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Analysis of Introduced Species as a form of Biological Weapon: part 2- Strategies for Discernment of an Attack and Countermeasures

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Abstract

One key issue with biological weapons (BW) using Non-Indigenous Species (NIS) (aka invasive species) is to differentiate a deliberate attack from a natural outbreak or accidental introduction via commerce or other means.

The methods to detect a Non-Indigenous Species based Biological Warfare (NIS BW) attack as well as discern accidental introductions from a deliberate attack are diverse and challenging. They include a multi-tiered analysis to rule out the following: possible routes of accidental releases from commercial trade; escapes from exotic breeders; releases of exotic pets; and release or dispersal of NIS from catastrophic storms, prevailing wind currents or animal migrations. Other keys to determine a deliberate NIS BW attack are detection of uncommon routes of entry; widespread dissemination of the NIS; extremely high rates of propagules found; or evidence of genetic alternation of NIS, especially to enhance invasiveness, reproduction, or colonization traits as well as human intelligence of a planned BW attack using the introduced organism; and evidence of culturing of the NIS organism by a nation state or terrorist facility.

In all cases of NIS BW, it is a violation of the Biological and Toxin Weapons Convention (BTWC) as the NIS is considered a biological agent used for hostile purposes. This paper also briefly explores other international treaties that would prohibit use of NIS BW.

The recommendations for countermeasures (either as prevention or as remediation) to a NIS BW attack include the following tasks; expansion of NIS databases, improvements to the APHIS Port Information Network (PIN) data collection and database availability, and enhancements to NIS research in experimental controlled field trials. Another important counterstrategy is to expand research on potential NIS organisms including enhancements to Environmental Niche Modeling software. The enhancements on the software and data processing accuracy would improve the predictive potential of these tools. Next, although NIS genomic mapping is still in its infancy, expansion of genomic maps of NIS organisms would serve several purposes. First, it would expand understanding of the role that genetic variability plays in invasion survival and colonization in naïve niches as well as locate specific genes necessary for successful invasion and colonization. Also, genomic maps would accelerate the development of gene-based diagnostics for NIS BW detection. Second, NIS genomic maps would help in the detection of genetically engineered NIS organisms. The detection of genetically altered NIS would strongly indicate that a NIS BW attack had occurred. Furthermore, regardless of whether the identity of the originator of the NIS BW attack was known or not, the revelation of a NIS BW attack with genetically engineered traits must be reported to the BTWC committee for follow up investigation.

Keywords: Agrobioterrorism; Berne protocols; Biodefense; Biological weapon; Biosecurity; Bioterrorism; Black biology; Bwtc; Counterstrategies; Detection; Environmental modification treaty; Environmental niche modeling; Exotic species; Garp; Introduced species; Invasive species

Methods to Discern or Detect a Deliberate Attack-Introduction

An attack using biological weapons (BW) has been defined as "the intentional use by the enemy, of live agent or toxins to cause death and disease among citizens, animals, and plants". Introduced species are non-native species introduced into a foreign ecosystem that successfully flourish and may damage the abiotic or biotic factors of that ecosystem. As discussed in the previous paper, the potential use of non-indigenous species (NIS) as a biological weapon has the potential to affect a nation's public health, ecosystems, agricultural commodities, and biofuel feed stocks and could be delivered by a hostile nation, criminal or terrorist groups or by a lone individual.

As previous research [1] supports the hypothesis that Non-Indigenous Species (NIS) can be used as a Biological Weapon (BW), the question arises as to how a deliberate release could be distinguished from an accidental release of NIS into a niche. Although much more research would be needed, at the present time, the following sections of this chapter provide some suggested approaches and protocols to differentiate accidental from deliberate releases of NIS. Although the following approaches are suggested strategies, they are based on previous known methods of NIS introduction and/or previous cases of BW attacks [1-3].

Detection via Nis Dispersal Modes

The means to rule out accidental from deliberate introductions of NIS include a variety of dispersal modes to rule out. These include analysis of whether any natural or human based methods or pathways exist for transport of the NIS into the naïve niche (i.e. dispersal modes) [4]. To rule out accidental release, investigators would need to examine (depending on the characteristics of the target niche) if any commercial

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carriers (e.g. cargo ships, aircraft, etc.) or products (including imported grain, lumber, etc) were introduced into the target niche area. For example, these investigations would examine for cargo ships releasing ballast water, lumber products with NIS attached to or inside of the wood, rubber tire shipments with NIS laden rain water, packing containers or dunnage with NIS present, or imported food grains or food products that may carry fungal spores, insect eggs, etc. Several examples of this type of NIS accidental introductions include the introduction of Dutch Elm Disease (*Ophiostoma ulmi*) along with the bark beetle vector into the US via a shipment of veneer logs from Europe [5]; the introduction of the Asian Long horned Beetle (*Anoplophora glabripennis*) via solid wood packing materials from China [6]; the introduction of the zebra mussel (*Dreissena polymorpha*) into the North American Great Lakes from shipping ballast water [7]; or the occasional establishment of NIS Asian Tiger Mosquito (*Aedes alvopictus*) into the US, Albania, Italy, and Australia, from shipments of wet used tires exported from Japan for recycling [8].

If no commercial or large scale deliveries could explain the introduction of NIS into the niche, then the next level of investigation would occur. The next approach would review if any unauthorized release of pets or hobbyist release could explain the presence of the NIS. Several examples of this type of NIS accidental introductions include *Caulerpa taxifolia* from the Oceanographic Museum of Monaco's aquatic tanks that were dumped into the Mediterranean Sea [9], the accidental release of the spiny-tailed black iguana (*Ctenosaura similis*) as exotic pets onto Gasparilla Island (Florida) [10], or the escape of Gambian Rats (*Cricetomys gambianus*) from an exotic pet breeder into Grassy Key, Florida [11].

