajectories for droplets sized 22.3, 54.3, and 104.7 μm were plotted. Predictions of droplet trajectories made by AGDISP were then compared to empirical data derived from crosswind field trials.

Table 2. Parameters of the BVA-13 spray-oil field characterization trials of the C-130H airplane ULV fuselage boom configurations.

Date of Trial	Application Rate (ml/ha)	Flow Rate (L/min)	Boom Pressure (kPa)	Average Wind Speed (km/hr), ground/altitude	Nozzle Configuration Size (number)	Temperature	Relative Humidity
4 Dec 04*	45.3	8.5	634	3.2/13.7	8005 (8)	16.1°C	51%
6 Dec 04*	45.3	8.5	641	3.2/17.7	8005 (8)	25.6°C	59%
8 Dec 04*	45.3	8.5	621	6.4/12.9	8001 (40)	24.4°C	79%
15 Feb 05*	45.3	8.5	483	1.6/6.4	8001 (40)	20.6°C	70%
16 Feb 05†	45.3	8.5	486	6.4/13.0	8005 (8)	18.3°C	70%
17 Feb 05†	45.3	8.5	472	7.5/12.1	8005 (8)	17.1°C	92%
4 Dec 04*	45.3	8.5	634	3.2/13.7	8005 (8)	16.1°C	51%

*Into-the-wind flight trials
†Crosswind flight trials

RESULTS

Flight parameters and meteorological conditions during the trials are given in Table 2. Meteorological conditions were acceptable during the into-the wind and

crosswind trials. Humidity and temperature were typical for ULV mosquito control operations. Boom pressure was relatively constant during the into-the-wind trials (621 – 641 kPa) in December, but was slightly lower during the February trials (472-486 kPa). Wind speeds measured at the ground were within acceptable ranges (1.6 to 6.4 km/hr) during the into-the-wind trials.

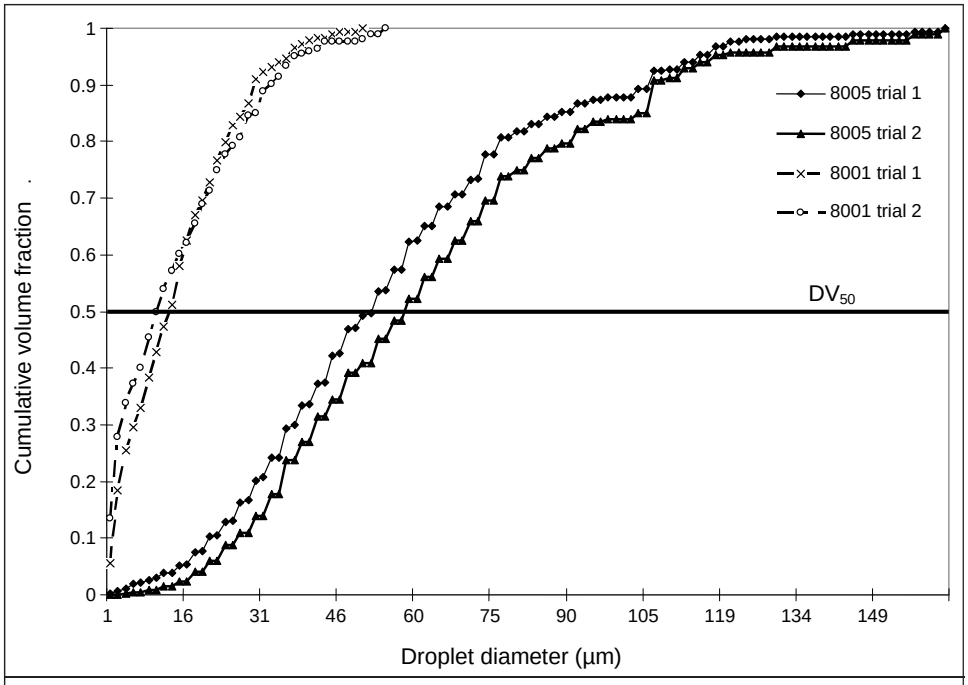

Figure 3. Drop size distribution for droplet spectra produced by 8001 and 8005 flat-fan nozzles used with Air Force C-130H fuselage boom characterization trials.

Over 7,000 droplets were measured in samples collected during the into-the-wind and crosswind field trials. The 2 nozzle types produced different cumulative volume curves during into-the-wind trials (Figure 3). Average volume median diameter (DV_{50}) was 11.4 μm (SE ± 1.0) for 8001 flat-fan nozzles and 54.3 μm (SE ± 2.2) for 8005 nozzles. Droplets from 8001 nozzles produced a narrow range of relatively small droplets: DV_{10} was 1.7 (SE ± 0.6) μm and DV_{90} was 30.8 μm (SE ± 1.6). The larger orifices of the 8005 nozzles delivered a wider range of droplet sizes: DV_{10} was 22.3 μm (SE ± 2.1), and DV_{90} was 104.7 μm (SE ± 0.6). Thus, the cumulative volume curve of 8005 nozzles generated a lower slope than the 8001 nozzles.

The distribution of drop sizes in spray clouds produced during the into-the-wind trials was also different between the 8001 and 8005 nozzles. The 8001 nozzles produced smaller droplets, with 40% of drops in the smallest class size and 75% were <7 μm (Figure 4). In contrast, 8005 nozzles generated a wide range of size classes with 1.2% of drops <7 μm and 22% of droplets in the 7 to 25 μm size range (Figure 4).

On February 16, 2005, the crosswind trials with a spray release height of 46 m had droplet sizes ranging from 42.5 μm to 10.1 μm (Table 3). The swath drifted much further when the spray was released at 91 m above ground on February 17 (Figure 5).

In both crosswind trials the largest droplets were deposited at the first collection station and the mean droplet size decreased downwind.

AGDISP Computer Model Predictions and Field-Trial Results

Droplet spectra data for 8005 nozzles and meteorological data from into-the-wind field trials were input into the AGDISP model to predict droplet fate (Table 1). The model predicted heavy deposition near the airplane when spraying at 46 m above ground with the fuselage booms (Figure 6). Deposition was predicted to reach a maximum of 13.8 ml/ha at 92 m from the release point and then rapidly dropped to 4.7 ml/ha by 300 m downwind. From this point, deposition gradually decreased to <0.5 ml/ha at the maximum predicted range (1,582 m). Average deposition over 1,582 m was predicted to be 3.5 ml/ha. Between the release point and the standard operational swath width

Table 3. Average BVA oil droplet size (μm) collected at stations downwind of release point, February 16, 2005. C-130H fuselage boom configuration; 8005 nozzles; crosswind - 6.4 km/hr; release height - 46 m.

	Distance Downwind							
	154 m	305 m	457 m	610 m	762 m	914 m	1,066 m	1,219 m
Mean±SE	42.5±2.5	24.9±2.6	13.9±2.8	7.8±0.5	8.4±1.2	5.7±0.8	6.0±0.3	6.2±0.4

of the C-130H (610 m) the model predicted an average deposition of 6.0 ml/ha.

When release height was increased to 91 m, the predicted deposition was more spread out (Figure 6). Deposition was first predicted at approximately 200 m downwind of the release point, when deposition gradually increased to peak at 3.5 ml/ha at 770 m. Deposition was predicted to remain within half of the maximum over the remaining model's predictive range.

When we modeled droplets sprayed from the four 8005 nozzles on C-130H fuselage booms, the AGDISP model predicted different trajectories for small (set at 22.3 μm, which is our DV_{10}) medium (54.3 μm, our DV_{50}) and large (104.7 μm, our DV_{90}) droplets and for sprays released at 46 m or 91 m above ground. Overall, AGDISP predictions followed standard ballistics such that smaller droplets released at greater altitudes drifted further than larger droplets released at lower heights. However, the model also predicted droplet trajectories would be affected by airplane vortices. For example, all droplets released on the windward side at 46 m were affected by airplane induced vortices, which first lifted them and then allowed them to drift downwards (Figure 7). In contrast, droplets released on the leeward side of the airplane were entrained in down-ward vortices that pushed them toward the ground until the vortices broke apart or droplets deposited. The model predicted that small droplets (eg, 22.3 μm) remain airborne approximately 450 m downwind of the release point, and most medium droplets (eg, 54.3 μm) reached the

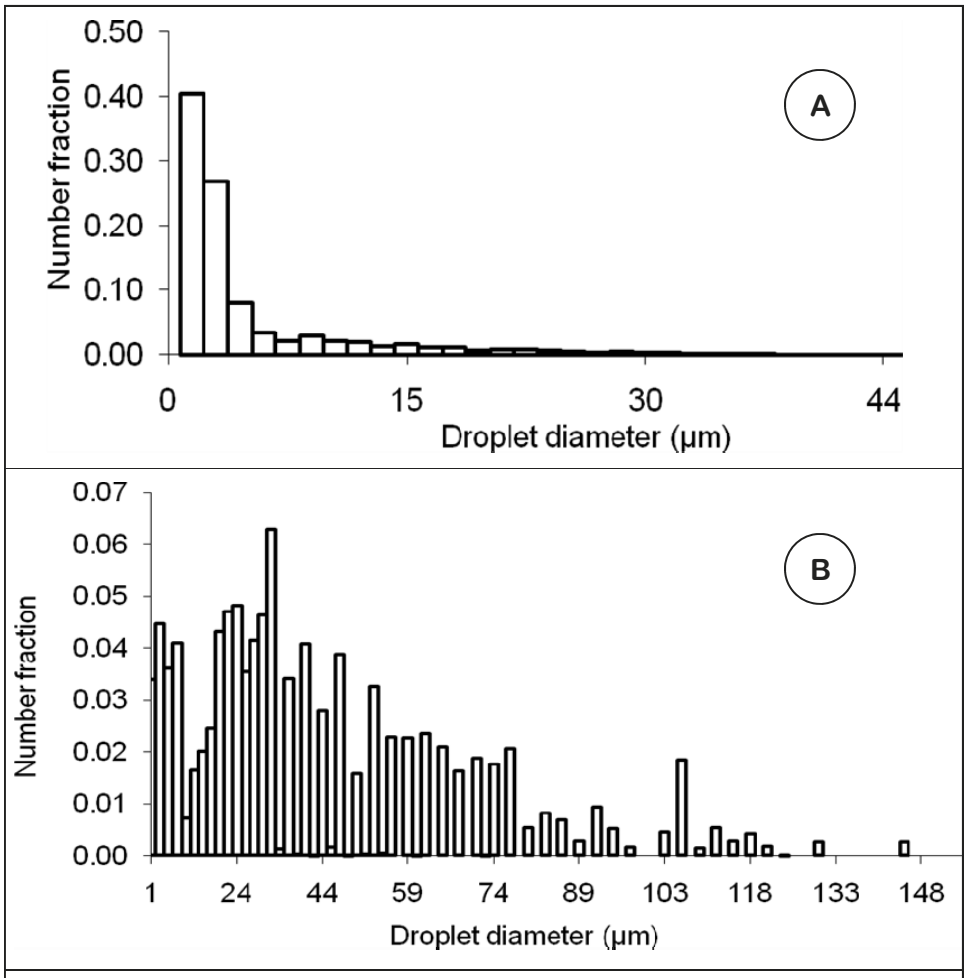

Figure 4. Distribution of drop sizes in spray cloud from (A) 8001 and (B) 8005 nozzles.

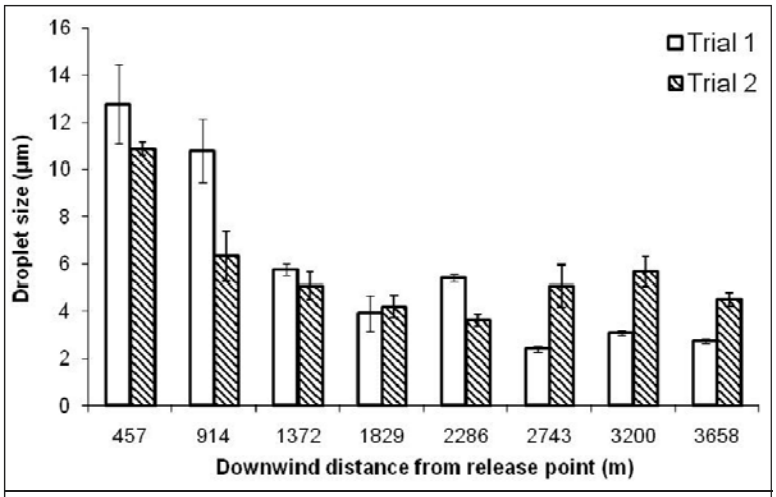

Figure 5. Average droplet size recovered from USAF C-130H fuselage mounted boom sprays using 8005 flat-fan nozzles at 91 m release height, February 17, 2005. (Bars are ±1 standard error.)

ground by 250 m. The large droplets (eg, 104.7 μm) also fit the previous pattern but reached the ground faster (approximately 130 m downwind) than the other size classes (Figure 7).

