Beating the Dengue Carrying *Aedes aegypti* Mosquito at its own Game

Success of mosquitoes as insect vectors of human disease is linked to their ability to seek out, pin-point and target human hosts in preference animal hosts, achieved by strong insect attraction to specific natural profiles of volatile chemicals in sweat excreted on to the skin surface of the human body.

AgriSense-BCS (United Kingdom) and BioGents GmbH (Germany) have used these natural phenomena to develop novel technologies for monitoring and controlling mosquito vectors and their deadly 'cargoes' like dengue fever. Highly specific chemical attractants of human origin were used to develop a commercial lure/dispenser for use in a unique insect trap, custom designed according to the intrinsic behavioural pattern of the target insect. The system is already providing an accurate and efficient monitoring of mosquito populations, by playing and beating these blood-sucking insects at their own game.

The lure is the result of years of research at the University of Regensburg in Germany, based on a specific blend of chemicals identified in human sweat and therefore highly specific for anthropophylic (human biting) insects. The attractant blend is contained in commercial lure/dispenser designed and produced by AgriSense BCS in South Wales. The lure/dispenser is designed for custom use in the BG-Sentinel trap, developed and produced by BioGents to mimic convection of volatile chemicals from a sweat-laden human body. Both companies are research-based with decades of experience in the design, development and deployment of insect attractants (lures) and custom-designed traps for insect pest monitoring and control.

Aedes aegypti – the most prolific vector of the arboviruses causing dengue and yellow fever (picture courtesy USDA - United States Department of Agriculture)

Focus on the Aedes Mosquito

Most research has been carried out with *Aedes*, a medically-important mosquito genus belonging to the Culcini. The group includes some of the most mobile, versatile and potentially lethal species of mosquito found anywhere in the world. They carry, transmit and spread the most notorious and lethal arboviruses (viruses carried and transmitted by insects) known to man. Assisted by international trade and travel and nurtured by global warming *Aedes* mosquitoes are moving into and surviving in hitherto uncongenial and, therefore, unexploited regions of the world.

tropical rainstorms leave plenty of sites for mosquito breeding

There are hundreds of different species of *Aedes* from those with simple nuisance status for tourists to others which vector life-threatening disease. *Aedes aegypti* with its traditionally tropical distribution stands head and shoulders above the rest as a vector of human disease. *Ae. aegypti* is the most important vector of the arboviruses causing yellow fever and dengue fever. The latter is now considered to be the most health (life) threatening and economically important arbovirus in the world, and responsible for an increasingly large number of deaths particularly in Asia and South America.

Ae. aegypti breeds primarily in man-made containers like earthenware jars, metal drums and concrete cisterns used for domestic water storage, as well as discarded plastic food containers, used automobile tyres and other items that collect rainwater. Such is the seriousness of Dengue carrying mosquitoes in Brazil that use of water containers to support fresh flowers in cemeteries and grave yards is banned.

Aedes mosquitoes are highly adaptable breeders, seeking out and selecting clean and fresh or only slightly polluted water, including puddles left after rainfall and in which eggs can survive when the puddle dries up. The day-time feeding behaviour of the female *Aedes* mosquito heightens and broadens health risks compared with night-time feeders like Culex pipiens, the so called 'house' mosquito, primarily responsible for spread of West Nile Virus (WNV) across the United States of America. Day-time feeding by *Aedes* makes this mosquito the ideal subject for the research and development into new insect attractants and trapping systems for pest monitoring and control.

Different methods of monitoring

A range of methods have traditionally been used to assess mosquito numbers and associated health hazards and risks, though few are sufficiently and universally sensitive for the level of accuracy required. Assessments are made using counts and records of larvae and pupae at breeding sites, or adults by searching homes and dwellings and vacuuming up all mosquitoes using special-back pack aspirators. This may prove accurate for species that stay in the dwelling after taking a blood meal, but the system will also 'suck up' nuisance species of no medical importance.

Custom-designed 'traps' that lure and encourage gravid females to lay eggs on or inside the trap surface is a major step forward, but there is no way of knowing which mosquito species has laid the eggs without laboriously hatching and identifying the larvae. The only way to accurately assess the real potential for disease transmission both temporally (in time) and spatially (in place) is to target adult female mosquitoes as they seek out human hosts for a blood meal. The air-borne adult female mosquito seeking a blood meal is a 'ravenous' beast, its 'single-minded' purpose achieved through attraction to the desired hosts. Mosquitoes take blood meals from the widest range of animals, including mammals and birds, but humans are invariably the preferred host.