After this approach has been ruled out, the next major review is whether the NIS entry could have been due to migration of the NIS or a carrier species OR the transport of the species into a naïve niche due to catastrophic storms (such as hurricanes) or prevailing wind currents. This analysis would include data from meteorological sources as well as review of migratory patterns of various carriers (such as birds or insects like butterflies or locust swarms) and Geographic Information Systems (GIS) to assist in the tracking analysis [12,13]. Several examples of this type of NIS accidental introduction include the discovery of a dead Gambian rat found 33 kilometers from Grassy Key (Florida) en route to mainland Florida on US highway 1 [14], a banded cattle egret with the NIS Tropical Bont Tick (*Amblyomma variegatum*) was found to have migrated to the Florida Keys from the island of Guadeloupe [15], the extensive spread of the NIS Asian Citrus Canker Disease (*Xanthomonas axonopodis pv. Citri*) in Southern Florida as a result of the 2005 Hurricane Wilma [16,17] the migration by wind currents of fungal spores of the NIS Wheat Stem Rust (*Puccinia graminis f. st. tritici*) strain Ug99 from the Sudan of Africa into Iran [14,18], and the wind transport of fungal spores of the NIS Tobacco Blue Mold (*Peronospora tabacina Adam*) across the US [19,20].

Pearson [3] brings up several valuable reasons used in the Biological and Toxin Weapons Convention (BTWC) protocols that have merit in this process. The protocol in working paper 262 (WP.262) describes reasons to discern a natural from an unusual outbreak of a disease and these reasons help State Parties of the BTWC to determine if a treaty violation investigation is warranted. The working paper states that an unusual outbreak of a disease is one in which the disease is unexpected from the "prevailing context for the host agent and environment parameters" [3]. These points can be applied in a similar fashion for NIS BW if the NIS is compared to the pathogen (host agent) and it is considered in light of the environmental parameters (which would include the naïve target niche).

The following reasons from Pearson could be equally applied to NIS BW (if one compared the term "epidemic" for a BW pathogen similar to the term "invasion" for an NIS).

Thus, the similar points are:

- The disease is being reported for the first time in the region and was never endemic.
- The epidemic occurs outside its normal anticipated season.
- The reservoir host or insect vector of the disease do not occur in or were previously eradicated from the affected region.
- The disease appears to be transmitted by an uncommon or unusual route.
- The epidemiological features of the disease suggest increased virulence of the organism manifested in the form of increased case fatality rate.
- The causative agent has a higher survival time even in the adverse environmental conditions and shows unusual resistance.
- Is capable of establishing new natural reservoirs to facilitate continuous transmission.
- The epidemiology of the disease suggests an abnormal reduction in the incubation period of the disease.
- When the characteristics of the causative agent differ from the known characteristics of that agent prevalent in the territory of the State Party [13].

Sequeira [2] describes the following points to help in determining that the NIS outbreak is intentional. The following criteria are used for pathogens or for other "introduced species". Sequeira notes that intentional introductions will differ from accidental introductions in the following ways (NOTE: this author added follow-up comments where applicable):

Use of non-traditional pathways; if evidence of delivery is via smuggling or aerial delivery.

Increase of the probability of survival of the pest in transit; NIS BW may require careful culturing and storage prior to distribution.

Widespread dissemination of the disease from disparate foci; multiple foci will lead to at greater success in invasion and colonization and strongly indicates intentional introduction.

Use of highly virulent strains; strains could also be genetically engineered to enhance survival (see below Black Biology).

High rates of inoculum; this concept follows with the propagule pressure concept-the more propagules-either in single or multiple dispersals- enhances the probability of successful NIS invasion and colonization.

Introduction into remote areas; remote areas favor the time lag necessary for colonization and reduce the risk of early detection and eradication efforts.

Targeting of susceptible production areas; as discussed in part 1 article [1], the best target niches would have biogeographic factors favoring invasion and colonization.

Targeting of susceptible natural environments; comments similar to part 1 article [1], except natural environments may also be determined by Genetic Algorithm for Rule-set Prediction (GARP) or similar Environmental Niche Modeling (ENM) analysis.

Release of multiple species simultaneously; this follows with an invasion meltdown approach [21] where several different organisms enhance the impact on the target niche and result in quicker alteration of the target niche.

Precise timing of releases to coincide with maximal colonization potential; if temporal or spatial factors are considered during the initial NIS BW analysis, then the NIS BW dispersal would favor the best environmental conditions for rapid and maximal colonization.

Sequeira also notes that the globalization of the economy has already taxed the existing USDA structures and resources, especially the Animal and Plant Health Inspection Service (APHIS) [2]. Hence, APHIS might not be prepared to handle an NIS BW attack as described in part 1 [1]. Sequeira mentions that use of GIS based monitoring as well as development of a rapid response strategy will enhance responses to bioterrorism threatening animal and plant production [2].

Asner et al. [22] describes an analysis of five NIS plants in Hawaiian ecosystems, using airborne remote sensing techniques High-Fidelity Imaging Spectrometers (HiFIS) along with Light Detection and Ranging Sensors (LIDAR). This analysis provided the research team with a mean to quantify NIS impacts on the 3D structure of the Hawaiian rain forests (including canopy and understory levels). The results demonstrated that airborne mapping can identify and track the spread of NIS plant species, analyze the ecological impact of the NIS as well as provide analysis of invasion meltdowns [22]. These techniques could be used to monitor present NIS invasions to assist in management of eradication efforts, but if expanded, could also be used to monitor for and provide early "first detection" of an NIS BW attack.