When the droplet trajectories were modeled from a release height of 91 m, trajectories were different than when released at 46 m (Figure 8). Overall, an increase in spiraling caused by vortices was predicted in all drop sizes; this was more pronounced in droplets from the windward side of the airplane. Most notable was that small and medium droplets from the leeward side of the airplane were not pushed to the ground, but were brought back in contact with droplets from the windward side and crossed paths with these droplets' trajectories (Figure 8).

DISCUSSION

These trials characterized droplet spectra and dispersal to evaluate if the USAF C-130H fuselage boom configuration is effective for adult mosquito control operations. Under optimal conditions, aerial spray operations produce droplets that adhere to mosquitoes but do not drift beyond the intended spray area.[25] We found that both flat-fan nozzles (8001, 8005) produced droplets within the optimal range for mosquito control (7 to 25 μm), but they had very different droplet size distributions.

In general, droplet distributions from flat-fan nozzles show that the largest numbers of drops are found in the smallest size classes while the greatest volume is found in the relatively scarce but larger size classes.[26] In this study, the 8001 nozzles produced a narrow spectrum of smaller droplets, and consequently, the ma -jority of the volume sprayed was comprised of small droplets (ie, $DV_{90} = 30.8$ μm). Although most droplets were within the most effective size range (7 to 25 μm), droplets < 7 μm comprised 35% of spray volume. In contrast, 8005 nozzles produced larger drops but a more even size class distribution (Figure 4).

We tested the fuselage boom configuration for its potential usefulness for ULV adult mosquito control with the C-130H airplane. The narrow droplet spectra and ideal DV_{50} of the 8001 nozzles would appear to make these a better choice for mosquito adulticiding. However, these nozzles produce many droplets considered too small for effective mosquito control. In addition, the small orifice size required 5 times more nozzles than 8005s to produce the desired flow-rate, and they often became clogged during field trials. The 8005 nozzles produced relatively large drops which equates to additional chemical waste if they deposit without contacting the target pest. Characterization tests with intermediate nozzle sizes (eg, 8002, 8003) would be useful to further examine the efficacy of fuselage booms for mosquito control.

We used rotating microscope slides to measure droplet sizes for 2 flat-fan nozzles, which is a widely used method to characterize droplet size.[9,27-29] A more precise method to determine droplet size is a wind tunnel equipped with laser-diffraction equipment. Recent wind tunnel studies with 8001 nozzles at wind speeds of 225 km/hr produced a DV_{50} of 55.2 μm, which is nearly 5 times larger than in our study.[30] Volume median diameter for 8005 nozzles at wind speeds of 225 km/hr were 87.7 μm, which were also larger than in our results. However, the Hornby et al[30] data suggests that increased wind shear at faster speeds can create smaller droplets. Since the C-130H airplane flew at 370 km/hr, this might have caused the smaller droplet size we measured with the 8005 nozzles. However, wind shear alone probably cannot account

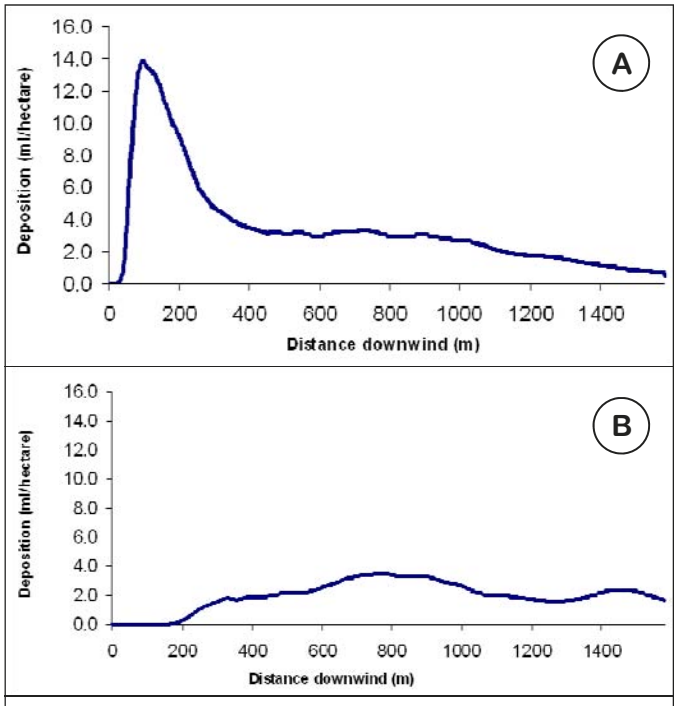

Figure 6. Predicted deposition from AGDISP model of droplets sprayed with C-130H fuselage mounted booms released at (A) 46 m and (B) 91 m above ground.

for the very small DV_{50} that we measured with the 8001 nozzles. A possible explanation is that minute oil droplets from other sources (eg, engine exhaust) were trapped on the microscope slides and were counted as sprayed material. This would be more likely to affect the data from the 8001 nozzles because they made smaller droplets than the 8005 nozzles. Adding florescent dye to the spray material (eg, Barber et al[22]) might help to distinguish between sprayed material and environmental contaminants in future studies. Also, we may have undercounted the largest possible droplets because these would be rare and might have dropped out of the air column before they impacted our sampling stations.

Making accurate predictions of pesticide droplet dispersal is desirable[19] and, therefore, we compared AGDISP model predictions to our downwind field data. The overall AGDISP predictions and empirical data followed a similar and expected pattern of larger drops falling first and smaller drops drifting further. For example, in crosswind field trials from a 46 m release height, the average droplet size measured at the second collection station (305 m) was ~25 μm (Table 3). In comparison, at 305 m downwind, AGDISP predicted that 22.3 μm droplets would still be airborne but that 54.3 μm droplets would have already reached the ground around 225 m downwind. Taking into consideration the influence that wind speed and direction can have on these small to medium-sized drops, the disparity between the model and the empirical data may be considered reasonably similar at this level of resolution. AGDISP predictions regarding trajectories of large droplets were also fairly closely confirmed by field trials. AGDISP predicted 104.7 μm droplets to reach ground level 130 m downwind (Figure 7), and many droplets of this size were collected at the 150 m collection station, although 104.7 μm droplets from the leeward side are not predicted to reach the ground until 430 m.

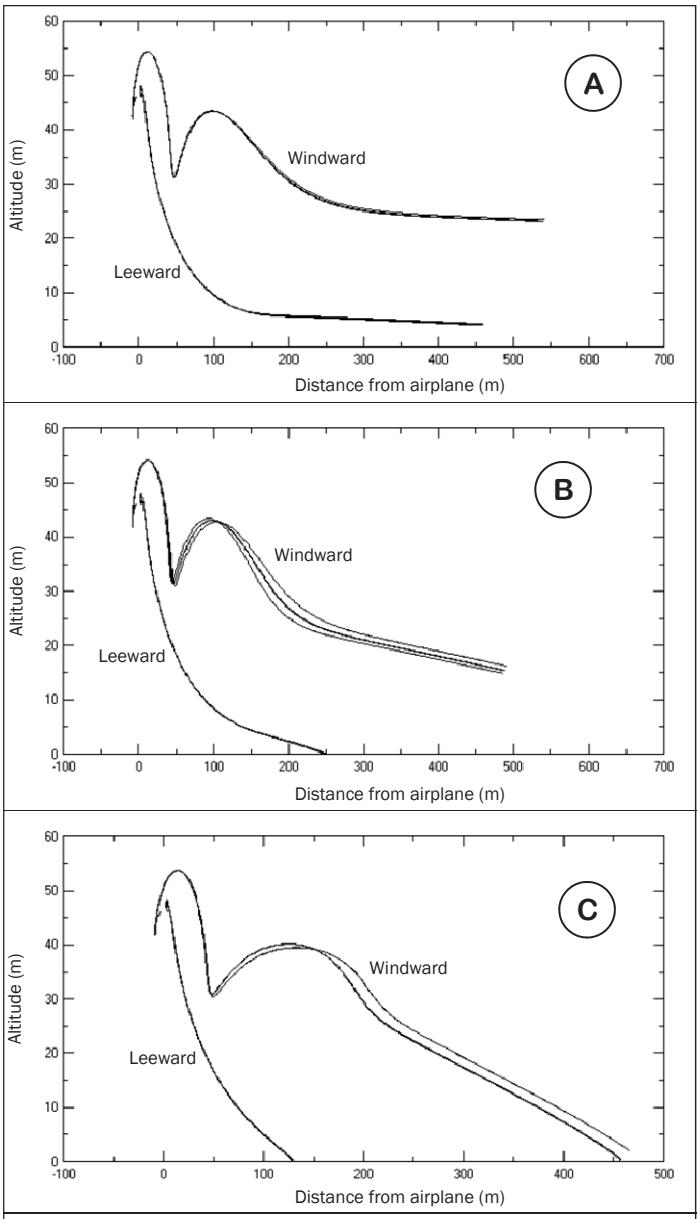

Figure 7. AGDISP model predicted trajectory of an (A) 22.3 μm droplet (DV_{10}), (B) 54.3 µm droplet (DV_{50}), and (C) 104.7 μm droplet (DV_{90}) from the C-130H airplane at 46 m release height. Windward and leeward annotations indicate the side of the airplane from which the droplets were released.

However, we found that some AGDISP predictions were different than observed in our field data. For instance, a greater disparity existed between AGDISP predicted trajectories and field data for droplets released from 90 m. The AGDISP model could not predict droplet fate past 400 m, but many droplets were still airborne at this distance. The AGDISP model also predicted further drift than we observed in the crosswind trials. For example, the trajectory path of all modeled droplet sizes from 91 m predicts droplets will still be aloft at approximately 500 m downwind from release. In contrast, we collected many droplets sizes at the 500 m sampling station during the field trials (Figure 5).

Our comparisons between predicted and empirical data indicate that the AGDISP model is more accurate at assessing the environmental fate of larger droplets and their movement closer to their release points. In general, AGDISP made the most accurate predictions of droplet fate at 46 m release height, but was less accurate for sprays released at 91 m above ground.

Fuselage sprays from 8005 nozzles produced wide pesticide swaths that suggest they would be appropriate for military aerial ULV operations where a minimum 600 m swath width is required.[31] Additional operational evaluations will be necessary to determine effective swath width for C-130H fuselage booms using sentinel mosquito mortality and various pesticides.

A potential reason for inaccuracies in AGDISP models is that the model is unable to use continuous weather data. Obviously, even modest changes in meteorological conditions (eg, wind direction) could have significant effects on droplet fate. Modeling ULV sprays at high altitudes is also difficult because the model does not calculate downwind drift past 3,600 seconds. This is an artifact from the model's origins in depositional spraying[19] and, subsequently, the development of better algorithms that accurately incorporate field conditions would improve the AGDISP model.

In conclusion, the fuselage boom configuration we tested would be desirable for use in military operations because setup and maintenance is simple and it produces less pesticide waste than the wing boom configuration. Our trials suggest that 8001 and 8005 flat-fan nozzles produce a droplet spectra and swath dispersal that would be effective for ULV mosquito control operations. However, we also found that small changes in wind speed and direction substantially affect droplet dispersion and deposition behavior. Therefore, continual monitoring of current meteorological conditions should be an ongoing consideration during ULV mosquito control operations. Increased wind speed and directional variability also make it difficult to predict insecticide dispersal characteristics with the currently available AGDISP modeling software.