Human volunteers are used as 'living, breathing, sweating' landing/collection sites for host seeking female mosquitoes. In doing so they are literally sacrificed to blood sucking mosquitoes and perhaps disease, through spending several hours in a test area and catching all female mosquitoes that land on their skin. Apart from being highly questionable, indeed unethical, the method cannot be properly standardised because different individuals have different capacities to attract mosquitoes.

Reservations about using the human body for such a 'landing/collection' system have been overtaken by availability of attractants and lures based on actual chemical components of human perspiration (sweat), and deployed in a trap that mimics release of volatile sweat chemicals from the human body.

New attractant and trapping system

Twin centre-pieces of this novel and commercially operating system are the lure/dispenser with its crafted blend of human sweat components for anthropophylic (human blood sucking) insects, and the unique and novel custom-designed trap. Components of the attractant were researched at the University of Regensburg in Germany and the controlled release technology for the commercial lure/dispenser developed by AgriSense-BCS.

AgriSense lure for the BG Sentinel trap

Chemicals contained in human sweat and deposited on human skin surfaces were identified and assessed for mosquito attractant property and potential. Researchers used the 'Y' tube bioassay system ('choice olfactometer) to determine the role and effect of these chemical factors in the chemical and sensory ecology of the mosquito species under test. Through sequential activation, upwind flight and odour choice, the research team were able to identify the chemicals and then copy and craft the most potent attractant blend for use in a commercial lure.

Emanations from vertebrate hosts including humans comprise complex blends of chemicals. Odour blends rather than individual constituent chemicals were shown to attract *Ae. aegypti*. The most highly attractive blends consisted of L-(+)-lactic acid, ammonia and specific fatty acids which were essential to maximise the attractant properties of the blend. In a direct competition bioassay one distinct blend was shown to be as attractive as an average human hand.

Some human odour samples were less attractive than others but addition of lactic acid significantly increased their attractiveness. While there was almost no response by *Ae. aegypti* to natural animal odour samples addition of lactic acid made these animal samples as attractive as natural human odour samples. Lactic acid as a component of the blend is the single most important factor in attraction of mosquitoes to human odour samples. Carbon dioxide was not essential to effectiveness of the blend but enhanced catching rate through synergism by instant sensitisation of *Ae. aegypti* to human skin odours. Water also displays synergistic effects.

The commercial lure produced by AgriSense is custom-designed (physically and chemically) as the attractant dispenser for deployment in a new suction trap designed, commercialised and patented by BioGents. The appropriately named 'BG Sentinel' trap mimics convection currents created and generated during heat exchange by the human body, as well as displaying attractive visual (black and white) clues, and releasing attractant chemicals from the lure over an extensive surface area.

An integral fan blows volatile chemicals upwards through the fabric gauze on top of the trap in a convection-like current, thus mimicking scents continually emitted by sweat on the skin surface of the human body. Any mosquitoes following the scent trail are visually attracted to the edge of the black suction column centrally placed in the top of the trap and surrounded by the white fabric gauze. Insects are subsequently forced into a net inside the trap by the downward suction forces generated by the trap's fan.

Application to Aedes and Dengue

Aedes turned out to be an easy 'lab animal' to work with. Day-flying eliminates the need to use the red light techniques required to observe and monitor activity of night-flying species like Culex and Anopheles. But this is not the reason why these researchers selected Aedes as prime target for monitoring and control of arthropod vectors of human disease.

Dengue in tandem with the Dengue Haemorragic Fever (DHF) complication is a killer disease, currently on the rampage across huge swathes of tropical and sub tropical Asia, Africa and America. The primary vector of Dengue is Ae. aegypti a predominantly urban species exploiting huge numbers of people crowded together in Third World city slums who are at continual risk. Preference for breeding indoors and requiring a minimum of fresh water, whether from leaking taps or other sources, aggravates already high levels of disease transmission. Disease incidence is highest during periods of high temperature and high rainfall. Evidence suggests the vector is continuing to adapt to its preferred urban environment by now using polluted water sources for breeding if a fresh water source does not exist.

Many other mosquitoes leave a raft of eggs in one pool of water but female *Ae. aegypti* hedges her bets by laying her eggs 'little and often' at many different sites. Other mosquitoes tend to feed from just one animal or human but female Ae. aegypti takes blood meals from several or more different hosts and has the potential to infect up to 10 people with Dengue. These factors also contribute to the status of *Ae. aegypti* as the primary vector of Dengue and DHF.