Detection via Human Intelligence (Humint)

First, as part of the method to detect a deliberate NIS BW attack, HUMINT would draw upon both aspects of intelligence organizations (international and domestic intelligence). The international component would include intelligence agencies (e.g. Central Intelligence Agency-CIA, Secret Intelligence Service-SIS –aka MI6), disarmament agencies (e.g. Defense Threat Reduction Agency-DTRA), INTERPOL, and other law enforcement/intelligence organizations that would investigate, discover, or report of research on weaponization efforts or actual NIS BW weaponization development. Domestic intelligence (US) would include border agents, biosecurity and law enforcement agencies (e.g. US Customs and Border Protection, APHIS, Federal Bureau of Investigation -FBI) that would review and interdict smuggling or illicit importation of known NIS species or large numbers (e.g. propagules) of organisms with questionable commercial, research, or scientific value. From the international arena, Pearson [3] notes that the actual discovery of an NIS BW might also come via international cooperation from organizations such as World Health Organization (WHO), Food and Agricultural Organization (FAO), and World Organization for Animal Health (OIE) that are striving to expand surveillance, communication, and monitoring of disease outbreaks.

It must be noted that both international and domestic intelligence organizations should be monitoring for evidence of Black Biology (aka genetically modification) [23] of NIS organisms as well. It is also important that intelligence agencies monitoring research into NIS (both covert and overt) in any instance of genetically engineering NIS as the research could have the potential to be misdirected into BW applications.

Evidence of Black Biology

Ainscough defines black biology as the use of recombinant DNA technology towards the development of Biological Weapons [23]. This is one factor of NIS research that international and domestic agencies must be constantly vigilant over. The presence of an actual NIS organisms with evidence of genomic enhancement, especially for genes not normally present in the genome of the NIS species (e.g. mammal with novel bacterial genes, plants carrying plasmids with animal toxins, unrelated species with novel weapons, etc.) would be cause for alarm. The actual genetic enhancement of NIS might include immunity to target niche diseases, enhanced allotropic effects of NIS (aka novel weapons) [24,25], enhanced reproduction of offspring, resistance to standard eradication pesticides or herbicides, or greater colonization traits. If intelligence or other agencies discovered a genetically enhanced NIS, it would warrant that the event and species be reported to the BTWC for further investigation regardless whether the origin of the NIS BW is known or not.

Government Investigation into Entry via Smuggled or Imported Pets or Food

As described by Kadlec [26], the introduction of NIS BW could occur as easily by bioterrorists as smuggling in tins of pate containing millions of grape louse (*Phylloxera vastratrix*) in a plot to destroy the California vineyards. Kadlec used this scenario to demonstrate that a dedicated attempt by bioterrorists could come from smuggling NIS laden products into the US and a planned distribution on targeted niches of vineyards with disastrous economic effects [26]. A National Research Council (NRC) report [26] states that some of the main trends that influence unintentional arrivals of NIS (especially plant pests) are the smuggling of contraband fruits, vegetables, and animal products coming from international flights into the US. Furthermore, since inland cities are now major points of disembarkation (as well as air terminals in smaller cities) for international travelers and air cargo, the interception of smuggled agricultural materials, including those with potential NIS has increased and the risk of invasions has also increased. Aside of air travel, seaports have become sites of smuggled organisms as only a fraction of the containers are opened for inspection at the port; many of the containers are not opened until final delivery at the inland site. This increases the risk of a NIS laden shipping containers leaking NIS or an NIS escaping upon unloading with a subsequent invasion occurring [27]. Finally, the NRC report admits that illegal transport of NIS organisms into the US has created a myriad of ports of entry that are very difficult to monitor. The NIS could come in via smuggled drugs, ornamentals, crops or other illegal products [27]. The report also notes that with the onset of the North American Free Trade Agreement (NAFTA); the smuggling of ornamentals and prohibited fruits and vegetables with NIS has increased [27].

Nevertheless, if the smuggling pathways can be ruled out for indeliberate motives, then the NIS entry may have been deliberate. Admittedly, this determination may be difficult and lack 100% certainty. If the smuggler is apprehended with the actual NIS BW, common sense may indicate that the NIS risk exists if the NIS agent serves no reasonable commercial, research, or scientific purpose and hence the smuggling action maybe to transport a potential NIS BW.

For a review of the protocol of analysis (Table 1) - Nis Dispersal Mode Analysis- A Strategy To Rule Out Accidental from Deliberate Introduction of Nis. Although further research and amendments to the protocol will enhance this process in the future, this strategy is offered as a start for further research and discussion.

Mode of Entry Analysis Questions	Answer Yes or No	Risk of Deliberate Action Possible NIS Bw?
#1-NIS arrived in commercial carriers, packaging materials, or commercial products (e.g. grain, lumber)?	NO	Uncertain-More info required
#2-NIS arrived by unauthorized release of pets, hobbyists, or escaped from exotic breeder facility?	NO	Uncertain-More info required
#3-NIS arrived or was spread via catastrophic storms, prevailing wind currents, or via migrating carrier organisms (e.g. birds, insects)?	NO	Uncertain-More Info required
#4-NIS has been found to be genetically engineered?	Yes	Risk high and NIS invasion requires further investigation and notification to BTWC. Risk moderate to high
#5-NIS found in smuggled food products or traveler's suitcases or packages?	NO	Harder to rule out with 100% certainty.
#6-NIS found in large numbers (propagules) in the smuggled products?	Yes	Further investigation warranted. Risk moderate
#7-NIS that was found in smuggled pathway serves little or no reasonable commercial, research, or scientific purpose?	Yes	Further investigation warranted. Risk moderate to high

Table 1: NIS dispersal mode analysis: a strategy to rule out accidental from deliberate introduction of NIS.

BTWC and Other Treaties

It is worthy to note that NIS BW-if discovered to be a deliberate attack- would be in violation of various international treaties.

Clearly the use of NIS BW would be a violation of the Biological and Toxin Weapons Convention (BTWC) [28]. As stated in Article I: "Each State Party to this Convention undertakes never in any circumstances to develop, produce, stockpile or otherwise acquire or retain:

(1) Microbial or other biological agents, or toxins whatever their origin or method of production, of types and in quantities that have no justification for prophylactic, protective or other peaceful purposes;

(2) Weapons, equipment or means of delivery designed to use such agents or toxins for hostile purposes or in armed conflict [28].

