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Exploration of Volatile Organic Molecules for Detection of the Brown Tree Snake and Other Non-Indigenous Species

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The Air Force Research Laboratory (AFRL/MLQ) at Tyndall AFB initiated a research effort for detection and monitoring of non-indigenous invasive animals. This effort's objective was to identify and develop reliable, cost-effective methods for identification and monitoring of non-indigenous invasive animals using sampling, sensor, and instrumentation technologies.

We were able to detect a myriad of VOCs from the snakes' cages. Most of these compounds could also be detected in background samplings around the building. While background remains a problem, the efforts we used to eliminate the bedding background tremendously improved our analysis. The data collected was thoroughly examined for unique compounds originating from the snake itself. Unfortunately the data was inconclusive. There was no evidence of a volatile chemical that could be consistently attributed to the presence of a snake.

If future work is considered, other analytical techniques should be considered to look at different classes of compounds. Future techniques could look at detection of some of the larger lipid molecules or other semi-volatile compounds. It may also be possible that molecules sorbed onto particulate matter or surfaces contacted by the snake are detectable. Ion mobility spectroscopy was unavailable to us but is a suggested technique to consider in the future.

EXPLORATION OF VOLATILE ORGANIC MOLECULES FOR DETECTION OF THE BROWN TREE SNAKE AND OTHER NON-INDIGENOUS SPECIES

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EXECUTIVE SUMMARY

The Brown Tree Snake (*Boiga irregularis,* Figure 1) is a species thought to have been introduced onto the island of Guam in the early 1950's through cargo shipped from New Guinea during World War II, possibly through military operations either on a Navy ship or an Army Air Corps aircraft. Since the snake has no natural predators on the island, the population of the snake has grown to the point where it has become a major concern. In some forested areas of Guam, Brown Tree Snake (BTS) population density is as high as 13,000 snakes per square mile.

Figure 1. Brown Tree Snake

The BTS has been responsible for the extermination of 9 of Guam's 12 native forest bird species, and the others persist in low numbers. Additionally, 2 of 3 native bat species and numerous species of lizards and small mammals have also disappeared, an ecological disaster. The BTS also causes frequent power outages while crawling on power lines seeking food.

The threat is that the BTS could become established on other islands. A major concern for the U.S. government is the prospect of these snakes spreading to Hawaii or the southern United States. Current control methods consist of snake traps and patrols of fence-lines to keep snakes from transportation hubs in Guam, establishing snake-free holding zones for cargo during exercises using temporary barriers, and source and destination inspection of cargo and aircraft using detection dogs.

Since these snakes tend to hide in confined spaces, an effective inspection method must be available to prevent the spread of this snake through outgoing cargo. Currently, the only effective means of detecting these snakes in confined spaces is through the use of trained canines. Although effective, trained canines are expensive, have relatively short working times, and require trained handlers. Cost for canine detection of the brown tree snake by the DoD alone has been estimated at $2M annually. HQ PACAF submitted high ranked Environmental Safety and Occupational

Health (ESOH) Need 1301, "Detect Brown Tree Snakes in Cargo and Craft to Prevent Spread to Other Areas of the Pacific and Mainland United States." A reliable, portable, cost-effective device capable of detecting and locating the BTS in and around aircraft, ships, and cargo would greatly enhance the efforts to control the BTS and prevent its spread to other locations. The Air Force Research Laboratory (AFRL/MLQ) at Tyndall AFB initiated a research effort for detection and monitoring of non-indigenous invasive animals. This effort's objective was to identify and develop reliable, cost-effective methods for identification and monitoring of non-indigenous invasive animals using sampling, sensor, and instrumentation technologies.

BACKGROUND

Non-Indigenous Invasive Species

"An invasion is under way that is undermining our nation's economy and endangering our most precious natural treasures. The intruders are alien species - non-native plants and animals introduced into this country either intentionally or by accident." (Stein, Bruce A. and Stephanie R. Flack, eds. 1996. America's Least Wanted: Alien Species Invasions of U.S. Ecosystems. The Nature Conservancy, Arlington, Virginia.)

The world today is a political environment of unrest causing more numerous interventions by peacekeeping military forces. The United States has committed itself to assisting the United Nations and other treaty nations to maintain and foster peace. This often requires short-notice deployments to staging areas and locations that have indigenous species not found in other locals. The military's global reach provides great opportunity for transport of unwanted species to and from different ecosystems. A capability to detect the intrusion of these unwelcome stowaways could prevent their transport to other locations where they have no natural balancing species.

Biological invaders carried by expanding worldwide military operations and commerce are colonizing and negatively impacting native ecosystems. In naturally evolved ecosystems, the species are kept in balance by competition, predation, and disease. Non-indigenous invasive species can degrade balanced ecosystems by altering their physical or chemical properties, depleting native wildlife by preying on them, or setting off cascading biological changes in the natural systems they invade. Non-indigenous species have been shown to cause major ecological and economic problems. Non-indigenous species have been implicated in the decline of many U.S. species federally listed as threatened or endangered. Some conservationists now rank invasive species among the top menaces to endangered species. Four approaches have been suggested for control of non-indigenous invasive species: prevention of additional introductions; early detection and eradication of new pests; control and management of established problem species; and protection and recovery of native species and ecosystems.