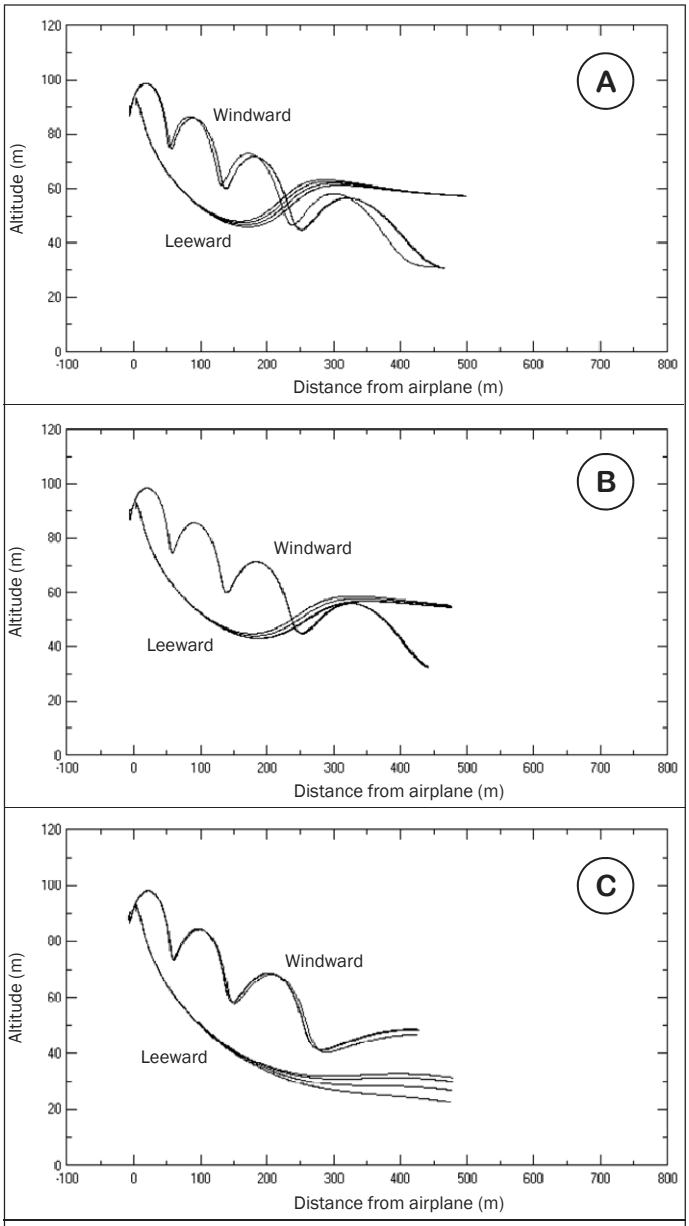

Figure 8. AGDISP model predicted trajectory of an (A) 22.3 μm droplet (DV_{10}), (B) 54.3 µm droplet (DV_{50}), and (C) 104.7 μm droplet (DV_{90}) from the C-130H airplane at 91 m release height. Windward and leeward annotations indicate the side of the airplane from which the droplets were released.

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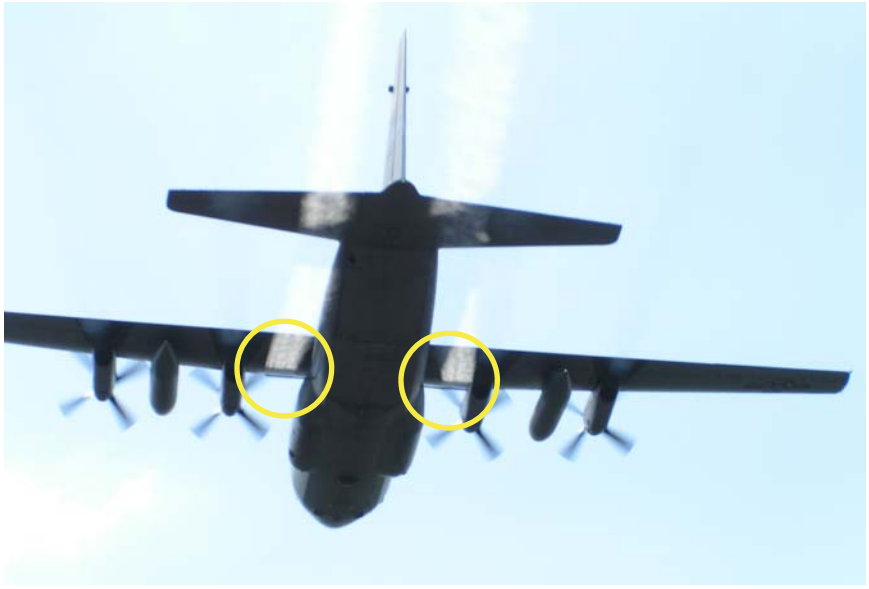

The fuselage-mounted spray booms (circled) on a USAF C130H airplane are shown in operation during evaluation flights at the Air Force Range, Avon Park, Florida.

The Armed Forces Pest Management Board: Force Multiplier Through Policy, Guidance, Research, and Information

Col William M. Rogers, USAF, BSC
Richard G. Robbins, PhD
CAPT Stanton E. Cope, USN, MSC

INTRODUCTION

The Armed Forces Pest Management Board (AFPMB), located at the Fort Detrick-Silver Spring (Maryland) Forest Glen Annex, is the Department of Defense's (DoD) lead agency in the prevention and control of arthropod-borne diseases, arthropod pests, and invasive species. Whether the issue is contingency operations, invasive species, or pest management research, the AFPMB is prepared to meet challenges head-on through the establishment of pest management policies, the encouragement and financial support of original research, and the exchange of up-to-the-minute scientific and technical information. No similar entity exists anywhere within DoD.

HISTORY

The AFPMB, originally chartered as the Armed Forces Pest Control Board, was established by *DoD Directive 5154.12** in 1956. Members have met on a regular basis since 1957. The Board currently meets twice a year at its Forest Glen location, and once a year at a US Department of Agriculture (USDA) laboratory where research of interest to DoD is reviewed with USDA investigators. The Board was redesignated as the Armed Forces Pest Management Board in 1979, and the AFPMB secretariat became a directorate of the Deputy Assistant Secretary of Defense (Environment) after *DoD Directive 6050.10** was implemented in 1985. Reestablished by *DoD Directive 4715.1E*[1] in 2005, the AFPMB operates under *DoD Instruction 4150.07*[2] and is now a directorate in the Office of the Deputy Under Secretary of Defense for Installations and Environment (DUSD (I&E)).

MISSION

The mission of the AFPMB is to recommend, coordinate, and develop policy, guidance, and the exchange of information on all matters and activities related to pest management and arthropod-borne disease control throughout DoD. The AFPMB ensures that environmentally sound and effective programs are implemented to support operating forces worldwide in the prevention of arthropod-borne diseases, and to prevent losses from pest attack on subsistence, supplies, and facilities. The AFPMB functions as DoD's scientific/research advisory body and coordination/liaison activity for pest management. In support of its mission, the AFPMB:

- Develops and recommends policy to the DUSD (I&E).
- Coordinates DoD pest management activities.
- Develops, issues, and maintains manuals and other guidance necessary to implement the technical requirements of the Federal Insecticide, Fungicide, and Rodenticide Act.[3]
- Implements DoD's plan for certification of pesticide applicators, and develops comprehensive training guidance for DoD pest management personnel.
- Coordinates DoD contingency disease vector and pest management with the Joint Staff,† the combatant commands, and other contingency planning organizations.
- Serves as an advisory body to the DoD components, and provides timely scientific and professional pest management advice.
- Develops and distributes technical information and guidance on pest management to the components by means of technical guides, disease vector ecology profiles, etc.

* Canceled, no longer in effect.
† US Joint Chiefs of Staff

- Reviews and approves any introduction, stockage, and deletion of pest management materiel (excluding disinfectants and biocides) by the Defense Logistics Agency in the DoD supply system.
- Coordinates and develops DoD's requirements for pest management research, development, testing, and evaluations.
- Carries out its mission with 3 new divisions: Operations, Research, and Information Services.

It is the AFPMB's vision that the DoD will be the federal leader for innovative prevention and management of disease vectors and pests. The strategy of the AFPMB is to support military readiness and preventive defense, and to demonstrate environmental leadership and avert future pollution problems by maximizing the use of nonchemical or least toxic chemical techniques to manage pests and disease vectors. In furtherance of this strategy, the AFPMB promotes integrated pest management, biopesticides, and the use of least toxic pesticides for installations and deployments, and advocates personal protection measures against arthropod bites and stings. The AFPMB has received numerous awards and citations for such efforts, including frequent recognition by the US Environmental Protection Agency's Pesticide Environmental Stewardship Program, which aims to "reduce pesticide use, protect human health, and preserve the environment."[4]

STRUCTURE

The AFPMB includes a directorate, council, and several committees:

Directorate – The full-time administrative body of the AFPMB currently consists of 7 active duty medical entomologists representing the US Army, Navy, and Air Force, 3 federal employees, and 3 contractors. Reorganized in January 2009, the permanent staff includes a director, deputy director, directorate support staff, contingency liaison officer, research liaison officer, information liaison officer, environmental biologist, and support personnel, as shown in Figure 1.

- Director – Supervises the directorate, and plans and conducts AFPMB operations.
- Deputy Director – Responsible for the day-to-day operations of the Directorate, the directorate support staff, facility maintenance, and personnel.
- Directorate Support Staff – Includes the financial resource manager, information technology manager, and administrative assistant.
- Contingency Liaison Officer (CLO) – In charge of the Operations Division and oversees the activities of the assistant CLO and the environmental biologist. The CLO interfaces with the Joint Staff, the unified commands, and component headquarters, playing a coordination role in DoD readiness planning for prevention and management of vector-borne diseases and invasive species.
- Research Liaison Officer – In charge of the Research Division and coordinates and assists in research activities pertinent to military pest management programs.
- Information Liaison Officer – In charge of the Information Services Division and responsible for the acquisition, analysis, and dissemination of information on arthropod-borne disease control, pest management, and natural resources for the AFPMB.

Council – The Council (voting Board members) is a part-time advisory body of senior pest management

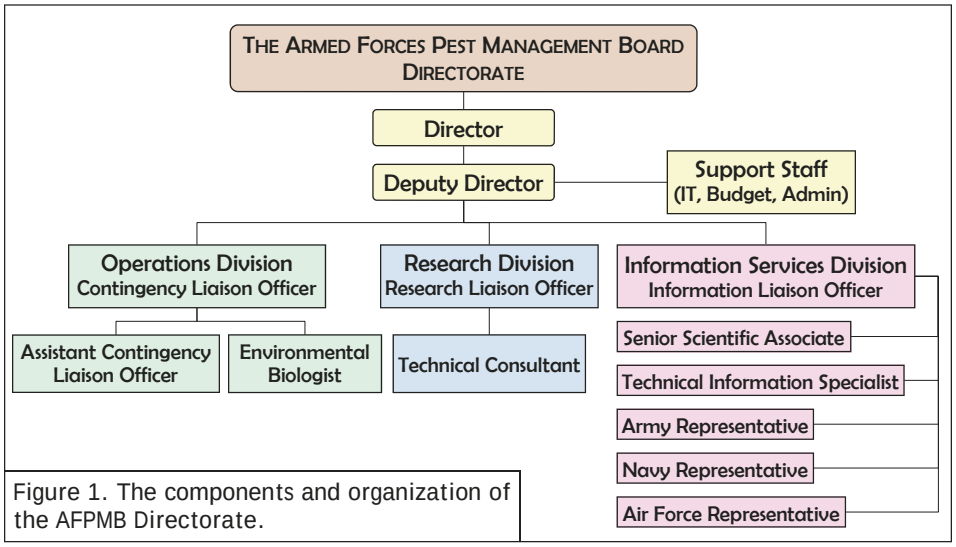

Figure 1. The components and organization of the AFPMB Directorate.

and natural resource professionals appointed from each of the military departments and the Defense Logistics Agency. It currently meets twice each year. Other federal agencies provide nonvoting representation.

Committees – The major business of the AFPMB is accomplished by committees consisting of members and agency representatives. Twice each year over 80 technical professionals representing a broad range of federal organizations and allied countries voluntarily meet at the AFPMB to contribute their expertise toward the resolution of vector-borne disease and pest management issues that are of concern to the Department of Defense.