The application of NIS in a BW approach would be considered as a "biological agent" that would be used in a "non-peaceful purpose". The delivery vehicles (e.g. biocruise, aerial sprayer, etc.) used to transport and disperse the NIS BW would also be in violation of the BTWC as they would be designed for delivery of such "biological agents" for "hostile purposes".

It must be also noted that the Sixth Conference of the BTWC reaffirmed: "…that the Convention is comprehensive in its scope and that all naturally or artificially created or altered microbial and other biological agents and toxins, as well as their components, regardless of their origin and method of production and whether they affect humans, animals or plants, of types and in quantities that have no justification for prophylactic, protective or other peaceful purposes, are unequivocally covered by Article I." [28].

Hence, this reaffirmation states that genetically altered organisms for BW -even NIS BW- would be in violation of the BTWC [28].

Beyond the BTWC, the application of NIS BW would also be in violation of other prior international treaties. For example, the Environmental Modification Treaty of 1977 (Article I & II) is clear in the prohibition of "…military or any other hostile use of environmental modification techniques having widespread, long-lasting or severe effects as the means of destruction, damage or injury to any other State Party" [29].

The convention clarifies the term "Environmental Modification Techniques" to include: "...any technique for changing -- through the deliberate manipulation of natural processes" as well as stating that this treaty covers the Earth's "biota, lithosphere, hydrosphere and atmosphere, or of outer space" [29].

Hence, any use of NIS BW which alters the ecosystems or niches as part of the invasion, colonization and adverse effects on the autochthonous biotic and abiotic components would be in violation of this treaty especially where it may result in severe or long-lasting damaging effects to the target niche.

Finally, The Berne Protocols (both I and II) were added to the Geneva Convention of 1949 and in Article 54 [30], it reinforces that the military are: "prohibited to attack, destroy, remove or render useless objects indispensable to the survival of the civilian population, such as food-stuffs, agricultural areas for the production of food-stuffs, crops, livestock, drinking water installations and supplies and irrigation works, for the specific purpose of denying them for their sustenance value to the civilian population or to the adverse Party, whatever the motive, whether in order to starve out civilians, to cause them to move away, or for any other motive" [30].

This would mean that NIS BW used to destroy agricultural productivity, water supplies, livestock, or even render an area uninhabitable by virtue of area denial properties of the NIS would be prohibited by this Treaty [30].

It is possible that as further research into NIS BW becomes better understood, a Confidence Building Meeting of the BTWC (in a future Conference) may be called to address language modifications, monitoring methods, and inspection protocols to review instances of NIS BW and how to reduce the risk of actual future NIS BW attacks.

Recommendations for Counterstrategies-Introduction

At present, there are limitations for counterstrategies to a Non-Indigenous Species based Biological Warfare (NIS BW) attack. The intent of this section is to discuss areas of research and organizational improvements that are necessary to enhance counterstrategies (e.g. detection, interception, quarantine, eradication or biocontrol) of a NIS BW attack. The concept of deterrence of BW as stated by Lebeda [31] is based on three components: military action via retaliation; diplomatic pressure via treaties, inspections, and verification; and defensive action via counteragents for treatment (e.g. drugs, vaccines, decontamination treatments, etc.) of BW exposure. In NIS BW, the only real differences from the Lebeda model of deterrence is that the counteragents for treatment would include eradication and biocontrol measure for NIS organisms causing non-human or non-agricultural damage (e.g. ecological damage). Furthermore, the counteragents concept takes into consideration that drugs and vaccines and other agents (e.g. pesticides, herbicides, antifungal compounds, etc.) exist and can respond to an NIS BW attack that causes morbidity or mortality to humans, livestock, or crops.

Yet, in the world of bioterrorism, asymmetrical warfare tactics as described by McKenzie and Kenneth [32], would favor use of a NIS BW to attack American (or any nation's) vulnerabilities to leverage the bioterrorists' weaknesses in number (i.e. inferior tactical or operational strength) to achieve a disproportionate effects on the targeted society. The resultant disproportionate effects from an NIS BW attack would create social chaos, psychological fear, ecological destruction, and economic damage that would undermine the will of a populace. Hence, the probability is higher that bioterrorists would use NIS BW than nation states.

The following areas would require further research to bolster the counterstrategies to an NIS BW attack. These areas include-but are not limited to-expansion of biogeographic data of many organisms-especially known NIS organisms; genomic analysis of NIS organisms-known and potential candidates; and enhancement of HUMINT on hostile NIS BW research; as well as expanded border protection and multi-government communication and cooperation efforts. In the following sections, some details of these various factors will be explored.

Border Containment and Control

Sequeira [2] describes how improvements in APHIS at the point of entry and a new pest advisory group as a well as the development of an emergency response structure would enhance the response to new invading pests. The response tools are integrated together with Geographic Information Systems (GIS) to determine the spread of NIS as well as review for trend abnormalities in invasion, colonization, and location of the NIS. Sequeira [2] notes that APHIS centers must evolve and communicate better with sister agencies (e.g. US Forest Service, FEMA (Federal Emergency Management Agency), EPA (Environmental Protection Agency), etc.) as well as include cooperation from Federal, State, and academic institutions. But, in the global economy, APHIS will need to network in data and monitoring exchange with other nations and international agencies especially in instances of NIS BW.

A report from the NRC in 2002 made a series of recommendations to enhance the scientific basis for predicting invasive potential of plants and plant pests [27]. Some of these recommendations have merit to counter NIS BW attacks and have been included in this chapter where they offer value. The NRC report [27] recommends that APHIS improve its Port Information Network (PIN)-a database which logs all APHIS interceptions of restricted organisms found at US entry points-by improving sampling protocols, methodology, as well as make the data available for scientific investigators. Furthermore, the NRC report urges improvements in APHIS's risk assessment of organisms upon arrival; improve the documentation process; that the risk assessment process becomes peer-reviewed; and update the process to capture new information and provide for improvements in expert judgment [27]. Finally, the NRC report recommends the USDA upgrade its imported plant evaluation procedures, including a multi-tiered evaluation of hazards that the potential NIS species might offer. This analysis would include use of controlled experimental field screening and life history and population data where establishment and rapid spread data of the species is lacking [27]. This author recommends a genomic mapping (similar to a genetic finger print of the species) be conducted –if it was not already performed-prior to deliberate release (See below Section 11- NIS GENOMIC RESEARCH).