In 1990, Congress mandated the establishment of the Aquatic Nuisance Task Force (Title 16, United States Code, Chapter 67, Subchapter III). Paragraph 4728 of the code directs the Task Force to "undertake a comprehensive, environmentally sound program in coordination with regional, territorial, State and local entities to control the brown tree snake." The resultant multi-agency Brown Tree Snake Control Committee includes representatives of the Armed Forces Pest Management Board (AFPMB). In April 1995, the Committee published a draft of the Brown Tree Snake Control Plan, which discusses control and detection protocols as well as recommendations for research and development programs for control and detection. The Air Force (PACAF) has the High Priority ESOH Need to "Detect Brown Tree Snakes in Cargo and Craft to Prevent Spread to Other Areas of the Pacific and the Mainland United States."

A Presidential Executive Order on Invasive Species was issued on 3 February 1999 to prevent the introduction of invasive species, provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause. This Executive Order references a number of other laws such as the National Environmental Policy Act, Lacey Act, and Sikes Act.

These provisions require thorough inspection of all aircraft and cargo departing Guam both at origin and at destination to prevent spread of the BTS. Along with the commercial airport, Guam is home to a Navy air base, a commercial and a naval shipping port, and an Air Force base. Agricultural quarantine and the Military Customs Inspection Program (MCIP) require that all aircraft leaving Guam be inspected for BTS. According to USDA, detection dogs (Jack Russell Terriers and Beagles) accomplish this but at a considerable cost and in a very limited number of areas. The actual confidence level associated with BTS detection by the dogs is good but could be improved and the inaccessibility of many areas within aircraft, ships, and cargo make their effectiveness uncertain. Of the snakes discovered by the dogs, 82% were in positions of high risk for export from Guam by being in the immediate vicinity of vessels and cargo-loading areas, or actually in cargo (Mike Pitzler, APHIS communication). Trained canines are expensive, have relatively short working times, and require trained handlers.

<u>Brown Tree Snake</u>

Snakes have been found as accidental stowaways in cargo or aircraft leaving Guam, and unless intercepted, may become established on other islands. A major concern for the U.S. government is the prospect of the snake spreading to other Pacific islands (including Hawaii) that do not have a native snake population, and therefore no natural predators and to the southern United States. Economic and ecological problems like those currently present on Guam are likely to develop if the BTS reaches other Islands. As an example, Hawaii, Saipan, Tinian, Rota, Kwajalein, Diego Garcia, Oahu, Pohnpei Okinawa, Cocos Island, Wake Island and in Texas on the U. S. mainland have all experienced accidental introductions of the BTS.

The increased public emphasis in this area often associates the military with the problem. Newsweek, 28 Jul 97, "2000, The Millennium Notebook: Invasion of the Tree Snakes and Other Coming Infestations" and The Washington Post, 23 Aug 97 article "Trouble in Paradise?" began, "Shortly after a huge transport plane unloaded its cargo at Hickam Air Force Base one day earlier this month, Airman John Gerist happened to spot a brownish, three-foot-long snake slither into a nearby canal and disappeared." This was the sixth snake sighting in two months on an island with no indigenous snake population (Washington Post, August 23, 1997). Brown tree snakes have been introduced to the mainland United States demonstrating the potential for introduction of the BTS to a survivable habitat in the United States. Crated in household goods shipped from Guam, a BTS arrived alive and well at Ingleside Naval Air Station, which is located on the north side of Corpus Christi Bay, Aransas County, Texas. The snake was positively identified as a BTS and destroyed. Also a BTS was discovered aboard cargo at Rota AB Spain after a flight from Guam. The aircraft had an interim landing at Tyndall AFB, Florida.

The snakes have interfered with electrical power transmission and communications, and have invaded homes and businesses. Snakes crawling on electrical and communication lines frequently cause power outages, impact communications, and damage electrical lines maintained by Guam Power Authority and Naval Public Works. Since 1978, more than 1200 power outages have been caused by snakes and the resulting damage to electrical equipment is a significant economic burden to nearly all civilian and military activities on Guam. Power interruptions cause a multitude of problems ranging from food spoilage to computer failures (BioScience, Oct 97).