The Executive Committee consists of 7 council representatives or alternates and comprises the senior pest management consultant from each military department, the entomology consultant to each military service's Surgeon, and the single Defense Logistics Agency representative. The Director, as ex officio, is a nonvoting member of the Committee.

There are 12 standing committees:

Contingency Advisory
Diagnostics
Education and Training
Equipment
Information Management
Medical Entomology
Natural Resources
Pesticides
Quarantine and Commodities Protection
Real Property Protection
Repellents
Research

The Contingency Advisory Committee comprises the senior medical entomologists from each service and functions to ensure delivery of vector-borne disease control support for the operating forces. Ad hoc subcommittees provide a task force effort on urgent issues.

Operations Division

The newly formed Operations Division (OD) combines the contingency and environmental biology/ invasive species areas into a single, cohesive group. The Division's primary mission is to provide consultation, guidance, and liaison with other DoD and non-DoD agencies and governments on a variety of operational pest management issues. The OD consists of the Contingency Liaison Officer, a senior military entomologist with substantial deployment experience who supervises the division; another military entomologist who serves as the Assistant CLO; and the Environmental Biologist, who is the liaison with the DoD natural resources program managers. The Operations Division is ready to assist customers in accomplishing their mission of protecting the health and safety of service members, civilians, and the environment.

The primary focus is to provide consultation and guidance to personnel and units, either deploying or already deployed, on any type of contingency, stabilization, or humanitarian and civic assistance mission. This guidance includes establishing contact between a customer and personnel already in the area of operation, referral to organizations that can provide the most recent entomological risk assessments, making recommendations on pesticides and application equipment, consulting on the implementation of personal protective measures, and advising on the handling of contract pest control operations. The OD is involved in vegetation control during contingency operations, control of agricultural pests impacting humanitarian and civic assistance efforts, assisting in the acquisition of bed nets for distribution, and treatment of uniforms with permethrin. These are a few of the many issues that may be addressed on a daily basis. The OD seeks feedback from deployed personnel to identify problems and implement appropriate steps toward their resolution through training, education, or the provision of equipment.

The Operations Division acts as a conduit to the AFPMB on contingency issues and is able to consult and coordinate with offices throughout the DoD and among our allies. Thus, the OD is able to assist customers in the rapid receipt of information needed to address their current situation. If a customer requires a piece of equipment or pesticide that is not included in the field set, they should contact the OD (email, letter, etc) and explain the specifics and purpose of the requirement. The Operations Division will then work with the requestor to determine if another item can be used, or if the request represents a new requirement which must be addressed. The OD also needs to know what obstacles a customer encountered during

deployment (ie, what prevented completion of the job). While the OD cannot help with command and control issues, it can provide guidance on how to approach these matters in order to gain command support. If the issue is significant, the OD has points of contact within each service's senior entomological leadership and the combatant commands, and potentially can work the issue through those avenues.

In addition to direct customer support, the OD addresses contingency issues at the DoD level by reviewing new policies and guidance to ensure that there are no unrealistic restrictions placed upon deployed personnel. Contingency operations encompass situations outside day-to-day, installation-level activities, such as armed conflict, disaster relief, and humanitarian support. Such unforeseen events are increasingly common, and each operation brings with it new pest management issues.

Environmental Biology, Invasive Species

The primary focus of the Environmental Biologist is as the liaison with DoD natural resource program managers and coordinator with federal natural resource working groups and the National Invasive Species Council. This working relationship fosters better partnership development among federal, state, and nongovernmental agencies in the management of invasive and nuisance animal and plant species. Through this relationship, the pest management and natural resources program experts are able to discuss areas of mutual interest and share their respective expertise.

The Operations Division works with both the DoD Legacy Program and the Strategic Environmental Research and Development Program to assist in the selection of proposed projects to aid DoD in the better management of invasive species problems that impact its mission and readiness. These issues can range from the control of the brown tree snake on Guam, to management of endangered species habitats on installations, and guidance on the problems of invasive species with regard to retrograde cargo preparation and shipment.

Research Division

The Research Division is managed by the Research Liaison Officer (RLO). The RLO is the principal AFPMB contact with the USDA's Agricultural Research Service and other pest management research programs, and helps to coordinate DoD research requirements for military pest management and vector control. Also, the RLO serves as the Directorate ex officio to the AFPMB Council and committees on all research matters.

The principal activity of the RLO is to administer the Deployed Warfighter Protection (DWFP) research program, a $5 million per year effort to develop new pesticides, new application equipment, and new methods of personal protection. The DWFP was recently profiled in this journal.[5]

Information Services Division

The Information Services Division (ISD) provides DoD customers with timely information on such topics as arthropod-borne disease, pest management, and invasive species. Managed by the Information Liaison Officer, the division also supports DoD through expert consultations and electronic publications retrieval via its online Literature Retrieval System.

The ISD evolved from the Defense Pest Management Information Analysis Center (originally the Military Entomology Information Service) which was established in 1962 as the information and communications branch of the Armed Forces Pest Control Board, the forerunner of today's AFPMB. Reorganized into the ISD in January 2009, it continues to collect, store, analyze, and disseminate published and unpublished information on arthropod vectors and pests, hazardous and invasive organisms, and natural resources and environmental biology that is important to the DoD. Services include technical consultations on vectors, pests, environmental biology, and natural resources; development and distribution of Disease Vector Ecology Profiles that provide summaries of the bionomics of disease vectors and data on hazardous animals and plants for individual countries or biogeopolitical areas (eg, the Middle East); Technical Guides (formerly Technical Information Memoranda) that provide guidance on specific issues of interest to the DoD pest management community (eg, the surveillance and control of ticks that transmit disease); interactive teaching programs in CD (tick morphology) and DVD (mosquito morphology) format; and provision of subject-specific bibliographies, utilizing in-house and proprietary databases. Authorized ISD customers are all DoD medical, pest management, and natural resources personnel, as well as other DoD staff

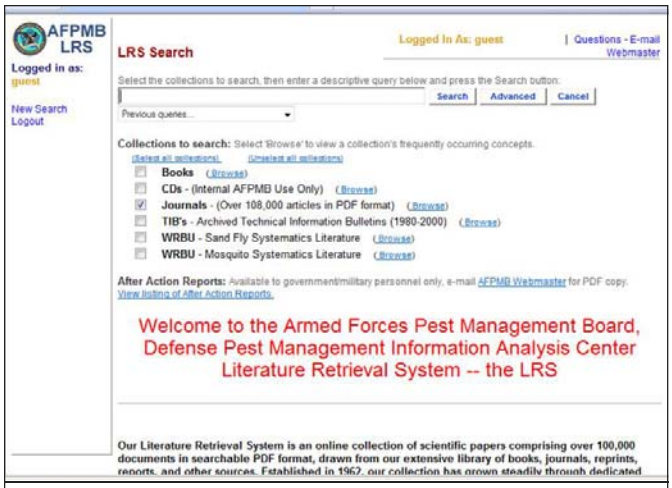

Figure 2. Literature Retrieval System basic search page.

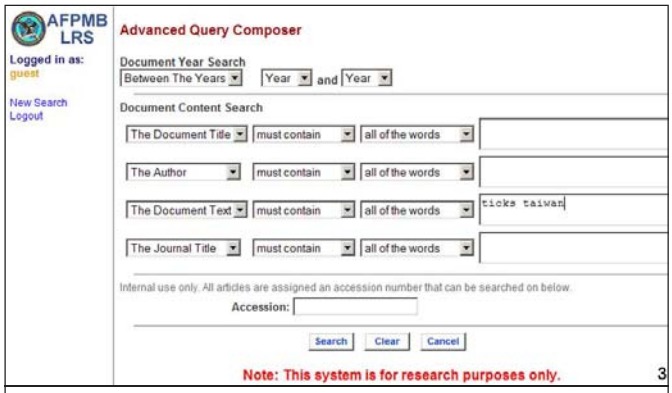

Figure 4. Simple search entries in the query composer.

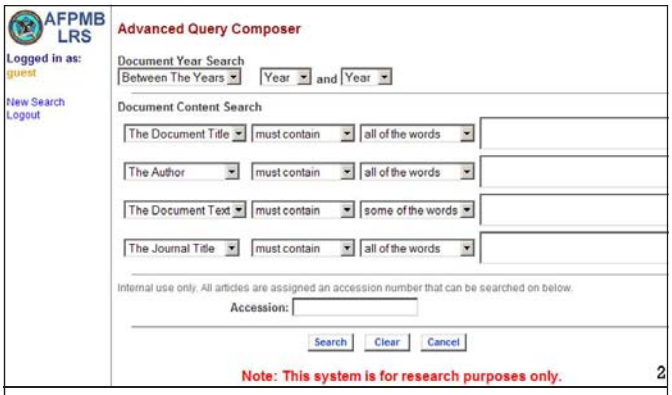

Figure 3. Literature Retrieval System advanced query composer page.

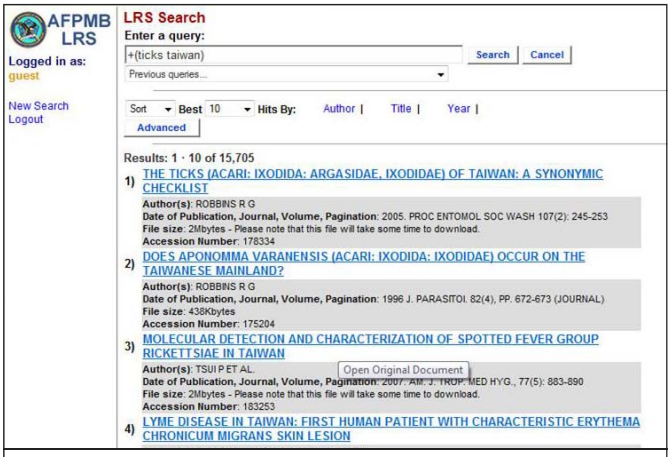

Figure 5. False positives result from simple query terms.

responsible for dealing with problems caused by disease vectors and pests. Also eligible are non-DoD members of the AFPMB, certain personnel of other federal or state agencies, and university faculty associated with the AFPMB.

Since its inception, ISD has maintained a reference library that is now one of the world's chief sources of information on what the late Ralph Audy called "medical zoology."[6] The current library contains a quarter million accessions, of which about 108,000 are available as full-text, digital PDF files, together with some 2,300 books, 100 CDs or DVDs, and active subscriptions to over 60 periodicals in biomedicine, parasitology, and natural resources management.

Central to ISD's mission of information dissemination is its online Literature Retrieval System, a Boolean search engine that formerly provided only bibliographic literature summaries, but, with the advent of the internet, is now capable of supplying copies of all papers (PDF format) accessed by ISD personnel from the serial scientific literature. Users start at the basic search page (http://lrs.afpmb.org/rlgn_app/ar_login/guest/guest), shown as Figure 2, where a journal search is the default option, although the book and CD collections as well as the former (1980-2000) AFPMB Technical Information Bulletin and papers in the online database of the Walter Reed Biosystematics Unit may also be searched, separately or concurrently. While any terms (authors, keywords, words from the title or text of a paper) can be typed into the basic search box, it is almost always preferable to select the Advanced tab which presents the advanced query composer page, Figure 3, where search parameters can be specified by terms in the document title, by author, by terms in the document text, by journal title, and by year range. Effective use of this system is dependent on practice and experience in its use. For example, one might search for papers on

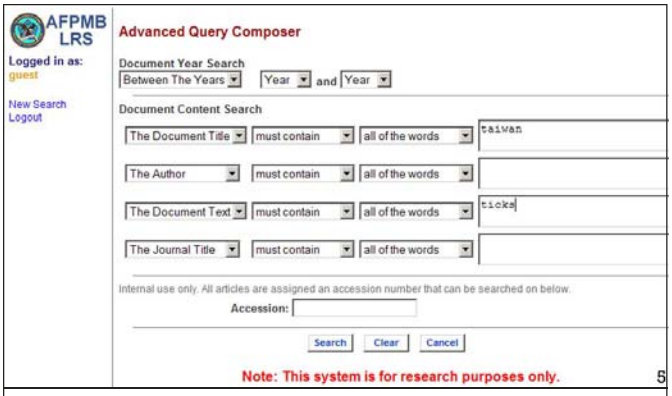

Figure 6. Narrow the query by moving "Taiwan" to The Document Title box to return only those documents focused on Taiwan which discuss ticks in the text.