Several points regarding the above proposals need to be considered here. The above recommendations were focused on plants and plant pest control, but could be applied to many other potential NIS organisms (e.g. animals, fungi, etc.). The requirements of a genomic map prior to introduction of a new species might lengthen the time a new potential plant-food crop or ornamental- is introduced to market; but the data would be valuable in the event the species is invasive or is a potential NIS BW agent in other areas of the world. Finally, the above processes may work more effectively in developed nations than in resource scare developing nations. Hence, as stated in the part 1 paper [1], developing nations would have a unique level of vulnerability to NIS BW attack due to their limited resources and diminished economic vigor.

Nis Organism Research

To help risk assessment research on NIS organisms, expanded use of Ecological Niche Modeling (ENM) is required. The present limitations to ENM can be overcome by expanding the available primary data of organisms (requiring biogeographic and ecological survey funding for NIS and other species) in their native niches. Furthermore, where organisms maybe deliberately imported into a country, a GARP analysis (or similar ENM analysis) is done prior to the deliberate release to ascertain if the organisms could be a potential NIS. If the ENM data is available, this could be used to counter an NIS BW attack by directing further monitoring (i.e. post-release of the NIS BW) in areas beyond the known target area and directing eradication and education resources to those sites to prevent colonization and spread of the NIS.

Furthermore, enhancements to the present ENM software (e.g. GARP, BIOCLIM) that would reduce the errors of commission and errors of omission as well as speed up the data analysis (i.e. decrease the time of delivery of a fine point mapping while increasing iteration rate) would be necessary. As computer CPU speeds increase and ENM programming improves, it would yield faster monitoring and discovery of potential NIS BW attacks as well as speed up the mapping for counterstrategies to contain and eradicate a NIS BW attack.

Recommendations by the NRC report [27] offer several areas of NIS research that would help in NIS BW counterstrategies. The report [27] recommends expanded research on host-pathogen associations, including host range, reproduction rates and mode of dispersal of the NIS. Also, the report suggests research on using NIS for biological control, including from the time of initial release, efficacy on the target pest and on non-target pests as well as the range of spread [27]. This research could provide data to the general process of NIS invasions for any species as well as provide supportive data on biocontrol techniques that could be applied as counterstrategies to an actual NIS BW attack using other related species. Finally, the NRC report recommends close monitoring of native US plants growing in botanical gardens and arboretums in other countries for evidence of species (e.g. pathogens, parasites, etc.) to which these US plants are susceptible [27]. It is the species attacking US native plants that must undergo a risk analysis for potential arrival as an NIS to US soil.

The same research recommendation could be applied to other native plants from other nations in US or other national botanical gardens for detection of pathogens or pests to those plants and hence the data could be collected into a database of potential NIS organisms. It would be important to note that the same research approach could be applied to native US animals in foreign zoos or nature preserves and monitor these animals for susceptible organisms (e.g. pathogens, parasites, etc.). These organisms would be analyzed for risk of invasive potential as well as the potential impact to native US animals (both wildlife and domesticated animals). Furthermore, it would be important to determine if any of these pathogenic organisms with invasive potential exhibit the potential for zoonotic behavior (i.e. jumping species to infect humans).

Expansion of Nis Databases

Databases on NIS do presently exist. Two of the most notable are the National Invasive Species Information Center (NISIC) [33] and the Global Invasive Species Database (GISD) [34].

Further research on possible ranges of invasiveness using GARP and other ENM tools would be an additional benefit for these databases. Also, accompanying the NIS data for these databases could be information on the biocontrol organisms for the NIS as well as the commercial suppliers of such biocontrol organisms. As the NRC recommendation [27] above mentioned about the APHIS PIN (Port Information Network), it would be valuable to link the PIN data to each specific NIS in the above databases. This might be useful to make a determination of an accidental or deliberate NIS incident (possibly NIS BW attack). One other recommended addition to the databases would be to include a genome map of the NIS species. Granted full genomic maps are not present for many species, but with the advancements in genotyping and rapid genome sequencing with robotic tools, it is inevitable that full maps of many NIS organisms will become available in the future.

An NRC report recommends [27] that regular updates of invasion organism information databases occur as well as use of email and the Internet to report first detection of an NIS invasion. This "first detection" communication must be expanded, readily available, and international in scope, especially as this "first detection" may herald the first signs of a NIS BW attack. Hence, accurate and up-to-date Internet NIS invasion reports will be critical for biosecurity and counterstrategies against NIS BW attacks. A further recommendation by the NRC report [27] is that a standardization for natural history of the NIS as well as the development of standardization measures for reporting NIS invasion impact. With a standardized measure of impact (i.e. ecological, economic, and social variables), the risk analysis and impact of an NIS BW attack could be better determined and more effective countermeasures to the attack could be enacted.

Nis Genomic Research

As stated in the previous section, the number of genomic maps of NIS organisms is small. Nevertheless, as rapid advances in genome sequencing technology continue and funding becomes available, the capability for full genome mapping of NIS organisms will occur. Expansion of genomic analysis of NIS organisms serves several purposes.

First, by providing a genome map, the NIS can be reviewed for vulnerabilities or genetic characteristics that may help in the detection, eradication or control (i.e. containment) efforts in invaded niches. Scorza [35] discusses how the genetic structure of the NIS population can affect the initial establishment and growth of the NIS population in the naïve niche. Scorza states that the greater the genetic variability of the founder stock (i.e. the NIS propagules invading the naïve niche); the less important are the similarities in ecosystems between the native niche and the naïve niche [35]. This principle allows for genetic diversity to enhance NIS survival by the natural selection from the ecosystem differences of the naïve site.