Detection and Control

Current detection and control methods include the use of U.S. Department of Agriculture (USDA) Animal Damage Control dogs to find non-indigenous invasive species in cargo and craft at source and destinations. Control methods consist of using pesticides, repellents, fumigants and snake traps and patrols to keep snakes from transportation hubs in Guam, establishing snake-free holding zones for cargo. Since these snakes tend to hide in confined spaces, an effective inspection method must be available to prevent the spread of this snake through outgoing cargo. Without monitoring the probability of introduction of the non-indigenous invasive animals into other areas is high. Failure to detect and control the non-indigenous invasive animals poses a major environmental threat to the United States and other countries, endangering ecosystems, wildlife, and economies. In addition to the potential financial cost of mitigating the problem once it has occurred, the public relations costs could be enormous.

The Air Force Research Laboratory (AFRL/MLQ) at Tyndall AFB initiated a research effort in 1998 for detection of non-indigenous invasive animals. Initial proof-of-concept work addressed chemical detection of BTS. The AFRL/MLQ funded the BTS sampling on Guam and the Armed Forces Pest Management Board funded initial studies at the U.S. Dept. Agriculture National Wildlife Research Center (USDA NWRC) Fort Collins, Colorado. A Joint AFRL and Air Mobility Command project was initiated in Feb 99 that continued work at the NWRC. If successful, detection and monitoring technology would enhance the ability of DoD and USDA personnel to perform inspections (USDA has about 1300 inspectors at 90 ports) to prevent further migration of harmful or noxious pest species. In addition to conservation of the ecology, this research would avoid the costs of active inspection, intervention and training programs.

CONCEPT

Reliable, portable, cost-effective devices capable of detecting and locating the non-indigenous invasive animals in and around aircraft, ships, and cargo would greatly enhance the efforts to control and prevent the spread of non-indigenous invasive animals to other locations, as well as enhance the capability to support increased activity associated with training exercises and contingency operations.

This effort's objective was to identify and develop reliable, cost-effective methods for identification and monitoring of non-indigenous invasive animals using sampling, sensor, and instrumentation technologies. Such technology would have the following advantages over currently used detection dogs:

- Greatly reduced operating costs
- Improved access - e.g., wheel wells, containerized cargo
- Increased availability
- Reduced training
- Flexibility - technician operable, ready anytime
- Complement other control methods
- Capability can be enhanced over time
- Broad application to different locations - mobility

These technologies would help prevent inadvertent introductions into new non-native habitats and provide monitoring for management and control of infestations. Without improved methods and technologies for controlling the non-indigenous invasive animals it may only be a matter of time before they become established in other locations. When this occurs, the financial and public relations costs to the military would be very high. Additionally, once this technology has been developed for one non-indigenous invasive animal, it may be adapted to other species to help prevent their transport to other ecosystems during military operations.

<u>Objective</u>

Techniques for rapid, cost-effective, and technically defensible detection are needed for pest management. The objective was to identify and develop reliable, cost-effective methods for identification and monitoring of non-indigenous invasive animals using sampling, sensor, and instrumentation technologies. This research effort was to provide for the early screening, detection, and management/control technologies to sustain Department of Defense operations, protect health and the ecology, reduce the cost of these operations and avoid the potential cost of mitigating a problem once it has occurred.

The technical objective of the research was to characterize the chemical emissions from non-indigenous invasive animals to identify indicator compounds and then demonstrate the ability to detect the species based on measuring those parameters. Prototype devices for detection of non-indigenous invasive animals in an operational environment would be developed. These real-time tools would feature a hand-held probe that would be placed in or near the space to be measured, and provide a qualitative/quantitative measurement of indicator parameters in near real-time. A cargo sampler, much like a dosimeter badge, would be an additional early product.

Canines have a long history of being used in search and detection operations, including airport inspections and police investigations. They have been used to assist in detecting a wide variety of targets. Targeted scents have included humans both alive and dead, explosives, accelerants, natural gas, drugs, agricultural produce, currency, cancerous tissues, gypsy moths, screwworms, and termites. In this effort we initiated a phased approach for investigating the feasibility of using analytical detection methods to supplement canines for the detection of the brown tree snake. The long-term project goal is development of a man-portable, cost effective, detection instrument to be used to detect the presence of the brown tree snake in confined spaces and in cargo. The developed technologies and methods would have broad potential for ready adaptation to detection of other non-indigenous invasive species.

Approach

Canine olfaction has been widely used for detection of many things as noted above, but is not completely understood. Since the detection dogs apparently locate the BTS primarily by scent, it is reasonable to surmise that the BTS and other animals exude specific molecules to the atmosphere that are recognized by the dogs. This effort investigated the detectability of animals and reviewed off-the-shelf and developing technology that could be used for their detection. This information could be used in subsequent efforts to demonstrate/validate and field a cost effective, transportable device to detect and locate non-indigenous invasive animals in cargo, aircraft and structures to provide a cost-effective solution for DoD and USDA needs.

The fact that trained canines are capable of detecting the brown tree snake from scent alone indicates that certain volatiles or airborne particulates are emitted from the snake and are detected through the olfactory system of the dog. The task in designing an "electronic dog nose" is to determine the characteristics of these volatiles and to devise methods of detection that provide adequate sensitivity and discrimination against potential interferents.