There are four different virus serotypes (DEN 1, DEN 2, DEN 3 and DEN 4) that cause Dengue. Recovery from one confers life long immunity to that serotype but only partial and temporary protection against the other three. What's more there is good evidence to suggest sequential infection increases the risk of contracting the more serious disease resulting in DHF.

Dengue is endemic in more than 100 countries spanning South East Asia and Western Pacific, Middle East and Eastern Mediterranean, Africa and the Americas, putting more than 2500 million people at risk. The WHO (World Health Organisation) estimates there could be as many as 50 million cases each year. Not only is the disease spreading into new areas and putting more people at risk, but is occurring in explosive outbreaks. The 609,000 cases (of which 15,000 were DHF) reported throughout the Americas during 2001 represented a doubling of cases over 5 years.

Dengue swept through South East Asia in 2007, despite aggressive attempts to control both vector and disease. It caused the worst ever outbreak, surpassing that in 1998 when 750,000 cases were recorded. Dengue is traditionally regarded as a disease of the poor and poverty stricken neighbourhoods but no country escaped the 2007 outbreak.

Singapore and Malaysia were fighting the disease alongside Laos and Cambodia. By August 2007 Indonesia had already recorded 100,000 cases and 1000 deaths, with Jakarta predicting 200,000 cases compared with 114,000 in 2006. Malaysia suffered a 50% increase in cases (56 deaths) with 1000 people infected weekly during June and July 2007. Vietnam, which traditionally suffers high rates of dengue, had recorded 33,000 (32 deaths), representing a 40% increase on 2006. Singapore has one of the lowest fatality rates in the region but 3,000 plus cases in 2007 translates into one of the highest infection rates on a per capita basis.

Australia has eliminated previous Dengue outbreaks in the northern tropical region around Cairns, but continues to be concerned about re-introduction given record numbers of cases elsewhere in the Asia Pacific Region. Particular concern was voiced in 2008 following renewed outbreaks in island nations around Australia. This led Dr Kevin Palmer the WHO representative in Samoa to claim money spent trying to stop a pandemic of the H5N1 subtype of highly pathogenic avian influenza (HPAI) in the Pacific would be better spent fighting the actual pandemic of Dengue Fever.

"There has been hundreds of millions of dollars spent on bird flu (avian influenza) and bird flu pandemic preparedness. Bird flu [pandemic] is something that hasn't happened and probably won't happen," he said. The Pacific is experiencing a dengue pandemic, and it would require only a very small amount of funds to really make a difference. This is a pandemic that is real, it is here, it is now, and having a direct effect on the health and the economy of the people that it has hit," Palmer said.

Fiji authorities said 966 people were affected by dengue in September 2008, causing 118 people to be treated in hospital. Kiribati had recorded 831 diagnosed cases, New Caledonia 866 and Samoa 427 by September 2008. There were also serious outbreaks of dengue fever in French Polynesia, the Cook Islands and elsewhere. Palmer pointed out how the numbers of people affected with avian influenza (compared with Dengue Fever) were miniscule.

Dengue has been described as an enigmatic virus, difficult to diagnose and impossible to quarantine. Symptoms and antibodies take many days to appear which makes Dengue difficult to detect and treat in time. The only logical alternative is to target the mosquito which carries and transmits the virus.

Evaluation and commercial application

The BG-Sentinel-lure combination has been tested in Brazilian cities for monitoring *Ae. aegypti* in classic urban environments. Female Aedes mosquitoes are active during the day and virtually impossible to catch and secure with conventional trapping systems using only carbon dioxide.

Field trials in the Brazilian city of Belo Horizonte showed the BG-Sentinel-lure system outperformed other traps (gas-powered carbon dioxide trap and visual-cue only trap) and was as efficient as the 'real thing' (live human 'landing/biting' collections). A six-month comparison between BG-Sentinel and a sticky ovitrap for gravid females showed BG-Sentinel to be far more efficient and sensitive for measuring density of *Ae. aegypti* populations.

The yellow arrows show the direction of the drawn-in air, the red arrows the air loaded with the attractants which are emitted through the permeable fabric.