A genomic map of the NIS would help support Scorza's concepts and this would support the propagule pressure concept necessary for any successful NIS BW attack. A genomic map of the NIS would provide a framework to determine the genetic variability of the NIS population and perhaps determine how the range of genetic variability of the population is related to the range of naïve niche colonization. This data would provide information on the determination of NIS BW spread, including the rate of colonization spread beyond the initial invasion niche.

Furthermore, genome maps of an NIS could be useful to determine if a species related to the NIS (e.g. by family or genus), could also have NIS potential and hence must be monitored for NIS BW applications (or applied to the APHIS PIN database banning the potential NIS from entry into the US).

Finally, as the genomes of pathogenic bacteria and potential BW agents have been completed sequenced [36-39], the data has been used for the various applications, including: the understanding of the physiology of the pathogen; the interaction of the host-pathogen relationship; and the development of diagnostics, drug therapies, and vaccines to the pathogen. One important development from genomic mapping is the development of genetic fingerprinting of BW agents for epidemiological and forensic investigation [40]. Linder, Huang and team [40] describe how various genetic fingerprinting techniques have been devised to indentify various strains of biological warfare agents. This information is critical not merely for diagnostic purposes, but for the forensic identification of the nation or source of the BW agent used in a BW attack. If the development of NIS genome maps occurred, the same forensic applications could be applied for the determination of a deliberate NIS BW attack.

For example, Schaad et al. [41] describes how real time Polymerase Chain Reaction (PCR) techniques have been developed for an array of bacterial, viral, and fungal pathogens (some have BW applications). Schaad et al. [41] notes that as sequencing techniques improve for a variety of organisms, the accuracy and reliance on PCR primers will improve and PCR real time diagnostics will become routine. As the genomes of NIS organisms are mapped, it is conceivable that diagnostic tests for NIS species and even specific strain identification will be developed. The quicker an NIS organism (from a BW attack) is identified, the faster counterstrategies can be enacted to halt the NIS invasion.

The second, and very critical, role for genomic analysis of NIS organisms is in the determination of whether the NIS was genetically altered. Lindler et al. [42] notes that the genotyping of pathogens would aid infectious disease specialists and Human Intelligence agencies (HUMINT) in the identification of BW agents as well as genetically engineered BW agents [42]. If an NIS invasion was found with the NIS genetically engineered, especially for enhanced invasion traits or novel weapons, then this evidence would be highly indicative that the NIS introduction was not accidental, but a deliberate NIS BW attack.

Black [43] discusses how genome projects can be used to create the next generation of biological weapons. Although Black focuses his paper on use of gene vectors for weapons development, his arguments are applicable to the issue of genetically engineered NIS BW. Black states that the prevention of the misuse of genome projects for military purposes will be next to impossible [43]. The author bases his argument on the following reasons: the long history of humanity using any technology possible for weapons development; that the progress of biotechnology will lead to more highly effective gene vectors and gene cloning for enhanced genetic engineering; and that the results of publicly funded genomic research is freely available via the Internet around the world. Black warns that genomic weapons and the technology to develop such weapons must be carefully monitored for any developments of military importance. The same monitoring must occur for NIS genetic research and any NIS weaponization research.

Although it is possible that NIS genome mapping would speed up the development of genetically altered NIS for BW purposes, the need for mapping NIS genomes could outweigh the threat as the genomic information would be essential to compare a native strain of NIS with potential invading NIS strains (especially if the invading strain is suspected to be genetically engineered). If a comparison of NIS genomes is performed, what signs or markers would indicate a genetically altered NIS?

One study by Allen et al. [44] describes using computational software designed to distinguish artificial vector signatures from background DNA of viral and bacterial genomes and natural plasmids. The tools can identify DNA oligomers unique to artificial vectors with high rates of sensitivity and specificity in microarray-based bioassays. These DNA signatures when applied to tests were successful in distinguishing artificial vectors from plasmids in a variety of bacteria strains, including human pathogens (e.g. *Enterococcus faecalis*, *Staphylococcus aureus*) and BW bacteria (e.g. *Yersinia pestis*) [44]. The authors state that the DNA signatures would be important in the detection of genetically altered bacteria in environmental samples [44,45]. With further research aimed at NIS genomes and improvements in the speed of data analysis, this type of vector detection could be applied to detect genetically engineered NIS organisms-both prokaryotes and eukaryotes.

Conclusions

As the previous paper [1] built up data and strategies to support the hypothesis that NIS could be used as a biological weapon, the challenge in this paper was focused on how to discern that an actual NIS invasion is a BW event.

The first approach examines the modes of dispersal of NIS as a possible explanation to an accidental or unintended NIS invasion. The commercial cargo transports, packing materials and even the cargo itself would have to be reviewed as a possible carrier of NIS. Beyond that approach, the next level of review would examine if the NIS gained entry to the niche by unauthorized releases of pets or an escape from exotic pet breeders. If that reasoning mode yielded no results, the next area of investigation would analyze if the NIS was spread or was introduced into a naïve niche via catastrophic storms (e.g. hurricanes), prevailing winds, or even "hitching a ride" on migrating organisms like birds or insects.

As this approach progresses, it must be noted that several researchers using established BW protocol for analysis of unusual outbreaks provide for an array of indicators that may warn of a deliberate BW attack or deliberate BW development. Many of these protocol points are equally applicable to NIS BW analysis.

Of course, the proper investigation and prevention of a NIS BW attack will involve human intelligence (HUMINT) organizations-both domestic and international. The organizations would need to be vigilant not merely to actual outbreaks (i.e. NIS invasions), but to the attempts to develop NIS BW systems or smuggling operations to import NIS agents for BW development and subsequent use.