The approach was to analyze air samples from known non-indigenous invasive animal environments and identify the airborne molecule(s) that indicate the animal's presence. After identifying compounds as suspect markers, the methods would be optimized for detection of those specific markers, and validated with additional sampling. When the indicator molecule(s) is isolated, off-the-shelf or emerging technology would be assessed and refined to reliably detect the indicator molecule(s).

Methods

The program had parallel paths, one focusing on sampling and identification of detectable compounds and the other on sensors and instrumentation. Methods that can measure low concentrations of vapors more quickly and quantitatively than currently available instrumental approaches (e.g., gas chromatography and organic vapor analyzers) are needed. Each of these approaches is inadequate for one or more of the following reasons: low sensitivity, susceptibility to interferences, slow response, and complicated operational and/or maintenance requirements. A user-friendly instrument that specifically responds to target compounds in the parts-per-billion volume (ppbv) range in less than one second is desired. The long-term program goal is development of field-portable, cost effective, detection instruments that can be used to detect the presence of non-indigenous animals in confined spaces and in cargo.

A comprehensive study of the volatile compounds that are emitted from the BTS was conducted in 1998-1999. The key to the effort is in the ability to successfully sample and measure the unknown volatile species. Several chemical sampling or trapping techniques were utilized to concentrate air samples taken in the vicinity of a BTS prior to analysis by gas chromatograph/mass spectrometers (GC/MS) and/or GC/MS/FTIR (Fourier Transform Infrared Spectroscopy). The proposed trapping techniques included solid-phase micro-extraction (SPME), cyro-trapping and chemical adsorption. These techniques are commonly used for collection and concentration of volatile organic compounds from the atmosphere and for laboratory analysis. Some physical extraction of the skin and collection of excrement was also conducted. These initial experiments with the BTS were conducted in Guam and at the National Wildlife Research Center (NWRC in Ft. Collins, CO)

The initial tasks involved performing a cursory study of prospective sensors and analytical instrumentation. This involved literature reviews as well as conducting discussions with experts in the field of trace vapor detection and identification and herpetology. Four potential vapor analysis instrumentation areas that were considered for investigation were (assumption is that the dogs are alerting in response to airborne molecules or particulates) ion mobility spectrometry (IMS), gas chromatography with a surface acoustic wave (SAW) detector, gas chromatograph/mass spectrometers (GC/MS) and a technology based upon Resonance Enhanced Multi-Photon Ionization (REMPI). Emphasis was on off-the-shelf or emerging technologies that show promise for portability and that can be implemented quickly.

Potential Analytical Instrumentation

Field-portable gas chromatograph/mass spectrometers (GC/MS) have been used primarily for monitoring volatile organic compounds. Due to its compact size and battery operation, the portable GC/MS system lacks some of the versatility of larger scale AC powered units. However, for compounds with molecular weights below 300 and boiling points below 200°C, the system provides a rapid, convenient method for trace vapor detection.

A number of portable gas chromatographs are available with various detectors such as surface acoustic wave and photo ionization detectors (PID). A vapor or particle sample is captured in a pre-concentrating trap. The sample is injected into a GC column where separation of chemical species occurs prior to detection by the detector. The system software identifies the suspect sample by comparing it to a database library of chemical signatures and calculates the concentration.

In addition to the more common technologies such as above, a chemical detection technology that holds significant promise is resonance enhanced multi-photon ionization (REMPI). The REMPI processes of interest here are of the so-called 1+1 variety. One photon is absorbed to create the initial molecular excited state, from which the second photon is absorbed to ionize the molecule. REMPI methods can sensitively test the airspace for the presence of certain hydrocarbon constituents.

Such instruments may be referred to as laser ionization detectors (LIDs), which can be contrasted with conventional photo ionization detectors (PIDs) that use far-ultraviolet lamps to create the ions. Of the baseline technologies for vapor sensing and analysis, LIDs most closely resemble organic vapor analyzers (OVAs). An OVA is little more than a gas chromatography detector connected to a small pump. OVAs sacrifice the specificity of GC methods to gain very fast response, typically a few seconds. The advantage of the LID approach is that the laser wavelength provides the specificity instead of the column. The LID technology bridges the gap between the specific detection of gas chromatography and the simplicity of an organic vapor analyzer.

User Interest and Field Studies

In 1998 a briefing on the BTS detection effort was presented at the BTS Coordination meeting. There was considerable discussion and expression of support for the detection of BTS by the chemical recognition concept as a valuable tool in the control program. A Hawaiian representative stated that there is not a more serious problem than the BTS, that it could kill Hawaii's economy. He stated that they are weak on inspecting incoming craft and cargo. The dog handlers performing the K-9 inspections for the USDA's Animal and Plant Health Inspection Service (APHIS) saw the value of the concept. By placing snake "hide-tubes" among cargo, the dog inspections on Guam have been tested and found to be approximately 70% effective. Effectiveness was more related to dog handler experience and thoroughness. USDA is focusing on research areas such as toxicants, fumigants, attractants, dog training, and permanent and temporary barriers that could be used during military operations.