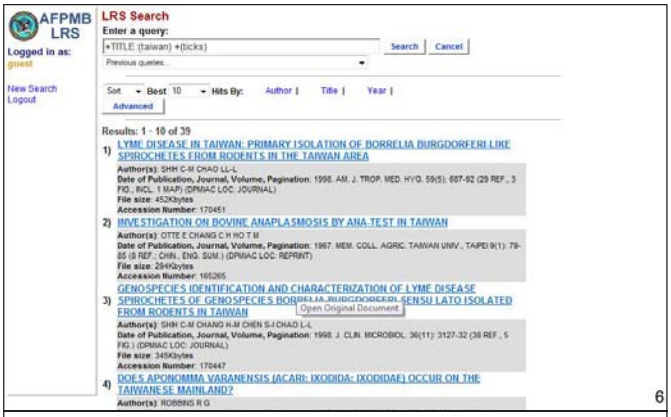

Figure 7. The modified query shown in Figure 5 produces a more manageable list of documents with virtually no false positive returns.

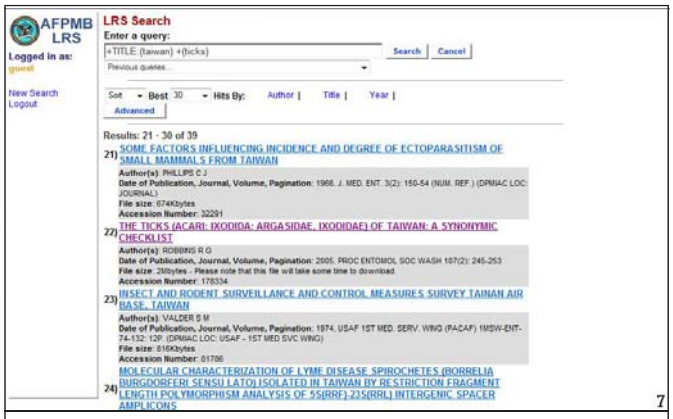

Figure 8. Click the hyperlinked title of a document to download a PDF formatted version.

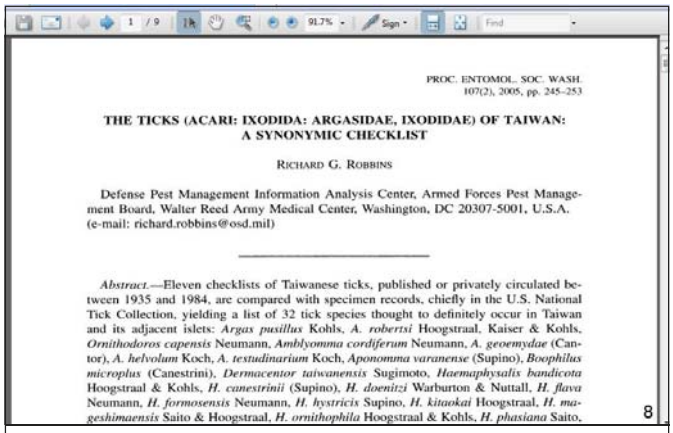

Figure 9. The selected, downloaded document can be saved, forwarded, or printed.

the ticks of Taiwan by typing the words "ticks" and "Taiwan" into The Document Text field (Figure 4) or into the basic search field. However, either method yields an unmanageable (and identical) number of hits, including many false positives (Figure 5). But in such a search it is evident that the focus is on Taiwan, which should therefore appear in The Document Title box, while "ticks" remains in The Document Text box (Figure 6). This strategy yields a much more manageable hit list, with virtually no false positives (Figure 7). Click the paper's hypertext title (Figure 8) to view the entire document (Figure 9), which can then be printed, saved, or emailed.

The Literature Retrieval System is not the only search tool available to ISD customers. Through contracts with ProQuest Dialog (Cary, NC) and Ovid Technologies, Inc. (New York, NY), customers enjoy free online access to such powerful biological databases as AGRICOLA (the National Agricultural Library's catalog and article citation database), BIOSIS (Biosciences Information Service), CAB (Commonwealth Agricultural Bureaux) Abstracts, and MEDLINE (the National Library of Medicine's premier bibliographic database). Hard copies of biomedical papers whose citations are retrieved via these proprietary databases are swiftly delivered (usually on a same-day basis) as web .pdf formatted documents through ISD's DOCLINE® contract with the National Library of Medicine. For nonbiomedical literature, the ISD staff has access to the stacks of the nearby National Agricultural Library and Library of Congress.

Living Hazards Database

Maintained by ISD, the Living Hazards Database (LHD), publicly accessible at the AFPMB's website, contains concise descriptive and bionomic information

on most of the animals that have been reported to cause serious injury or death in humans. The LHD may be searched by species scientific name or by country, and is worldwide in scope, with each venomous animal discussed under the headers Identification, Description, Habitat, Activity and Behavior, and Venom Characteristics. Digital images are included for most of the 500+ listed venomous animals, which serve as aids to initial identification. Because the focus of the LHD is on injury by envenomization, most of the animals depicted are snakes. Birds, mammals, and plants are not currently included in the LHD.

The LHD has been designed to materially assist medical, paramedical, and first-responder teams supporting US military and associated personnel throughout the world. The ISD therefore carefully reviews the LHD several times each year, and updates information and images whenever new material is published or in response to corrections by recognized technical experts.

CONCLUSION

The history of warfare has repeatedly shown that casualties during contingency operations are often caused by diseases, most of which are arthropod-borne. The AFPMB is a force multiplier in the ceaseless campaign against arthropod-borne diseases, providing policies, guidance, research support, and information for US and allied Warfighters worldwide. Concurrently, the AFPMB ensures environmental stewardship and safety through careful management of pesticide products and the control of invasive species. Like the vectors and pests that it was established to combat, the AFPMB is an evolving entity, but one honed by over 50 years of accomplishment to face the challenges of the new century.

ACKNOWLEDGEMENT

We thank our AFPMB colleagues LTC Jamie A. Blow, USA; Peter J. Egan, PhD; and Harold J. Harlan, PhD, for their assistance in preparing the sections on contingency pest management, environmental biology/ invasive species, and the Living Hazards Database, respectively.

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Evaluation of Efficacy and Duration of the Stick Camouflage Face Paint with 30% Deet Against Mosquitoes in Belize

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John Paul Benante
Nicole C. Close, PhD
Nicole L. Achee, PhD

ABSTRACT

A combination of insect repellent, *N,N*-diethyl-3-methylbenzamide (deet), with camouflage face paint in a newly designed stick formulation was evaluated on human volunteers under field conditions in Belize during February 2007. The formulation provided over 90% protection against mosquitoes for 8 hours and at least 80% protection for 12 hours, with 100% protection for 2 to 4 hours after application.

INTRODUCTION

Historically, disease and nonbattle injuries, particularly vector-borne diseases, have resulted in more casualties than have combat operations.[1] The impact of vector-borne disease on military operations is well-documented from the Revolutionary and American Civil Wars.[2] The incidence of these diseases in recent operations[3-8] demonstrates that the threat has not diminished. Currently, the US military has a presence in over 130 countries[9] and is involved in a wide-range of operations, including stability and support operations, small scale contingency operations, and major theater wars. Military personnel remain exposed to a wide range of vectors and vector-borne diseases. The very nature of military operations (rapid deployments, troop movements, fluidity of the modern battlefield) often makes the conduct of area-wide vector control operations impossible. In many tactical situations (eg, assault, bivouac, and entrenchment), vector control measures cannot be instituted, or are insufficient to prevent disease transmission. Thus, personal protective measures (PPMs) have been, and remain the first and best line of defense for military personnel against all vector-borne diseases.

There are many types of PPMs available to military personnel, including permethrin for uniform and bed net treatment, and the Extended Duration Topical Insect and Arthropod Repellent (EDTIAR) with 33% *N,N*-diethyl-3-methylbenzamide (deet) in a polymer formulation.[10] The Department of Defense Insect Repellent System, consisting of a permethrin-treated uniform, EDTIAR on exposed skin, and a properly worn uniform, is a system that, when employed properly, is very effective at protecting Soldiers from vector-borne disease threats.[11] The EDTIAR is the standard military repellent which is designed to be used in all situations for protection against vector threats.

Since concealment is important in many tactical situations, Soldiers use camouflage face paint. Although EDTIAR can be used in conjunction with the standard camouflage face paint, it requires 2 separate applications, an initial application of EDTIAR followed by the camouflage face paint.[12] Recognizing the special needs of tactical situations, the Walter Reed Army Institute of Research (WRAIR) Repellent Program, in collaboration with Amon Re, Inc (Thomasville, Georgia), developed combined camouflage face paint with 30% deet.[13] The addition of insect repellent to the camouflage face paint replaces 2 items with one, saves space, reduces the weight of the individual survival kit, and saves application time.

An Operational Requirements Document (ORD) dated May 28, 1999 (internal use document) was issued to guide development of camouflage face paint combined with 30% deet in both compact and stick form. The compact form, with 5 colors of face paint (black, green, loam, sand, and white) was developed and

tested for efficacy and Soldier acceptability,[14] and endorsed by the Armed Forces Pest Management Board. It was recommended for assignment of a national stock number (6840-01-493-7334) in 2001. Development of the stick formulations to meet the requirements of the ORD began in 2006.

The purpose of the study was to evaluate the repellent efficacy and duration of the stick camouflage face paint formulated with 30% deet (SCFPwD). The study was essential for further advanced development and processing of this product into the military supply system.

MATERIALS AND METHODS

Study Site

Belize (formerly British Honduras) is a Central American country with a geographic area of 22,966 sq km and a population of approximately 290,000.[15] The climate and general ecology of Belize is favorable for year round transmission of malaria. Extensive marshes, swamps, and rivers provide continuous larval habitats for malaria vector species, even during the dry seasons. Malaria incidence is significantly higher in the southern and western provinces of Toledo, Cayo, and Stann Creek than in the northern provinces of Corozal and Orange Walk.[16] The attack rate of malaria is less than 1% per month, concentrated primarily in the south.[17] The study was conducted in the town of San Roman in the northern Belize province of Orange Walk (Figure 1) from February 20 to February 22, 2007. The study site in San Roman (18° 17.8' N, 88° 30.6' W) was located about 100 m from the Rio Nueve (New River) in an open field (approximately 300 m^2) bordered on one side by a semiwooded area. Weather conditions during the 3 nights of collection were seasonal, with an average temperature of 24ºC, 87% relative humidity, and wind speeds about 2 kph.

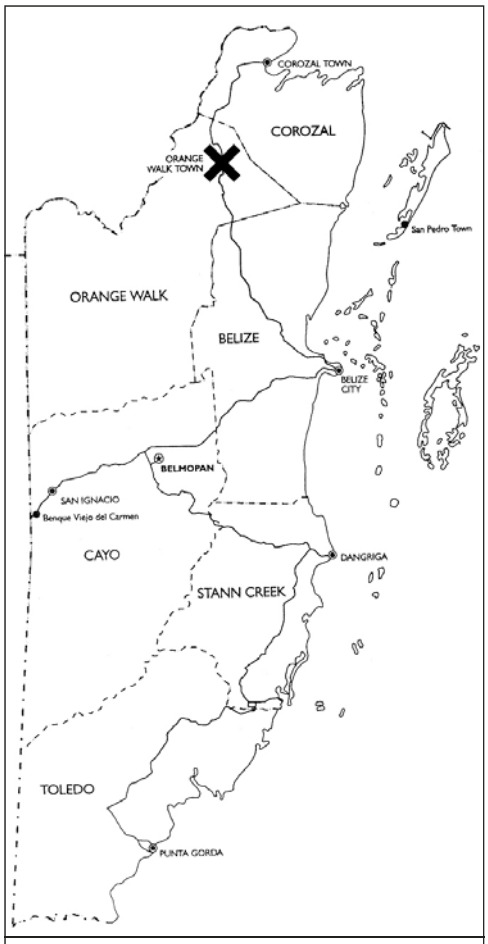

Figure 1. Location of the study site was in the Orange Walk province of Belize in Central America.