One key warning sign of potential NIS BW development would be research into or discovery of genetically engineered NIS (e.g. Black Biology). If NIS were genetically engineered to improve its invasiveness or colonization, this should raise a "red flag" to the intelligence and law enforcement network as well MUST be reported to BTWC (even if no actual BW event has yet occurred).

Finally, along borders of nations (including the US), one of the key challenges to NIS biosecurity is interception of smuggled NIS. Whether by tourist suitcases or air cargo or container ship, the illicit importation can create a powerful challenge in efforts to prevent NIS agents from entry into a nation. Aside of the US, the issue of smuggling is a great concern to many other nations with large scale trade and human travel exchanges-especially in this age of the "global marketplace".

A table reviewing an NIS dispersal mode analysis is provided as a proposed strategy to rule out accidental from deliberate NIS introductions. It is hoped that as more research and better techniques for detection develop, this protocol can be amended for improved efficacy in NIS BW determination.

Furthermore, if NIS can be used as a form of biological warfare, it is worth noting that language in several international conventions and treaties prohibit the use of NIS BW to damage ecosystems, incite disease on plants, food crops, livestock, and humans or act to drive out civilians from land due to the presence of the NIS invasion and colonization (i.e. area denial weapons application).

From detection and determination of a NIS BW, the next step would be further research and expansion of techniques to help develop counterstrategies to NIS BW attacks. One key means to prevent NIS BW attacks is enhanced border or port of entry prevention. Furthermore, improvements in the PIN database which records interceptions of NIS is necessary to enhance research on NIS introductions as well as assisting in preventing NIS entry. Beyond interception of NIS, APHIS as well as international biosecurity agencies must communicate, cooperate, and exchange data (i.e. real-time data exchange) on threats or potential NIS organisms that could result in potential NIS invasions. Also, research must be expanded on NIS potential of organisms via research field testing and coordination of native organisms in foreign lands (e.g. botanical gardens or arboretums) exposed to organisms with the potential to be an NIS in naïve ecosystems. Although national and international databases exist on NIS organisms, enhancements to databases could include listings of the potential invasiveness using ENM as well as genomic mapping of NIS organisms and listing biocontrol organisms and commercial suppliers of such organisms. This would assist authorities in providing tools for rapid response to a detected NIS BW attack.

Although the genomic mapping era is still in its infancy, rapid developments in DNA mapping techniques along with robotic tools will eventually lead to a greater number of NIS genomes that are sequenced. As a result, the genomic database of the NIS will be useful for researchers to study and identify the genetic traits to invasiveness, colonization, novel weapons, and habitat adaptation. This information will provide tools for counterstrategies against NIS BW attacks; perhaps via development of tools for early detection, eradication methods, or halting colonization.

Also, from genetic mapping of NIS organisms, researchers would perhaps be able to determine the origin of NIS species (i.e. nation of origin based on genetic fingerprinting) and be able to determine if the NIS organism was genetically altered and what specific alternations have occurred. In short, the tools for NIS research must be expanded if they are going to help counter future NIS BW attacks.