The BTS In-Process Review on Guam provided tours of the control operations at the Navy and commercial ports, warehouse facilities, commercial airport, and on Andersen AFB. On Guam, efforts are focused on controlling the snakes from being transported in cargo and on craft leaving Guam to high-threat areas such as Hawaii, Commonwealth of the Northern Marianas Islands (CNMI), and mainland US. Trapping, fence-line searches, dog inspections, and education are the only tools currently available. A temporary barrier has been developed for use around cargo areas during military exercises. The USDA District Supervisor on Guam, commented that there was an increasing demand from locations receiving cargo shipped from Guam that the cargo be certified "snake free." USDA cannot currently comply with this requirement. This need could possibly be addressed by an "indicator badge" or "sensor tag."

The addition of a "BTS cargo sampler," similar in concept to a personal dosimeter badge, that could be inserted into cargo containers, pallets, cargo holds, etc, and sample for chemical markers indicating the presence of a BTS during the entire shipping period is needed. When received at the destination, the sampler would be removed and checked for the chemical markers indicating the presence or absence of a snake and then appropriate action taken. It is anticipated that the device could be processed for reuse. This would augment other inspection methods such as the use of dogs, and be especially useful in situations where cargo could not be inspected prior to shipment, and for cargo with especially sensitive destinations. Sampling technology and methodology is a key research issue and directly leads to concept addressing the cargo sampler need. Emphasis was on currently-off-the-shelf (COTS) technologies or emerging technologies that have been demonstrated in the field and are approaching commercialization. All participants at the meeting felt this could be a valuable tool. This was very useful information in considering design requirements for sensor development.

While on Guam, swipe samples from the skin of nine Brown Tree Snakes (Figure 2) were collected and brought back to the Air Force Research Laboratory located at Tyndall AFB, FL for chemical analyses by a gas chromatograph/mass spectrometer (GC/MS) to identify potential chemical(s) that the detection dogs may be alerting. The swipes were 100% cotton cloths that had been cleaned and placed in bottles to prevent contamination and loss of sample upon their return to the laboratory. Of the nine swipes sent to Guam, three were saturated with hexane, three were saturated with methanol, and three were sent dry. Thought was that the different properties of the various solvents might result in the collection of different classes of compounds from the snake.

Of particular interest upon analysis of the swipe samples, was the detection of a volatile organic compound in the headspace of the sealed sample bottles. This compound was detected in all but one of the sample bottles that had snake swipes, but not in any of the bottles that were not exposed to a snake. Results from all three types of swipes were not significantly different. The compound appears to be a cyclic dienedione compound which is smaller, yet similar to known snake sex pheromones reported in the literature. Unfortunately, after additional analyses the compound appeared to be contamination from preservatives in laboratory gloves used in handling samples. Figure 3 shows the gas chromatogram indicating the detection of the volatile compound and Figure 4 it's mass spectrum that assisted in the confirmation of its identity.

Figure 2. Brown Tree Snake in Guam

Figure 3. Gas Chromatogram Showing Presence of Volatile Compound in BTS Swipe

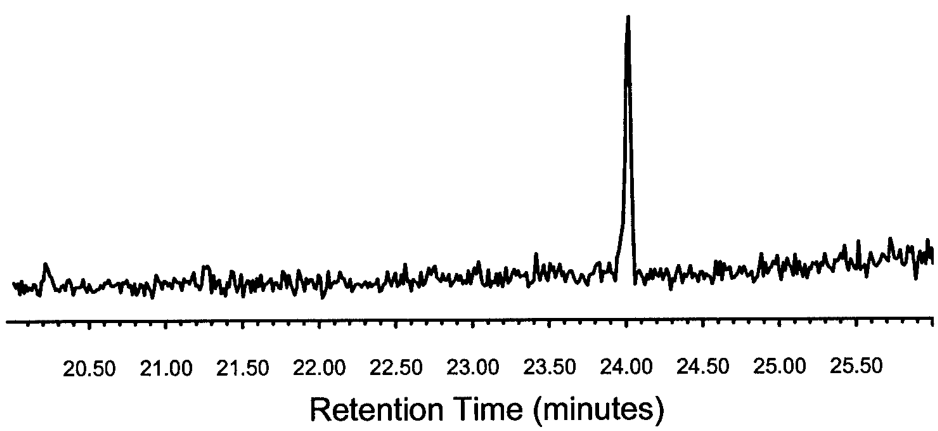
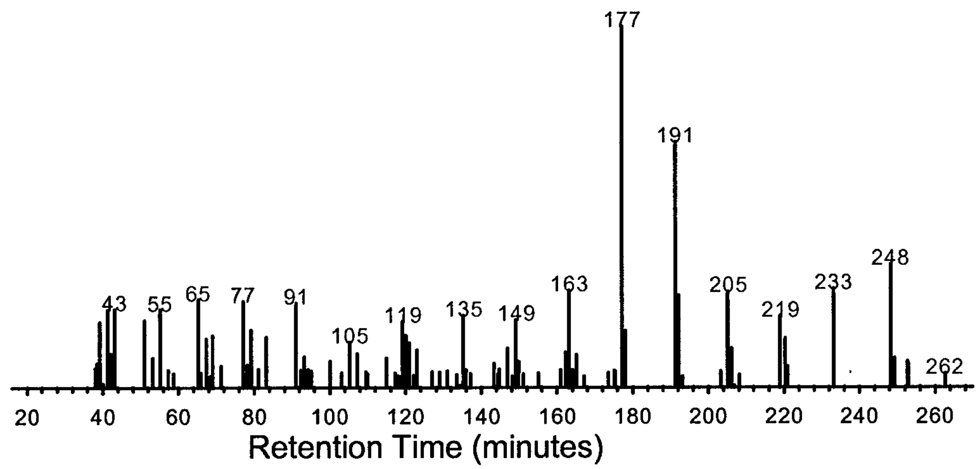