Test Material

The SCFPwD is a camouflage face paint in a hardened, tube-shaped form, comprised of the following ingredients: Puresyn ME300, Puresyn 1000 (Polydecene), *N,N*-diethyl-3-methylbenzamide, Multiwax W835 (microcrystalline wax), white beeswax, light candelilla wax, propylparaben, Cab-O-Sil M5 (silica), Thixcin R (trihydroxystearin), Tenox 6 (Zea mays (corn) oil, glyceryl oleate, propylene glycol, BHA, BHT, propyl gallate, citric acid), titanium dioxide, black iron oxides, red iron oxides, yellow iron oxides, chromium oxide greens, ultramarines, and zinc oxide. The manufacturer of this product, Iguana, Inc (Thomasville, GA), received a formulation amendment dated July 11, 2007, from the Environmental Protection Agency (EPA). A formulation amendment is intended for products that are identical or substantially similar to a currently registered product. This product has the same EPA registration number (66306-11) and label as the camouflage face paint with 30% deet in the compact, originally approved on March 27, 2001.

Volunteers

Volunteers in this study were recruited, screened, and enrolled under a human-use protocol reviewed and approved by the WRAIR Institutional Review Board.[18] Volunteers were recruited from the villages of Orange Walk, August Pine Ridge, and San Roman, with the assistance of bilingual field liaisons. Interested parties were briefed on the nature of study participation. Participating volunteers signed informed consent forms prior to any study related procedures in accordance with research guidelines for studies involving humans.[19] Volunteer recruitment, screening, and enrollment occurred February 17 through February 20, 2007.

Study Design and Procedure

All 5 colors of the camouflage face paint with 30% deet were evaluated on human volunteers at 6 time points: 2, 4, 6, 8, 10, and 12 hours postapplication. Peak biting activity occurred between 6 PM and 9 PM; therefore, only 2 mosquito challenges could occur each night, one at 6 PM and one at 8 PM. Three study nights were required in order for each volunteer to be challenged at all 6 time points. Therefore, a staggered application design was employed. Application times were at 8 AM, 12 noon, and 4 PM each day for 3 days. At the start of the study, volunteers were randomly assigned to a treatment group: black, green, loam, sand, or white camouflage face paint (n = 5 per treatment). Each volunteer rotated through all of the treatment times during the course of the study to measure all postapplication time points during peak biting activity.

A 600 cm^2 treatment area from above the ankle to below the knee was determined for each volunteer. Five equally spaced circumference measurements were taken along each lower leg, averaged and divided into 600 to get the length of the exposed area which was defined by an indelible marker on both legs. Each day of the study, the marked area from ankle to knee on one leg was treated with the assigned SCFPwD color and the same marked area on the opposite leg was left untreated to serve as a control. The EPA Product Performance Test Guidelines[19] recommend using between 1.0 g and 1.5 g of lotion or cream product over 600 cm^2 of skin surface area for testing repellents. Using these guidelines, 1.5 g of the SCFPwD was applied evenly on the treatment leg, which was similarly repeated on subsequent nights of testing. Treatment application alternated between the left and right legs of each volunteer every night of the trial to minimize the number of bites on any one leg that served as the control.

From February 20 through February 22, 2007, volunteers performed landing collections at the study site in San Roman starting at 6 PM and ending at 8:30 PM each night. During the mosquito challenges, volunteers were covered (long sleeve shirts, long pants, jackets of Bug-Out® insect barrier material (Bug-Out Outdoorwear, Inc, Cedarville, IA), and footwear) except for the exposed experimental areas (treatment and control) on each leg. All mosquitoes landing in the marked areas of the exposed lower legs were counted and mouth-aspirated for a 20-minute test period or until 20 mosquitoes were counted and collected on the control leg. If 20 mosquitoes were collected from the control leg before the 20 minutes elapsed, the time was recorded and the challenge completed for the volunteer at that challenge point. If mosquito landing rates were low (<20 mosquitoes in 20 minutes), the testing period was extended for an additional 10 minutes. Collected mosquitoes were placed into screen-topped cartons marked with collection data (date, time of collection, collector number, etc). All insects were killed, labeled, and stored for identification. One of the coauthors, Dr Achee, identified the anophelines, and a taxonomist of the WRAIR Biosystematics Unit performed the remaining identifications.

Table 1. Distribution of species across total mosquitoes collected from human volunteers at San Roman, Orange Walk, Belize (20-22 February 2007).

Species	Number Collected	%
Anopheles albimanus	2,742	75.02
Mansonia titillans	243	6.65
Anopheles vestitipennis	137	3.75
Anopheles punctimacula	124	3.39
Aedes scapularis	87	2.38
Anopheles gabaldoni	81	2.22
Culex erraticus	54	1.48
Anopheles darlingi	49	1.34
Psorophora confinnis	33	0.90
Coquillettidia venezuelensis	32	0.88
Anopheles crucians	23	0.63
Culex sp	15	0.41
Culex coronator	11	0.30
Aedes serratus	5	0.14
Psorophora varipes	5	0.14
Psorophora sp	5	0.14
Aedes sp	4	0.11
Culex corniger/lactator	2	0.05
Culex quinquefasciatus	2	0.05
Aedes aegypti	1	0.03
Total	3,655	100.00

Analysis

Data were analyzed by calculating percent protection (PP) of a particular subject, at a particular time point, using a particular camouflage paint stick color. Percent protection was calculated for each volunteer at each time point as: $PP = 100 \times ((LC - LT)/(LRC))$ where LC represents the number landing on the bare skin control and LT the number

landing on the leg treated with SCFPwD. The PP values were not normally distributed; therefore, the arcsine transformation was applied to the calculated PP values to stabilize the variance. The transformed data were then analyzed using a mixed effects model, with both fixed and random effects. Fixed effects were stick color and duration of repellent. A variable representing the association of data within subjects was included to account for the variability of repeated measurements on subjects (random effects). The model was run first with the interaction term: stick color × duration. If the interaction term was not significant, the model was run again without the interaction term. The data analysis was generated using SAS System for XP Pro Platform, Version 9.1.3 (SAS Institute Inc, Cary, NC).

Table 2. Number of mosquitoes landing and landing rate (no. landing/minute) on treatment and control legs at each collection point over three nights of testing in San Roman, Orange Walk, Belize (2007).

		Feb 20	Feb 21	Feb 22	All Nights
6 PM	No. landing (mean ± SE)				
	Treatment	1.88 ± 0.76	2.32 ± 0.67	3.32 ± 1.46	2.51 ± 0.59
	Control	23.04 ± 1.19	22.36 ± 1.39	25.56 ± 1.44	23.65 ± 0.78
	Landing rate (mean ± SE)				
	Treatment	0.09 ± 0.04	0.17 ± 0.05	0.11 ± 0.05	0.12 ± 0.03
	Control	1.08 ± 0.06	1.50 ± 0.06	0.97 ± 0.05	1.18 ± 0.04
	Total landing	623	617	722	1962
8 PM	No. landing (mean ± SE)				
	Treatment	0.80 ± 0.26	3.96 ± 1.67	2.00 ± 0.91	2.25 ± 0.65
	Control	20.88 ± 0.44	21.72 ± 0.84	21.76 ± 0.85	21.45 ± 0.42
	Landing rate (mean ± SE)				
	Treatment	0.08 ± 0.03	0.22 ± 0.07	0.14 ± 0.06	0.14 ± 0.03
	Control	1.62 ± 0.15	1.76 ± 0.13	1.42 ± 0.16	1.60 ± 0.08
	Total landing	**542**	**642**	**594**	**1,778**

RESULTS AND DISCUSSION

Twenty-four male and 2 female volunteers participated in this study. Of those enrolled, 46% were hispanic, 31% mestizo, and 23% other. The mean age of the volunteers was 29.7 years (range, 18-57 years). No adverse reactions or events were reported during the study and there were no deviations from the approved human-use protocol.

During the 3 nights of collection (February 20 through February 22, 2007), over 3,600 mosquitoes from 20 species were collected from human volunteers, with *Anopheles albimanus* Wiedemann constituting the majority (75%) of those collected (Table 1). The number of mosquitoes landing per volunteer was consistent over each night and between challenge points (Table 2). The biting pressure, expressed as landing rate (number landing as a function of time), was also consistent in the control over each night and between challenge points (Table 2). The overall landing rates on control legs, averaged over all 3 nights, were 1.10 ± 0.04 (mean ± SE) and 1.29 ± 0.08 (mean ± SE) at 6 PM and 8 PM, respectively. Thus, the biting pressure under which the study was conducted exceeds that recommended by the EPA.[20]

Percent protection (PP) of the SCFPwD was at least 85%, with 4 of the 5 colors demonstrating an overall PP of at least 90% (Figure 2). Overall PP was the highest for green (0.94 ± 0.03, mean ± SE) and the lowest for sand (0.86 ± 0.04, mean ± SE). Percent protection was near 100% at 2 hours and 4 hours

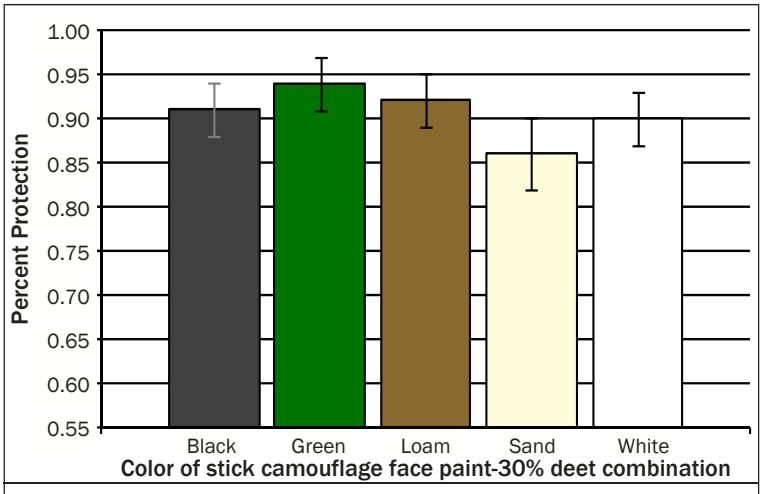

Figure 2. Percent Protection for each of the 5 camouflage face paint stick colors averaged over all 6 postapplication collection time points.

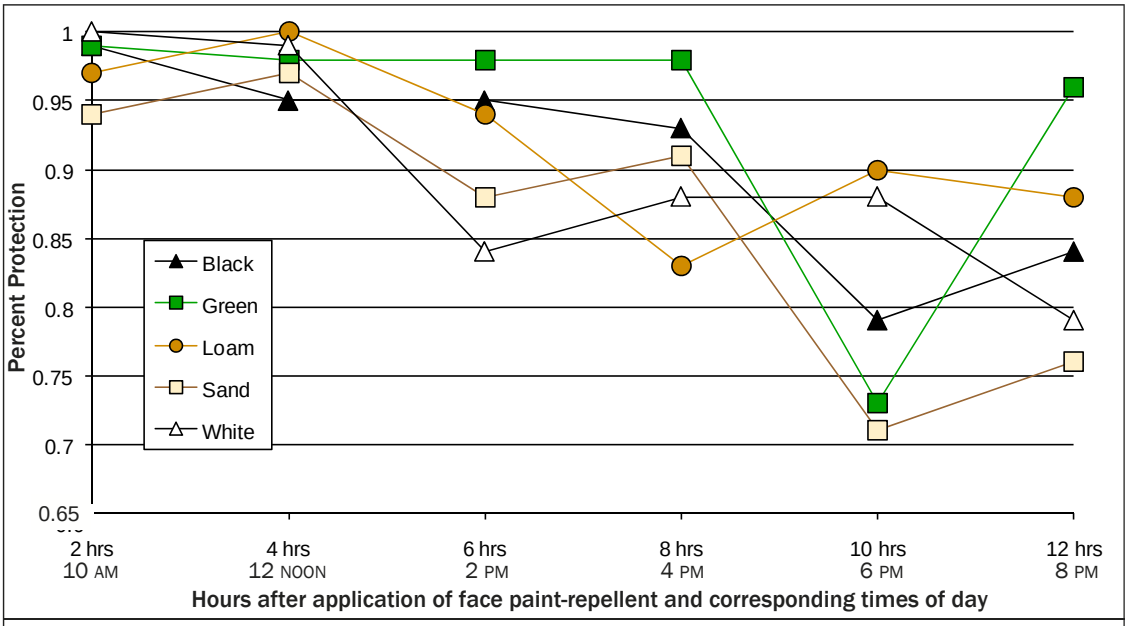

Figure 3. Percent Protection over time of each of the 5 camouflage face paint stick colors.