References

1. Roberge LF (2013) Analysis of Introduced Species as a Form of Biological Weapon: Part 1-Theory and Approaches. Biosafety 2: 107.
2. Sequeira R (1999) "Safeguarding Production Agriculture and Natural Ecosystems against Biological Terrorism: A U.S. Department of Agriculture Emergency Response Framework". In Food and Agricultural Security: Guarding Against Natural Threats and Terrorist Attacks Affecting Health, Annals of the New York Academy of Sciences, New York 894: 48-67.
3. Pearson GS (2001) "The Importance of Distinguishing Between Natural and Other Outbreaks of Disease". Scientific and Technical Means of Distinguishing Between Natural and Other Outbreaks of Disease, the Netherlands: Kluwer Academic Publishers 1-20.
4. Horn FP, Breeze RG (1999) Agriculture and Food Security. In Food and Agricultural Security: Guarding Against Natural Threats and Terrorist Attacks Affecting Health (1st Edn) Annals of the New York Academy of Sciences, New York 894: 9-17.
5. Mack RN (2003) "Global Plant Dispersal, Naturalization, and Invasion: Pathways, Modes, and Circumstance". Invasive Species: Vectors and Management Strategies. Washington, DC: Island Press 3-30.
6. Palm ME, Rossman AY (2003) "Invasion Pathways of Terrestrial Plant-Inhabiting Fungi". Invasive Species: Vectors and Management Strategies, Washington, DC: Island Press 31-43.
7. Meyer DA (2011) "SPECIAL REPORT: Asian Longhorned Beetle." The Entomology and Forest Resources Digital Information Work Group. Invasive. org, Nov 1998.
8. Fofonoff PW, Ruiz GM, Steves B, Carlton JT (2003) In Ships or On Ships? Mechanisms of Transfer and Invasion for Nonnative Species to the Coasts of North America. Invasive Species: Vectors and Management Strategies 152-182.
9. Kiritani K, Yamamura K (2003) Exotic Insects and their Pathways for Invasion. Invasive Species: Vectors and Management Strategies 44-67.
10. Meinesz A (1999) Killer Algae. Chicago: University of Chicago Press.
11. Stoddard G (2011) The Lizard King. Hemispheres Magazine 90-95.
12. Perry ND, Britta H, Winston H, Roel LL, Craig R, et al. (2006) New Invasive Species in Southern Florida: Gambian Rat (Cricetomys Gambianus). Journal of Mammalogy 87: 262-264.
13. Irey M, Gottwald TR, Graham JH, Riley TD, Carlton G (2006) Post-hurricane analysis of citrus canker spread and progress towards the development of a predictive model to estimate disease spread due to catastrophic weather events. Plant Health Progress.
14. Amri A, Yahyaoui A, Nazari K, Hodson (2011) Wheat rust population dynamics and its implication on global wheat production: Case study of Ug99.
15. Witmer GW, Snow NP, Burke PW (2010) Potential attractants for detecting and removing invading Gambian giant pouched rats (Cricetomys gambianus). Pest Manag Sci 66: 412-416.
16. Burridge MJ, Simmons LA, Peter TF, Mahan SM (2002) Increasing Risks of Introduction of Heartwater into the American Mainland Associated with Animal Movements. In The Domestic Animal/Wildlife Interface: Issues for Disease Control, Conservation, Sustainable Food Production, and Emerging Diseases, Annals of the New York Academy of Sciences, New York 969: 269-274.
17. Gottwald TR, Irey M (2007) Post-hurricane analysis of citrus canker II: Predictive model estimation of disease spread and area potentially impacted by various eradication protocols following catastrophic weather events. Plant Health Progress.
18. Food and Agricultural Organization of the United Nations (2008) Wheat Killer Detected In Iran: Dangerous Fungus on the Move from East Africa To The Middle East. Science Daily.
19. Main CE, Keever T, Holmes GJ, Davis JM (2001) Forecasting Long-Range Transport of Downy Mildew Spores and Plant Disease Epidemics. APSnet Features.
20. Main CE, Davis JM (1989) Epidemiology and biometeorology of tobacco blue mold. In: Blue Mold of Tobacco. W. E. McKean, ed. American Phytopathological Society, St. Paul, MN, 201-215.
21. Simberloff D, Betsy VH (1999) Positive Interactions of Nonindigenous Species: Invasional Meltdown? Biological Invasions 1: 21-32.
22. Asner GP, Hughes RF, Vitousek PM, Knapp DE, Kennedy-Bowdoin T, et al. (2008) Invasive plants transform the three-dimensional structure of rain forests. Proc Natl Acad Sci U S A 105: 4519-4523.
23. Ainscough MJ (2002) Next Generation Bioweapons: Genetic Engineering and Biological Warfare. The Gathering Biological Warfare Storm 165-186.
24. Callaway R, Ridenour WM (2004) Novel weapons: Invasive Success and the Evolution of Increased Competitive Ability. Frontiers in Ecology and the Environment 2: 436-443.
25. Callaway RM, Cipollini D, Barto K, Thelen GC, Hallett SG, et al. (2008) Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. Ecology 89: 1043-1055.
26. Kadlec RP (1998) "Biological Weapons for Waging Economic Warfare. BATTLEFIELD OF THE FUTURE: 21st century warfare issues . Ed. Barry R. Schneider, Lawrence E. Grinter. , Maxwell Air Force Base, Alabama: Air University Press, 1998, 251-266.
27. Predicting Invasions of Nonindigenous Plants and Plants Pests (2002) National Research Council. Washington, DC: National Academy Press.
28. (2009) "Additional understandings and agreements reached by review conferences relating to each article of the biological weapons convention". United Nations-The Biological Weapons Convention. United Nations.
29. (2008) "Convention on the Prohibition of Military or Any Other Hostile Use of Environmental Modification Techniques". U.S. Department of State.
30. (2005) "Protocol Additional to the Geneva Conventions of 12 August 1949, and relating to the Protection of Victims of International Armed Conflicts (Protocol I)". International Committee of the Red Cross.
31. Lebeda FJ (1997) Deterrence of biological and chemical warfare: a review of policy options. Mil Med 162: 156-161.
32. McKenzie JR, Kenneth F (2000) "The Revenge of the Melians: Asymmetric Threats and the Next QDR: McNair Paper 6" Air War College, Maxwell air force base, Montgomery, Alabama .
33. (2011) "National Invasive Species Information Center (NISIC)". National Agricultural Library.
34. (2011) "Global Invasive Species Database (GISD)". Invasive Species Specialist Group (ISSG). IUCN Species Survival Commission.
35. Scorza R (1983) "Ecology and Genetics of Exotics." In Exotics, Pests, Plants, and North American Agriculture. New York, Academic Press 219-238.
36. Guzmán E, Romeu A, Garcia-Vallve S (2008) Completely sequenced genomes of pathogenic bacteria: a review. Enferm Infecc Microbiol Clin 26: 88-98.
37. Seshadri R, Read TD (2005) "Genomic Efforts with Biodefense Pathogens." In Biological Weapons Defense: Infectious Diseases and Counterbioterrorism, Humana Press 417-433.
38. Andersson Siv GE, Forsman M (2005) "Genomics for Biodefense: Exploiting the Francisella tularensis Genome Sequence". In Biological Weapons Defense: Infectious Diseases and Counter bioterrorism Humana Press 435-452.
39. Linder LE (2005) "Yersinia pestis as an Emerged Pathogen: What Lessons Can Be Learned?" In Biological Weapons Defense: Infectious Diseases and Counterbioterrorism. Ed. Luther E. Lindler, Frank J. Lebeda, George W. Korch: Totowa, NJ, Humana Press 481-505.
40. Linder LE, Huang, XZ (2005) "Genetic Fingerprinting of Biodefense Pathogens for Epidemiology and Forensic Investigation". In Biological Weapons Defense: Infectious Diseases and Counterbioterrorism. Humana Press 453-480.
41. Schaad NW, Frederick RD, Shaw J, Schneider WL, Hickson R, et al. (2003) Advances in molecular-based diagnostics in meeting crop biosecurity and phytosanitary issues. Annu Rev Phytopathol 41: 305-324.
42. Linder LE, Choffnes E, Korch GW (2005)"Definition and Overview of Emerging Threats." In Biological Weapons Defense: Infectious Diseases and Counterbioterrorism. Ed. Luther E. Lindler, Frank J. Lebeda, George W. Korch: Totowa, NJ, Humana Press 351-360.
43. Black JL 3rd (2003) Genome projects and gene therapy: gateways to next generation biological weapons. Mil Med 168: 864-871.
44. Allen JE, Gardner SN, Slezak TR (2008) DNA signatures for detecting genetic engineering in bacteria. Genome Biol 9: R56.
45. Couch D, Captain USNR (2003) The U.S. Armed Forces Nuclear, Biological and Chemical Survival Manual. New York, NY: Basic Books.