Figure 4. Mass Spectrum of Compound Detected in Headspace of BTS Swipe Sample



At this point much sampling and analysis work needed to be accomplished to isolate a compound that is uniquely released by the BTS. If it can be confirmed, additional research must be conducted to determine if the compound is exclusive to the BTS or snakes, in general or is it a common compound to many animals. Confirmation of a volatile compound would open the door for the possibility of a chemical detection system for the Brown Tree Snake.

Other compounds similar to compounds known to be present on many types of animals were detected in solvent extractions of the swipe samples. These generally are not as easily sampled and detected due to their low volatility and commonality. The fact that chemical compounds were detected from simple swipe samples was a measure of success.

To continue two other series of experiments to measure volatile organic chemical (VOC) emissions from the Brown Tree Snake (BTS) were conducted. Personnel from AFRL/MLQ visited the National Wildlife Research Center (NWRC) in Fort Collins, CO on two occasions in 1999 to conduct these experiments. The NWRC houses several Brown Tree Snakes in their animal care facility. The combination of their excellent animal care facilities and analytical laboratories was perfect for our research efforts.

On the first visit, approximately 40 samples were collected and analyze on-site during the week. This allowed identification of problem areas and redirection of efforts. Also collected were five air canister samples, approximately 30 absorbent trap samples and several swipe samples for analysis in at Tyndall AFB (AFRL/MLQ) laboratories.

As discussed above the purpose was to collect samples in a variety of ways and to conduct experiments designed to measure VOCs emitted by the BTS. On-site sampling and analysis were conducted using solid-phase microextraction (SPME) and gas chromatography/mass spectrometry (GC/MS). This technique proved to be invaluable in that it identified a large VOC signature emitting from the bedding used in the snakes' cages as shown in Figure 5. The signature was a series of hydrocarbons whose molecular chain length ranged from approximately fourteen to twenty carbons atoms. The bedding was a pellet-shaped material made from recycled newspaper that most likely used paraffin as a binder. This signature at a reduced level was detected on the snake even when the bedding was removed from its cage (Figure 6). Procedures were modified to minimize this signature in subsequent samplings but there was still no clear detection of a VOC that could be attributed to the snake. Various swipe samples were collected from the skin and excrement of the snake. Some of these were analyzed on-site by SPME/GC/MS and two significant peaks were detected. Identification could not be confirmed. The remaining swipe samples were returned to Tyndall AFB for analysis.