(Table 3), gradually declining over time (Figure 3) to 0.85 ± 0.05 (mean ± SE) at 12 hours (Table 3). Percent protection was lowest (0.80 ± 0.06, mean ± SE) at the 10 hour time point (Table 3) due to a decrease for 3 of the colors, as shown in Figure 2. A decrease in PP over time is expected as the product's effectiveness declines; however, a more marked decrease in only 3 of the colors (green, sand, black) was unexpected. Furthermore, PP increased for those 3 colors at the 12 hour time point (Figure 2) and overall PP increased to 85% (Table 3). This decrease and subsequent increase in PP could be explained by the fact that all 10-hour challenges took place at 6 PM, the very beginning of the activity period. Landing rates on the controls were slightly lower at 6 PM than at 8 PM on all 3 nights (Table 2). Therefore, as the repellent wears off or loses effectiveness over the course of the day, small differences in landing rates, such as seen at 10 versus 12-hour challenges, will be more obvious or amplified giving the appearance of a possible interaction between the 2 fixed effects, color and duration (time). However, in the mixed effects model with the interaction term, duration was the only significant factor ($F_{5,120} = 5.44$, $P < 0.001$) while neither color ($F_{4,120} = 1.26$, $P = 0.29$) nor duration × color ($F_{20,120} = 0.53$, $P = 0.95$) were significant (Table 4). When the model was run without the interaction term, duration was again highly significant ($F_{5,140} = 4.54$, $P < 0.0001$) and color was not significant ($F_{4,140} = 1.35$, $P = 0.25$) (Table 4).

Table 3. Number of mosquitoes landing on treatment and control legs and percent protection averaged over each postapplication collection timepoint.

Time*	N†	Variable	Mean ± SE
2	25	Control	23.28 ± 1.51
		Treatment	0.44 ± 0.18
		Percent Protection	0.98 ± 0.01
4	25	Control	20.40 ± 0.28
		Treatment	0.44 ± 0.15
		Percent Protection	0.98 ± 0.01
6	25	Control	23.08 ± 1.33
		Treatment	2.20 ± 0.77
		Percent Protection	0.92 ± 0.02
8	25	Control	21.48 ± 0.64
		Treatment	2.12 ± 0.92
		Percent Protection	0.91 ± 0.04
10	25	Control	24.60 ± 1.25
		Treatment	4.88 ± 1.48
		Percent Protection	0.80 ± 0.06
12	25	Control	22.48 ± 1.03
		Treatment	4.20 ± 1.66
		Percent Protection	0.85 ± 0.05

*Number of hours after application of face paint
†Number of volunteers who participated

The new SCFPwD meets the threshold specifications of the ORD, to provide a minimum of 8 hours of protection. It provided maximum protection for 4 hours and substantial protection for 8 hours when tested against the malaria vector *Anopheles albimanus* under field conditions in Belize. Data collected during this field study,

Table 4. The results of the mixed-effects model ANOVA run with and without the interaction term.

Effect	*df*	F-value	*P*
Model with Interaction			
Duration	5,120	5.44	0.0002
Color	4,120	1.26	0.2905
Duration × Color	20,120	0.53	0.9491
Model without Interaction			
Duration	5,140	5.84	<0.0001
Color	4,140	1.35	0.2551

along with a laboratory study with mosquitoes and sand flies and Soldier acceptability surveys, will be compiled and submitted to the Armed Forces Pest Management Board for recommendation for a national stock number. Upon approval, the product, shown in Figure 5, will be available to all military personnel as part of the personal protective measures used to protect against arthropod bites.

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The SCFPwD used in this study was supplied by Iguana, Inc at no cost to WRAIR.

Figure 5. Tubes of the camouflage face paint with 30% deet product used in the study.

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Comparison of the Attractiveness of Organic Infusions to the Standard CDC Gravid Mosquito Trap

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ABSTRACT

Field experiments were conducted in southeastern Texas in 2008 to compare the attractiveness of selected gravid-trap infusions to ovipositing female mosquitoes. Comparisons were made among the following infusions: Bermuda grass, oak leaves, acacia leaves, rabbit chow (alfalfa pellets) and green algae. Experiments were conducted at 6 trap locations in Fort Sam Houston military reservation in San Antonio, Texas. Four (Bermuda grass, acacia leaves, oak leaves, and algae) of the 5 infusions were effective in collecting *Culex quinquefasciatus, Cx nigripalpus,* and *Cx erraticus*. However, Bermuda grass attracted the greatest numbers of the mosquito species. *Aedes albopictus* female mosquitoes were collected in moderate numbers during this study; however the infusions were not determined to be significantly different from one another in their attractiveness for this species.

INTRODUCTION

Mosquito-borne pathogens are generally acquired from vertebrate hosts and transmitted by blood-feeding female mosquitoes. Since pathogen acquisition and subsequent disease transmission require at least 2 blood meals and one oviposition cycle to be completed, screening gravid female mosquitoes for virus infection provides the best estimate of the infected population.[1] Multiple techniques and methods are used to conduct mosquito surveillance, such as incandescent light traps, ultraviolet light traps, electric nets, CO_2 traps, counterflow traps, human bait counts, oviposition traps, and Centers for Disease Control and Prevention (CDC) gravid traps. Of all the mosquito surveillance traps and methods, the CDC gravid trap is the only one that specifically targets and attracts gravid females.[2] Therefore, the likelihood of arbovirus detection significantly increases when using this trap. According to some surveillance studies, collections from gravid traps were estimated to contain from 57%[3] to 95%[4] gravid females. The CDC gravid trap, shown in Figure 1, consists of a plastic rectangular basin that holds an infusion of organic matter that is attractive to some species of ovipositing mosquitoes. A vertical hollow cylinder with a battery-powered fan is mounted approximately 2.5 cm over the infusion. The action of the fan creates an upward, unidirectional air flow that vacuums the mosquitoes to the top of the tube and into a collection net.

Figure 1. The CDC gravid trap configuration (with battery) used in the study.

Both laboratory and field studies have shown that gravid mosquitoes exhibit significant preferences when selecting aquatic sites for oviposition.[5,6] The physical characteristics such as color, substrate texture, odorants, and chemical cues were some of the factors identified to influence oviposition.[7] Additional studies have indicated that gravid females of different species used different factors for acquisition and selection of oviposition sites. For example, *Aedes albopictus*

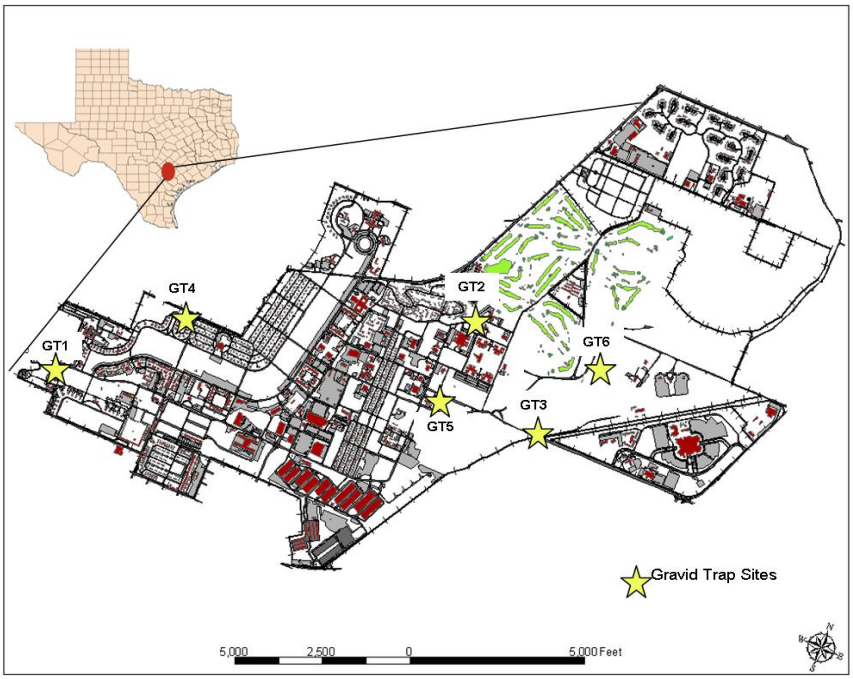

Figure. 2. Location of the study area (Fort Sam Houston, Texas) and the 6 sampling locations of the mosquito gravid traps (Sep 3 to Nov 22, 2008).

(Skuse) females exhibited a strong preference to oviposit in artificial containers such as tires. Organic matter dissolved in water has been found to attract different mosquito species.[8] *Aedes aegypti* (L) females were attracted to horse manure infusions[8] and *Aedes triseriatus* (Say) females to tree-hole water.[9] Infusions made with materials such as hay,[10] grass,[11] alfalfa pellets, bulrush,[12] manure,[8] and oak leaves were reported as attractive substrates for *Culex* species. The purpose of this study was to compare the relative attractiveness of several organic infusions to different mosquito species in southeastern Texas.

Materials and Methods

Study Site

The field study was conducted at six locations on Fort Sam Houston military reservation (29.42°N, 98.49°W, 195 m elevation) (Figure 2). Fort Sam Houston is a US Army base located in the northeast of San Antonio, Texas. The installation comprises approximately 3,000 acres and has a population of 35,000 people. Mosquito activity was prevalent throughout the installation from July to October 2008.

Trapping Techniques

A total of 6 mosquito gravid trapping sites were selected for this study (Figure 2). Trap site one was placed on the west side of the installation near the military horse stables adjacent to an active creek that is surrounded by urban terrain (residential). Trap two was placed in the northeast side of the installation near a low lying drainage channel that divides two urban communities. Trap three was placed near a large creek located on the edge of a recreational picnic area. Trap four was placed next to a house in a populated neighborhood, which had no immediate proximity to water. Trap five was placed at the edge of the wood line near an active athletic complex. Trap six was placed next to a tree near the golf course. The location of each trap was not less than 900 m from other traps. The traps used for this study were CDC gravid traps (model 1712, John W. Hock Company, Gainesville, FL). The tray of each gravid trap was filled ¼ full with a different infusion bait (rabbit chow, Bermuda grass, live oak leaf *Quercus virginiana* (Mill), acacia leaf *Acacia schaffneri* (Wats), and filamentous green algae). All infusions except the green algae were prepared using 227 g of substrate added to 4 L of tap water. Each mixture was placed in closed Rubbermaid (Newell Rubbermaid Inc, Atlanta, GA) plastic containers and left outside in the sun to incubate for 5 days prior to each trap week. The algae was collected from fresh water ponds and was incubated at outdoor temperatures (27°C - 29°C) for 7 days prior to each trapping week. The green algae infusion consisted of 133 g of wet algae mixed with 4 L of pond water. Trapping was conducted 3 days per week from September 3 to November 22, 2008. The trapping cycle was divided into 2 experiments. Experiment one was conducted at locations one, two, and three during the first 6 weeks and experiment two was conducted at locations four, five, and six during the last 6 weeks. Trapping sessions commenced at 8 AM on Wednesday and ended 8 AM on Saturday. After the completion of each trap week, each infusion was rotated to a different trap site to ensure each infusion was tested once at each trap location. All of the mosquitoes collected were counted and identified to species using the morphological keys by Darsie and Ward.[13]

Statistical Analysis

All data were analyzed using Stata release 10 for Windows (StataCorp LP, College Station, TX). Collection data was converted using the logarithmic scale. Counts of zero were replaced with a value of 0.5 before taking the natural log. Histograms and normal quantile plots of the log counts were examined and no substantial deviations from normality were observed. Log-transformed counts of female mosquitoes collected by gravid traps were analyzed by 2-way analysis of variance (ANOVA) for variation among the most significant infusions, after adjusting for species. Multiple comparisons among infusions were made using Tukey HSD (honest significant difference) test at a 95% confidence level. A 3-factor ANOVA was also conducted to determine if the different trap sites affected the species of mosquitoes and the different infusions.