BG Sentinel trap in position

BG-Sentinel trapping system is more efficient in sensitivity as well as absolute numbers of mosquitoes caught. Traps deployed for several months at a single location in Belo Horizonte showed catch rates rising perceptibly at the beginning of the wet season. However, the trap was sufficiently sensitive to catch enough mosquitoes during the dry season and enable assessment of these much lower seasonal populations.

Trials are part of the on-going strategy to develop and deploy early warning systems for Dengue fever and to monitor effectiveness of any control measures. Both early warning systems and control measure monitoring systems require ultra-sensitive trapping systems that can lure and secure mosquitoes even when they are in low numbers, and therefore detect small early-stage breeding populations.

Another major benefit afforded by the BG-Sentinel is better use of human resources and ability to increase the intensity of pest monitoring. Brazilian city authorities have only been able to carry out mosquito egg/larval surveys at a rate of four per district in any one calendar year.

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But mosquitoes have a development cycle of just two weeks which means there is no way of providing a completely up to date picture of disease risk with just one 'scout' or survey every 12 weeks. This new technology provided by the BG-Sentinel and armed with the attractant blend will allow authorities to survey weekly with the same level of human resources.

The system is being evaluated in the coastal city of Cairns in tropical northern Queensland, where previous Dengue outbreaks have occurred (including 1998 and 2003) although all were quickly stamped out. That said the Queensland State and national government are continually worried about re-introduction and want to develop a reliable and efficient 'early warning system'. They require an ultra sensitive trapping system like 'BG-Sentinel trap - human sweat lure' to detect small populations of *Aedes* mosquitoes in the very earliest stages of development. The University of Queensland and the Brisbane Public Health Department have already tested the system with exceptionally good results. The United States military with personnel in many Dengue infected areas of the world are testing the BG Sentinel system in a range of countries including Thailand, Indonesia and Peru.

First field trials evaluated the system for mosquito monitoring only, but work has been underway in Brazil to use the system as a control 'tool', reducing incidence of Dengue by exploiting 'weak points' in the *Ae. aegypti*/dengue fever life cycle and transmission period. Researchers are fine-tuning the strategy to hit the disease just as much as the vector for preventing transmission of Dengue from mosquito vector to human host.

It only takes 7 days for *Ae. aegypti* eggs to develop into mosquitoes but 12 days for an adult female mosquito (having acquired infected blood) to transmit the disease to a new host. This is considered a long time for adult *Aedes* mosquitoes to remain alive. Population models show control authorities will not have to trap that many mosquitoes to exert a significant effect on disease transmission.

The research group in Germany has funding to test the 'theory' in Brazil where they are going into villages and deploying as many traps as possible to see if they can break the transmission cycle. Early calculations show that removing just 35 per cent of the *Aedes* mosquito population should reduce level of Dengue by 80 per cent. Two key factors in the calculations are the relatively long period (12 days) required before the mosquito becomes infective, after sucking up infected blood, and corresponding short life expectancy of the same adult mosquitoes.

Plans are underway to extend application of 'BG-Sentinel-lure system' to monitoring and control of other species of *Aedes* mosquito responsible for carrying different diseases, as well as *Aedes* carriers of Dengue into new hitherto disease-free temperate areas of the world. The BG-Sentinel-lure is the only system that effectively traps and monitors *Ae. polynesiensis* (Polynesian tiger mosquito), that carries and transmits the parasitic roundworm (nematode) to cause filariasis in the Asia-Pacific region. *Ae. polynesiensis* is an important vector of Dengue in this particular region. The US military are evaluating the system for deployment in Iraq and Afghanistan against sand flies (*phlebotominae*), which carry and transmit leishmaniasis.

New Modular Indoor Air Quality Area Monitor

GrayWolf Sensing Solutions (USA) introduces a new Area Monitor for Indoor Air Quality (IAQ) and for other environmental monitoring applications. The WolfPack is a fully modular system: plug in one, up to three GrayWolf DirectSense® probes with up to six sensors installed per probe. Add a particle counter, airspeed probe, ΔP or other sensors: up to 21 parameters may be monitored simultaneously. The same probes, as part of the modular design, may alternately be connected to a Windows Mobile Pocket PC® or Vista® OS mobile PC for hand-held, walkthrough surveys, spot checking and spot logging.

Available parameters include: TVOCs, Carbon Dioxide, %RH, °C/°F, Particulates, auto-zeroing Differential Pressure and Air Velocity. Sensors for these parameters all exhibit rapid response and are highly accurate. In addition, up to 18 specific electrochemical gas sensors (CO, O_3, NH_3, NO_2, NO, H_2S, SO_2, Cl_2, HCN, HCl, etc.) are available.