Figure 5. Overnight Sample from BTS in Cage with Bedding

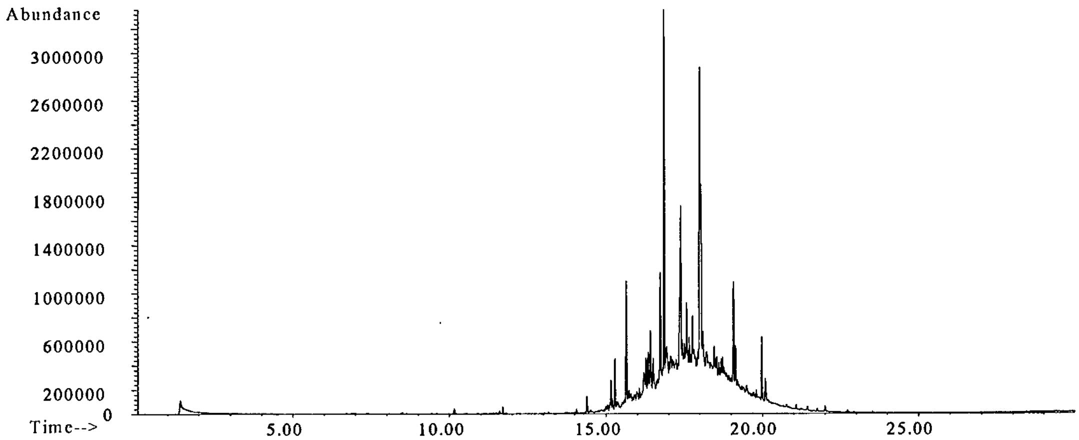
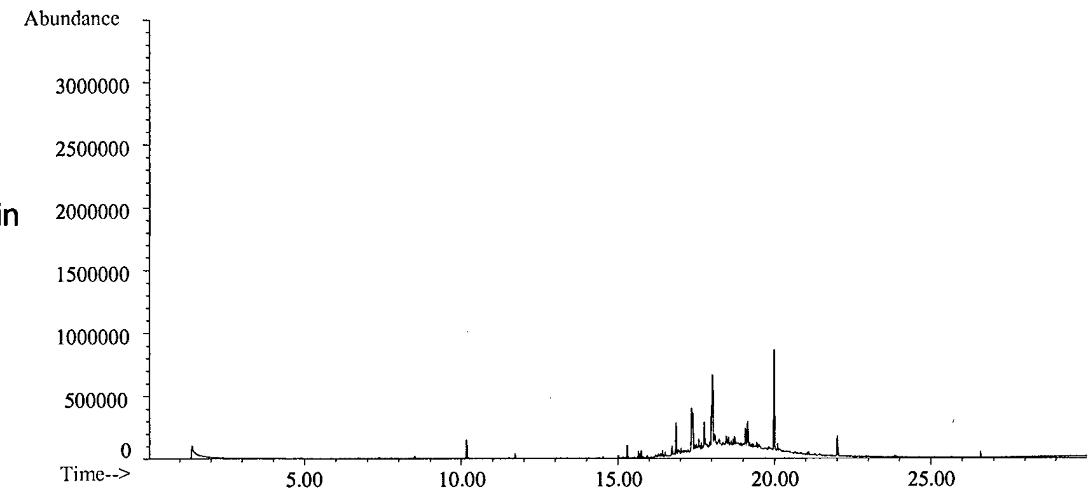


Figure 6. Overnight Sample from BTS in "Clean" Cage, No Bedding



Analysis at Tyndall AFB failed to detect these compounds due to suspected bacterial decay quickly degrading the samples. Air samples were also collected in canisters and onto carbon and Tenax traps that were analyzed in Tyndall AFB (AFRL/MLQ) laboratories. These samples revealed similar "bedding" signatures as previously mentioned. Overall the experimental plan went well but the short amount of time, approximately four days, at NWRC's facility was a challenge. A myriad of VOCs was detected from the snakes' cages. Most of these are believed to have originated from the bedding. Efforts to clean the snake and its cage reduced but did not eliminate these artifact compounds. The data collected were thoroughly examined for unique compounds originating from the snake itself but no significant compounds were detected. If minor compounds were present the dominant signature of the bedding likely masked them.

A second trip to NWRC conducted additional experiments to measure VOC emissions from the BTS. Previous data showed that no additional information was gained by canister and absorbent trap sampling so only on-site sampling using SPME as our sampling tool and analyses were conducted using GC/MS. On this trip approximately 35 samples were collected and analyzed on-site during three days. Expressed liquid samples were also collected from each of the snakes and these samples were returned to Tyndall AFB (AFRL/MLQ) laboratories for analysis.

As discussed, previous experiments identified a large VOC signature emitting from the bedding used in the snakes' cages. We minimized potential background problems by having the NWRC personnel remove all bedding material from the BTS cages prior to our arrival. In addition, a stainless steel sampling box measuring approximately 0.25 ft^3 was manufactured and used to conduct "clean" emission samplings. A snake was captured and placed in the box where SPME fibers could be exposed for a period of time. A typical chromatogram is shown in Figure 7.