RESULTS

A total of 2,003 females of 14 species of mosquitoes in 3 genera (*Aedes, Culex, Orthopodomyia*) were collected. The Table shows the mosquitoes that were attracted to the 5 different infusion types. The most common mosquitoes collected were *Culex quinquefasciatus* Say (35%), *Cx erraticus* Dyar and Knab (34%), *Cx nigripalpus* Theobald (20%), *Cx interrogator* Dyar and Knab (6%), and *Aedes albopictus* (3%). The average numbers of *Ae albopictus* and *Cx interrogator* collected were significantly lower than those for the other species. Most of the mosquitoes collected (51%) were from traps containing the Bermuda grass infusion. Based on Tukey's multiple comparison procedure, traps baited with Bermuda grass ($F = 23.57$, $df = 3$, $P < 0.0010$) had a significantly higher number of mosquitoes than the other infusion types tested. Following Bermuda grass (51%), oak leaves (18%), algae (15%), and acacia leaves (12%) attracted the bulk of the remaining mosquito population. However, these 3 infusion types were not significantly different from each other. Of the 3 genera represented by the collected mosquitoes, 96% were *Culex*. Fifty-three percent of the collected *Culex* species were attracted to traps containing Bermuda grass infusion. Rabbit chow infusion collected the least number of *Culex* species (4%). The genus *Aedes* represented 4% of the total mosquitoes collected. Most of the *Aedes* (39%) were collected by acacia leaf baited infusions. Traps baited with rabbit chow infusions attracted the least number of *Aedes* (3%).

The total number of mosquitoes collected for each infusion by experiments one and two is presented in Figure 3. Experiment two had significantly lower numbers compared to experiment one. Bermuda grass infusion performance was still superior over all other infusions during this period. Averaging across species and sites, there was a significant main effect of infusion in both experiment one ($P = 0.0017$) and experiment two ($P < 0.0001$). In both experiments, the Bermuda grass infusions attracted the highest number of mosquitoes. In experiment one, the algae and oak infusions attracted nearly as many mosquitoes as the Bermuda grass infusion, and the differences among these infusions were not statistically significant. The Bermuda grass infusion attracted significantly more mosquitoes than the acacia infusion in experiment one ($P = 0.0025$). In experiment two, significantly more mosquitoes were collected using the Bermuda grass infusion than any of the other infusions ($P < 0.001$ for all comparisons). There was a significant site to infusion interaction in both experiment one ($P = 0.0070$) and experiment two ($P = 0.0074$), indicating that the differences among infusions varied by site. Overall, in all sites, except site two (experiment one), the Bermuda grass infusion attracted the highest number of mosquitoes. After adjusting for multiple comparisons, the differences between Bermuda grass

Distribution by species of female mosquitoes collected by 5 different infusion types during 2 trapping occasions in 2008 (Sep 3 to Oct 11, Oct 15 to Nov 22) at Fort Sam Houston Military Reservation, Texas.

Mosquito Species	Infusion Type				
	Bermuda	Rabbit Chow	Acacia	Oak	Algae
Aedes aegypti	0	0	0	1	1
Aedes albopictus	6	2	26	8	17
Aedes triseriatus	0	0	0	2	0
Aedes vexans	3	0	2	2	0
Aedes zoosophus	1	0	0	0	0
Aedes atropalpus	0	0	0	1	0
Culex coronator	0	0	0	0	1
Culex erraticus	450	26	60	79	64
Culex interrogator	38	3	19	42	24
Culex nigripalpus	198	24	43	63	76
Culex quinquefasciatus	336	30	84	154	107
Culex salinarius	0	0	4	0	3
Culex tarsalis	0	0	0	2	0
Orthopodmyia alba	0	0	0	0	1
Total	1,032	85	238	354	294

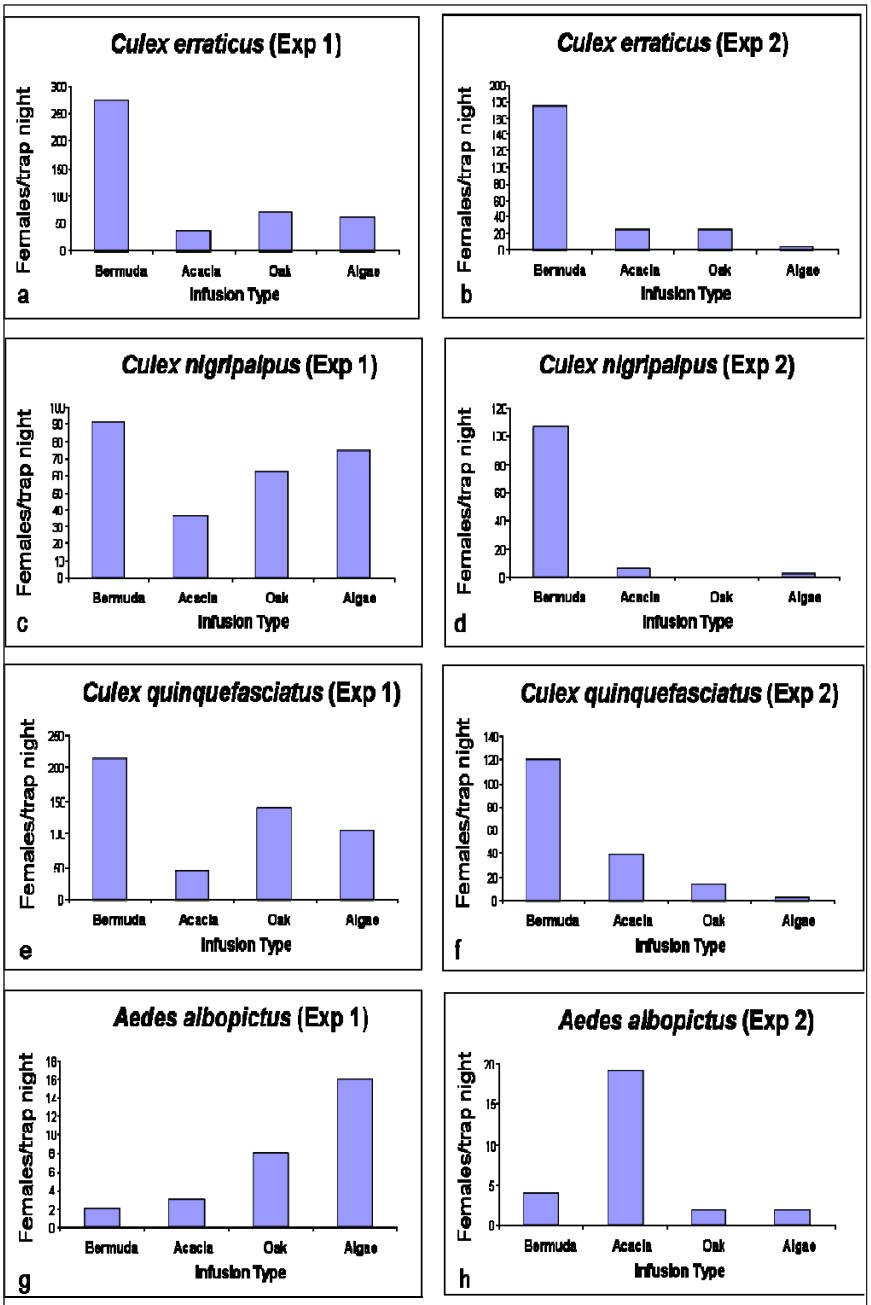

Figure 3. Total numbers of *Cx erraticus* (a, b), *Cx nigripalpus* (c, d), *Cx quinquefasciatus* (e, f), and *Ae albopictus* (g, h) female mosquitoes collected by gravid traps with 4 different experimental infusions during 2 trapping occasions: experiment one (Exp 1), Sep 3-Oct 11, 2008, and experiment two (Exp 2), Oct 15-Nov 22, 2008.

and the other infusions were not statistically significant in sites one, two and three, but were significant ($P < 0.05$) in sites four, five, and six (experiment two). Significant species to infusion interaction was observed in experiment two ($P = 0.0024$) but not in experiment one ($P = 0.0712$). This finding suggests that the relative effectiveness of the 4 (Bermuda grass, oak leaves, acacia leaves, and algae) infusions was similar for all species in experiment one. In experiment two, after adjusting for multiple comparisons, there was no significant difference among infusion with respect to the number of *Ae albopictus* mosquitoes attracted. The Bermuda grass infusion attracted significantly more of both *Cx erraticus* and *Cx nigripalpus* than any other infusion, and attracted significantly more *Cx quinquefasciatus* than any infusion except acacia.

Discussion

Our data supports reports from several other studies that Bermuda grass infusion is highly attractive to *Cx quinquefasciatus*.[2,7,14,15] The Bermuda grass infusion baited trap collected 2 to 3 times more *Cx quinquefasciatus* than all of the other infusions. Over half of the *Culex* species collected in this study were in the traps that contained Bermuda grass infusion. The Bermuda grass infusion also attracted more *Cx nigripalpus* and *Cx erraticus* mosquitoes than the other infusions. In fact, the numbers of *Cx erraticus* collected with the Bermuda grass infusion were higher than the numbers of *Cx quinquefasciatus*. Burkett-Cadena and Mullen[16] reported, in contrast to our results, that small numbers of *Cx erraticus* were collected with gravid trap, and inferred that the gravid trap was not a useful tool in collecting females of *Cx erraticus*. In their study, they used oak leaves, pine straw, red (dyed) hardwood mulch, and composted manure to make 4 different infusions. Similar to their results, the traps baited with oak leaf infusion used in our study collected modest numbers of *Cx erraticus* (Figure 3). Therefore, it is likely that the low numbers of *Cx erraticus* collected in their study were impacted by the selection of infusions. The infusions selected

for this study collected low numbers of *Cx coronator* Bayer, *Cx interrogator*, *Cx salinarius* Coq. and *Cx tarsalis* Coquillett. With the exception of *Cx interrogator*, they all were commonly collected (in the vicinity of the trap sites) in moderate numbers using the CDC standard light trap. It is possible that the age of the infusions impacted the collection of these common species. Isoe et al[17] showed that *Cx tarsalis* and *Cx quinquefasciatus* attraction to Bermuda grass infusion was affected by age. They suggested that *Cx tarsalis* preferred relatively clean water for oviposition sites, whereas *Cx quinquefasciatus* preferred more polluted and turbid waters. The oak, algae, and acacia infusions used in this study were the least turbid. *Culex coronator*, *Cx salinarius* and *Cx tarsalis* were only attracted to these 3 infusions. After the Bermuda grass infusion, the oak leaf infusion attracted the second highest number of mosquitoes. In addition, the oak leaf infusion attracted 11 different mosquito species, while the Bermuda grass infusion attracted 7 species.

Aedes albopictus was the only non-*Culex* mosquito species collected by all 5 infusions used in this study. Overall, the numbers of *Ae albopictus* were low. However, their presence in the traps was common when compared with other *Aedes* species collected during this study. Excluding the algae infusion, the acacia infusion attracted 2 to 3 times more *Ae albopictus* than the other 3 infusions. However, the mean number was low. This lack of preference for a certain type of infusion is most likely due to their oviposition behavior. Mogi and Mokry[18] referred to this behavior as skip oviposition. This occurs when female mosquitoes lay their eggs in several containers as opposed to laying their entire clutch in one container.[19-21]

The rabbit chow infusion yielded the lowest results from all the infusions. Similar reports of low responses to this infusion were reported by Isoe and Millar,[15] Allan et al,[14] and Jackson et al.[22] In contrast to the other infusions, the rabbit chow infusion attracted significant numbers of flies (Calliphoridae). It is possible that the attraction of these flies was antagonistic to the trapping efficiency. The high levels of calliphorid flies around the traps created an intense situation in which the flies were fighting over the oviposition media. The trapping location that had the most significant issues with the flies was the one at the horse stables. Ironically, the rabbit chow attracted the least amount of mosquitoes at this location.

In conclusion, Bermuda grass, acacia leaves, oak leaves, and algae infusions were effective as gravid trap baits for the attraction of *Cx quinquefasciatus*, *Cx nigripalpus*, and *Cx erraticus*. Additional studies focusing on the concentration of organic material and age of these infusions might show how to increase attraction rates of low density gravid trap species such as *Ae albopictus* and *Cx tarsalis.*

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