The WolfPack has a tactile color screen and is powered by an embedded WinCE® operating system running GrayWolf's WolfSense® 2009 application software. WolfSense 2009 offers a broad range of user-friendly features. A unique workflow feature handholds users through data-logging set-up; easily customized for very specific applications, allowing less experienced operators to take advantage of more advanced functions. On-board features also include: Video help, Parameter details (typical sources, typical background levels, health effects, etc.), Sensor info (basic sensor care, cross-interferences, specifications, etc.), Application related documents, In-situ text notation, In-situ audio notation…and much more.

Once measurements have been trend-logged over time; review, analyze and report on the data and field-collected notes with the included WolfSense PC software. Optional Advanced Report Generator software automates the entire reporting process.

Reader Reply Card no 41

Infrared Camera Sees Sulphur Hexafluoride

The introduction of **FLIR Systems'** (Hong Kong) ThermaCAM® GasFind IR gas leak detector has allowed companies to incorporate infrared into their leak detection and repair programmes (LDAR) providing a far quicker and more efficient method than traditional toxic vapour analysis.

This success has led to further product development and the introduction of an infrared camera specifically for detecting greenhouse gases that are not visible in the mid-wave infrared band; sulphur hexafluoride and anhydrous ammonia are typical examples. This new camera is the ThermaCAM® GasFind IR LW. It is based on the standard model but as its suffix suggests it operates in the longwave infrared band.

Sulphur hexafluoride (SF6) is a relatively expensive man-made chemical released by electrical substations where it is used as an electrical insulator in equipment that transmits and distributes electricity. It is also used as a cover gas in the magnesium industry, in the manufacture of semiconductors, for thermal and sound insulation, as a tracer gas for ventilation efficiency and in medical applications. Although SF6 is extensively recycled leakage is still a concern. Excessive exposure can harm human health and of the internationally monitored greenhouse gases it has, by far, the greatest global warming potential – 23,000 times that of CO_2. This gas is included in the European Pollutant Emission Register, a vast database that catalogues European industrial pollution. It requires affected industrial plants to identify leaks from a range of 50 pollutants that they emit into the air or water in quantities over a given threshold. The ThermaCAM® GasFindIR LW allows plant managers to see, and hence, reduce the emission of sulphur hexafluoride with ease. Although the detection of SF6 is a key selling point for this new camera it is not by any means the only gas that it can detect. It is designed to see a wide range of other gases too including anhydrous ammonia that is widely used as a refrigerant in large cold-storage facilities.

The new ThermaCAM® GasFindIR LW actually shows any leakage point or points of sulphur hexafluoride gas in real time. It can detect small leaks from several metres away and big leaks from hundreds of metres away and in trials has proved able to see leaks that have eluded a traditional active laser system. In addition to making leaks visible, the camera is able to scan large areas quickly, greatly increasing inspection speed and improving overall plant safety. It can also be used without stopping any operation or process.

Reader Reply Card no 42

H2scan Corporation Receives the Korea Occupational Safety & Health Agency (KOSHA) Certificate of Compliance

H2scan Corporation (USA) announce the Company has received a Certificate of Compliance from the Korea Occupational Safety & Health Agency (KOSHA) for its HY ALERTA™ 1600 Intrinsically Safe Area Hydrogen Monitor and HY-OPTIMA™ 1700 Intrinsically Safe Process Hydrogen Analyser. They are also ATEX Certified (Atmosphere Explosive).

H2scan's Vice President of Sales and Business Development Cyril Berg stated, "Having these two units receive the KOSHA Certificate of Compliance in addition to their current ATEX certification is an important step for us to penetrate the Korean market. It will provide us and our Korean distributor, Young Heung Corporation, the necessary leverage in selling these products."

H2scan's HY-ALERTA 1600 Intrinsically Safe Area Hydrogen Monitor provides fixed area hydrogen specific measurement for hydrogen concentrations as low as 4000 ppm and can be scaled to any concentration up to 5% hydrogen by volume. Additionally, its hydrogen specific sensor technology has no cross sensitivity to other combustible gases, thus eliminating false alarms and ensuring safety system reliability. With a 10 year product life expectancy, it also proves to be fiscally prudent.

Reader Reply Card no 43