Figure 7. One-hour Sample of BTS in Stainless Steel Box

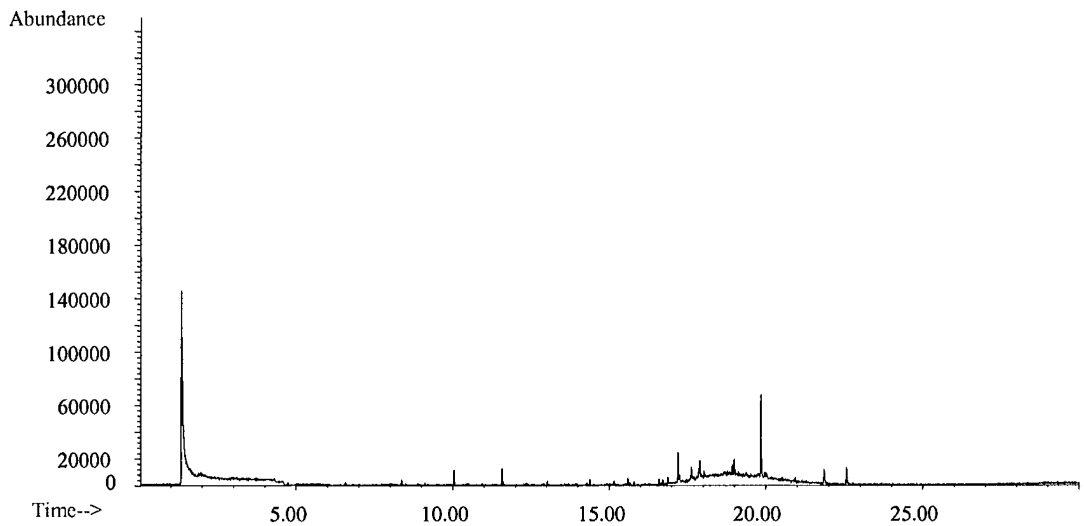
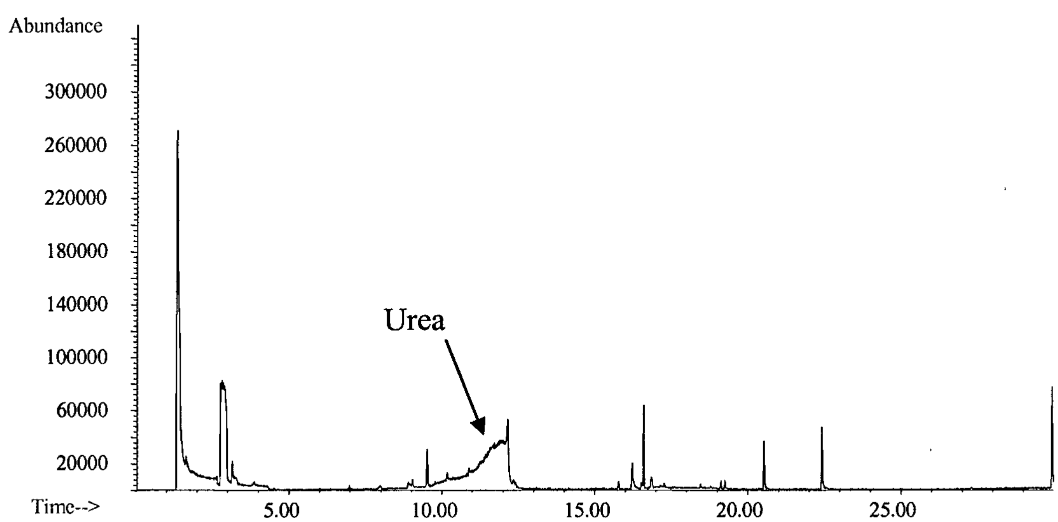


Figure 8. Direct Injection of BTS Excrement



The box was cleaned thoroughly between samplings and showed no signature of its own. Miscellaneous peaks in Figure 7 were also detected in background samplings. Four sets of one-hour emission samples were collected from four different snakes during the week. During each sampling three different SPME fiber types were exposed. The fiber types used were polydimethylsiloxane, carboxen/polydimethylsiloxane and polyacrylate. Many additional VOC samples were collected directly from the snakes' cages, typically overnight samplings. Expressed liquid samples were collected from each of the snakes. These samples were returned to Tyndall AFB for analysis. To avoid the problem we experienced on the previous trip with biodegradation we stored and transported these samples at approximately 4°C. One liquid sample was analyzed on-site by direct injection of the liquid into the GC/MS as shown in Figure 8. This analysis revealed the presence of urea and a few other previously undetected compounds. Overall the experimental plan went well but background (not bedding this time) remains a problem. It is becoming evident that typical VOC analysis may not work for us. At this time there doesn't appear to be a strong VOC candidate for identification of the Brown Tree Snake.

Conclusions/Recommendations:

We were able to detect a myriad of VOCs from the snakes' cages. Most of these compounds could also be detected in background samplings around the building. While background remains a problem, the efforts we used to eliminate the bedding background tremendously improved our analysis. The data collected was thoroughly examined for unique compounds originating from the snake itself. Unfortunately the data was inconclusive. There was no evidence of a volatile chemical that could be consistently attributed to the presence of a snake. The snakes' excrement did emit volatile compounds and the direct analysis of the excrement revealed the presence of several organic chemicals. However, these compounds were not detected in "clean" environments such as when the BTS was sampled in the stainless steel sampling box.

If future work is considered, other analytical techniques should be considered to look at different classes of compounds. We know, for example, that urea is present in the liquid excrement but we detect no trace of it in the headspace. Future techniques could look at detection of some of the larger lipid molecules or other semi-volatile compounds. It may also be possible that molecules sorbed onto particulate matter or surfaces contacted by the snake are detectable. Ion mobility spectroscopy was unavailable to us but is a suggested technique to consider in the future.